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"RUSSIAN JOURNAL OF TRANSPLANTOLOGY AND ARTIFICIAL ORGANS" – АНГЛОЯЗЫЧНАЯ ВЕРСИЯ «ВЕСТНИКА ТРАНСПЛАНТОЛОГИИ И ИСКУССТВЕННЫХ ОРГАНОВ»

Прошедший 2019 год был отмечен двумя юбилейными датами для «Вестника трансплантологии и искусственных органов»: 20-летием его издания и 25-летием основания В.И. Шумаковым первого в нашей стране профильного журнала по трансплантологии «Трансплантология и искусственные органы», который и был предшественником нашего «Вестника».

В 2019 году мы перешли к качественно новому этапу развития «Вестника…» – изданию полнотекстовых версий на английском языке, которые доступны на интернет-сайте http://journal.transpl.ru.

Проблема трансляции результатов научных исследований для международной аудитории не только не утрачивает, но приобретает все большую актуальность. Одним из путей ее решения является издание журнала на английском языке с публикацией наиболее перспективных исследований ученых из неанглоязычных стран.

Переходу к выпуску нашего журнала на двух языках – русском и английском – предшествовала многолетняя работа, направленная на обеспечение высокого качества научных публикаций, соответствия международным стандартам издания. Включение в существующие системы идентификации, индексации, цитирования началось с 2014 года, с получения так называемого цифрового идентификатора объекта (Digital Object Identifier) – DOI. Далее журнал был включен в интернет-каталог «Directory of Open Access Journals» (DOAJ), индексирующий журналы и представляющий их в открытом доступе, что является актуальным для открытости результатов иссле-

"RUSSIAN JOURNAL OF TRANSPLANTOLOGY AND ARTIFICIAL ORGANS": ENGLISH VERSION

The year 2019 was marked by two jubilee events for the Russian Journal of Transplantology and Artificial Organs (Journal). First was the 20th anniversary of its publication and the second was the 25th anniversary of the foundation of Russia's first profile journal "Transplantology and Artificial Organs" dedicated to transplantology by Valery Shumakov. It was the predecessor of our Journal.

In our pursuit to position the Journal as a driving force in the global transplantation and artificial organ industry, the year 2019 saw us transitioning into a new game-changing phase – the Journal started publishing full-text versions of research papers in English, which are available at http://journal.transpl.ru.

The problem of communicating our research results to an international audience is becoming an ever-pressing challenge. One way to address this problem is to publish the journal papers in English, featuring the most promising research by non-English speaking scientists.

The transition to publishing our journal in two languages, Russian and English, was the result of many years of efforts focused on delivering high quality in scientific papers and ensuring compliance with international publication standards. Inclusion in existing identification, indexing, and citation systems began in 2014 when we obtained our Digital Object Identifier (DOI). The journal was further included in the Directory of Open Access Journals (DOAJ) that indexes and provides access to high quality, open access, peer-reviewed journals. This is crucial for accessibility of re-

дований в международном информационном пространстве. Работа по включению журнала в базу данных Scopus увенчалась успехом в 2016 году.

Среди журналов по направлению «трансплантация», индексируемых в Web of Science, «Вестник трансплантологии и искусственных органов», несмотря на короткий срок пребывания в международной базе, уже занял место в третьем квартиле.

Вскоре мы сможем проанализировать, как издание англоязычной версии отразится на рейтинге нашего «Вестника» в кругу близких по тематике профильных изданий, насколько журнал на английском языке укрепит статус «Вестника» как международного издания и в то же время насколько эффективно позволит представлять исследования неанглоязычных ученых в международном научном сообществе.

Нельзя не признаться, что осуществление такого качественно нового этапа, как переход к двуязычному изданию, требует от всех участников процесса больших усилий и накладывает на нас дополнительную ответственность.

Мы надеемся, что этот проект окажется полезным и будет поддержан коллегами – авторами и читателями.

search results in the global information space. Measures taken to get the journal indexed in Scopus paid off in 2016.

Among transplantation-related journals indexed in Web of Science, our Journal, despite the short period it had stayed in the international database, has already taken a place in the third quartile.

Soon we will be able to analyze how our English publications will reflect on our Journal's rating among other similar specialized publications. We will also see how much the English version of the journal will boost the Journal's status as an international publication, and at the same time, how efficiently possible it would be in presenting the works of non-English-speaking scientists to the international scientific community.

We cannot but admit that moving on to such a new groundbreaking phase – transition to bilingual publication – requires a great deal of effort and commitment from all stakeholders.

We hope that this project will prove useful and be supported by fellow authors and readers.

С уважением
академик РАН С.В. Готье

Sincerely
Academician of the RAS S.V. Gautier

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ORGAN DONATION AND TRANSPLANTATION IN THE RUSSIAN FEDERATION IN 2019

12TH REPORT FROM THE REGISTRY OF THE RUSSIAN TRANSPLANT SOCIETY

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Objective: to monitor current trends and developments in organ donation and transplantation in the Russian Federation based on the 2019 data. **Materials and methods.** Heads of organ transplant centers were surveyed. Data obtained over years from federal subjects of the Russian Federation and from organ transplant centers in the country were analyzed and compared. **Results.** Based on data retrieved from the 2019 Registry, only 46 kidney, 31 liver and 17 heart transplant centers were functioning in Russia. In 2019, there were 6,878 potential recipients in the kidney transplant waitlist. This represents 13.7% of the 50,000 dialysis patients in the country. Donation activity in 2019 reached 5.0 per million population; multi-organ procurement rate was 71.6%; 2.9 organs on average were procured from one effective donor. In 2019, there were 10.0 kidney transplants per million population, 4.0 liver transplants per million population and 2.3 heart transplants per million people. Same year, the number of transplant surgeries performed in Russia rose 10.7% from the previous year. Moscow and Moscow Oblast alone have 13 functioning organ transplantation centers. They account for half of all kidney transplant surgeries and 70% of all liver and heart transplants performed in the country. Organ recipients in the Russian Federation have exceeded 16,000 in number. **Conclusion.** Organ transplantations in Russia keep on increasing – 10–15% per year. Donor and transplant programs are also becoming more effective and efficient. However, the demand for organ transplants far exceeds the current supply of available organs in the Russian Federation. Peculiarities of the development of organ donation and organ transplantation in Russia in 2019 were associated with some factors, such as structure and geographical location of transplant centers, waitlisting of patients, funding sources and amount, and management of donor and transplant programs. The national transplantation registry will be developed taking into account new monitoring and analysis challenges.

Keywords: organ donation, kidney, liver, heart, lung, pancreas transplantation, transplant center, waitlist, registry.

INTRODUCTION

Current trends and developments in organ donation and transplantation in Russia are monitored via the National Registry under the auspices of the organ transplant commission of the Russian Ministry of Health and the Russian Transplant Society. Previous reports have been published in 2009–2019 [1–10].

Information contained in the Registry is sent to the following international registries: International Registry of Organ Donation and Transplantation (IRODaT), Registry of the European Renal Association – European Dialysis and Transplant Association (ERA-EDTA Registry), and Registries of the International Society for Heart and Lung Transplantation (ISHLT Registries).

Since 2016, the National Registry has been serving as a tool for ensuring quality control and data integrity in the information system used for recording donor organs, human tissues, and information about donors and recipients. The information system was created by executive order No. 355n of the Ministry of Health of Russia, dated June 8, 2016.

Annual reports of the Registry contain not only statistical data for the reporting period, but also systems analysis of the data with an assessment of the current situation in transplantology, challenges, trends and prospects for further development in this healthcare sector.

Since 2019, the Registry has also been popularly used for monitoring the implementation of departmental target program "Organ Donation and Transplantation in the Russian Federation", approved via executive order No. 365 of the Russian Ministry of Health, dated June 4, 2019.

Data entered in the Registry is collected via a questionnaire survey of the relevant officials at all transplant centers in the Russian Federation. Data gathered over

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years from Russian regions, transplant centers and from international registries was analyzed and compared.

The task team wishes to express its gratitude to all the regular and new participants in the registry who have provided data, as well as to the Russian Ministry of Health and the Central Research Institute for Health-care Organization and Informatization of the Russian Ministry of Health.

TRANSPLANT CENTERS AND WAITING LISTS

As of December 31, 2019, there were 58 organ transplantation centers functioning in Russia (60 in 2018). Kidney transplant was performed at 46 of these 58 centers, liver transplantation in 31, heart transplantation in 17, pancreatic transplantation in 2, and lung transplantation in 3 centers.

Of the 58 functioning transplantation centers: 21 institutions are subordinate to the federal government, including 13 institutions from the Russian Ministry of Health, 2 from the Russian Ministry of Science and Higher Education, 5 from the Federal Biomedical Agency (FMBA), 1 from the Russian Ministry of Defense, and 37 institutions are run by the federal subjects of the Russian Federation.

The structure of transplantation centers, taking into account their departmental affiliation to kidney, liver and heart transplantation programs in the Russian Federation in 2019 is presented in Fig. 1.

Major contributions to transplantation programs in Russia came from the medical organizations owned by federal subjects of the Russian Federation and national medical research centers of the Russian Ministry of Health.

Medical organizations owned by federal subjects of the Russian Federation account for about 62.7% of the total number of kidney transplants, 53.1% of liver, and 18.5% of heart transplants performed in Russia.

Approximately 21.7% of the total number of kidney transplants, 29.1% of liver transplants, and 76.1% of heart transplants performed in Russia were carried out at national medical research centers of the Russian Ministry of Health.

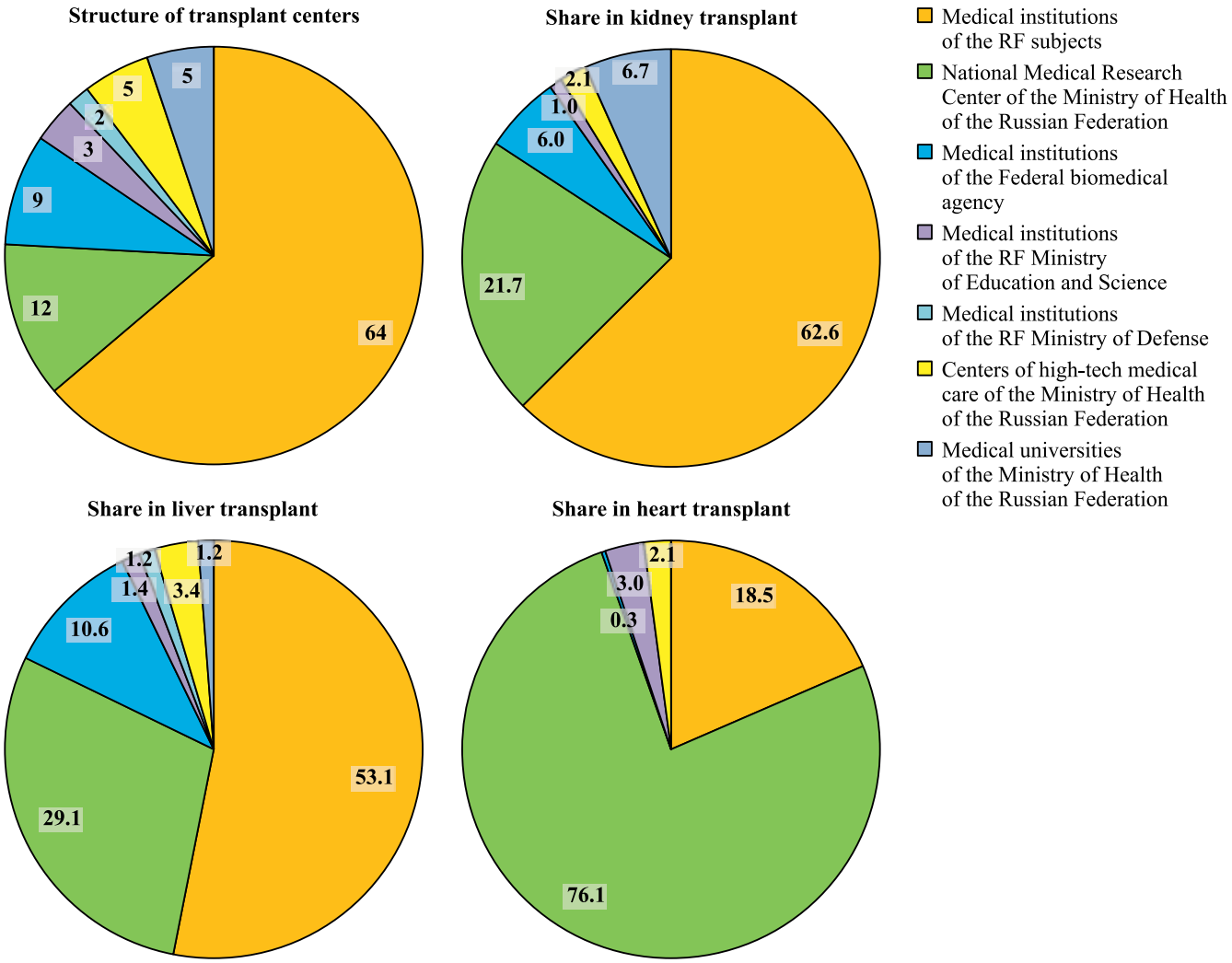

Fig. 1. Contribution of the centers of organ transplantation taking into account their departmental accessory to kidney, liver and heart transplantation programs in the Russian Federation in 2019, %

Other transplant centers in the country accounted for 15.7% of total number of kidney transplants, 17.8% of liver transplants, and 5.4% of all heart transplants performed in Russia.

The 58 transplantation centers operating in the Russian Federation are located in 32 federal subjects of the Russian Federation with a total population of 99.5 million people. Of these centers, 13 are in Moscow and Moscow Oblast, while 7 centers are operating in St. Petersburg and Leningrad Oblast (Fig. 2).

53 federal subjects of the Russian Federation with a population of 47.3 million people do not have any transplant centers on their territory, despite existing need for organ transplantation (primarily patients on renal replacement therapy) and an unused donor organ resource [11].

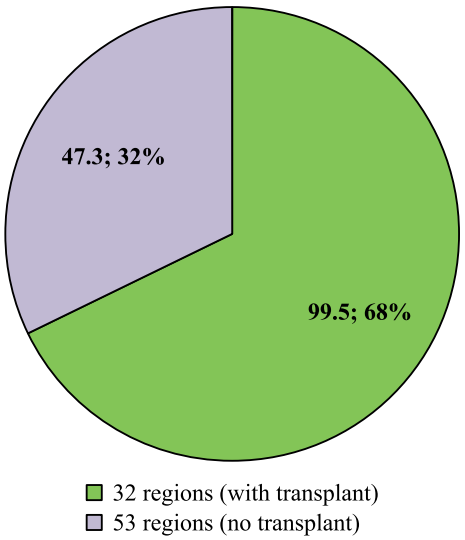

Fig. 2. Geography of the centers of organ transplantation in the Russian Federation in 2019

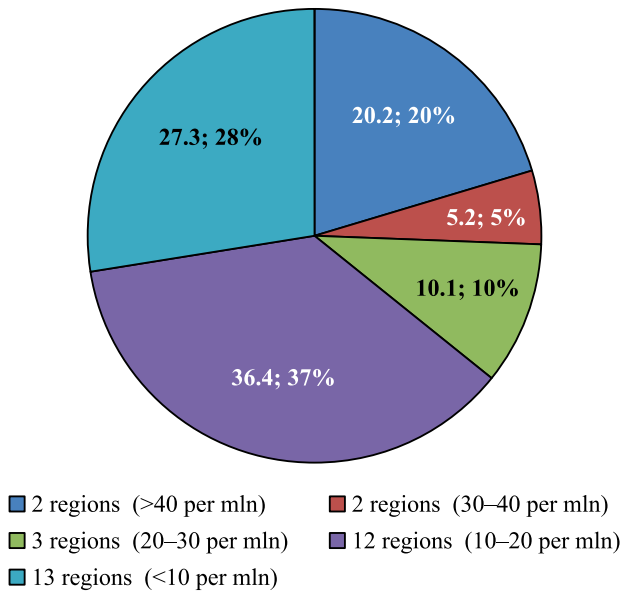

Fig. 3. The population of the Russian Federation living in regions with different availability of medical care for organ transplantation

The transplantation activity of centers located in the federal subjects of the Russian Federation varies considerably. A significant part of the country's population still lives in regions with low accessibility of medical assistance for organ transplantation (Fig. 3).

As the geographical coverage of transplant programs in Russia expands, the vector of managerial decisions aimed at increasing the availability and quality of transplant care for the population will shift from extensive replication of such programs in the federal subjects of the Russian Federation to higher effectiveness of existing programs. Moreover, the potential for extensive replication of transplant programs in the federal subjects of the Russian Federation has not yet been exhausted.

Table 1 presents data on the number of waitlisted transplant candidates at transplant centers.

In 2019, Russia had 6,878 potential recipients on the kidney transplant waiting list, i.e. 13.7% of the total number of patients on hemodialysis and peritoneal dialysis (approximately 50,000). Of these, 2,053 were waitlisted in 2019 for the first time. In Moscow and Moscow Oblast, 2,335 potential recipients (33.9% of waitlisted candidates in the country) were on the kidney transplant waitlist. The waiting list in the Russian Federation had a 0.9% mortality rate (62 patients) in 2019.

There were 2,060 potential recipients on the liver transplant waiting list in 2019; 889 were waitlisted for the first time in 2019. In Moscow and Moscow oblast, 665 potential recipients (32.3% of the waiting list in the country) were included in the liver transplant waitlist. Liver transplant waitlist mortality in Russia in 2019 was 8.2% (170 patients).

There were 789 patients waitlisted for heart transplantation in 2019; 303 of them were included in the waiting list for the first time in 2019. In Moscow, the heart transplantation waiting list had 317 potential recipients (40.2% of the waiting list in the country). Heart transplant waitlist mortality in Russia was 6.7% (53 patients).

The dynamics of waitlist indicators for organ transplantation in Russia from 2012 to 2019 is presented in Table 2.

From 2012 to 2019, the number of patients in the kidney transplant waiting list in Russia increased by almost twice, the liver transplant waiting list increased by 4.2 times, while that of heart transplant increased by 2 times. Meanwhile, the average waiting time for organ transplantation remained unchanged. On the contrary, waitlist mortality fell by 64.0%, 31.1%, and 13.0% for kidney, liver and heart transplants respectively.

Based on data obtained on the number of candidates waitlisted for kidney transplant and on the transplant activity of medical organizations in 2019, the kidney transplant waiting time in the federal subjects of the Russian Federation was calculated (Fig. 4).

Table 1

Waiting lists for organ transplantation in the regions of the Russian Federation in 2019

Federal district, region, population in 2019 (mln)* Transplant	Russian Federation	Central Federal District					Southern Federal District			North Caucasian Federal District	Northwestern Federal District		Siberian Federal District						Urals Federal District				Volga Federal District								Far Eastern Federal District
		Moscow & Moscow Region	Belgorod Oblast	Voronezh Oblast	Ryazan Oblast	Tula Oblast	Krasnodar Kraj	Volgograd Oblast	Rostov Oblast	Stavropol Kraj	Saint Petersburg and Leningrad region	Arkhangelsk Oblast without Nenets Autonomous District	Novosibirsk Oblast	Kemerovo Oblast	Irkutsk Oblast	Omsk Oblast	Altai Kraj	Krasnoyarsk Kraj	Sverdlovsk Oblast	Tyumen Oblast without autonomous districts	Khanty-Mansiisk autonomous district – Yugra	Chelyabinsk Oblast	Samara Oblast	Saratov Oblast	Nizhny Novgorod Oblast	Republic of Tatarstan	Republic of Bashkortostan	Orenburg Oblast	Perm Kraj	Ulyanovsk Oblast	Sakha Republic (Yakutia)
	146.8	**12.6**	**1.5**	**2.3**	**1.1**	**1.5**	**5.6**	**2.5**	**4.2**	**2.8**	**5.4**	**1.1**	**2.8**	**2.7**	**2.4**	**1.9**	**2.3**	**2.9**	**4.3**	**1.5**	**1.7**	**3.5**	**3.2**	**2.4**	**3.2**	**3.9**	**4.0**	**2.0**	**2.6**	**1.2**	**1.0**
		7.6									**1.8**																				
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
KIDNEY																															
Transplant Centers	**46**	12	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1
New patients in the waiting list in 2019	**2053**	806	8	12	29	13	75	25	48	6	260	10	59	72	25	42	22	62	30	12	42	12	61	33	71	78	44	30	26	38	12
Total number of patients in the waiting list in 2019	**6878**	2335	54	111	46	13	393	133	127	6	375	70	146	182	74	102	111	179	253	97	153	150	272	129	502	293	241	104	117	38	72
Patients in the waiting list as of December 31, 2019	**5339**	1584	42	95	32	10	356	110	92	0	262	66	104	109	51	98	90	140	210	68	140	137	226	108	475	254	197	78	112	31	62
Parients in the waiting list died in 2019	**62**	15	4	2	3	1	1	2	1	0	5	0	2	0	1	0	4	2	0	3	1	1	2	2	3	0	4	1	0	2	0
LIVER																															
Transplant Centers	**31**	6	1	0	1	0	2	0	1	1	4	0	1	1	1	1	1	2	1	0	1	1	1	0	1	1	1	0	0	0	1
New patients in the waiting list in 2019	**889**	457	2	0	31	0	22	0	66	2	43	0	35	18	20	5	8	25	29	0	7	4	6	0	44	48	15	0	0	0	2
Total number of patients in the waiting list in 2019	**2060**	665	69	0	31	0	74	0	157	2	247	0	79	72	20	5	44	45	127	0	7	29	14	0	196	62	95	0	0	0	20
Patients in the waiting list as of December 31, 2019	**1288**	238	66	0	5	0	46	0	116	0	201	0	35	59	1	4	42	25	97	0	3	27	2	0	179	41	85	0	0	0	16
Parients in the waiting list died in 2019	**170**	51	0	0	4	0	15	0	24	0	11	0	4	5	5	0	0	5	15	0	0	1	8	0	5	8	6	0	0	0	3

End of table 1

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
HEART																															
Transplant Centers	**17**	4	1	0	0	0	1	0	1	0	1	0	1	1	0	0	1	2	1	0	0	1	0	0	0	1	1	0	0	0	0
New patients in the waiting list in 2019	**303**	133	4	0	0	0	43	0	9	0	34	0	19	16	0	0	7	9	14	0	0	1	0	0	0	8	6	0	0	0	0
Total number of patients in the waiting list in 2019	**789**	317	14	0	0	0	101	0	25	0	70	0	49	67	0	0	9	35	50	0	0	10	0	0	0	16	26	0	0	0	0
Patients in the waiting list as of December 31, 2019	**405**	80	12	0	0	0	88	0	17	0	38	0	25	46	0	0	7	18	34	0	0	8	0	0	0	11	21	0	0	0	0
Parients in the waiting list died in 2019	**53**	10	1	0	0	0	2	0	2	0	7	0	9	11	0	0	0	3	5	0	0	1	0	0	0	1	1	0	0	0	0
PANCREAS																															
Transplant Centers	**3**	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
New patients in the waiting list in 2019	**14**	10	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total number of patients in the waiting list in 2019	**110**	106	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Patients in the waiting list as of December 31, 2019	**100**	97	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parients in the waiting list died in 2019	**0**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
LUNGS																															
Transplant Centers	**3**	2	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
New patients in the waiting list in 2019	**46**	45	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total number of patients in the waiting list in 2019	**80**	79	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0
Patients in the waiting list as of December 31, 2019	**48**	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parients in the waiting list died in 2019	**7**	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		0		0	0	0	0	0	0	0	0

* http://www.gks.ru/free_doc/new_site/population/demo/Popul2019.xls.

Table 2

The indicators connected with the waiting list of organ transplantation in the Russian Federation during the period from 2012 to 2019

Parameter	2012	2013	2014	2015	2016	2017	2018	2019
Number of patients in kidney transplant waiting list	3276	4172	4636	4167	4818	5401	6219	6878
Average waiting time, years	4.4	5.6	5.5	5.5	5.7	5.5	4.6	4.7
Mortality while in the waiting list, %	2.5	3.0	1.2	2.0	1.6	1.4	0.9	0.9
Number of patients in liver transplant waiting list	488	765	949	1062	1260	1535	1830	2060
Average waiting time, years	3.5	5.0	5.4	5.5	5.5	5.0	3.6	3.5
Mortality while in the waiting list, %	11.9	8.8	9.3	10.8	6.7	9.2	8.4	8.2
Number of patients in heart transplant waiting list	399	402	428	434	497	692	823	789
Average waiting time, years	3.0	2.5	2.6	2.4	2.3	2.7	2.9	2.3
Mortality while in the waiting list, %	7.7	12.4	10.5	9.2	7.4	6.1	5.8	6.7

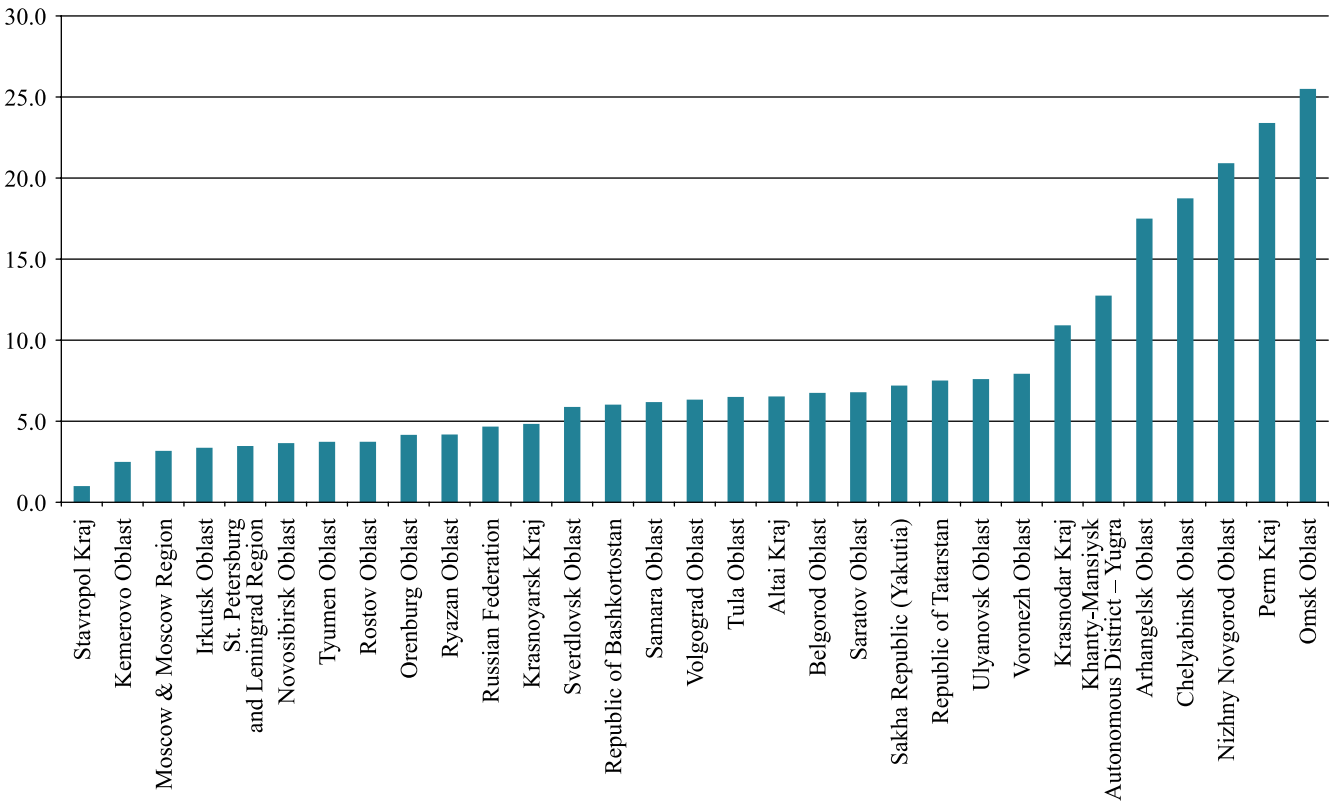

Fig. 4. Estimated waiting period for kidney transplantation in the regions of the Russian Federation in 2019, years

Long waiting times for kidney transplantation typically indicate low activity of donor and transplantation programs in the federal subjects and (or) insufficient work with the waiting list. A long waiting list has quite predictable negative consequences – higher number of patients (recipients) with associated diseases and complications of renal replacement therapy, with additional risks when treating with a kidney transplant, and the cost of such treatment. Moreover, there is significant financial cost for long-term medical and drug support for waitlisted candidates.

Lack of sufficient number of waitlisted candidates is an obstacle to optimal immunological selection of a donor/recipient pair. It poses the risk of not using a suitable organ for transplantation due to absence of a recipient, and also does not allow justifying and planning the need for kidney transplantation medical care.

In 2019, 2427 organ transplants were performed in Russia, or 16.5 per million population; 227 of them were pediatric transplants. In 2018, it was 2193 transplants or 14.9 per million population). See Tables 3 and 4.

Based on data obtained from the Federal Registry for High-Tech Medical Care, 2,119 (87.3%) organ transplant

Table 3

Organ donation and transplantation in the Russian Federation in 2019

Parameter	Qty (abs.)	Per 1 mln*
Organ donation		
Organ donors, total	1062	7,2
Cadaver donors	732	5,0
Live (relative) donors	330	2,2
Organ transplant		
Organs transplanted, total	2427	16,5
of these, in juveniles	227	1,5
Kidney,	1473	10,0
incl. cadaver	1290	8,8
from live donor	183	1,2
of these, in juveniles	101	0,7
Liver,	584	4,0
incl. cadaver	437	3,0
from live donor	147	1,0
of these, in juveniles	113	0,8
Heart	335	2,3
of these, in juveniles	11	0,1
Pancreas	10	0,1
Lungs	23	0,2
of these, in juveniles	2	0,0

* – The RF population in 2019: 146.8 mln (http://www.gks.ru/free_doc/new_site/population/demo/Popul2019.xls).

Table 4

Transplantation activity in the Russian Federation in 2019

No.	Transplant center, region, federal district	Total	Kidney, total	Kidnay, cadaver	Kidney, relative	Liver, tota	Liver, cadaver	Liver, relative	Heart	Pancreas	Lungs	Heart/lungs complex	Small intestine
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	FGBU V.I. Shumakov National Medical Research Center for Transplant and Artificial Organs, RF Ministry of Health, Moscow, Central Federal District	**646**	**240**	172	68	**170**	85	85	**212**	**6**	**16**	**2**	**0**
2	N.A. Lopatkin Research Institute of Urology and Interventional Radiology, branch of FGBU Scientific Medical Research Center for Radiology, RF Ministry of Health, Moscow, Central Federal District	**53**	**53**	39	14	**0**	0	0	**0**	**0**	**0**	**0**	**0**
3	FGBU Russian Children's Clinical Hospital, RF Ministry of Health, Moscow, Central Federal District	**31**	**31**	31	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
4	FGBNU B.V. Petrovsky Russian Scientific Center for Surgery, Moscow, Central Federal District	**23**	**15**	7	8	**8**	0	8	**0**	**0**	**0**	**0**	**0**
5	FGBU A.I. Burnazyan State Scientific Center of the Russian Federation - Federal Medical Biophysical Center, FMBA of Russia, Moscow, Central Federal District	**59**	**14**	11	3	**45**	15	30	**0**	**0**	**0**	**0**	**0**
6	FGBU A.N. Bakulev National Medical Research Center for Cardiovascular Surgery, RF Ministry of Health, Moscow, Central Federal District	**3**	**0**	0	0	**0**	0	0	**3**	**0**	**0**	**0**	**0**

Continuation table 4

1	2	3	4	5	6	7	8	9	10	11	12	13	14
7	FGBU National Medical Research Center for Hematology, RF Ministry of Health, Moscow, Central Federal District	**3**	**3**	3	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
8	FGANU National Medical Research Center for Children's Health, RF Ministry of Health, Moscow, Central Federal District	**23**	**23**	2	21	**0**	0	0	**0**	**0**	**0**	**0**	**0**
9	GBUZ, Moscow S.P. Botkin City Clinical Hospital of the Moscow Healthcare Department, Moscow, Central Federal District	**75**	**50**	50	0	**25**	25	0	**0**	**0**	**0**	**0**	**0**
10	GBUZ, Moscow N.V. Sklifosovsky Research Institute of Emergency Medicine of the Moscow Healthcare Department, Moscow, Central Federal District	**354**	**230**	229	1	**103**	103	0	**12**	**3**	**6**	**0**	**0**
11	GBUZ, Moscow Scientific Research Institute of Emergency Pediatric Surgery and Traumatology of the Moscow Healthcare Department, Moscow, Central Federal District	**3**	**3**	3	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
12	GBUZ MO M.F. Vladimirsky Moscow Regional Research Clinical Institute, Moscow, Central Federal District	**74**	**49**	47	2	**25**	17	8	**0**	**0**	**0**	**0**	**0**
13	FGBU Federal Clinical High MedTech Center of the Federal Medical and Biological Agency, Moscow Region, Central Federal District	**26**	**25**	18	7	**0**	0	0	**1**	**0**	**0**	**0**	**0**
14	GBUZ Belgorod St. Joasaph Regional Clinical Hospital, Belgorod, Central Federal District	**12**	**8**	8	0	**3**	3	0	**1**	**0**	**0**	**0**	**0**
15	BUZ VO Voronezh Regional Clinical Hospital No. 1, Voronezh, Central Federal District	**14**	**14**	14	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
16	GUZ TO Tula Regional Clinical Hospital, Tula, Central Federal District	**2**	**2**	2	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
17	GBU RO Ryazan Regional Clinical Hospital, Ryazan, Central Federal District	**13**	**11**	11	0	**2**	2	0	**0**	**0**	**0**	**0**	**0**
18	GBUZ SK Stavropol Regional Clinical Hospital, Stavropol, North Caucasian Federal District	**8**	**6**	6	0	**2**	2	0	**0**	**0**	**0**	**0**	**0**
19	GBUZ S.V. Ochapovsky Regional Clinical Hospital No. 1, Krasnoyarsk Kraj Department of Healthcare, Krasnodar, Southern Federal District	**58**	**36**	32	4	**11**	11	0	**11**	**0**	**0**	**0**	**0**
20	GBUZ Regional Clinical Hospital No. 2, Krasnoyarsk Kraj Department of Healthcare, Krasnodar, Southern Federal District	**2**	**0**	0	0	**2**	2	0	**0**	**0**	**0**	**0**	**0**
21	GBUZ Volzhsky Regional Uronephrological Center, Volzhsky, Southern Federal District	**21**	**21**	20	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
22	GBU RO Rostov Regional Clinical Hospital, Rostov-on-Don, Southern Federal District	**57**	**34**	34	0	**17**	16	1	**6**	**0**	**0**	**0**	**0**
23	FGBU A.M. Granov Russian Scientific Center for Radiology and Surgical Technologies, RF Ministry of Health, St. Petersburg, Northwestern Federal District	**20**	**0**	0	0	**20**	20	0	**0**	**0**	**0**	**0**	**0**
24	FGBU V.A. Almazov National Medical Research Center, RF Ministry of Health, St. Petersburg, Northwestern Federal District	**25**	**0**	0	0	**0**	0	0	**25**	**0**	**0**	**0**	**0**
25	GBOU VPO I.P. Pavlov Saint Petersburg State Medical University, RF Ministry of Health, St. Petersburg, Northwestern Federal District	**51**	**46**	36	10	**4**	4	0	**0**	**0**	**1**	**0**	**0**

Continuation table 4

1	2	3	4	5	6	7	8	9	10	11	12	13	14
26	GBU I.I. Dzhanelidze Saint Petersburg Research Institute of Emergency Medicine, St. Petersburg, Northwestern Federal District	**46**	**41**	41	0	**4**	4	0	**0**	**1**	**0**	**0**	**0**
27	GBUZ Leningrad Regional Clinical Hospital, St. Petersburg, Northwestern Federal District	**18**	**18**	18	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
28	FGBVOU VO S.M. Kirov Military Medical Academy, St. Petersburg, Northwestern Federal District	**7**	**0**	0	0	**7**	7	0	**0**	**0**	**0**	**0**	**0**
29	SPB GUZ City Mariinsky Hospital, St. Petersburg, Northwestern Federal District	**3**	**3**	3	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
30	GBUZ Arkhangelsk Oblast E.E. Volosevich First City Clinical Hospital, Arkhangelsk, Northwestern Federal District	**4**	**4**	4	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
31	GBU RS(Y) Republican Hospital No. 1 – National Center of Medicine, Yakutsk, Far Eastern Federal District	**11**	**10**	4	6	**1**	1	0	**0**	**0**	**0**	**0**	**0**
32	FGBU E.N. Meshalkin National Medical Research Center, RF Ministry of Health, Novosibirsk, Siberian Federal District	**15**	**0**	0	0	**0**	0	0	**15**	**0**	**0**	**0**	**0**
33	GBUZ NSO State Novosibirsk Regional Clinical Hospital, Novosibirsk, Siberian Federal District	**80**	**40**	36	4	**40**	29	11	**0**	**0**	**0**	**0**	**0**
34	FGBNU Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Siberian Federal District	**10**	**0**	0	0	**0**	0	0	**10**	**0**	**0**	**0**	**0**
35	GBUZ S.V. Belyaev Kemerovo Regional Clinical Hospital, Kemerovo, Siberian Federal District	**73**	**73**	73	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
36	MBUZ M.A. Podgorbunsky City Clinical Hospital, Kemerovo, Siberian Federal District	**11**	**0**	0	0	**11**	11	0	**0**	**0**	**0**	**0**	**0**
37	GBUZ Irkutsk Regional Clinical Hospital, Irkutsk, Siberian Federal District	**36**	**22**	22	0	**14**	14	0	**0**	**0**	**0**	**0**	**0**
38	FGBUZ West Siberian Medical Center, FMBA of Russia, Omsk, Siberian Federal District	**1**	**0**	0	0	**1**	1	0	**0**	**0**	**0**	**0**	**0**
39	BUZOO A.N. Kabanov Omsk City Clinical Hospital No. 1, Omsk, Siberian Federal District	**4**	**4**	4	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
40	KGBUZ Regional Clinical Hospital, Altai Kraj (Barnaul), Siberian Federal District	**21**	**17**	16	1	**2**	2	0	**2**	**0**	**0**	**0**	**0**
41	FGBU Federal Center for Cardiovascular Surgery, Krasnoyarsk, Siberian Federal District	**7**	**0**	0	0	**0**	0	0	**7**	**0**	**0**	**0**	**0**
42	FGBU Federal Siberian Research and Clinical Center of FMBA of Russia, Krasnoyarsk, Siberian Federal District	**29**	**25**	24	1	**4**	4	0	**0**	**0**	**0**	**0**	**0**
43	KGBUZ Regional Clinical Hospital, Krasnoyarsk, Siberian Federal District	**30**	**12**	12	0	**11**	10	1	**7**	**0**	**0**	**0**	**0**
44	GBUZ SO Sverdlovsk Regional Clinical Hospital No. 1, Yekaterinburg, Ural Federal District	**69**	**43**	41	2	**15**	15	0	**11**	**0**	**0**	**0**	**0**
45	GBUZ Chelyabinsk Regional Clinical Hospital, Chelyabinsk, Ural Federal District	**10**	**8**	8	0	**1**	1	0	**1**	**0**	**0**	**0**	**0**
46	GBUZ TO Regional Clinical Hospital No. 1, Tyumen, Ural Federal District	**26**	**26**	26	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
47	BU Regional Clinical Hospital, Khanty-Mansiysk, Ural Federal District	**16**	**12**	10	2	**4**	4	0	**0**	**0**	**0**	**0**	**0**
48	GBOU VPO Samara State Medical University, RF Ministry of Health, Samara, Volga Federal District	**47**	**44**	44	0	**3**	3	0	**0**	**0**	**0**	**0**	**0**

End of table 4

1	2	3	4	5	6	7	8	9	10	11	12	13	14
49	GBOU VPO V.I. Razumovsky Saratov State Medical University, RF Ministry of Health, Saratov, Volga Federal District	**8**	**8**	0	8	**0**	0	0	**0**	**0**	**0**	**0**	**0**
50	GUZ Regional Clinic Hospital, Saratov, Volga Federal District	**11**	**11**	11	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
51	FBUZ Privolzhsky District Medical Center, FMBA of Russia, Nizhny Novgorod, Volga Federal District	**36**	**24**	22	2	**12**	9	3	**0**	**0**	**0**	**0**	**0**
52	GAUZ Republican Clinical Hospital, Ministry of Health, Republic of Tatarstan, Kazan, Volga Federal District	**52**	**39**	30	9	**13**	13	0	**0**	**0**	**0**	**0**	**0**
53	GAUZ Interregional Clinical Diagnostic Center, Kazan, Volga Federal District	**4**	**0**	0	0	**0**	0	0	**4**	**0**	**0**	**0**	**0**
54	GBUZ G.G. Kuvatov Republican Clinical Hospital, Ufa, Volga Federal District	**44**	**40**	40	0	**4**	4	0	**0**	**0**	**0**	**0**	**0**
55	GBUZ Republican Cardiology Outpatient Clinic, Ufa, Volga Federal District	**7**	**0**	0	0	**0**	0	0	**7**	**0**	**0**	**0**	**0**
56	GBUZ PK Perm Regional Clinical Hospital, Perm, Volga Federal District	**5**	**5**	0	5	**0**	0	0	**0**	**0**	**0**	**0**	**0**
57	GUZ E.M. Chuchkalov Ulyanovsk Regional Clinical Center for Specialized Types of Medical Care, Ulyanovsk, Volga Federal District	**5**	**5**	5	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
58	MBUZ City Clinical Emergency Hospital No. 1, Orenburg, Volga Federal District	**25**	**25**	21	4	**0**	0	0	**0**	**0**	**0**	**0**	**0**
Всего за 2019 год		**2427**	**1473**	**1290**	**183**	**584**	**437**	**147**	**335**	**10**	**23**	**2**	**0**

surgeries were performed in 2019 using funds from the compulsory medical insurance system, allocated for provision of high-tech medical care on organ transplant (there were 1732 transplant surgeries (79.0%) in 2018). See Fig. 5.

Since 2010, when funding was included in the Registry as an indicator, the number of organ transplants performed using the funds allocated for provision of high-tech medical care on organ transplant has increased 2.7 times. At the same time, the share of organ transplants performed using these funds has increased by 29.0%.

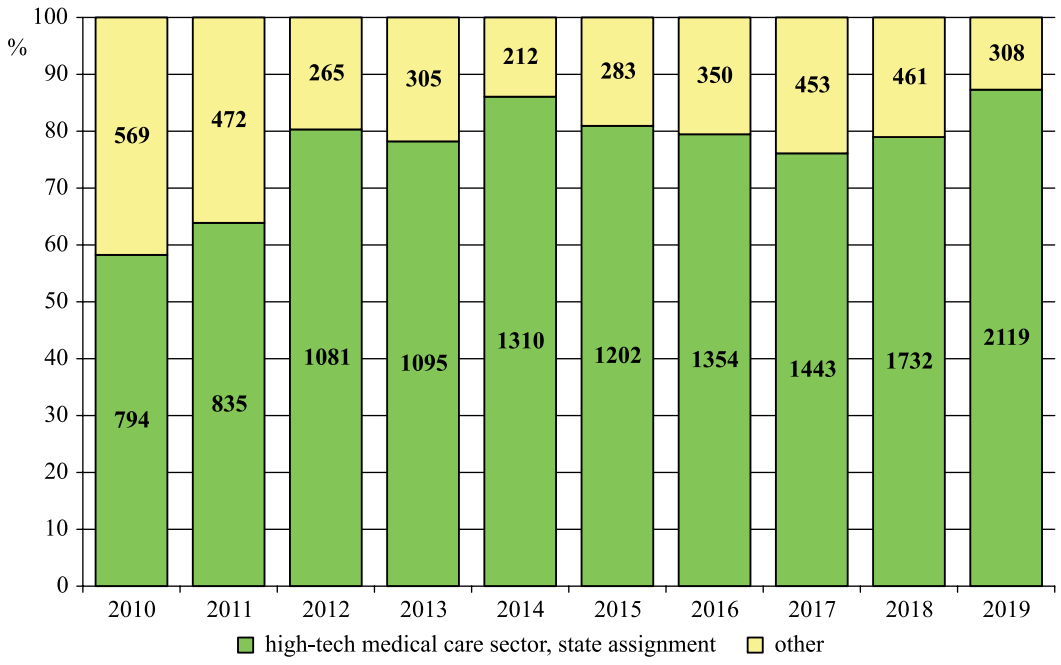

Fig. 5. Financing of transplantation in the Russian Federation in 2010–2019

In 2019, 56 (96.5%) of the 58 transplant centers participated in implementation of state assignment on provision of high-tech medical care for organ transplant.

The financial cost per unit of high-tech medical care for transplantation in 2019 was:

– 923,200 rubles for kidney, pancreas, kidney-pancreas, small bowel, lung transplants;
– 1,171,200 rubles for heart and liver transplants;
– 1,673,420 rubles for heart-lung transplants.

(Resolution No. 1506 of the Government of the Russian Federation, dated December 10, 2018).

ORGAN DONATION

In 2019, donor programs were implemented in 31 (out of 85) federal subjects of the Russian Federation with a population of 96.9 million people. In Perm Oblast, only living-related donor kidney transplants were performed.

There were a total of 732 effective deceased donors in 2019, or 5.0 per million population, which is 93 more donors than in 2018 (639). See Table 5.

In 2019, 43.4% (318) of effective donors in the country came from Moscow and Moscow Oblast alone. Last year's figure was 44.7% (286).

Donor activity per population of federal subjects implementing donor programs (96.9 million) reached 7.5 per 1 million population.

The highest donor activity was recorded in Moscow (22.0), Kemerovo Oblast (14.8), Ryazan Oblast (11.8), St. Petersburg (9.8), Tyumen Oblast (8.7), Novosibirsk Oblast (8.2), Samara Oblast (7.8), and Irkutsk Oblast (6.7). Low donor activity in 2019 was noted in Omsk Oblast (1.1; amid recession), Chelyabinsk Oblast (1.1; recession), Stavropol Krai (1.1; beginning of the program), and Tula Oblast (1.3; beginning of the program). See Table 6.

In 2019, the donor programs of federal subjects were multidirectional in nature (See Table 7).

In 17 federal subjects, effective donors increased in 2019 to 146 donors. Donor activity had a major increase in Moscow (+59), St. Petersburg (+19), Ryazan Oblast (+11), Republic of Tatarstan (+11), Kemerovo donors (+10), and Irkutsk donors (+9).

In 5 federal subjects, effective donors decreased to 48 in 2019. A considerable fall in organ donor activity was observed in Moscow Oblast (–27), Krasnoyarsk Krai, including the FMBA program (–11), and Leningrad Oblast (–8).

In 2019, the practice of brain death pronouncement continued to expand in Russia. There were 692 effective brain-dead donors (601 in 2018) – 94.5% (94.0% in 2018) of the total pool of effective donors. See Fig. 6.

In 25 federal subjects of the Russian Federation, organ donor programs worked only with brain-dead donors (24 federal subjects participated in 2018). At the same time, there were no organ donor programs in the country that did not follow the guidelines for determining human death based on brain death diagnosis.

The low proportion of brain-dead donors in the donor program of Kemerovo Oblast, 47.5% (36.7% in 2018), is not consistent with the modern level of technology development. Moreover, it hampers efficient use of donor resources. This therefore needs to be significantly corrected through targeted implementation and supervision of implementation of the guidelines for determining brain death.

A total of 524 multi-organ procurements were done in 2019, which is more than the 430 recorded in changed from 2017 to 2018. Multi-organ procurements accounted for 71.6% (67.3% in 2018).

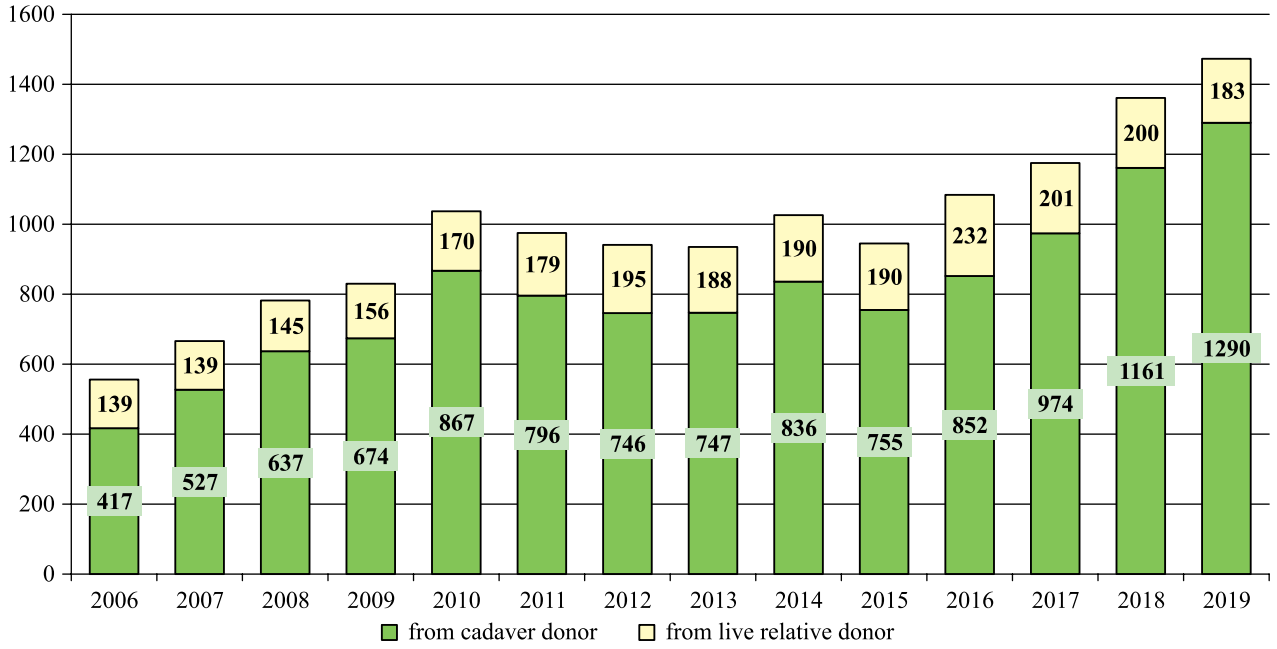

Fig. 6. Structure of effective donors in the Russian Federation in 2006–2019

Table 5

The indicators connected with the organ donation activity in the regions of the Russian Federation in 2019

Nos.	Region	Organ Donation Coordination Center, region	Population (mln)	Number of donor bases	Effective donors (abs., per 1 mln)		incl. with diagnosed brain death (abs., %)		incl. multi-organ donors (abs., %)		Organs prepared, total	incl. kidneys	organs / donors ratio	prepared kidneys, %
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Moscow	Moscow Coordination Center for Organ Donationa, Moscow (GBUZ, Moscow S.P. Botkin City Clinical Hospital of the Moscow Healthcare Department)	12.6	17	277	22.0	273	98.6	229	82.7	893	507	3.2	91.5
2	Moscow Region	GBUZ MO M.F. Vladimirsky Moscow Regional Research Clinical Institute, Moscow	7.5	33	41	5.5	39	95.1	34	82.9	140	72	3.4	87.8
3	Belgorod Oblast	GBUZ Belgorod St. Joasaph Regional Clinical Hospital, Belgorod	1.5	1	4	2.7	4	100.0	4	100.0	12	8	3.0	100.0
4	Voronezh Oblast	BUZ VO Voronezh Regional Clinical Hospital No. 1, Voronezh	2.3	10	8	3.5	8	100.0	2	25.0	18	14	2.3	87.5
5	Tula Oblast	GUZ TO Tula Regional Clinical Hospital, Tula	1.5	1	2	1.3	2	100.0	2	100.0	5	4	2.5	100.0
6	Ryazan Oblast	GBU RO Ryazan Regional Clinical Hospital, Ryazan, Central Federal District	1.1	1	13	11.8	13	100.0	11	84.6	39	24	3.0	92.3
7	Krasnodar Kraj	GBUZ S.V. Ochapovsky Regional Clinical Hospital No. 1, Department of Healthcare of Krasnodar Kraj, Krasnodar	5.6	2	23	4.1	23	100.0	11	47.8	54	30	2.3	65.2
8	Volgograd Oblast	GBUZ Volga Regional Uronephrological Center, Volzhsky	2.5	11	10	4.0	10	100.0	0	0.0	20	20	2.0	100.0
9	Rostov Oblast	GBU RO Rostov Regional Clinical Hospital, Rostov-on-Don	4.2	1	21	5.0	21	100.0	20	95.2	65	40	3.1	95.2
10	Stavropol Kraj	GBUZ SK Stavropol Regional Clinical Hospital, Stavropol, North Caucasus Federal District	2.8	1	3	1.1	3	100.0	3	100.0	9	6	3.0	100.0
11	St. Petersburg	Organ and Tissue Donation Center, St. Petersburg(GBU I.I. Dzhanelidze Saint Petersburg Research Institute of Emergency Medicine)	5.4	14	53	9.8	47	88.7	40	75.5	143	86	2.7	81.1
12	Leningrad Oblast	GBUZ Leningrad Regional Clinical Hospital, St. Petersburg	1.8	1	7	3.9	7	100.0	3	42.9	16	12	2.3	85.7
13	Arkhangelsk Oblast	GBUZ E.E. Volosevich Arkhangelsk Region First City Clinical Hospital, Arkhangelsk, Northwestern Federal District	1.1	1	5	4.5	5	100.0	4	80.0	12	8	2.4	80.0
14	Novosibirsk Oblast	GBUZ NSO State Novosibirsk Regional Clinical Hospital, Novosibirsk	2.8	10	23	8.2	23	100.0	22	95.7	65	38	2.8	82.6
15	Kemerovo Oblast	GBUZ S.V. Belyaev Kemerovo Regional Clinical Hospital, Kemerovo	2.7	15	40	14.8	19	47.5	16	40.0	100	70	2.5	87.5
16	Irkutsk Oblast	GBUZ Irkutsk Regional Clinical Hospital, Irkutsk	2.4	1	16	6.7	16	100.0	14	87.5	36	22	2.3	68.8
17	Omsk Oblast	BUZOO A.N. Kabanov Omsk City Clinical Hospital No. 1, Omsk	1.9	2	2	1.1	2	100.0	2	100.0	6	4	3.0	100.0
18	Altai Kraj	KGBUZ Regional Clinical Hospital, Barnaul	2.3	1	8	3.5	8	100.0	8	100.0	26	16	3.3	100.0

End of table 5

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
19	Krasnoyarsk Kraj	KGBU Krasnoyarsk Clinical Hospital, Krasnoyarsk	2.9	12	13	4.5	13	100.0	11	84.6	33	17	2.5	65.4
20	Sverdlovsk Oblast	GBUZ SO Sverdlovsk Regional Clinical Hospital No. 1, Yekaterinburg	4.3	8	24	5.6	24	100.0	20	83.3	69	43	2.9	89.6
21	Chelyabinsk Oblast	GBUZ Chelyabinsk Regional Clinical Hospital, Chelyabinsk	3.5	1	4	1.1	4	100.0	2	50.0	11	8	2.8	100.0
22	Tyumen Oblast	GBUZ TO Regional Clinical Hospital No. 1, Tyumen	1.5	1	13	8.7	13	100.0	5	38.5	31	26	2.4	100.0
23	Khanty-Mansi Autonomous Okrug – Yugra	BU Regional Clinical Hospital, Khanty-Mansiysk	1.7	8	5	2.9	5	100.0	4	80.0	14	10	2.8	100.0
24	Samara Oblast	GBOU VPO Samara State Medical University, RF Ministry of Health and Social Development, Samara	3.2	5	25	7.8	19	76.0	5	20.0	52	46	2.1	92.0
25	Saratov Oblast	GUZ Regional Clinical Hospital, Saratov	2.4	1	10	4.2	10	100.0	1	10.0	21	20	2.1	100.0
26	Nizhny Novgorod Oblast	FBUZ Privolzhsky District Medical Center, FMBA of Russia, Nizhny Novgorod	3.2	9	12	3.8	12	100.0	12	100.0	32	22	2.7	91.7
27	Republic of Tatarstan	GAUZ Republican Clinical Hospital, Ministry of Health of the Republic of Tatarstan, Kazan	3.9	2	15	3.8	15	100.0	13	86.7	47	30	3.1	100.0
28	Republic of Bashkortostan	GBUZ G.G. Kuvatov Republican Clinical Hospital, Ufa	4.1	12	24	5.9	24	100.0	7	29.2	51	40	2.1	83.3
29	Orenburg Oblast	MBUZ City Clinical Emergency Hospital No. 1, Orenburg	2.0	2	11	5.5	11	100.0	7	63.6	28	21	2.5	95.5
30	Sakha Republic (Yakutia)	GAU RS(Y) Republican Hospital No. 1 – National Center of Medicine, Yakutsk	1.0	1	3	3.0	3	100.0	1	33.3	5	4	1.7	66.7
31	Departmental program of FMBA of Russia	FGBU GNTs A.I. Burnazyan Federal Medical Biophysical Center, FMBA of Russia, Moscow	–	28	1	–	1	100.0	1	100.0	4	2	4.0	100.0
32	Departmental program of FMBA of Russia	FGBU Federal Siberian Research Clinical Center, FMBA of Russia, Krasnoyarsk	–	5	16	–	15	93.8	10	62.5	42	24	2.6	75.0
		Total	**146.8**	**218**	**732**	**5.0**	**692**	**94.5**	**524**	**71.6**	**2099**	**1294**	**2.9**	**88.4**

Table 6

Rating of regions donor activity in 2019

RF subject (region)	Population in 2019, mln	Effective donors per 1 mln population		Rating	
		2019	2018	2019	2018
Moscow	12.6	22.0	17.3	1	1
Kemerovo Oblast	2.7	14.8	11.1	2	2
Ryazan Oblast	1.1	11.8	1.8	3	25
St. Petersburg	5.4	9.8	6.3	4	7
Tyumen Oblast	1.5	8.7	8.7	5	4
Novosibirsk Oblast	2.8	8.2	6.1	6	8
Samara Oblast	3.2	7.8	7.2	7	6
Irkutsk Oblast	2.4	6.7	2.9	8	22
Republic of Bashkortostan	4.1	5.9	4.9	9	11
Sverdlovsk Oblast	4.3	5.6	5.6	10	9
Orenburg Oblast	2.0	5.5	4.0	11	14
Mosow Region	7.5	5.5	9.1	12	3
Rostov Oblast	4.2	5.0	4.5	13	12
Arkhangelsk Oblast	1.1	4.5	4.5	14	13
Krasnoyarsk Kraj*	2.9	4.5	5.5	15	10
Saratov Oblast	2.4	4.2	3.3	16	21
Krasnodar Kraj	5.6	4.1	3.6	17	18
Volgograd Oblast	2.5	4.0	3.6	18	17
Leningrad Oblast	1.8	3.9	8.3	19	5
Republic of Tatarstan	3.9	3.8	1.0	20	28
Nizhny Novgorod Oblast	3.2	3.8	3.8	21	16
Voronezh Oblast	2.3	3.5	3.5	22	20
Altai Kraj	2.3	3.5	3.5	23	19
Sakha Republic (Yakutia)	1.0	3.0	4.0	24	15
Khanty-Mansi Autonomous Okrug – Yugra	1.7	2.9	2.4	25	24
Belgorod Oblast	1.5	2.7	2.7	26	23
Tula Oblast	1.5	1.3	–	27	–
Chelyabinsk Oblast	3.5	1.1	1.1	28	27
Stavropol Kraj	2.8	1.1	0.7	29	29
Omsk Oblast	1.9	1.1	1.6	30	26
Russia (85 RF subjects)	146.8	5.0	4.3	–	–

Note. Without taking into account the donor program Federal Siberian Scientific and Clinical Center of the Federal Medical and Biological Agency, Krasnoyarsk

In Moscow and Moscow Oblast, there were 263 multi-organ donors, which is 50.2% of the total number of multi-organ donors in Russia (239 donors, 56.2% in 2017).

There were 18 organ donor programs involving a high share of multi-organ procurements (more than 70%). In 6 of the programs, multiple organs were procured from all (100%) the patients.

In 2019, the average number of organs procured from one donor remained the same with that of 2018 – 2.9 procurements. The highest number of organ procurements were, as before, performed at federal subjects where extrarenal organs were transplanted and (or) at federal subjects where there was interregional coordination: Moscow Oblast (3.4), Altai Krai (3.3), Moscow (3.2), Rostov Oblast (3.1), and Republic of Tatarstan (3.1). There was low procurement in the Republic of Sakha (Yakutia) (1.7) and in Volgograd Oblast (2.0)).

In 2019, the rate of procurement and use of donor kidneys was 88.4% (in 2018 – 91.9%). In 17 federal subjects this indicator was in the optimal range of 90–100%, in 9 regions between 80–90% and in 4 programs it was less than 80% (Krasnodar Krai (65.2%), Irkutsk Oblast (68.8%), Krasnoyarsk Krai (65.4%), Republic of Sakha (Yakutia) (66.7%)).

In 2019, 330 organs were procured from living related donors – 31.1% of 1062 (the total number of procurements). In 2018, there were 364 procured organs or 36.3% of 1003).

Table 7

Deceased organ donors (effective donors) in 2006–2019

Nos.	Region	2006	2007		2008		2009		2010		2011		2012		2013		2014		2015		2016		2017		2018		2019	
		Efefctive donors	Efefctive donors	Change per year (abs.)	Efefctive donors	Change per year (abs.)	Efefctive donors	Change per year (abs.)	Efefctive donors	Change per year (abs.)	Efefctive donors	Change per year (abs.)	Efefctive donors	Change per year (abs.)	Efefctive donors	Change per year (abs.)	Efefctive donors	Change per year (abs.)	Efefctive donors	Change per year (abs.)	Efefctive donors	Change per year (abs.)	Efefctive donors	Change per year (abs.)	Efefctive donors	Change per year (abs.)	Efefctive donors	Change per year (abs.)
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
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
2	Moscow Region	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
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
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
5	Tula Oblast																										2	+2
6	Ryazan Oblast																								2	+2	13	+11
7	Krasnodar Kraj						3	+3	39	+36	52	+13	42	–10	41	–1	23	–18	25	+2	24	–1	19	–5	20	+1	23	+3
8	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
9	Rostov Oblast																		1	+1	7	+6	13	+6	19	+6	21	+2
10	Stavropol Kraj																								2	+2	3	+1
11	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
12	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
13	Arkhangelsk Oblast																								5	+5	5	0
14	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	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
16	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
17	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
18	Altai Kraj														3	+3	5	+2	4	–1	4	0	8	+4	8	0	8	0
19	Krasnoyarsk Kraj																3	+3	6	+3	18	+12	27	+9	16	прим.	13	–3
20	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

End of table 7

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
21	Chelyabinsk Oblast								6	+6	2	–4	7	+5	6	–1	10	+4	9	–1	11	+2	8	–3	4	–4	4	0
22	Tymen Oblast																						4	+4	13	+9	13	0
23	Khanty-Mansi Autonomous Okrug – Yugra																						3	+3	4	+1	5	+1
24	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
25	Saratov Oblast														4	+4	7	+3	7	0	7	0	7	0	8	+1	10	+2
26	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
27	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
28	Republic of Bashkortostan								2	+2	7	+5	14	+7	18	+4	19	+1	14	+5	20	+6	22	+2	20	–2	24	+4
29	Orenburg Oblast																		3	+3	8	+5	9	+1	8	–1	11	+3
30	Sakha Republic (Yakutia)																				2	+2	4	+2	4	0	3	–1
31	FMBA, Moscow														6	+6	11	+5	14	+3	16	+2	9	–7	5	–4	1	–4
32	FMBA, Krasnoyarsk																								24	прим.	16	–8
	RF total	**225**	**300**	**+75**	**364**	**+64**	**381**	**+17**	**487**	**+106**	**470**	**–17**	**412**	**–58**	**420**	**+8**	**465**	**+45**	**434**	**–31**	**499**	**+53**	**564**	**+65**	**638**	**+74**	**732**	**+94**

Note. Donor activity of the donor program of the Federal Siberian Scientific and Clinical Center of the Federal Medical and Biological Agency, Krasnoyarsk is presented as a separate program.

KIDNEY TRANSPLANTATION

A total of 1,473 kidney transplantations were performed (10.0 per million population) in 2019, which is more than in previous years. See Fig. 7.

Kidney transplant surgeries were performed at 46 centers.

There were 1,290 deceased-donor kidney transplants in 2019, which is 129 (+11.1 %) more transplants than in 2018 (1,161). There were 183 living-related donor kidney transplants in 2019 (200 in 2018).

Table 8 and Fig. 8 show the kidney transplant centers where the highest number of kidney transplants were done as of the end of 2019.

The activity of kidney transplant centers in 2019 varied widely. Five centers performed over 50 transplant surgeries each, 11 centers conducted 30 to 50 operations within the year, 11 centers carried out 15 to 29 surgeries,

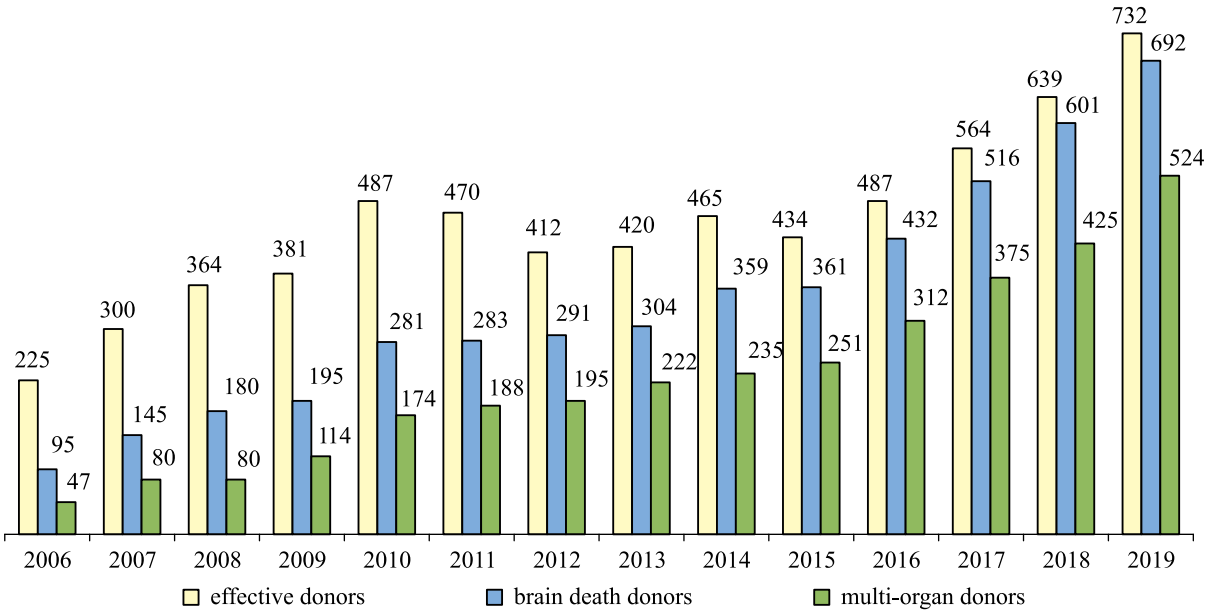

Fig. 7. Kidney transplantation in the Russian Federation in 2006–2019

Table 8

The medical organizations – leaders in number of transplantations of a kidney

Rank	Centers leading in numbers of kidney transplant	Kidney transplants in 2019
1	FGBU V.I. Shumakov National Medical Research Center for Transplant and Artificial Organs, RF Ministry of Health, Moscow, Central Federal District	240
2	GBUZ, Moscow, N.V. Sklifosofsky Research Institute of Emergency Medicine, Moscow Healthcare Department, Moscow, Central Federal District	230
3	GBUZ S.V. Belyaev Kemerovo Regional Clinical Hospital, Kemerovo, Siberian Federal District	73
4	N.A. Lopatkin Research Institute of Urology and Interventional Radiology – branch of FGBU Scientific Medical Research Center for Radiology, RF Ministry of Health, Moscow, Central Federal District	53
5	GBUZ, Moscow S.P. Botkin City Clinical Hospital, Moscow Healthcare Department, Moscow, Central Federal District	50
6	GBUZ MO M.F. Vladimirsky Moscow Regional Research Clinical Institute, Moscow, Central Federal District	49
7	GBOU VPO I.P. Pavlov Saint Petersburg State Medical University, RF Ministry of Health, St. Petersburg, Northwestern Federal District	46
8	GBOU VPO Samara State Medical University, RF Ministry of Health, Samara, Volga Federal District	44
9	GBUZ SO Sverdlovsk Regional Clinical Hospital No. 1, Yekaterinburg, Ural Federal Distric	43
10	GBU I.I. Dzhanelodze Saint Petersburg Research Institute of Emergency Medicine, St. Petersburg, Northwestern Federal District	41
	TOTAL	**869**
	59.0% of total kidney transplants in the RF (1473)	

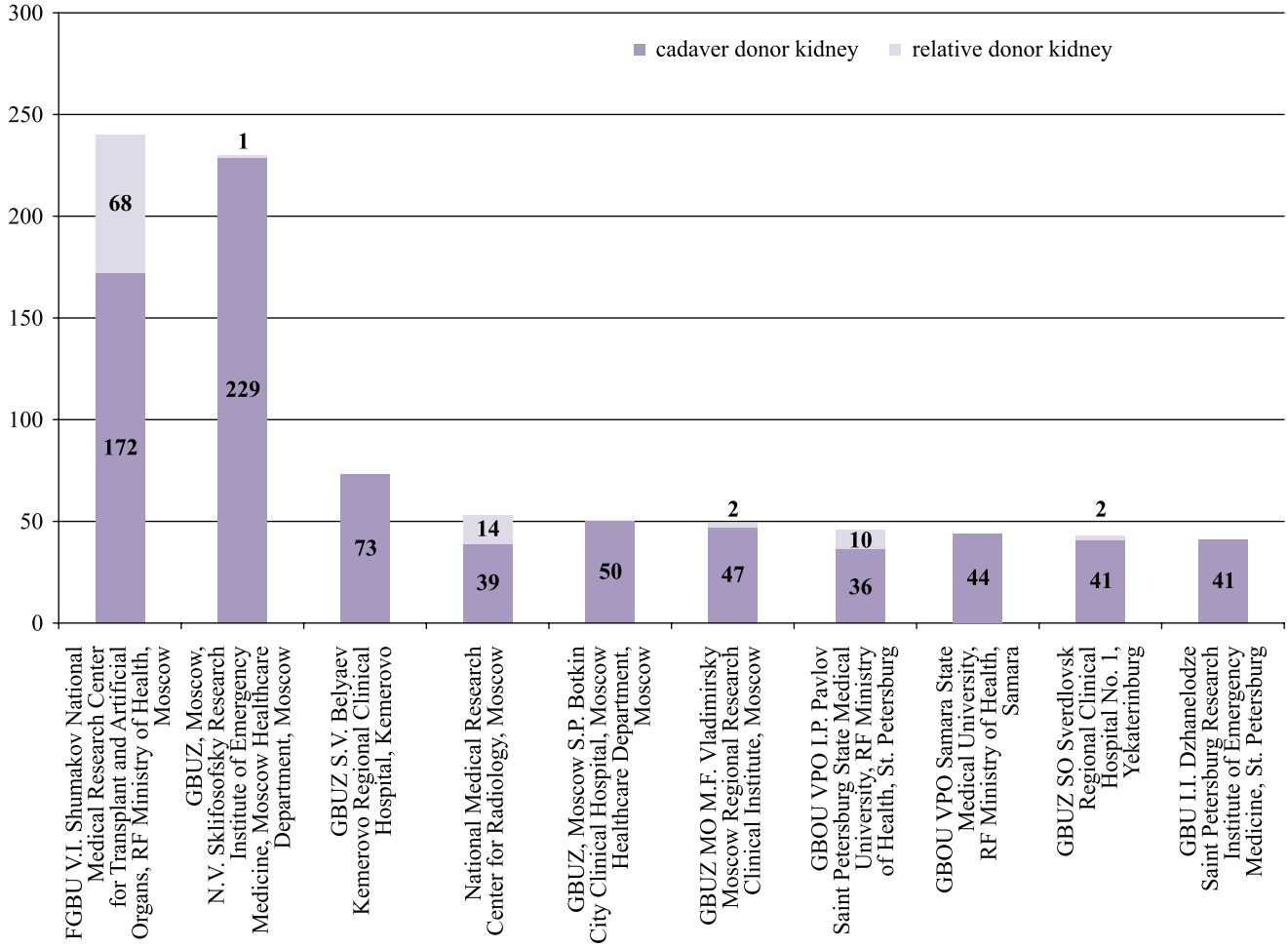

Fig. 8. The medical organizations – leaders in number of transplantations of a kidney

while the remaining 19 centers performed less than 15 kidney transplant surgeries.

All the 12 kidney transplant centers located in Moscow and Moscow Oblast performed half (50.0%, 736 surgeries) of all kidney transplantations performed in the country (50.3%, 685 surgeries in 2018).

Of these, 4 centers carried out 50 or more kidney transplants within the year. The 4 centers were Shumakov National Medical Research Center of Transplantology and Artificial Organs (Shumakov Center) (240 kidney transplants), Sklifosovsky Research Institute of Emergency Care (Sklifosovsky Institute) (230), National Medical Research Center for Radiology (53), and Botkin City Clinical Hospital (50).

In 2019, 28 centers out of 46 performed related-donor kidney transplants. A total of 183 transplants were performed (200 in 2018). In the same year, Moscow and Moscow Oblast accounted for 8 centers that performed 124 related kidney transplants or 66.3% of the total number of related kidney transplants in Russia (117 or 58.5% in 2018). Two centers performed 20 or more related kidney transplants – Shumakov Center (68 operations) and the National Medical Research Center for Children's Health (21). The average frequency of living-donor kidney transplants in 2019 was 12.4% of the total number of kidney transplants performed (14.7% in 2018).

In 2019, 6 centers carried out pediatric kidney transplants. A total of 101 kidney transplants were done (89 in 2018), of which 96 (95.0%) were in Moscow – Shumakov Center (36), Russian Children's Clinical Hospital (31), and National Medical Research Center for Children's Health (23). See Fig. 9.

EXTRARENAL ORGAN TRANSPLANT

In 2019, 335 heart transplants were performed (2.3 per million population), of which 11 were pediatric transplants. This was more than the figure recorded in previous years, particularly in 2018 (282), +18.8%.

Heart transplants were performed at 17 centers.

The Shumakov Center (Moscow) accounted for 63.3% (212 heart transplant surgeries) of the total number of heart transplants in the Russian Federation. The successful heart transplant program in this center, along with new programs, continues to drive the overall positive trend in the increasing number of heart transplants recorded so far in the country from 2009 to 2019.

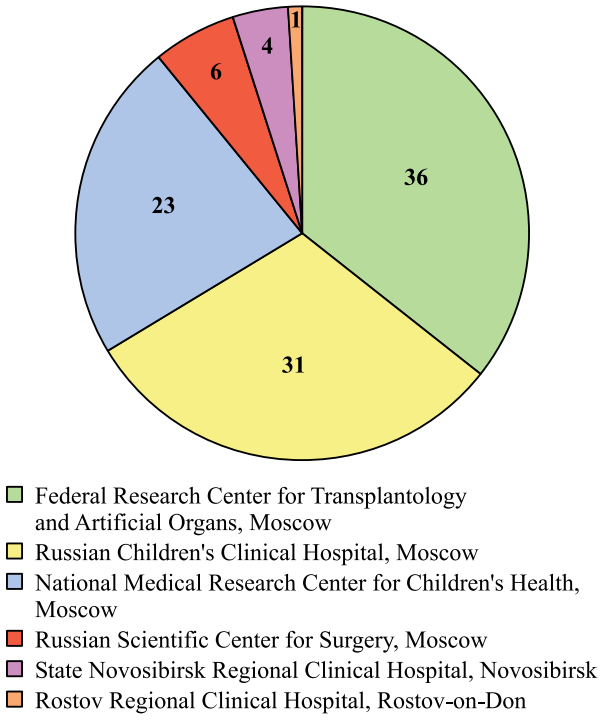

Fig. 9. Pediatric kidney transplantation in the Russian Federation in 2019

Table 9 and Fig. 10 show thoracic organ transplant centers that performed the highest number of heart and lung transplants as of the end of 2019.

Apart from Shumakov Center, 6 other transplantation centers carried out 10 or more heart transplants in 2019. They were the Almazov National Medical Research Centre in St. Petersburg (25 heart transplants), Meshalkin National Medical Research Center in Novosibirsk (15), Sklifosovsky Institute in Moscow (12), Ochapovsky Regional Clinical Hospital No. 1 in Krasnodar (11), Sverdlovsk Regional Clinical Hospital No. 1 (11), and the Research Institute for Complex Problems of Cardiovascular Diseases in Kemerovo (10).

In 2019, lung transplants were performed at 3 transplantation centers. A total of 23 transplants were conducted (25 in 2018), of which 2 were pediatric lung transplantations. They were Shumakov Center (16 lung transplants), Sklifosovsky Institute (6), and Pavlov First St. Petersburg State Medical University in St. Petersburg (1). In 2019, Shumakov Center also performed 2 heart-lung transplants.

In 2019, a total of 584 liver transplants were carried out (4.0 per million population). This was more than in previous years, particularly in 2018 (505), +15.6%.

Liver transplants were performed at 31 centers.

In 2019, two new liver transplant programs were launched – four and two deceased-donor liver transplants were performed at District Clinical Hospital (Khanty-Mansiysk) and Ryazan Regional Clinical Hospital (Ryazan), respectively.

The 6 Moscow-based transplant centers accounted for 64.4% (376 liver transplants) in 2019 (68.7%, 347 transplants in 2018).

Table 10 and Fig. 11 show the liver transplant centers that performed the highest number of liver transplants as of the end of 2019.

In 2019, 7 transplant centers performed 20 or more liver transplants each: Shumakov Center (170), Sklifosovsky Institute (103), Burnazyan Federal Medical and Biophysical Center (45), State Novosibirsk Regional Clinical Hospital (40), Botkin City Clinical Hospital

Table 9

The medical organizations – leaders in number of transplantations of thoracic organs

Rank	Centers leading in numbers of heart transplants	Heart transplants in 2019
1	FGBU V.I. Shumakov National Medical Research Center for Transplant and Artificial Organs, RF Ministry of Health, Moscow, Central Federal District	214
2	FGBU V.A. Almazov National Medical Research Center, RF Ministry of Health, St. Petersburg, Northwestern Federal District	25
3	FGBU E.N. Meshalkin National Medical Research Center, RF Ministry of Health, Novosibirsk, Siberian Federal District	15
4	GBUZ, Moscow, N.V. Sklifosofsky Research Institute of Emergency Medicine, Moscow Healthcare Department, Moscow, Central Federal District	12
5	GBUZ SO Sverdlovsk Regional Clinical Hospital No. 1, Yekaterinburg, Ural Federal Distric	11
6	GBUZ S.V. Ochapovsky Regional Clinical Hospital No. 1, Healthcare Department, Krasnodar Kraj, Krasnodar, Southern Federal District	11
7	FGBNU Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Siberian Federal District	10
8	FGBU Federal Center for Cardiovascular Surgery, Krasnoyarsk, Siberian Federal District	7
9	KGBU Krasnoyarsk Clinical Hospital, Krasnoyarsk, Siberian Federal District	7
10	GBUZ Republican Cardiology Outpatient Clinic, Ufa, Volga Federal District	7
	TOTAL	**319**
	94.7% of the total heart transplants in the RF (337)	

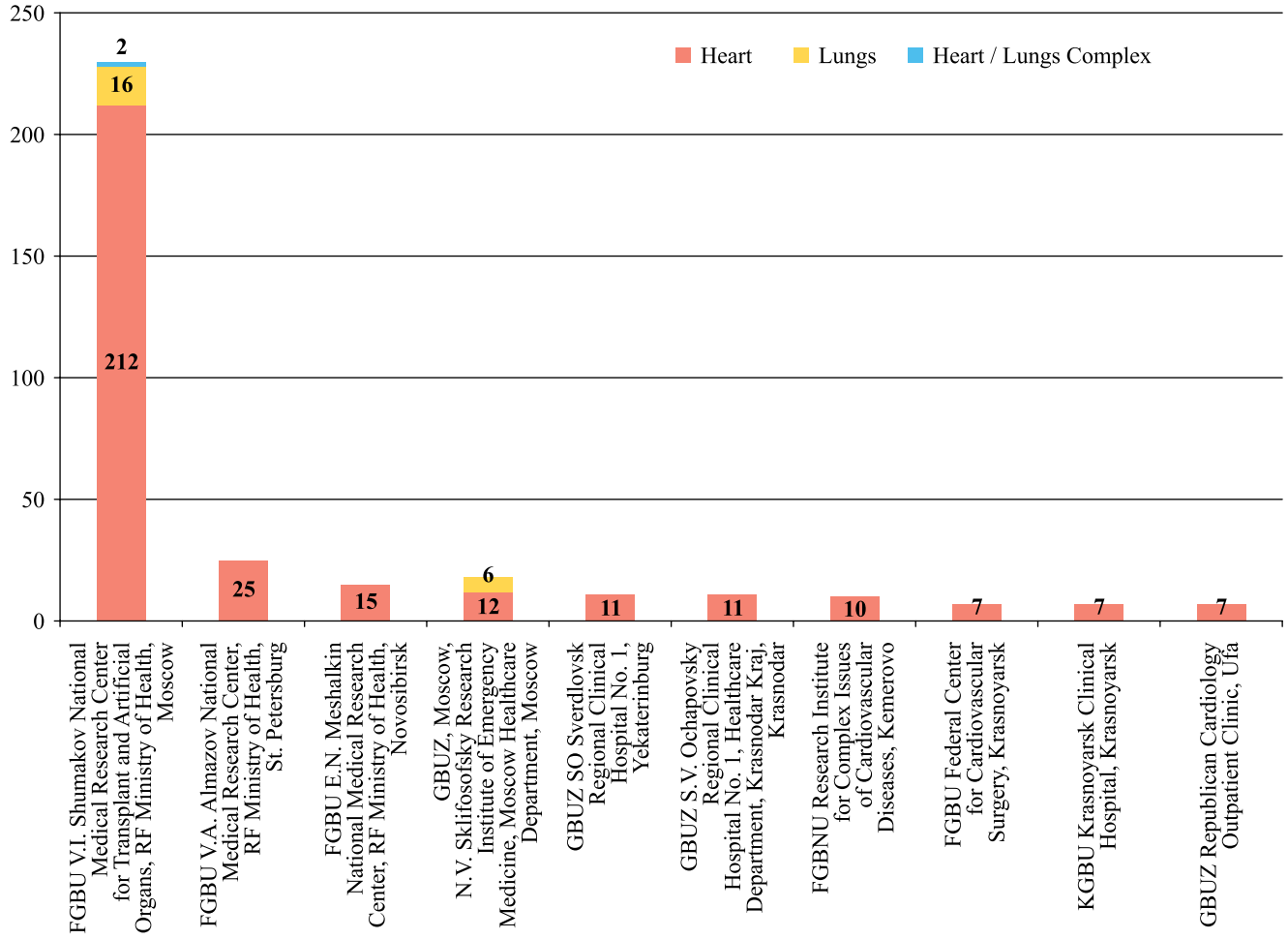

Fig. 10. The medical organizations – leaders in number of transplantations of thoracic organs

Table 10

The medical organizations – leaders in number of transplantations of a liver

Rank	Centers leading in numbers of liver transplants	Liver transplants in 2019
1	FGBU V.I. Shumakov National Medical Research Center for Transplant and Artificial Organs, RF Ministry of Health, Moscow, Central Federal District	170
2	GBUZ, Moscow, N.V. Sklifosofsky Research Institute of Emergency Medicine, Moscow Healthcare Department, Moscow, Central Federal District	103
3	FGBU A.I. Burnazyan State Scientific Center of the Russian Federation – Federal Medical Biophysical Center, FMBA of Russia, Moscow, Central Federal District	45
4	GBUZ NSO State Novosibirsk Regional Clinical Hospital, Novosibirsk, Siberian Federal District	40
5	GBUZ MO M.F. Vladimirsky Moscow Regional Research Clinical Institute, Moscow, Central Federal District	25
6	GBUZ, Moscow S.P. Botkin City Clinical Hospital, Moscow Department of Healthcare, Moscow, Central Federal District	25
7	FGBU Russian Scientific Center for Radiology and Surgical Technologies, RF Ministry of Health, St. Petersburg, Northwestern Federal District	20
8	GBU RO Rostov Regional Clinical Hospital, Rostov-on-Don, Southern Federal District	17
9	GBUZ SO Sverdlovsk Regional Clinical Hospital No. 1, Yekaterinburg, Ural Federal District	15
10	GBUZ Irkutsk Regional Clinical Hospital, Irkutsk, Siberian Federal District	14
	TOTAL	**474**
	81.2% of the total liver transplants in the RF (584)	

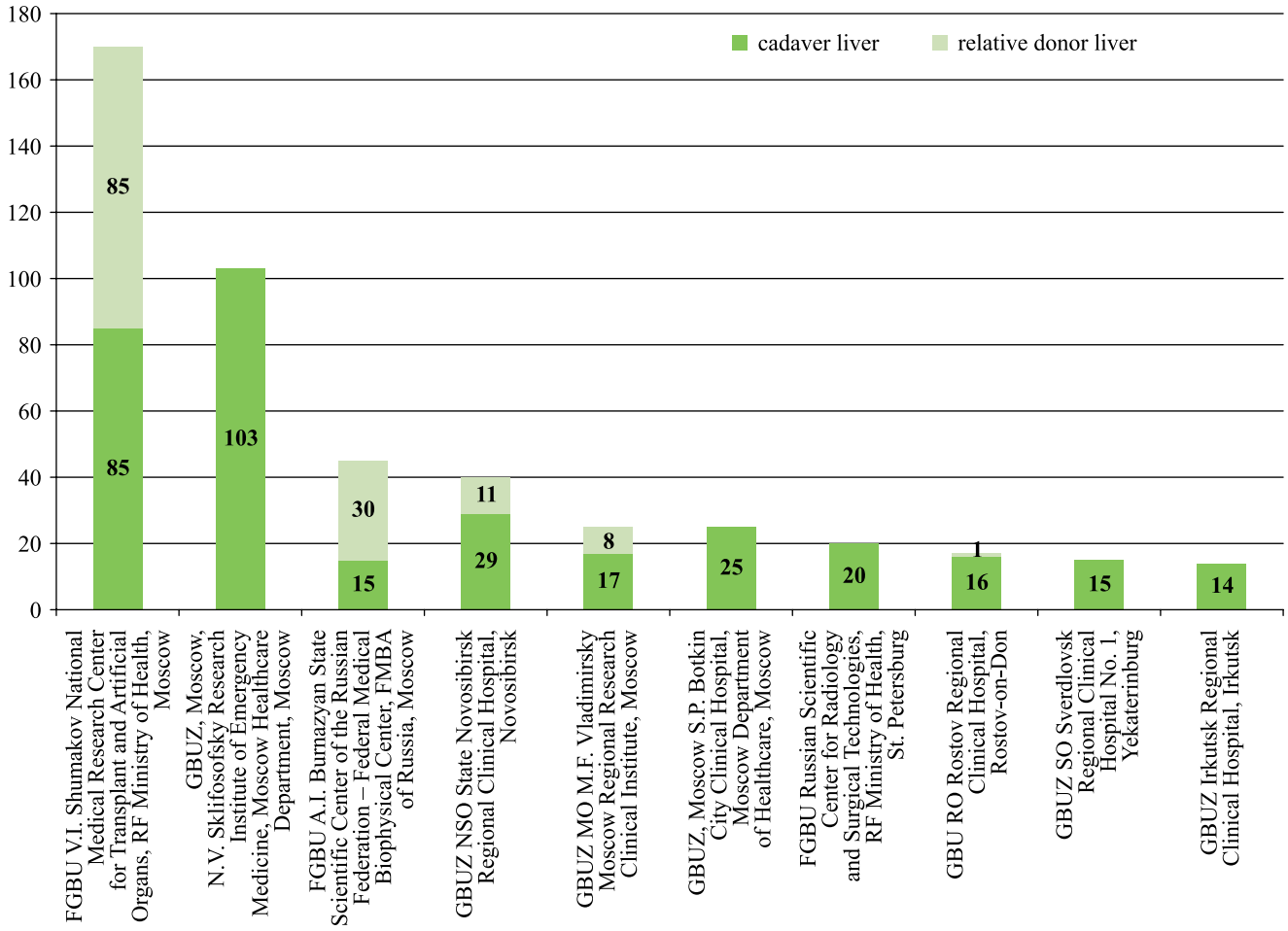

Fig. 11. The medical organizations – leaders in number of transplantations of a liver

(25); Vladimirsky Moscow Regional Research Clinical Institute (25), and the Granov Russian Research Center of Radiology and Surgical Technologies (20).

Related liver transplants were performed at 8 centers. Living-related transplants accounted for 147 surgeries (25.2%). In 2018, there were 9 centers that performed 164 related liver transplants (32.5%).

In 2019, 113 pediatric (mostly young children) liver transplants were carried out (133 in 2018). Three centers performed pediatric liver transplants: Shumakov Center (98), Petrovsky National Research Centre of Surgery (8) and State Novosibirsk Regional Clinical Hospital (7). See Fig. 12.

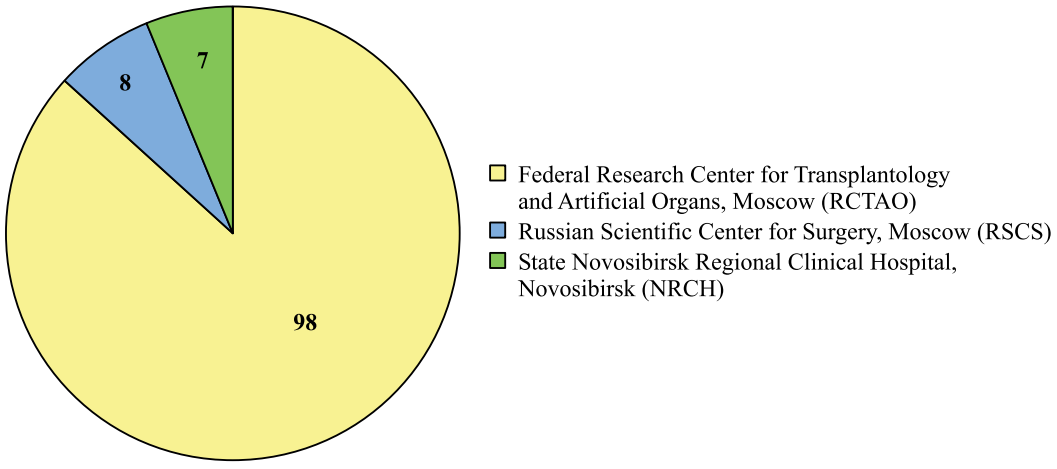

Fig. 12. Pediatric liver transplantation in the Russian Federation in 2019

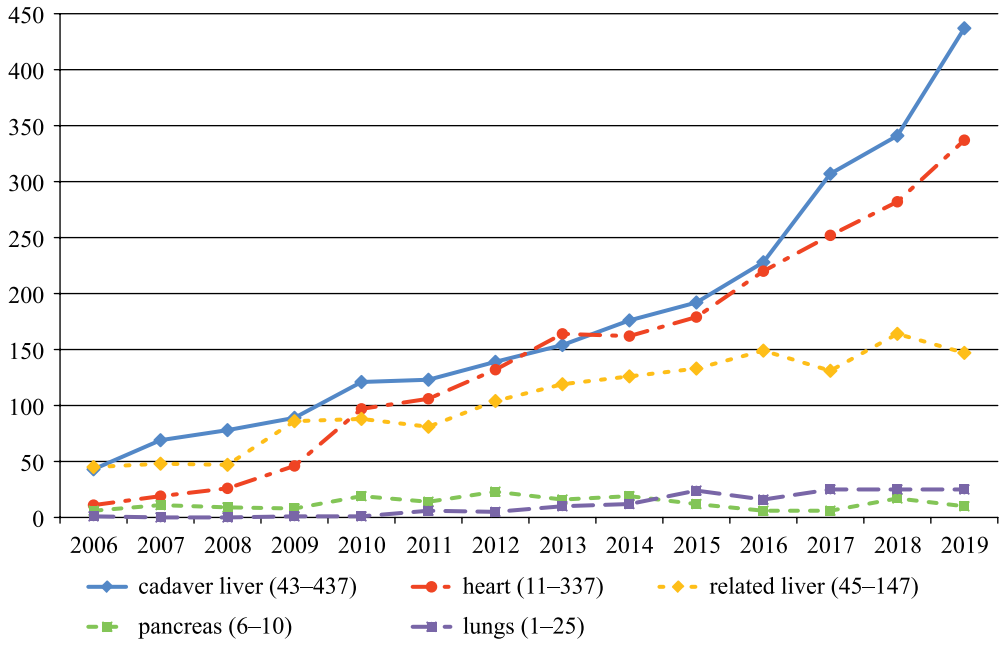

Fig. 13. Nonrenal solid organ transplantation in 2006–2019

In 2019, pancreas transplants were performed at 2 centers. A total of 10 pancreas transplant surgeries were carried out (17 in 2018) – 9 of them were kidney-pancreas transplants.

There were 954 extrarenal transplants performed in 2019 – 39.3% of the total number of transplants (2427). In 2018, it was 832 (37.9% of 2193 transplants). Transplant centers located in Moscow and Moscow Oblast remain key players in extrarenal transplantation in the country. In 2019, these centers accounted for 637 extrarenal transplants (66.8%) performed in the country; it was 593 (71.3%) in 2018.

Over the observation period (from 2006 to 2019), the number of extrarenal organ transplants in Russia increased by 848 (9 times). See Fig. 13.

Extrarenal transplantations increased by 23.3% in the total number of transplants performed.

Table 11 contains information on the number of organ transplants performed in the Russian Federation from 2006 to 2019.

ORGAN TRANSPLANT RECIPIENTS

Information concerning the number of organ transplant recipients in Russia (from 2013 to 2018), obtained from the Federal Registry of the Ministry of Health of the Russian Federation (see Executive Order No. 2323-r of the Government of the Russian Federation dated October 23, 2017; Resolution No. 404 of the Government of the Russian Federation dated April 26, 2012), is presented in Table 12.

According to information from the Federal Registry, there were 17,637 organ transplant recipients in Russia as of 2019 (120.1 per million population). Among these recipients, 11,880 (80.9 per million population) had kidney transplants, 3,032 (20.6 per million population) received liver transplants, while 1,355 (9.2 per million) were heart transplant recipients.

Over the 7 years of observation (since 2013), the number of organ transplant recipients in Russia has increased by 7,257 (84.8%).

CONCLUSION

Results recorded in 2019 show organ donors and organ transplants in Russia continue to increase in number – by 10–15% per year (2,427 transplant surgeries in 2019). Moreover, the potential for both quantitative and qualitative development of donor and transplantation programs in the federal subjects of the Russian Federation is far from being fully utilized. Organ transplantation demand among the population significantly exceeds supply.

Last year, the following features of the development of donor and organ transplantation programs in the Russian Federation became apparent.

The main contributors to kidney, liver and heart transplantation programs in the Russian Federation were medical institutions of the federal subjects of the Russian Federation (37) and national medical research centers (7). The way these transplant programs would develop at these organizations will determine the general trends in organ donation and organ transplantation in the country.

About 67.8% of the population lives in federal subjects of the Russian Federation where, one way or the other, there is medical assistance on organ transplantation. This is certainly one of the positive results of the many years of progressive development of the sector,

Table 11

Organ transplantation in the Russian Federation in 2006–2019

Nos.	Organ	2006	2007		2008		2009		2010		2011		2012		2013		2014		2015		2016		2017		2018		2019	
		Abs.	Abs.	Change per year	Abs.	Change per year	Abs.	Change per year	Abs.	Change per year	Abs.	Change per year	Abs.	Change per year	Abs.	Change per year	Abs.	Change per year	Abs.	Change per year	Abs.	Change per year	Abs.	Change per year	Abs.	Change per year	Abs.	Change per year
1	Kidneys, 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
2	incl. cadaver	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
3	from live relative 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
4	Livers, 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
5	incl. cadaver	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
6	from live relative 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
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
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
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
10	Heart/ lungs complex										2	+2	2	0	1	–1	0	–1	0	0	0	0	0	0	3	+3	2	–1
11	Small intestine														1	+1	1	0	0	–1	0	0	0	0	0	0	0	0
	Total	662	813	+151	942	+129	1060	+118	1363	+303	1307	–56	1345	+38	1400	+55	1522	+122	1485	–37	1704	+219	1896	+192	2193	+297	2427	+234

Table 12

Number of patients with transplanted organs in the Russian Federation in 2013–2019

ICD-10 Code	Patients in register												
	2013	2014		2015		2016		2017		2018		2019	
		abs.	rel. (%)	abs.	rel. (%)	abs.	rel. (%)	abs.	rel. (%)	abs.	rel. (%)	abs.	rel. (%)
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
Z94.1 Heart transplant status	416	520	25.0	639	22.9	803	25.7	952	18.6	1164	22.3	1355	16.4
Z94.2 Lung transplant status	2	3	50.0	4	33.3	5	25.0	8	60.0	28	250.0	26	–7.1
Z94.4 Liver transplant status	1150	1406	22.3	1649	17.3	1948	18.1	2152	10.5	2632	22.3	3032	15.2
Z94.8 Other transplanted organ and tissue status (bone marrow, intestine, pancreas, stem cells)	334	467	39.8	654	40.0	808	23.5	909	12.5	1135	24.9	1344	18.4
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

popularization and introduction of an organ transplantation method at federal subjects of the Russian Federation. At the same time, 27.3 million people live in 13 federal subjects of the Russian Federation with low availability of organ transplantation services (less than 10 organ transplants per million population). Therefore, boosting transplantation activity at such federal subjects (Omsk Oblast, Chelyabinsk Oblast, Stavropol Krai, etc.) is one of the key development tasks in the coming years.

Over half of the organ transplant waitlists in federal subjects of the Russian Federation requires revision and optimization in terms of number of patients. Adequate provision of organ transplantation care begins with selection of patients on the waiting list. Here, the average waiting times for organs influences both transplant outcome and economic component of medical care. The inclusion of a waitlisted patient subsystem in the state system for donor organs, donors and recipients, which is planned for 2020, would ensure transparency and increase the efficiency of the activities of transplant centers with regards to transplant waitlists.

In Russia, medical care on organ transplantation is provided in the overwhelming majority of cases (87.3% in 2019) through funds from the compulsory medical insurance system, allocated for provision of high-tech medical care on organ transplant. Without adequate increase in financial support, the 10–15% annual growth in the number of organ transplants in the Russian Federation, observed in recent years, becomes unrealistic. Therefore, in order to further increase the number of organ transplantations in the federal subjects (to meet the real need of the population), funding appears to be a crucial factor along with effective administration of donor and transplantation programs by health care authorities in federal subjects of the Russian Federation.

The essential difference, observed in 2019, between donor and transplantation activity in federal subjects, and the unstable development over the years, which depends on a number of subjective factors, confirm the urgency of two development tasks: first, to increase and balance the level of donor and transplantation activity across federal subjects of the Russian Federation; secondly, to increase the stability of programs to subjective and other factors. There are successful organizational models for implementation at the federal subjects of the Russian Federation.

In 2019, a technology for determining human death based on brain death diagnosis was widely used in medical organizations running donor transplant programs (94.5% of effective donors). The only program that is lagging behind on this indicator is the Belyaev Kemerovo Regional Clinical Hospital – 47.5% with 40 effective donors in 2019.

The number of kidney transplants in the Russian Federation continues to increase (in 2019 there were 1473 transplants, +8.2%), but the rate of development in most federal subjects does not match the increase in the number of patients receiving renal replacement therapy via dialysis. This problem should be taken into account when planning the tasks and the scope of medical care on kidney transplant, including in regional health development programs.

The number of extrarenal organ transplants in the Russian Federation also continues to rise. This reflects the need for such technologies for health care and confirms their effectiveness. Liver and heart transplantation programs are an indicator of the level of development of medical technology in the federal subjects of the Russian Federation.

The number of pediatric organ transplants in the country remains approximately at the same level every

year (227 transplants in 2019). The number of pediatric liver transplants (110–130) meets the identified need of the population for this intervention method.

The number of pediatric kidney transplants (90–110) is currently limited by the geographic location of transplant centers – 95% of pediatric kidney transplant operations are performed in Moscow. To increase the number of pediatric kidney transplant operations, 4–5 more corresponding programs need to be launched at leading transplant centers in the country's federal districts (Northwestern Federal District, Southern Federal District, Volga Federal District, Ural Federal District and Siberian Federal District).

The Transplantation Registry, including its analytical part, complements the state recording system for donor organs, donors and recipients (executive order No. 355n of the Ministry of Health of Russia, dated June 8, 2016). The Registry remains popular as a tool for supporting management decisions on assessing the state, challenges and trends in donor and transplant programs in the federal subjects of the Russian Federation. In 2020, there are plans to improve the Registry in order to ensure monitoring of the implementation of departmental target program "*Organ Donation and Transplantation in the Russian Federation*" (approved via executive order No. 365 of the Russian Ministry of Health, dated June 4, 2019). Improvement of the Registry is also aimed at monitoring the interaction of regional health authorities and medical organizations with the Shumakov Center with regards to running and developing donor and transplant programs.

The authors declare no conflict of interest.

REFERENCES

1. Organ donation and transplantation in Russian Federation in 2009. *Transplantology 2009: results and prospects*. Vol. I / Ed. by S.V. Gautier. M.–Tver: Triada, 2010: 408, 8–20.
2. Organ donation and transplantation in Russian Federation in 2006–2010. *Transplantology: results and prospects*. Vol. II. 2010 / Ed. by S.V. Gautier. M.–Tver: Triada, 2011: 464, 18–32.
3. Organ donation and transplantation in Russian Federation in 2011 (IV report of National Registry). *Transplantology: results and prospects*. Vol. III. 2011 / Ed. by S.V. Gautier. M.–Tver: Triada, 2012: 416, 14–37.
4. Organ donation and transplantation in Russian Federation in 2012. (V report of National Registry). *Transplantology: results and prospects*. Vol. IV. 2012 / Ed. by S.V. Gautier. M.–Tver: Triada, 2013: 304, 8–28.
5. Organ donation and transplantation in Russian Federation in 2013 (VI report of National Registry). *Transplantology: results and prospects*. Vol. V. 2013 / Ed. by S.V. Gautier. M.–Tver: Triada, 2014: 352, 32–57.
6. Organ donation and transplantation in Russian Federation in 2014 (VII report of National Registry). *Transplantology: results and prospects*. Vol. VI. 2014 / Ed. by S.V. Gautier. M.–Tver: Triada, 2015: 488, 44–75.
7. Organ donation and transplantation in Russian Federation in 2015 (VIII report of National Registry). *Transplantology: results and prospects*. Vol. VII. 2015 / Ed. by S.V. Gautier. M.–Tver: Triada, 2016: 488, 38–71.
8. Organ donation and transplantation in Russian Federation in 2016 (IX report of National Registry). *Transplantology: results and prospects*. Vol. VIII. 2016 / Ed. by S.V. Gautier. M.–Tver: Triada, 2017: 368, 33–66.
9. Organ donation and transplantation in Russian Federation in 2017 (X report of National Registry). *Transplantology: results and prospects*. Vol. IX. 2017 / Ed. by S.V. Gautier. M.–Tver: Triada, 2018: 392, 26–63.
10. Organ donation and transplantation in Russian Federation in 2018 (XI report of National Registry). *Transplantology: results and prospects*. Vol. X. 2018 / Ed. by S.V. Gautier. M.–Tver: Triada, 2019: 504, 46–85.
11. Assessment of requirement of the population in the organ transplantation, the donor resource and planning of the effective network of the medical organizations (the centers of transplantation). *Transplantology: results and prospects*. Vol. IV. 2012 / Ed. by S.V. Gautier. M.–Tver: Triada, 2013: 304, 30–40.

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A FIVE-YEAR LIVER TRANSPLANT EXPERIENCE IN ROSTOV OBLAST

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Objective: to reflect on a 5-year experience in liver transplant surgery at the Rostov Regional Clinical Hospital. **Materials and methods.** Liver transplant was performed in Rostov Oblast in July 2015 for the first time. There were 52 liver transplant surgeries performed in the region by the end of February 2020. Cirrhosis due to viral hepatitis is the leading indication for liver transplantation in 33.3% of patients. The average age of recipients was 43.5 ± 15.8 years. Male recipients accounted for 59.6% of cases. Nine recipients got liver transplants from blood relatives, while 43 recipients received an organ from post-mortem donors. For two patients, liver graft was obtained by splitting the liver into two lobes using the *in situ* split technique. **Results.** The average duration of surgery was 5.14 ± 1.92 hours. Blood loss during surgery did not exceed 1400 ml. Up to 93% of lost blood was recovered using the reinfusion system. The need for red blood cell transfusion was observed in 48.1% of cases. Fresh frozen plasma was transfused in all cases. Early postoperative complications were observed in 15 patients (29.4%), and some of them had several complications simultaneously. Biliary and vascular complications, which were eliminated by minimally invasive methods and open surgeries, had a significant influence on liver transplant outcome. In-hospital mortality was 5.6%. The causes of death were intra-abdominal bleeding (1), portal vein thrombosis (1) and biliary sepsis (1). Four more people died in the long term after being discharged from hospital: lung cancer (1), graft rejection (1) and fungal sepsis (2). **Conclusion.** Liver transplant outcome depends on the skills and experience of the specialists implementing this program. Post-transplant in-hospital and long-term mortality depends on the presence and nature of complications, and on the possibility of early treatment.

Keywords: liver transplantation, surgical complications, hospital mortality.

INTRODUCTION

Today, environmental and socio-economic issues are playing major roles in the health of the population. Conditions of the digestive system, particularly cirrhosis, is among the major diseases leading to reduced working capacity, frequent hospitalization and rapid disability in adults [1].

Cirrhosis as the final stage of chronic liver disease comes with many complications, sometimes requiring urgent treatment, such as surgical interventions [2, 3]. But as practice has shown, it is not always possible to save a patient's life, being limited only by "half measures" – *f* palliative interventions aimed at eliminating the complications of portal hypertension, hepatic encephalopathy, hepatorenal syndrome and other life-threatening conditions [4].

At present, liver transplantation (LT) is the only effective treatment for cirrhosis in the final stages, when all other treatment methods have been unsuccessful. For more than half a century of existence, this operation has shown to be effective due to high 5-year survival rates of patients, reaching 80–90% [5, 6]. Nevertheless, active use of transplant surgery as a method of treating patients with end-stage cirrhosis all over the world is hampered by organ shortages [7]. This often increases the waiting time for operation, increases waitlist mortality, and those patients who survive often approach transplantation in a critical condition, which worsens surgery outcome both in the perioperative period and in the long-term post-transplant period [8].

Supportive therapy (immunosuppressive, antibacterial, etiotropic) and specific postoperative surgical complications remain the stumbling blocks on the way to achieving excellent transplantation outcomes [9]. First of all, these include problems associated with biliary anastomotic leak and decreased patency of blood vessels. Despite the fact that these complications take a small share in the overall structure, their specificity is often fraught with more serious consequences for the patient, primarily graft loss.

Considering the above, the purpose of the work was to conduct a retrospective generalized analysis of the outcomes of liver transplants performed by us over five years and assess the implementation of the regional program.

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MATERIALS AND METHODS

The first liver transplant performed in Rostov Oblast was at the Rostov Regional Clinical Hospital in July 2015. This event was preceded by multilateral organizational and practical training, generalization of the experience of domestic and foreign transplant centers, which resulted in the creation of a regional transplant center and beginning of implementation of the corresponding program.

At present, there are slightly above 3000 people observed for cirrhosis in Rostov Oblast. Waitlisted candidates for liver transplantation reached 350 in number.

As of the end of February 2020, 51 waitlisted candidates underwent liver transplantation. The indications for surgery were cirrhosis of various origins. However, viral hepatitis became the main cause of end-stage liver disease in patients – 17 (33.3%) cases (Fig. 1).

One patient underwent retransplantation due to graft dysfunction caused by hepatic vein thrombosis. So, a total of 52 liver transplants were performed.

The average age of recipients was 43.5 ± 15.8 years. Male recipients accounted for 59.6% (31 people) of cases. Forty-one patients (78.9%) received whole organs from deceased donors; 10 patients (19.2%) received the right lobe; 9 recipients got liver transplants from their blood relatives; there was 1 deceased donor; 1 patient received the left lobe of a cadaveric liver (1.9%). For 50 transplant cases, the liver or part of it was procured according to the standard protocol. For 2 patients, liver graft was procured by splitting the liver into two lobes using the *in situ* split technique (Fig. 2).

Before performing liver transplant operations, we, of course, carefully examined all patients. Laboratory tests, along with general clinical indicators, included an assessment of the functional state of the liver. We performed complete virological tests: HIV, hepatitis B, C,

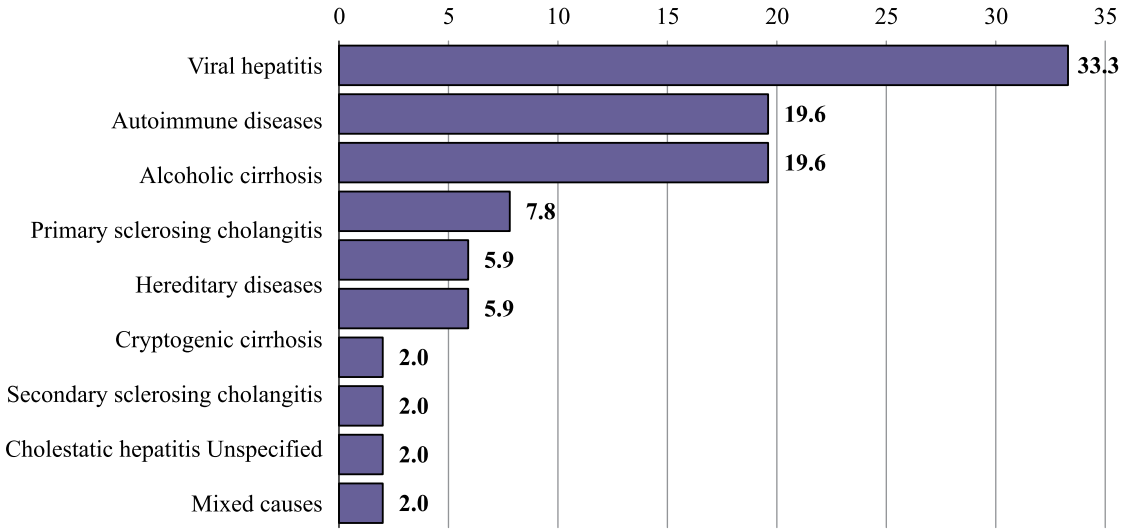

Fig. 1. Distribution of liver recipients according to the etiology of cirrhosis, %

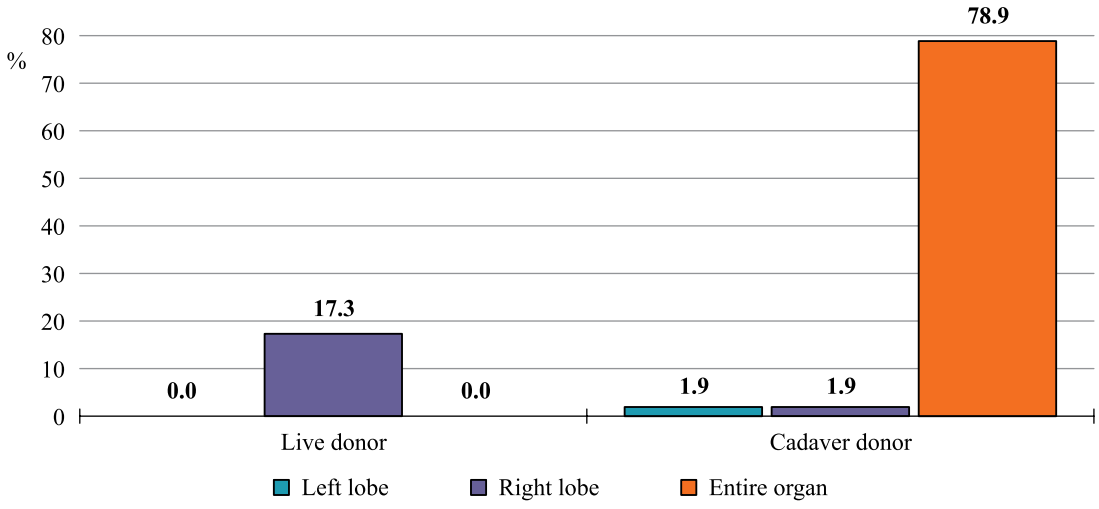

Fig. 2. Distribution of donor organs

D, G viruses, herpes viruses, cytomegalovirus (CMV), with detection of specific antibodies by ELISA and the activity of these viruses in PCR. Instrumental diagnostics included ECG, echocardiography, spirograph, esophagogastroduodenoscopy, chest CT scan, colonoscopy (irrigoscopy), triplex ultrasound of the veins and arteries of the lower limbs. In addition, clinical and instrumental diagnostic assessment of portal blood flow, liver structure assessment (ultrasound with dopplerography, triple-phase CT scan and magnetic resonance imaging (MRI) with bolus contrast, indirect liver elastometry) were performed.

After the surgery, CT scan, MRI, ultrasound scan, selective celiacography and minimally invasive diagnostic manipulations on the bile ducts were used, including transpapillary and percutaneous transhepatic interventions.

RESULTS

We performed liver transplant surgeries on all patients in accordance with ethical and legal standards. Average duration of operations was 5.14 ± 1.92 hours. Blood loss during surgery did not exceed 1400 mL (1076.1 ± 191.8 mL). Using the reinfusion system, we were able to recover up to 93% of lost blood (on average 996.5 ± 177.5 mL of blood), while 48.1% of cases required red blood cell transfusion during operation – an average of 238.7 ± 133.1 mL transfused blood in the next postoperative hours. In all cases, fresh frozen plasma was transfused with 1394.7 ± 303.1 mL in average transfusion volume.

Side-to-side cavo-caval anastomosis was performed in 35 (67.3%) cases, end-to-side cavo-caval anastomosis in 11 (21.2%) patients, piggyback anastomosis in 4 (7.7%) patients, and anastomosis using classical method was done in 2 (3.8%) cases. End-to-end arterial anastomosis was formed with common hepatic artery in 38 (73.1%) cases. A similar technique was used for lobar arterial anastomosis in 11 (21.2%) patients: right lobar arterial anastomosis in 10 patients, and left lobar arterial anastomosis in 1 patient. Due to the peculiarities of arterial blood supply to the graft, three cases (5.8%) required Y-shaped arterial reconstruction during anastomosis of the common hepatic artery and accessory hepatic artery. Portal reconstruction was carried out in a standard way, end-to-end. In 11 (21.2%) patients, the recipient's portal vein was anastomosed with the lobar vein of the graft. It should be noted that thrombectomy was performed in 3 recipients due to Yerdel's type I–II portal vein thrombosis (2 patients with Yerdel's type II portal vein thrombosis and 1 patient with type I).

End-to-end biliary anastomosis was formed in 39 (75.0%) cases. Roux-en-Y biliodigestive anastomosis was formed in 3 (5.8%) cases, including during retransplantation.

In the early postoperative period, surgical complications were noted in 15 (29.4%) patients, while several patients had several complications at once. In the "vascular" complications group, we often encountered intra-abdominal bleeding irrespective of the type of transplantation performed (Table 1).

Both minimally invasive techniques (41.7%) and relaparotomy (58.3%) were used for vascular complications management. In 2 clinical cases, common hepatic artery thrombosis developed on postoperative day 5 and day 7. When performing selective angiography of the celiac trunk in these patients, hepatic artery occlusion in the proximal third was determined, without hemodynamically significant stenosis of the trunk itself and the splenic artery with its branches (Fig. 3, a). Thrombolysis and stenting in these patients restored blood flow in the common hepatic artery (Fig. 3, b).

In 2 more cases, right hepatic artery stenosis was found, which has a significant effect on hemodynamics. This complication was eliminated by vascular stenting (Fig. 4).

Endovascular technique also helped to eliminate right hepatic vein stenosis in a patient after related liver transplant (Fig. 5).

Hepatic vein thrombosis in a patient after related transplantation led to graft dysfunction and death, which required retransplantation (Fig. 6).

Table 1

Characteristics of the vascular complications in different types of liver transplantation

Complication	Transplant				Total (n = 51)	
	Relative (n = 9)		Cadaver (n = 42)			
	n	%	n	%	n	%
Abdominal bleeding	2	22.2	2	4.8	4	7.8
Common hepatic artery thrombosis	1	11.1	1	2.4	2	3.9
Right hepatic artery coarctaction	0	0	2	4.8	2	3.9
Portal vein thrombosis	1	11.1	0	0	1	2.0
Inferior vena cava thrombosis	0	0	1	2.4	1	2.0
Transplant hepatic veins thrombosis	1	11.1	0	0	1	2.0
Right hepatic vein coarctaction	1	11.1	0	0	1	2.0
Total	**6**	**66.7**	**6**	**14.3**	**12**	**23.5**

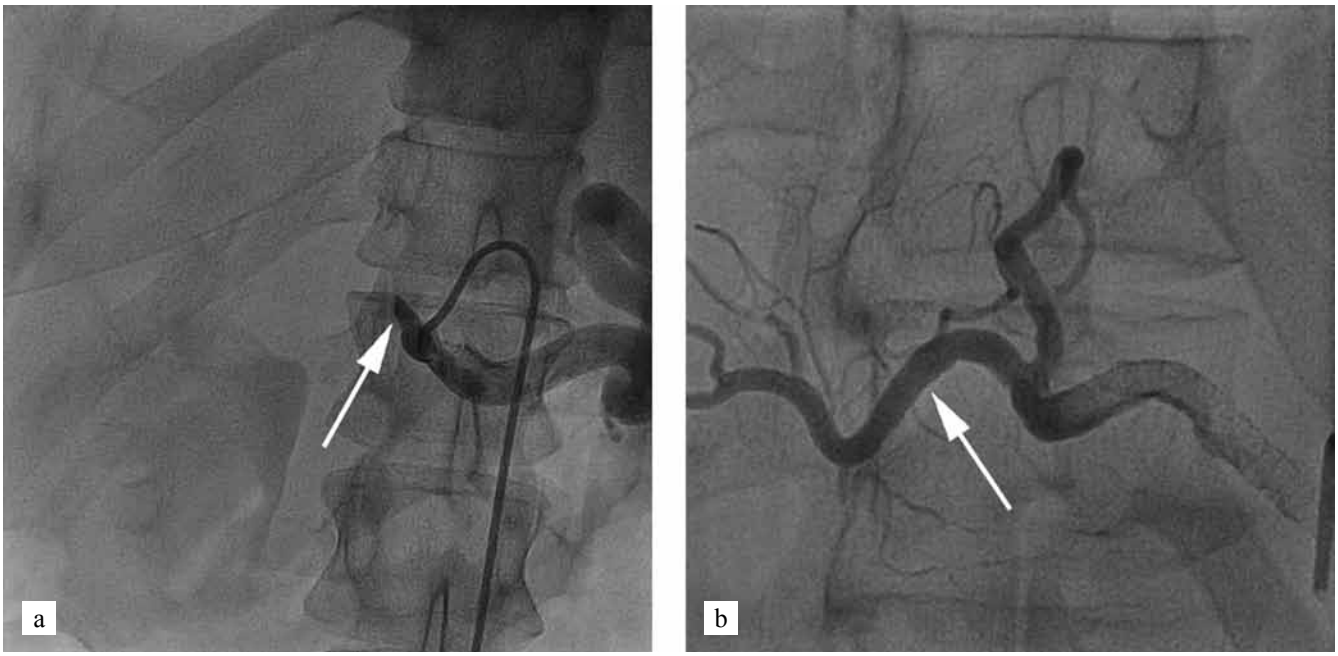

Fig. 3. The common hepatic artery thrombosis. Angiography: a – zone of occlusion; b – after stenting

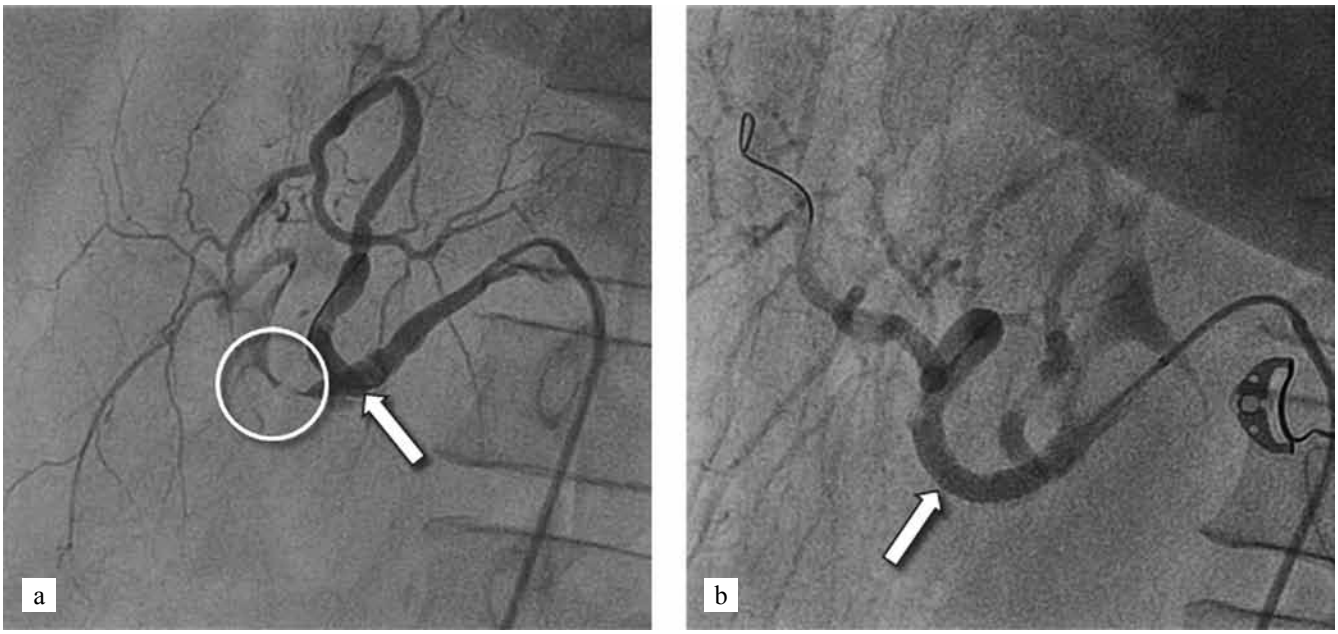

Fig. 4. The right hepatic artery stricture. Angiography: a – zone of stenosis; b – after stenting

Relaparotomy was done due to the technical failure of minimally invasive techniques in eliminating blood clots in the inferior vena cava and portal veins, as well as intra-abdominal bleeding (see Table 1).

Post-liver transplant biliary complications occurred in 8 (15.7%) patients (Table 2).

In 3 cases of biliary anastomosis incompetence, an ultrasound-guided percutaneous drainage of bilomas was carried out, of which percutaneous transhepatic cholangiostomy was additionally performed in one case. In one patient, partial biliary anastomosis incompetence was accidentally discovered during relaparotomy for intra-abdominal bleeding. Interestingly, there were no clinical signs of biliary incompetence at the time of re-intervention. In this case, anastomosis was dissociated, and a Roux-en-Y biliodigestive anastomosis was formed.

To eliminate biliary anastomotic strictures arising after the operation, we used various combinations of minimally invasive and open surgical interventions. For example, antegrade anastomosis was performed in one case, while in another two cases, antegrade anastomosis was supplemented with percutaneous transhepatic cholangiostomy. In another clinical case (after split transplantation), treatment was divided into two phases. At

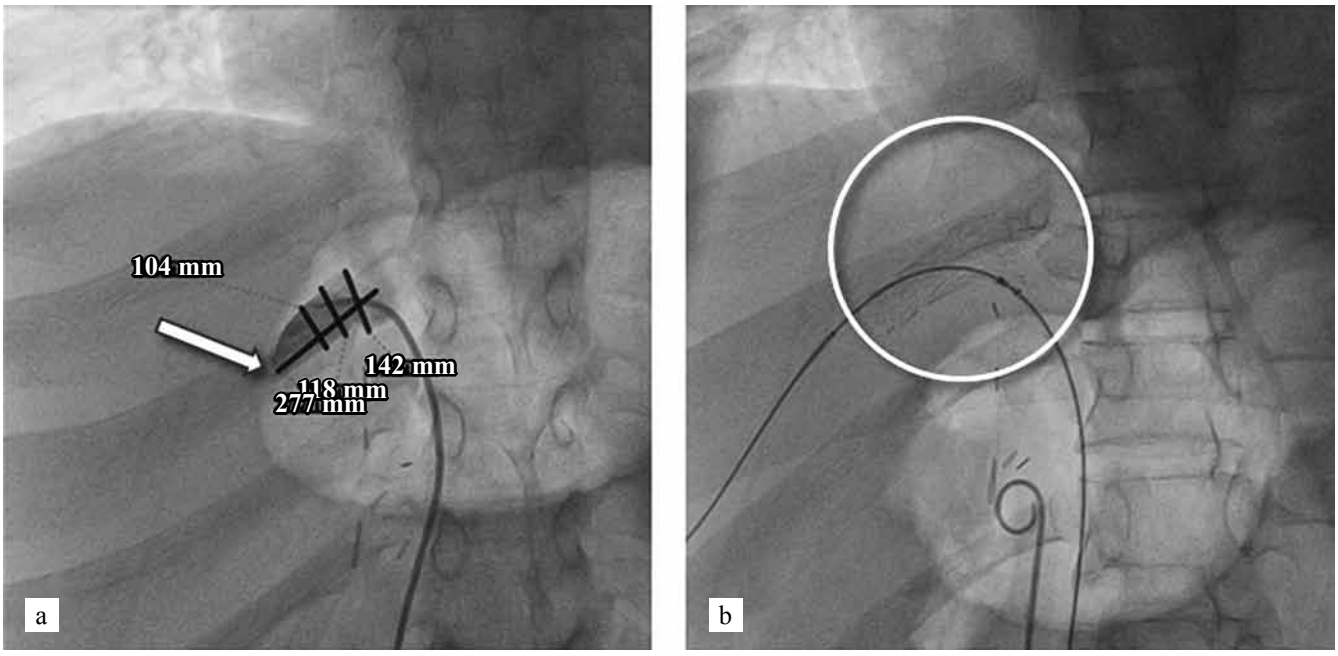

Fig. 5. The right hepatic vein stenosis. Angiography: a – zone of stenosis; b – stenting of the vein

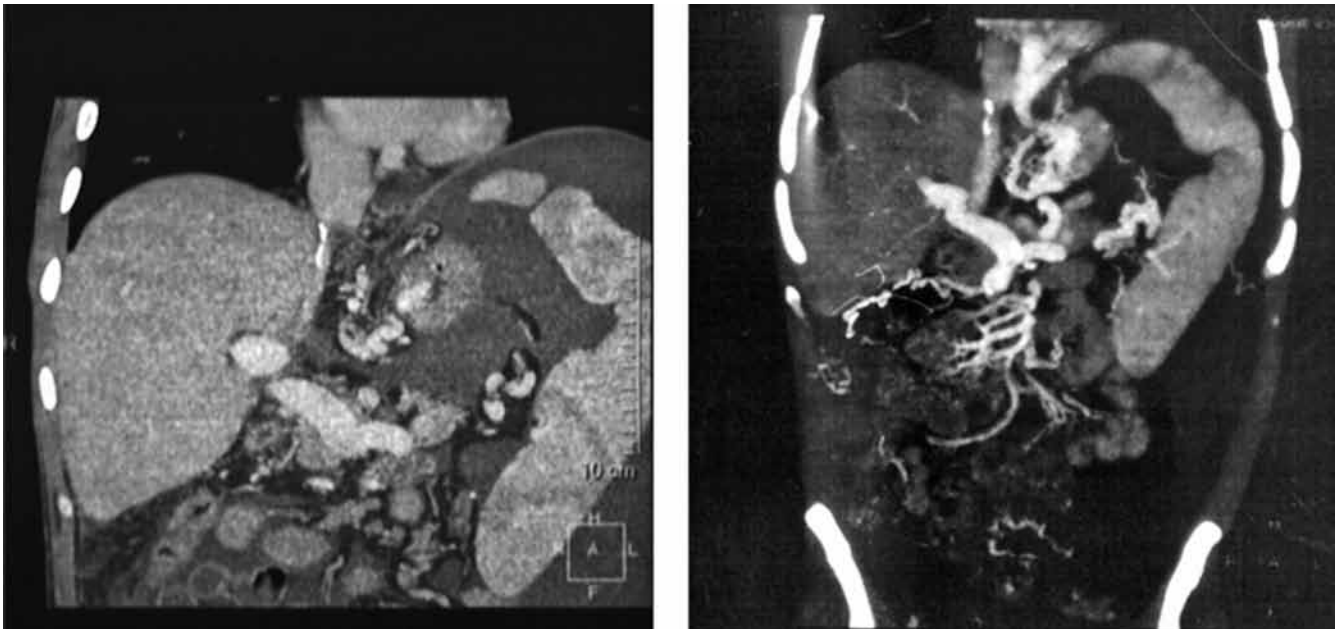

Fig. 6. CT with bolus contrast. The liver transplant acute failure on the background of hepatic vein thrombosis. The transplantation of the right lobe of the liver from a related donor due to liver cirrhosis in the outcome of mixed HBV + HDV infection, class C, decompensation stage. MELD 36. UNOS 1B

the first phase, we performed percutaneous transhepatic cholangiostomy (Fig. 7), and after reaching acceptable levels of bilirubinemia, we performed bile duct reconstruction, forming a biliodigestive fistula (Fig. 8).

Thus, timely measures taken to eliminate early postoperative complications saved the lives of 94.1% of liver transplant recipients. Unfortunately, three patients died from intra-abdominal bleeding (1), portal vein thrombosis (1), and biliary sepsis (1).

The average hospital stay after liver transplantation was 26.7 ± 2.2 days.

Speaking about the therapy given to recipients after organ transplantation, we note that immunosuppression was selected on an individual basis. In 89% of cases of related transplantation, monotherapy using calcineurin inhibitors (long-acting tacrolimus or cyclosporine) was administered. In the case of organ transplantation from a deceased donor, dual or triple therapy, which included calcineurin inhibitor, mycophenolic acid, and methyl-

Table 2

Characteristics of the biliary complications in different types of liver transplantation

Complication	Transplant				Total (n = 51)	
	Relative (n = 9)		Cadaver (n = 42)			
	n	%	n	%	n	%
Biliar anastomotic leak	4	44.4	0	0	4	7.8
Biliar anastomotis Biliar anastomotic	1	11.1	3	7.1	4	7.8
Total	**5**	**55.6**	**3**	**7.1**	**8**	15.7

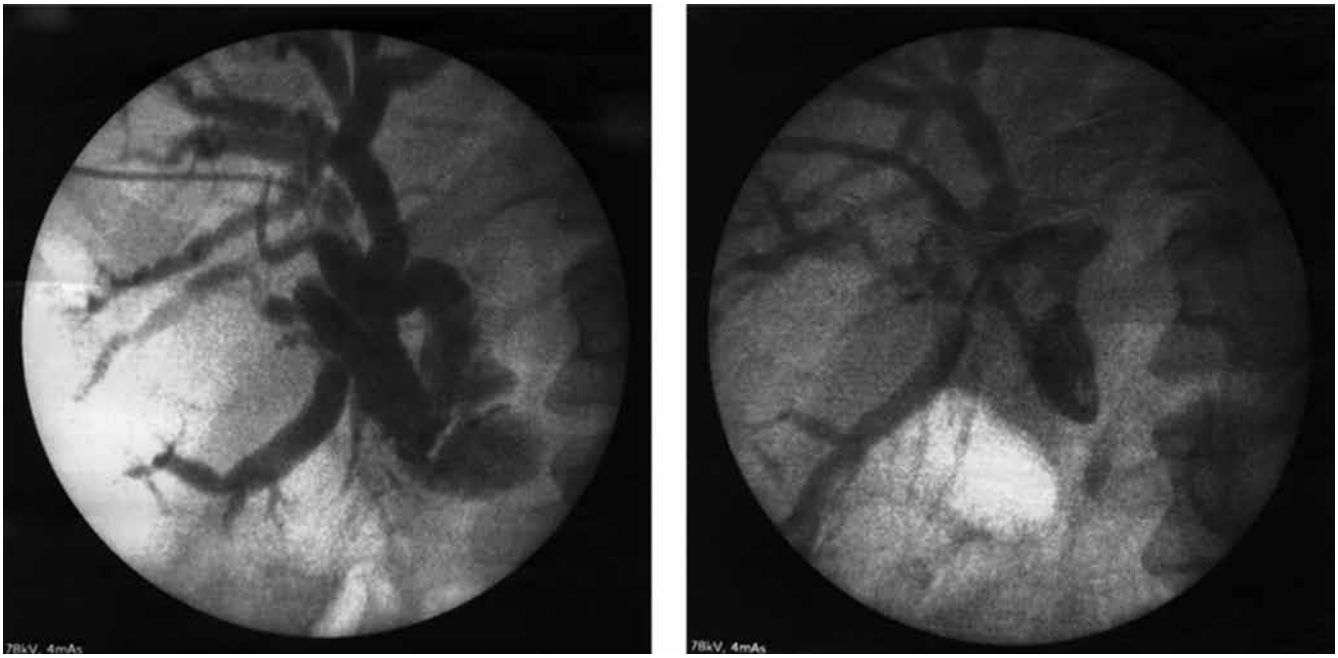

Fig. 7. Biliary anastomosis stricture with expansion of the intrahepatic ducts of the liver transplant in a patient after split transplantation of the right lobe. Transcutaneous transhepatic biliary drainage

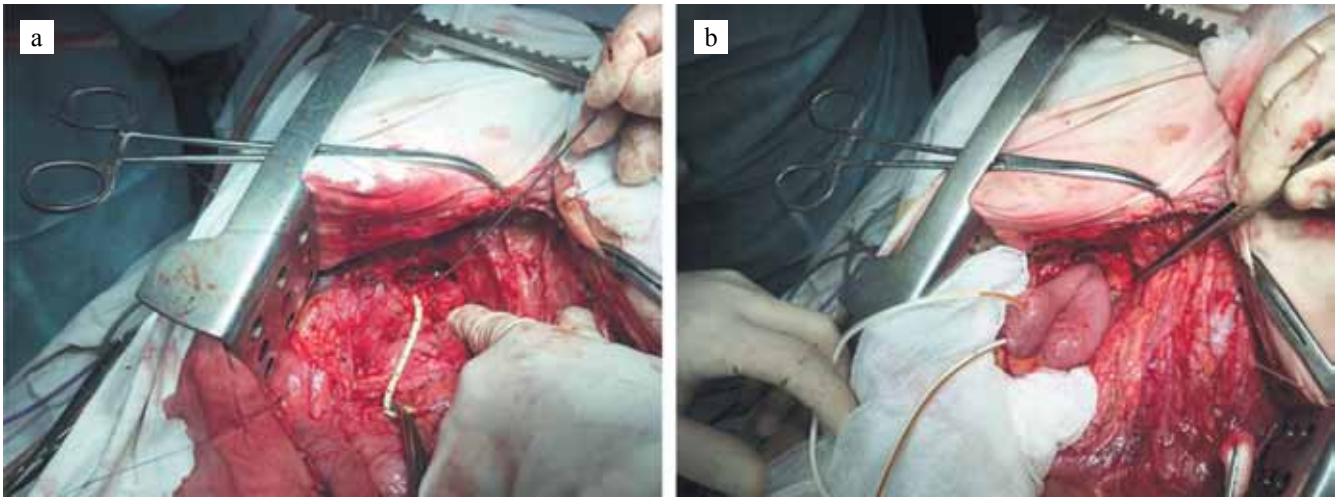

Fig. 8. Reconstruction of the biliary anastomosis: a – stage of separation of the anastomosis; b – the final type of operation (hepaticoejunostomy)

prednisolone, was administered. Pulse therapy with glucocorticosteroids was administered in 5.9% of cases due to graft rejection crisis. In 9 cases (17.6%), in connection with kidney failure and detected oncopathology, therapy was converted with the addition of an mTOR inhibitor (everolimus).

Currently, the follow-up for liver transplant recipients is from 1 month to 4.6 years. Among the long-term post-transplant complications experienced by patients were: autoimmune return (4), drug-induced nephropathy (9), arterial hypertension (7), drug-induced diabetes (5), obesity (5), generalized systemic atherosclerosis (1), lung adenocarcinoma (1), fungal sepsis (2), and testicular seminoma (1). Four more people died during this period from lung cancer (1), biliary sepsis (1), and fungal sepsis (2).

DISCUSSION

Transplantation is presently the only effective method for improving the survival rate in end-stage liver disease. However, despite the society's awareness of the necessity and justification of this technology, the stumbling block for further development of transplantation remains religious, moral and ethical issues, as well as the expediency of huge material costs. Lack of understanding among the public of the importance and humanity of transplant programs hinders rapid development of such programs, including in our region, Rostov Oblast.

A successful transplantation outcome depends not only on the transplant surgeon's skills, but also on the recipient's initial condition caused by underlying disease and associated pathology, as well as the "quality" and functional state of the donated organ. For example, we did not use organs from elderly donors. Therefore, we were able to avoid early graft dysfunction or non-function in almost all cases.

It should be noted that liver transplantation can become successful only with the active participation of a wide range of specialists at all stages of treatment. In this regard, a hepatologist managing the waiting list and monitoring the recipient's condition after liver transplantation, with constant correction of immunosuppressive and symptomatic therapy, plays an important role. Equally important is a qualified morphological and immunohistochemical graft assessment and diagnosis of complications.

The greatest concern on the effectiveness of liver transplantation comes from postoperative complications. Early postoperative vascular thrombosis is fraught with acute graft failure and loss. Biliary and bacterial-infectious complications, including fungal, often lead to patients' death, which has been the case in our practice.

CONCLUSION

Outcomes in liver transplantation depend on the skills and experience of the specialists implementing it. Post-transplant in-hospital and long-term mortalities depend on the presence and nature of complications, and on the possibility of early treatment.

The authors declare no conflict of interest.

REFERENCES

1. *Malayeva YeG, Tsyrul'nikova AN, Tsitko YeV, Khudyakov IA, Men'shakova MN, Kobruseva LA*. Tsirroz pecheni vchera i segodnya. *Aktual'nyye problemy meditsiny. Sbornik nauchnykh statey Respublikanskoy nauchno-prakticheskoy konferentsii s mezhdunarodnym uchastiyem: v 5 tomakh*. Gomel'skiy gosudarstvennyy meditsinskiy universitet, redkollegiya: A.N. Lyzikov [i dr.]. 2019; 131–133.
2. *Ivashkin VT, Mayevskaya MV, Pavlov ChS, Fedosyina YeA, Bessonova YeN, Pirogova IYu, Garbuzenko DV*. Treatment of liver cirrhosis complications: Clinical guidelines of the Russian Scientific Liver Society and Russian gastroenterological association. *Ross. zhurn. gastroenterol., gepatol., koloproktol*. 2016; 26 (4): 71–102.
3. *Van Thiel DH, Schade RR, Starzl TE et al.* Liver Transplantation in Adults. *Hepatology*. 1982; 2 (5): 637–640. doi: 10.1002/hep.1840020517.
4. *Kockerling D, Nathwani R, Forlano R et al.* Current and future pharmacological therapies for managing cirrhosis and its complications. *World J Gastroenterol*. 2019; 25 (8): 888–908.
5. *Burra P, Freeman R.* Trends in liver transplantation 2011. *J Hepatol*. 2012; 56 (1): 101–111. doi: 10.1016/S0168-8278(12)60011-7.
6. *Ravaioli M, Grazi GL, Dazzi A et al.* Survival benefit after liver transplantation: a single European center experience. *Transplantation*. 2009; 88 (6): 826–834. doi: 10.1097/TP.0b013e3181b26807.
7. *Toniutto P, Zanetto A, Ferrarese A et al.* Current challenges and future directions for liver transplantation. *Liver Int*. 2017; 37 (3): 317–327. doi: 10.1111/liv.13255.
8. *Koch DG, Tillman H, Durkalski V et al.* Development of a Model to Predict Transplant-free Survival of Patients With Acute Liver Failure. *Clin Gastroenterol Hepatol*. 2016; 14 (8): 1199–1206. doi: 10.1016/j.cgh.2016.03.046.
9. *Popov AY, Lishchenko AN, Davydenko MN, Porkhanov VA*. Analysis of post-liver transplantation complications. *Russian Journal of Transplantology and Artificial Organs*. 2015; 17 (2): 107–110. (In Russ.). doi: 10.15825/1995-1191-2015-2-107-110.

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COMPARATIVE ANALYSIS OF INDUCTION IMMUNOSUPPRESSIVE THERAPY PROTOCOLS IN RENAL TRANSPLANT RECIPIENTS (RETROSPECTIVE REVIEW)

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Objective: to evaluate the clinical efficacy and outcomes of kidney transplants performed using an alternative immunosuppressive therapy protocol that is based on double induction. **Materials and methods.** We examined 296 cases of kidney transplants performed in 295 patients between January 1, 2004 and December 31, 2018. Based on induction immunosuppressive therapy regimen, the patients were divided into two groups. Group 1 included patients who underwent transplantation from January 1, 2004 to June 30, 2013 and who used the standard induction immunosuppression protocol. Group 2 included patients who did transplant surgeries between the period January 7, 2013 and December 31, 2018 and who received the "double" induction protocol being analyzed. The method of dividing patients into these groups is associated with routine implementation of the analyzed protocol at the transplantation center since July 1, 2013. **Results.** Graft and recipient survival rates at all follow-up periods were higher in the group of patients who received the "double" induction immunosuppressive protocol than in the standard group. The studied protocol provides initially better and more stable graft function than in standard therapy. This is especially valuable in centers experiencing difficulties in assessing pre-transplant immunological risk. The graft and recipient survival rates achieved by the analyzed protocol are more pronounced in deceased-donor kidney transplantation. **Conclusion.** Positive results obtained from retrospective analysis of the protocol under study justify a prospective randomized study.

Keywords: kidney transplantation, recipient survival, immunosuppressive protocols, double induction immunosuppressive therapy.

IMPORTANCE OF THIS ISSUE

Kidney transplantation (KT) is currently the standard treatment for end-stage chronic kidney disease (ESCKD). It increases life expectancy, improves quality of life and provides social rehabilitation for kidney recipients [1]. Taking into account the economic efficiency of KT in comparison with other modalities of renal replacement therapy, its effective development at the state and regional levels stabilizes the entire health care system, enabling the most rational use of funding sources [2]. Despite improvements in immunosuppressive and adjuvant medication therapies and significant progress so far achieved in recent years in post-transplant survival rates, all recipients in the long-term period develop graft rejection to some extent, resulting in shortened duration of the graft function [3]. The initial state of the donor organ, the degree of immunological compatibility, the duration of cold, primary ischemia and secondary, warm ischemia, and the severity of reperfusion injuries equally play important roles in the long-term survival of kidney grafts and recipients [4]. Several of these factors lead to early graft dysfunction. Nonspecific lesions significantly increase the level of immune response, which requires increased doses of calcineurin inhibitors (CNIs), which have a nephrotoxic and additional damaging effect on the kidney graft, reducing its reparative capacity. Research has shown that delayed graft function is associated with a more pronounced incidence of acute rejection response [5]. Moreover, standard induction regimens are not always justified [6]. These factors make us look for new approaches to induction immunosuppressive therapy

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(IST) [7, 8] that would help to reduce additional damage, provide effective immunosuppression with delayed administration of calcineurin inhibitors, and reliable, long-term survival of patients and grafts [9].

Objective: to evaluate the clinical efficacy and outcomes of kidney transplants performed using an alternative double-induction immunosuppressive therapy.

The following tasks have been formulated to achieve this goal.

1. To compare kidney recipient and graft survival in the group that received the standard IST protocol and the group that received the double-induction IST protocol.
2. To assess kidney graft function in patients who received the standard IST and those who received the double-induction IST protocol.
3. To identify those patients that are expected to get better outcomes from double-induction IST regimen in comparison with the standard IST regimen.
4. To establish the structure of complications leading to adverse outcomes in kidney transplant recipients, depending on the IST regimen.

RESEARCH METHODOLOGY AND METHODS

The work was performed as a retrospective, open, nonrandomized, single-center, controlled study of the outcomes of kidney transplantations for a follow-up period covering January 1, 2002 to September 30, 2019. Clinical laboratory and instrumental research methods were used in the work.

MATERIALS AND METHODS

The study examined 296 kidney transplant surgeries performed in 295 patients from January 1, 2004 to December 31, 2018 at the kidney transplant department of the Republican Clinical Hospital, Kazan (Fig. 1). Based on the goal and objectives of the study, all patients were divided into two groups according to the IST regimen received. Group 1 included patients who underwent kidney transplant surgery within the period January 1, 2004 to June 30, 2013 and who used the standard IST protocol. Group 2 included patients who did transplant surgeries within the period January 7, 2013 and December 31, 2018 and who received the double-induction IST protocol. The method of dividing patients into these groups was associated with routine implementation of the protocol being analyzed at the transplantation center since July 1, 2013. Demographic indicators and structure of groups are presented in Table 1.

In group 1, immunosuppression therapy was administered according to the following protocol: pulse methylprednisolone therapy, basiliximab, calcineurin inhibitor with selection of dosage according to the drug concentration in blood, mycophenolic acid preparations. Accompanying therapy: proton-pump inhibitors, ganciclovir at a dose selected according to the glomerular filtration rate, then replaced with valganciclovir and co-trimoxazole. Perioperative antibiotic prophylaxis began 30 minutes before operation and lasted for 5–7 days (Table 2).

In group 2, immunosuppression was administered according to the following protocol: pulse methylprednisolone therapy, basiliximab, anti-thymocyte immunoglobulin. From day 4, the patients switched to basic immunosuppressive therapy, which included methylprednisolone, calcineurin inhibitor with dosage selection based on drug concentration in blood. Mycophenolic acid was administered from the day the lymphocyte count was

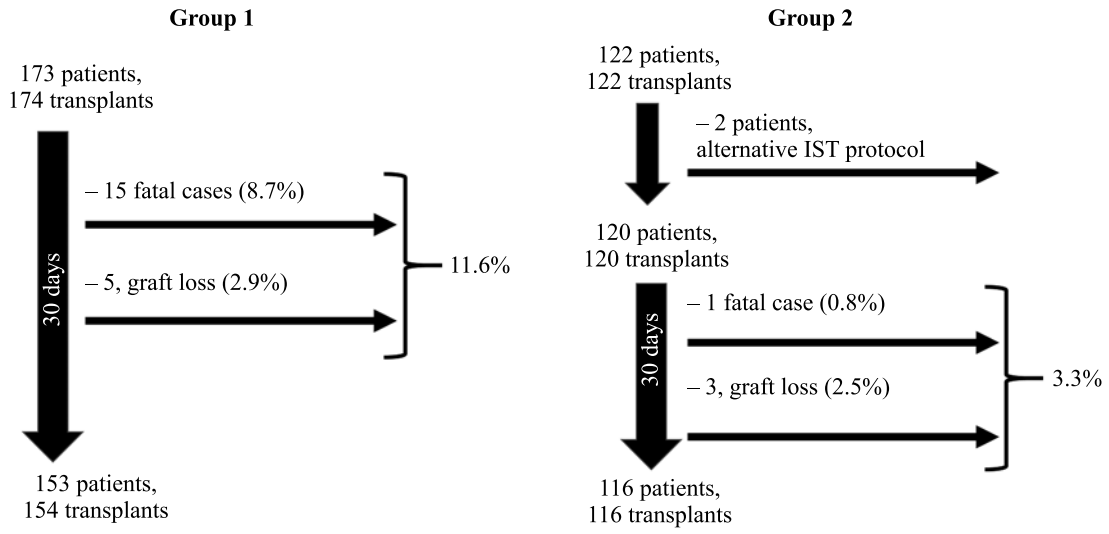

Fig. 1. Study design

Table 1

Demographic parameters and group structure

Parameters	Group 1	Group 2
Enrolled	01.01.04–30.06.13	01.07.13–31.12.18
Patients / Transplants, n	173 / 174	120 / 120
Age of recipients (years)	36 ± 1.0	34.5 ± 1.0
Age of recipients at KT from live donor (years)	28.1 ± 1.0	30.6 ± 0.9
Age of recipients at KT from cadaver donor (years)	43.1 ± 1.3	43.3 ± 1.7
KT from live donor / KT from cadaver donor	0.9 / 1	2.2 / 1
Male / Female	1.5 / 1	1.7 / 1
Diabetic nephropathy in TCKD structure (%)	9.8	8.3
Mismatch*	3.9 ± 1.0**	3.4 ± 1.0**

Note. * – antigens were determined only by A and B locus. Allocation of organs from deceased donors based on the less mismatches of A, B locus and negative Cross match results. ** – for the Mismatch analysis in cases of related kidney donation and coincidence of one antigen in A and B locus, one antigen in Dr locus was regarded as coinciding, in cases of deceased donation, antigens at the Dr locus always have been regarded as mismatching.

Table 2

Therapy protocol for patients of group No. 1

Days	–1	0	1	2	3	4	5	6	7	8	9	10	…
Methylprednisolone (mg)		500	500	500	250	250	250	24	24	24	24	24	↓
Basiliximab (mg)		20	–			20	–						
CNI inhibitor		±	+	+	+	+	+	+	+	+	+	+	↑↓
Mycophenolates			+	+	+	+	+	+	+	+	+	+	↑↓
Esomeprazole (mg)		40	40	40	40	40	–						
Omeprazole (mg)	–						20	20	20	20	20	20	+
Co-trimoxazole (mg)		480	480	480	480	480	480	480	480		480		6 months
Ganciclovir / valganciclovir (mg)		250	Under renal function control										200 days
Antibiotic therapy (amoxicillin / clavulanic acid)		+	+	+	+	+	+	?	?	–			
LMWH	±	+	+	+	+	+	+	+	+	+	+	+	–

Table 3

Therapy protocol for patients of group No. 2

Days	–1	0	1	2	3	4	5	6	7	8	9	10	…
Methylprednisolone (mg)		500	250	125	125	125	16	16	16	16	16	16	↓
Basiliximab (mg)		20	–			20	–						
Chloropyramine (mg)		20	20	20	20	–							
Paracetamol (mg)			500	500	500	–							
Anti-thymocyte immunoglobulin (mg)		50	50	50	50	–							
CNI inhibitor	–					Under blood concentration control							↑↓
Mycophenolates	–					Under leukocyte count control							↑↓
Esomeprazole (mg)		40	40	40	40	40	–						
Omeprazole (mg)		–					20	20	20	20	20	20	+
Co-trimoxazole (mg)		480	480	480	480	480	480	480	480	–	480	–	6 months
Ganciclovir / valganciclovir (mg)		250	Under renal function control										200 days
Antimycotics		+	+	+	+	–							
Antibacterial therapy (amoxicillin / clavulanic acid)		+	+	+	+	+	+	?	?	–			
LMWH	±	+	+	+	+	+	+	+	+	+	+	+	–

above 4×10^9/L. Accompanying therapy was similar and differed in the use of micafungin (Table 3).

Patients in both groups were monitored based on outcomes as of September 30, 2019. Patient deaths were analyzed, and kidney function indicators of recipients were studied for 12 months after transplantation.

RESULTS AND DISCUSSION

Due to the fact that to assess the efficacy and safety of the double-induction IST protocol, we used retrospective analysis method, and patients were divided according to IST protocol used on the basis of routine use of the study protocol since July 1, 2013. We excluded all cases of adverse outcomes that occurred in the first 30 days after surgery. The chosen approach allows minimizing the influence of such historically dependent factors as existing levels of anesthetic and intensive care support, and surgical technique. Results obtained (Fig. 1) show that adverse events associated with graft death or graft loss within a 30-day postoperative period was 11.6% in the group of patients who received the standard IST protocol, and 3.3% in the double-induction group. As a result, the number of subjects that continued the study in the first and second groups was 153 and 116 patients, respectively.

To assess the efficacy of immunosuppressive therapy protocols used for this general population, Kaplan–Meier curves for patient and graft survival were constructed (Fig. 2, 3).

The diagrams presented show that recipient survival and graft survival are higher in group 2 (double-induction IST protocol) by 4% and 10% respectively at year 3 of follow-up. The relative heterogeneity of the compared groups, which involves a different ratio of the number of transplants performed from a living relative and from a deceased donor, necessitated a separate analysis of the outcomes of recipient survival depending on the source of the donor organ (Fig. 4, 5).

When these data are compared over 6 years after operation, it can be seen that the differences in outcomes for living-donor transplant for up to 36 months are insignificant. Meanwhile, in deceased-donor kidney transplantation, at month 36 of follow-up, the double-induction IST protocol achieves a more than 10% patient survival and 20% graft survival. These data suggest that the more effective the double-induction IST protocol is, the more severely compromised the donor organ is and the higher the HLA incompatibility, which is typical for deceased organ donation.

In evaluating the effect of the protocol on kidney graft function, we analyzed the serum creatinine levels in kidney recipients who received an organ from a living donor in the period from 3 to 12 months (Fig. 6).

As can be seen from the data obtained, graft function, for a period of up to one year, was better with the double-induction protocol, showing lower average creatinine levels in recipients and a smaller degree of variation in this indicator. Similar results were obtained for recipients who received an organ from a deceased donor (Fig. 7).

Over the entire follow-up period, including in the first 30 days after transplantation, 51 patients died in the first group, and 8 in the second group. Cardiovascular disease was the main cause of death among patients who received the standard IST protocol (Fig. 8), whereas infectious complications was the main cause of death in the group that received the double-induction IST protocol (Fig. 9).

The prevalence of infectious complications in the double-induction group is probably due to a shorter follow-up period for these patients, with absolute values

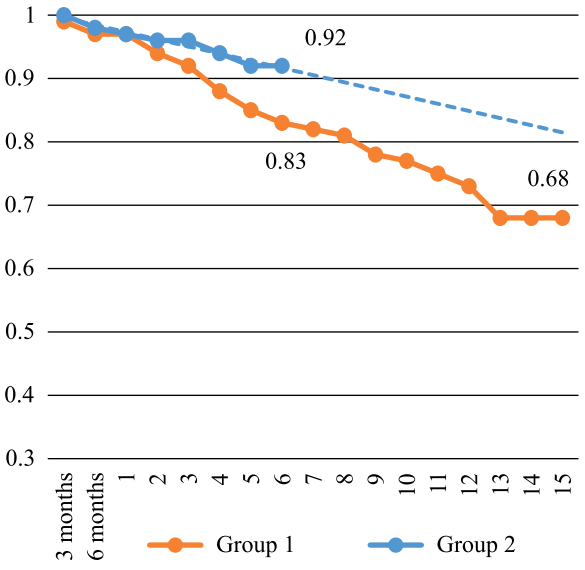

Fig. 2. Recipients survival

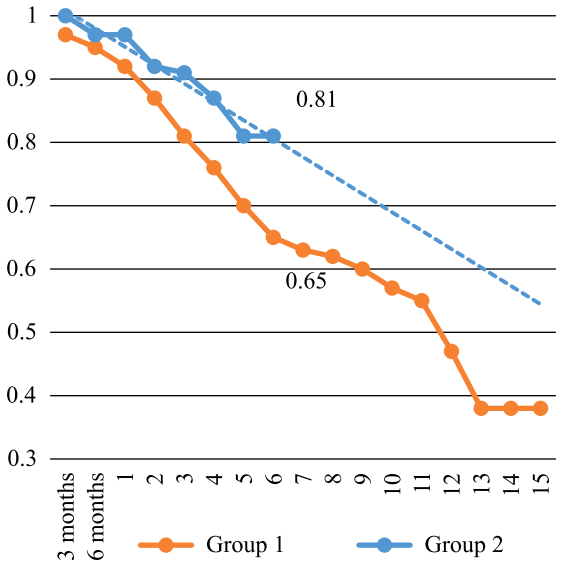

Fig. 3. Transplant survival

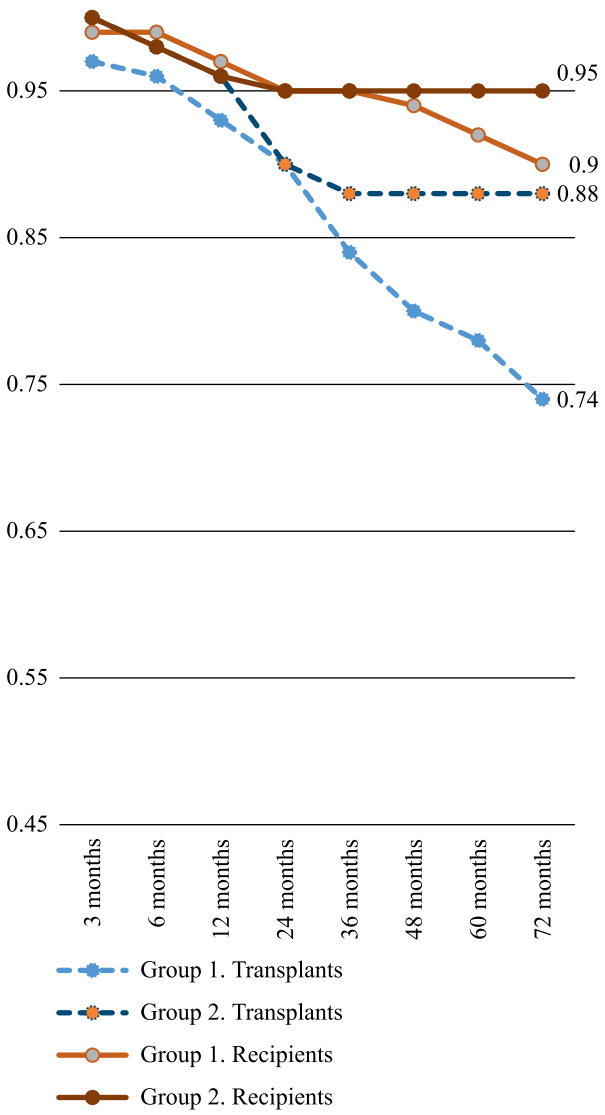

Fig. 4. Recipients and transplants survival after transplantation from alive donor

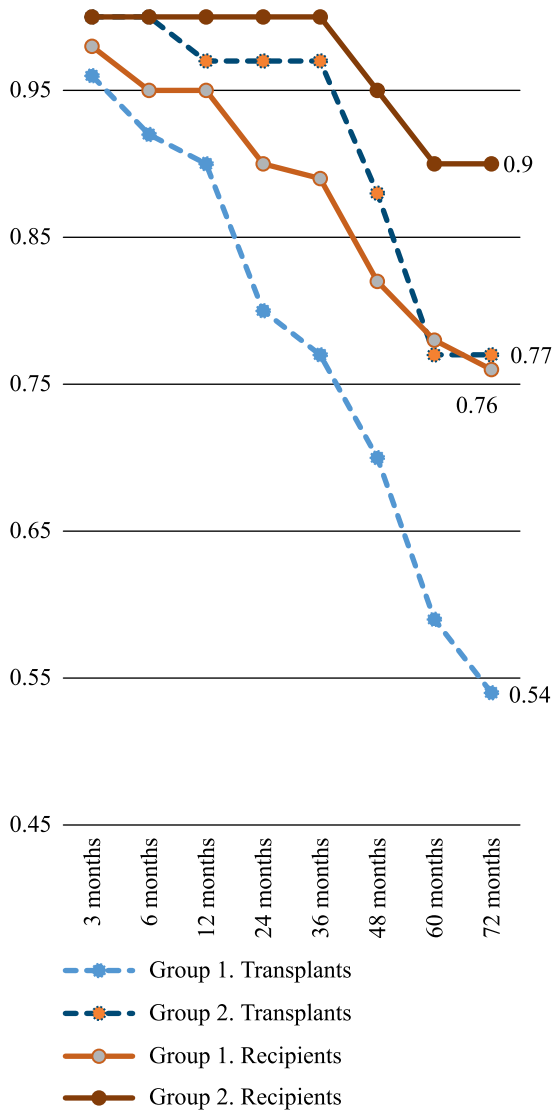

Fig. 5. Recipients and transplants survival after transplantation from deceased donor

of 15 cases in the standard IST group versus 5 in the study group.

Based on data obtained, it can be suggested that the observed results in the double-induction group were achieved due to the following factors: a) lower white blood cell count, leading to reduced severity of immunological response in the early postoperative period; b) pronounced induction immunosuppression makes it possible to delay CNI administration and maintain their lower concentration in the future, thereby reducing the negative nephrotoxic effect of CNIs in the kidney graft; c) against the background of depletion of lymphocytes most actively responding to donor antigens, basiliximab effectively inhibits interleukin-2 receptors in newly maturing and recruited CD4+ lymphocytes.

FINDINGS

1. Graft survival and recipient survival at all follow-up periods are higher in the double-induction IST group than in the standard IST group.
2. Double-induction IST protocol provides an initially better and stable kidney graft function compared to the standard IST protocol. This is especially valuable for centers experiencing difficulties in assessing pretransplant immunological risk (this makes it possible to prolong graft half-life to 5 years in group 2 compared to group 1).
3. The advantages of the double-induction protocol in recipient and graft survival is observed in deceased-donor kidney transplant to a greater extent.
4. In relative terms, infectious complications are the prevailing cause of mortality in kidney recipients who received the double-induction IST protocol. This is probably down to the shorter follow-up period for

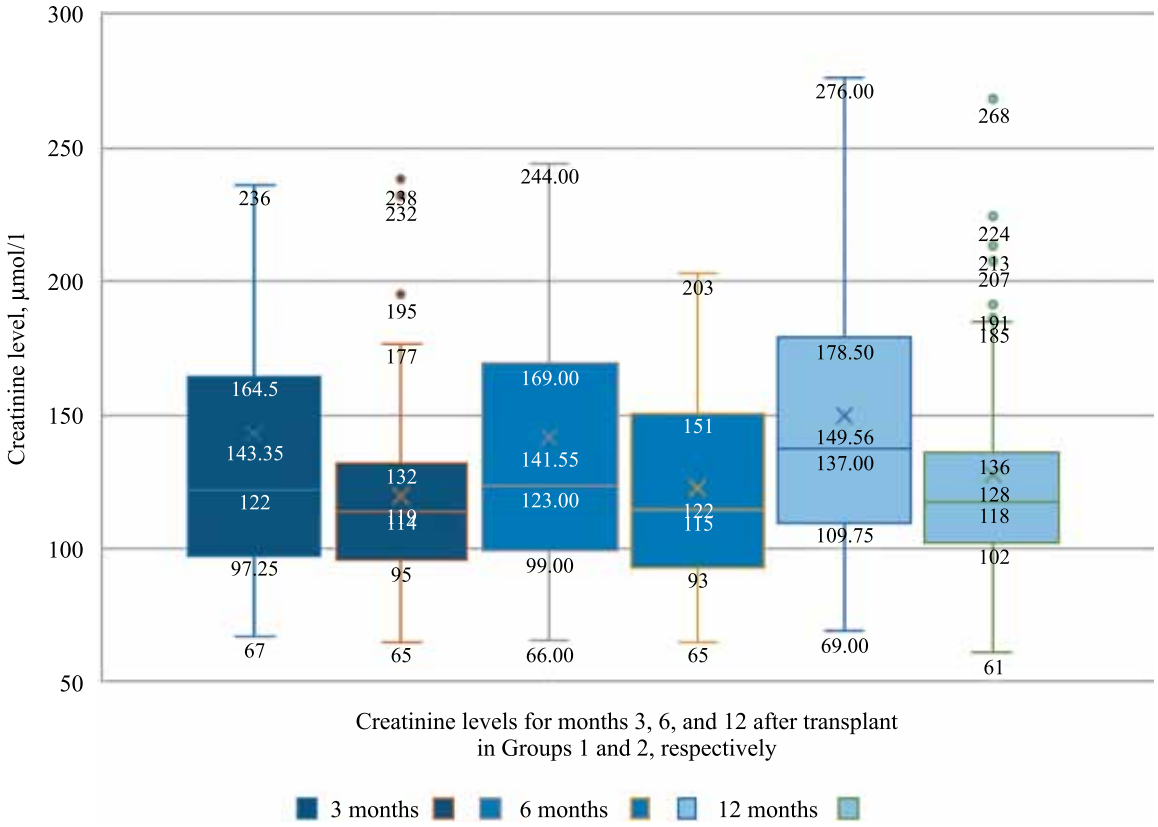

Fig. 6. Renal function in kidney recipients from alive donors

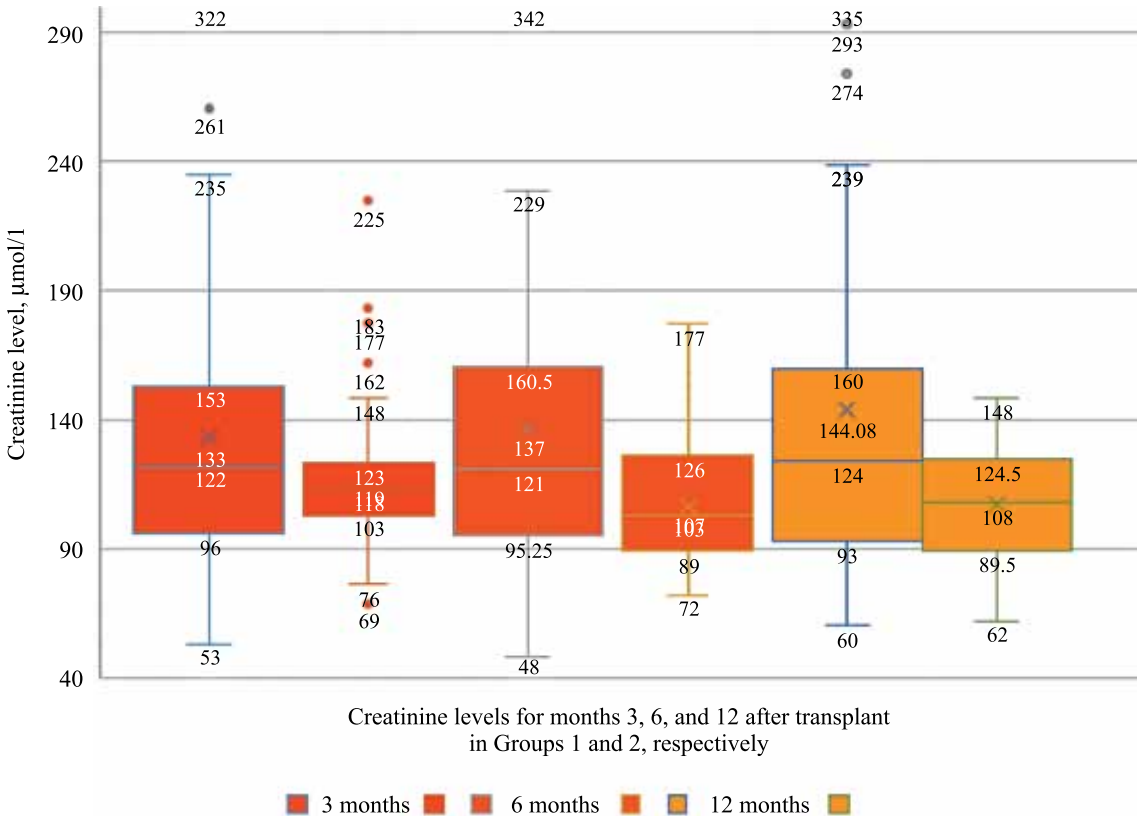

Fig. 7. Renal function in kidney recipients from deceased donors

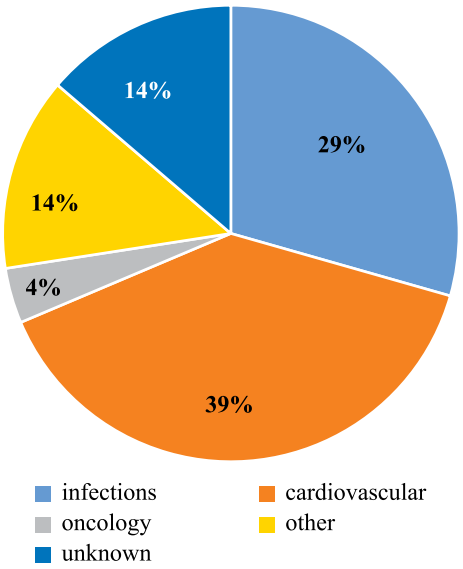

Fig. 8. The structure of mortality in group No. 1 (n = 51)

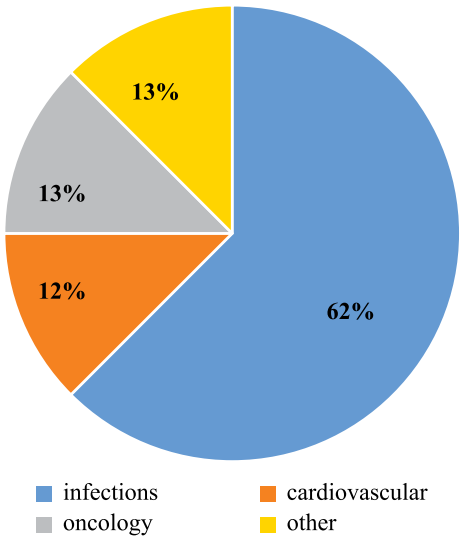

Fig. 9. The mortality structure in group No. 2 (n = 8)

this group, and possibly requires an assessment of whether or not baseline immunosuppression should be reduced.

The authors declare no conflict of interest.

REFERENCES

1. *Gautier SV.* Transplantologiya XXI veka: vysokie tekhnologii v meditsine i innovatsii v biomeditsinskoy nauke. *Vestnik transplantologii i iskusstvennykh organov*. 2017; 3: 10–32.
2. *Shilov EM, Kotenko ON, Shilova MM, Mil'chakov KS, Gabaev MA, Khal'fin RA.* Sostoyanie nefrologicheskoy sluzhby: zamestitel'naya pochechnaya terapiya v Rossiyskoy Federatsii v period s 2015-go po 2019 g. *Klinicheskaya nefrologiya*. 2020; 1: 6–14.
3. *Suslov DN.* Osnovnye printsipy immunosupressivnoy terapii posle transplantatsii pochki. *Transplantologiya*. 2018; 1: 42–49.
4. *Danovich GM.* Transplantatsiya pochki (perevod s angliyskogo pod red. Ya.G. Moysyuka.). M.: GOETAR-Media, 2013. 848.
5. *Rayniene T, Asakiene E, Zelvis A, Trofimenko II.* Vliyanie otsrochennoy funktsii transplantata na vyzhivaemost' v techenie goda posle transplantatsii. *Nefrologiya*. 2005; 4: 63–66.
6. *Hellemans R, Bosmans J-L, Abramowicz D.* Induction Therapy for Kidney Transplant Recipients: Do We Still Need Anti-IL2 Receptor Monoclonal Antibodies? *Am J Transplant*. 2017 Jan; 17 (1): 22–27.
7. *Pinchuk AV.* Induktsionnaya immunosupressiya pri transplantatsii pochki: Timoglobulin® (obzor literatury). *Transplantologiya*. 2014; 2: 31–43.
8. *Arzumanov SV, Tiptsov DV, Mitish AE, Gramotnev AK.* Opyt primeneniya razlichnykh protokolov induktsionnoy terapii istoshchayushchimi antitelami pri transplantatsii pochki. *Transplantologiya*. 2015; 2: 14–19.
9. *Brennan DC, Schnitzler MA.* Longterm results of rabbit antithymocyte globulin and basiliximab induction. *N Engl J Med*. 2008; 359 (16): 1736–1738.

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INDICATORS OF MONOCYTE-DERIVED COMPONENT OF THE IMMUNE SYSTEM IN PATIENTS WITH SATISFACTORY RENAL GRAFT FUNCTION

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Objective: to study the indicators of the monocyte-derived component of the immune system in kidney transplant recipients with satisfactory early and delayed renal transplant function. **Materials and methods.** The study involved 76 kidney transplant recipients. Concentrations of serum creatinine (sCr), serum urea (sUr) and serum cystatin C (sCysC) were measured. $CD14^{+mid/high}$ and $CD14^{+low}$ were isolated from $CD14^{+}$ monocytes. CD64- and CD86-expressing cell counts were determined for each subpopulation. Immunological examination was performed before surgery, as well as at days 1, 3, 7, 30, 90, 180 and 360 after surgery. **Results.** There was significant imbalance between the two monocyte subpopulations before transplantation and in the early post-transplant period (first 3 months). By the end of a 6-month follow-up period, the percentage of $CD14^{+}$ cells had normalized. The dynamics of the subclasses of CD86-expressing monocytes in the post-transplant period is somewhat different from the dynamics of the total count for these monocytes. However, by the end of a 6-month follow-up period, these biomarkers returned to normal for the group of healthy individuals ($CD14^{+mid/high}CD86^{+}$ $p_{180} = 0.079$; $CD14^{+low}CD86^{+}$ $p_{180} = 0.789$). $CD14^{+low}CD64^{+}$ level was significantly higher in the kidney transplant group than in the control group during the entire follow-up period ($p_0 = 0.0006$, $p_1 = 0.0001$, $p_7 = 0.005$, $p_{30} = 0.005$, $p_{90} = 0.007$, $p_{180} = 0.0002$, $p_{360} = 0.001$). On the other hand, $CD14^{+mid/high}CD64^{+}$ count for up to 180 days was not significantly different from that of the control group ($p_0 = 0.561$, $p_1 = 0.632$, $p_7 = 0.874$, $p_{30} = 0.926$, $p_{90} = 0.912$), with subsequent significant increase by day 360 of follow-up ($p_{180} = 0.01$, $p_{360} = 0.003$). We observed a negative correlation between $CD14^{+low}CD86^{+}$ level at day 0 and sCr levels at day 7 ($r = –0.4$; $p = 0.008$) and day 360 ($r = –0.34$; $p = 0.042$) and sCysC level at day 7 ($r = –0.57$; $p = 0.014$). A negative correlation was also found between $CD14^{+low}CD86^{+}$ at day 1 and sCr levels at day 7 ($r = –0.4$; $p = 0.005$) and day 360 ($r = –0.39$; $p = 0.02$). There was positive correlation between the $CD14^{+low}CD64^{+}$ subpopulation index at day 0 and sCr ($r = 0.54$; $p = 0.008$) and sCysC ($r = 0.6$; $p = 0.008$) levels at day 7, and also between the $CD14^{+low}CD64^{+}$ count at day 1 and sCr ($r = 0.55$; $p < 0.0001$) and sCysC ($r = 0.58$; $p = 0.004$) levels at day 7. $CD14^{+mid/high}CD64^{+}$ at day 0 negatively correlated with sCysC level at day 360 ($r = –0.85$; $p = 0.015$), while $CD14^{+mid/high}CD64^{+}$ at day 7 positively correlated with sCysC level at day 360 ($r = 0.50$; $p = 0.016$). **Conclusion.** Before transplant surgery, $CD14^{+mid/high}$, $CD14^{+mid/high}CD86^{+}$, and $CD14^{+low}CD86^{+}$ counts were reduced, while those of $CD14^{+low}$, $CD14^{+mid/high}CD64^{+}$ and $CD14^{+low}CD64^{+}$ were increased. By the 6-month follow-up, all these subpopulations except $CD14^{+mid/high}CD64^{+}$ had reached values for healthy people. Positive correlation between $CD14^{+mid/high}$, $CD14^{+low}CD64^{+}$, $CD14^{+mid/high}CD86^{+}$, $CD14^{+mid/high}CD64^{+}$ counts in the early post-transplant period and sCr/sCysC levels in long-term follow-up, as well as negative correlation between $CD14^{+low}$, $CD14^{+low}CD86^{+}$ counts in the early post-transplant period and sCr/sCysC levels in long-term follow-up can serve as a predictor of renal graft function.

Keywords: kidney transplantation, $CD14^{+}$ monocytes.

INTRODUCTION

Post-transplant immunologic monitoring in kidney transplant recipients is essential for improving transplant outcomes. However, many factors influencing the recipient's immune response make interpretation of immunological test results difficult. This problem is especially acute when evaluating the efficacy and toxicity of immunosuppressive therapy, predicting kidney transplant function, correcting secondary immunodeficiency in these patients with frequent and severe infectious complications and malignant tumors.

Patients are known to have post-transplant disorders in the basic functioning of both the acquired immune system and innate immune system associated with mononuclear phagocytic cells. In this regard, the study of the peculiarities of the subpopulation composition of peripheral blood monocytes in patients with uneventful post-transplant period seems to be quite logical and justified.

Monocytes are an important cell type for studying aseptic inflammation occurring in kidney transplants during reperfusion. Depending on expression of the high-

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affinity receptor for lipopolysaccharide (LPS) (CD14), it is common to distinguish the classical subpopulations of $CD14^+$ monocytes, transient $CD14^+CD16^+$ and non-classical $CD14^{+high}CD16^+$. As is known, $CD14^{+high}$ monocytes are commonly referred to as "classical", representing a larger population of less mature cells, while $CD14^{+low}$ monocytes are usually referred to as "pro-inflammatory" [1]. Monocyte subpopulations differ in the expression of molecules mediating the recognition, phagocytosis and presentation of antigens, which determines their functional characteristics. Although the roles of various monocyte subpopulations are not clearly defined, non-classical monocytes have both pro-inflammatory and anti-inflammatory properties, while classical and intermediate monocytes play a major role in phagocytosis and inflammation [2–4].

Classical monocytes $CD14^+CD16^-$ constitute about 80–85% of the total monocyte population. These monocytes are characterized by increased phagocytic activity, including those opsonized by complement components, due to the expression of scavenger receptors and the CD11b/CD18 receptor (CR3) [5, 6]. Also, the $CD14^+CD16^-$ subpopulation expresses high affinity receptor CD64, which in non-activated cells is associated with immunoglobulin G. The CD64 receptor is also associated with interferon-gamma receptor (IFNγ). In turn, the CD64-IgG bond maintains signaling pathways in a preactivated state and provides rapid signal transduction when binding to the IFNγ receptor [1, 7, 8]. Due to the less pronounced expression of HLA-DR and co-stimulatory molecules CD80 and CD86, this monocyte has less important properties of antigen presentation compared to other subpopulations [*5*].

The other two monocyte groups belong to minor subpopulations. Transient monocytes $CD14^+CD16^+$ are formed as a result of activation and differentiation of classical monocytes [5]. Receptors CD64 and CD16, CD14 and TLR, HLA-DR and co-stimulatory molecules are expressed on the surface of these cells. Therefore, their main functions are IgG-mediated phagocytosis, antigen presentation, PAMP recognition and cytokine synthesis [5, 6, 9].

In turn, expression of scavenger receptors and CR3 on transient monocytes is less dense than on classical ones [6].

With minimum density, $CD14^+$ and scavenger receptors express non-classical monocytes, which reduces their role in recognition of the LPS + LPS-binding protein complex and elimination of apoptotic cells. However, nonclassical monocytes express HLA-DR and co-stimulatory molecules with maximum density. Therefore, antigen presentation is one of their main functions [5, 6].

In our study, we have investigated the features of the monocyte subpopulation phenotype in patients after kidney transplantation. We have determined the relationship between monocyte content in peripheral blood and indicators reflecting the kidney transplant function with a satisfactory post-transplant period.

MATERIALS AND METHODS

The study was performed at the Republican Research Centre for Radiation Medicine and Human Ecology, Gomel, Belarus. It involved kidney transplant recipients (KTR) who underwent kidney transplantation for stage 5 chronic kidney disease at the surgical department (transplantation, reconstructive and endocrine surgery) of the Republican Research and Practical Center of the Republic of Moldova. A group (main group) was formed consisting of 76 subjects that met the following criteria: primary renal transplantation, induction therapy with anti-CD25 monoclonal antibodies, triple immunosuppressive therapy during the first 12 months of follow-up; kidney transplant function was satisfactory at postoperative day 7 and during the first 12 months.

All patients were tested for serum concentrations of creatinine, urea, and cystatin C. Immunological tests were carried out before surgery, as well as at postoperative day 1, 3, 7, 30, 90, 180 and 360.

There were 49 (64.47%) male and 27 (35.53%) female patients in this group. The median age (Me) was 46.89 ± 1.37 [44.16; 49.63] years. Before transplantation, 76.32% of patients were on long-term hemodialysis, while 23.68% were on peritoneal dialysis. Average cold ischemia time was 11.87 ± 0.43 hours. A negative result of direct cross-match was observed in 100% of cases.

When the creatinine level at postoperative day 7 was below 300 μmol/L, the function was considered primary – primary graft function (PGF), with values equal to or exceeding 300 μmol/L, and if there were indications for dialysis in the first postoperative week, the state was classified as renal graft dysfunction (RGD) [10]. A satisfactory renal graft function after a year was characterized by a 150 μmol/L blood creatinine level, as well as absence of episodes of graft rejection and the need for dialysis in the first year of follow-up [11].

The comparison group included 90 apparently healthy patients. Post-transplant follow-up period lasted for 12 months. The clinical study was carried out in accordance with the 1975 Declaration of Helsinki and approved by the Ethics Committee of the Republican Research Centre for Radiation Medicine and Human Ecology (Protocol No. 5 dated December 2, 2013).

Patients received induction therapy with anti-CD25 monoclonal antibodies, calcineurin inhibitors in combination with mycophenolate (89.5%) or azathioprine (10.5%), as well as corticosteroids. Anti-CD25 monoclonal antibodies were administered twice at 20 mg, at day 0 and day 4. Calcineurin cyclosporine was used as an inhibitor in 73.7% of patients, while 26.3% received tacrolimus.

The recipients underwent immunological tests on a FacsCanto II flow cytometer (Becton Dickinson and

Company, BD Biosciences, USA) complete with a sample preparation station using CD14PC7, CD64 FITC, CD86PE monoclonal antibodies (Beckman Coulter, USA) by mono-, two- and six-parameter analysis according to the manufacturer's instructions using multiple translational gating.

Determination of the relative and absolute monocyte counts

Blood was taken from the median cubital vein into tubes with anticoagulant EDTA. To determine the expression of monocyte surface markers by flow cytometry, a sample was prepared using the no-wash technique. CD14PC7, CD64 FITC, and CD86 PE monoclonal antibodies (Beckman Coulter, USA) were added to 100 μL of blood in the quantity recommended by the manufacturer. The mixture was incubated for 15 minutes in the dark at room temperature. Lysis solution OptiLyse B was used for lysis of red blood cells. Samples were analyzed on a FACS CantoII flow cytometer (BD, USA). Up to 20,000 events were accumulated. The monocyte population was determined as CD14+ cells. Depending on the CD14 expression density, two subpopulations were identified among $CD14^+$ monocytes: $CD14^{+mid/high}$ (classical) and $CD14^{+low}$ (nonclassical). For each of the subpopulations, the relative CD64- and CD86-expressing cell count was determined. The absolute content of these subpopulations was calculated using the data obtained from a general blood test carried out from this tube on the same day.

The results were statistically processed using the Statistica 10.0 software package. Descriptive statistics of the qualitative features are presented by absolute and relative frequencies, and quantitative statistics in the format: mean (confidence interval) – M [Confidence –95%; +95%] and median (interquartile range) – Me [Q25; Q75]. To compare the values, we used a numerical characteristics method (Mann–Whitney U test, Wilcoxon Matched Pairs Test) with estimation of the distribution of variables. Correlation analysis of indicators was evaluated using Spearman rank-order correlations. The results were considered statistically significant with a less than 0.05 significance level.

RESULTS

Results presented in Table 1 were obtained during analysis of biochemical indicators of renal function.

When studying the indices of peripheral blood monocytes (of kidney transplant recipients) expressing the main differentiating marker, the LPS receptor CD14, on their surface, the monocytes were clearly divided into two subpopulations: $CD14^{+mid/high}$ and $CD14^{+low}$ (Tables 2 and 3).

In the KTR group before transplantation, the $CD14^{+low}$ level was significantly higher than in the comparison group ($p_{0Mann–Whitney\ U\ Test} = 0.0003$). There was a negative trend in the dynamics of this subpopulation at day 1 relative to the preoperative level ($p_{0.1Wilcoxon\ Matched\ Pairs\ Test} = 0.001$) and the achievement of the result of the comparison group ($p_{1Mann–Whitney\ U\ Test} = 0.289$), restoring and even significantly exceeding the control result by day 7 ($p_{7Mann–Whitney\ U\ Test} = 0.001$; $p_{30Mann–Whitney\ U\ Test} = 0.038$; $p_{90Mann–Whitney\ U\ Test} = 0.001$).

We noted a slight decrease in this subpopulation at day 180 and day 360, but not lower than that of the comparison group ($p_{180Mann–Whitney\ U\ Test} = 0.72$; $p_{360Mann–Whitney\ U\ Test} = 0.279$). As for the comparison group with the preoperative count of this subpopulation and its relative dynamics, we found decreased $CD14^{+low}$ monocytes at day 1 (p0.1Wilcoxon Matched Pairs Test = 0.001) and an increase in this subpopulation from day 7 ($p_{0.7Wilcoxon\ Matched\ Pairs\ Test} = 0.028$, $p_{0.30Wilcoxon\ Matched\ Pairs\ Test} = 0.028$, $p_{0.90Wilcoxon\ Matched\ Pairs\ Test} = 0.005$, $p_{0.180Wilcoxon\ Matched\ Pairs\ Test} = 0.001$, $p_{0.360Wilcoxon\ Matched\ Pairs\ Test} = 0.023$). The dynamics of the $CD14^{+low}$ monocyte subpopulation is shown in Fig. 1.

$CD14^{+low}$ monocytes are antigen-presenting, which are responsible for increased production of pro-inflammatory cytokines – interleukins-1, -6, tumor necrosis factor. An increase in their count can serve as a marker of acute and exacerbation of chronic infectious diseases [12]. The $CD14^{+low}$ monocyte count over time decreased

Table 1

Biochemical parameters of renal function in recipients of kidney transplant and comparison group (Me [Q25; Q75])

Group	Urea, mmol/l	Creatinine, μmol/l	Cystatin C, mg/l
RTR0	19.2 [16.8; 22.2]	649.50 [569.0; 927.5]	5.94 [3.71; 6.39]
RTR1	17.0 [15.0; 21.9]	466.5 [354.0; 616.5]*	2.51 [1.99; 3.77]*
RTR7	10.35 [7.8; 14.5]*	148.0 [114.0; 196.5]*	1.33 [1.19; 1.92]*
RTR30	10.7 [8.1; 13.4]*	115.0 [102.0; 136.0]*	1.37 [1.22; 1.75]*
RTR90	8.2 [6.5; 10.1]*	99.0 [86.0; 129.5]*	1.46 [1.23; 1.68]*
RTR180	8.2 [6.1; 10.0]*	106.0 [86.0; 125.0]*	1.49 [1.16; 1.77]*
RTR360	7.2 [5.9; 10.6]*	106.5 [84.0; 130.0]*	1.51 [1.26; 1.72]*

Note. * – $p < 0.05$ compared to preoperative level. ** – RTR = renal transplant recipient.

Table 2

Indices of $CD14^{+low}$ monocyte subpopulations in recipients of kidney transplant and comparison group (Me [Q25; Q75])

Group	Unit	Monocyte subpopulations		
		$CD14^{+low}$	$CD14^{+low}CD86^{+}$	$CD14^{+low}CD64^{+}$
control	rel x %	3.7 [1.9; 5.5]	93.80 [88.2; 96.4]	82.00 [50.0; 91.3]
	10^9 cell/l	0.02 [0.01; 0.03]	0.02 [0.01; 0.02]	0.008 [0.006; 0.019]
RTR0	rel x %	7.50 [6.52; 8.04]*	78.27 [69.19; 90.36]*	96.14 [94.69; 97.59]*
	10^9 cell/l	0.02 [0.01; 0.02]	0.011 [0.011; 0.026]	0.016 [0.014; 0.032]
RTR1	rel x %	3.2 [2.28; 3.74]**	58.53 [48.7; 66.85]*	96.46 [94.69; 98.55]*
	10^9 cell/l	0.01 [0.01; 0.01]	0.004 [0.004; 0.005]	0.007 [0.006; 0.008]
RTR7	rel x %	5.57 [4.87; 6.39]*.**	81.43 [72.36; 90.51]*	93.94 [91.69; 95.39]*
	10^9 cell/l	0.03 [0.01; 0.04]	0.021 [0.01; 0.02]	0.024 [0.014; 0.031]*
RTR30	rel x %	6.08 [5.48; 7.13]*.**	71.27 [59.92; 75.80]*	96.94 [95.09; 98.39]*
	10^9 cell/l	0.04 [0.02; 0.05]	0.022 [0.017; 0.04]	0.037 [0.018; 0.051]
RTR90	rel x %	6.68 [6.21; 7.57]*.**	71.66 [66.36; 79.60]*	93.92 [92.39; 95.21]*
	10^9 cell/l	0.04 [0.03; 0.05]	0.032 [0.02; 0.04]	0.04 [0.028; 0.048]*
RTR180	rel x %	4.48 [3.43; 5.09]**	91.90 [88.31; 96.24]	97.13 [95.52; 98.18]*
	10^9 cell/l	0.02 [0.01; 0.03]	0.02 [0.016; 0.03]	0.022 [0.018; 0.034]*
RTR360	rel x %	4.79 [3.15; 7.0]**	85.03 [77.04; 90.12]*	94.96 [92.72; 96.64]*
	10^9 cell/l	0.03 [0.02; 0.04]	0.026 [0.018; 0.04]	0.029 [0.018; 0.043]*

Note. Here and in the Table 3: * – $p < 0.05$ relative to the matched control group; ** – $p < 0.05$ compared to preoperative level.

Table 3

Indices of $CD14^{+mid/high}$ monocyte subpopulations in recipients of kidney transplant and comparison group (Me [Q25; Q75])

Group	Unit	Monocyte subpopulations		
		$CD14^{+mid/high}$	$CD14^{+mid/high}CD86^{+}$	$CD14^{+mid/high}CD64^{+}$
control	rel x %	95.5 [93.6; 98.1]	98.6 [97.6; 99.6]	97.2 [96.3; 98.5]
	10^9 cell/l	0.42 [0.37; 0.56]	0.41 [0.37; 0.56]	0.41 [0.36; 0.54]
RTR0	rel x %	92.50 [91.96; 93.48]*	99.40 [99.02; 99.81]*	97.80 [96.22; 99.15]
	10^9 cell/l	0.18 [0.16; 0.37]	0.18 [0.16; 0.43]	0.172 [0.152; 0.43]
RTR1	rel x %	96.8 [96.26; 97.72]**	98.33 [98.02; 98.75]	97.80 [96.22; 99.15]
	10^9 cell/l	0.39 [0.28; 0.42]	0.36 [0.16; 0.19]	0.52 [0.39; 0.73]
RTR7	rel x %	94.43 [93.61; 95.13]**	98.83 [98.52; 99.20]	97.56 [96.22; 99.00]
	10^9 cell/l	0.18 [0.16; 0.39]	0.18 [0.16; 0.44]	0.155 [0.08; 0.45]
RTR30	rel x %	93.92 [92.87; 94.52]**	81.46 [76.42; 90.30]*	97.70 [96.00; 99.17]
	10^9 cell/l	0.29 [0.18; 0.39]**	0.28 [0.14; 0.49]	0.36 [0.16; 0.50]
RTR90	rel x %	93.32 [92.43; 93.79]*.**	94.63 [94.24; 95.0]*	97.38 [96.22; 98.95]
	10^9 cell/l	0.18 [0.16; 0.39]	0.17 [0.15; 0.43]	0.179 [0.15; 0.39]
RTR180	rel x %	95.52 [94.91; 96.58]**	97.19 [95.01; 98.69]	98.86 [98.19; 99.3]*
	10^9 cell/l	0.19 [0.16; 0.39]	0.18 [0.15; 0.45]	0.18 [0.15; 0.45]
RTR360	rel x %	95.21 [93.0; 96.85]**	97.80 [97.13; 98.57]	99.47 [99.13; 99.7]*
	10^9 cell/l	0.17 [0.15; 0.19]	0.15 [0.15; 0.19]	0.16 [0.16; 0.18]

relative to the preoperative level. However, during the entire follow-up period, we noted that the level in the comparison group was exceeded. This was due to the influence of a complex of factors, including surgical intervention, antigenic conflict, and immunosuppressive therapy.

The $CD14^{+high}$ monocytes are considered to be "classical", representing a larger population of less mature cells that provide antimicrobial protection as a result of phagocytic activity [13]. In our study, a reduced pre-transplantation level of the $CD14^{+mid/high}$ subpopulation ($p_{0Mann–Whitney\ U\ Test} = 0.0005$) was noted (Table 3, Fig. 2).

Starting from postoperative day 1, the level of this subpopulation reached that of the comparison group and remained so until 3 months of follow-up, briefly decreased at day 90 of follow-up and restored by day 180 ($p_{1Mann–Whitney\ U\ Test} = 0.207$, $p_{7Mann–Whitney\ U\ Test} = 0.528$, $p_{30Mann–Whitney\ U\ Test} = 0.077$, $p_{90Mann–Whitney\ U\ Test} = 0.003$, $p_{180Mann–Whitney\ U\ Test} = 0.858$, $p_{360Mann–Whitney\ U\ Test} = 0.426$). Although the entire follow-up period showed a signi-

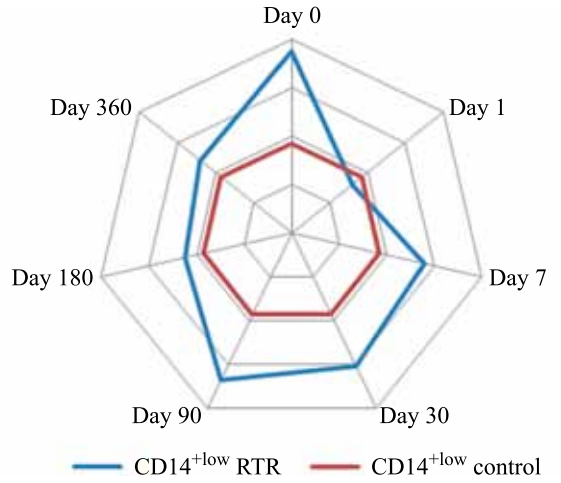

Fig. 1. Dynamics of $CD14^{+low}$ monocytes in recipients of kidney allograft during the first year of observation (CG)

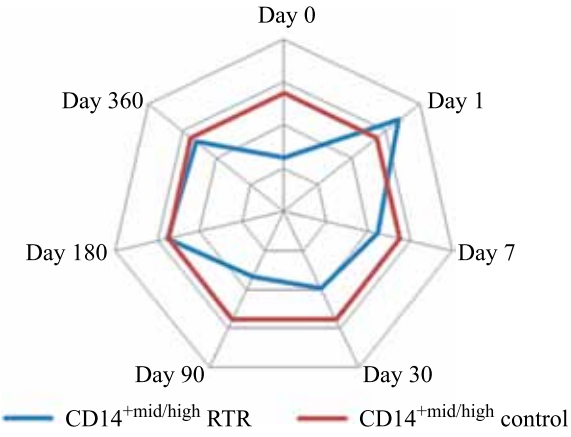

Fig. 2. Dynamics of $CD14^{+mid/high}$ monocytes in recipients of kidney allograft during the first year of observation

ficant upward trend relative to the preoperative level ($p_{0.1Wilcoxon\ Matched\ Pairs\ Test} = 0.001$, $p_{0.7Wilcoxon\ Matched\ Pairs\ Test} = 0.0003$, $p_{0.30Wilcoxon\ Matched\ Pairs\ Test} = 0.003$, $p_{0.90Wilcoxon\ Matched\ Pairs\ Test} = 0.028$, $p_{0.180Wilcoxon\ Matched\ Pairs\ Test} = 0.005$, $p_{0.360Wilcoxon\ Matched\ Pairs\ Test} = 0.001$).

Based on results obtained, it can be stated that before transplantation and in the early post-transplant period (first 3 months), two monocyte subpopulations were found to have a significant imbalance. By month 6 of follow-up, the percentage of $CD14^{+}$ cells had normalized.

In performing correlation analysis of the level of subpopulations $CD14^{+low}$ and $CD14^{+mid/high}$ with indicators characterizing kidney function, we found a negative correlation between the relative count of $CD14^{+low}$ before transplantation and creatinine level at day 1 ($r = –0.60$; $p = 0.008$) and between the absolute count at day 7 with creatinine level at year 1 ($r = –0.85$; $p = 0.016$). Also, a high level of $CD14^{+low}$ at day 30 of follow-up negatively correlated with serum cystatin C levels at year 1 ($r = –0.81$; $p = 0.015$). In turn, the preoperative $CD14^{+mid/high}$ level and the serum creatinine level at day 1 had a positive correlation ($r = 0.60$; $p = 0.008$), while the $CD14^{+mid/high}$ level after 1 month of follow-up and serum cystatin C levels at year 1 had a positive correlation ($r = 0.8$; $p = 0.015$). Thus, the negative correlation between the $CD14^{+low}$ level at the early stages of examination and the creatinine/cystatin C levels at 12 months of follow-up, and the positive correlation between the $CD14^{+mid/high}$ level at the early stages of examination and the creatinine/cystatin C levels at 12 months of follow-up suggest that these indicators could be used for predictive purposes.

We analyzed the count of $CD14^{+mid/high}$ and $CD14^{+low}$ subpopulations expressing CD86, which is a costimulatory ligand of the CD28 and CD152 molecules. The interaction between these molecules contributes to either positive or negative regulation of the immune response [14].

In the KTR group, almost the entire follow-up period, with the exception of day 180, revealed a significantly lower $CD14^{+low}CD86^{+}$ monocyte subpopulation than in the comparison group ($p_{0Mann–Whitney\ U\ Test} = 0.0004$, $p_{1Mann–Whitney\ U\ Test} < 0.0001$, $p_{7Mann–Whitney\ U\ Test} = 0.001$, $p_{30Mann–Whitney\ U\ Test} = 0.0001$, $p_{90Mann–Whitney\ U\ Test} < 0.0001$, $p_{180Mann–Whitney\ U\ Test} = 0.789$, $p_{360Mann–Whitney\ U\ Test} = 0.0006$). As for the dynamics relative to the preoperative level, the trend towards a reduced $CD14^{+low}CD86^{+}$ count persisted up to day 360, when there were no differences with the preoperative period ($p_{0.1Wilcoxon\ Matched\ Pairs\ Test} = 0.001$, $p_{0.7Wilcoxon\ Matched\ Pairs\ Test} = 0.0003$, $p_{0.30Wilcoxon\ Matched\ Pairs\ Test} = 0.028$, $p_{0.90Wilcoxon\ Matched\ Pairs\ Test} = 0.005$, $p_{0.180Wilcoxon\ Matched\ Pairs\ Test} = 0.001$, $p_{0.360Wilcoxon\ Matched\ Pairs\ Test} = 0.307$) (Fig. 3).

In turn, the $CD14^{+mid/high}CD86^{+}$ monocyte subpopulation had less pronounced dynamics. It decreased significantly only at day 30 and 90 relative to the preoperative level ($p_{0.30Wilcoxon\ Matched\ Pairs\ Test} = 0.028$,

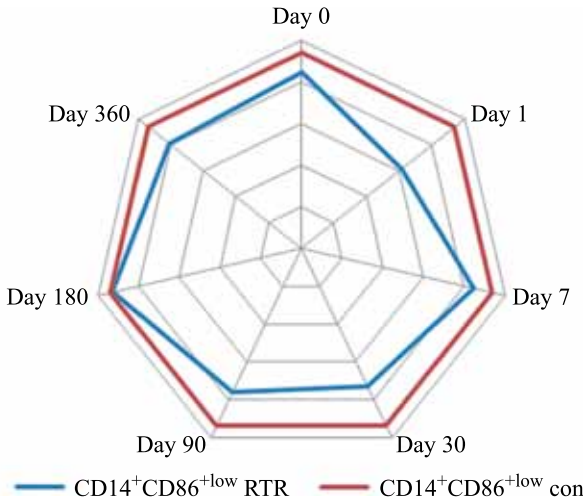

Fig. 3. Dynamics of $CD14^{+low}CD86^{+}$ monocytes in recipients of kidney allograft during the first year of observation

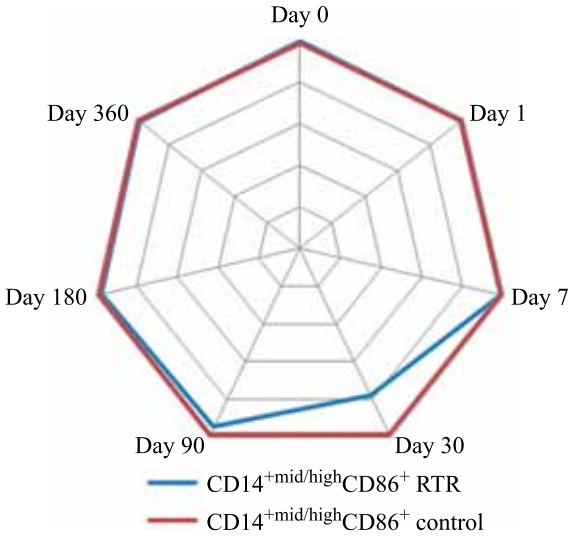

Fig. 4. Dynamics of $CD14^{+mid/high}CD86^{+}$ monocytes in recipients of kidney allograft during the first year of observation

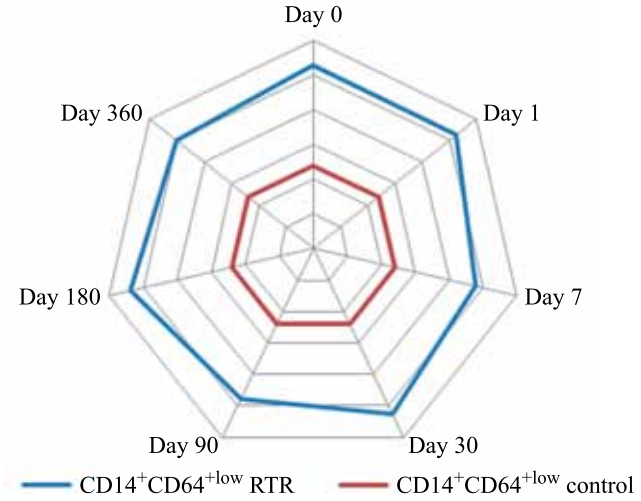

Fig. 5. Dynamics of $CD14^{+low}CD64^{+}$ monocytes in recipients of kidney allograft during the first year of observation

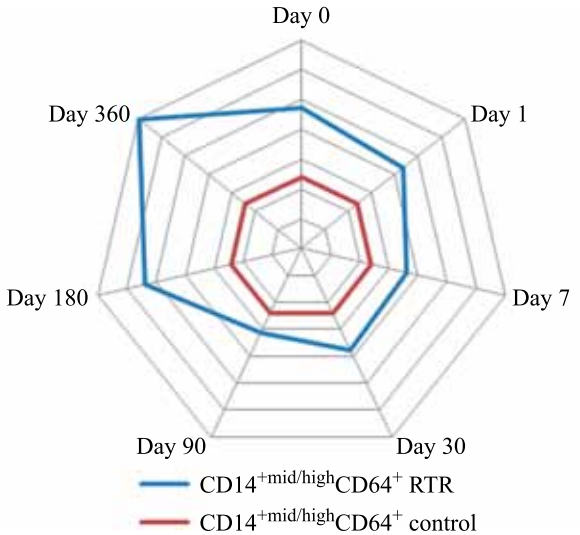

Fig. 6. Dynamics of $CD14^{+mid/high}CD64^{+}$ monocytes in recipients of kidney allograft during the first year of observation

$p_{0.90Wilcoxon\ Matched\ Pairs\ Test} = 0.005$). In the comparison group, the $CD14^{+mid/high}CD86^{+}$ cell count before transplantation was slightly higher ($p = 0.02$). But later there was a decrease in this subpopulation and recovery at month 6 of follow-up ($p_{1Mann–Whitney\ U\ Test} = 0.528$, $p_{7Mann–Whitney\ U\ Test} = 0.479$, $p_{30Mann–Whitney\ U\ Test} < 0.0001$, $p_{90Mann–Whitney\ U\ Test} = 0.002$, $p_{180Mann–Whitney\ U\ Test} = 0.079$, $p_{360Mann–Whitney\ U\ Test} = 0.209$) (Fig. 4).

Based on the observed results, it can be stated that the dynamics of the two studied subclasses of CD86-expressing monocytes in the post-transplantation period slightly differs from the dynamics of the total count of these monocytes. But at month 6 of follow-up, not only was the preoperative level restored, but these indicators normalized relative to the groups of healthy individuals.

With a stable constancy, the count of minor subpopulation of monocytes expressing $CD14^{+low}CD64^{+}$, the high-affinity receptor for IgG, prevailed in the KTR group relative to the comparison group throughout the follow-up period ($p_{0Mann–Whitney\ U\ Test} = 0.0006$, $p_{1Mann–Whitney\ U\ Test} = 0.0001$, $p_{7Mann–Whitney\ U\ Test} = 0.005$, $p_{30Mann–Whitney\ U\ Test} = 0.005$, $p_{90Mann–Whitney\ U\ Test} = 0.007$, $p_{180Mann–Whitney\ U\ Test} = 0.0002$, $p_{360Mann–Whitney\ U\ Test} = 0.001$) (Fig. 5).

Some differences were revealed in the dynamics of classical monocytes expressing the IgG receptor. Before day 180, the $CD14^{+mid/high}CD64^{+}$ count did not significantly differ from the comparison group ($p_{0Mann–Whitney\ U\ Test} = 0.561$, $p_{1Mann–Whitney\ U\ Test} = 0.632$, $p_{7Mann–Whitney\ U\ Test} = 0.874$, $p_{30Mann–Whitney\ U\ Test} = 0.926$, $p_{90Mann–Whitney\ U\ Test} = 0.912$). Then, a progressive increase in the level of this subpopulation led to maximum count of the cells at day 360 of follow-up ($p_{180Mann–Whitney\ U\ Test} = 0.01$, $p_{360Mann–Whitney\ U\ Test} = 0.003$) (Fig. 6).

Correlation analysis of the above subpopulations with renal graft function indicators revealed the following. The $CD14^{+low}CD86^{+}$ count at day 0 was negatively correlated with creatinine level at day 7 and day 360 ($r = –0.4$; $p = 0.008$ and $r = –0.34$; $p = 0.042$, respectively) and with cystatin C level at day 7 ($r = –0.57$; $p = 0.014$). The $CD14^{+low}CD86^{+}$ count at day 1 was also found to be negatively correlated with creatinine levels at day 7 and day 360 ($r = –0.4$; $p = 0.005$ and $r = –0.39$; $p = 0.02$, respectively). There was a positive correlation between the $CD14^{+low}CD64^{+}$ count at day 0 and creatinine and cystatin C levels at day 7 ($r = 0.54$; $p = 0.008$ and $r = 0.6$; $p = 0.008$, respectively). The $CD14^{+low}CD64^{+}$ count at day 1 and creatinine and cystatin C levels at day 7 were also positively correlated ($r = 0.55$; $p < 0.0001$ and $r = 0.58$; $p = 0.004$, respectively).

There was a positive correlation between the $CD14^{+mid/high}CD86^{+}$ count at day 0 and day 7 and the cystatin C level at day 360 ($r = 0.48$; $p = 0.019$ and $r = 0.36$; $p = 0.033$, respectively).

As for the $CD14^{+mid/high}CD64^{+}$ subpopulation, there were no significant correlations with the creatinine level over the entire follow-up period, but correlations

were found with cystatin C level. The $CD14^{+mid/high}CD64^{+}$ count was negatively correlated at day 0 and positively correlated at day 7 with cystatin C level at day 360 (r = 0.-85; p = 0.015 and r = 0.50; p = 0.016, respectively.

DISCUSSION

At the pre-transplantation stage of comparing monocyte subpopulations with the group of healthy individuals, it was revealed that the classical $CD14^{+mid/high}$ monocyte count reduced, while the non-classical $CD14^{+low}$ count increased in the KTR group. Similar changes were found in studies conducted earlier to investigate the dynamics of these subpopulations in other types of surgical interventions, particularly in patients with coronary heart disease who underwent coronary artery bypass grafting under artificial blood circulation [15].

The most pronounced changes in the two main monocyte subpopulations – $CD14^{+mid/high}$ and $CD14^{+low}$ – were observed at day 1. The sharp increase in the $CD14^{+mid/high}$ subpopulation may be due to the positive correlation between the content of classical monocytes and the concentration of interleukin-6 (IL-6) [2]. According to the authors, an increase in the absolute count of classical monocytes and IL-6 is an indirect criterion for assessing the degree of activation of endothelium, an active producer of growth factors myeloid germ and IL-6 [2]. With regard to the secretion of IL-6 in the early post-transplant period, a number of researchers noted that during transplantation of brain-dead donor kidneys, an IL-6 release can be expected within the first 4–6 hours after reperfusion. The peak time of IL-6 release depends on the influence of many factors, such as the warm and cold ischemia time, type of donor, and characteristics of the initial graft function. Moreover, the authors found that the absence of such a reaction is a poor prognostic sign [16]. Therefore, the peak of increase in classical monocytes at day 1 and restoration of their preoperative level at day 7 is prognostically favorable for the patient.

The peculiarities of the dynamics of monocyte subpopulations $CD14^{+mid/high}$, $CD14^{+low}$, $CD14^{+mid/high}CD86^{+}$, $CD14^{+low}CD86^{+}$, $CD14^{+mid/high}CD64^{+}$, and $CD14^{+low}CD64^{+}$ revealed in our study were that all significant differences with the comparison group were erased at month 6 after transplantation, except for the minor subpopulation $CD14^{+mid/high}CD64^{+}$, whose count increased maximally by year 1 of follow-up. At various stages of the study, the $CD14^{+mid/high}$, $CD14^{+low}CD64^{+}$, $CD14^{+mid/high}CD86^{+}$, $CD14^{+mid/high}CD64^{+}$ counts in the early post-transplant period were found to be positively correlated with creatinine and cystatin levels at long-term follow-up, and the $CD14^{+low}$, $CD14^{+low}CD86$ counts in the early post-transplant period were negatively correlated with creatinine and cystatin levels at long-term follow-up. The exception was the minor subpopulation $CD14^{+mid/high}$, which expresses the high-affinity IgG receptor. There were no correlations between this monocyte subpopulation and creatinine levels. However, there was a positive correlation between this subpopulation, which is a precursor of inflammatory macrophages [17], at day 0, day 1, and day 7 with serum cystatin C level at day 90, day 180 and day 360 of follow-up.

Considering that $CD14^{+mid/high}CD64^{+}$ are an important link in the innate immune system, particularly in implementation of phagocytic function, the results obtained in our study should be further explored.

So, the revealed relationships between minor monocyte subpopulations and laboratory indicators of kidney graft function indicate the possibility of using indicators of these subpopulations on postoperative day 1 in order to predict the functional state of the graft. Considering that the study was conducted in patients with satisfactory renal graft function at year 1 of follow-up and a comparable immunosuppressive therapy, the described dynamics of monocyte subpopulations can be used for immunological monitoring in the post-transplant period.

CONCLUSION

The revealed features of the monocytic immunity link in kidney recipients with satisfactory early and delayed graft function included reduced pre-transplant count of classical monocytes $CD14^{+mid/high}$, $CD14^{+mid/high}CD86^{+}$, $CD14^{+low}CD86$ and increased count of $CD14^{+low}$, $CD14^{+mid/high}CD64^{+}$, $CD14^{+low}CD64^{+}$ relative to those of healthy individuals. In the post-transplant period, all indicated subpopulations, with the exception of $CD14^{+mid/high}CD64^{+}$, reached normal values by month 6 of follow-up. The presence of negative correlations between the $CD14^{+low}$, $CD14^{+low}CD86^{+}$ count in the early post-transplant period and the creatinine/cystatin C levels in the long-term follow-up, and the positive correlations between the $CD14^{+mid/high}$, $CD14^{+low}CD64^{+}$, $CD14^{+mid/high}CD86^{+}$, $CD14^{+mid/high}CD64^{+}$ count in the early post-transplant period and the creatinine/cystatin C levels in the long-term follow-up can be used as prognostic factors for delayed kidney graft function.

The authors declare no conflict of interest.

REFERENCES

1. *Bezbradica JS, Rosenstein RK, DeMarco RA, Brodsky I, Medzhitov R.* A role for the ITAM signaling module in specifying cytokine-receptor function. *Nat Immunol*. 2014; 15 (4): 333–342. doi: 10.1038/ni.2845.
2. *Kalashnikova AA, Voroshilova TM, Chinenova LV, Davydova NI, Kalinina NM.* Monocyte subsets in healthy adults and sepsis patients. *Medical Immunology (Russia)*. 2018; 20 (6): 815–824. (In Russ.). doi: 10.15789/1563-0625-2018-6-815-824.
3. *Ziegler-Heitbrock L, Hofer TP.* Toward a refined definition of monocyte subsets. *Front Immunol.* 2013; 4: 23. doi: 10.3389/fimmu.2013.00023.
4. *Liang EC, Rossetti M, Sidwell T, Groysberg V, Sunga G, Korin Y et al.* Differences in Proinflammatory

Cytokines and Monocyte Subtypes in Older as Compared With Younger Kidney Transplant Recipients. *Transplant Direct*. 2018; 4 (3): e348. doi: 10.1097/TXD.0000000000000762.

5. *Mukherjee R, Kanti Barman P, Kumar Thatoi P, Tripathy R, Kumar Das B., Ravindran B.* Non-Classical monocytes display inflammatory features: Validation in sepsis and System Lupus Erythematous. *Sci Rep*. 2015; 5: 13886. doi: 10.1038/srep13886.
6. *Skrzeczynska-Moncznik J, Browska M, Loseke S, Grage-Griebenow E, Zembala M, Pryjma J.* Peripheral blood $CD14^{hlgh}CD16^{+}$ monocytes are main producers of IL-10. *Scand J Immunol*. 2008; 67 (2): 152–159. doi: 10.1111/j.1365-3083.2007.02051.x.
7. *Swisher JF, Feldman GM.* The many faces of FcyRI: implication for therapeutic antibody function. *Immunol Rev*. 2015; 268 (1): 160–174. doi: 10.1111/imr.12334.
8. *Van der Poel CE, Spaapen RM, van de Winkel JGJ, Leusen JHW.* Functional characteristics of the high affinity IgG receptor, FcyRI. *J Immunol*. 2011; 186 (5): 2699–2704. doi: 10.4049/jimmunol.1003526.
9. *Lee J, Tam H, Adler L, Ilstad-Minnihan A, Macaubas C, Mellins ED.* The MHC class II antigen presentation pathway in human monocytes differs by subset and is regulated by cytokines. *PLoS ONE*. 2017; 12 (8): e0183594. doi: 10.1371/journal.pone.0183594.
10. *Cantaluppi V, Dellepiane S, Tamagnone M, Medica D, Figliolini F, Messina M et al.* Neutrophil Gelatinase Associated Lipocalin Is an Early and Accurate Biomarker of Graft Function and Tissue Regeneration in Kidney Transplantation from Extended Criteria Donors. *PLoS ONE*. 2015; 10 (6): 1–19. doi: 10.1371/journal.pone.0129279.
11. *Massart A, Ghisdal L, Abramowicz M, Abramowicz D.* Operational Tolerance in Kidney Transplantation and Associated Biomarkers: Serendipitous tolerance in kidney recipients. *Clinical & Experimental Immunology*. 2017; 189 (2): 138–157. doi: 10.1111/cei.12981.
12. *Loems Ziegler-Heitbrock.* The CD14+ CD16+ blood monocytes: their role in infection and inflammation. *J Leuk Biol*. 2007; 81: 584–592.
13. *Kim OY, Monsel A, Bertrand M, Coriat P, Cavaillon J-M, Adib-Conquy M.* Differential down- regulation of HLA-DR on monocyte subpopulations during systemic inflammation. *Critical Care*. 2010; 14: R61. doi: 10.1186/cc8959.
14. *Novikov DK, Novikov PD.* Klinicheskaya immunopatologiya. M.: Med. lit., 2009; 448.
15. *Golovkin AS, Matveeva VG, Kudryavtsev IV, Grigor'ev EV, Velikanova EA, Bairakova YuV.* Subpopulyatsii monotsitov krovi pri neoslozhnennom techenii perioperatsionnogo perioda koronarnogo shuntirovaniya. *Meditsinskaya immunologiya*. 2012; 14 (4–5): 305–312. doi: 10.15789/1563-0625-2012-4-5-305-312.
16. *Vatazin AV, Zulkarnaev AB, Kruglov EE, Astakhov PV, Krstic M, Gubarev KK i dr.* Interleukin-6 profile in early postoperative period after kidney transplantation. *Russian Journal of Transplantology and Artificial Organs*. 2013; 15 (1): 51–59. (In Russ.). doi: 10.15825/1995-1191-2013-1-51-59.
17. *Yarilin AA*. Immunologiya. M.: GEOTAR-Media, 2010; 752.

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PREVALENCE AND RISK FACTORS OF POST-KIDNEY TRANSPLANT HYPERPARATHYROIDISM: A SINGLE-CENTER STUDY

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Objective: to assess the prevalence of hyperparathyroidism (HPT) and the factors affecting its development in kidney transplant recipients. **Materials and methods.** The single-center observational cohort study included 97 kidney transplant recipients – 40 men, 57 women, age 50 ± 9 years. Inclusion criteria: more than 12 months of post-transplant period, 3 months of stable renal transplant function. Non-inclusion criterion: therapy with vitamin D, with its alternatives or with cinacalcet. Dialysis ranged from 0 to 132 months (median 18); 46% of patients had pre-operative secondary HPT. A comprehensive laboratory study included evaluation of serum concentrations of parathyroid hormone (PTH), 25-OH vitamin D, calcium, phosphorus, magnesium, total alkaline phosphatase (ALP) activity, albumin, creatinine and daily proteinuria. At the dialysis stage, the target PTH range of 130–585 pg/ml was used, in the post-transplant period – ≤130 pg/ml. Glomerular filtration rate (eGFR) was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula. **Results.** Patients were divided into two groups based on PTH threshold level (130 pg/ml): the first with HPT (PTH >130 pg/ml, median 203), the second without HPT (PTH ≤130 pg/ml, median 101). Both groups were comparable in terms of gender, age, primary renal disease, dialysis modality, post-transplant follow-up, and immunosuppressive therapy regimen. In group 1 and group 2 recipients, dialysis therapy, pre-transplant median PTH level, incidence of reoperation and incidence of immediate renal graft function were 30 (14; 50) and 14 (6; 28) months ($p = 0.004$), 681 (538; 858) and 310 (182; 556) pg/ml ($p < 0.001$), 17% and 2% ($p = 0.028$), 51% and 80% ($p = 0.005$), respectively. At the time of the study, 72% of group 1 recipients had eGFR <60 ml/min, versus 36% of group 2 ($p < 0.001$). Among HPT biochemical parameters, there were differences for ionized serum calcium (1.32 ± 0.07 versus 1.29 ± 0.04 mmol/l, $p = 0.017$) and ALP activity (113 ± 61 versus 75 ± 19 u/l, $p = 0.021$). Serum vitamin D in both groups reduced in equal measures – 14 ± 4 and 15 ± 6 ng/ml. **Conclusion.** Persistent HPT in the long-term post-transplant period reaches 48.5%. Risk factors for its development included dialysis for more than 18 months, pre-operative secondary HPT, repeated kidney transplantation, delayed graft function, and eGFR <60 ml/min.

Keywords: kidney transplantation, hyperparathyroidism, kidney graft function.

INTRODUCTION

Kidney transplantation is the best modality of renal replacement therapy for patients with stage 5 chronic kidney disease (CKD). It provides a high level of medical and social rehabilitation for such patients. According to recent domestic and foreign publications, there is a steady increase in the number of kidney transplants and a high one-year and five-year survival rate for kidney transplants and recipients [1–3].

Successful kidney transplantation eliminates complicated endocrine and metabolic disorders, particularly secondary hyperparathyroidism (HPT), a characteristic and common complication of CKD. Secondary HPT, an endocrinopathy manifested by excessive secretion of parathyroid hormone (PTH), is closely associated with changes in hormonal homeostasis, calcium and phosphate metabolism, and bone metabolism caused by decreased renal function [4, 5]. Spontaneous resolution of HPT occurs in over half of kidney transplant recipients. However, this process is slow, especially with the initial suboptimal function of the graft, and it does not occur in all recipients. In the first months of the postoperative period, decreased functional mass of the parathyroid glands (PTG) leads to rapid decrease (about half) in blood levels of PTH, followed by slower process, since paratyrocytes have a longer lifespan, and only about 5% of cells are renewed annually [6, 7].

Post-transplant HPT has a significant impact on recipient and graft survival and quality. Elevated PTH levels (more than 140 pg/mL in 2.5–3 months after surgery) were found to be associated with cardiovascular complications, bone fractures, graft loss, and increased risk of overall mortality [8–12]. According to several sources, HPT prevalence among kidney transplant recipients varies widely: it is higher in the first year after surgery and lesser afterwards. Some authors have reported about 40–50% of recipients with blood PTH above 130 pg/mL in the first postoperative year, others – only about 18%; a few years after kidney transplantation, from 17% to one third of recipients had HPT [8, 13–15]. Various risk fac-

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tors for post-transplant HPT are analyzed – convincing data has already been obtained for some, while studies are still ongoing for others [13, 15–18]. One should take note of the scarcity of information on post-kidney transplant HPT, which is presented exclusively by foreign publications. Russian studies aimed at analyzing the frequency, possible risk factors of post-kidney transplant HPT, and approaches to its prevention and correction, have almost never been carried out, despite the fact that the number of such operations is increasing annually.

The aim of this study was to assess the prevalence of HPT among kidney transplant recipients and to identify the risk factors of post-kidney transplant HPT.

MATERIALS AND METHODS

The cohort, observational study was carried out at the kidney transplantation surgical department of Vladimirsky Moscow Regional Research Clinical Institute. The study included 97 cadaver kidney recipients. Their characteristics are presented in Table 1.

The inclusion criteria for the study were: 12 months post-transplant period and above, and at least a 3-month stable kidney graft function. The non-inclusion criterion was therapy with vitamin D, its substitutes or cinacalcet. All patients received cadaver kidneys. Induction immunosuppressive therapy included basiliximab administration (40 mg total dose) and methylprednisolone (1.5 g total dose), base dose prednisolone (30 mg/day, followed by a dose reduction to a maintenance dose of 5–10 mg/day), calcineurin inhibitor (cyclosporin A, tacrolimus under control of plasma concentration of the drug), and mycophenolate.

A comprehensive laboratory study included determination of blood levels of PTH (two consecutive measurements) and vitamin D (25-OH vitamin D) (in the autumn-winter period) by chemiluminescent immunoassay using the ARCHITECT system (USA), serum electrolyte concentrations, total ALP activity, albumin, nitrogen metabolism parameters and protein concentration in urine using standard methods. The target ran-

Table 1

Demographic and clinical characteristics of patients at the time of kidney transplantation

Parameter	All patients(n = 97)
Age, years	50 ± 9
Male / Female, n (%)	40/57 (41/59)
Body mass index, kg/cm^2	25.8 ± 4.3
Primary renal disease, n (%)	
Chronic glomerulonephritis, incl. at systemic lupus erythematosis and widespread vasculitis	56 (58)
Congenital hereditary nephropathy (incl. polycystic kidney)	28 (29)
Chronic interstitial nephritis	5 (5)
Other/unknown nephropathy	8 (8)
Dialysis mode, n (%)	
Hemodialysis	60 (62)
Peritoneal dialysis	22 (23)
Hemodialysis + Peritoneal dialysis	8 (8)
No dialysis	7 (7)
Duration of dialysis therapy, months	18 (6; 35)
Renal regrafting, n (%)	9 (9)
Hyperparathyroidism before kidney transplant, n (%)	45 (46)
– mild (PTH 595–800 pg/ml)	26 (27)
– moderate (PTH 801–1000 pg/ml)	5 (5)
– severe (PTH >1000 pg/ml)	14 (14)
Posttransplant period at the moment of examination, months	21 (12; 37)
Renal graft function, n (%)	
Immediate	64 (66)
Delayed	33 (34)
Min. blood creatinine level after operation, μmol/l	114 ± 35
eGFR in 1 month (at discharge from the hospital), ml/min	66 ± 22
Maintenance immunosuppression, n (%)	
Steroids	94 (97)
Cyclosporin A	15 (16)
Tacrolimus	82 (85)
Mycophenolate group drugs	97 (100)

Note. PTH – parathyroid hormone; eGFR – estimated glomerular filtration rate (according to the formula CKD-EPI).

ge of blood PTH levels at the stage of dialysis therapy was chosen to be 130–585 pg/mL [19], and in the post-transplant period – not exceeding the upper limit of the reference range (11–65 pg/mL) twice (≤130 pg/mL) [8, 15]. Serum calcium concentration was adjusted for changes in plasma albumin concentration [20]. Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration [19]) formula.

The material was statistically analyzed using the SPSS Statistics 17.0 software package (SPSS Inc, USA). Distribution of features was assessed by the Kolmogorov–Smirnov test. Description of quantitative features was presented as arithmetic mean and standard deviation (M ± SD) in normal distribution, and as median, 25% and 75% quartiles [Me (Q25–Q75)] in asymmetric distribution. Qualitative features were presented as fractions (%) and absolute numbers (n). For quantitative data comparison, we used the Mann–Whitney U test (to compare differences between independent variables) and the chi-square test for classification of qualitative characteristics. The strength of the relationship between quantitative attributes was estimated using the Spearman's rank correlation coefficient. In comparing the probability, depending on the presence or absence of a risk factor, the relative risk was calculated with determination of the confidence interval boundaries (95% CI). The critical values for statistical hypotheses testing in this study was assumed to be 0.05 significance level.

RESULTS

Plasma PTH levels in the observed patients ranged from 57 to 520 pg/mL; it was within the reference range (11–65 pg/mL) only in four patients. All patients were divided into two groups based on PTH levels. The first group included 47 (48.5%) recipients with >130 pg/mL PTH level, while the second group included 50 (51.5%) recipients with PTH level ≤130 pg/mL. Thus, the HPT rate in this cohort of kidney recipients was 48.5%. It turned out to be the same with different lengths of post-transplant period – from one year to six years (Fig. 1). Comparative characteristics of patient groups are presented in Table 2.

Both groups of patients were comparable by gender, age, body mass index, primary kidney disease, and dialysis therapy modality. Both groups had the same length of post-transplant follow-up until inclusion in the study and a comparable supportive immunosuppressive therapy regimen. Recipients diagnosed with HPT had a longer period of dialysis therapy and a repeated kidney transplantation in their history. A large proportion of patients from this group had secondary HPT while on the kidney transplant waiting list, as well as a higher average PTH levels in the blood; there was direct association between the pre- and post-transplant PTH levels ($r = 0.551$, $p < 0.001$). The groups were also found to have differences

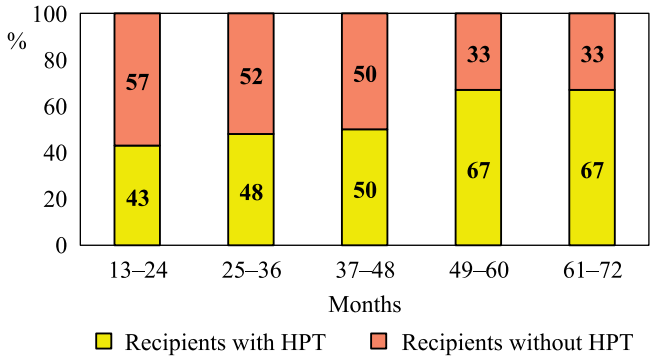

Fig. 1. The frequency of HPT at various times after kidney transplantation

in initial renal graft function. By the time the patients were examined, essential differences in graft function between both groups persisted: the median serum creatinine level in kidney recipients with HPT was 135 (110; 173) μmol/L, in recipients without HPT – 110 (80; 124) μmol/L ($p = 0.0002$), median eGFR was 50 mL/min (34; 63) and 62 mL/min (49; 84) respectively ($p = 0.0007$), daily proteinuria was 0.3 g (0.2; 0.5) and 0.2 g (0.1; 0.2) respectively, ($p = 0.04$) (Fig. 2). There was a positive

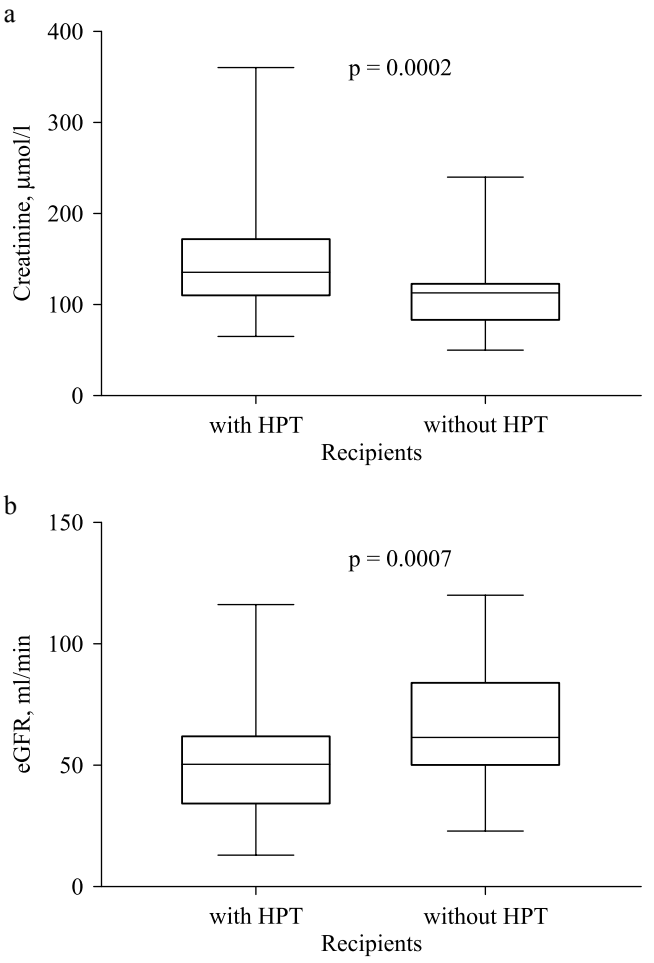

Fig. 2. Renal transplant function in recipients with and without HPT: a – blood creatinine concentration; b – estimated glomerular filtration rate

Table 2

Clinical characteristics in renal transplant recipients with and without hyperparathyroidism

Parameter	Renal transplant recipients		p
	PTH >130 pg/ml (n = 47)	PTH ≤130 pg/ml (n = 50)	
PTH, pg/ml	203 (164; 302)	101 (83; 114)	<0.001
Age, years	44 ± 9	45 ± 9	n/a
Male / Female, n	22/25	18/32	n/a
Body mass index, kg/cm^2	24.2 ± 4.6	25.3 ± 4.1	n/a
Primary renal disease, n (%)			
Chronic glomerulonephritis, incl. at systemic lupus erythematosis and widespread vasculitis	30 (64)	26 (52)	
Congenital hereditary nephropathy (incl. polycystic kidney)	10 (21)	18 (36)	n/a
Chronic interstitial nephritis	4 (9)	1 (2)	
Other/unknown nephropathy	3 (6)	5 (10)	
Dialysis mode, n (%)			
Hemodialysis	31 (66)	29 (58)	
Peritoneal dialysis	8 (17)	14 (28)	n/a
Hemodialysis + Peritoneal dialysis	5 (11)	3 (6)	
No dialysis	3 (6)	4 (8)	
Duration of dialysis therapy, months	30 (14; 50)	14 (6; 28)	0.004
Renal regrafting, n (%)	8 (17)	1 (2)	0.028
Patients with PTH >585 pg/ml, n (%)	33 (70)	12 (24)	<0.001
Blood PTH, pg/ml	681 (538; 858)	310 (182; 556)	<0.001
Posttransplant period at the moment of examination, months (min–max)	26 (14; 44)	19 (15; 35)	n/a
Patients, n (%) with duration:			
13–24 months	22 (47)	29 (58)	n/a
25–36 months	10 (21)	11 (22)	
37–48 months	5 (11)	5 (10)	
49–60 months	8 (17)	4 (8)	
61–72 months	2 (4)	1 (2)	
Renal graft function, n (%)			
Immediate	24 (51)	40 (80)	0.005
Delayed	23 (49)	10 (20)	
Min. blood creatinine level after operation, μmol/l	133 ± 42	97 ± 22	0.002
eGFR in 1 month (at discharge from the hospital), ml/min	57 ± 21	74 ± 20	<0.001
Maintenance immunosuppression, n (%)			
Steroids	46 (98)	48 (96)	
Cyclosporin A	4 (9)	11 (22)	n/a
Tacrolimus	43 (91)	39 (78)	
Mycophenolate group drugs	47 (100)	50 (100)	

Note. PTH – parathyroid hormone; eGFR – estimated glomerular filtration rate (according to the formula CKD-EPI); n/a – unreliable differences.

correlation between serum PTH and minimum (after kidney transplantation) serum creatinine ($p < 0.001$), serum creatinine at the time of examination ($p < 0.001$), and daily proteinuria ($p = 0.003$). Serum PTH was found to be negatively correlated with eGFR one month after transplantation ($p = 0.001$) and at the time of patient examination ($p < 0.001$).

Separation of patients into stages of CKD showed the following (Fig. 3). Prevalence of stage 1 CKD was an order of magnitude lower among recipients with HPT than among recipients without HPT ($p = 0.014$). In total, 34 (72%) patients with HPT had eGFR <60 mL/min versus 18 (36%) patients in the non-HPT group ($p < 0.001$).

Risk factors for post-transplant HPT were dialysis therapy lasting for more than 18 months and presence of secondary HPT at this stage, repeated kidney transplantation, delayed graft function, eGFR (transplanted kidney) <60 mL/min in the long term (Table 3).

During laboratory examination, recipients from the first group more often showed changes in calcium and phosphate metabolism resulting in hypercalcemia and hypophosphatemia, as well as elevated serum activity

of total ALP (Table 4). Serum PTH levels positively correlated with serum ionized calcium ($p < 0.001$) and ALP activity ($p = 0.003$), but did not correlate with the total serum calcium, phosphorus and magnesium concentrations. Serum vitamin D levels in both groups reduced in equal measure – half of the patients had moderate (insufficient), while the other half had significant (deficiency) reduction.

DISCUSSION

Discussing the prevalence of post-transplant HPT is quite complicated. Available information on this issue is scarce and quite contradictory. This is partly due, firstly, to restoration of hormonal-metabolic balance during the first year after successful kidney transplantation, and secondly, to lack of a clear definition of the target range of PTH in contrast to predialysis and dialysis patient populations. Data were obtained indicating a higher target blood PTH levels in kidney transplant recipients than in patients with CKD with similar GFR values. Therefore, a reliable assessment of the PTG function is carried out no earlier than 12 months after kidney transplantation. Blood PTH level >130 pg/mL is used as a diagnostic threshold for sustained HPT [8, 10, 13]. Our study, under

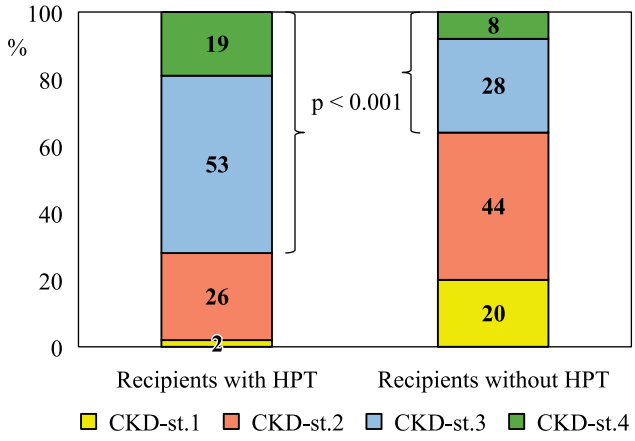

Fig. 3. CKD stages in recipients with and without HPT

Table 3

Impact of various factors on the development of hyperparathyroidism in patients after kidney transplantation

Factor	Rate in group with HPT (n = 47)	Rate in group without HPT (n = 50)	Relative risk [CI 95%]	p
Duration of dialysis therapy >18 months	31 (66%)	19 (38%)	1.736 [1.169; 2.659]	0.0082
PTH >585 pg/ml	33 (70%)	12 (24%)	2.926 [1.785; 5.046]	<0.0001
Kidney regrafting, n (%)	8 (17%)	1 (2%)	8.511 [1.467; 51.52]	0.0137
Renal graft function, n (%)	23 (49%)	10 (20%)	1.567 [1.584; 9.868]	0.0049
eGFR <60 ml/min, n (%)	34 (72%)	18 (36%)	2.009 [1.362; 3.089]	0.0005

Table 4

Biochemical parameters of hyperparathyroidism in renal transplant recipients

Parameter	Renal transplant recipients		p
	PTH >130 pg/ml (n = 47)	PTH ≤130 pg/ml (n = 50)	
PTH, pg/ml	203 (164; 302)	101 (83; 114)	<0.001
Ionized calcium, mmol/l	1.32 ± 0.07	1.29 ± 0.04	0.017
Hypercalcemia (Ca^{++} >1.31 mmol/l), n (%)	18 (38)	4 (8)	<0.001
Total Ca, mmol/l	2.4 ± 0.1	2.4 ± 0.1	n/a
Hypercalcemia (Ca >2.6 mmol/l), n (%)	8 (17)	1 (2)	0.03
Phosphorus, mmol/l	1.02 ± 0.20	1.01 ± 0.11	n/a
Hypophosphatemia (P <0.81 mmol/l), n (%)	11 (23)	3 (6)	0.032
Alkaline phosphatase (total), U/l (ref. 31–120 U/l)	113 ± 61	75 ± 19	0.021
Hyperenzymemia, n (%)	8 (17)	1 (2)	0.028
Magnesium, mmol/l	0.79 ± 0.08	0.76 ± 0.07	n/a
Hypomagnesemia(Mg <0.70 mmol/l), n (%)	8 (17)	7 (14)	n/a
Uric acid, µmol/l (ref. 150–420 µmol/l)	404 ± 62	375 ± 63	0.068
Hyperuricemia, n (%)	18 (38)	10 (20)	0.078
Vitamin D (calcidiol), ng/ml	14 ± 4	15 ± 6	n/a
Reference value (vitamin D >30 ng/ml), n (%)	1 (2)	1 (2)	
Deficiency (vitamin D 15–30 ng/ml), n (%)	21 (45)	21 (42)	
Hypovitaminosis D (vitamin D <15 ng/ml), n (%)	25 (53)	28 (56)	

Note. PTH – parathyroid hormone; n/a – unreliable differences.

the indicated conditions, established that HPT prevalence in kidney transplant recipients in the long-term period (1–6 years after surgery) was 48.5%. Similar data have also been given by other authors [8, 15]. This suggests that HPT is a problem not only for dialysis patients, but also for kidney transplant recipients. High prevalence of HPT at kidney transplant centers emphasizes the importance of dynamic monitoring of PTG function and related parameters of mineral and bone metabolism. The need for regular laboratory testing is also driven by the fact that HPT has no early clinical manifestations.

The leading risk factor associated with post-kidney transplant HPT is the existence of secondary HPT at the preoperative period [8, 13, 17]. The result of our study is fully consistent with this conclusion. Patients with pre-kidney transplant moderate/severe HPT have a high probability of disease persistence even with optimal graft function. Postoperative HPT is due to formation of parathyroid nodular hyperplasia formed at the stage of dialysis therapy, which is accompanied by reduced expression of calcium-sensing receptors and vitamin D receptors and is not capable of complete involution after successful kidney transplantation. Tertiary HPT attracts the most attention from nephrologists that are observing kidney transplant recipients. Its distinguishing feature is hypercalcemia, whose clinical manifestations vary from complete absence to severe damage to the cardiovascular, musculoskeletal, nervous systems and graft. Occurring in 5–10–15% of cases in the first year after kidney transplantation, hypercalcemia is associated with elevated levels of blood PTH preoperatively and postoperatively [21–24]. We also observed patients with increased serum calcium, who were more in the first group than in the second group. However, a positive correlation was established only between PTH and ionized calcium, which emphasizes the need to determine this fraction, since measuring total calcium can underestimate hypercalcemia diagnosis [6].

Another major factor in development/progression of post-transplant HPT, established in the course of our study, is the suboptimal function of the transplanted kidney in the early postoperative period or formed in subsequent years. Obviously, in both cases it leads to the same complex of hormonal-metabolic disorders and HPT formation by the same mechanisms as in CKD progression [4, 5]. There are publications supporting the interdependence of kidney transplant function and PTG function; there are as well contrary opinions on the absence of such a relationship [23, 25, 26]. This situation is possible in recipients with tertiary HPT, which can occur with a well-functioning graft.

PTH secretion is closely related to vitamin D and magnesium content in the body – their low serum concentrations stimulate the PTG function [27]. Decreased serum magnesium after kidney transplantation due to inhibition of its tubular reabsorption is initiated by calcineurin inhibitors and is more characteristic of the early postoperative period [28]. Our study, which was carried out in the long-term after kidney transplantation, revealed no relationship between PTH and magnesium levels in the blood. At the same time, it is known that serum magnesium levels may not accurately reflect the level of total body magnesium and that a normal serum magnesium level does not rule out magnesium deficiency [27]. There was also no relationship between plasma concentrations of PTH and vitamin D, in contrast to the study by Timalsina S. et al. [18]. Moreover, vitamin D levels in all recipients with both normal and hyperfunctional PTG were lower than the target range accepted in the general population [29]. However, serum vitamin D (calcidiol) levels may not accurately reflect the level of its active form, D-hormone (calcitriol), in the blood, but is only an optimal indicator of its availability in the body.

The possible role of high body mass index in recipients in post-transplant HPT is being considered. The basis for this was the data on the action of leptin stimulating PTH secretion [30]. In their study, Perrin P. et al. [8] found a significant difference in body mass index in patients with normal and increased PTG function three months after kidney transplantation. However, we did not find such a difference, which is probably due to the small sample and/or long-term follow-up.

CONCLUSION

Persistent hyperparathyroidism (secondary/tertiary) is a common disease in the long-term post-kidney transplant period. Its risk factors include prolonged dialysis therapy, preoperative secondary HPT, repeated kidney transplantation, delayed graft function, and eGFR <60 ml/min. Dynamic outpatient monitoring of renal transplant recipients requires regular monitoring of PTG function and biochemical parameters of HPT. Implementation of rational preventive and therapeutic measures for post-transplant HPT includes proper management of secondary HPT in the preoperative period and maintenance of serum PTH level, corresponding to the kidney graft function. Actual clinical practice confirms that the recommended tactics are valid [31].

The authors declare no conflict of interest.

REFERENCES

1. *Gautier SV, Khomyakov SM*. Donation and organ transplantation in the Russian Federation in 2018. 11th report of National Register. Vestnik transplantologii i iskusstvennikh organov. *Russian Journal of Transplantology and Artificial Organs*. 2019; 21 (3): 7–32. doi: 10.15825/1995-1191-2019-3-7-32. [In Russ, English abstract].
2. *Bikbov BT, Tomilina NA*. The contingent and treatment quality indicators in patients on replacement therapy of end stage renal disease in the Russian Federation in

1998–2013 years. Report of the register of renal replacement therapy of the Russian Dialysis Society. Part II. *Nefrologiya i dializ. Russian Journal of Nephrology and Dialysis*. 2016; 18 (2): 98–164. [In Russ, English abstract].
3. *Wang JH, Skeans MA, Israni AK*. Current status of kidney transplant outcomes: dying to survive. *Adv Chronic Kidney Dis*. 2016; 23 (5): 281–286. doi.org/10.1053/j.ackd.2016.07.001.
4. *Cunningham J, Locatelli F, Rodriguez M*. Secondary hyperparathyroidism: pathogenesis, disease progression and therapeutic options. *Clin J Am Soc Nephrol*. 2011; 6: 913–921. doi: 10.2215/CJN.06040710.
5. *Jamal SA, Miller PD*. Secondary and tertiary hyperparathyroidism. *J Clin Densitometry: Assessment of Skeletal Health*. 2013; 16 (1): 64–68. doi.org/101016/j.jocd.2012.11.012.
6. *Evenepoel P*. Recovery versus persistence of disordered mineral metabolism in kidney transplant recipients. *Semin Nephrol*. 2013; 33: 191–203. doi: 10.1016/j.semnephrol.2012.12.019.
7. *Lou I, Foley D, Odorico SK*. How well does renal transplantation cure hyperparathyroidism? *Ann Surg*. 2015; 262 (4): 653–659.
8. *Perrin P, Caillard S, Javier RM, Braun L, Heibel F, Borni-Duval C et al*. Persistent hyperparathyroidism is a major risk factor for fractures in the five years after kidney transplantation. *Am J Transplant*. 2013; 13: 2653–2663. doi: 10.1111/ajt.12425.
9. *Hiemstra TF, Brown AJ, Chaudhry AN, Walsh M*. Association of calcium, phosphate and parathyroid hormone with renal allograft function: A retrospective cohort study. *Am J Nephrol*. 2013; 37: 339–345.
10. *Bleskestad IH, Bergrem H, Leivestad T, Hartmann A, Gøransson LG*. Parathyroid hormone and clinical outcome in kidney transplant patients with optimal transplant function. *Clin Transplant*. 2014; 28: 479–486. doi: 10.1111/ctr12341.
11. *Pihlstrom H, Dahle DO, Mjoen G, Pilz S, Marz W, Abedini S et al*. Increased risk of all-cause mortality and renal graft loss in stable renal transplant recipients with hyperparathyroidism. *Transplantation*. 2015; 99 (2): 351–359. doi.org/10.1097/tp.00000 00000000583.
12. *Moiz A, Javed T, Garces J, Dornelles A, Staffeld-Coit C*. Posttransplant nephrocalcinosis is associated with poor renal allograft function: a single-center experience. *Ochsner J*. 2015 Spring; 15 (1): 25–29.
13. *Evenepoel P, Claes K, Kuypers D, Maes B, Bammens B, Vanrenterghem Y*. Natural history of parathyroid function and calcium metabolism after kidney transplantation: A single-centre study. *Nephrol Dial Transplant*. 2004; 19: 1281–1287. doi: 10.1093/ndt/gfh128.
14. *Muirhead N, Zaltman JS, Gill JS, Churchill DN, Poulin-Costello M, Mann V et al*. Hypercalcemia in renal transplant patients: prevalence and management in Canadian transplant practice. *Clin Transplant*. 2014; 28 (2): 161–165. doi.org/10.1111/ctr.12291.
15. *Wolf M, Weir MR, Kopyt N, Mannon RB, Visger JV, Deng H et al*. A prospective Cohort Study of mineral metabolism after kidney transplantation. *Transplantation*. 2016; 100 (1): 184–193. doi: 10.1097/TP.0000000000000823.
16. *Tillmann F-P, Wächtler C, Hansen A, Rump LC, Quack I*. Vitamin D and cinacalcet administration pre-transplantation predict hypercalcaemic hyperparathyroidism post-transplantation: a case-control study of 355 deceased-donor renal transplant recipients over 3 years. *Transplantation Research*. 2014; 3: 21–26. doi: 10.1186/s13737-014-0021-5.
17. *Nakai K, Fujii H, Ishimura T, Fujisawa M, Nishi S*. Incidence and risk factors of persistent hyperparathyroidism after kidney transplantation. *Transplantation Proceedings*. 2017; 49, Issue 1: 53–56. doi.org/10.1016/j.transproceed.2016.10.011.
18. *Timalsina S, Sigdel MR, Baniya S, Subedee S*. Status of vitamin D and parameters of calcium homeostasis in renal transplant recipients in Nepal: a cross sectional study. *BMC Nephrol*. 2018; 22; 19 (1): 290–295. doi: 10.1186/s12882-018-1088-x.
19. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Supp*. 2013; 3 (1): 1–150. doi: 10.1038/ki.2009.188.
20. National kidney foundation. K/DOQI Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease. *Am J Kidney Dis*. 2003; 42 (Suppl. 3): S1–S202.
21. *Kawarazaki H, Shibagaki Y, Fukumoto S, Kido R, Ando K, Nakajima I et al*. Natural history of mineral and bone disorders after living-donor kidney transplantation: A one-year prospective observational study. *Ther Apher Dial*. 2011; 15 (5): 481–487. doi: 10.1111/j.1744-9987.2011.00932.x.
22. *Torregrosa JV, Barros X*. Management of hypercalcemia after renal transplantation. *Nefrologia*. 2013; 33 (6): 751–757.
23. *Amin T, Coates TP, Barbara J, Hakendorf P, Karin N*. Prevalence of hypercalcaemia in a renal transplant population: A single centre study. *Int J Nephrology*. 2016: 7126290. doi.org/10.1155/2016/7126290.
24. *Nanmoku K, Shinzato T, Kubo T, Shimizu T, Yagisawa T*. Prevalence and predictors of early hypercalcemia after kidney transplantation: a nested case-control study within a cohort of 100 patients. *Clin Exp Nephrol*. 2019; 23 (2): 268–274. doi: 10.1007/s10157-018-1627-6.
25. *García V, Sánchez-Agesta V, Agüera LM, Calle O, Navarro MD, Rodríguez A, Aljama P*. Influence of pre-kidney transplant secondary hyperparathyroidism on later evolution after renal transplantation. *Transplantation*. 2018; 102: S535.
26. *Al-Moasseb Z, Aitken E*. Natural history of serum calcium and parathyroid hormone following renal transplantation. *Transplant Proc*. 2016; 48 (10): 3285–3291. doi: 10.1016/j.transproceed.2016.09.050.
27. *Erem S, Atfi A, Razzaque MS*. Anabolic effects of vitamin D and magnesium in aging bone. *Journal of Steroid Biochemistry and Molecular Biology*. 2019; 193: 8p.105400. doi.org/10.1016/j.jsbmb.2019.105400.

28. *Kalantar-Zadeh K, Molnar MZ, Kovesdy CP, Mucsi I, Bunnapradist S*. Management mineral and bone disorder after kidney transplantation. *Curr Opin Nephrol Hypertens*. 2012; 21 (4): 389–403. doi: 10.1097/MNH.0b013e3283546ee0.
29. *Holick MF, Binkley NC, Bischoff-Ferrari HA et al.* Endocrine Society. Evolution, treatment and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011; 96: 1911–1930.
30. *Grethen E, Hill KM, Jones R, Cacucci BM, Gupta CE, Acton A et al*. Serum leptin, parathyroid hormone, 1,25-dihydroxy vitamin D, fibroblast growth factor 23, bone alkaline phosphatase, and sclerostin relationships in obesity. *J Clin Endocrinol Metab*. 2012; 97: 1655–1662. doi: 10.1210/jc.2011-2280.
31. *Perrin P, Kiener C, Javier RM, Braun L, Cognard N, Gautier-Vargas G et al*. Changes in chronic kidney disease-mineral and bone disorders and associated fractures after kidney transplantation. *Transplantation*. 2017; 101 (8): 1897–1905. doi: 10.1097/TP.0000000000001449.

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ELECTROKINETIC, OXIDATIVE AND AGGREGATION PROPERTIES OF RED BLOOD CELLS IN THE POSTOPERATIVE PERIOD FOLLOWING KIDNEY TRANSPLANTATION

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Objective: to study the electrokinetic and aggregation properties, as well as the pro-oxidant and antioxidant processes in red blood cells following kidney transplantation in donors and in recipients in the postoperative period. **Materials and methods.** Blood from 12 recipients and 5 kidney donors over time – before transplantation, as well as at week 1, months 1, 2, 7, 10 and 12 after surgery, as well as from 8 healthy volunteers who formed the control group. We used microelectrophoresis to measure the electrophoretic mobility of red blood cells, characterizing the electrokinetic properties of cells. Aggregation was calculated microscopically by counting unaggregated red blood cells. Malondialdehyde concentration was measured spectrophotometrically at its absorbance maximum at 530 nm by reaction with thiobarbituric acid. Catalase activity was analyzed by reducing hydrogen peroxide in the sample spectrophotometrically at 240 nm wavelength. The obtained values were compared using the Mann–Whitney U test. **Results.** Decreased electrophoretic mobility of red blood cells within 2 months after transplantation was associated with increased malondialdehyde concentration and erythrocyte aggregation, decreased catalase activity in kidney recipients, followed by restoration of indicators to the control values. Electrophoretic mobility of red blood cells decreased, while malondialdehyde concentrations increased in donors after surgery. However, the increase was less pronounced than in recipients. The changes indicate that the postoperative period causes changes at the cellular level both in donors and in recipients. This is manifested by decreased stability of erythrocyte membrane structure, which is largely determined by lipid peroxidation processes. At the systemic level, a change in the electrophoretic mobility of red blood cells indicates a stress reaction before and after kidney transplantation in recipients within 2 months after surgery, and in donors in 1–2 months in the postoperative period with gradual increase in the body's resistance. **Conclusion.** Kidney transplantation is manifested at the cellular and systemic levels. At the cellular level, there is decreased stability of the membrane structure, which is largely determined by lipid peroxidation processes. At the systemic level, a change in the electrophoretic mobility of red blood cells indicates a stress reaction with gradual increase in the body's resistance. The data obtained demonstrate changes in the functional properties of red blood cells both in kidney transplant recipients and in donors. These changes need to be taken into account when carrying out therapeutic measures.

Keywords: kidney transplantation, red blood cells.

INTRODUCTION

Kidney transplantation is the best modality of renal replacement therapy for patients with irreversible acute and chronic kidney diseases [1]. The life expectancy of patients with a transplanted kidney exceeds that of those treated with hemodialysis and peritoneal dialysis. It provides a high quality of life [2, 3]. However, organ transplantation continues to be a complicated surgical procedure with the risk of developing several major complications [4]. Cardiovascular disease is the most common cause of morbidity and mortality in patients with a transplanted kidney [5]. Incidence of acute rejection crises, arterial hypertension, proteinuria, hyperglycemia, and anemia are also considered as risk factors for allograft dysfunction [6–8]. Besides, arterial thrombosis and venous thrombosis are distinguished, which in most cases occur in the first week after transplantation, although they can also appear in a longer time frame [9].

It should be considered that renal toxicity, ischemia-reperfusion injury and immunological disorders of the kidney lead to increased formation of reactive oxygen species [10]. In addition, some immunosuppressants increase oxidative stress, especially compounds from calcineurin inhibitors, and thus indirectly increase the risk of complications [11–12]. Impaired oxidative balance plays a huge role in the patient's homeostasis. Pathogenesis of

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arising and developing disorders in this case includes oxygen deficiency, activation of free radical oxidation – lipid peroxidation stimulation, which leads to changes in the structure and function of cell membranes, damage to cellular and subcellular structures, and aggravation of the pathological state [13, 14]. Metabolic, circulating and hemodynamic factors have a greater impact on the functional state of the kidney graft of the post-traumatic period [15].

The functional state of red blood cells of kidney donors and recipients in the postoperative period was analyzed considering that red blood cells (RBCs) play an important role in maintaining oxygen homeostasis in the body, which, if disrupted, causes tissue hypoxia with development of free radical processes. At the same time, we could not find studies on the state of red blood cells in kidney recipients. Moreover, there is scarce information on the state of donors not only at the cellular, but also at the organ level. The aim of this work was to study the electrokinetic, aggregation properties, as well as the pro-oxidant and antioxidant processes in red blood cells of kidney donors and recipients in the postoperative period.

MATERIALS AND METHODS

The study investigated the blood of kidney recipients and donors in the postoperative period. Kidney explantation and transplantation operations were carried out at Volga Regional Medical Center, Federal Medical and Biological Agency (FMBA), where such medical interventions have been performed since 2006 [16]. All patients gave their voluntary consent in a form approved via executive order No. 517n of the Russian Ministry of Health, dated August 11, 2017. The study was approved by the local ethics committee of FMBA. The study included 12 patients aged 40 to 54 years, who underwent kidney transplantation. Five people donated their kidneys, and 8 people were healthy volunteers who made up the control group. Blood for analysis was taken from the median cubital vein over time – before surgery, at postoperative week 1, at postoperative month 1, 2, 7, 10, and 12.

Electrokinetic and aggregation properties were determined by measuring the RBC electrophoretic mobility and via optical measurements of RBC aggregation. RBC electrophoretic mobility was determined by microelectrophoresis using a cytoferometer in our modification [17]. The time it took the RBCs to travel a distance of 100 μm was recorded in a Tris-HCl pH 7.4 buffer at 8 mA current intensity. The RBC electrophoretic mobility magnitude was determined by the formula: U = S/TH, where S is the distance covered by the cells, T is the time taken by the cells to cover a distance of S, H is the potential gradient. The magnitude of the potential gradient was determined by the formula: H = I/gχ, where I is the current intensity, g is the chamber cross section, and χ is the specific electrical conductivity of the medium. RBC aggregation was studied by optical microscopy by counting single RBCs and their aggregates (Derjugina, 2006). A blue dextran T-2000 solution (GE Healthcare firm, 20 mg/mL) in a Tris-HCl buffer (pH 7.4) was used as an aggregation stimulator. The washed RBCs were diluted with a dextran solution (at a 1:10 volume ratio) and the number of non-aggregated RBCs was counted in the Goryaev's chamber. The total number of RBCs in the sample was counted in an isotonic NaCl solution. The level of aggregation (A) was calculated by the formula: A = 100% – (the number of free (non-aggregated) RBCs × 100%/total number of RBCs).

Pro-oxidant and antioxidant properties of RBCs were evaluated by malondialdehyde (MDA) levels and activity of catalase in RBCs [18]. Serum MDA levels in RBCs were determined spectrophotometrically at 530 nm absorption maximum during reaction with thiobarbituric acid. Molar extinction coefficient ($E = 1.56 \times 10^{-5}$ $M^{-1}cm^{-1}$) was used to calculate serum MDA level. Catalase activity was analyzed by reducing hydrogen peroxide (H_2O_2) in the sample. Measurements were carried out spectrophotometrically immediately after addition of H_2O_2 in the sample cuvette and 20 seconds after addition at 240 nm wavelength.

The data obtained is presented as mean values ± mean error. The obtained values were compared using the Mann–Whitney U test. Differences between groups were considered significant at ≤0.05 significance level.

RESULTS AND DISCUSSION

Results of studies have shown that in kidney transplant recipients, RBC electrophoretic mobility was significantly reduced relative to the physiological norm within two months after surgery. It was then restored to the level of the control group (Fig. 1). It should be noted that RBC electrophoretic mobility also reduced prior to

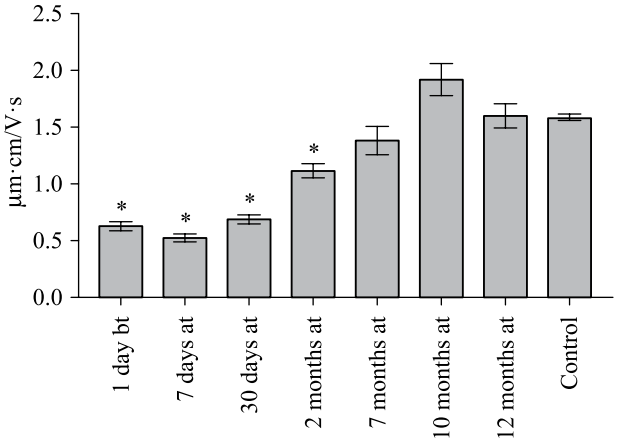

Fig. 1. Dynamics of electrophoretic mobility of erythrocytes in patients with kidney transplantation

Note. Here and in other figures: bt – before transplant; at – after transplant; * – statistically significant differences to control ($p < 0.05$)

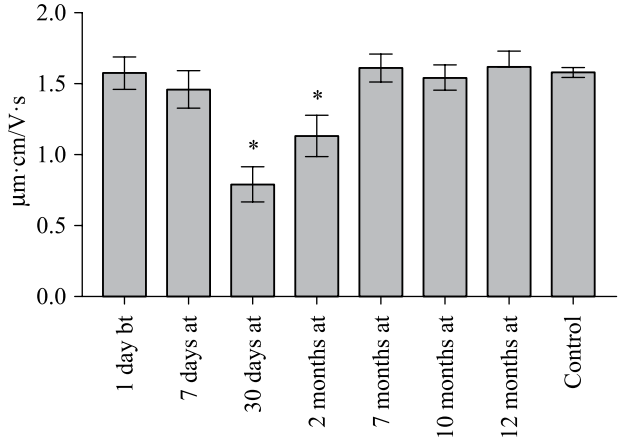

Fig. 2. Dynamics of electrophoretic mobility of erythrocytes of kidney donor

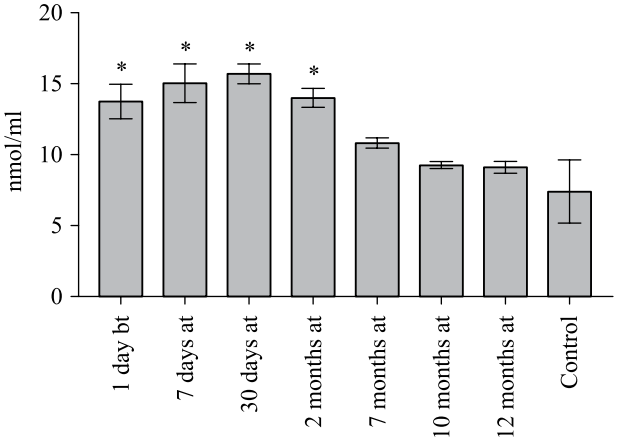

Fig. 3. Dynamics of aggregation of erythrocytes in patients with kidney transplantation

surgery. Kidney donors had reduced RBC electrophoretic mobility at postoperative month 1 and 2 (Fig. 2).

Decreased RBC electrophoretic mobility under various extreme influences and conditions is a reflection of the general nonspecific reaction of the body to a stimulus and a stress-response severity criterion [19, 20]. Analysis of RBC electrophoretic mobility reveals changes in adrenal functional activity and diagnoses of the direction of processes associated with activation or inhibition of nonspecific stress resistance of the body [20, 21]. Moreover, decreased RBC electrophoretic mobility accompanies activation of the sympathoadrenal system, whereas its increase is associated with activation of the pituitary-adrenal system and higher resistance of the body [21]. Thus, RBC electrophoretic mobility at different stages of the postoperative period reflects a stress response in recipients and donors, which is recorded before month 2, with gradual activation of stress-regulating reactions and triggering of adaptive mechanisms at month 7 after operation.

RBC surface charge determines the aggregation characteristics of cells, which have a significant role in blood flow under microcirculation conditions. A study of RBC aggregation properties revealed that kidney transplant recipients had increased RBC aggregation at month 2 of the post-transplant period ($p \leq 0.001$) (Fig. 3). In kidney donors, the RBC aggregation properties did not statistically significantly change (Fig. 4).

In turn, RBC surface characteristics are determined by the properties of cell membranes, whose state largely depends on pro-oxidant and antioxidant processes. A study of Serum MDA levels showed that in kidney transplant recipients, MDA red blood cells concentration before month 2 was significantly higher than that of the control group (Fig. 5). Subsequent measurements of Serum MDA levels from month 7 revealed no differences from the physiological norm. It was shown that in kidney donors, increased levels of MDA were observed

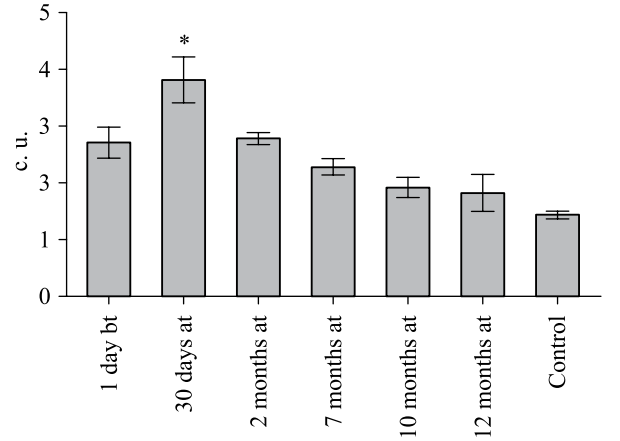

Fig. 4. Dynamics of aggregation of kidney donor erythrocytes

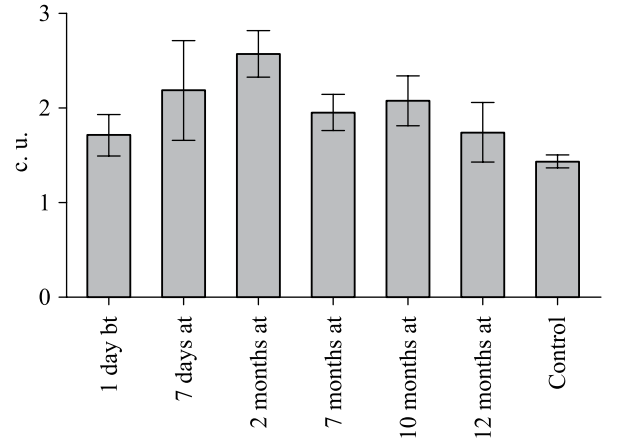

Fig. 5. The concentration of malone dialdehyde in erythrocytes in patients with kidney transplantation

on postoperative day 7–30, followed by restoration to normal values (Fig. 6).

In turn, analysis of catalase activity in RBCs showed that the activity decreased in kidney recipients during

the first month after surgery (p = 0.0040) (Fig. 7). No changes in catalase activity were observed in kidney donors (Fig. 8).

Analysis of results indicates that, during kidney transplant surgery, there is decreased RBC electrophoretic mobility, accompanied by increased aggregation and lipid peroxidation. In kidney donors, decreased RBC electrophoretic mobility is less pronounced and occurs against a background of increased serum MDA levels. Intensification of lipid peroxidation of cell membranes leads to compaction or destruction of the lipid bilayer, increased microviscosity, impaired functional activity of enzymes, changes in membrane permeability and surface charge, which can affect cell viability [22]. At the same time, it should be noted that decreased RBC negative charge reduces the suspension stability of blood and increases RBC aggregation, which slows down blood flow and ultimately leads to adverse changes in macrorheological blood parameters [23]. Since the studied groups had no increased catalase activity in response to increased oxidative stress, it can be assumed that the observed changes are mediated by the body's systemic response. Thus, activation of the sympathoadrenal system is accompanied by increased formation of reactive oxygen species during adrenaline autoxidation [24]. Interaction of adrenaline with RBC receptors activates phospholipases and increases lipid peroxidation. A breach in the structure of RBCs would lead to lower ability to effectively participate in tissue perfusion and oxygen delivery to cells.

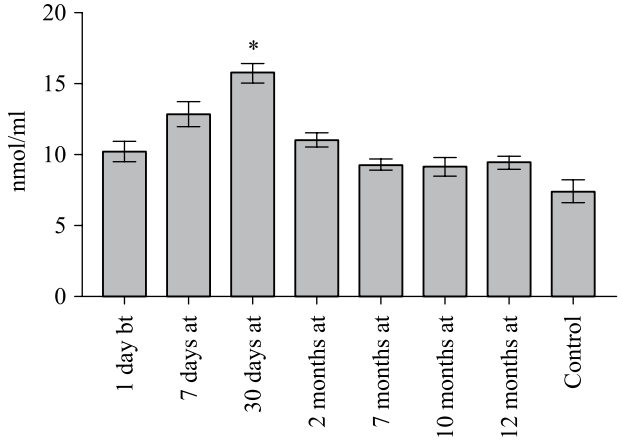

Fig. 6. The concentration of malone dialdehyde in erythrocytes of kidney donor

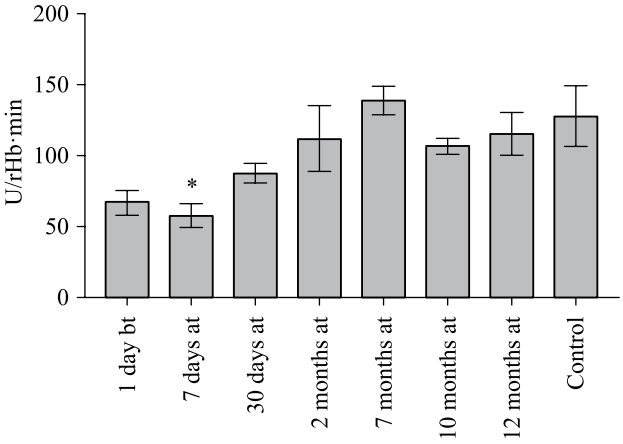

Fig. 7. The activity of catalase of erythrocytes in patients with kidney transplantation

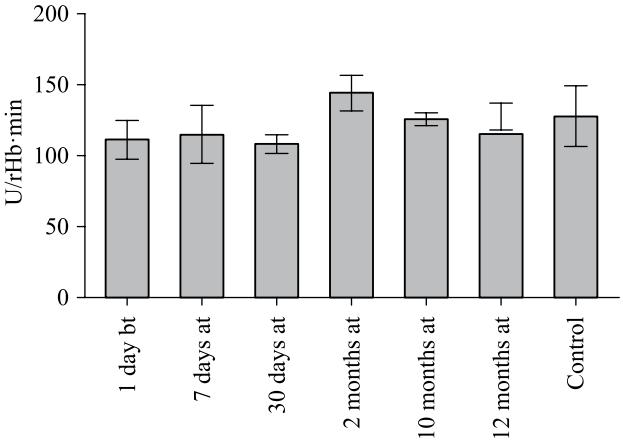

Fig. 8. The activity of catalase of erythrocytes of kidney donor

Ischemia leads to decreased energy metabolism due to depletion of macroergic phosphate reserves. Subsequently, perversion of specific intracellular metabolism, disturbances in enzymatic activity, intensification of anaerobic glycolysis, and pH changes are observed. Changes in enzymatic activity under hypoxia destabilize cell membranes and organelle membranes. This reduces membrane permeability, disrupts the functioning of ion pumps, and causes intracellular electrolyte disturbances. Normally functioning transplant cells reduce in number [15].

It is known that cell metabolism under ischemic conditions leads to lysis of cell membranes with the release of a large number of enzymes and vasoactive substances negatively affecting the recipient's state [25].

In turn, restoration of RBC electrophoretic mobility reflects decreased stress response and is observed with increased activity of the pituitary-adrenal system [26]. Cortisol plays a key role in maintaining homeostasis of the entire hypothalamic-pituitary-corticoid complex, which is responsible for development of non-specific mechanisms of the body's reactivity [27]. Corticosteroids cause an antioxidant effect [28], and it can be assumed that decreased serum MDA levels are mediated by increased corticosteroid concentration during development of adaptation processes.

So, this study has shown that kidney transplantation comes with some consequences both at the cellular and at the systemic level. At the cellular level, decreased stability of the membrane structure, which is largely dependent on lipid peroxidation processes, leads to reduced RBC electronegativity. At the systemic level, changes in

RBC electrophoretic mobility indicates a stress response with gradual increase in body resistance. Data obtained suggests there are changes in the functional properties of RBCs in kidney recipients and donors, which must be taken into account in therapeutic interventions.

The authors declare no conflict of interest.

REFERENCES

1. *Sayegh MH, Carpenter CB*. Transplantation 50 years later – progress, challenges, and promises. *The New England Journal of Medicine.* 2004; 351 (26): 2761–2766. doi: 10.1056/NEJMon043418.
2. *Kiyokazu Akioka, Sirou Takahara, Seiji Ichikawa, Norio Yoshimura, Takahiro Akiyama, Shinichi Ohshima.* Factors predicting long-term graft survival after kidney transplantation: multicenter study in Japan. *World Journal of Surgery.* 2005; 29 (2): 249–256. doi: 10.1007/s00268-005-7531-8.
3. *Stolyar AG.* Effect of anemia on the results of kidney transplantation. *Clinical medicine (Russian Journal).* 2015; 93 (12): 24–27. [In Russ, English abstract].
4. *Roufosse C, Simmonds N, Clahsen-van Groningen M, Haas M, Henriksen KJ, Horsfield C et al.* Reference Guide to the Banff Classification of Renal Allograft Pathology. *Transplantation.* 2018; 102 (11): 1795–1814. doi: 10.1097/TP.0000000000002366.
5. *Campbell P.* Clinical relevance of human leukocyte antigen antibodies in liver, heart, lung and intestine transplantation. *Current opinion in organ transplantation.* 2013; 18 (4): 463–469. doi: 10.1097/MOT.0b013e3283636c71.
6. *Vanrenterghem Y.* Anaemia after renal transplantation. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association – European Renal Association.* 2004; 19 (5): 54–58. doi: 10.1093/ndt/gfh1057.
7. *Molnar MZ, Czira M, Ambrus C, Szeifert L, Szentkiralyi A, Beko G et al.* Anemia is associated with mortality in kidney-transplanted patients. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association – European Renal Association.* 2007; 7 (4): 818–824. doi: 10.1093/ndt/gfh1057.
8. *Vatazin AV, Zulkarnaev AB.* Kidney Transplantation as an Optimal Treatment for Chronic Kidney Disease. *The Journal of General Medicine.* 2013; 3: 47–52. [In Russ, English abstract].
9. *Robertson AJ, Nargund V, Grey DW, Morris PJ.* Low dose aspirin as prophylaxis against renal-vein thrombosis in renal-transplant recipients. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association – European Renal Association.* 2000; 15 (11): 1865–1868. doi: 10.1093/ndt/15.11.1865.
10. *Gnjatic S, Wheeler C, Ebner M, Ritter E, Murray A, Altorki NK et al.* Seromic analysis of antibody responses in non-small cell lung cancer patients and healthy donors using conformational protein arrays. *Journal of Immunological Methods.* 2009; 1–2 (341): 50–58. doi: 10.1016/j.jim.2008.10.016. Epub 2008 Nov 28.
11. *Colvin MM, Cook JL, Chang P, Francis G, Hsu DT, Kiernan MS et al.* Antibody-Mediated Rejection in Cardiac Transplantation: Emerging Knowledge in Diagnosis and Management. *Circulation.* 2015; 18 (131): 1608–1639. doi: 10.1161/CIR.0000000000000093.
12. *An C, Akankwasa G, Liu J, Wang D, Cheng G, Zhang J et al.* Urine markers of renal tubular injury in idiopathic membranous nephropathy: A cross sectional study. *Clinica chimica acta; international journal of clinical chemistry.* 2019; 492: 7–11. doi: 10.1016/j.cca.2019.01.015.
13. *Belyakov NA, Semesko SG.* Antioxidant activity of human biological fluids: methodology and clinical value. *Efferentnaya terapiya.* 2005; 11 (1): 5–21. [In Russ, English abstract].
14. *Lobanova NA, Borovkov NN.* The disputable issues of influence of uraemic intoxication and free-radical processes on the development of anaemia of patients at terminal stage of chronic renal insufficiency. *Medical Almanac.* 2010; 3 (12): 152–155. [In Russ, English abstract].
15. *Vatazin AV, Zulkarnaev AB, Kantaria RO, Artemov DV.* Humoral pathogenesis ischemia/reperfusion injury factors in renal transplantation. *Ural'skij medicinskij zhurnal.* 2012; 13 (105): 94–97. [In Russ, English abstract].
16. *Romanov SV, Abaeva OP, Alexandrova OYu, Smirnova GYu.* Issues and perspectives of building a regional system of donor services (on the example of Nizhny Novgorod region). *Russian Journal of Transplantology and Artificial Organs*. 2019; 21 (1): 57–63. doi: 10.15825/1995-1191-2019-1-57-63. [In Russ, English abstract].
17. *Boyarinov GA, Yakovleva EI, Zaitsev RR, Bugrova ML, Boyarinova LV, Solov'eva OD et al.* Pharmacological Correction of Microcirculation in Rats Suffering from Traumatic Brain Injury. *Cell and Tissue Biology.* 2017; 11 (1): 65–72. doi: 10.1134/S1990519X17010023.
18. *Deriugina AV, Shumilova AV.* An influence of cytoflavin on oxidative stress and activity of Na/K-ATPase of erythrocytes after brain trauma. *Neuroscience and Behavioral Physiology.* 2017; 11: 51–55. doi: 10.17116/jnevro201711711151-55. [In Russ, English abstract].
19. *Kozinec GI, Popova OV, Budnik MI, Shmarov DA, Pogorelov VM, Procenko DD.* Elektricheskij zaryad kletok krovi. M.: Prakticheskaya medicina, 2007; 208.
20. *Krylov VN, Deryugina AV, Pleskova SN.* Electrophoretic mobility and morphometry of the rat erythrocytes at the stress effects. *Sovremennye tehnologii v medicine.* 2010; 4: 23–26. [In Russ, English abstract].
21. *Deryugina AV, Martusevich AA, Veselova TA.* Molecular and cellular mechanisms of stress response realization in the organism. *Izvestiya Ufimskogo nauchnogo centra Rossijskoj Akademii nauk.* 2015; 3: 58–63. [In Russ, English abstract].
22. *Alyasova AV, Terentiev IG, Tsybusov SN, Vedunova MV, Mishchenko TA, Shakhova KA et al.* Novel notions of the mechanisms of action of doxorubicin and ozone on malignant hepatic cells. *Sovremennye tehnologii v medicine* 2017; 9 (2): 145–149. doi: 10.17691/stm2017.9.2.18. [In Russ, English abstract].

23. *Sprague R, Ellsworth M, Stephenson A, Kleinhenz M.* Deformation-induced ATP release from red blood cells requires CFTR activity. *American Physiological Society.* 2010; 26 (2): 168–174.
24. *Bizenkova MN, Romantseva MG, Chesnokova NP.* Metabolic effects of antioxidants in acute anoxic hepoxia. *Fundamental research.* 2006; 1: 17–21. [In Russ, English abstract].
25. *Gol'din MM, Evseev AK, El'kov AN, Pinchuk AV, Tsar'kova TG.* Development and efficacy evaluation of the electrochemical predictor of complications in patients after kidney transplantation. *Transplantologiya. The Russian Journal of Transplantation.* 2015; 3: 6–10. [In Russ, English abstract].
26. *Antipenko EA, Derugina AV, Gustov AV.* An effect of cytoprotective therapy on stress resistance and compensatory abilities of patients with chronic cerebral ischemia. *S.S. Korsakov Journal of Neurology and Psychiatry.* 2015; 115 (12): 74–78. doi: 10.17116/jnevro201511511274-78. [In Russ, English abstract].
27. *Kalyi VV.* Synthesis and metabolism of glucocorticoid hormones in patients of young age groups having laryngeal cancer. *The Siberian Medical Journal.* 2010; 25 (1): 15–16. [In Russ, English abstract].
28. *Sergeev P.V.* Steroidnye gormony. M.: Nauka, 1984; 240.

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FLUORESCENCE IMAGING IN EVALUATING THE REVASCULARIZATION OF HETEROTOPICALLY TRANSPLANTED PRIMATE TRACHEA SEGMENT

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Objective: to assess the potentials of using indocyanine green fluorescence angiography in evaluating revascularization of tissue-engineered construct that was obtained from the decellularized biological matrix of primate trachea, including using mesenchymal stem cells, after heterotopic tracheal allotransplantation. **Material and methods.** Tracheas were obtained from two male hamadryas baboons. After decellularization, 4 cm segments of tracheas were implanted under the lateral part of the latissimus dorsi in two healthy primates, one after recellularization with mesenchymal stem cells (animal 1), and the second without recellularization (animal 2). Immunosuppressive therapy was not performed. Blood flow in the transplanted segment of the trachea was evaluated 60 days after transplantation by surgical isolation of the flap of the latissimus dorsi with the transplanted segment of the trachea, while maintaining blood flow through the thoracodorsal artery. Indocyanine green near-infrared fluorescence angiography was visualized using a FLUM-808 multispectral fluorescence organoscope. **Results.** Sixty days after implantation, the tracheal cartilaginous framework macroscopically appeared to be intact in both animals, tightly integrated into the muscle tissue. The framework retained its natural color. After intravenous injection of indocyanine green, the tracheal vessels were visualized in both animals. Intercartilaginous vessels and portions of the cartilaginous semi-rings devoid of vessels were clearly distinguished. The entire implanted segment was almost uniformly vascularized. No local disruptions in blood supply were observed. The fluorescence brightness of the tracheal vessels was 193 ± 17 cu and 198 ± 10 cu in animals 1 and 2, respectively. The average muscle brightness in the implantation zone was 159 ± 9 cu and 116 ± 8 cu in animals 1 and 2, respectively. **Conclusion.** Indocyanine green fluorescence angiography is characterized by high-contrast images and high sensitivity. This facilitates vascular patency visualization and allows to assess the degree of neoangiogenesis after experimental transplantation of the tracheal segment, at different stages of experiment, without euthanizing the animal.

Keywords: trachea, tissue engineering, transplantation, angiography, fluorescence.

INTRODUCTION

Tracheal diseases, both tumor and benign, often require surgical treatment. Radical surgery is technically feasible where what is being resected is just less than 50% of the length of the trachea. This involves circular resection of the corresponding section of the trachea. Most surgeons believe that resection of a longer trachea comes with a significant risk of complications and is considered unrealizable [1]. A radical solution to this problem may be to replace the affected part or the entire trachea with a cadaveric donor organ or tissue-engineered construct (TEC) [2]. Regenerative medicine is a promising new interdisciplinary field of research and clinical practice. Its methods avoid the need for life-long postoperative immunosuppressive treatment. Tissue engineering involves the modeling and creation of biological or synthetic scaffolds of the trachea in order to replace the affected organ. It is crucial to ensure that the matrix of the bioengineered organ could repeat the mechanical and biological properties of the extracellular matrix of the native organ, could have a three-dimensional structure that facilitates attachment, growth and reproduction of the corresponding cell type, could ensure cell migration and influx of growth factors, could support neoangiogenesis and adequate reinnervation. Unfortunately, there is currently negative experience in the clinical use of tissue-engineered trachea based on both synthetic and decellularized matrices, including in the case of repopulation with the recipient's stem cell. Most attempts at one-time allotransplant of donor organs have also ended unsatisfactorily. This was associated with the difficulty of ensuring adequate graft vascularization in the postoperative period [3]. Attempts at ensuring revascularization of the transplanted trachea with the

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greater omental flap, transplanting it together with the thyroid gland, and applying microvascular anastomosis, were mostly accompanied by major complications and lysis of the donor trachea [4, 5].

Existing limited clinical experience shows that the main reasons for the failure of the tracheal graft is the lack of sufficient formation of an epithelial lining on its inner surface, as well as the loss of frame function due to lysis, primarily tracheal cartilage lysis. To prevent such complications, it is necessary at least to achieve adequate revascularization of the transplanted organ. Some researchers believe that the most reliable way to achieve higher positive outcomes in tracheal transplantation is to base the method on preliminary heterotopic implantation of the donor organ in the recipient's well-vascularized tissue (greater omentum, muscles) perfused by a vascular pedicle [6]. This method allows prefabrication of the TEC to increase its survival rate and reduce the risk of complications.

At the same time, common visual inspection will not be able to prove whether in vivo revascularization was achieved. This problem can be solved via indocyanine angiography, based on systemic injection of indocyanine green dye (ICG) into the bloodstream, followed by observation of the zone of interest in an infrared fluorescence light [7–9].

Objective: to assess the potentials of using indocyanine green fluorescein angiography in evaluating revascularization of a tissue-engineered trachea obtained from decellularized biological matrix of primate trachea, including using mesenchymal stem cells, after heterotopic tracheal allotransplantation.

MATERIALS AND METHODS

Procurement of donor trachea, recellularization of tracheal acellular matrix, and surgical interventions on hamadryas baboons were carried out at the Research Institute of Medical Primatology, Sochi. Tracheal implantation and explantation, as well as methodological and hardware support for infrared fluorescein angiography, were carried out by employees of Pavlov First St. Petersburg State Medical University. Donor tracheal decellularization was carried out at the regenerative medicine fundamental research laboratory of Kuban State Medical University, Krasnodar. The study protocol was approved by the local ethics committee of the Research Institute of Medical Primatology, Sochi.

Tracheas were procured from two male hamadryas baboons that died from natural causes at the age of 6 and 7 years; the animals weighed 9 and 10 kg, respectively. Within 60 minutes after their death, pathological and anatomical examination, excision and explantation of the cervical and thoracic trachea were performed under aseptic conditions. The material was transported to the laboratory for decellularization within 24 hours of explantation in sterile containers containing phosphate-buffered saline with addition of a 2% solution of antibiotic and antimycotic at a temperature not exceeding 4 °C.

Both tracheas were decellularized according to a single protocol: after removal of connective tissue and washing with phosphate-buffered saline, the trachea was placed in a specialized bioreactor ORCA (Harvard Apparatus, USA) on a rotating platform. The procedure included 3 cycles of treatment with detergents and enzymes for 24 hours each: 4% sodium deoxycholate solution in combination with 0.002 M Na2-EDTA solution (Sigma Aldrich, USA), then 1% sodium dodecyl sulfate solution and porcine pancreatic DNase-I 2000 IU/200 mL of phosphate buffer with calcium and magnesium (Sigma Aldrich, USA; Gibco, Life Technologies, USA). Decellularization was completed by washing the trachea in a 10% solution of chlorhexidine digluconate in a phosphate buffer with a three-fold change of solution every 8 hours.

After completion of decellularization, the biological scaffold was transported to the laboratory of the Research Institute of Medical Primatology within 12 hours in a sterile container with a phosphate buffer containing an antibiotic-antimycotic. Two healthy male hamadryas baboons, aged 1 year and weighing about 5 kg, were selected as recipients of the tissue-engineered trachea. Sections of donor trachea, 4 cm each, were implanted under the lateral portion of the latissimus dorsi. A tracheal section was implanted in one primate (animal 1) after recellularization with mesenchymal stem cells, the other primate (animal 2) was implanted without recellularization. Stem cell preparation and the recellularization process were carried out according to the protocol described earlier [3].

All operations were carried out according to the general plan: after epilation of the right lateral thoracic wall, under general intravenous anesthesia (ulnar vein), about a 7 cm long skin incision was made, the outer surface of the latissimus dorsi was isolated and a donor trachea section was sutured using three Prolen 4.0 ligatures, and the implant was completely circularly enveloped with the muscle (Fig. 1). The wound was sutured without leaving drainage tightly. Within 5 days after operation, the animals received antibiotic prophylaxis with ceftriaxone – 300,000–500,000 IU/day (depending on body weight); 2 mL of ketorol was also intramuscularly injected over 3 days. No immunosuppressive therapy or corticosteroid therapy was administered in the postoperative period.

The presence of blood flow in the transplanted segment of the trachea was assessed 60 days after transplantation. Under general anesthesia, a latissimus dorsi flap with the transplanted segment of the trachea was isolated with preservation of blood flow through the thoracodorsal artery (Fig. 2); the zone of interest was determined intraoperatively by palpation, as well as by localization of the filaments that fixed the tracheal areas. Then, an ICG solution (1 mg in 10 mL of water for injection) was

injected intravenously. Infrared fluorescein angiography was visualized using multispectral fluorescence organoscope FLUM-808 [8, 9], which included a fiber-coupled infrared diode laser (λ = 808 nm, 5W power) to excite ICG fluorescence, and a small-sized multispectral television system mounted on a tripod that records images in four parts of the spectrum, including in a 820–850 nm infrared range in which ICG is emitted. The camera is connected via a USB 3.0 port to a computer running the specialized RSScam program. This system can record photo and video images with a 960 × 960 pixels resolution at 25 frames per second and quantify the intensity of infrared fluorescence in the zone of interest.

RESULTS

All surgical interventions were performed based on a predetermined plan. No intra- or postoperative complications were noted. None of the animals died during the chronic experiment.

Tracheal decellularization quality, determined during routine histological examinations and in the quantitative determination of residual DNA, was found to be satisfactory and sufficient to continue the experiment. Hematoxylin and eosin staining of the decellularized trachea did not reveal the presence of intact nuclei and cells both in the mucous membrane and in the submucosal layer. However, single cells with significantly damaged nuclei remained in the cartilaginous part.

60 days after heterotopic implantation, the implanted tracheal fragments were found in both animals, the cartilaginous tracheal framework appeared to be intact macroscopically, tightly integrated into the muscle tissue, and of native color (Fig. 3, a); at the same time, the membranous wall of the trachea, and, accordingly, the lumen of the trachea, were absent. In animal 1, the extent of cicatricial changes in muscle tissue was minimally expressed, tracheal contours were even. In animal 2, the cicatricial process was expressed in the implantation zone, there were adhesions with neighboring muscles, the edges of the implanted trachea were not clearly defined palpatorically. In both animals, about half the length of the implanted trachea was separated from the latissimus dorsi, these areas were the areas of interest during fluorescein angiography imaging.

Immediately after introduction of ICG, a strong glow in the operating wound was detected in the infrared mode, while localization of the glow corresponded to the selected area of the latissimus muscle. In both animals, the tracheal vessels were visualized; inter-cartilage vessels and sections of cartilaginous half-rings devoid of vessels were clearly distinguished (Fig. 3, b). The entire implanted segment is almost evenly vascularized, there were no local blood supply disturbances. Fluorescence intensities of the tracheal vessels in animal 1 and animal 2 were 193 ± 17 and 198 ± 10 standard units, respectively. The average muscle brightness in the implantation zone of

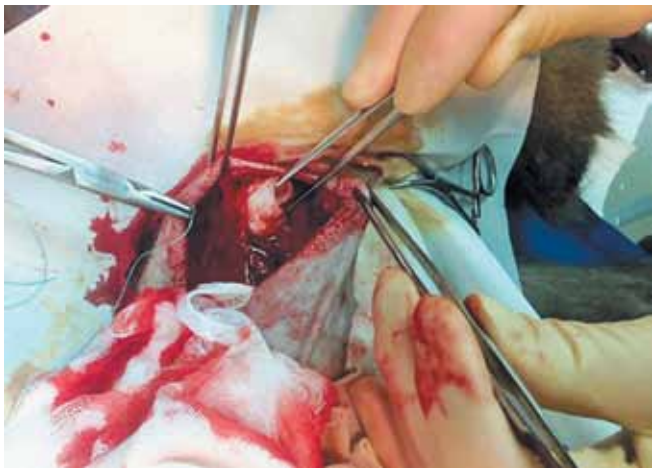

Fig. 1. Implantation of a decellularized trachea

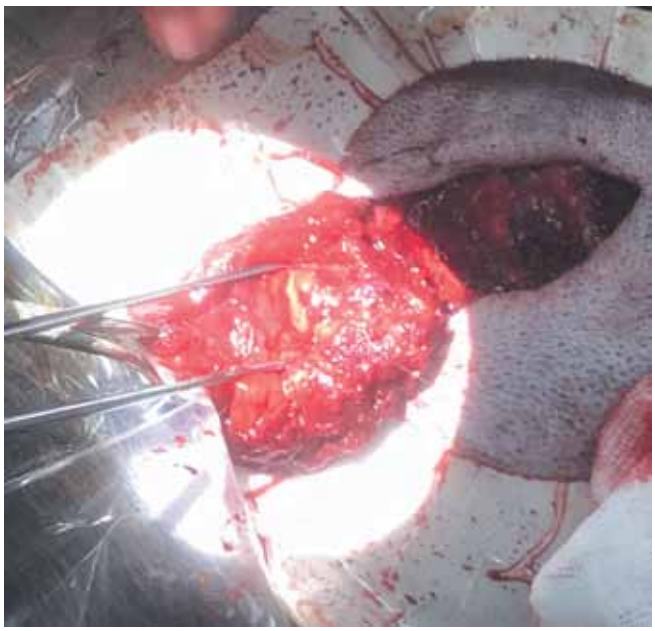

Fig. 2. Dissection of the latissimus dorsi flap with a transplanted segment of the trachea 60 days after heterotopic transplantation

animal 1 and animal 2 was 159 ± 9 and 116 ± 8 IU standard units, respectively; that is, the fluorescence intensity of the implanted trachea was the same in both animals, and noticeably higher compared to the brightness of the surrounding muscle areas. Subsequently, within approximately 60 seconds, fluorescence intensity decreased both in the muscle and in the implanted trachea.

DISCUSSION

Regenerative medicine offers a method for replacing a totally damaged trachea with a tissue-engineered organ for those patients who were previously considered incurable. The advantage of this approach is that immunosuppressive therapy is completely avoided in the postoperative period [2]. However, it should be noted that to date, the mechanisms of regulation of regenera-

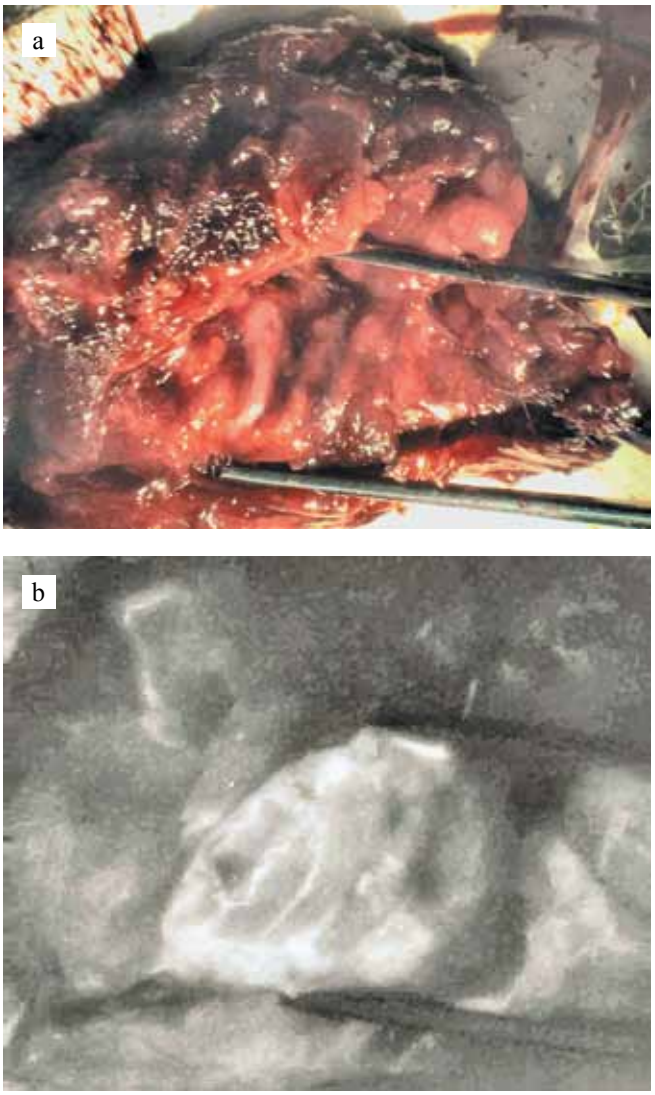

Fig. 3. Macroscopic evaluation of the implanted segment of the trachea (animal 1): a – in white light; b – in the light of ICG infrared fluorescence

tive processes in the body itself are not fully understood yet. This essentially complicates improving therapeutic approaches, which in itself is a very difficult task and requires more than one decade of scientific research and observation. The authors of the study planned to get an answer to the question about the possibility of prefabricating a tissue-engineered trachea during heterotopic transplantation in order to restore blood flow in the transplanted organ. This is one of the most important aspects of restoring organ function. They also had plans to explore the possibilities of a relatively new method of vascular imaging using indocyanine green fluorescence angiography. Existing vascular bed imaging methods using classical dyes with tissue tropicity (e.g. trypan blue), unfortunately, do not allow for a lifetime assessment of vascular patency. Moreover, the animal would need to be removed from the experiment at each stage of the study. This does not allow us to track neoangiogenesis process over time during TEC transplantation. These shortcomings are absent in infrared fluorescence imaging of hidden (from the eyes) features of the state of tissues in functioning organs. This imaging is based on the use of the fluorescence properties of ICG [9].

The results obtained allow us to suggest that implantation of a recellularized and unrecellularized tissue-engineered trachea is accompanied by inclusion of the transplanted segment of the trachea into the bloodstream 60 days after implantation. The pronounced fibrotic changes in the implantation zone in animal 2 may indicate a more pronounced reaction of the recipient's immune system to the implantation of a donor trachea in the absence of recellularization. Blood flow restoration mechanism in a transplanted trachea is quite complex and consists of many factors [10]. It is fundamentally important that fluorescein angiography is an informative method for assessing tracheal revascularization [11]. The fluorescence intensity of muscles and transplanted segments was almost the same.

It can be stated that in both animals there was neoangiogenesis stimulation regardless of the recellularization of mesenchymal stem cells. Of course, this is not enough for formation of a complete organ structure for the implanted trachea, but the indocyanine green angiography technique can be used to monitor blood flow restoration and an orthotopically transplanted organ, including during a tracheoscopy. The recently developed video endoscopic system for bronchoscopy, which provides a fluorescence imaging regime in the infrared region of the spectrum, can serve as an instrumental basis for such studies, including in clinical conditions [5].

CONCLUSION

Indocyanine green fluorescence angiography is characterized by high image contrast and high sensitivity. Therefore, visualizing the patency of the vasculature and assessing the extent of neoangiogenesis after experimental transplantation of a tracheal segment at different stages of the experiment can be possible without animal euthanasia.

The authors declare no conflict of interest.

REFERENCES

1. *Parshin VD, Vyzhigina MA, Rusakov MA, Parshin VV, Titov VA, Starostin AV*. Postreanimatsionnyy rubtsovyy stenoz trakhei. Sovremennoye sostoyaniye problemy – uspekhi, nadezhdy i razocharovaniya. *Anesteziologiya i reanimatologiya*. 2016; 61 (5): 360–366. doi: http://dx.doi.org/10.18821/0201-7563-2016-61-5-360-366.
2. *Baranovskiy DS, Demchenko AG, Oganesyan RV, Lebedev GV, Berseneva DA, Balyasin MV i dr*. Polucheniye beskletochnogo matriksa khryashcha trakhei dlya tkaneinzhenernykh konstruktsiy. *Vestnik RAMN*. 2017; 72 (4): 254–260. doi: 10.15690/vramn723.
3. *Gubareva YeA, Kuyevda YeV, Sotnichenko AS, Gilevich IV, Gumenyuk IS, Nakokhov RZ i dr*. Perspektivy

ispol'zovaniya mul'tipotentnykh mezenkhimal'nykh stromal'nykh kletok makak rezusov dlya sozdaniya tkaneinzhenernykh konstruktsiy. *Meditsinskiy vestnik Severnogo Kavkaza*. 2017; 12 (1): 82–86. doi: 10.14300/mnnc.2017.12023.

4. *Parshin VD, Lyundup AV, Tarabrin YeA, Parshin VV*. Otdalennyy rezul'tat transplantatsii trakhei: uspekh i nereshennyye problemy. *Khirurgiya. Zhurnal im. N.I. Pirogova*. 2018; 11: 11–19. doi: 10.17116/hirurgia201811111.
5. *Levashev YN, Akopov AL, Mosin IV.* The possibilities of greater omentum usage in thoracic surgery. *European journal of cardio-thoracic surgery*. 1999; 15 (4): 465–468. doi: 10.1016/S1010-7940(99)00041-X.
6. *Delaere P, van Raemdonck D.* Tracheal replacement. *J Thorac Disease*. 2016; 8 (Suppl. 2): 186–196. doi: 10.3978/j.issn.2072-1439.2016.01.85.
7. *Akopov AL, Papayan GV, Yefimov AN, Nikityuk DB, Yaprintsev VO, Magruk MA*. Infrakrasnaya fluorestsentnaya angiografiya pri transplantatsii trakhei v eksperimente. *Byulleten' eksperimental'noy biologii i meditsiny*. 2017; 164 (10): 519–522.
8. *Papayan GV, Akopov AL.* Fluorescence diagnostics in the near-IR: Apparatus, application. *JOT*. 2016; 83 (9): 536–542. doi: 10.1364/JOT.83.000536.
9. *Papayan GV, Akopov AL*. Potentials of indocyanine green near-infrared fluorescence imaging in experimental and clinical practice. *Photodiagnosis Photodyn Ther*. 2018; 24: 292–299. doi: 10.1016/ j.pdpdt.2018.10.011.
10. *Nakokhov RZ, Gubareva YeA, Kuyevda YeV, Sotnichenko AS, Gumenyuk IS, Mogil'naya GM, Kade AKh*. Mekhanizmy angiogeneza pri transplantatsii tkaneinzhenernykh konstruktsiy. *Vestnik transplantologii i iskusstvennykh organov*. 2017; 19 (4): 141–145. doi: 10.15825/1995-1191-2017-4-141-145.
11. *Papayan GV, Akopov AL, Goncharov SYe, Struy AV, Kazakov NV*. Videoendoskopicheskaya sistema dlya fotodinamicheskoy teranostiki tsentral'nogo raka legkogo. *Opticheskiy zhurnal*. 2019; 86 (7): 27–34. doi: 10.17586/1023-5086-2019-86-07-27-34.

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MICRORNA EXPRESSION LEVELS IN LUNG RECIPIENTS: CORRELATIONS WITH CLINICAL AND LABORATORY DATA

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Objective: to evaluate the expression levels of miRNA (miR-27, miR-101, miR-142, miR-339 and miR-424) and its relationship with clinical and laboratory parameters in lung transplant recipients. **Materials and methods.** The study included 57 lung recipients aged 10 to 74 years (35 ± 15), including six children (9%) – four boys 10, 12, 13 and 17 years and girls 13 and 14 years old – and 51 adult recipients, including 30 men (62.5%). The control group was made up of 14 healthy individuals that were not significantly different by gender and age. Expression levels of the microRNAs studied in blood plasma were determined via quantitative polymerase chain reaction (PCR). Correlations of miRNA expression levels with complete blood count and biochemical blood test indicators were analyzed. **Results.** Patients with end-stage chronic respiratory failure (potential lung recipients) were found to have significantly higher expression levels of miR-27, miR-101 and miR-339 in plasma than the healthy individuals ($p = 0.02$, $p = 0.03$ and $p = 0.01$, respectively). The expression level of miR-339 correlated with the age of potential lung recipients ($p = 0.04$). It was a negative correlation ($r = –0.46$). The expression levels of the other four miRNAs were age independent. The average expression level of miR-424 in lung recipients in the long-term period after lung transplant was higher than in waitlisted patients ($p = 0.03$). Analysis of the relationship between miRNA expression levels and external respiration function in the long-term post-transplant period showed that miR-142 expression level ($r = 0.61$; $p = 0.04$) positively correlates with the Tiffeneau-Pinelli index. This strong correlation, which exceeds 85%, indicates the presence of restrictive lung diseases. A year and more after transplantation, it was found that in the recipients, there were close positive correlations between miR-27, miR-142, miR-424 expression levels and blood leukocyte concentration, as well as between the miR-142 expression level and the sCD40L concentration during this period. **Conclusion.** A comparative study of the expression level of miRNAs (miR-27, miR-101, miR-142, miR-339 and miR-424) in the blood plasma of patients suffering from end-stage chronic lung diseases of various origin and in lung recipients enables us to conclude that further studies of the miRNA panels are needed in order to assess their effectiveness as potential molecular and genetic markers of post-transplant complications.

Keywords: lung transplantation, biomarker, miRNA, miR-27, miR-101, miR-142, miR-339, miR-424, sCD40L, chronic respiratory failure.

The recent significant increase in the survival rates of solid organ recipients have come with the challenge of providing long-term follow-up for recipients in order to detect early post-transplant complications, assess graft condition, and provide immunosuppression control. There are presently a considerable number of studies aimed at finding minimally invasive laboratory technologies for early preclinical diagnosis of complications in solid organ recipients. Changes in blood levels of some specific biomarker molecules involved in pathophysiological processes and acting as indicators of the risk of associated adverse events, are an objective reflection of the systematic nature of the processes occurring in the recipient's body [1].

Lung transplantation is a radical but effective remedy in severe respiratory failure. Transplanted lung biopsy followed by histological examination of the biopsy material allows to verify the graft condition. However, this comes with risks and limitations characteristic of invasive interventions. In recent years, miRNAs have been actively studied as potential biomarkers of post-transplant complications. MiRNAs are a family of small non-coding RNAs, about 22 nucleotides (18–25) in length, acting as regulatory elements of post-transcriptional genes. MicroRNAs inhibit protein synthesis by blocking translation by base pairing with complementary ribonucleic acid (RNA), thereby leading to degradation of a specific target [2]. It has been estimated that

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miRNAs may be able to regulate more than 30% of the protein-coding genes in the human genome. Moreover, miRNAs play a key role in regulating the functions of both healthy and damaged cells. They are closely related to various biological processes, including development and differentiation of hematopoietic cells, apoptosis and proliferation. It has been shown that expression of certain miRNAs is associated with a number of conditions, such as autoimmune diseases, malignant neoplasms, and graft rejection [3–5].

Small signaling molecules are studied in terms of their potential significance in the pathogenesis of post-transplant complications and use of targeted therapy as potential targets for solid graft rejection [2, 6, 7]. Recent studies have shown that data on changes in the expression levels of certain types of miRNAs in solid organ recipients can be useful for early diagnosis and monitoring of post-transplant complications, including rejection and fibrosis of heart, kidney, liver, and lung transplants [8–11].

The aim of this study was to evaluate the expression levels of miRNA (miR-27, miR-101, miR-142, miR-339 and miR-424) and its relationship with clinical and laboratory parameters in lung transplant recipients.

MATERIALS AND METHODS

The study included 57 lung recipients aged 10 to 74 years (35 ± 15 average). From 2014 to 2019, they underwent lung transplant surgeries at the Shumakov National Medical Research Center of Transplantology and Artificial Organs. Among them were 6 children (9%) – 4 boys aged 10, 12, 13 and 17 years and 2 girls aged 13 and 14 years. There were also 51 adult recipients, aged 18 to 74 (37 ± 14) years, including 30 (62.5%) male patients. The diseases that caused respiratory failure and determined the indications for transplantation were cystic fibrosis (n = 22), chronic obstructive pulmonary disease (COPD) (n = 15), primary pulmonary hypertension (n = 9), pulmonary fibrosis (n = 6), lymphangioleiomyomatosis (n = 3) and bronchiectasis (n = 2). Maximum follow-up for lung transplant recipients was 1,808 days (median 294 [85; 545]). The control group consisted of 14 healthy individuals.

Scheduled examination of the patients was done in accordance with the clinical recommendations of the Russian Transplant Society. It included complete physical examination, general and biochemical blood tests, as well as virological and bacteriological studies. When studying external respiration function by spirometry, we measured the forced expiratory volume in one second (FEV_1) and forced vital capacity (FVC), and calculated the Tiffeneau-Pinelli index (FEV1/FVC × 100%). All patients received basiliximab induction. Immunosuppressive therapy included tacrolimus, mycophenolic acid and methylprednisolone preparations. Everolimus was administered if necessary [12].

Venous blood plasma served as the material for studying miRNA expression (1 to 3 samples from each patient, 1.22 on average). Peripheral blood samples were collected in disposable tubes with anticoagulant ethylenediaminetetraacetic acid (EDTA), centrifuged for 10 minutes at 3,000 rpm. After that, the blood plasma was isolated from the cell pellet and immediately frozen at –20 °C. Total RNA was isolated from 100 μl of blood plasma using SerumPlasma kits (Qiagen, USA) with preliminary addition of 1.6×10^8 copies of synthetic microRNA cel-miR-39 (Qiagen) after plasma incubation with Qiazol phenolic mixture. Cel-miR-39 was used in real-time as an internal control of the efficiency of RNA isolation, complementary DNA (cDNA) synthesis, and quantitative polymerase chain reaction (PCR). MiRNA expression intensity was expressed in relative units that are equivalent to $2^{-\Delta Ct}$, where ΔCt are the working values of product cycle change relative to the internal control of cel-miR-39 expression. Statistical analysis of data obtained was performed using standard statistical processing methods – Microsoft Office Excel software and Statistica v.13.0 application package, StatSoftInc (USA). Data are represented by the values of the median and interquartile range for nonparametric variables. Expression values obtained were checked for distribution normality. Spearman rank-order correlation coefficient and Mann–Whitney U test were used to compare independent variables. Critical significance level was taken to be 5%, i.e., the null hypothesis was rejected at $p < 0.05$.

RESULTS AND DISCUSSION

Comparative analysis showed that the expression level of three out of the five miRNAs (miR-27, miR-101 and miR-339) in candidates waitlisted for lung transplantation was significantly higher than in healthy individuals ($p = 0.02$, $p = 0.03$ and $p = 0.01$, respectively, Fig. 1).

Results obtained from measurement of miRNA expression levels are presented as median and interquartile range values, which is due to the distribution of values that is different from normal distribution. Data are given in relative units. There were no significant differences in the expression values of miR-142 and miR-424 in potential lung recipients and in healthy individuals ($p = 0.16$ and $p = 0.06$, respectively).

No significant differences were found in the expression levels of miR-27, miR-101, miR-142, miR-339 and miR-424 in men and women ($p = 0.37$, $p = 0.85$, $p = 0.98$, $p = 0.27$ and $p = 0.34$, respectively). The expression of four out of the five miRNAs was age independent; miR-339 expression level correlated with the age of potential lung recipients ($p = 0.04$), and the correlation was inverse ($r = –0.46$).

Fig. 2 shows comparative data on the change in the expression value of each of the five miRNAs in recipients in the early (n = 27) and long-term (n = 44) post-transplant period. The early post-transplant period in-

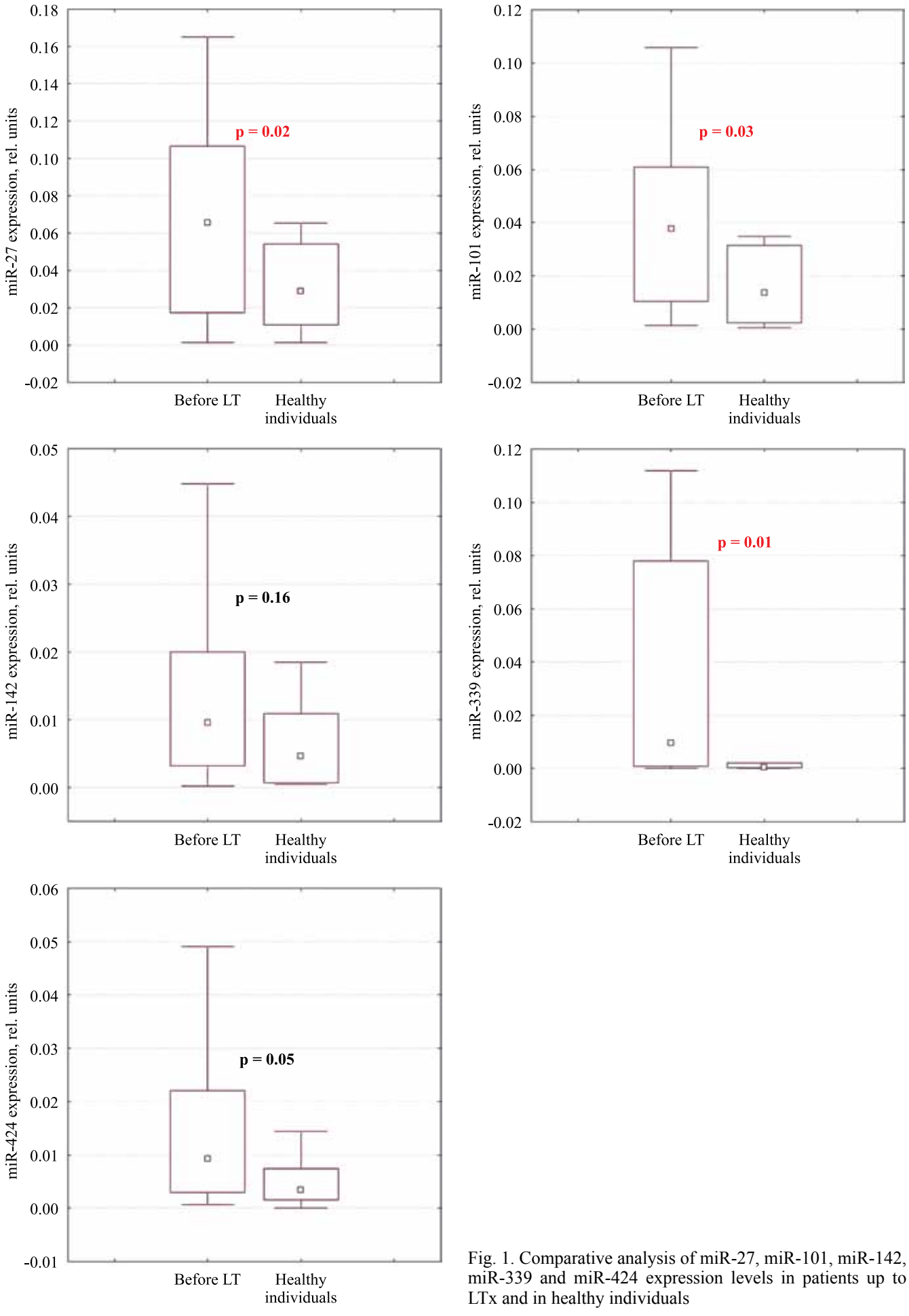


Fig. 1. Comparative analysis of miR-27, miR-101, miR-142, miR-339 and miR-424 expression levels in patients up to LTx and in healthy individuals

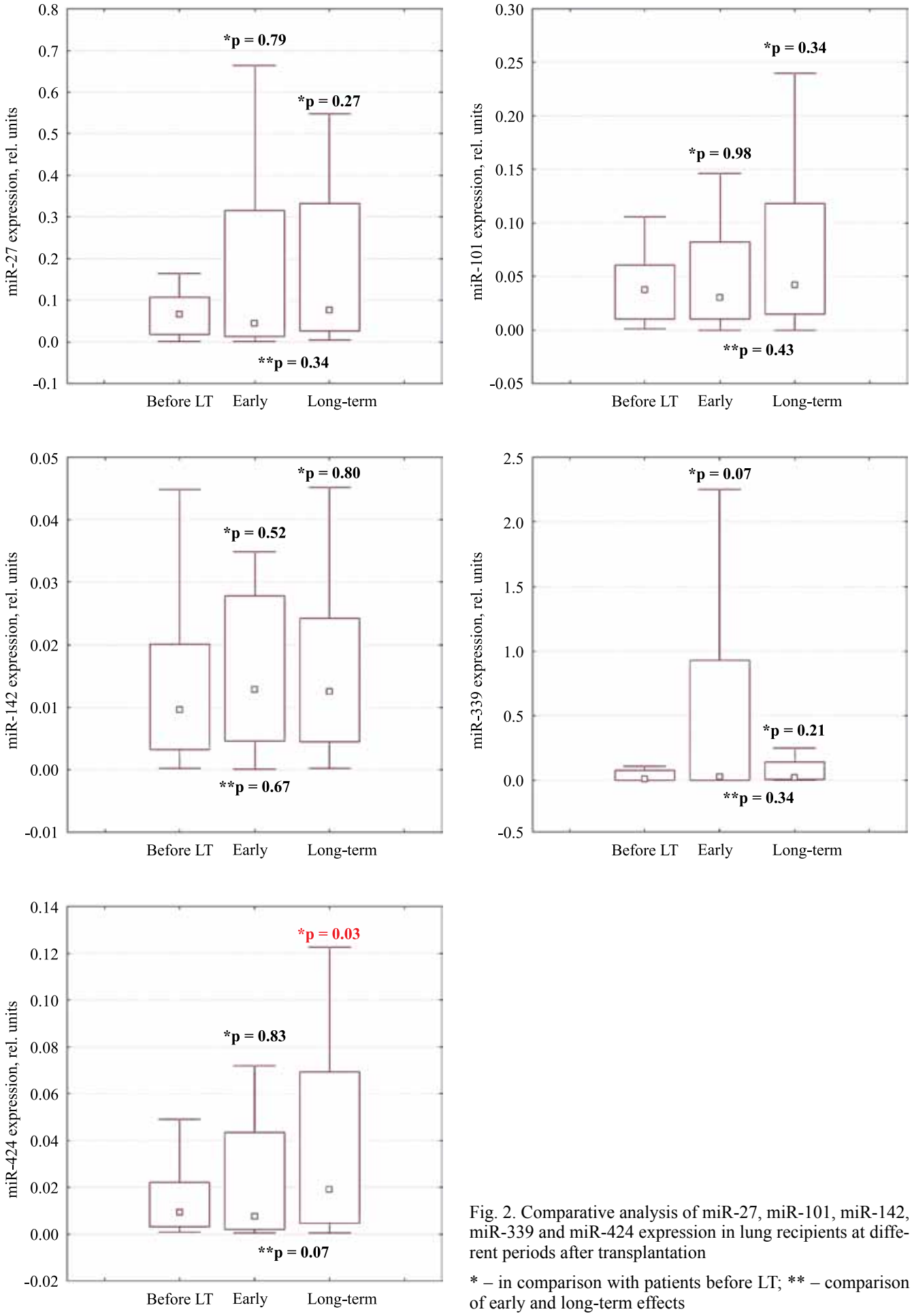


Fig. 2. Comparative analysis of miR-27, miR-101, miR-142, miR-339 and miR-424 expression in lung recipients at different periods after transplantation

* – in comparison with patients before LT; ** – comparison of early and long-term effects

Table

Comparative analysis of microRNAs expression in lung recipients with and without postoperative complications

microRNA	Without complications	Complications			
		infectious	*p	obstructive	*p
miR-27	0.07 [0.03; 0.25]	0.06 [0.03; 0.11]	0.64	0.07 [0.03; 0.28]	0.98
miR-101	0.04 [0.01; 0.08]	0.05 [0.03; 0.13]	0.81	0.04 [0.02; 0.14]	0.58
miR-142	0.01 [0.01; 0.02]	0.01 [0.004; 0.01]	0.44	0.01 [0.01; 0.02]	0.94
miR-339	0.02 [0.01; 0.07]	0.02 [0.004; 0.06]	0.83	0.03 [0.004; 0.15]	0.98
miR-424	0.02 [0.01; 0.05]	0.07 [0.04; 0.07]	0.50	0.03 [0.03; 0.07]	0.86

Note. * – in comparison with the group without complications.

cluded recipients who were examined a median of 33 [23; 68] days after transplant surgery. The long-term post-transplant period included recipients examined 511 [388; 930] days after lung transplant surgery.

The average expression level of miR-424 in lung recipients in the long-term post-transplant period was significantly higher than in patients awaiting transplantation ($p = 0.03$). In all other cases, there were no significant differences in miRNA expression levels in recipients in the early and long-term post-transplant periods, as in patients awaiting lung transplant.

The Table presents a comparative analysis of the expression levels of individual miRNAs in recipients with complications associated with obstructive airway processes (n = 14), infectious complications (n = 6), as well as in recipients who were not diagnosed with these complications. The average expression level of each of the five miRNAs in the long-term post-transplant period represented by the median values [interquartile range], did not significantly differ in recipients with and without complications (see Table).

It cannot be ruled out that with increased number of observations, significant differences (in the expression of individual miRNAs) associated with post-transplant complications can be revealed.

At the same time, when studying the relationship between the expression level of each of the five miRNAs and the follow-up data obtained, it was found that the expression level of miR-27 inversely correlated with the patient's body mass index (BMI). The indicated dependence was revealed both in lung recipients in the long-term post-transplant period and in waitlisted patients with respiratory failure (Fig. 3).

The miR-27 expression level is higher in underweight patients; we found no correlation between the expression values of miR-101, miR-142, miR-339, miR-424 with the BMI.

When studying the external respiration function parameters in lung recipients, there was no significant correlation between the miR-27, miR-101, miR-142, miR-339, miR-424 expression levels and FEV_1 at a long-term post-transplant period ($p = 0.25$, $p = 0.64$, $p = 0.59$, $p = 0.14$ and $p = 0.48$, respectively). However, in the long-term post-transplant period, there was significant positive correlation between the expression level of miR-142 ($r = 0.61$; $p = 0.04$) and the Tiffeneau-Pinelli index.

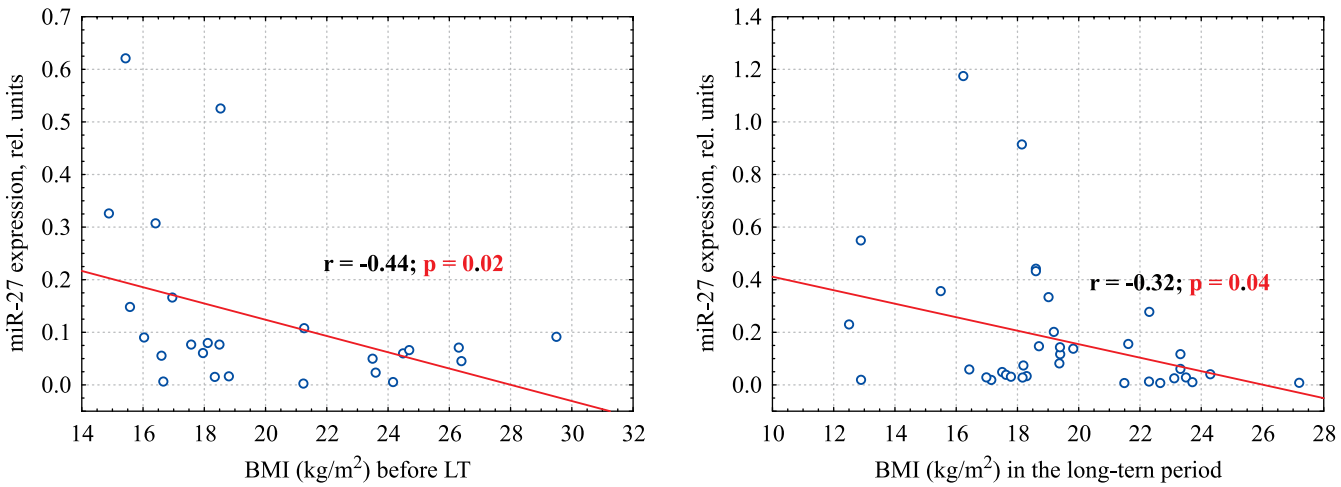


Fig. 3. Correlation of miR-27 expression with body mass index (BMI) in long-term lung recipients and in potential lung recipients

The value, which is more than 85%, suggests restrictive respiratory tract disorders (Fig. 4).

A positive correlation was found between white blood cell count and the expression levels of miR-27 (r = 0.63; p = 0.0002), miR-142 (r = 0.44; p = 0.04) and miR-424 (r = 0.56; p = 0.001) in recipients in the long-term post-transplant period (Fig. 5).

Analysis showed there were no significant correlations between microRNA expression levels and total plasma protein, albumin, creatinine, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) activity. In the long-term post-transplant period, there was moderate inverse correlation between ALT activity and miR-27 (r = –0.44; p = 0.01), and between AST and miR-27 (r = –0.41; p = 0.02) and miR-142 (r = –0.43; p = 0.04).

In lung recipients, the expression level of each of the five miRNAs in the long-term post-transplant period did not depend on blood levels of tacrolimus and everolimus. At the same time, concentration of the soluble CD40 ligand lymphocyte stimulating factor (sCD40L) correlated with the expression levels of miR-27 (r = 0.52; p = 0.02) and miR-142 (r = 0.53; p = 0.02) in the plasma of recipients in the long-term post-transplant period (Fig. 6).

No significant dependence of sCD40L concentration on the expression levels of other miRNAs has been established.

A number of recent studies have identified miRNAs as potential biomarkers of post-transplant complications

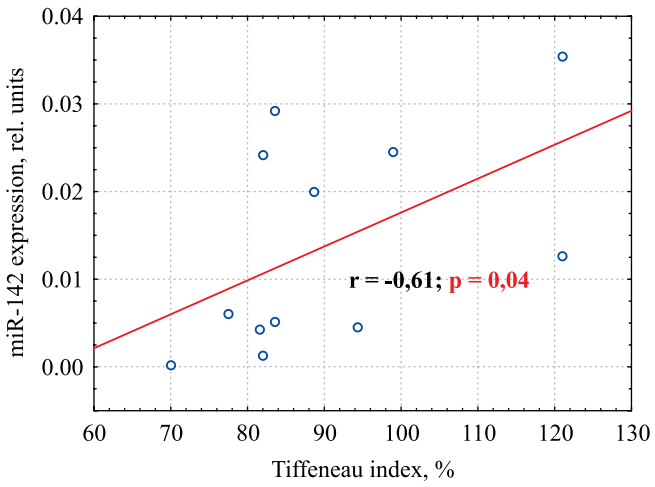
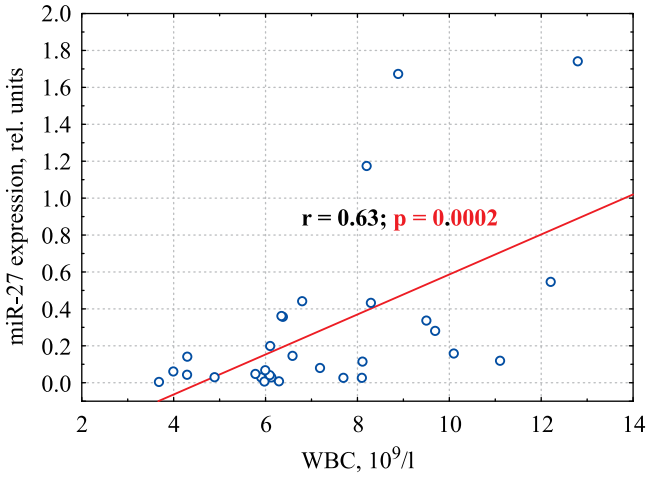
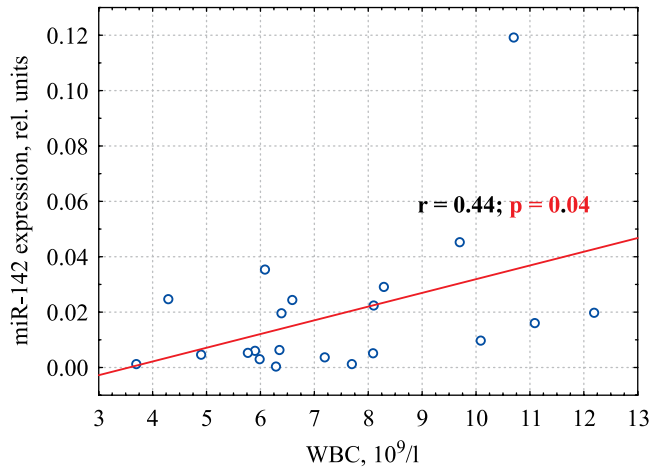


Fig. 4. Correlation of Tiffeneau index (%) and miR-142 expression in lung recipients in the long-term period





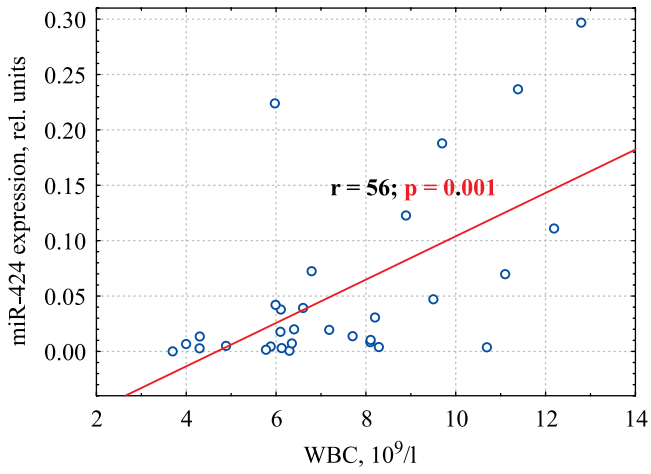


Fig. 5. Correlation of miR-27, miR-142 and miR-424 expression with leukocyte levels in lung recipients

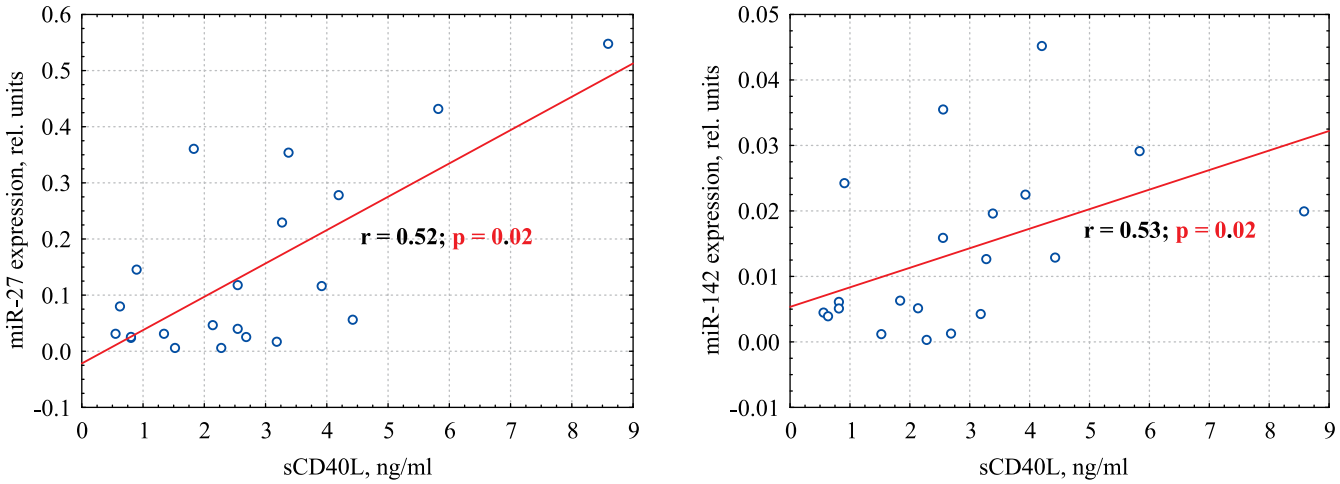


Fig. 6. Correlation of sCD40L and miR-27, miR-142 expression in lung recipients in the long-term period

[13]. Thus, significant differences in the expression levels of individual miRNAs (miR-10a, miR-31, miR-92a, miR-101, miR-142-3p, miR-155, etc.) were noted in heart recipients with and without acute cellular rejection [8]. Studies of the diagnostic potential for measuring miRNA expression in the plasma of kidney recipients revealed a number of molecules (miR-150, miR-192, miR-200b and miR-423-3p) associated with renal allograft rejection and allograft dysfunction [5]. Specific miRNAs were found (miR-122, miR-148a and miR-194), whose expression levels increase significantly in blood serum upon liver allograft rejection [9].

In experimental studies, the regulatory role and diagnostic significance of miRNAs in the development of post-lung transplant complications were established. It was shown that miR-124, through regulation of monocyte chemotactic protein-1 expression, affects proliferation and activation of pulmonary adventitial fibroblasts [14], which is of great importance in chronic graft dysfunction. Another study using an experimental rat lung transplantation model showed increased expression of miR-146a and miR-155 in obliterative bronchiolitis [15]. Similar data on possible diagnostic significance in lung graft rejection were obtained in the study of miR-376-5p, miR-338-3p, miR-16 and miR-195 [16]. It was shown that miRNA-199b regulates the severity of immune responses during lung graft rejection [17].

A significant increase in the expression of miR-21, miR-29a, miR-103, and miR-191 was observed in the blood serum of patients with end-stage respiratory disease at different times after lung transplantation [18].

Recent results from the study of microRNAs in solid organ recipients suggest that they could be characterized as potential biomarkers of post-transplant complications [19]. However, due to the small number of studies, there is a need to further study the participation of this group of molecules in biological processes in lung recipients.

The objective of this study was to establish a relationship between miRNA expression levels and changes in clinical and laboratory parameters in patients with severe respiratory failure and in lung transplant recipients, with subsequent determination of the possibility of using the microRNAs as potential biomarkers of post-transplant complications. MicroRNAs that regulate expression of genes potentially associated with post-transplant complications, primarily graft rejection, infection, and fibrosis, were selected for the study.

It was found that in potential lung recipients, expression levels of three of the five miRNAs (miR-27, miR-101 and miR-339) are higher than in healthy individuals. This may suggest that they are involved in the pathological process. We could not detect any significant changes in the expression level of each of the miRNAs, as compared to waitlisted patients and recipients in the early and long-term post-transplant period.

A comparative analysis of the expression level of the miRNAs in recipients with and without infectious-mediated, obstructive complications in the long-term postoperative period also did not reveal any major differences. It cannot be excluded that the absence of differences in the average expression levels between the indicated groups may be due to the small number of observations, especially if we take into account the wide range of variations in the miRNA expression value, as well as the wide variety of factors that can influence these parameters, as in the early and in the long-term post-transplant period.

A fairly large number of different types of miRNAs have been identified and characterized to date. Selected for the present study were five miRNAs that presumably play a role in the development of lung diseases, cardiovascular disease and/or are potentially significant for diagnosis of post-transplant complications in lung recipients [13]. The mechanisms of influence of individual miRNAs on transplant immunity and various aspects of

the relationship between a graft and the recipient's body have not been studied enough. Most of the available information is based on the results of experimental studies [13, 15, 20]. However, available data on the involvement of individual microRNAs in regulation of immune response to transplantation, in the processes of acute participation and chronic rejection, in their influence on the functions of fibroblasts, dendritic cells, T-lymphocytes are the basis for in-depth studies of the role of signaling molecules in the pathogenesis of post-transplant complications, with subsequent development of fundamentally new approaches to diagnosis and treatment [6, 14, 19].

In the present study, it was shown that the expression level of miR-27 in lung recipients is associated with a number of clinical and laboratory parameters – body mass index, hepatic transaminase activity, and white blood cell count. According to published data, miR-27 and its isoforms participate in regulation of metabolic processes and fibrosis by inhibiting the expression of transforming growth factor β (TGF-β) [12, 21].

The expression level of miR-142 also correlates with white blood cell counts and AST activity in lung recipients. MiR-142 is expressed on T lymphocytes, which play a major role in acute cellular rejection. The fact that miR-142 originates from immune cells, and not from transplant tissue, suggests the possibility of predicting rejection even before damage to the organ itself. Moreover, miR-142 has recently been shown to have a regulatory role in the pathogenesis of inflammatory lung diseases via suppression of macrophage activation [12, 22]. In this context, the dependence we found between miR-142 expression and Tiffeneau-Pinelli index, reflecting the restrictive pathology caused by alveolar disease of the bronchopulmonary system, may be of particular interest.

The presence of a direct relationship between expression levels of miR-27 and miR-142 and the blood concentration of soluble CD40 ligand, whose role in solid organ transplantation is mediated through co-stimulation of T-lymphocytes [23], may, on one hand, indicate one of the mechanisms regulating lung transplant functioning, and on the other hand, serve as the basis for development of diagnostic approaches.

Our study also established a connection between miR-424 expression and white blood cell count in lung recipients. MiR-424 is secreted by pulmonary arterial endothelial cells and plays an important role in the pathogenesis of pulmonary hypertension. It has recently been shown that miR-424 has a protective effect on alveolar epithelial cells in acute respiratory distress syndrome; this action is mediated through the nuclear factor kappa B (NF-kB) [24].

A comparative study (undertaken in this present work) of the level of miRNA expression (miR-27, miR-101, miR-142, miR-339 and miR-424) in the blood plasma of patients suffering from end-stage chronic lung diseases of various origins in lung recipients in early and long-term post-transplant period allows us to conclude that further study of the microRNA panel and evaluation of their effectiveness as potential molecular genetic markers in the observation of waitlisted patients and lung recipients are necessary.

The correlation found between miRNA expression levels and clinical, functional, and laboratory parameters may suggest that miRNAs play a role in regulation of graft-recipient relationships. It also shows that further investigation of the involvement of the five miRNAs in the immunological mechanisms of graft damage and their diagnostic effectiveness in post-transplant complications is needed.

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The authors declare no conflict of interest.

REFERENCES

1. *Hamilton BCS, Kukreja J, Ware LB, Matthay MA.* Protein Biomarkers Associated With Primary Graft Dysfunction Following Lung Transplantation. *Am J Physiol Lung Cell Mol Physiol*. 2017; 312 (4): 531–541. doi: 10.1152/ajplung.00454.2016.
2. *Harris A, Krams SM, Martinez OM.* MicroRNAs as immune regulators: implications for transplantation. *American Journal of Transplantation*. 2010; 10 (4): 713–719.
3. *Bartel DP.* MicroRNAs: target recognition and regulatory functions. *Cell*. 2009; 136 (2): 215–233.
4. *Amrouche L, Rabant M, Anglicheau D.* MicroRNAs as biomarkers of graft outcome. *Transplantation Reviews*. 2014; 28 (3): 111–118.
5. *Vahed SZ, Zonouzi AP, Mahmoodpoor F.* Circulating miR-150, miR-192, miR-200b, and miR-423-3p as Non-invasive biomarkers of chronic allograft dysfunction. *Archives of Medical Research*. 2017; 48 (1): 96–104.
6. *Sarma NJ, Tiriveedhi V, Ramachandran S.* Modulation of immune responses following solid organ transplantation by microRNA. *Experimental and Molecular Pathology*. 2012; 93 (3): 378–385.
7. *Carlo S Di, Rossi E, Politano G.* Identification of miRNAs potentially involved in bronchiolitis obliterans syndrome: a computational study. *PLoS One*. 2016; 11 (8): 1–22.
8. *Duong Van Huyen JP, Tible M, Gay A*. MicroRNAs as non-invasive biomarkers of heart transplant rejection. *European Heart Journal*. 2014; 35 (45): 3194–3202.
9. *Farid WRR, Pan Q, Van der Meer AJP.* Hepatocyte-derived microRNAs as serum biomarkers of hepatic injury and rejection after liver transplantation. *Liver Transplantation*. 2012; 18 (3): 290–297.
10. *Velikij DA, Gichkun OE, Shevchenko AO.* MikroRNK: rol' v razvitii serdechno-sosudistyh zabolevanij, perspektivy klinicheskogo primeneniya. *Klinicheskaya laboratornaya diagnostika*. 2018; 63 (7): 403–409.

11. *Liu X, Dong C, Jiang Z.* MicroRNA-10b downregulation mediates acute rejection of renal allografts by derepressing BCL2L11. *Experimental Cell Research*. 2015; 333 (1): 155–163.
12. Obshcherossiyskaya obshchestvennaya organizatsiya transplantologov "Rossiyskoe transplantologicheskoe obshchestvo". Klinicheskie rekomendatsii. Transplantatsiya legkikh i kompleksa "serdtse–legkie". 2016.
13. *Hamdorf M, Kawakita S, Everly M.* The Potential of MicroRNAs as Novel Biomarkers for Transplant Rejection. *Journal of Immunology Research*. 2017; 2017: 1–12. doi: 10.1155/2017/4072364.
14. *Wang D, Zhang H, Li M.* MicroRNA-124 controls the proliferative, migratory, and inflammatory phenotype of pulmonary vascular fibroblasts. *Circulation Research*. 2014; 114 (1): 67–78.
15. *Wang J, Cao H, Hong X.* MicroRNA screening and functional study of obliterative bronchiolitis in a rat model simulating lung transplantation. *Genetics and Molecular Research*. 2015; 14 (4): 19309–19316.
16. *Dong M, Wang X, Zhao HL.* Integrated analysis of transcription factor, microRNA and LncRNA in an animal model of obliterative bronchiolitis. I*nternational Journal of Clinical & Experimental Pathology.* 2015; 8 (6): 7050–7058.
17. *Xu Z, Nayak DK, Benshoff N.* De novo-developed antibodies to donor MHC antigens lead to dysregulation of microRNAs and induction of MHC class II. *The Journal of Immunology*. 2015; 194 (12): 6133–6143.
18. *Zhu L, Xu H, Lu W.* MiR-199b-5p regulates immune-mediated allograft rejection after lung transplantation through the GSK3β and NF-κB pathways. *Inflammation*. 2018; 41 (4): 1524–1535. doi: 10.1007/s10753-018-0799-2.
19. *Shan J, Feng L, Luo L.* MicroRNAs: potential biomarker in organ transplantation. *Transplant Immunology*. 2011; 24 (4): 210–215.
20. *Zhang W, Zhou T, Ma SF.* MicroRNAs implicated in dysregulation of gene expression following human lung transplantation. *Translational Respiratory Medicine*. 2013; 1 (12): 1–9.
21. *Ma M, Yin Z, Zhong H, Liang T, Guo L.* Analysis of the expression, function, and evolution of miR-27 isoforms and their responses in metabolic processes. *Genomics*. 2019; 111 (6): 1249–1257. doi: 10.1016/j.ygeno.2018.08.004.
22. *Zhang D, Lee H, Wang X, Groot M, Sharma L, Dela Cruz CS, Jin Y.* A potential role of microvesicle-containing miR-223/142 in lung inflammation. *Thorax*. 2019; 74 (9): 865–874. doi: 10.1136/thoraxjnl-2018-212994.
23. *Shevchenko OP, Khalilulin TA, Shevchenko AO.* Predictive value of PAPP-A, sCD40L, anti-HLA antibodies detected by Elisa and Luminex in heart transplant recipients. *The Journal of Heart and Lung Transplantation*. 2013; 33 (4): 135.
24. *Cheng D, Zhu C, Liang Y, Xing Y, Shi C.* MiR-424 overexpression protects alveolar epithelial cells from LPS-induced apoptosis and inflammation by targeting FGF2 via the NF-κB pathway. *Life Sci*. 2020; 242: 117213. doi: 10.1016/j.lfs.2019.117213.

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ASSESSMENT AND MONITORING OF LIVER GRAFT VIABILITY AND INITIAL FUNCTION USING INTERSTITIAL MICRODIALYSIS

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Assessing the viability and monitoring the function of liver graft in the early postoperative period are critical clinical tasks. One possible solution is to determine the changes in concentration of blood glucose, its metabolites and glycerol in the graft using interstitial microdialysis. **Objective:** to study the dynamics of interstitial glucose, lactate, pyruvate and glycerol in the early post-liver transplant period – depending on the initial graft function (IGF) – and to compare with the results of standard laboratory blood tests. **Materials and methods.** Four selected clinical observations of deceased donor liver transplantation are presented. Two of the observations showed normal IGF, one observation – early allograft dysfunction (EAD), complicated by hepatic artery thrombosis (HAT), while one observation demonstrated primary non-function (PNF). Collection of microdialysis samples began after arterial reperfusion of the liver graft and continued continuously for 7 days or until death. Standard blood biochemistry and coagulation tests were performed at least once a day. **Results.** With normal IGF and a smooth postoperative period, interstitial concentrations of glucose, lactate, pyruvate and glycerol remained stable throughout the observation period, ranging from 5 to 20 mmol/L, 1.1 to 7.5 mmol/L, 90 to 380 μmol/L, and 10–100 μmol/L, respectively. EAD was associated with initially higher levels of glucose, lactate, and pyruvate. With HAT development, there was a rapid (within 2–4 hours) five-fold increase in interstitial concentration of lactate with simultaneous decrease in glucose and pyruvate levels to 0.1 mmol/L and 11 μmol/L, respectively. In the case of PNF, there was an initially high concentration of interstitial lactate – 16.4 mmol/L, which increased further to 35.5 mmol/L. Glucose concentration was close to 0. Changes in interstitial glucose, its metabolites and glycerol concentrations chronologically preceded the corresponding changes in peripheral blood composition by 3–5 hours. **Conclusion.** Microdialysis measurement of interstitial glucose, lactate, pyruvate and glycerol concentrations facilitates real-time monitoring of liver graft viability and function. The high sensitivity of the method could help in accelerating diagnosis of vascular complications (HAT in particular), as well as graft dysfunction with other causes. Therefore, the method is feasible in clinical practice.

Keywords: microdialysis, liver transplantation, early allograft dysfunction, hepatic artery thrombosis, primary graft non-function.

INTRODUCTION

Assessing the initial liver graft function is an extremely important clinical task, especially in organ transplantation from an expanded criteria donor. The key points are to determine as early and objectively as possible the degree of ischemia-reperfusion injury and the reversibility of graft dysfunction. There is no doubt that initial graft function depends not only on the donor's parameters, but also on the severity of the recipient's condition, the peculiarities and complexity of the surgical intervention and anaesthetic support.

Conventionally, graft function is assessed by determining a number of laboratory indicators in peripheral blood (aspartate transaminase (AST) and/or alanine aminotransferase (ALT), bilirubin, prothrombin time, prothrombin index, international normalized ratio (INR)), sometimes taking into account such clinical signs as severity of encephalopathy, bile production rate, and need for fresh frozen plasma (FFP) infusion. This requires dynamic follow-up and collection of laboratory samples within postoperative days 2–7 [1]. It should be emphasized that the relatively low sensitivity and specificity of individual signs of graft dysfunction require their combined analysis. Sometimes, multidirectional trends in changes in the set of laboratory indicators and possible inconsistency with the clinical picture lead the need to extend the follow-up period, thereby delaying diagnosis. It must be borne in mind that changes in the peripheral blood composition, reflecting the liver graft function, occur with a certain time delay, and the use of renal replacement therapy, albumin dialysis, FFP and clotting factor concentrate in patients with severe graft dysfunction and coagulopathy may create a false impression of restored liver function.

So far, several methods have been developed and tested in the clinic for direct assessment of liver function, including during liver transplantation. They include

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determination of the maximal liver functional capacity by clearance of C^{13} methacetin (LiMAx test) [2, 3], measurement of liver function and perfusion by indocyanine green clearance (LIMON test) [4, 5], and interstitial microdialysis [6]. The first two methods are based on non-invasive measurement of the concentration of substances that are specifically metabolized (C^{13} methacetin) or secreted (indocyanine green) by the liver.

Microdialysis is a more versatile technology, as it allows to measure the concentration of almost any substance in the intercellular space of the tissue under study. It is based on passive diffusion of substances through a semipermeable membrane by concentration gradient. To assess the viability and functional state of the liver, like any other tissue of the body, glucose, lactate and pyruvate concentrations are measured and correlated in the intercellular fluid. This allows to determine the nature (anaerobic or aerobic) and the rate of glucose metabolism. Moreover, the analysis can be complemented by studying glycerol (a product of degradation of membrane phospholipids) levels, which reflects the degree of cytolysis.

The aim of this work is to study the characteristics of glucose metabolism in a liver graft in the early post-transplant period, to determine typical patterns of changes in interstitial concentrations of glucose, lactate, pyruvate, and glycerol, and to compare them with results from routine laboratory methods used for diagnosing early liver graft dysfunction or primary non-function.

MATERIALS AND METHODS

Study design

Since August 2017, the Center for Surgery and Transplantology, Burnazyan Federal Medical and Biophysical Center, Moscow has been conducting a non-randomized, single-center, observational study titled "Study of the characteristics of glucose metabolism in a liver graft for early diagnosis of dysfunction". The study is approved by the institution's local ethics committee and the Expert Council of the Russian Science Foundation (project No. 17-75-10010). The work does not provide for changes in patients' selection criteria for transplantation, surgery technique, anesthetic treatment, treatment and follow-up tactics in the postoperative period, as well as choice of drugs and doses. The study includes male and female subjects aged 18 years and above, who underwent a liver transplant surgery and has a microdialysis catheter installed in them during the operation. The study does not include patients operated on for acute liver failure, who underwent multivisceral transplantation and received a liver fragment from a living related donor.

The following events and conditions were established as endpoints: 1) early allograft dysfunction (EAD) according to the criteria by K. Olthoff et al., 2010 [6]; 2) primary graft non-function (PGNF) according to UNOS criteria [7]; 3) graft loss (recipient death or retransplantation); 4) recipient death. The severity of the patient's pre-transplant condition was assessed using the well-known and commonly used scales MELD, MELD-Na, and Child-Pugh. For donors, the donor risk index (DRI) was calculated [8].

The study lasted for 7 days (160–175 hours) after completion of liver transplant operation. During this period, microdialysis samples of the intercellular fluid of the graft were continuously collected. The peripheral blood composition (acid-base state, electrolyte levels, biochemical analysis, coagulogram) was examined at least once a day. The recipients had a 6-month follow-up period after the end of the study.

Receiving, collecting and analyzing intercellular fluid samples

The principle of the method and its use in liver transplantation are detailed in [9–12]. The microdialysis catheter (61 Hepatic Microdialysis Catheter, M Dialysis AB, Sweden) is a 1 mm diameter polyurethane tube with two concentrically located lumens. The outer surface of the 3 cm long terminal part of the catheter is made of a semipermeable membrane (Fig. 1).

During transplantation, before wound suturing, the catheter is placed by puncture in segment IV of the liver, fixed to the falciform ligament, and is brought to the anterior abdominal wall through a counter-opening. A standard isotonic solution (Perfusion Fluid T1, M Dialysis AB, Sweden) is fed through the inner lumen of the catheter using a micropump (106 MD Pump, M Dialysis AB, Sweden) at 0.3 μL/min. When the perfusion fluid reaches the semipermeable membrane, passive transport of substances from the intercellular fluid into the catheter cavity begins by a concentration gradient. The resulting solution is continuously evacuated at the same rate through the second lumen of the catheter and collected in a microtube. Microtubes are changed (i.e. individual samples are obtained) at least once every 3 hours. Glucose, lactate, pyruvate and glycerol concentrations are measured using a set of standard reagents on analyzer ISCUS Clinical Microdialysis Analyzer (M Dialysis AB, Sweden) within 24 hours from the time the sample is received.

Clinical cases

Four clinical cases were selected to demonstrate and discuss the first results of the study, depending on the initial graft function:

Case No. 1 (normal initial graft function)

A 51-year-old male recipient diagnosed with cirrhosis resulting from hepatitis C, Child-Pugh B. Was waitlisted for liver transplant for 4 months. Preoperative MELD-Na score was 13.

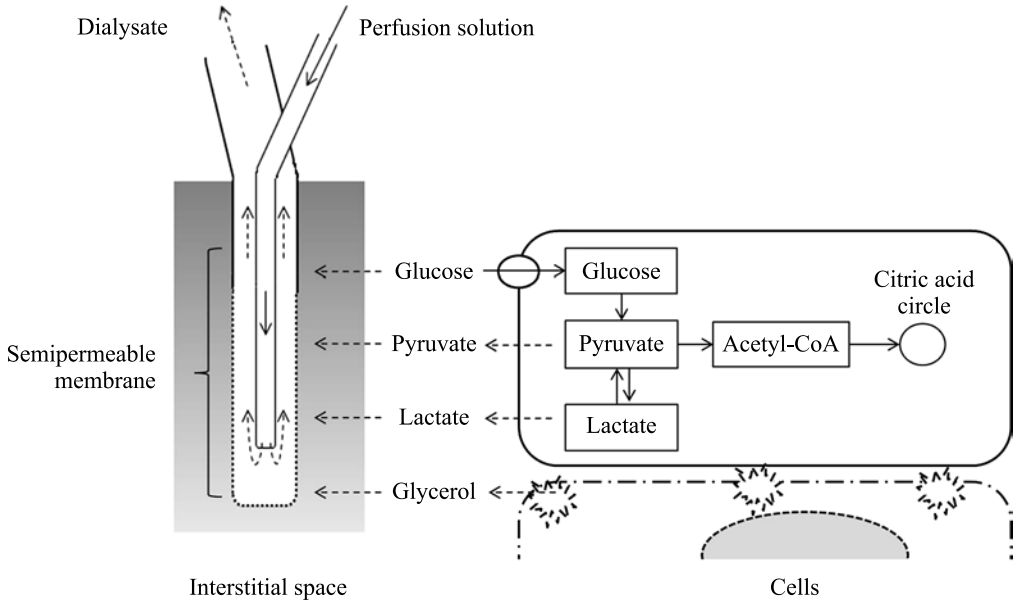

Fig. 1. Microdialysis catheter – the circuit and device operation principle

A 39-year-old male donor, 1.24 DRI. Brain death resulted from traumatic brain injury (TBI). Length of ICU stay under mechanical ventilation was 2 days before multi-organ procurement was performed. Laboratory findings: AST 26 IU/L, ALT 17 IU/L, total bilirubin 6 µmol/L, creatinine 100 µmol/L, and Na 145 mmol/L. Stable hemodynamics (blood pressure (BP) 120/70 mm Hg) against the background of noradrenaline (norepinephrine) infusion at 700 ng/kg/min.

Visual quality assessment of liver graft: normal color, rounded edge of the right lobe, sharp edge of the left lobe, no parenchymal edema, normal consistency, satisfactory perfusion quality, steatosis ≤30% (retrospectively via histological examination – 0%).

Cold ischemia time was 8.5 hours. Transplant surgery lasted for 7 hours. The anhepatic phase lasted for 80 minutes. Warm ischemia time was 48 minutes. IVC clamping time was 50 minutes. Intraoperative plasma transfusion volumes: red blood cells 900 mL, FFP 3,100 mL, blood reinfusion 543 mL.

Trachea was extubated within the first 24 hours. Laboratory MELD score at 24 hours following transplantation was 13. Maximum AST/ALT level at day 7 was 215 IU/L, total bilirubin at day 7 was 22 µmol/L, INR at day 7 was 1.1. Patient was discharged from the hospital on postoperative day 14.

Case No. 2 (normal initial graft function)

A 64-year-old female recipient diagnosed with hepatocellular carcinoma (the size and number of nodes meet the UCSF criteria [13]) against the background of cirrhosis resulting from hepatitis C, Child-Pugh C. She had a history of myocardial infarction, whose duration is uncertain and acute ischemic stroke in the basin of left middle cerebral artery in August 2016. Was waitlisted for liver transplant for 10 months. Preoperative MELD-Na score was 13.

A 25-year-old male donor, 1.27 DRI. Brain death resulted from TBI. Length of ICU stay under mechanical ventilation was 1 day before multi-organ procurement was performed. Laboratory findings: AST 46 IU/L, ALT 40 IU/L, total bilirubin 11 µmol/L, creatinine 85 µmol/L, Na 148 mmol/L. Stable hemodynamics (BP 120/80 mmHg) without vasopressors and inotropes.

Visual quality assessment of liver graft: normal color, sharp edge, no parenchymal edema, normal consistency, excellent perfusion quality, no steatosis (retrospectively via histological examination – 0%).

Cold ischemia time was 11 hours. Transplant surgery lasted for 7.5 hours. The anhepatic phase lasted for 40 minutes. Warm ischemia time was 30 minutes, IVC clamping time was 40 minutes. Intraoperative plasma transfusion volumes: red blood cells 570 mL, FFP 2300 mL, blood reinfusion 0 mL.

Trachea was extubated within the first 24 hours. Laboratory MELD score at 24 hours following transplantation was 10. Maximum AST/ALT level at day 7 was 544 IU/L, total bilirubin at day 7 was 10 µmol/L, INR at day 7 was 1.1. Patient was discharged from the hospital on postoperative day 20.

Case No. 3 (early allograft dysfunction, hepatic artery thrombosis)

A 45-year-old male recipient diagnosed with cirrhosis resulting from primary sclerosing cholangitis, Child-Pugh class B. Was waitlisted for liver transplant for 8 months. Preoperative MELD-Na score was 16.

A 42-year-old male donor, 1.41 DRI. Brain death resulted from TBI. Length of ICU stay under mechanical ventilation was 2 days before multi-organ procurement was performed. Laboratory findings: AST 40 IU/L, ALT 35 IU/L, total bilirubin 8 μmol/L, creatinine 70 μmol/L, Na 154 mmol/L. Stable hemodynamics (BP 120/80 mmHg) against the background of noradrenaline (norepinephrine) infusion at 200 ng/kg/min.

Visual quality assessment of liver graft: normal color, sharp edge, no parenchymal edema, normal consistency, excellent perfusion quality, steatosis ≤30% (retrospectively via histological examination – 0%).

Cold ischemia time was 9 hours. Transplant surgery lasted for 6.5 hours. The anhepatic phase lasted for 50 minutes. Warm ischemia time was 40 minutes, IVC clamping time was 45 minutes. Intraoperative plasma transfusion volumes: red blood cells 620 mL, FFP 2140 mL, platelet concentrate – 1 dose, blood reinfusion 942 mL.

Trachea was extubated within the first 24 hours. Laboratory MELD score at 24 hours following transplantation was 20 баллов, AST – 5254 IU/L, INR – 1.58. On postoperative day 2, AST increased to 6461 IU/L, INR went up to 1.99. Control ultrasound examination could not locate the hepatic artery, hepatic artery thrombosis is suspected. CT scan with intravenous contrast confirmed hepatic artery thrombosis. Emergency angiography was performed; attempts at thrombus extraction from the right hepatic artery and the anastomotic area, brown thrombotic masses were obtained, which, however, did not restore antegrade blood flow. Balloon angioplasty restored blood flow in the proximal sections of the left and right hepatic arteries without filling the distal bed. The patient was placed on the waiting list for urgent liver transplantation. Due to intensified encephalopathy and respiratory failure, repeated tracheal intubation was performed, mechanical ventilation was resumed. Due to absence of a suitable donor organ, despite ongoing intensive therapy, the patient died on postoperative day 5.

Case No. 4 (primary graft non-function)

A 41-year-old male recipient diagnosed with cirrhosis resulting from secondary biliary cirrhosis, Child-Pugh class B. Was waitlisted for liver transplant for 2 months. Preoperative MELD-Na score was 17.

A 61-year-old male donor, 1.41 DRI. Brain death resulted from acute stroke. Length of ICU stay under mechanical ventilation was 2 days. Laboratory findings: AST 68 IU/L, ALT 73 IU/L, total bilirubin 10 μmol/L, creatinine 74 μmol/L, Na 137 mmol/L. Stable hemodynamics (BP 130/90 mmHg) against the background of noradrenaline (norepinephrine) infusion at 700 ng/kg/min.

Visual quality assessment of liver graft: normal color, sharp edge, no parenchymal edema, normal consistency, excellent perfusion quality, steatosis ≤30% (retrospectively via histological examination – 0%).

Cold ischemia time was 11 hours. Transplant surgery lasted for 11 hours. The anhepatic phase lasted for 50 minutes. Warm ischemia time was 45 minutes, IVC clamping time was 50 minutes. Intraoperative plasma transfusion volumes: red blood cells 930 mL, FFP 1790 mL, blood reinfusion 2,139 mL.

Intraoperatively, after blood flow was started, hypotension developed, which required increasing the doses of vasopressors and inotropes, lactic acidosis (pH 7.19, lactate 18 mmol/L), massive coagulopathic diffuse bleeding. Hemostasis for 4 hours. After the end of surgery and the patient transferred to the ICU, multiple organ failure progressed, the patient died on postoperative day 1.

RESULTS

In all the cases, even with severe coagulopathy, insertion and removal of microdialysis catheters were not accompanied by bleeding from the liver parenchyma and other complications. The installed catheter and the micropump attached to the anterior abdominal wall did not interfere with activation of patients, did not cause them discomfort (cases 1 and 2), and did not create inconveniences during diagnostic tests and postoperative wound care.

Fig. 2, a, b, c show the data on the dynamics of interstitial levels of glucose and its metabolites (lactate and pyruvate), as well as standard laboratory tests used to evaluate graft function – level of aminotransferases, total bilirubin and INR (Fig. 2, d, e, f).

With an uneventful postoperative period and good initial graft function (cases 1 and 2), moderate hyperglycemia (10–15 mmol/L) and successive decrease in lactate levels to 1.5 mmol/L were observed in the peripheral blood of recipients within the first 24–48 hours, and less in the next 24 hours. For seven days, measured glucose and lactate concentrations in the intracellular fluid of the liver were higher than in the peripheral blood, ranging from 5 to 20 mmol/L (average 9.3) and from 1.1 to 7.5 (average 2.4), respectively. Interstitial pyruvate levels ranged from 90 to 380 μmol/L (175 μmol/L average).

In case 3, early graft dysfunction was diagnosed 24 hours after the end of operation based on increase in AST level to 5254 IU/L. At the same time, within the first 24 hours, there was reductions in the initially high levels of glucose (21.3 mmol/L), lactate (14.7 mmol/L) and pyruvate (582 μmol/L) to the values recorded in the cases in the absence of graft dysfunction. The exact time hepatic artery thrombosis developed is difficult to establish because diagnosis was made based on ultrasound and CT scan results, performed 37 and 38 hours after surgery. Decrease in interstitial levels of glucose and pyruvate with simultaneous increase in lactate was noted from the 30th hour, while at the time of angiography (43 hours)

and re-intubation (48 hours) they were 2.2 mmol/L, 41 μmol/L, 23.6 mmol/L and 0.1 mmol/L, 11 μmol/L, 28.3 mmol/L, respectively. A day later (72 hours after transplantation), there was transient increase in interstitial glucose levels (from 0.0 to 6.2 mmol/L) and decrease in lactate levels (from 27.2 to 19.9 mmol/L). We have no reliable explanation for this phenomenon. However, this chronologically coincided with the commencement of noradrenaline (norepinephrine) infusion. Later on, until the onset of death (96 hours), interstitial levels of glucose and pyruvate were close to 0, lactate – to 30 mmol/L.

In case 4, after graft reperfusion, hypotension unresponsive to high-dose vasopressor, severe uncorrected metabolic disorders, severe coagulopathy, massive diffuse bleeding, and acute renal injury developed and increased. The first microdialysis test was obtained 6.5 hours after the start of blood flow through the hepatic artery, and the next 3 were collected with an interval of 2 hours. The test results showed no aerobic glucose metabolism.

It should be emphasized that interstitial levels of glucose, lactate and pyruvate are not physiological constants and may vary interrelatedly within relatively wide ranges. To interpret the observed changes, it is customary to operate not with absolute values of indicators, but to calculate coefficients reflecting the lactate-to-pyruvate (L/P) ratio and the lactate-to-glucose (L/G) ratio. An increase in these coefficients reflects the insufficiency of aerobic glycolysis due to mitochondrial ischemia or dysfunction with normal oxygen delivery. Thus, with normal graft function, L/P and L/G ratios ranged from 10 to 20 and from 0.1 to 0.9, respectively. With hepatic artery thrombosis, a 10-fold increase in L/P ratio and an increase in L/G ratio by 500 times were noted. In the case of PGNF, these coefficients, already at the first measurement, exceeded the norm by more than 10 times and demonstrated further rapid growth (Fig. 3).

Special attention should be paid to the issue of glucose-to-lactate ratio, determined simultaneously in the intercellular fluid of the graft and in the peripheral blood (Fig. 4). With normal graft function (cases 1 and 2 – green markers), these values were close to each other in most measurements, but sometimes differed by 5–7 mmol/L (glucose) and 2–5 mmol/L (lactate), which obviously cannot be neglected. In EAD complicated by hepatic artery thrombosis (case 3 – blue markers), after cessation of adequate arterial blood flow to the graft against the background of a relatively normal blood glucose level (4.5–15.0 mmol/L), its interstitial level was close to 0, while the interstitial lactate level, on the contrary, was 5–6 times higher than that measured in blood. For PGNF (case 4 – red markers), significant differences were also observed in glucose and lactate levels measured in the graft tissue and in the blood.

Glycerol, being a product of degradation of membrane phospholipids, is considered a marker of cytolysis. Its

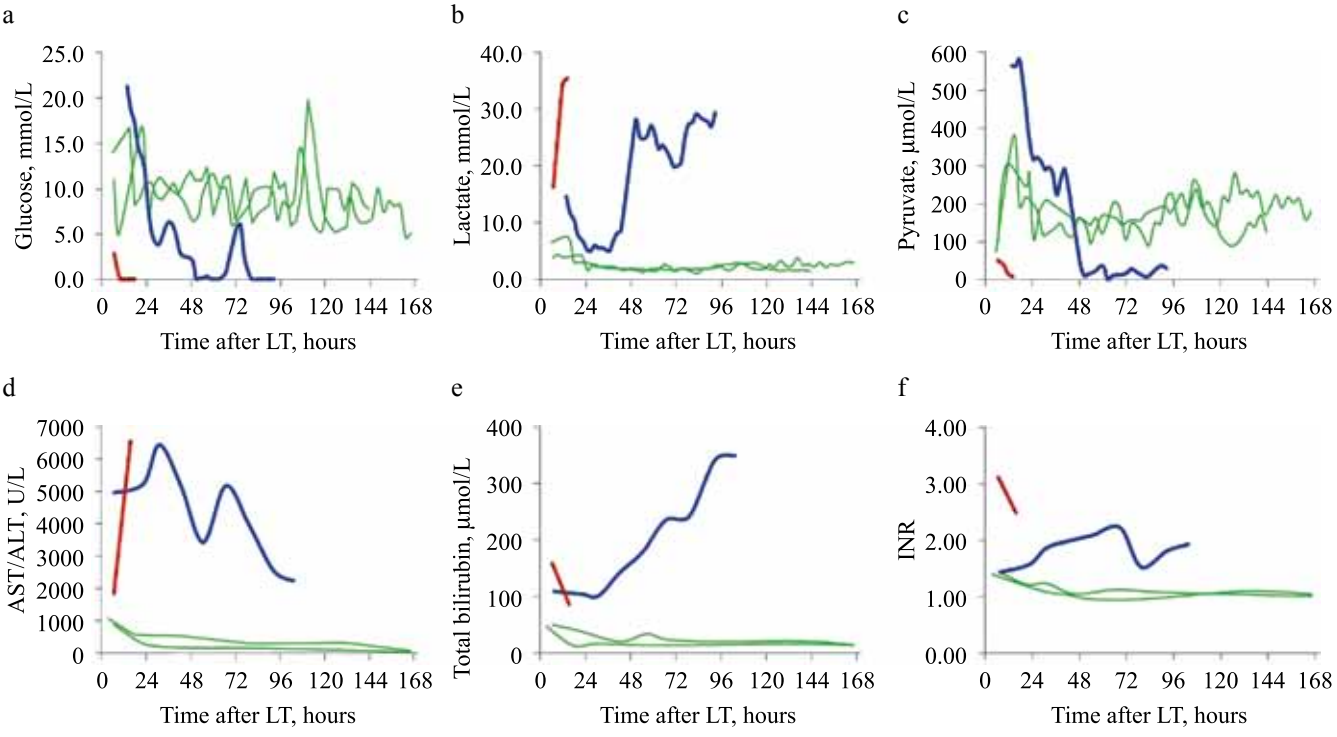

Fig. 2. First post-transplant week dynamics of interstitial graft glucose metabolism parameters (a, b, c) and standard peripheral blood liver function tests (d, e, f). Green lines – normal initial graft function (Cases 1, 2); blue lines – early graft dysfunction complicated with hepatic artery thrombosis on postoperative day 2 (Case 3); red lines – primary non-function graft (Case 4). AST/ALT – maximum of aspartate- or alanine-aminotransferases; INR – international normalized ratio; LT – liver transplantation

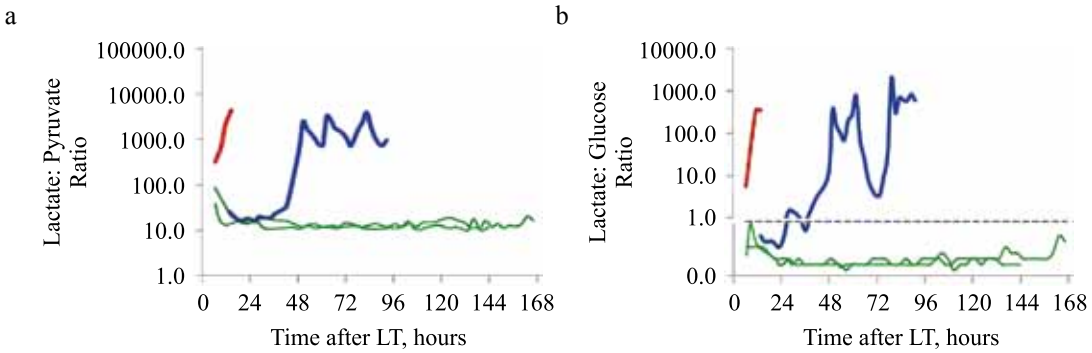

Fig. 3. First post-transplant week dynamics of Lactate : Pyruvate Ratio (a) and Lactate : Glucose Ratio (b). Green lines – normal initial graft function (Cases 1, 2); blue lines – early graft dysfunction complicated with hepatic artery thrombosis on postoperative day 2 (Case 3); red lines – primary non-function graft (Case 4). LT – liver transplantation

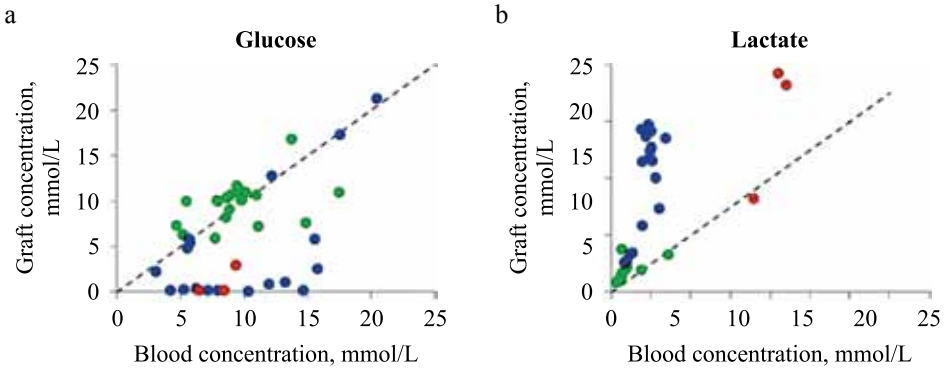

Fig. 4. Glucose (a) and Lactate (b) concentrations in peripheral blood and graft interstitial fluid

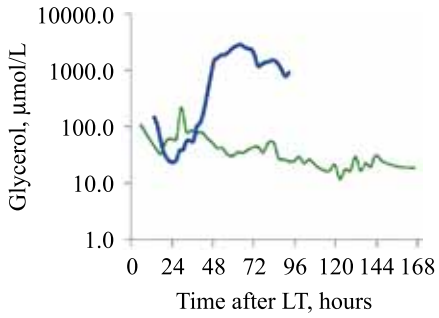

Рис. 5. First post-transplant week dynamics of interstitial graft glycerol concentration. Green line – normal initial graft function (Case 2); Blue line – early graft dysfunction complicated with hepatic artery thrombosis on postoperative day 2 (Case 3)

level measured in the intercellular fluid of a transplanted organ is a quantitative characteristic of this process. Fig. 5 shows the dynamics of glycerol levels in normal graft function (case 2) and in EAD with hepatic artery thrombosis (case 3).

Destruction of hepatocytes due to graft thrombosis and ischemia led to increase in interstitial levels of glycerol by more than 15 times. At the same time, increase in AST levels (Fig. 1, d, blue curve) was more restrained – from 5,254 to 6,461 IU/L (1.2 times), which makes glycerol more sensitive as a marker of cytolysis. Indicators of interstitial glycerol levels in normal graft function and during an uneventful postoperative period do not seem to exceed 100 μmol/L.

DISCUSSION

Microdialysis is a method for studying the composition of interstitial fluid, widely used both in fundamental research [14] and in clinical (mainly neurosurgical) practice [15]. Despite the availability of experimental and clinical results demonstrating the possibility of using microdialysis in liver [9–12, 16, 17] and kidney [18–20] transplantation, as well as positive assessments made by the authors on the prospects for further research in this direction, it should be admitted that the place for this technology in organ transplantation has not yet been determined. It can be assumed that widespread use of the method is hindered by the limited availability of equipment and consumables, high cost of a single analysis compared to standard laboratory tests, the need to install a catheter into the organ parenchyma (invasive method) and train personnel to work with new equipment, and most importantly, the lack of clearly formulated guidelines for correct interpretation of results, their diagnostic value and association with surgery outcomes. That is why the main goal of the study was to find an answer to

the question about the expediency of studying glucose metabolism indicators in a liver transplant and introducing microdialysis technology into real clinical practice.

Two of the presented cases – early hepatic artery thrombosis and PGNF – are examples of rare (3–8% [21] and 1–9% [1], respectively) but typical post-liver transplant complications. In both cases, results from interstitial determination of glucose metabolism indicators did not contradict the data from standard laboratory and instrumental studies. Moreover, in case 3, taking into account the indicators of interstitial microdialysis, disturbances in blood supply to the graft could be suspected 6–7 hours earlier, which could speed up the diagnostic search and initiation of therapeutic measures. In case 4, when graft non-function became the cause of patient death, the rate of increase and severity of metabolic disorders, coagulopathy and multiple organ failure allowed to establish the PGNF diagnosis even intraoperatively without any additional tests. However, the clinical course of severe and irreversible liver graft dysfunction is not always so lightning-fast, and then, glucose metabolism indicators of the graft may be crucial when choosing retransplantation as the only option to save the patient's life.

In two other cases, the postoperative period was uneventful, the function of both grafts was stable and normal, and the level of interstitial glucose, its metabolites, and glycerol was within relatively narrow limits, starting from the first hours after surgery till the end of the study.

The cases presented demonstrate the clinical significance and expediency of prolonged monitoring of liver graft function in the early postoperative period using interstitial microdialysis. The high sensitivity of the method allows to quickly diagnose graft dysfunction, accelerate implementation of diagnostic measures aimed at clarifying the origin of the dysfunction, and to launch therapeutic actions immediately. The question about the need to include microdialysis in the list of routine diagnostic studies in liver transplantation is still open. Apparently, the optimal strategy is to intraoperatively decide to place a catheter if the operating surgeon has reasonable assumptions that graft dysfunction has developed or that there is increased risk of vascular complications.

We consider the use of microdialysis not only in the postoperative period, but also in the work of the donor service to be a promising area for clinical trials. For hypothermic organ preservation by static storage, blood glucose, lactate and pyruvate levels must be stable and close to zero. This would indicate achievement of the preservation goal – stopping metabolic processes in the graft tissue. Glycerol level and dynamics would allow to objectively assess the severity of ischemic injury. Together with other data, this would allow to reasonably use it for transplantation from expanded criteria donors or to abandon transplantation due to high risk of developing PGNF.

CONCLUSION

Microdialysis allows for continuous monitoring of the viability and functional state of the transplanted liver in the early post-transplant period. The high sensitivity of this method makes it possible to accelerate diagnosis of vascular complications (particularly thrombosis) or graft dysfunction of other origins. Clarification of the boundary values of interstitial levels of glucose, lactate, pyruvate and glycerol in various conditions and complications, as well as application of the method at the preservation stage, should be subjected to future research.

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The authors declare no conflict of interest.

REFERENCES

1. *Chen XB, Xu MQ.* Primary graft dysfunction after liver transplantation. *Hepatobiliary Pancreat Dis Int*. 2014 Apr; 13 (2): 125–137. Review. PubMed PMID: 24686540.
2. *Stockmann M, Lock JF, Malinowski M, Seehofer D, Puhl G, Pratschke J, Neuhaus P.* How to define initial poor graft function after liver transplantation? – a new functional definition by the LiMAx test. *Transpl Int*. 2010 Oct; 23 (10): 1023–1032. doi: 10.1111/j.1432-2277.2010.01089.x. PubMed PMID: 20444241.
3. *Lock JF, Schwabauer E, Martus P, Videv N, Pratschke J, Malinowski M et al.* Early diagnosis of primary nonfunction and indication for reoperation after liver transplantation. *Liver Transpl*. 2010 Feb; 16 (2): 172–180. doi: 10.1002/lt.21973. PubMed PMID: 20104485.
4. *Levesque E, Saliba F, Benhamida S, Ichaï P, Azoulay D, Adam R et al.* Plasma disappearance rate of indocyanine green: a tool to evaluate early graft outcome after liver transplantation. *Liver Transpl*. 2009 Oct; 15 (10): 1358–1364. doi: 10.1002/lt.21805. PubMed PMID: 19790157.
5. *De Gasperi A, Mazza E, Prosperi M.* Indocyanine green kinetics to assess liver function: Ready for a clinical dynamic assessment in major liver surgery? *World J Hepatol*. 2016 Mar 8; 8 (7): 355–367. doi: 10.4254/wjh.v8.i7.355. Review. PubMed PMID: 26981173; PubMed Central PMCID: PMC4779164.
6. *Olthoff KM, Kulik L, Samstein B, Kaminski M, Abecassis M, Emond J et al.* Validation of a current definition of early allograft dysfunction in liver transplant recipients and analysis of risk factors. *Liver Transpl*. 2010 Aug; 16 (8): 943–949. doi: 10.1002/lt.22091. PubMed PMID: 20677285.
7. United Network For Organ Sharing. United Network for Organ Sharing Liver Disease Severity Score Committee. 2014. www.unos.org.
8. *Feng S, Goodrich NP, Bragg-Gresham JL, Dykstra DM, Punch JD, DebRoy MA et al.* Characteristics associated

with liver graft failure: the concept of a donor risk index. *Am J Transplant*. 2006 Apr; 6 (4): 783–790. PubMed PMID: 16539636.

9. *Nowak G, Ungerstedt J, Wernerman J, Ungerstedt U, Ericzon BG.* Clinical experience in continuous graft monitoring with microdialysis early after liver transplantation. *Br J Surg.* 2002 Sep; 89 (9): 1169–1175. PubMed PMID: 12190684.
10. *Silva MA, Murphy N, Richards DA, Wigmore SJ, Bramhall SR, Buckels JA et al.* Interstitial lactic acidosis in the graft during organ harvest, cold storage, and reperfusion of human liver allografts predicts subsequent ischemia reperfusion injury. *Transplantation*. 2006 Jul 27; 82 (2): 227–233. PubMed PMID: 16858286.
11. *Haugaa H, Thorgersen EB, Pharo A, Boberg KM, Foss A, Line PD et al.* Early bedside detection of ischemia and rejection in liver transplants by microdialysis. *Liver Transpl*. 2012 Jul; 18 (7): 839–849. doi: 10.1002/lt.23425. PubMed PMID: 22407878.
12. *Haugaa H, Almaas R, Thorgersen EB, Foss A, Line PD, Sanengen T et al.* Clinical experience with microdialysis catheters in pediatric liver transplants. *Liver Transpl*. 2013 Mar; 19 (3): 305–314. doi: 10.1002/lt.23578. PubMed PMID: 23193034.
13. *Yao FY, Ferrell L, Bass NM, Watson JJ, Bacchetti P, Venook A et al.* Liver transplantation for hepatocellular carcinoma: expansion of the tumor size limits does not adversely impact survival. *Hepatology*. 2001 Jun; 33 (6): 1394–1403. PubMed PMID: 11391528.
14. *Hammarlund-Udenaes M.* Microdialysis as an Important Technique in Systems Pharmacology – a Historical and Methodological Review. *AAPS J*. 2017 Sep; 19 (5): 1294–1303. doi: 10.1208/s12248-017-0108-2. Epub 2017 Jul 31. Review. PubMed PMID: 28762127.
15. *Zeiler FA, Thelin EP, Helmy A, Czosnyka M, Hutchinson PJA, Menon DK.* A systematic review of cerebral microdialysis and outcomes in TBI: relationships to patient functional outcome, neurophysiologic measures, and tissue outcome. *Acta Neurochir* (Wien). 2017 Dec; 159 (12): 2245–2273. doi: 10.1007/s00701-017-3338-2.
16. *Nowak G, Ungerstedt J, Wernerman J, Ungerstedt U, Ericzon BG.* Metabolic changes in the liver graft monitored continuously with microdialysis during liver transplantation in a pig model. *Liver Transpl*. 2002 May; 8 (5): 424–432.
17. *Gillispie A, Rooyackers O, Wernerman J, Nowak G.* Effect of extended cold ischemia time on glucose metabolism in liver grafts: experimental study in pigs. *J Hepatobiliary Pancreat Surg*. 2007; 14 (2): 183–188.
18. *Keller AK, Jorgensen TM, Ravlo K, Nielsen TK, Olsen LH, Stolle LB.* Microdialysis for detection of renal ischemia after experimental renal transplantation. *J Urol*. 2009 Oct; 182 (4 Suppl): 1854–1859. doi: 10.1016/j.juro.2009.03.015
19. *Fonouni H, Esmaeilzadeh M, Jarahian P, Rad MT, Golriz M, Faridar A et al.* Early detection of metabolic changes using microdialysis during and after experimental kidney transplantation in a porcine model. *Surg Innov*. 2011 Dec; 18 (4): 321–328. doi: 10.1177/1553350610392063.
20. *Khubutia MSh, Zhuravel SV, Kozlov IA, Romanov AA, Goncharova II.* Microdialysis – a new method of monitoring of the transplanted cadaveric kidneys function. *Anesteziol Reanimatol*. 2015 Jan-Feb; 60 (1): 69–72. [In Russ].
21. *Bekker J, Ploem S, de Jong KP.* Early hepatic artery thrombosis after liver transplantation: a systematic review of the incidence, outcome and risk factors. *Am J Transplant*. 2009 Apr; 9 (4): 746–757. doi: 10.1111/j.1600-6143.2008.02541.x. Epub 2009 Mar 2. Review. PubMed PMID: 19298450.

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NUMERICAL ANALYSIS OF THE EFFECT OF THE DESIGN OF AXIAL-FLOW PUMP CANNULA TIP ON STAGNATION AND RECIRCULATION ZONES IN THE LEFT VENTRICLE

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Objective: to analyze the inflow cannula of an implantable axial-flow blood pump for a long-term left ventricular assist system in order to minimize thromboembolic complications. **Materials and methods.** Hemodynamics was considered for 4 different designs of the inflow cannula, from 0 mm to 25 mm long. Areas at the base of the cannula received the most attention. Analysis was performed using the *OpenFOAM* software. **Results.** It was revealed that sizes of stagnation and recirculation zones directly depended on the length of the cannula when placed in the left ventricle. Accordingly, longer cannula increases the risk of thrombosis. **Conclusion.** The design of an inflow cannula determines the likelihood of thrombosis in the cannula. Longer inflow cannula increases stagnation and recirculation zones. This provides a basis for a search for other possible modifications.

Keywords: end-stage heart failure, artificial circulatory support, left ventricular bypass, inflow cannula, thromboembolic complications.

INTRODUCTION

Cardiovascular diseases (CVDs) are one of the leading causes of death in developed countries. According to various sources, over 1 million people die annually from CVDs in Russia and in the USA. Most heart diseases are amenable to drug treatment, but in end-stage heart failure (ESHF), heart transplantation is the only way out. However, organ shortages limit treatment options for such patients. In recent decades, this has led to the appearance of a new technology for treating patients with heart failure by means of long-term mechanical circulatory support (MCS) using implantable centrifugal axial-flow rotary blood pumps. Despite successful application of such systems in clinical practice, thrombolytic complications remain one of the main problems, which are a consequence of the combined effect of shear stress, as well as formation of stagnation and blood recirculation zones. In this case, the area of the inflow cannula is the most critical site of thrombus formation [1–4]. The causes of blood clots are not fully known. However, several factors that affect ventricular hemodynamics and the safety of the cannula can be identified:

1) Position of the cannula relative to the heart, including its tilt angle [2, 5–7];
2) Shape and size of the cannula [8–11];
3) Coating of the cannula surface [1, 12].

In this paper, the influence of the tip length of an inflow cannula on formation of stagnation and recirculation zones is investigated via numerical analysis.

MATERIALS AND METHODS

Direct-type blood intake cannulas are used in most foreign-made long-term MCS systems which incorporate the rotary assisted circulatory support (HeartMate II, HeartMate III, Jarvik 2000), as well as in the Russian-made assisted circulatory support (ACS) AVK-N. Fig. 1 shows the AVK-N cannula (Russia).

This design is fairly simple, symmetrical, and performs its function well. However, the clinical experience of using this cannula has shown a high probability of thrombosis at the pump inlet (Fig. 2) [1, 13].

Our preliminary analysis of relevant literature showed that the imperfect design of the cannula in terms of local hemodynamics can lead to negative consequences:

1) obstruction of blood flow due to thrombocytic masses and connective tissue [14];
2) suction of the cannula lumen to the ventricular wall [15];
3) formation of blood clots at the site of the cannula's contact with the myocardium [16].

In this work, we conducted studies to assess the effect of the length of the inflow cannula of an axial-flow blood pump on intraventricular hemodynamics. Fig. 3 shows the models of cannulas studied in this work.

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Fig 1. AVK-N input cannula

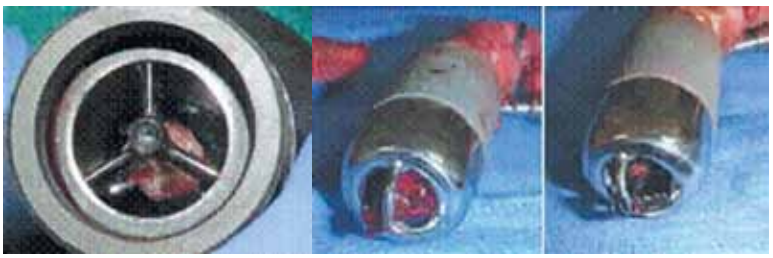

Fig 2. Pump inlet thrombosis

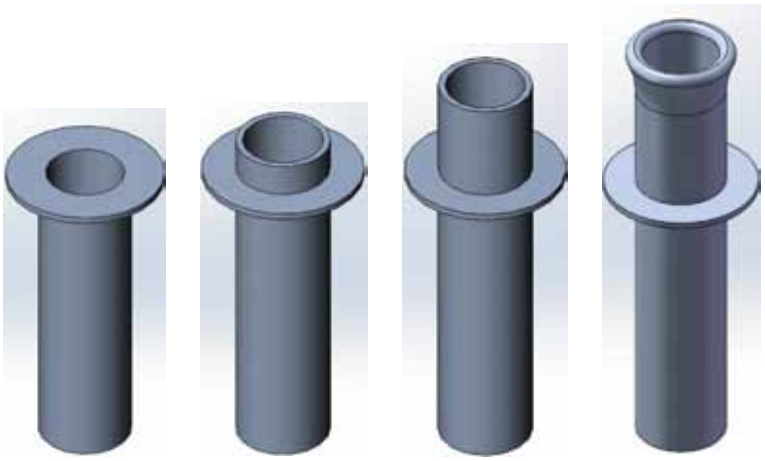

Fig 3. Cannulas with different tip length (from left to right) 0 mm, 5 mm, 15 mm and 25 mm AVK-N

Blood flow through the left ventricle (LV) with a cannula placed inside it is simulated in the absence of wall movement, in ESHF conditions, when the LV is practically not reduced, and the aortic valve is closed.

The LV geometry is shown in Fig. 4. Each cannula model is placed in the LV, forming the required volume for numerical simulation. The presence and size of stagnation and recirculation zones for different cannula lengths in the LV cavity were investigated. Zero blood flow speed and closed trajectories are the criteria for such zones.

Simulation is done using the OpenFOAM software. Blood parameters are set by the Navier-Stokes equation for a viscous liquid (the icoFoam utility included in OpenFOAM). A 120-mmHg pressure is set at the outlet of the model, then the computational process is started until a stationary flow regime is configured.

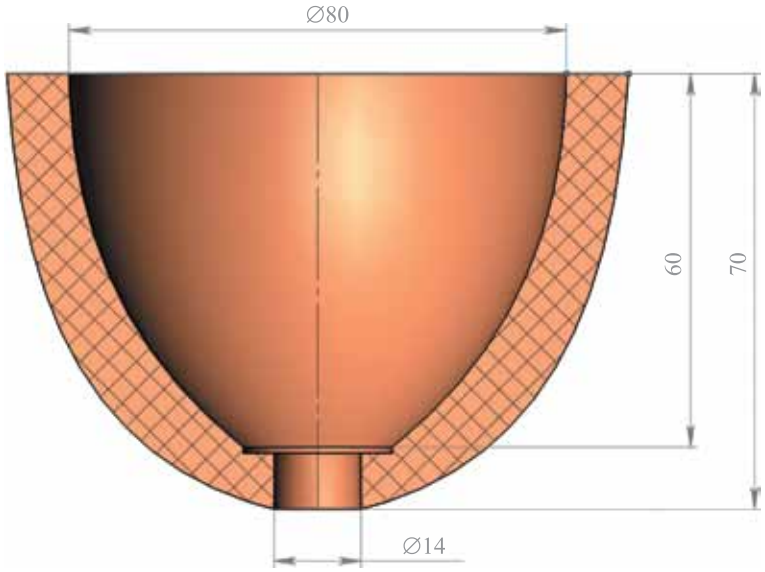

Fig 4. The geometry of the ventricle model

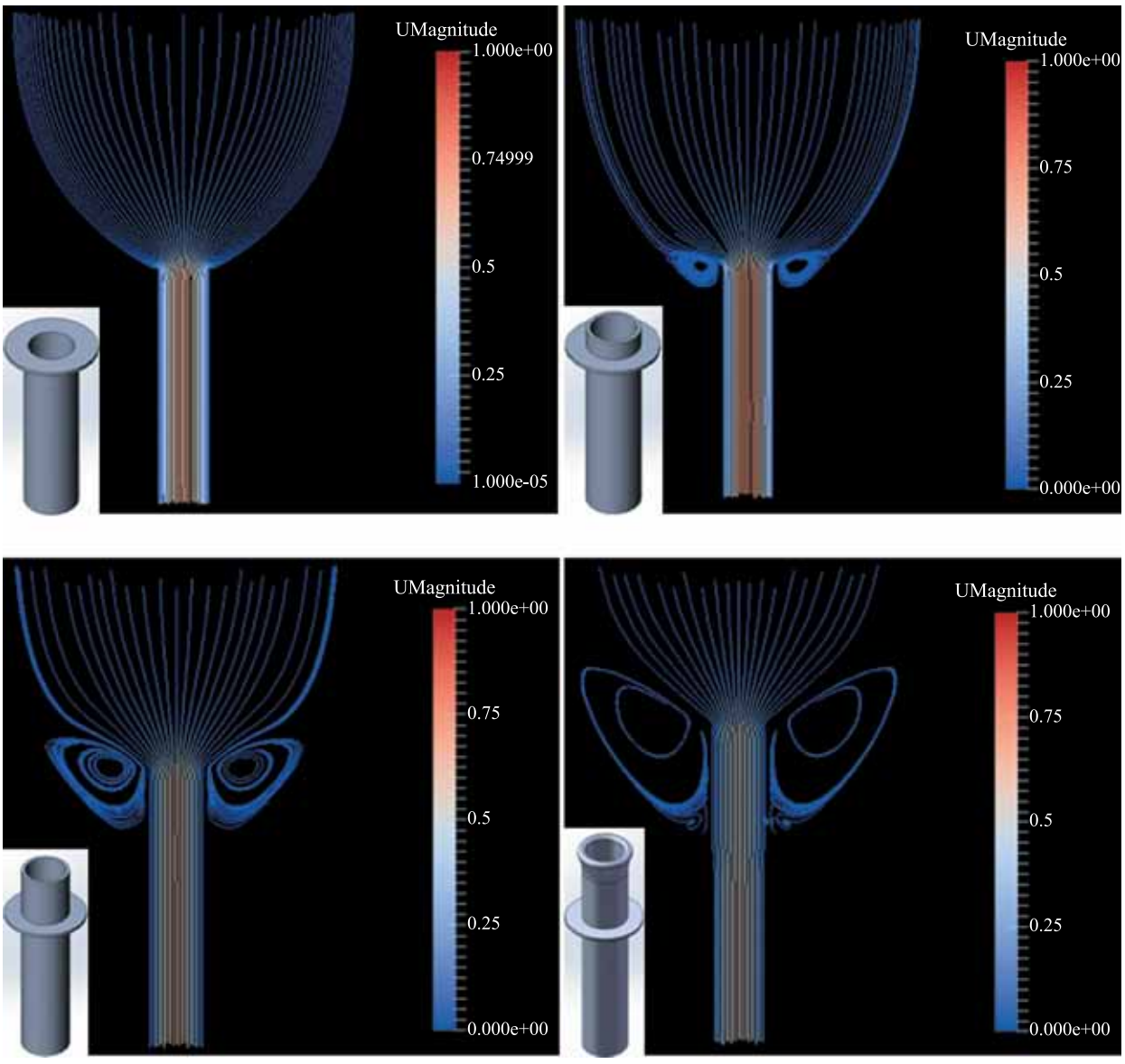

Fig 5. Results of modeling blunt tip cannulas

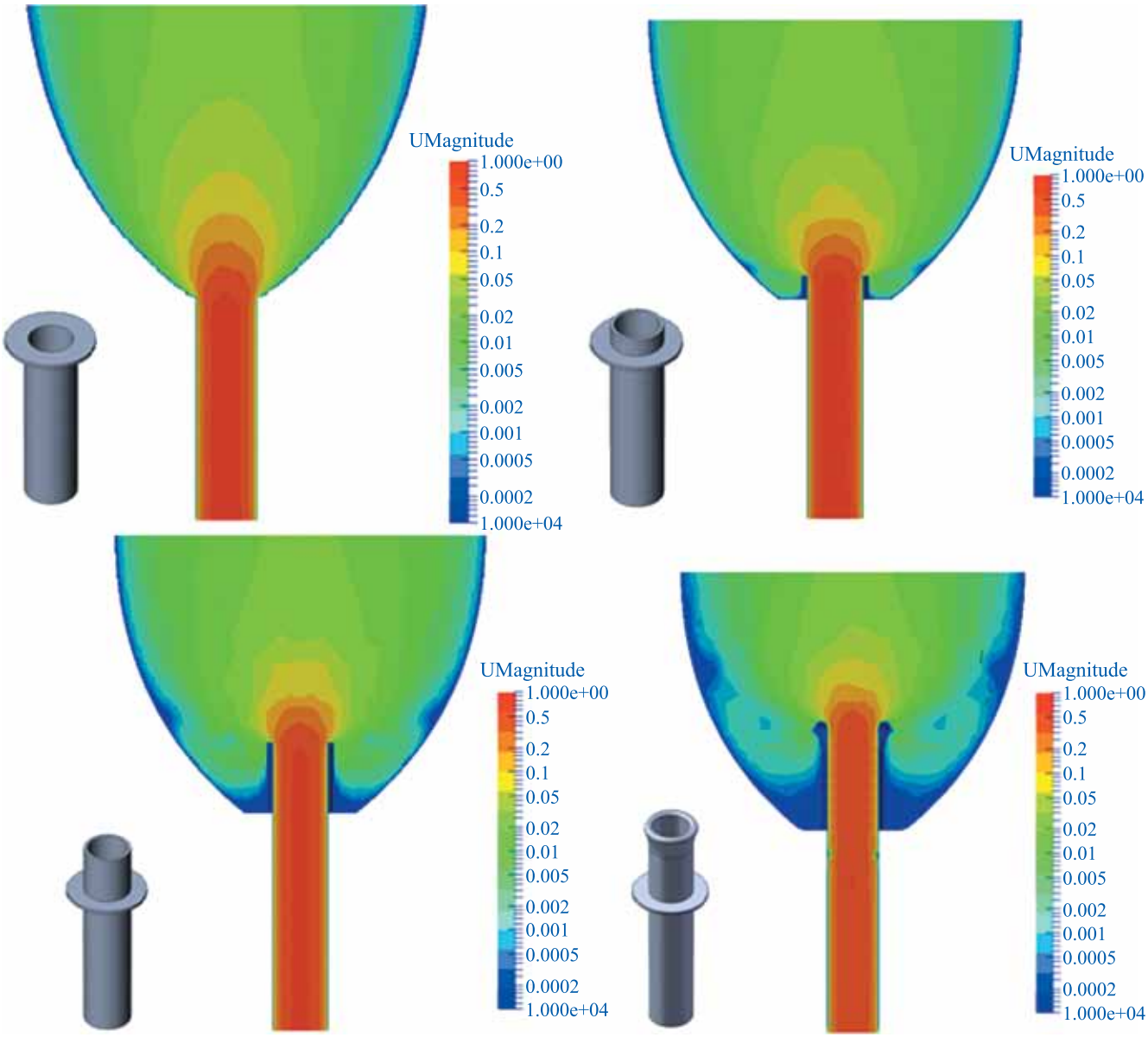

Fig. 6. Results of modeling blunt tip cannulas. Absolute speed

RESULTS

Simulation results for straight cannulas are shown in Fig. 5. The lines in the figure are fluid flow lines. The color corresponds to the local velocity on a scale from 0 m/s (blue) to 1 m/s (red).

There is clear tendency that the size of the recirculation zones increases with longer tip length of the cannula. For a 0 mm-tip length cannula, there is no such recirculation zone, but for a 25 mm-cannula (AVK-N), the zone is maximum. Velocity profiles and contours are shown in Fig. 6. Here you can see a similar relationship with stagnation zones. The more the cannula protrudes, the larger the stagnation zone.

DISCUSSION

The direct cannula with zero tip length allows for minimal stagnation and recirculation zones. In this case, a cannula's tip length other than zero, is the source of appearance of stagnation and recirculation zones inside the LV. The size of these zones is proportional to the tip length. Besides, increased cannula protrusion leads to increased hydraulic resistance of the system. In spite of this, a cannula with zero tip length under typical conditions can become overgrown with connective tissue over time [14]. This process can be blocked by using systems for enhanced pulsatile flow generation [17].

CONCLUSION

Results obtained show that an inflow cannula design has a direct influence on the likelihood of thrombus formation in the cannula. It is clearly obvious that stagnation and recirculation zones tend to be larger with longer cannula tip. This makes it necessary to search for other possible inflow cannula configurations. At the

same time, for a zero-tip-length cannula with very good hemodynamic parameters, implementation of such a design requires further research to minimize the clogging of the cannula lumen with connective tissue.

The authors declare no conflict of interest.

REFERENCES

1. *Arie Blitz*. Pump thrombosis – A riddle wrapped in a mystery inside an enigma. *Ann Cardiothorac Surg*. 2014; 3 (5): 450–471. doi: 10.3978/j.issn.2225-319X.2014.09.10.
2. *Bachman TN, Bhama JK, Verkaik J, Vandenberghe S, Kormos RL, Antaki JF. In vitro* Evaluation of Ventricular Cannulation for Rotodynamic Cardiac Assist Devices. *Cardiovasc Eng Technol*. 2011 Sep; 2 (3): 203–211. doi: 10.1007/s13239-011-0050-x. PMID: 22563363; PMCID: PMC3343032.
3. *Frazier OH, Forrester MD, Gemmato C, Gregoric ID.* Urgent pump exchange for stroke resulting from a distorted HeartMate XVE inflow conduit. *J Heart Lung Transplant*. 2007 Jun; 26 (6): 646–648. doi: 10.1016/j.healun.2007.03.012. Epub 2007 Apr 16. PMID: 17543792.
4. *Uriel N, Han J, Morrison KA, Nahumi N, Yuzefpolskaya M, Garan AR et al.* Device thrombosis in HeartMate II continuous-flow left ventricular assist devices: a multifactorial phenomenon. *J Heart Lung Transplant*. 2014 Jan; 33 (1): 51–59. doi: 10.1016/j.healun.2013.10.005. Epub 2013 Nov 28. PMID: 24290832.
5. *Imamura T, Adatya S, Chung B, Nguyen A, Rodgers D, Sayer G et al.* Cannula and Pump Positions Are Associated With Left Ventricular Unloading and Clinical Outcome in Patients With HeartWare Left Ventricular Assist Device. *J Card Fail*. 2018 Mar; 24 (3): 159–166. doi: 10.1016/j.cardfail.2017.09.013. Epub 2017 Oct 2. PMID: 28982636; PMCID: PMC5856593.
6. *Imamura T, Nguyen A, Chung B, Rodgers D, Sarswat N, Kim G et al.*Association of Inflow Cannula Position with Left Ventricular Unloading and Clinical Outcomes in Patients with HeartMate II Left Ventricular Assist Device. *ASAIO J*. 2019 May/Jun; 65 (4): 331–335. doi: 10.1097/MAT.0000000000000823. PMID: 29933250; PMCID: PMC6342671.
7. *Shiose A, Kim HI, Takaseya T, Kobayashi M, Fujiki M, Chen JF et al.* Performance of extracorporeally adjustable ventricular assist device inflow cannula. *Ann Thorac Surg*. 2010 Nov; 90 (5): 1682–1687. doi: 10.1016/j.athoracsur.2010.06.081. PMID: 20971290.
8. *Ong C, Dokos S, Chan B, Lim E, Al Abed A, Bin Abu Osman NA et al.* Numerical investigation of the effect of cannula placement on thrombosis. *Theor Biol Med Model*. 2013 May 16; 10: 35. doi: 10.1186/1742-4682-10-35. PMID: 23680359; PMCID: PMC3673905.
9. *Gregory SD, Zwischenberger J, Wang D, Liao S, Slaughter M.* Cannula design. *Mechanical Circulatory and Respiratory Support*. S.D. Gregory, M.C. Stevens, J.F. Fraser (Eds.). London UK: Academic Press, 2017: 567–596. https://doi.org/10.1016/B978-0-12-810491-0.00018-7.
10. *Wong KC, Büsen M, Benzinger C, Gäng R, Bezema M, Greatrex N et al.* Thoughts and Progress. *Artificial Organs*. 2014; 38: 810–817. doi: 10.1111/aor.12369.
11. *Liu G, Zhou J, Hu S, Sun H, Chen H, Zhang Y et al.* Numerical simulation of LVAD inflow cannulas with different tip. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi*. 2013 Feb; 30 (1): 141–148. Chinese. PMID: 23488155.
12. *Yamada Y, Nishinaka T, Mizuno T, Taenaka Y, Tatsumi E, Yamazaki K*. Neointima-inducing inflow cannula with titanium mesh for left ventricular assist device. *J Artif Organs*. 2011 Dec; 14 (4): 269–275. doi: 10.1007/s10047-011-0586-4. Epub 2011 Jul 7. PMID: 21735150.
13. *Saito S, Sakaguchi T, Miyagawa S et al.* Jarvik 2000 biventricular assist device conversion from old pin-shaped bearing pumps to new conical bearing pumps. *J Artif Organs*. 2013; 16: 105–109.
14. *Ootaki Y, Saeed D, Ootaki C, Akiyama M, Fumoto H, Horai T et al.* Development of the DexAide Right Ventricular Assist Device Inflow Cannula. *ASAIO Journal*. 2008 Jan-Feb; 54 (Issue 1): 31–36.
15. *Akira Shiose, Hyun-Il Kim, Tohru Takaseya, Mariko Kobayashi, Masako Fujiki, Ji-Feng Chen et al.* Performance of Extracorporeally Adjustable Ventricular Assist Device Inflow Cannula. *Ann Thorac Surg*. 2010; 90: 1682–1687. doi: 10.1016/j.athoracsur.2010.06.081.
16. *Chitaru Kurihara, Minoru Ono, Takashi Nishimura, Aya Saito, Tsuyoshi Taketani, Motoyuki Hisagi et al.* Use of DuraHeart support for more than 1 year as the first successful bridge to heart transplantation in Japan. *J Artif Organs*. 2011; 14: 67–69. doi: 10.1007/s10047-010-0524-x.
17. *Buchnev AS, Kuleshov AP, Drobyshev AA, Itkin GP.* Hemodynamic evaluation of a new pulsatile flow generation method in cardiopulmonary bypass system. *Russian Journal of Transplantology and Artificial Organs*. 2019; 21 (3): 69–75. [In Russ, English abstract]. https://doi.org/10.15825/1995-1191-2019-3-69-75.

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FIRST EXPERIENCE IN IMPLANTATION OF A MECHANICAL CIRCULATORY SUPPORT DEVICE BASED ON A DISK-TYPE PUMP: AN ACUTE EXPERIMENT

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Objective: to carry out the implantation of an artificial left ventricle of the heart based on a disk-type pump in an acute experiment on a large mammal (mini-pig). **Materials and methods.** To test the surgical technique of implantation and assess the biocompatibility of the apparatus for mechanical support of blood circulation based on a viscous friction pump, an acute experiment was conducted on an animal. A large mammal (mini-pig weighing 90 kg) was used as an experimental model. The implantation of the pump was performed extracorporeally according to the scheme "the apex of the left ventricle – the descending thoracic aorta". During the experiment, invasive blood pressure, central venous pressure, cardiac arrhythmias, body temperature, blood gas composition, activated coagulation time were monitored. Under the control of transesophageal echocardiography, the pump operation mode was set with parameters – speed 2400–2600, productivity 4 ± 0.5 l/min, average IAD – 70–80 mm Hg. **Results.** In the course of the experiment, the fundamental possibility of using the developed disk-type pump as a device for supporting blood circulation was proved. For 4 hours, the pump provided adequate hemodynamic parameters with an average productivity of 4 ± 0.5 l/min and 2500 rpm. After 4 hours of operation of the pump in the conditions of inactivated heparin (AST – 114 sec), no blood clots were found between the pump disks. **Conclusion** The hemodynamics feature of the disk pump allows you to develop sufficient performance parameters to ensure adequate blood circulation. The mechanism of action of the "boundary layer" minimizes the risk of blood clots in the pump cavity. However, the topographic and anatomical features of the pig's body do not allow experiments with a long observation period.

Keywords: heart failure, mechanical support of the heart, Tesla disk pump, left ventricular bypass system.

INTRODUCTION

Over the past decade, the use of circulatory assist devices in clinical practice has increased significantly [1]. According to the 25th report of the International Society for Heart and Lung Transplantation in 2008, every third heart transplant was performed in the second stage after implantation of the left ventricular assist device (LVAD) [2]. Already in 2014, however, half of the world's heart transplants were performed against the background of previously implanted LVADs [3]. At the same time, in recent years, the need for LVAD has sharply increased with the growing number of the patients with implanted MCSs as the final method of treating end-stage CHF [4]. That is why domestic research in the development and implementation of circulatory support systems is the most relevant and highly demanded.

Earlier bench tests of the hemolytic properties of disk pumps have shown good outcomes, suggesting the basic possibility and high safety of implanting a living organism with a MCS based on a viscous friction pump [5].

MATERIALS AND METHODS

To test the surgical technique of implantation and preliminarily assess the biocompatibility of the viscous friction pump MCS, an acute animal experiment was performed. A large mammal (90 kg mini-pig) was used as an experimental model. The niпpe before the experiment, the animal was deprived of food with unlimited access to water. The animal was premedicated in a vivarium with i.m. atropine / Zoletil solution dosaged for weight and height. With the animal asleep, the surgical site was prepared. The experiment was carried out under endotracheal anesthesia with sevoflurane and muscle relaxation (pipecuronium bromide).

During the experiment, invasive blood pressure (IBP) by catheterization of the right carotid artery, central venous pressure (CVP) by catheterization of the right jugular vein, cardiac arrhythmias (electrocardiography), body temperature, blood gas composition, and activated coagulation time (ACT) were monitored. To correct hy-

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povolemic disorders, infusion therapy with crystalloid and colloid solutions was performed.

Preparing the pump for implantation

On a sterile table, $^1/_2$-inch lines were fixed to the pump inlet and outlet. Then the pump was filled with saline at low speed (500–1000 rpm), carefully removing air emboli (Fig. 1).

Main stage of implantation

The animal was placed in right lateral decubitus. Anterolateral thoracotomy was performed in the VI intercostal space from the left with partial subperiosteal resection of the 6th rib. The LVAD lines were passed through the formed subcutaneous canals paravertebrally. After systemic heparinization (3 mg/kg) and lateral compression of the thoracic aorta, an end-to-side anastomosis was formed between the 12 mm Dacron vascular graft and the descending thoracic aorta with 5/0 thread. The outflow line was connected to the pump outlet. The inflow cannula was implanted on the beating heart through the avascular zone of the left ventricular apex. The inflow line was connected to the corresponding pump connection. Under the control of transesophageal echocardiography, the pump operation mode was set at 2,400–2,600 rpm, 4 ± 0.5 l/min flow rate, 70–80 mm Hg average IBP (Fig. 2).

Fig. 1. Stage of preparation (refueling) of the pump

Initial bench tests of the hemolytic properties of the disc pump with human blood have shown a low level of hemolysis [4].

Besides finalizing the surgical technique of implanting a disk-type pump MCS, the experiment was aimed at investigating the thrombogenicity of the inner surface of the pump and its moving parts. For this, the pump was operated in tough conditions. In 30 minutes

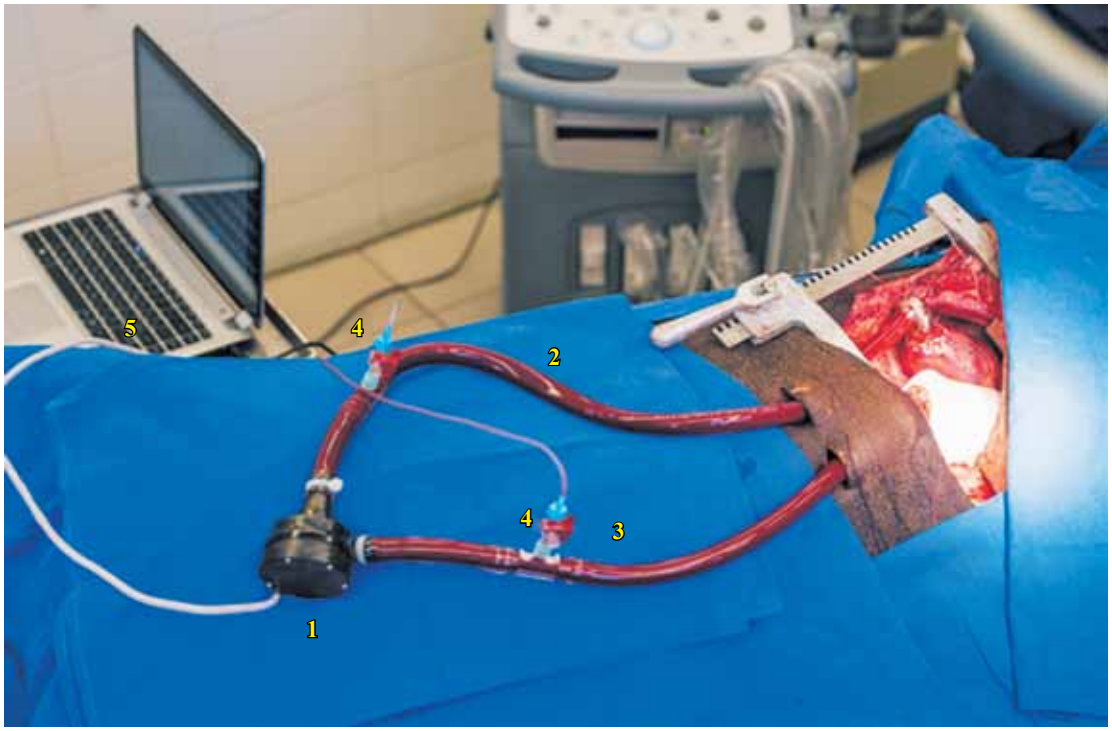

Fig. 2. General view of the wound and the working LVAD: 1 – pump; 2 – supply line; 3 – outlet line; 4 – port for measuring pressure; 5 – control unit

after reaching the calculated parameters, heparin was completely inactivated (ACT – 114 s). The pump has been working without interruptions for 4 hours with the absence of pulse peaks on sphygmogram (mean IBP 70–80 mm Hg), and blood gas parameters showed the adequacy of systemic perfusion. Every 30 minutes, blood was sampled to determine free hemoglobin level and gas composition (Table).

Table

Indicators of the main parameters of homeostasis during the experiment

Parameters	Time, min							
	30	60	90	120	150	180	210	240
IBP, mm Hg.	90	100	110	115	105	100	95	105
HR	76	86	83	78	80	86	89	85
SpO_2, %	98	97	98	97	95	98	98	98
Free Hb, mg/%	1.5	2.0	2.1	2.0	2.3	2.2	2.2	2.0
pH	7.4	7.5	7.4	7.4	7.4	7.4	7.4	7.5
cLac, mmol/l	2.0	2.5	3.0	2.0	3.5	2.0	3.0	3.0

After 4 hours, the animal was euthanized in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, March 18, 1986), and the pump was explanted. After dismantling the pump casing and visual assessment, a white, organized and tightly fixed thrombus was found on the rotor base; however, no blood clots were found between the discs suggesting a sufficiently high biocompatibility of the viscous friction pump with respect to the animal's cardiovascular system.

DISCUSSION

The first experiment on the implantation of a disk pump has resulted in the approved implantation protocol considering the topographic and anatomical features of the animal's body (mini-pig), thus promoting a series of future experiments to study the long-term operation of LVAD based on a viscous friction pump. The principal possibility of a disk-type pump to replace the function of the left heart compartment has been proved. The results of blood biochemical and gas analysis confirm that the operating parameters of the pump (2,600 rpm, 4 ± 0.5 l/min productivity) allow for adequate perfusion support of the organism. At the first implantation of a disc pump as an LVAD in an acute experiment, the first assumptions were made about a high degree of biocompatibility of the coating of the inner surface of the pump housing and discs. After 4 hours of pump operation in inactivated heparin (ACT – 114 s), no blood clots were found between the pump discs. This allows us to assume the possibility of implementing the most sparing scheme of anticoagulant therapy after LVAD implantation based on a disk-type pump and significantly reduce the risk of serious adverse events.

The authors declare no conflict of interest.

REFERENCES

1. *Halilulin TA i dr.* Osobennosti implantacii sistemy podderzhki nasosnoj funkcii serdca AVK-N v kachestve "mosta" k transplantacii serdca. *Vestnik transplantologii i iskusstvennyh organov*. 2018; 20 (1): 13–22.
2. *Nangia G, Borges K, Reddy KR.* Use of HCV-infected organs in solid organ transplantation: An ethical challenge but plausible option. *Journal of viral hepatitis*. 2019.
3. *Koloskova N.N.* Terminal'naya serdechnaya nedostatochnost'. Otbor pacientov dlya postanovki v "list ozhidaniya" na transplantaciyu serdca / 14.01.05. – kardiologiya // 14.01.26. – serdechno-sosudistaya hirurgiya. M., 2011.
4. *Lund LH et al.* The registry of the International Society for Heart and Lung Transplantation: thirty-third adult heart transplantation report – 2016; focus theme: primary diagnostic indications for transplant. *The Journal of Heart and Lung Transplantation*. 2016; 35 (10): 1158–1169.
5. *Medvedev AE et al.* Implanted system of mechanical support of the disk-based heart pump viscous friction. *AIP Conference Proceedings*. AIP Publishing, 2018; 2027 (1): 030149.

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HYDRODYNAMIC PERFORMANCE OF A NOVEL SUTURELESSPROSTHETIC AORTIC VALVE

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The aim of the study was an in vitro hydrodynamic study of the developed prosthetic heart valve of the second generation, designed to carry out an implantation using "valve-in-valve" method. **Material and methods.** Prototypes of the developed prosthesis were studied under simulated physiological conditions of the heart using a Vivitro Labs pulse duplicator (Canada) in a comparative aspect with "UniLine" clinical commercial aortic valve bioprosthesis (Russia). Samples were tested by simulating sutureless implantation procedure. **Results.** The developed valves showed satisfactory hydrodynamic characteristics – for all cases of "implantation" from the position of the average trans-prosthetic gradient (6.1–11.1 mm Hg) and the effective orifice area (1.60–1.81 cm^2). The analysis of the regurgitation fraction allowed us to determine the optimal sizes for implantation using "valve-in-valve" method, which subsequently will form the basis of sizing guidelines for size selection. A qualitative analysis of the leaflet's work demonstrated the presence of slight asymmetry for a number of prostheses – in case of mismatch of sizes when simulating "valve-in-valve" procedure. **Conclusion.** The tests demonstrate the viability of the developed design from the standpoint of hydrodynamic efficiency and determines the basic rules of selecting a prosthesis for reimplantation relative to the primary valve.

Keywords: hydrodynamic efficiency, aortic valve prosthesis, trans-prosthetic gradient, effective orifice area.

INTRODUCTION

In Russia, the number of procedures for implanting heart valves bioprostheses is increasing, exceeding 2000 units/year [1]. However, in contrast to the existing advantages of bioprostheses associated with more sparing antithrombotic therapy, there is a need for repeated interventions resulting from the dysfunction development. It has been shown that the period of freedom from reoperation is 7.8 years on average [2–4]. The Valve-in-Valve Registry reports the global results of clinical use of bioprostheses of higher duration, i.e. 9 years [5, 6]. However, in general, the period can be considered short compared to mechanical prostheses. At the same time, it was shown that re-intervention is associated with a higher complications risk, and most importantly, increased mortality of up to 11.5% [1, 7, 8], due to the volume and duration due to the need to remove the dysfunction prosthesis and its subsequent replacement. to a "new" one by reprosthetics. Such aspects may limit the scope of the bioprostheses use due to some degree of leveling the advantages of valves based on biological materials. Considering the annual growth in the number of heart valve replacement surgery, the search for solutions to the problems of repeated interventions remains an urgent research task in the field of cardiac surgery in terms of the development of new constructions. A possible solution could be transcatheter prostheses, the experience of which with prosthesis-to-prosthesis implantation demonstrates satisfactory clinical results [9]. However, such specific limitations as higher cost, required qualification of the operating team and its equipment, the impossibility of direct access to the implantation site for excision of calcifications, as well as specific complications [10] do not allow this technology to enter the routine practice of heart valve replacement [11].

The Research Institute for Complex Issues of Cardiovascular Diseases develops a minimally invasive sutureless heart valve prosthesis intended for repeated interventions and installed as a "prosthesis to prosthesis" [12]. In this approach, there is no need for complete removal of the prosthesis with developed dysfunction and reapplication of fixing sutures on the "new" one, which allows to reduce the volume of the surgical wound in the area of the aortic root and the time of its clamping. On the other hand, open access to the operating site provides the partial excision of affected tissue with massive calcification and / or pannus. The main functional feature of the developed heart valve prosthesis from the point of view of its efficiency, safety and, ultimately, long-term results of reprosthetics lies in the hydrodynamic parameters of the structure [13]. Besides, the peculiarity of prosthesis-to-prosthesis implantation creates constructive stenosis, i.e. a deliberate decrease in the geometric area of the orifice due to the "new frame + old frame" design imposes increased requirements on hydrodynamics (Fig. 1, e). Considering the factors described above, the development focused on this very characteristic, the assessment of the hydrodynamic characteristics of the

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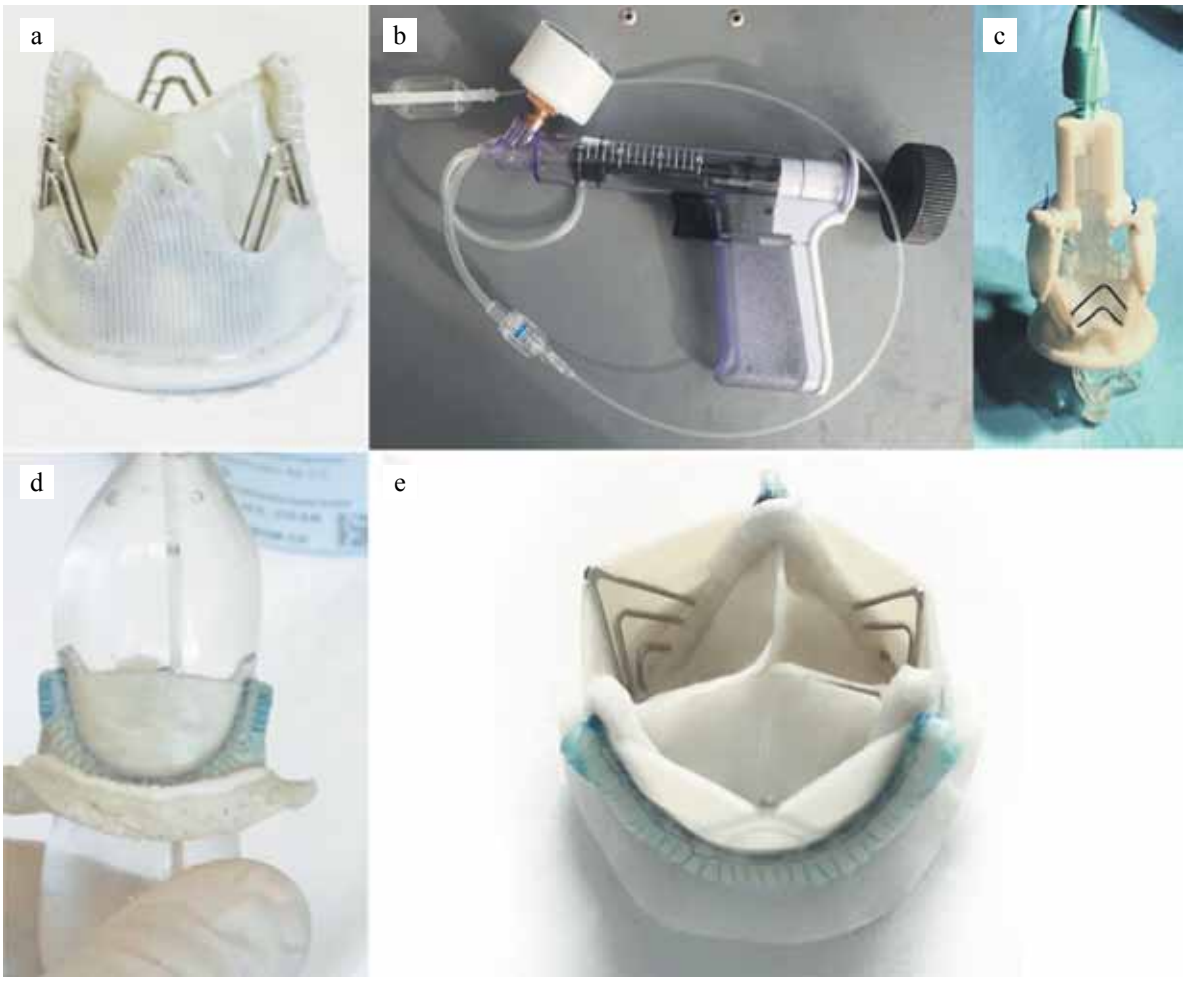

Fig. 1. Material and methods: a – design of the developed prosthesis; b – a delivery system comprising a balloon catheter and a high pressure syringe-defuser; c – re-implantation system with holder; d – the result of "valve-in-valve" implantation immediately after balloon dilation; e – the same, in the final state before the hydrodynamic study

experimental prosthesis under conditions of simulation of the prosthesis-to-prosthesis procedure.

MATERIALS AND METHODS

Prosthesis

From the point of design, the prosthesis under development is a mesh stent-like structure made of stainless steel, where the main components are mounted, i.e. a synthetic casing and a biological xenopericardial valve device stabilized with ethylene glycol diglycidyl ether with additional anticalcium treatment. The design of the supporting frame based on bar elements (stent) allows changing the outer diameter from preimplantation (15 mm) to target (17–21 mm) depending on the diameter of the prosthesis for reoperation. The use of medical grade stainless steel (AISI 316LVM) provides satisfactory biocompatibility even for "bare" elements of the support frame and maintains the final geometry at the target site [14]. Outside, the prosthesis is covered with a synthetic covering which turns into a single-row cuff made of a similar material. Both components ensure the sealing of the contact point of the two prostheses to reduce the risk of paraprosthetic fistula (Fig. 1, a). The device is implanted without sutures using balloon technology into the supporting frame of a failed heart valve prosthesis of the "prosthesis-to-prosthesis" type by connecting a high-pressure syringe to the catheter (Fig. 1, b).

Study methods

Considering the prosthesis is intended for prosthesis-to-prosthesis implantation, the study of its hydrodynamic efficiency was performed in two successive stages.

1. Study of the hydrodynamic parameters of the original "primary" prosthesis. For this, 3 clinical frame bioprostheses UniLine (CJSC NeoCor, Russia) were used, of 21, 23 and 25 standard sizes (TP), intended for clinical use in the aortic position. The prostheses consist of a rigid polymer support frame with mounted cusp and sheathing of biological material – cattle xenopericardium preserved with diglycidyl ether of ethylene glycol (DEE). The prosthesis involves suture installation; therefore, a biological sewing cuff is located at the base of the supporting frame (Fig. 1, b).

2. Study of the prosthesis under development. At this stage, the studied prosthesis of the corresponding diameter was "implanted" into the UniLine bioprostheses studied at the previous stage. For "implantation", a valvuloplasty balloon of our own design was used at a pressure of 4 atm with a syringe indeflator (Fig. 1, b). For each standard size of the original UniLine prosthesis, two variants of the studied prostheses were used, conventionally designated as -2TP and TP3. The stent-like mesh frame does not have a final standard size due to the design features; however, the valve device mounted on it provides tight locking only for a certain diameter of the "new" prosthesis. Thus, the prototypes of prostheses were created with two variants of the folding device for each UniLine standard size (21, 23, 25). The current stage of the study has made it possible to evaluate the most suitable standard size for implantation.

Hydrodynamic parameters were assessed with the Vivitro Labs (Canada) pulsating flow device at simulation of the heart physiological mode for 10 cycles in a steady state:

a) "beats" of the chamber simulating ventricle – 70 bpm;
b) pressure in the chamber simulating aorta – 120/80 mm Hg;
c) mean pressure in the chamber simulating aorta – 100 mm Hg;
d) minute volume – 5 l;
e) duration of systole – 35% cycle.

In the study, we assessed:

a) mean transprosthestic gradient as averaged over 10 work cycles the pressure difference "before" and "after" the bioprosthesis, measured using appropriate sensors in the chambers simulating the ventricle and aorta;
b) effective orifice area as the passage orifice area obtained from pressure and flow data by the formula (1):

$$EOA = 1{,}94 \sqrt{\frac{\int_{t_2}^{t_1} (q(t))^2 dt}{\int_{t_2}^{t_1} \Delta p(t) dt}} \quad (1)$$

where $q(t)$ – volume flow, l/s; $\Delta p(t)$ – transprosthestic gradient, mm Hg; t_1 – direct flow start time, s; t_2 – direct flow stop time, s;

c) regurgitation volume as the volume of fluid passing through the valve prosthesis in the opposite direction;
в) additionally, to qualitatively assess the operation of the valve device, video recording of the functioning of the prostheses was performed with the FastVideo-250 high-speed camera (Russia), followed by image analysis for the maximum opening and closure states.

All the described parameters were recorded for UniLine bioprostheses ("Before") and after installation of the developed prosthesis ("After").

RESULTS

Quantitative parameters

The obtained quantitative parameters showed an increase in the average transprosthestic gradient for -3TP relative to the primary one by 11.03–27.32% (Fig. 2). In this case, the maximum growth was noted for the UniLine-23 mm: from 6.83 to 9.40 mm Hg (2.57 increase). On the other hand, for -2TP implantation, the average transprosthestic gradient decreased by 10.64–33.22%. The maximum decrease was observed for the UniLine-25 mm: from 9.47 to 7.11 mm Hg (2.36 decrease).

The effective orifice area (Fig. 2), a parameter featuring the operation of the prosthesis in general, changed insignificantly for all prostheses' combinations: an

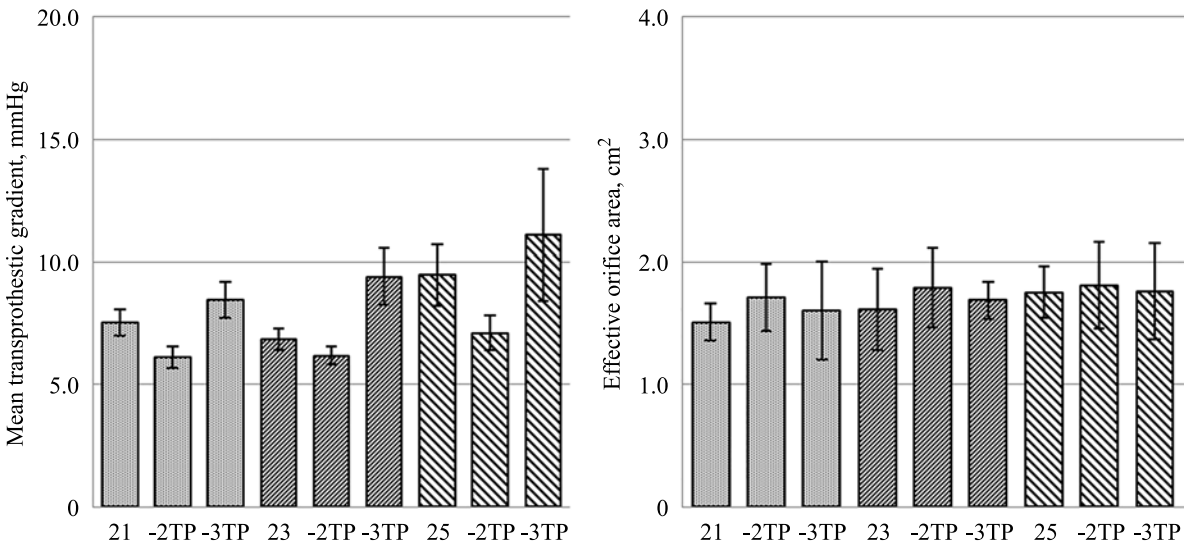

Fig. 2. Quantitative characteristics of the bioprostheses before and after implantation – average gradient and effective orifice area, grouped according to the initial standard sizes of "UniLine" prosthesis -21, 23, 25 mm. TP – size

increase by 0.40–11.70% relative to the primary one was recorded.

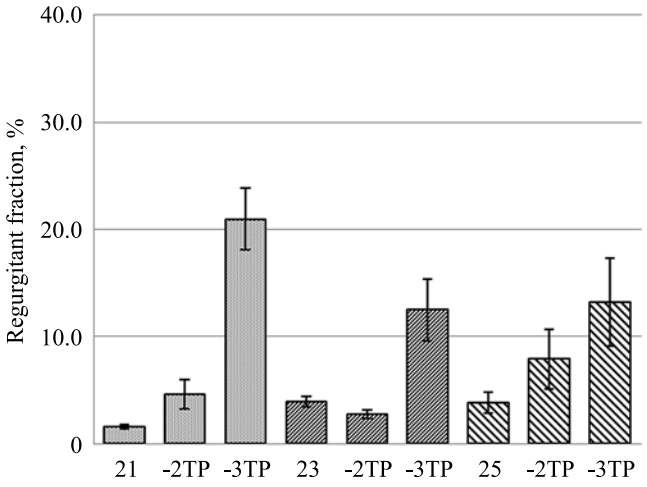

Fig. 3. The results of the assessment of the regurgitation fraction of the prostheses before and after "valve-in-valve" procedure. TP – size

The most notable parameter that changed after "prosthesis to prosthesis" implantation was the regurgitation fraction (Fig. 3). In all cases, the -3TP implantation option led to significant increase in this parameter in the most negative case (21 mm), 20.95% of the stroke volume accounted for the liquid reflux. The -2TP variant showed the best values of the regurgitation fraction in all cases (Fig. 3).

Qualitative assessment

The qualitative analysis of the prostheses performance before and after implantation showed a symmetrical, uniform opening of the cusp device. It was noted that for the -3TP, the closure state of the valves had greater symmetry and did not have such defects as twisting in the coaptation zone in comparison with the -2TP variant (Fig. 4). It is noteworthy that in the primary "Before" state, the UniLine bioprosthesis cusp has a slight barrel-like effect of the valves in the open state, while this was not observed for the experimental prosthesis.

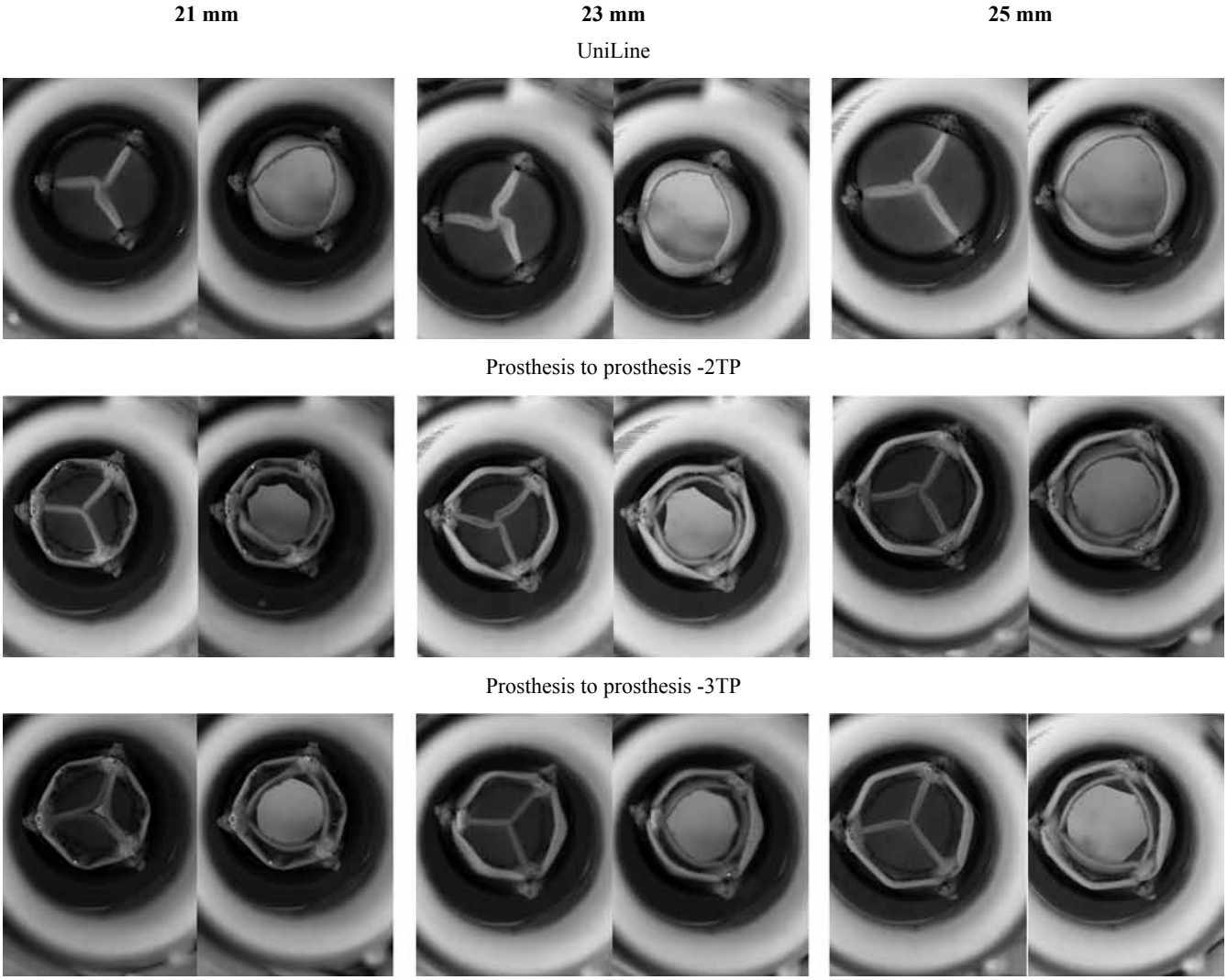

Fig. 4. Comparison of the quality work of the leaflet device before and after the implantation of the prosthesis for closed and open conditions. TP – size

DISCUSSION

The prosthesis under development shoes satisfactory hydrodynamic performance in terms of the transprothestic gradient, effective orifice area and regurgitation fraction in a comparative aspect relative to the original UniLine prostheses. It is noteworthy that with an adequate choice of the standard size of the experimental prosthesis, it is possible to achieve generally insignificant reductions in hydrodynamic efficiency. The supporting frame of the newly installed prosthesis has a nonzero thickness, narrowing the geometrical opening; at the same time, the higher efficiency of the experimental cusp device does not allow the flow parameters to change significantly, i.e. the effect of mutual compensation of negative "stenosing" and positive "productive" effects occurs. Th effect is presumably due to the use of a balloon-expandable stent-like design, which simulates the effect of balloon valvuloplasty, a significant increase in the lumen of the valve with dysfunction.

In this case, the issue of an adequate choice of standard size for reprosthetics (for UniLine bioprostheses) is caused by the need to simultaneously ensure maximum performance and safety of the valve for reprosthetics. The conducted study, on the one hand, shows the advantages of the -2TP option in terms of quantitative parameters; however, the quality -3TP valve device differs markedly in the positive sense. The analysis showed the presence of a slight asymmetry (twisting) of the closure state of the -2TP prostheses, which could potentially worsen over time due to the effect of biomaterial stretching [15, 16]. In the case of -3TP, the occurrence of a significant fraction of regurgitation (up to 20.95%) is due to insufficiently tight closure of the valves and thus, the formation of transvalvular regurgitation. In this variant, the above-described biomaterial stretching can lead to a positive effect: the leveling of high values of the regurgitation fraction by creating a tight closure of the coaptation zone without the effects of asymmetry of the valves. It is worth noting that the symmetry of the coaptation zone positively effects the prosthesis durability due to more even distribution of stress without the occurrence of local extreme values (stress concentration) [17, 18]. However, in the modern cardiac surgery practice, the intervention success is assessed immediately after the prosthesis is installed, and high values of the regurgitation will be regarded as failure of the dysfunction correction operation; therefore it is necessary to use a more "reliable" option, i.e. -2TP.

In general, the results obtained for both primary UniLine bioprostheses in comparison with frame valves, and experimental samples relative to transcatheter analogs are consistent with the literature data on studies of domestic and foreign prostheses. Thus, the hydrodynamic characteristics of the UniLine prostheses are comparable to those for Carpentier-Edwards PERIMOUNT and Medtronic Hancock® valves of similar standard sizes (21–25 mm) [19]: the average transprothestic gradient is 5.8–6.2 and 11.9–18, 1 mmHg with the effective orifice area 1.82–2.12 and 1.20–1.49 cm^2, respectively. Besides, the obtained results are in compliance with the clinical hemodynamic parameters of Medlab-KT which is principally similar to the domestic development (CJSC NPP MedInzh) which, nevertheless, is intended for transcatheter implantation – the average transprothestic gradient is 8.41 ± 4.21 mm Hg [20].

Another group of similar devices which the results of the present experiment are potentially necessary to be compared to are transcatheter prostheses used for "prosthesis-to-prosthesis" implantation, mainly Edwards Lifesciences SAPIEN and Medtronic CoreValve™ bioprostheses [21]. The transprothestic gradient according to the results of functional studies is shown to be 7.7–16.9 mm Hg which is slightly higher than the mean transprothestic gradient in primary transcatheter prosthetics: 0–10 mm Hg [20, 22, 23]. Nevertheless, these results are considered satisfactory in terms of clinical efficacy expressed in a decrease in the NYHA functional class [24].

CONCLUSION

The design of the prosthesis under development has shown its consistency in terms of functional characteristics, both in comparison with the original UniLine prosthesis and with literature data. However, the study demonstrated the need for careful selection of the appropriate valve size to minimize safety risks and dangers of significant reduction in hydrodynamic efficiency, considering the prosthesis-to-prosthesis implantation technique.

The work was performed within the framework of the fundamental research topic of the Research Institute for Complex Issues of Cardiovascular Diseases No. 0546-2015-0011, Pathogenetic substantiation of the development of implants for cardiovascular surgery based on biocompatible materials with the implementation of a patient-oriented approach using mathematical modeling, tissue engineering, and genomic predictors.

The authors declare no conflict of interest.

REFERENCES

1. *Bokeriya LA, Gudkova RG.* Serdechno-sosudistaya khirurgiya – 2015. Bolezni i vrozhdennye anomalii sistemy krovoobrashcheniya [Tekst]; Sektsiya po serdechno-sosudistoy khirurgii uchenogo soveta Ministerstva zdravookhraneniya Rossiyskoy Federatsii [i dr.]. M.: Izd-vo NTsSSKh im. A.N. Bakuleva RAMN, 2002–2015. 225.
2. *Rogulina NV, Odarenko YuN, Kokorin SG, Barbarash LS.* Mekhanicheskie i biologicheskie protezy v khirurgii izolirovannogo poroka aortal'nogo klapana. *Evraziyskiy kardiologicheskiy zhurnal*. 2016; 3: 42–43.

3. *Odarenko YuN, Kokorin SG, Stasev AN, Rogulina NV, Burago AYu, Barbarash LS.* 25-letniy opyt primeneniya ksenoaortal'nykh epoksiobrabotannykh bioprotezov v khirurgii mitral'nykh porokov. *Evraziyskiy kardiologicheskiy zhurnal*. 2016; 3: 45–46.
4. *Kudryavtseva YuA, Nasonova MV, Akent'eva TN, Burago AYu, Zhuravleva IYu.* Rol' shovnogo materiala v kal'tsifikatsii kardiovaskulyarnykh bioprotezov. *Kompleksnye problemy serdechno-sosudistykh zabolevaniy*. 2013; 4: 22–27.
5. *Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP 3rd, Guyton RA et al*. ACC/AHA Task Force Members. 2014 AHA/ACC Guideline for the Management of Patients With Valvular Heart Disease: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation*. 2014; 129 (23): 2440–2492. doi: 10.1161/CIR.0000000000000029.
6. *Klyshnikov KYu, Ovcharenko EA, Kudryavtseva YuA, Barbarash LS.* Reprotezirovanie klapanov serdtsa po metodike "protez-v-protez". *Rossiyskiy kardiologicheskiy zhurnal*. 2016; 11 (139): 73–80. doi: 10.15829/1560-4071-2016-11-73-80.
7. *Balsam LB, Grossi EA, Greenhouse DG, Ursomanno P, Deanda A, Ribakove GH et al*. Reoperative valve surgery in the elderly: predictors of risk and long-term survival. *Ann Thorac Surg*. 2010; 90 (4): 1195–1200. doi: 10.1016/j.athoracsur.2010.04.057.
8. *Maganti M, Rao V, Armstrong S, Feindel CM, Scully HE, David TE*. Redo valvular surgery in elderly patients. *Ann Thorac Surg*. 2009; 87 (2): 521–525. doi: 10.1016/j.athoracsur.2008.09.030.
9. *Imaev TE, Komlev AE, Kolegaev AS i dr*. Sovremennoe sostoyanie problemy transkateternogo reprotezirovaniya klapanov serdtsa po metodike "klapan-v-klapan". *Consilium Medicum*. 2016; 18 (5): 89–92.
10. *Regeer M, Merkestein L, de Weger A, Kamperidis V, van der Kley F, van Rosendael P et al.* Left bundle branch block after sutureless, transcatheter, and stented biological aortic valve replacement for aortic stenosis. *EuroIntervention*. 2017; 12 (13): 1660–1666.
11. *Alekyan BG, Grigor'yan AM, Staferov AV, Karapetyan NG.* Rentgenendovaskulyarnaya diagnostika i lechenie zabolevaniy serdtsa i sosudov v Rossiyskoy Federatsii – 2017 god. *Endovaskulyarnaya khirurgiya*. 2018. 5 (2): 93–240. doi: 10.24183/2409-4080-2018-5-2-93-240.
12. *Barbarash LS, Klyshnikov KYu, Ovcharenko EA, Stasev AN, Kokorin SG.* Patent na PM "Biologicheskiy protez dlya reprotezirovaniya klapanov serdtsa" № 156774. Opubl. 20.11.2015. Byul. № 32: 11–13.
13. *Ovcharenko EA, Klyshnikov KYu, Savrasov GV, Glushkova TV, Barbarash LS.* Issledovanie gidrodinamicheskoy funktsii maloinvazivnogo bioproteza klapana aorty. *Kompleksnye problemy serdechno-sosudistykh zabolevaniy*. 2016; 5 (2): 39–45.
14. *Jeewandara TM, Wise SG, Ng MKC*. Biocompatibility of Coronary Stents. *Materials*. 2014; 7 (2): 769–786. doi: 10.3390/ma7020769.
15. *Butany J, Collins MJ.* Analysis of prosthetic cardiac devices: a guide for the practising pathologist. *J Clin Pathol*. 2005 Feb; 58 (2): 113–124. Review.
16. *Soares SJ, Feaver KR, Zhang W, Kamensky D, Aggarwal A, Sacks MS*. Biomechanical Behavior of Bioprosthetic Heart Valve Heterograft Tissues: Characterization, Simulation, and Performance. *Cardiovasc Eng Technol*. 2016; 7 (4): 309–351. doi: 10.1007/s13239-016-0276-8.
17. *Avanzini A, Battini D.* Structural analysis of a stented pericardial heart valve with leaflets mounted externally. *Proc Inst Mech Eng H*. 2014 Oct; 228 (10): 985–995. doi: 10.1177/0954411914552309. Epub 2014 Sep 23. PubMed PMID: 25252695.
18. *Martin C, Sun W*. Simulation of long-term fatigue damage in bioprosthetic heart valves: effects of leaflet and stent elastic properties. *Biomech Model Mechanobiol*. 2014 Aug; 13 (4): 759–770. doi: 10.1007/s10237-013-0532-x.
19. *Marquez S, Hon RT, Yoganathan AP.* Comparative hydrodynamic evaluation of bioprosthetic heart valves. *J Heart Valve Dis*. 2001; 10 (6): 802–811.
20. *Bazylev VV, Voevodin AB, Rosseykin EV.* Dvukhletniy opyt ispol'zovaniya otechestvennogo transkateternogo proteza aortal'nogo klapana "MEDLAB". *Byulleten' NTsSSKh im. A.N. Bakuleva RAMN "Serdechno-sosudistye zabolevaniya"*. 2017; 18 (S6): 131.
21. *Bapat V.* Technical pitfalls and tips for the valve-in-valve procedure. *Ann Cardiothorac Surg*. 2017; 6 (5): 541–552. doi: 10.21037/acs.2017.09.13.
22. *Reul RM, Ramchandani MK, Reardon MJ.* Transcatheter Aortic Valve-in-Valve Procedure in Patients with Bioprosthetic Structural Valve Deterioration. *Methodist Debakey Cardiovasc J*. 2017; 13 (3): 132–141. doi: 10.14797/mdcj-13-3-132.
23. *Dvir D, Webb JG, Bleiziffer S, Pasic M, Waksman R, Kodali S et al.* Valve-in-Valve International Data Registry Investigators. Transcatheter aortic valve implantation in failed bioprosthetic surgical valves. *JAMA*. 2014 Jul; 312 (2): 162–170. doi: 10.1001/jama.2014.7246.

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ROBOT-ASSISTED KIDNEY TRANSPLANTATION. FIRST EXPERIENCE

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Kidney transplantation is the preferred renal replacement therapy for patients with end-stage renal disease. Traditional surgical approaches consisting of vascular and urinary outflow reconstruction during kidney transplant have been sufficiently studied and standardized. However, surgical techniques are still evolving. The objective of this clinical report is to focus the attention of kidney transplant surgeons and specialists on the currently trending robot-assisted kidney transplantation (RAKT) as a minimally invasive procedure for surgical treatment of patients with end-stage renal disease. In our first experience, good primary graft function was achieved. This shows that RAKT is a surgical option. With considerable number of surgeries and experience, RAKT outcomes would be improved significantly.

Keywords: robotics, minimally invasive surgery, robotic surgery, kidney, chronic kidney disease, end-stage renal disease, kidney transplantation, robot-assisted kidney transplantation.

INTRODUCTION

Though open surgery is the method of choice for kidney transplantation in adult recipients, minimally invasive surgical techniques is not the least to gain their foothold. At this, laparoscopic and robot-assisted nephrectomy from live related donors has largely replaced conventional surgery since the late 1990s [1]. These techniques have established themselves as a standard approach, providing the same function of the graft, same rejection rate, same urological complications, equivalent survival of the patient and the graft, reducing the number of analgesia episodes to relieve postoperative pain, achieving good cosmetic outcomes, and shortened hospital stay [2–4].

András Hoznek was the first to perform RAKT [5]. Currently, more than 500 such operations have been described globally [6], while in Russia the RAKT experience is minimal [7–9]. The RAKT has the following advantages: better visualization, ease of manipulation of instruments, precision accuracy, minimal surgical and infectious postoperative complications, especially in obese patients [10, 11]; however, it is tied to longer operation time and thermal ischemia [12, 13] which, as a result, can affect the aggravation of reperfusion injury and restoration of the graft function [14].

From January to April 2020, at the Petrovsky National Research Centre of Surgery, four RAKTs were performed: 3 from deceased donors, 1 from a relative.

In this article, we present the of the technique and give a description of one of the clinical cases.

The technique used is quite standard for such interventions. The result was obtained for the first robotic operations; it is comparable to those in open surgery and requires further observation.

A 50-year-old male with terminal stage renal disease due to glomerulonephritis. The disease was identified at an examination for bilateral pneumonia in February 2017. The gradual deterioration of renal function led to the development of the terminal stage, and on August 17, 2017, planned renal replacement therapy through an arteriovenous fistula started, and on December 17, 2019, a robotic kidney allotransplantation from a deceased donor was performed. The donor was a 50-year-old female, dead of a hemorrhagic stroke. The selection parameters are presented in Table 1.

SURGICAL TECHNIQUE

The patient was fixed in Trendelenburg position with head end inclined at 20–30° and the table rotated and inclined left at 20–30°. The patient cart (da Vinci System, Intuitive Surgical, USA) with manipulators was located to the right of the operating table at the patient's feet. The most suitable location of the port points was chosen as a basis, similar to their location at a radical prostatectomy. At this, there were some differences, including those from the methodology developed in the course of the IDEAL study (Innovation, Development, Exploration,

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Table 1

Donor and recipient selection: blood group, HLA genotype, HLA incompatibility, cross-compatibility test (crossmatch testing)

Selection parameter	Recipient	Donor
Gender	Male	Female
Age	50	50
Blood type	0(I) Rh(+)	0(I) Rh(–)
HLA phenotype	A 2.10 B 15.15 Dr 6.6	A 2.19 B 15.16 Dr 4.2
Incompatibility	A 19 B 16 Dr 4.2 (MM4)	
Cross-match reaction	negative	

Assessment, Long-term study) [15], and similarities to the method described by Ugo Boggi et al. [16]: the camera port (12 mm) was located slightly higher and to the left of the navel. The working ports (8 mm) were located in an arc deviated to the left at 8 cm from each other. An assistant port was located in the left iliac area. An assistant port in the form of a retractor with an iris diaphragm (Seal Cap Assembly Dextrus, Ethicon, USA) was introduced through 7-cm Pfannenstiel incision (Fig. 1).

Intraoperatively: the 1st step, isolating the iliac vessels (external iliac vein and external iliac artery) on the right. Then, through the assistant manual port, a donor kidney was placed in the abdominal cavity on an ice draped cushion (Fig. 2).

The graft (left kidney) with 1 artery and 1 vein was placed in the right iliac fossa without craniocaudal inversion. Anastomoses were formed: the graft vein "end-to-side" of the external iliac vein with Prolene 5/0, the graft artery "end-to-side" of the external iliac artery with Prolene 5/0 (Fig. 3).

With the blood flow, the graft was evenly filled with blood, turned pink, with the satisfactory turgor. The extravesical Leach-Gregoire anastomosis of the ureter with the bladder on the JJ-stent was formed with PDS 5/0. At the last stage, the graft extraperitonization was performed. At the endo-ultrasound control with color Doppler mapping (CDM) using a robotic drop-in ultrasound transducer (BK medical, Denmark), the blood flow in the graft was evenly distributed (Fig. 4).

The console time – 140 min, the vascular anastomoses formation – 45 min, blood loss – 20 ml.

Induction therapy: basiliximab. Immunosuppressive therapy: tacrolimus from day 1, prednisolone from day 1, mycophenolate mofetil from day 3. 1st day: 5,400

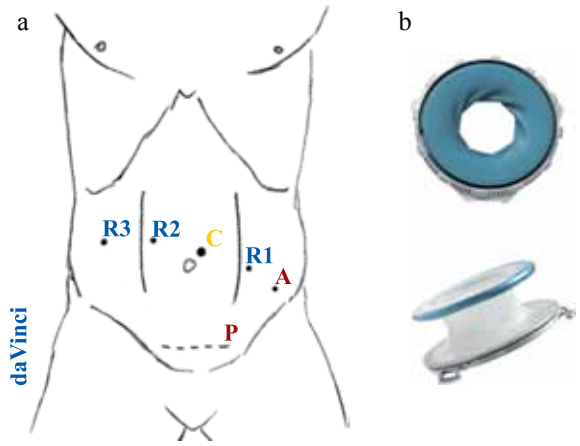

Fig. 1. Robotic kidney transplantation: a – illustration of port placement; b – retractor with sealing cap (Seal Cap "Dextrus"). C – 12-mm camera port; R1, R2, R3 – 8-mm robotic ports, corresponding original numbering of da Vinci manipulators; A – 10-mm assistant port; P – suprapubic incision for retractor with sealing cap; da Vinci – patient cart placement

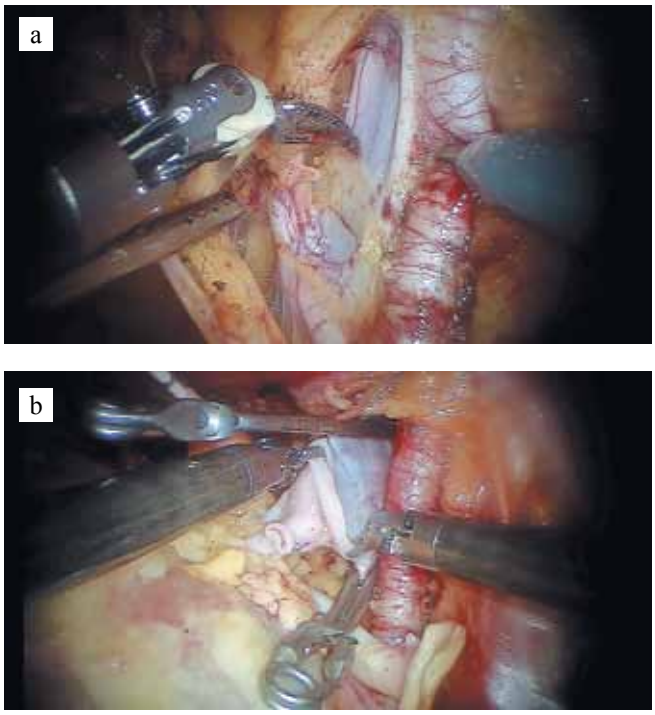

Fig. 2. The initial stage of the operation (preparation for future iliac vessels anastomoses): a – skeletonized iliac vessel bed (artery and vein); b – external iliac vein clamped with a robotic bulldog clamp, try-on before venotomy

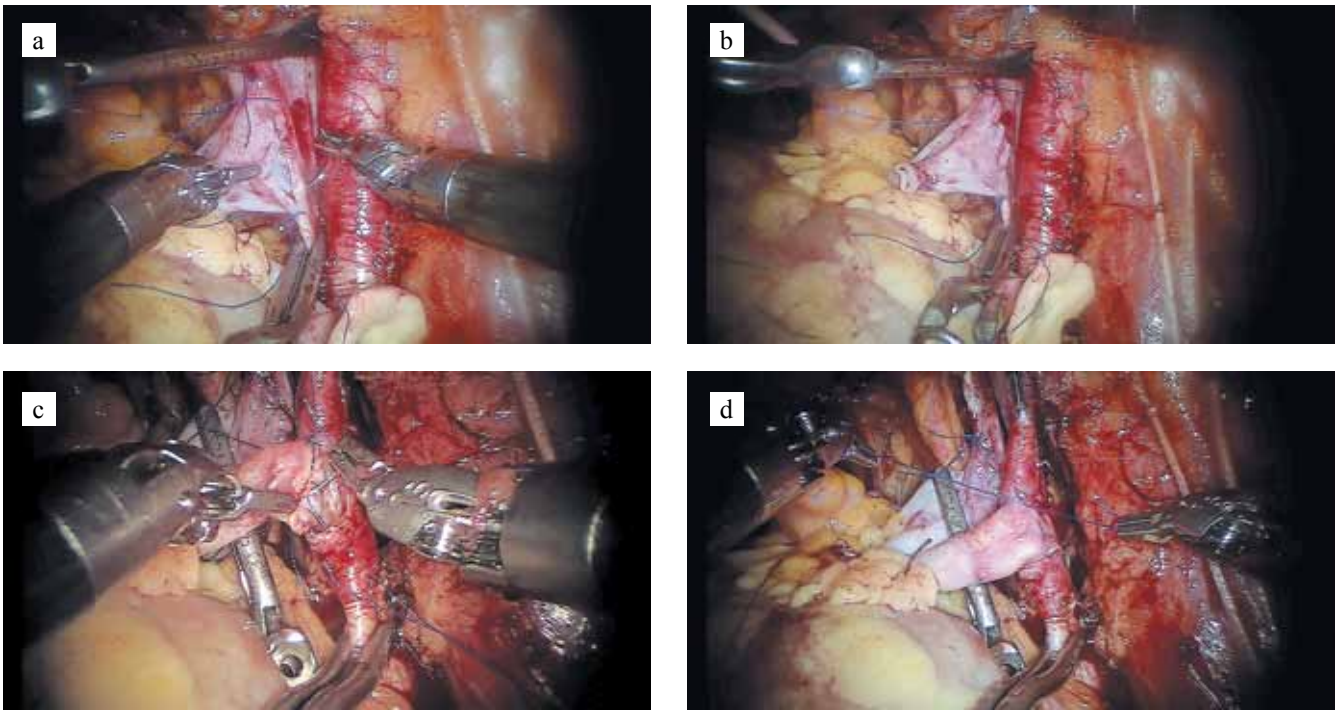

Fig. 3. Stage of vascular anastomoses: a, b – end-to-side venous anastomosis (the external iliac vein clamped with a robotic bulldog clamp): the graft vein – the external iliac vein; c, d – arterial anastomosis end to side (the external iliac artery clamped with a robotic bulldog clamp): the graft artery – the external iliac artery

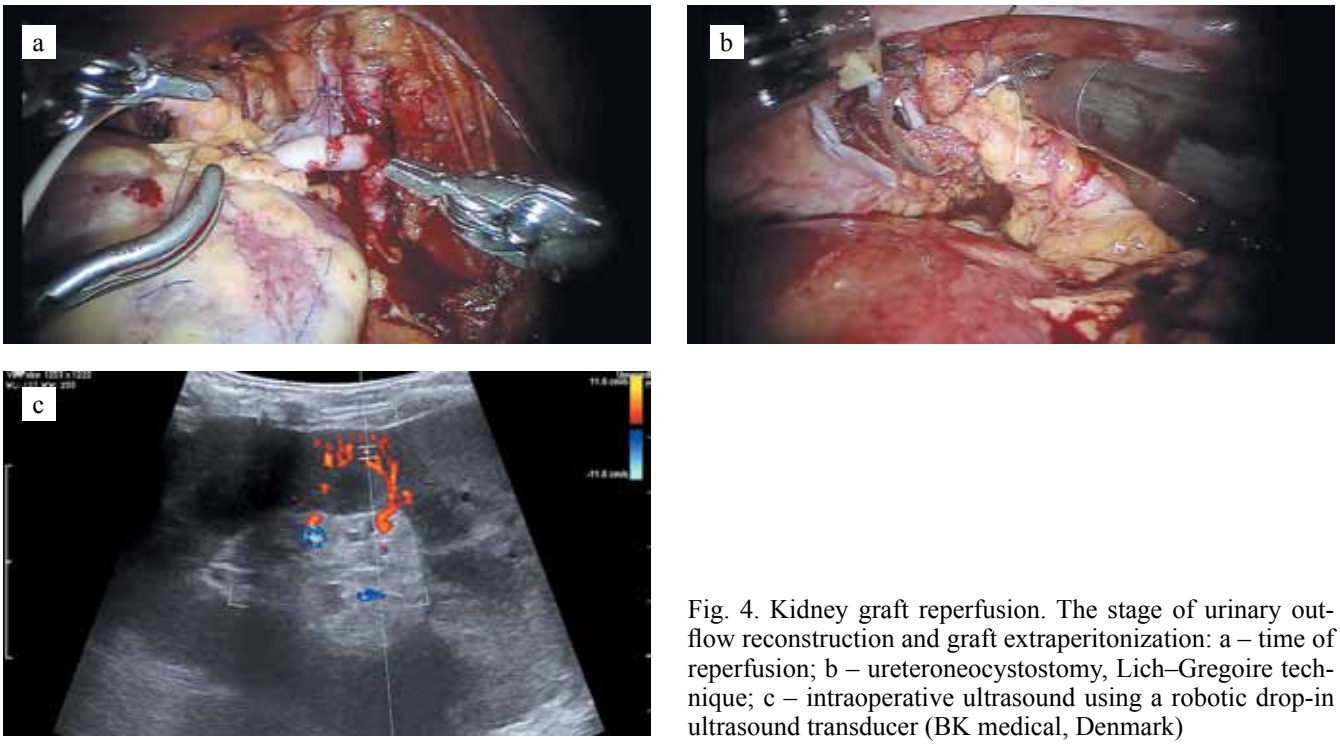

Fig. 4. Kidney graft reperfusion. The stage of urinary outflow reconstruction and graft extraperitonization: a – time of reperfusion; b – ureteroneocystostomy, Lich–Gregoire technique; c – intraoperative ultrasound using a robotic drop-in ultrasound transducer (BK medical, Denmark)

ml of urine. Creatinine on the 1st day: 629 µmol/l (7.1 mg%), glomerular filtration rate (GFR-EPDI) 8.15 ml/min/1.73 m^2. Creatinine suboptimization (<3 mg%) on the 5th day. The patient was discharged on the 14th day with creatinine 109 µmol/l (1.2 mg%), GFR-EPDI 67.6 ml/min/1.73 m^2. Ultrasound: the graft thickness – 6.2 cm, the cortical layer thickness – 0.68 to 0.8 cm, the sinus – 3.1 cm. The pyramids are not changed. The pelvis cavity – 1.4 cm. The parenchyma is not changed. The cortical layer is not changed. The accumulation of fluid around the kidney was not detected. CDM: blood flow – satisfactory (Table 2).

Table 2

Doppler indices in the graft after surgery (14 days)

	Vs, m/s	Vd, m/s	Ri
Renal artery	0.72	0.13	0.82
Interlobar artery	0.27	0.06	0.78
Arcuate artery	0.21	0.04	0.81

Table 3

Doppler indices in the graft after surgery (2.5 month)

	Vs, m/s	Vd, m/s	Ri
Renal artery	0.76	0.16	0.79
Renal artery	0.4	0.1	0.75
Arcuate artery	0.14	0.03	0.8

Examination in 2.5 months after surgery: the graft function – satisfactory: creatinine 111.7 μmol/l (1.3 mg%), GFR-EPDI 66.35 ml/min/1.73 m^2. The patient continues to receive three-component immunosuppressive therapy – tacrolimus, mycophenolate mofetil, and prednisolone. Ultrasound (Fig. 5): the graft – 6.2 cm thick, the cortical layer – 0.7 cm thick, the sinus – 2.9 cm. The pyramids are not changed. Moderate expansion of the pelvis. The parenchyma is not changed. The cortical layer is not changed. The fluid accumulation around the kidney was not detected. Blood flow CMD parameters are shown in Table 3.

CT: low location of the graft in the pelvic cavity, the contrast uniform distribution in the arterial phase. The performed retrograde cystography did not reveal signs of vesicoureteral reflux (Fig. 6).

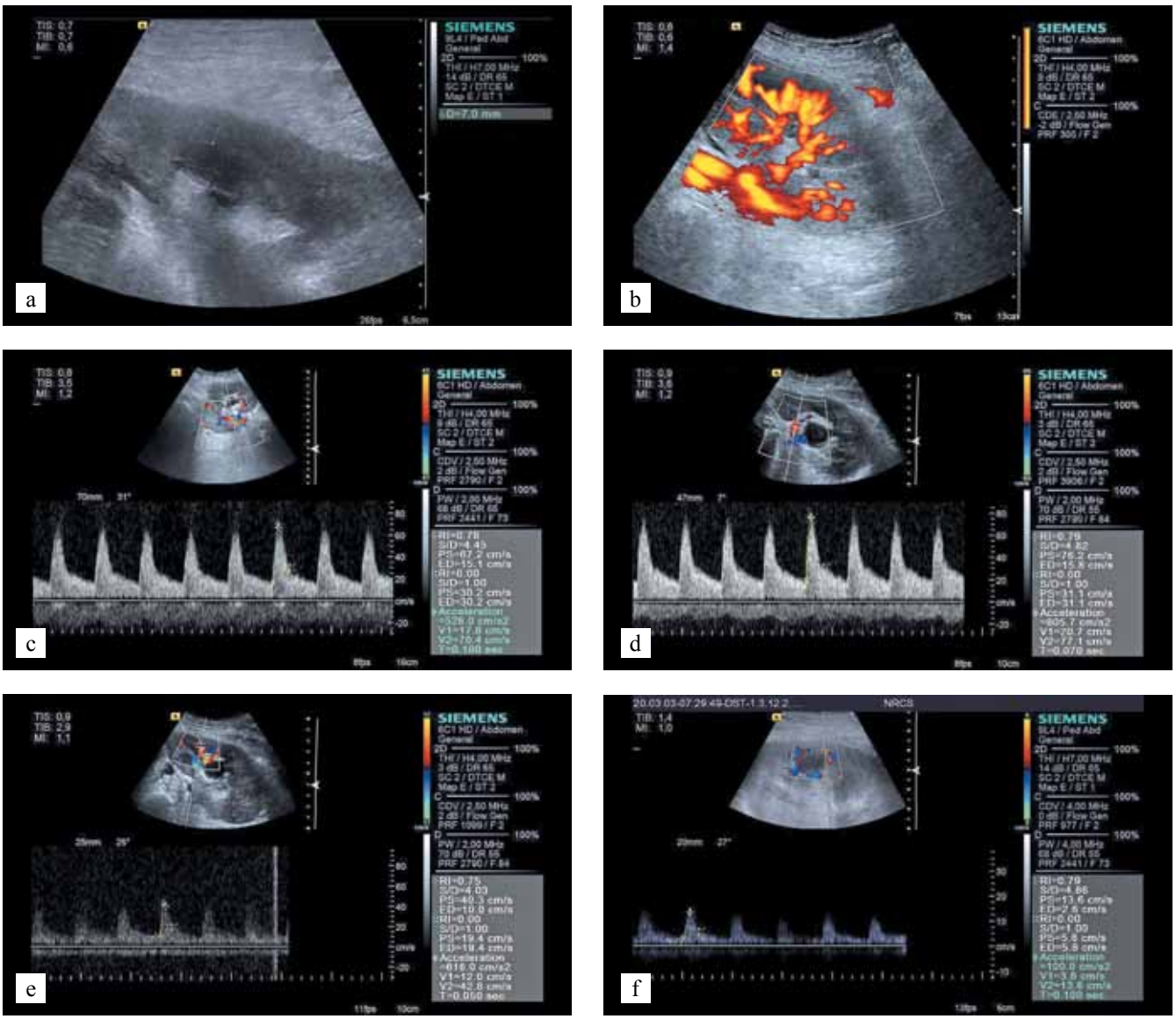

Fig. 5. Ultrasonography, vascular Doppler of the renal transplant 2.5 months after transplantation: a – renal cortex; b – a picture in the power doppler mode; c, d – renal artery; e – interlobar artery; f – arc artery

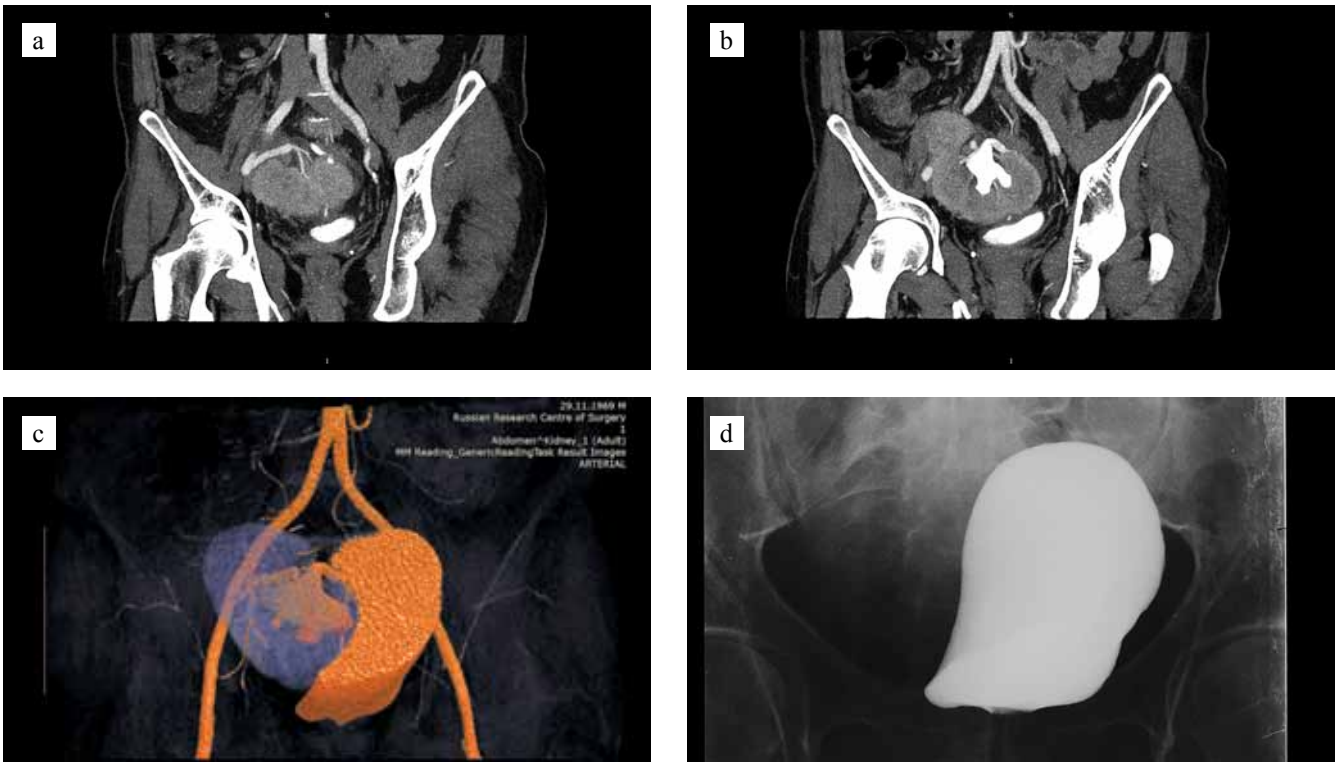

Fig. 6. X-ray diagnostic examination: a, b – computed tomography scans of arterial and uro phase; c – 3d-reconstruction; d – retrograde cystography

All operations were performed using a similar technique. In one case, thrombosis of the venous anastomosis was noted after the start of the blood flow; venotomy and thrombectomy were performed without conversion.

DISCUSSION

In the last decade, along with the growing interest in minimally invasive surgery using robotic systems, the efficiency of its usage has also increased. Organ transplantation also does not stand aside. Thus, along with laparoscopic donor nephrectomy, which has become part of everyday practice, the RAKT is gaining popularity. Performing such a surgical intervention, both from a technical and logistic point of view, is possible from both deceased and live donors, both in a standard situation and in the presence of various anomalies of the donor organ [17]. However, several studies note that surgeons with extensive experience in robotic surgery have minimal or no learning curve for RAKT, regardless of their previous experience in open transplantation, in contrast to experienced fellow surgeons who are familiar with traditional transplant methods [18]. For the traditional surgeon, as with many other robotic procedures, the learning curve can be a significant limitation for large-scale mastery of complex techniques. However, despite the fact that at present, according to the current literature, the generalized results of open and robotic transplantation can be comparable, this problem requires further research.

CONCLUSION

The present clinical observation is comparable with the early experience of the RAKT implementation in other transplant centers [19]. The absence of postoperative complications, minimal use of analgesics, early activation, discharge of the patient, and, above all, satisfactory functioning of the graft are good outcomes of the first experience of performing such a high-tech surgery.

The authors declare no conflict of interest.

REFERENCES

1. *Martin GL, Guise AI, Bernie JE, Bargman V, Goggins W, Sundaram CP.* Laparoscopic Donor Nephrectomy: Effects of Learning Curve on Surgical Outcomes. *Transplant Proc*. 2007 Jan-Feb; 39 (1): 27–29. PMID: 17275468. doi: 10.1016/j.transproceed.2006.10.006.
2. *Ahmed A Shokeir.* Open Versus Laparoscopic Live Donor Nephrectomy: A Focus on the Safety of Donors and the Need for a Donor Registry. *J Urol*. 2007 Nov; 178 (5): 1860–1866. PMID: 17868736. doi: 10.1016/j.juro.2007.07.008.
3. *Horgan S, Galvani C, Gorodner MV, Jacobsen GR, Moser F, Manzelli A et al.* Effect of Robotic Assistance on the "Learning Curve" for Laparoscopic Hand-Assisted Donor Nephrectomy. *Surg Endosc*. 2007 Sep; 21 (9): 1512–1517. PMID: 17287916. doi: 10.1007/s00464-006-9140-5.
4. *Janki S, Klop KWJ, Hagen SM, Terkivatan T, Betjes MGH, Tran TCK et al.* Robotic surgery rapidly and suc-

cessfully implemented in a high volume laparoscopic center on living kidney donation. Int J Med Robot. 2017 Jun; 13 (2). PMID: 26987773. doi: 10.1002/rcs.1743.

5. *Hoznek A, Zaki SK, Samadi DB, Salomon L, Lobontiu A, Lang P et al.* Robotic assisted kidney transplantation: an initial experience. *J Urol*. 2002 Apr; 167 (4): 1604–1606. PMID: 11912372.
6. *Stiegler P, Schemmera P*. Robot-Assisted Transplant Surgery – Vision or Reality? A Comprehensive Review. *Visc Med*. 2018 Feb; 34 (1): 24–30. PMID: 29594166. doi: 10.1159/000485686.
7. *Medvedev VL, Volkov SN, Palaguta GA, Chernov KE*. Pervyy opyt allotransplantatsii trupnoy pochki s ispol'zovaniem robotizirovannoy sistemy "Da Vinci". *Daydzhest urologii*. 2016; 2: 2–6.
8. *Medvedev VL, Volkov SN, Palaguta GA, Chernov KE*. Allotransplantacija trupnoj pochki s ispol'zovaniem robotizirovannoj sistemy "Da Vinci" s transvaginal'nym zavedeniem transplantata (Pervonachal'nyj otechestvennyj opyt). *XVI Kongress Rossijskogo obshhestva urologov*. 2016; 296–297.
9. *Medvedev VL, Volkov SN, Palaguta GA, Chernov KE*. Allotransplantacii trupnoj pochki s ispol'zovaniem robotizirovannoj sistemy "Da Vinci". *Vestnik Bashkirskogo gosudarstvennogo universiteta*. 2019; 5: 173–176.
10. *Tzvetanov I, Bejarano-Pineda L, Giulianotti PC, Jeon H, Garcia-Roca R, Bianco F et al.* State of the art of robotic surgery in organ transplantation. *World J Surg*. 2013; 37: 2791–2799. PMID: 24101021. doi: 10.1007/s00268-013-2244-x.
11. *Giulianotti P, Gorodner V, Sbrana F, Tzvetanov I, Jeon H, Bianco F et al.* Robotic transabdominal kidney transplantation in a morbidly obese patient. *Am J Transplant*. 2010; 10: 1478–1482. PMID: 20486912. doi: 10.1111/j.1600-6143.2010.03116.x.
12. *Oberholzer J, Giulianotti P, Danielson KK, Spaggiari M, Bejarano-Pineda L, Bianco F et al.* Minimally invasive robotic kidney transplantation for obese patients previously denied access to transplantation. *Am J Transplant*. 2013; 13: 721–728. PMID: 23437881. doi: 10.1111/ajt.12078.
13. *Territo A, Mottrie A, Abaza R, Rogers C, Menon M, Bhandari M et al.* Robotic kidney transplantation: current status and future perspectives. *Minerva Urol Nefrol*. 2017; 69: 5–13. PMID: 28009142. doi: 10.23736/S0393-2249.16.02856-3.
14. *Weissenbacher A, Oberhuber R, Cardini B, Weiss S, Ulmer H, Bosmuller C et al.* The faster the better: anastomosis time influences patient survival after deceased donor kidney transplantation. *Transpl Int*. 2015; 28: 535–543. PMID: 25557890. doi: 10.1111/tri.12516.
15. *Sood A et al.* Ontogeny of a surgical technique: Robotic kidney transplantation withregional hypothermia. *International Journal of Surgery.* 2016; 25: 158–161. doi: 10.1016/j.ijsu.2015.12.061.
16. *Boggi U, Vistoli F, Signori S, D'Imporzano S, Amorese G, Consani G et al.* Robotic renal transplantation: first European case. *Transplant International*. 2011; 24: 213–218. PMID: 21091963. doi: 10.1111/j.1432-2277.2010.01191.x.
17. *Siena G, Campi R, Decaestecker K, Tuğcu V, Sahin S, Alcaraz A et al.* Robot-assisted Kidney Transplantation with Regional Hypothermia Using Grafts with Multiple Vessels After Extracorporeal Vascular Reconstruction: Results from the European Association of Urology Robotic Urology Section Working Group. *Eur Urol Focus*. 2018 Mar; 4 (2): 175–184. PMID: 30049659. doi: 10.1016/j.euf.2018.07.022.
18. *Sood A, Ghani KR, Ahlawat R, Modi P, Abaza R, Jeong W et al.* Application of the statistical process control method for prospective patient safety monitoring during the learning phase: robotic kidney transplantation with regional hypothermia (IDEAL phase 2a-b). *Eur Urol*. 2014 Aug; 66 (2): 371–378. PMID: 24631408. doi: 10.1016/j.eururo.2014.02.055.
19. *Breda A, Territo A, Gausa L, Tuğcu V, Alcaraz A, Musquera M et al.* Robot-assisted Kidney Transplantation: The European Experience. *Eur Urol*. 2018 Feb; 73 (2): 273–281. PMID: 28916408. doi: 10.1016/j.eururo.2017.08.028.

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KIDNEY TRANSPLANTATION USING COMPLEMENT INHIBITOR IN A PATIENT SUFFERING FROM ATYPICAL HEMOLYTIC-UREMIC SYNDROME ASSOCIATED WITH FACTOR H ANTIBODIES: SUCCESSFUL PREVENTION OF RECURRENCE OF THE UNDERLYING DISEASE

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Atypical hemolytic-uremic syndrome (aHUS) is an extremely rare complement-mediated disease that belongs to the group of thrombotic microangiopathies (TMA). It often reoccurs after kidney transplantation (KT). Previously, KT was considered contraindicated in both children and adults with aHUS due to high (up to 50% and above) incidence of early graft loss associated with post-transplant recurrent TMA. Introduction of specific complement inhibitor therapy into clinical practice has improved outcomes in patients with aHUS and has significantly reduced the risk of post-transplant recurrence of underlying disease. We describe the clinical observation of a 20-year-old female patient with aHUS associated with antibodies to factor H, a major regulator of complement activation. The patient underwent KT and eculizumab was used for prophylactic purposes. In the postoperative period, the patient developed ureteral necrosis that required reconstructive surgery, followed by graft pyelonephritis. Despite post-operative complications, which were highly likely to trigger uncontrolled complement activation, TMA recurrence was avoided due to early treatment of the complications and prophylactic use of complement inhibitor therapy.

Keywords: atypical hemolytic-uremic syndrome, eculizumab, kidney transplantation, complement-activating conditions.

Atypical hemolytic uremic syndrome (aHUS) is an extremely disease that belongs to the group of thrombotic microangiopathies (TMA) often recurring after kidney transplantation (KT) [1–3]. The aHUS development is caused by excessive activation of the alternative complement pathway on the cell surface in the microvasculature associated with genetic factors (mutations of genes encoding complement regulator proteins) or with the appearance of antibodies to the most important regulator of complement activity, factor H [4, 5]. Clinically, the disease is expressed as thrombocytopenia, non-immune microangiopathic hemolytic anemia, and damage to target organs, primarily the kidney, and has a poor prognosis. Patients with aHUS caused by mutations in the factor H genes (*CFH*), factor I (*CFI*), factor B (*CFB*), C3 component of complement or thrombomodulin (THBD), with delayed diagnosis and/or no specific treatment in over 50% of cases reach the fifth stage of chronic kidney disease (CKD) or die in the first 3–5 years from the disease onset [6].

Until recently, aHUS has been mainly treated with plasma therapy: plasma exchange and/or fresh frozen plasma infusion, with antibody aHUS in combination with immunosuppression, though the efficacy of this therapy was insufficient. The emergence of a specific complement-blocking drug eculizumab, which is a monoclonal antibody against the C5 component of complement created new prospects for aHUS treatment. Eculizumab has been shown to improve outcomes in aHUS patients, including anti-factor H antibody-associated aHUS [7–9].

Despite recent advances in aHUS treatment, some patients with this disease develop stage 5 CKD requiring renal replacement therapy. Earlier, before the complement block therapy and the introduction of the principles of post-transplant prophylaxis into clinical practice, KT was considered contraindicated in both children and adults with aHUS due to the high incidence (up to 50% and more) of early graft losses associated with TMA relapses after transplantation [10, 11]. The development of aHUS recurrence is facilitated by a number of peri-transplantation factors contributing to the complement activation and the endothelium damage: ischemia-reperfusion injury, humoral transplant rejection, toxicity of calcineurin inhibitors and mTOR inhibitors in case of

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exceeding therapeutic concentrations in the blood, and infectious complications including active cystic viral infections [12]. Surgical complications and repeated surgical interventions can also trigger aHUS recurrence after KT. Nevertheless, the genetic profile of the complement system is considered to be the main factor determining TMA development after transplantation, that is why it is used to stratify the recurrence risk [10].

The risk of aHUS recurrence after KT is considered high in the presence of a previous early recurrence in the patient or patient's relatives, mutations in the *C3* and *CFB* genes, identification of pathogenic mutations of other genes involved in aHUS development; medium with an isolated *CFI* mutation, low level of antibodies to factor H, no identified mutations, or when a mutation with an unknown effect is detected; low with an isolated *MCP* mutation or a long period of negative anti-CFH antibodies [13]. According to modern concepts, the aHUS patients with high and medium recurrence risk should be prevented at KT with eculizumab that has shown high efficacy in preventing post-transplant TMA, including reoperations [2, 13–15]. In recipients who have not received preventive complement blocking therapy, eculizumab can be successfully used as a "salvage therapy" in case of already developed post-transplant aHUS recurrence [16]. The issue of the possibility and timing of discontinuation of eculizumab after KT is very difficult and not yet resolved, since the time of onset and severity of recurrence after complement blocking termination in aHUS patients are unpredictable, and a decision on drug withdrawal requires more accurate risk stratification and effective monitoring strategies that have not yet been developed [17]. Patients with high recurrence risk, especially those who have lost their first renal graft due to recurrent aHUS, are not candidates for termination of complement blocking treatment. Here, we present the clinical case of KT in a patient with antibody aHUS.

***Patient L**., born 1997, fell ill in June 2003 (at the age of 5.5), with anemia, thrombocytopenia, and acute kidney injury (AKI) developed after angina. In July 2003, the girl in serious condition was hospitalized in the ICU of the Center for Gravitational Blood Surgery and Hemodialysis of the St. Vladimir Children's City Clinical Hospital (Moscow) with the diagnosed aHUS, intensive therapy, repeated sessions of hemodiafiltration and plasmapheresis were performed. During the therapy, incomplete clinical and laboratory remission was achieved, the serum creatinine dropped to 170–180 μmol/l. In 1.5 months, in September 2003, a repeated TMA episode developed, requiring plasma therapy (plasmapheresis and infusion of fresh frozen plasma).*

Over the next ten years, the girl was observed by a nephrologist with the GFR of 44–48 ml/min/1.73 m^2, while anemia with a hemoglobin level of 90–100 g/L and thrombocytopenia (PLT CNT 100–180 × 10^9/l) were constantly noted. Persistent arterial hypertension gradually developed; BP increased to 160/85 mm Hg. To correct blood pressure and nephroprotection, the patient received ACE inhibitors for a long time. At this, the GFR level remained stable, but in 2013–2015, a relatively rapid decrease in creatinine clearance began, from 45 to 25 ml/min/1.73 m^2 in two years.

In May 2015, 12 years after the disease onset, the patient was re-admitted to the Center for Gravitational Blood Surgery and Hemodialysis of St. Vladimir Children's City Clinical Hospital (Moscow). To exclude thrombotic thrombocytopenic purpura, the activity of metalloproteinase ADAMTS13 in blood plasma was tested, which was 78% of the activity level of this enzyme in control plasma. Hemolytic activity of complement was increased – CH50 – 1:256. Since it became possible to conduct this study in Russia, the patient's level of antibodies to factor H was determined for the first time, amounting to 1,041% of the corresponding indicator in the control serum obtained by mixing serum samples from healthy donors. The study was repeated after 3.5 months: the level of antibodies to factor H was 1,975%. Thus, the antibody character of aHUS was confirmed.

In September 2015, a kidney biopsy was performed. Severe sclerotic changes in the glomeruli and tubules were revealed against the background of renal dysplasia and specific changes in the arteries – hypertrophy of the muscle layer of arterioles, myxomatosis, and intimal sclerosis. Morphology of the renal tissue: focal global and segmental glomerulosclerosis, most likely secondary. Cystic renal dysplasia. Comments: changes in minor vessels do not contradict the aHUS diagnosis (Fig. 1, 2).

Considering rather rapid decline in renal function, the signs of microangiopathic hemolytic anemia, it was decided to begin targeted complement-blocking therapy with eculizumab to arrest the TMA activity, and inhibit the CKD progression. In October 2015, the patient reached the age of 18 and came under the supervision of nephrologists of the Moscow Vladimirsky Moscow Regional Research Clinical Institute. After vaccination against meningococcal infection in November 2015, eculizumab was started according to the generally accepted scheme for adults: 4 injections of 900 mg with an interval of 7 days, the fifth injections a week later at a dose of 1,200 mg and further 1,200 mg with an interval of 2 weeks. Against the background of therapy, for the first time in many years of observation, the disappearance of anemia and the normalization of the PLT count were noted: in December 2015, hemoglobin 125 g/l, erythrocytes 4.6 × 1012/l, hematocrit 0.38, platelets 210 × 10^9/l, though remaining signs of serious kidney damage with decreased renal function – daily proteinuria 4.36 g, serum urea 15.8 mmol/l, creatinine 330 μmol/l, GFR in Rehberg test 20.5 ml/min. Complement blocking therapy was continued, unfortunately, with interruptions due to disruptions in receiving the drug.

Due to a further gradual decrease in GFR to 14–15 ml/min/1.73 m^2, an arteriovenous fistula was formed in an elective manner in October 2016. In December 2016 – January 2017, the patient suffered a severe acute respiratory viral infection that provoked a breakdown of the residual renal function with a rapid increase in serum creatinine to 700 µmol/l, urea to 35 mmol/l. Thus, in January 2017, the programmed hemodialysis was started. As there were no extrarenal aHUS manifestations, eculizumab therapy was discontinued. In 6 months after the start of dialysis therapy, the patient was included in the KT waiting list.

On December 14, 2017, a kidney from a brain death donor was transplanted. Since the risk of aHUS recurrence in the post-transplant period is regarded as high (early TMA recurrence in history, persistent high titers of antibodies to factor H), preventive administration of eculizumab was prescribed. The first 900 mg injection was performed 4 hours before reperfusion of the donor organ, the second injection, also at 900 mg, on the first day after KT (12 hours after reperfusion), other 4 injections with 7 day interval, 900 mg each (days 8, 15, 22, and 29 after transplantation), then 1,200 mg on the 35th day, and 1,200 mg with an interval of 2 weeks (Fig. 3).

Immunosuppression included induction with basiliximab on days 0 and 4 after KT, administration of methylprednisolone 500 mg on the operating table, tacrolimus per os at a single dose initially of 0.15 mg/kg/day, mycophenolate mofetil 2 g/day (decrease to 1 g/day 2 weeks after surgery), prednisolone 30 mg/day with a gradual dose reduction. Prevention of infectious complications was performed with a cephalosporin group antibiotic, valganciclovir from day 10 after KT, and trimethoprim-sulfamethoxazole.

The function of the transplanted kidney was primary: from day 1 after KT, the diuresis exceeded 2 L, after 3 days the serum creatinine level was 170 µmol/l, after 5 days and later, 70–80 µmol/l. In the early postoperative period, hemoglobin values were 86–102 g/l, PLT 139–228 × 10^9/l, daily proteinuria 0.16–0.25 g. The concentration of tacrolimus in blood was quite stable, 12.6–6.8–9.3–7.8–6.2–7.9 ng/ml.

On day 13 after KT, the elective removal of the ureteral stent was performed without any technical problems.

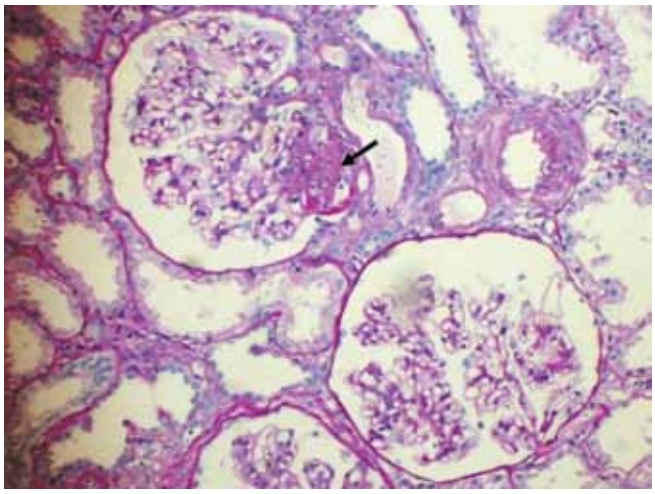

Fig. 1. Biopsy of the native kidney: the glomerulus with segmental sclerosis (arrow) and no proliferative lesions. PAS*100

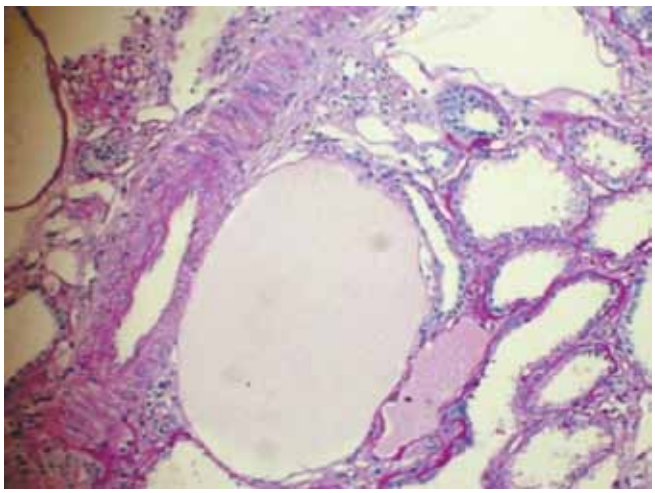

Fig. 2. Biopsy of the native kidney: microcystic transformation of tubulus. A small artery is unremarkable. PAS*100

Two days later, pain appeared in the lower abdomen, urine output decreased to less than 1 l/day, creatinine clearance decreased (GFR from 90 to 68 ml/min in 2 days), but the creatinine level increased slightly, from 80 to 90 µmol/l. Ultrasound of the graft revealed urinary leakage (Fig. 4). An urgent surgery was performed, revision of the postoperative wound and the graft: the

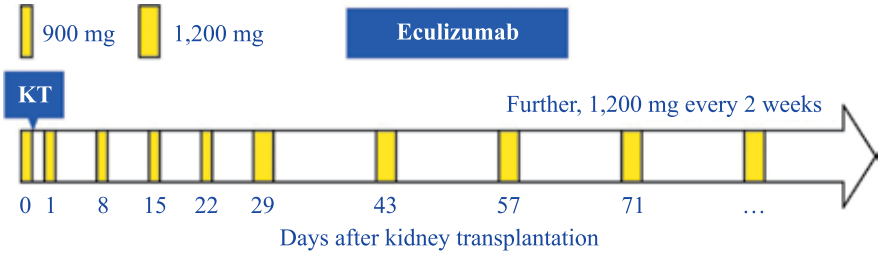

Fig. 3. Scheme of prophylactic use of eculizumab for kidney transplantation in a patient with atypical hemolytic-uremic syndrome

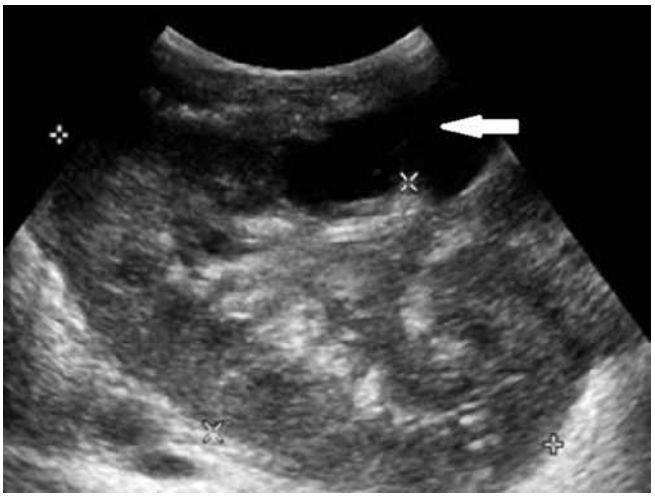

Fig. 4. Ultrasonogram of the renal transplant: urinary leakage (arrow)

transplanted kidney was pink, with normal turgor. The ureter was exposed from the bladder to the pelvis, pale gray, with necrosis areas. The neopieloureteroanastomosis with the ureter of the patient's left kidney using a stent was performed, after which the restoration of urine output was noted. Aggravation of anemia, thrombocytopenia, LDH growth was not observed.

After reconstructive surgery, Enterococcus faecium 10^5 CFU/ml, sensitive to vancomycin, was cultured from urine. It was treated with this antibiotic, after which the urine became sterile. On day 35 after KT, the patient was discharged from the hospital in a satisfactory condition with a diuresis of 2.1–2.3 l/day, normal urine analysis, daily proteinuria 0.08 g, serum urea level 4.3 mmol/l, creatinine 70 μmol/l, LDH 192 U/l. Postoperative anemia persisted, hemoglobin varied within 86–93 g/l, as PLT remained within the normal range – 199–246 × 10^9/l. The tacrolimus concentration in blood at discharge was 7.9 ng/ml.

On day 53, the ureteral elective removal was performed. On the day of surgery, hemoglobin 111 g/l, PLT 169 × 10^9/l; urine analysis: leukocytes 2–3 fov, serum urea 6.4 mmol/l, creatinine 70 μmol/l. On day 3 after stent removal, the body temperature increased to 38.5 °C; nebulous urine, leukocyturia. Serum hemoglobin was 117 g/l, leukocytes 15.8 × 10^9/L, but a decrease in PLT to 85 × 10^9/l was noted which was regarded as a possible onset of aHUS recurrence. Against the background of antibacterial therapy with carbapenems, another infusion of eculizumab at 1,200 mg was performed without complications. The symptoms of graft pyelonephritis were stopped quickly, PLT count increased to 159–180 × 10^9/l and did not decrease anymore. The patient's condition remained satisfactory; the renal graft function was not impaired. The patient was discharged for outpatient treatment with normal clinical and biochemical parameters and a normal ultrasound of the renal graft.

The follow-up examination in spring 2020: hemoglobin 129 g/l, hematocrit 0.39, PLT 253 × 10^9/l. Urine analysis: no protein. Daily proteinuria 0.01 g. The serum creatinine level 88 μmol/l, tacrolimus concentration in blood 6.9 ng/ml. No graft pyelonephritis episodes. The patient continues to receive standard immunosuppressive therapy with tacrolimus, prednisolone, and a drug from the mycophenolate group, as well as complement blocking therapy with eculizumab at 1,200 mg every two weeks.

DISCUSSION

The presented case shows the possibility of a long-term aHUS course with reduced but relatively stable renal function, despite the continuing insufficiently controlled activation of the complement system. After the first two clear TMA episodes, the pathogenetic treatment of which was performed with plasma therapy, there was a partial restoration of renal function. Subsequently, the patient received only nephroprotective therapy with ACE inhibitors for ten years, while GFR remained at 44–48 ml/min. However, anemia and moderate thrombocytopenia persisted, which indirectly confirms the presence of permanent subclinical microangiopathic hemolysis. Eculizumab after the onset of a rapid decline in renal function improved hematological parameters but did not prevent the progression of renal failure to its terminal stage, which is consistent with the data of global studies on the most complete recovery of renal function only with early initiation of targeted therapy [18, 19].

At KT, despite the most careful approach to the operation as such and the postoperative management of patients, it is impossible to completely prevent the excess of target concentrations of calcineurin inhibitors in blood and the development of surgical, urological, and infectious complications in some patients. At the same time, it is well known that any of these conditions promotes complement activation and can serve as a trigger for aHUS recurrence with impaired renal graft function and even its complete loss, as well as life-threatening systemic manifestations [10, 12, 20]. We considered the thrombocytopenia that the patient developed on the background of graft pyelonephritis as a possible onset of a TMA episode. Of course, thrombocytopenia could be caused by other reasons, cytomegalovirus infection, mycophenolate overdose, sepsis, disseminated intravascular coagulation syndrome, and heparin use. However, the patient did not have any of these conditions at that moment. Against the background of graft pyelonephritis, in the absence of other causes, the platelet count usually does not decrease, there is a normal platelet level or even thrombocytosis. In addition, platelet count normalization was very rapid after the next eculizumab infusion. Thus, the use of medication blocking the activation of

the complement system in combination with the timely correction of complications (surgical, medical), makes it possible to prevent aHUS recurrence, which we observed in our patient. Neither near-total ureteral necrosis nor subsequent graft pyelonephritis caused extensive TMA recurrence due to adequate treatment, including continued planned eculizumab administration without deviating from the scheme.

Besides the prevention and treatment of aHUS recurrence after KT, the successful use of eculizumab in transplantology for the correction of ischemia-reperfusion injury, the treatment of antibody-mediated graft rejection in combination with other drugs, the prevention of TMA after transplantation in patients with catastrophic antiphospholipid syndrome in history has been described, but these indications are still not registered, and further research in this direction is required [21].

Though immunosuppressive therapy may, to some extent, prevent exacerbation of aHUS associated with antibodies to factor H, for our patient, discontinuing complement blocking therapy is not considered, at least in the near future, due to the high risk of TMA recurrence in post-transplant period.

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REFERENCES

1. *Loirat C, Fremeaux-Bacchi V.* Atypical hemolytic uremic syndrome. *Orphanet J Rare Dis.* 2011; 6: 60. doi: 10.1186/1750-1172-6-60.
2. *Campistol JM, Arias M, Ariceta G, Blasco M, Espinosa L, Espinosa M et al.* An update for atypical haemolytic uraemic syndrome: diagnosis and treatment. A consensus document. *Nefrologia.* 2015; 35 (5): 421–447. doi: 10.1016/j.nefro.2015.07.005.
3. *Kozlovskaya NL, Prokopenko EI, Emirova KhM, Serikova SYu.* Klinicheskije rekomendacii po diagnostike i lecheniju atipichnogo gemolitiko-uremicheskogo sindroma. *Nefrologija i dializ*. 2015; 17 (3): 242–264.
4. *Dragon-Durey MA, Sethi SK, Bagga A, Blanc C, Blouin J, Ranchin B et al.* Clinical features of anti-factor H autoantibody-associated hemolytic uremic syndrome. *J Am Soc Nephrol.* 2010; 21: 2180–2187. doi: 10.1681/ASN.2010030315.
5. *Reusz G.* Atypical hemolytic uremic syndrome caused by primary complement defects. *Orv Hetil.* 2018; 159 (23): 929–936. doi: 10.1556/650.2018.31044.
6. *Constantinescu AR, Bitzan M, Weiss LS, Christen E, Kaplan BS, Cnaan A et al.* Non-enteropathic hemolytic uremic syndrome: causes and short-term course. *Am J Kidney Dis.* 2004; 43: 976–982. doi: 10.1053/j.ajkd.2004.02.010.
7. *Davin JC, van de Kar N.* Advances and challenges in the management of complement-mediated thrombotic microangiopathies. *Ther Adv Hematol.* 2015; 6 (4): 171–185. doi: 10.1177/2040620715577613.
8. *Diamante Chiodini B, Davin JC, Corazza F, Khaldi K, Dahan K, Ismaili K et al*. Eculizumab in anti-factor H antibodies associated with atypical hemolytic uremic syndrome. *Pediatrics.* 2014; 133 (6): e1764–e1768. doi: 10.1542/peds.2013-1594.
9. *Brocklebank V, Johnson S, Sheerin TP, Marks SD, Gilbert RD, Tyerman K et al.* Factor H autoantibody is associated with atypical hemolytic uremic syndrome in children in the United Kingdom and Ireland. *Kidney Int.* 2017; 92 (5): 1261–1271. doi: 10.1016/j.kint.2017.04.028.
10. *Zuber J, Le Quintrec M, Morris H, Frémeaux-Bacchi V, Loirat C, Legendre C et al.* Targeted strategies in the prevention and management of atypical HUS recurrence after kidney transplantation. *Transplant Rev (Orlando).* 2013; 27 (4): 117–125. doi: 10.1016/j.trre.2013.07.003.
11. *Le Quintrec M, Zuber J, Moulin B, Kamar N, Jablonski M, Lionet A et al.* Complement genes strongly predict recurrence and graft outcome in adult renal transplant recipients with atypical hemolytic and uremic syndrome. *Am J Transplant.* 2013; 13 (3): 663–675. doi: 10.1111/ajt.12077.
12. *Jager NM, Poppelaars F, Daha MR, Seelen MA.* Complement in renal transplantation: The road to translation. *Mol Immunol.* 2017; 89: 22–35. doi: 10.1016/j.molimm.2017.05.014.
13. *Goodship TH, Cook HT, Fakhouri F, Fervenza FC, Frémeaux-Bacchi V, Kavanagh D et al.* Atypical hemolytic uremic syndrome and C3 glomerulopathy: conclusions from a "Kidney Disease: Improving Global Outcomes" (KDIGO) Controversies Conference. *Kidney Int.* 2017; 91 (3): 539–551. doi: 10.1016/j.kint.2016.10.005.
14. *Loirat C, Fakhouri F, Ariceta G, Besbas N, Bitzan M, Bjerre A et al.* HUS International: An international consensus approach to the management of atypical hemolytic uremic syndrome in children. *Pediatr Nephrol.* 2016; 31 (1): 15–39. doi: 10.1007/s00467-015-3076-8.
15. *Davin JC, Gracchi V, Bouts A, Groothoff J, Strain L, Goodship T et al*. Maintenance of kidney function following treatment with eculizumab and discontinuation of plasma exchange after a third kidney transplant for atypical hemolytic uremic syndrome associated with a CFH mutation. *Am J Kidney Dis.* 2010; 55 (4): 708–711. doi: 10.1053/j.ajkd.2009.08.011.
16. *Matar D, Naqvi F, Racusen LC, Carter-Monroe N, Montgomery RA, Alachkar N.* Atypical hemolytic uremic syndrome recurrence after kidney transplantation. *Transplantation.* 2014; 98 (11): 1205–1212. doi: 10.1097/TP.0000000000000200.
17. *Macia M, de Alvaro Moreno F, Dutt T, Fehrman I, Hadaya K, Gasteyger C et al*. Current evidence on the discontinuation of eculizumab in patients with atypical haemolytic uraemic syndrome. *Clin Kidney J.* 2017; 10 (3): 310–319. doi: 10.1093/ckj/sfw115.

18. *Licht C, Greenbaum LA, Muus P, Babu S, Bedrosian CL, Cohen DJ et al.* Efficacy and safety of eculizumab in atypical hemolytic uremic syndrome from 2-year extensions of phase 2 studies. *Kidney Int.* 2015; 87 (5): 1061–1073. doi: 10.1038/ki.2014.423.
19. *Walle JV, Delmas Y, Ardissino G, Wang J, Kincaid JF, Haller H.* Improved renal recovery in patients with atypical hemolytic uremic syndrome following rapid initiation of eculizumab treatment. *J Nephrol.* 2017; 30 (1): 127–134. doi: 10.1007/s40620-016-0288-3.
20. *Java A, Edwards A, Rossi A, Pandey R, Gaut J, Delos Santos R et a*l. Cytomegalovirus-induced thrombotic microangiopathy after renal transplant successfully treated with eculizumab: case report and review of the literature. with eculizumab: case report and review of the literature. *Transpl Int.* 2015; 28 (9): 1121–1125. doi: 10.1111/tri.12582.
21. *Grenda R, Durlik M.* Eculizumab in renal transplantation: a 2017 Update. *Ann Transplant.* 2017 12; 22: 550–554. doi: 10.12659/AOT.905917.

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CLINICAL TRIALS FOR CELLULAR THERAPY PRODUCTS: CONCLUSIONS REACHED BY FOREIGN REGULATORY BODIES

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Currently, the problem of adopting viable human cell-based drugs – biomedical cell products (BCPs) – in medical practice in the Russian Federation includes, among others, lack of experience in clinical trials for such drugs and insufficient expert assessment under the national state registration procedure. In global practice, by the beginning of 2020, there were over 30 cellular therapy products (human cellular- and tissue-based products) known to have undergone clinical trials for sales licenses from regulatory bodies in the United States, European Union, Japan, and South Korea. Most cellular therapy products are intended for treatment of severe orphan diseases and life-threatening conditions that currently cannot be treated by traditional drugs or methods. The aim of this study is to analyze the global experience in clinical trials for cellular therapy products and also to examine conclusions reached by regulatory authorities with regards to issuance of sales licenses for the products. Particular attention was paid to clinical trials that subsequently led to granting of sales license (state registration). In reviewing such trials, we also focused on the types and number of clinical trials, the number of patients involved in the clinical trials, conclusions made by expert regulatory agencies on the efficacy, safety and risk/benefit ratio. Most of the products were approved for use based on uncontrolled phase II clinical trials. In the clinical trial, apart from the historical group and the placebo-controlled group, there was also a control group that received nothing. The number of patients in most clinical trials was limited, especially for drugs intended for treatment of rare genetic diseases, as well as drugs approved for use in Japan.

Keywords: biomedical cell product, human cellular- and tissue-based products, cell therapy, clinical trials, regulatory findings.

INTRODUCTION

In compliance with the Russia Federal Law No. 180-ФЗ of June 23, 2016 On Biomedical Cell Products, at the biomedical expertise as part of the state registration, the BCPs registration dossier should include a report on the results of clinical trials (CT) the portion of which has been conducted in the Russian Federation in medical institutions accredited to conduct BCP CTs. The list of medical organizations accredited to conduct BCP CTs already contains over 30 institutions [1]. To date, there are no BCP CTs for with permits were issued by the Ministry of Health of Russia in accordance with the Rules of Good Clinical Practice for Work with BCPs approved by the Order of the Ministry of Health of Russia No. 669н of September 22, 2017.

The international practices present, along with the experience of conducting CTs of drugs based on human cells and tissues (BCP analogues), the review of their results assessed by regulatory agencies. In total, there are over 30 drugs of the kind approved for use in medical practice around the world. These can be conditionally divided into the following groups:

1) for treatment of oncology diseases (*Immuncell-LC, CreaVax-RCC* – South Korea; *KYMRIAH, Yescarta* – European Union/EU, US);
2) for treatment of genetic diseases (*Strimvelis, Zynteglo* – EU);
3) for GVHD (graft versus host disease) treatment (*Prochymal,* Canada; *Zalmoxis,* EU; *Temcell,* Japan);
4) for regenerative medicine: treatment of the knee joint cartilage injuries *(MACI,* US; *Spherox,* EU; *JACC,* Japan; *Chondron, Cartistem* – South Korea; *Cartogen,* Australia, Singapore); burns, wounds, scars, diabetic ulcers, etc. (*JACE,* Japan; *Holoderm, KeraHeal, Cure-skin, Kaloderm, KeraHeal-Allo* – South Korea; *Gintuit,* US; *Holoclar,* EU); heart diseases (*HeartSheet,* Japan; *Hearticellfram-AMI,* South Korea); fistulae in Crohn's disease (*Cupistem,* South Korea; *Alofisel,* EU); for elimination of nasolabial creases in cosmetology (*Laviv,* US); for bone reconstruction (*RMS ossron,* South Korea).

The purpose of the present study is to review global experience on CTs of the cell therapy drugs and assessment of their results by regulatory agencies to obtain marketing authorization.

To analyze the global experience in conducting CTs of the drugs based on human cells and tissues (BCP

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analogues), a database of privately and publicly funded clinical studies conducted around the world (ClinicalTrials.gov) was mainly used; there, CTs were searched by the drugs commercial names and their international non-proprietary names (INNs) as well as regulatory permission documents for medical use drugs on the official websites of regulatory bodies of the US, EU, and Japan. It should be noted that open source information is not available for all drugs, in particular, data is limited on the drugs registered in South Korea, currently the leader in the field of cell therapy drugs approved for medical use. Particular attention was paid to CTs resulting in the drug marketing authorization (state registration), CT types and numbers, the number of patients included in the CT, the conclusions made by experts of the regulatory agencies on the efficacy, safety and the expected risk/benefit ratio. The CTs were analyzed for 14 BCP analogues.

PRODUCTS BASED ON CHIMERIC ANTIGEN RECEPTOR TECHNOLOGY (US AND EU)

Some of the latest cell therapy drugs authorized for the US (2017) and EU countries (2018) markets are KYMRIAH (Novartis) and Yescarta (Kite Pharma, Gilead), the products for adoptive immunotherapy based on chimeric antigen receptors (CAR). In the United States, both drugs were assigned the orphan diseases treatment status and the Breakthrough Therapy designation based on the FDA conclusions about the potential significant advantage of CAR therapy over existing therapies [2, 3]. In the European Union, KYMRIAH and Yescarta were the first drugs approved under the Priority Medicines scheme (PRIME) which implies an accelerated procedure for evaluating the CT applications and marketing authorization [4, 5].

As of the second half of 2019, the majority of CTs of KYMRIAH (Novartis) and Yescarta (Kite Pharma, Gilead) in the international CT database ClinicalTrials.gov have the status of "active" or "recruiting" (Table 1).

KYMRIAH was approved for market by the US regulatory agency for the treatment of recurrent/ refractory (r/r) acute lymphoblastic leukemia (ALL) and diffuse large B-cell lymphoma (DLBCL) on the results of two CTs, "ELIANA" (r/r ALL) and "JULIET" (DLBCL) (Table 1), aimed at assessing therapeutic *KYMRIAH* efficacy upon reaching the general remission rate, including complete and partial remission, within 3 months after administration, as well as the response duration [2, 6]. Marketing approval for *Yescarta* in the US and EU was obtained on the basis of one CT, "ZUMA-1" (Table 1).

When considering *KYMRIAH* registration in the EU, the regulatory agency used the comparative data of the "ELIANA" and "JULIET" CTs on the use of the drug manufactured in the EU and in the US [4].

Besides, data of the "JULIET" and "ZUMA-1" CTs on the efficacy as indicated for DLBCL were compared with the results of CTs of maintenance drugs (SCHOLAR-1, PIX301, and CORAL) [4, 7–10].

In general, when evaluating CTs of the products based on chimeric antigen receptors, the following points should be noted:

- *KYMRIAH* and *Yescarta* got marketing authorization based on uncontrolled Phase II CTs without providing long-term observations followed by annual update (trimming) of data;
- CTs assessing different drug doses were absent, the doses were selected on the basis of preclinical studies and literature data (*KYMRIAH*: 0.2–5 × 10^6 CAR-T positive T cells/kg body weight with a weight under 50 kg, 0.1–2.5 × 10^8 CAR-T positive T cells -cells/kg body weight over 50 kg; *Yescarta*: from 2 × 10^6 to 2 × 10^8 CAR-T positive T cells/kg body weight);
- the main risk factors for the drugs are cytokine release syndrome (CRS) and neurotoxicity, besides, there is a high rate of adverse events of class 3 and higher;
- patients were included by phases; in the CT of *Yescarta*, the patients were divided into cohorts depending on the type of non-Hodgkin lymphoma;
- not all patients included in the CT received therapy, the main causes being technical reasons, death of patients, physician's decisions, and others.

F.L. Locke et al. [9] present a comparative analysis of some aspects of CTs of the drugs based on chimeric antigen receptors including *lisocabtagene maraleucel* not yet licensed for sale (Table 2).

The US and EU regulatory agencies concluded that the efficacy of *KYMRIAH* (r/r ALL) and *Yescarta* (DLBCL) in comparison with existing therapies has been proven; serious adverse events (SAEs), in particular CRS and neurotoxicity, were considered manageable, and the risk valuation and identification strategy ensured higher benefits than risks associated with these SAEs [2–6]; long-term CT was a prerequisite for the drugs to be brought to market. For example, the requirements for post-marketing trials of *Yescarta* contain the following: a multicenter prospective CT of safety should be performed, including 1,500 subjects, with an observation interval of 3 months after drug administration for 5 years, and the total duration of observation of 15 years [6].

As for the use of *KYMRIAH* indicated for DLBCL, despite "modest" efficacy compared to conservative treatment, the response duration was found to be clinically significant. However, in June 2018, the opinion of 12 experts from the European Medical Agency (EMA) from different countries (Norway, Sweden, Netherlands, Italy, Greece, Romania, and Spain) was published, who did not agree with the marketing authorization of the drug according to DLBCL indication: "Due to the high degree of uncertainty in the obtained efficacy results for the DLBCL indication, the potential benefit cannot be determined for this population. Thus, the benefit/risk cannot be established and is thus not positive. As a con-

Table 1

Clinical trials of *KYMRIAH* and *Yescarta*, ClinicalTrials.gov Search Results 09/15/2019

Nos.	CT ID, particulars	Indications	Status	Start Date – End Date	Number of patients (age, years)	Number of countries / medical centers
KYMRIAH (tisagenlecleucel)						
1	NCT02445222, long-term	r/r ALL, DLBCL	Enrollment	11.2015 – 05.2035	620 (3 and older)	11/45
2	NCT02435849, B2202 **(ELIANA);** phase II, uncontrolled	r/r ALL	Active	04.2015 – 11.2022	81 (3–30)	11/ 25
3	NCT02445248, CCTL019C2201 **(JULIET)**; phase II, uncontrolled	DLBCL	Active	07.2015 – 02.2023	116 (18 and older)	10/28
4	NCT02228096, CCTL019-B2205J; phase II, uncontrolled	r/r ALL	Active	08.2014 – 12.2022	64 (3–21)	1 (US)/12
5	NCT02030834; phase IIA, parallel control	NHL	Active	02.2014 – 01.2020	63 (18 and older)	1 (US)/1
Yescarta (*Axicabtagene Ciloleucel, KTE-C19)*						
1	NCT02625480 (ZUMA-4); phase I/II	r/r ALL	Enrollment	02.2016 – (07.2021) 01.2036	100 (2–21)	4/ 21
2	NCT02926833 (ZUMA-6); phase I/II (combined with Atezolizumab)	DLBCL	Active	09.2016 – (02.2019) 08.2033	37 (18 and older)	1 (US)/4
3	NCT02348216, KTE-C19-101 **(ZUMA-1)**; phase I/II	r/r NHL*	Enrollment	01.2015 – (05.2020) 10.2034	250 (18 and older)	6/36
4	NCT03391466, KTE-C19-107 (ZUMA-7); phase III (compared to standard treatment)	r/r DLBCL	Enrollment	12.2017 – (01.2022) 01.2035	350 (18 and older)	9/49
5	NCT03704298, KTE-C19-111 (ZUMA-11); phase I/II (combined with Utomilumab)	r/r DLBCL	Enrollment	11.2018 – (01.2021) 06.2035	48 (18 and older)	1 (US)/3
6	NCT03761056, KTE-C19-112 (ZUMA-12); phase II (first-line therapy)	DLBCL	Enrollment	12.2018 – (07.2020) 12.2034	40 (18 and older)	1 (US)/1
7	NCT03105336, KTE-C19-105 (ZUMA-5); phase II (treatment, expected response 70%)	r/r NHL	Enrollment	06.2017 – (03.2020) 03.2034	80 (18 and older)	2/19
8	NCT02601313, KTE-C19-102 (ZUMA-2); phase II	MCL	Enrollment	11.2015 – (06.2019) 03.2034	130 (18 and older)	3/32
9	NCT02614066, KTE-C19-103 (ZUMA-3); phase I/II	r/r ALL	Enrollment	03.2016 – (01.2020) 03.2034	100 (18 and older)	5/32

Note. r/r – refractory/recurrent form; ALL – acute lymphoblastic leukemia; DLBCL – diffuse large B-cell lymphoma; MCL – mantle cell lymphoma; * Non-Hodgkin types of B-cell lymphomas (NHL – non-Hodgkin lymphoma): Refractory Diffuse Large B Cell Lymphoma (DLBCL), Relapsed Diffuse Large B-Cell Lymphoma, Transformed Follicular Lymphoma (TFL), Primary Mediastinal B-cell Lymphoma (PMBCL), High Grade B-cell Lymphoma (HGBCL).

sequence of the above considerations, and the regulatory environment where both indications were submitted under the same application, the below mentioned delegates disagree with the granting of the marketing authorization including both indications on the ground that the potential benefit is considered not to be sufficiently demonstrated for the DLBCL indication" [4].

PRODUCTS BASED ON HUMAN CELLS AND TISSUES PERMITTED FOR USE IN THE EU

In the EU today, two drugs (*Holoclar* and *Zalmoxis*) have conditional registrations based on limited data with the provision to annually update the efficacy and safety reports [11–13].

Holoclar (*Holostem Terapie Avanzate S.R.L.*), a limbal stem cell-based drug for the treatment of eye burns,

Table 2

Comparative characteristics of some aspects of clinical trials of drugs based on chimeric antigen receptors

Clinical study	JULIET	TRANSCEND	ZUMA-1
Drug	*KYMRIAH*	*lisocabtagene maraleucel*	*Yescarta*
Number of patients included	165	134	119
Number of patients receiving therapy	111 (67%)	114 (85%)	108 (91%)
Chemotherapy before use	Yes	Yes	No
Cytokine release syndrome 3/4	22% of 111 patients	1%	11% (12 of 108)
Neurological events	12%	11 (15%) of 73 patients	32% (35)

including chemical burns, received conditional registration from the EMA on the benefit/risk assessment based on the results of two retrospective uncontrolled studies (on the retrospective medical records, HLSTM01 and HLSTM02), with 200 patients since 1998. In addition, the developer must provide additional data from the prospective HLSTM03 study by December 2020 [11, 14–16].

In the CT HLSTM01, *Holoclar* efficacy was evaluated on 104 patients aged 13 to 79 years who received treatment in the presence of moderate and severe limbal stem cell deficiency 12 months after the drug administration. At the time of the drug administration, the average condition duration from the moment of injury was 18 years (median 10 years). A total of 75 cases (72.1%) of effective drug use are reported. These results were confirmed by an independent peer review of pre- and post-*Holoclar* implantation images of patients' eyes based on the assessment of superficial neovascularization [12].

The most serious adverse responses to *Holoclar* are corneal perforation and ulcerative keratitis, which may occur within 3 months of the drug implantation. As the side effects of Holoclar treatment are generally controllable, EMA experts concluded that the benefits outweigh the risks and recommended that it be approved for use in the EU. *Holoclar* has been given the so-called "conditional approval" [11, 12].

Zalmoxis (*MolMed SpA*) based on genetically modified cells (allogeneic T cells genetically modified with a replication-defective γ-retroviral vector encoding a truncated nerve growth factor receptor ΔLNGFR and herpes simplex virus thymidine kinase HSV-TK Mut2) to restore bone immunity after bone marrow transplantation in cancer patients was classified as an unsecured medical need and registered on the basis of two CTs (NCT00423124, NCT00914628) compared to retrospective (historical) control. The control (retrospective comparison) group included patients who underwent haploidentical transplantation and underwent graft versus host disease (GVHD) prevention by two most widely used methods, depletion of T cells in the graft and cyclophosphamide and immunosuppressants after transplant. In total, the effect of drug treatment in 37 patients (23 in NCT00423124 and 14 in NCT00914628) was comparable to the data of 140 patients from retrospective groups who received treatment in 2005–2013. Differences were observed in mortality from recurrences within 1 year: 22% in the drug group and 43% in the control group [13].

The EMA experts' conclusions regarding the benefit-risk of using *Zalmoxis*, given the limited treatment options and poor prognosis for cancer patients and hematopoietic stem cell transplant recipients, included the following aspects:

1. Annual update of the status of included patients and participation of new health institutions. It is important to prove that the sampling size appears to be sufficient for statistically significant differences in overall survival between retrospective comparison and the control groups [13, 17].
2. *Zalmoxis* efficacy has been proven in terms of one-year overall survival growth and reduced mortality from recurrences as compared with the control group.
3. *Zalmoxis* safety profile is considered acceptable. The main risk is GVHD which can be successfully treated with ganciclovir that affects the drug's genetically modified T cells [13, 17].

Alofisel (Darvadstrocel) for the treatment of complex pararectal fistulas in patients with Crohn's disease (adipose tissue stem cells) was approved by the EMA based on placebo-controlled ADMIRE-CD CT (NCT01541579) Phase III: a total of 212 patients were randomized, 205 received local injections of the drug or placebo into the lesion. The patients did not respond to standard treatments such as antibiotics, immunosuppressants, or therapy with tumor necrosis factor (anti-TNF) inhibitors. During the CT, the patients received adjuvant treatment with immunosuppressants (18%), anti-TNF drugs (33%), or with both (28%) [18].

The drug received marketing authorization in the presence of limited data on safety and a small effect compared to control (remission of the disease after 24 weeks in the experimental group occurred 15% more often than in the placebo group, and 17% after 52 weeks), given that the benefits of its use (mainly efficacy) for the treatment of complex anal fistulas that did not respond to standard treatments outweigh the risks. The data on *Alofisel* safety are limited, though there is sufficient information on the nature of side effects. Given the "modest" efficacy compared to standard treatments and the presence of only one large-scale CT (NCT01541579), the benefit/risk data on the drug use will be obtained in the ongoing

multicenter, placebo-controlled phase III CT Cx601-303 (NCT03279081) [19, 20].

Spherox (Co.don AG), a chondrocyte-based drug for the treatment of osteochondral lesions of the knee joint, was approved for use on the basis of the prospective, uncontrolled, phase II CT (with 4-year follow-up) with 75 patients 4–10 cm^2 grade III/IV focal defects of the knee cartilage using three doses of the drug. In all 3 dose groups, there was a significant improvement ($\alpha < 0.05$) on the Knee Injury and Osteoarthritis Outcome Score (KOOS) after 12, 24, 36 and 48 months compared to the state before the drug [21].

Besides, a multicenter, prospective, randomized, controlled phase III CT is ongoing to compare the efficacy and safety of treatment with *Spherox* of cartilage defects (1 to less than 4 cm^2) in the knee condylus and 5-year micro fracturing treatment. The micro fracturing treatment is not recommended for the restoration of large defects (no more than two defects in cartilage of 1 to 1.5 cm^2). The main efficacy data in this CT are based on interim analyzes in 12 and 24 months after treatment. The final report on this CT should be submitted by March 1, 2021 [22].

The EMA has found *Spherox* safety profile acceptable and most of the expected side effects related to surgery (way of the drug administration); the CT should be continued for long-term effects for 5 years [22, 23].

Further, we should dwell turn our attention to the CTs recognized by the EMA regulatory agency during the state registration of a drug for the treatment of such genetic disease as the severe combined immunodeficiency associated with the adenosine deaminase (ADA) gene defect. The drug, *Strimvelis (GSK)*, is based on genetically modified cells and is classified by the disease as an unmet medical need. All CTs of *Strimvelis* were non-randomized, single-group, open; intra-subject comparison was performed before and after treatment; the survival endpoint was compared with the retrospective (historical) control [24, 25]. Given the rarity of the disease (less than 50 children per year in the US and the EU in total), this was accepted as acceptable by the experts of the EU regulatory agency. A total of one main CT (AD 1115611), several pilot CTs (AD 1117056, AD 1117054, AD 1117064) have finished and one CT (AD 1115611, long-term) is ongoing (CTs have been launched since 2000). Given the rarity of the disease, in the marketing application, the applicant provided data on *Strimvelis* efficacy for 12 patients from the main CT and for 6 patients from the pilot CTs [25, 26].

The primary efficacy endpoint in the main study (AD1115611) was survival: 100% survival was observed for all patients with the mean follow-up of 6.9 years, which is higher than the most recent published historical comparative index of 67% overall survival seen in 15 marrow bone transplant patients with the mean follow-up of 6.5 years [24]. The survival rate also exceeds 86 and 83% for HLA-compatible transplant recipients (n = 42) and related donors (n = 12), respectively.

The EMA experts concluded that the evidence on *Strimvelis* efficacy is beyond doubt, but the problem is the use of the drug in centers other than the one where the CT of the drug was performed, due to the short shelf life of the product (6 hours).

As for the safety, there is a risk of insertional mutagenesis due to the use of a retroviral vector in the product manufacture; nevertheless, to date, no cases of leukemia, myelodysplastic syndrome or malignant neoplasm have been noted. The possible occurrence of an autoimmune disease is an issue that can be addressed with adjuvant standard therapy. Accordingly, from a clinical point of view, the benefit/risk ratio of *Strimvelis* is considered positive. However, the registration conditions were to conduct post-marketing CT analysis for the emergence of replication-competent retroviruses, assessment of genotoxicity and immunogenicity, and forming a register of patients treated with *Strimvelis* [25–27].

Zynteglo (bluebird bio) based on genetically modified hematopoietic stem cells transduced with a lentiviral vector encoding the βA-T87Q-globin gene, has received the EMA conditional approval for marketing under PRIME, as it is aimed at treating a serious, recognized orphan disease for which there is an unmet medical need. The stated *Zynteglo* indication is the treatment of patients over 12 years of age with transfusion β-thalassemia (TDT), without β0/β0 genotype, for whom HSC transplantation is required without related donors.

The drug received conditional marketing authorization based on a clinical program comprising 5 CTs: two phase I/II CTs (HGB-205 and HGB-204), three phase II CTs (HGB-207, HGB-212, and a long-term CT LTF-303). As of December 13, 2018, a total of 32 patients, excluding the β0/β0 genotype have been treated (11 adolescents and 21 adults). Age ranged from 12 to 35 years old. At the end of 2018, the follow-up after drug administration continued for about 60 months. Continuing long-term CT LTF-303 includes all patients from previous CTs for 15-year follow-up. The EMA recognized the benefit of the drug treatment in the study population despite the limited number of patients participating in the CT and the lack of long-term safety data. The safety profile includes adverse events (AEs) associated with mobilization, apheresis, and conditioning. As for the use of a lentiviral vector for genetic modification of cells, there is a theoretical risk of insertional mutagenesis. Additional data on *Zynteglo* efficacy and safety will come from ongoing CTs (HGB-207 and HGB-212) as well as long-term patient monitoring in the CT (LTF-303 and REG-501) [28]

CELL THERAPY PRODUCTS AUTHORIZED FOR USE IN THE US

In the US, *Laviv (Fibrocell)*, a drug based on fibroblasts has been approved for use in cosmetology to eliminate nasolabial creases. The registration application for *Laviv* was first filed in 2009 and contained the results of two Phase III CTs (NCT00655356, NCT00649428) conducted between 2006–2009. In total, the drug was used in 210 patients with 211 as control (saline administration). In 2009, the marketing application was rejected because the drug was not intended for a life-threatening condition and safety standards had to be set at a high level. The US regulatory agency required a histological examination of tissue in the area of the drug injection, which was subsequently performed. Based on the CT results, the regulatory agency concluded that the drug had a favorable risk-benefit ratio. However, post-marketing CTs have been recommended by regulators to assess the risks of skin cancer in the injection site and the risk of autoimmune reactions. Fibrocell conducted post-marketing research (NCT02120781) [29]. It should be noted that since September 2016, this technique has no longer been present in the US market, the manufacturer retained its patent rights but reduced the production of the drug due to low demand [30].

CELL THERAPY PRODUCTS AUTHORIZED FOR USE IN JAPAN

In Japan, since November 2013, the clinical use of gene and cell therapy products has been allowed for 7 years, subject to availability of data indicating their efficacy and safety in phase I CT [31].

In Japan, a distinctive feature of CTs of drugs approved for medical use is the small number of patients.

HeartSheet (Terumo Corporation) based on myoblasts for the treatment of severe heart failure in patients who do not respond to standard therapies, was conditionally released on the basis of the CT M-51073-21 with retrospective control with 7 patients; active control was not used due to the complexity of the drug implementation (heart surgery). The primary endpoint of efficacy was the change in left ventricular ejection fraction (LVEF) from baseline to week 26 after transplantation: in 5 patients this parameter did not change, decreasing in 2 patients [32, 33]. However, due to the improvement in exercise tolerance (walking), the effectiveness of this therapy was recognized in 5 out of 7 patients.

The Japanese Pharmaceuticals and Medical Devices Agency made the following conclusions:

1. The CTs of the drug were initially planned as research-purposed; however, the drug has potential therapeutic efficacy for patients who do not respond to standard treatments.
2. A retrospective comparison with patients who receiving other treatments between 2007 and 2014 and have been included in the University of Tokyo Hospital database did not reveal significant differences in condition between the 2 patient groups (*HeartSheet* drug group and other treatment group) within two years after using the drug.
3. As of October 30, 2014, 6 out of 7 patients survived for more than 1 year after using the drug. In two clinical studies (MP0604 and HM0801), 19 more patients were treated with the drug, 2 died in 2.5 years after transplantation, but a causal relationship between deaths and the drug was ruled out. As of September 2015, 14 out of 17 patients were alive for more than 2 years after transplantation, the longest life expectancy was 7 years.
4. Long-term safety of the drug: the pre-clinical trial of the drug efficacy in mini-pigs, drug cells are not detected 13 weeks after transplantation. In the unregistered CTs (MP0604 and HM0801) in humans, cells were not detected on biopsy within several months after transplantation. These results indicate that the drug does not remain in the body for a long time and therefore is unlikely to create safety problems for a long period after transplantation. Due to one case of colon cancer, it is necessary to evaluate the effect of the drug for malignant tumors [32].

JACE (Japan Tissue Engineering Co., Ltd., J-TEC) based on keratinocytes was approved for seven years in 2007 for the treatment of severe burns (over 30% of total body surface) according to the results of the CT J-TEC003 (2004) which includes 2 patients [34]. By 2013, the drug preparation was reported taking place in 4.5 years (since January 2009, drug treatment has been covered by the national health insurance) for 370 burn patients; 35% of the products were ultimately not used due to patients' death or for other reasons; During this time, *JACE* was used in over 240 patients aged from 1 to 80 years [35]. In November 2015, wound treatment after removal of the Giant congenital melanocytic nevi (GCMN) was added to the indications based on the CT 3SI-GCMN001 with 8 patients. According to experts from the Japanese regulatory agency, despite the small number of patients participating in this CT, the presented results are acceptable for the drug registration as a new treatment option for wounds after GCMN removal, since *JACE* efficacy was shown in 95% of cases [36].

The conclusions of the safety experts included the following:

1. At GCMN, the onset of malignant melanoma is possible; however, the disease develops for several years. The long-term follow-up of 52 weeks of the patients after using *JACE* is too short to detect a tumor caused by the drug; however, given that the drug does not include genetically modified cells, the manifestation of tumorigenic potential of cells is unlikely. Besides, the development of tumors was not detected when using the drug for the treatment of burns. Therefore, a

follow-up of 52 weeks to assess the safety of the drug for GCMN treatment was taken as acceptable. Safety data collection will continue after the completion of the CT.

2. Due to the risks associated with xenotransplantation of 3T3-J2 cells obtained from the mouse embryos which are used in the manufacture of the drug as feeder cells, the applicant must ensure the 30-year preservation of the final product and the protocol of use [34].

In January 2016, *JACE* was approved with a re-registration period of 10 years for two indications for use [34, 36].

The registration application was submitted in Japan for *Temcell* (JCR Pharmaceuticals Co., Ltd, previously registered in Canada as *Prochymal*®, Osiris Therapeutics Inc., US) based on mesenchymal stem cells (MSC) of bone marrow (BM). The filed evaluation of the efficacy and safety of the drug included the results from Japan's uncontrolled phase I/II CT (JR-031-201) and its continuation in the CT (JR-031-202) as well as the phase II/III CT (JR-031-301) with acute GVHD patients refractory to corticosteroids; 39 patients were enrolled in three CTs. The reference data presented were complemented by the results of the comparative CT of *Prochymal* conducted by Osiris Therapeutics Inc. (US) with 163 patients (81 controls) [37, 38].

The conclusions of the Japanese regulatory agency experts included the following:

1. Based on the benefits and risks of *Temcell* that were demonstrated in the submitted CTs, *Temcell* can be considered a second-line acute GVHD therapy for patients who do not respond to corticosteroids as a first-line therapy.
2. Since the data on *Temcell* safety is limited, for all patients treated with *Temcell*, the following information should be provided: on deaths, underlying disease recurrences, risks of malignant tumors with the exception of the underlying disease, risks of tumorigenic and carcinogenic properties, risks associated with intravenous infusion of allogeneic cells (events possibly associated with circulatory disorders due to cell embolism and thrombus formation; events possibly associated with intravascular hemolysis; events possibly associated with an immune response); on gastrointestinal bleeding and others [37].

CELL THERAPY PRODUCTS AUTHORIZED FOR USE IN SOUTH KOREA

ClinicalTrials.gov database contains data on 4 CTs of *Immuncell-LC (Green Cross Cell)* based on activated T-lymphocytes for the treatment of hepatocellular carcinoma, but only for one the results are published: CT Phase II/III (NCT00699816) of the indications for use of the drug. 114 patients received the drug, the control group included 112 people that did not receive therapy. The conclusions of the regulatory agency were based on the prolongation of disease-free and overall survival at the drug use for treatment of hepatocellular carcinoma with mild and moderate AEs (Table 3) [39].

Table 3

Data on *Immuncell-LC* (NCT00699816) efficacy

Group	*Immuncell-LC*	Control
Number of patients		
Started CT	115	115
Ended CT	114	112
Not ended CT	1	3
Dropped out due to protocol violence	1	3
Recurrence-free survival in ... months, percentage		
12	79.9	65.1
24	72.5	53.8
36	60.9	44.3
48	49.6	39.6
Total survival in ... months, percentage		
1	2	3
12	100.0	98.0
24	100.0	91.8
36	97.5	88.1
48	95.9	84.8
Median overall survival	n/a	

Adverse events were mild or moderate. Overall, AEs occurred more frequently in the immunotherapy group (62%) than in the control group (41%).

Hearticellgram-AMI (Pharmicell) is approved for the treatment of patients with acute myocardial infarction (MI) through improving the left ventricle function after intracoronary administration; autologous to BM MSC. There are the results of one CT phase II/III (NCT01392105) with parallel control: the experimental group (33 patients) and the control group (36 patients). During the observation period, 58 patients have completed the CT (3 patients from the experimental group and 8 from the control group dropped out to the protocol violation by corticosteroids administration). The conclusions of the regulatory agency included the following: intracoronary infusion of human BM MSCs for 1 month is safe with a slight improvement in left ventricular ejection fraction (LVEF) after 6 months [40].

One of the main issues in the use of MSCs in MI is the time limitation of use of the autologous MSCs in the acute phase: it is impossible to use autologous MSCs immediately, since the collection and cultivation of cells takes more than 3 weeks. However, the optimal time for SC therapy has not been precisely determined. The possible interval for achieving maximum efficacy appears to be between acute inflammatory response and scar formation. Several experimental studies and clinical subgroup analyses suggest that stem cell therapy may be effective during the first month after MI, but not in the acute phase

(24 h after IM). Further randomized CT should confirm the optimal treatment time (NCT01652209) [40].

CONCLUSION

Thus, the analysis of the global experience of conducting CTs and the subsequent approval by the regulatory agencies of the safety and efficacy data for drugs based on human cells – BCPS analogues showed that:

– predominantly, the products were approved for use on the basis of uncontrolled CT II phases;
– in some cases, historical control, placebo, or inclusion to the CT of groups without product usage;
– in most CTs, the number of patients did not exceed 100, with the exception of the cosmetics product (*LAVIV*). The CT had a limited number of patients, especially for drugs for rare genetic diseases (*Strimvelis,* 18 patients) and approved for use in Japan (*HeartSheet,* 7 patients, *JACE,* 2 and 8 patients).

An analysis of reviewing CTs by foreign regulatory agencies showed that the products received marketing authorization considering:

– limited data on efficacy and safety given the inclusion in the CT of a limited number of patients and the timing of the studies;
– "modest" efficacy (*Alofisel* – remission in the experimental group by 15% more often than in the control group) or efficacy with "limitations" (*Zalmoxis* – decrease in mortality from recurrences and increase in one-year survival; *Hearticellgram-AMI* – LVEF (primary point) changed insignificantly, with the increase in exercise tolerance).

The decisions on risk/benefit by EU and US regulators are mainly based on the of the classification as an unmet medical need for national health care or the lack of available treatments for patients who do not respond to standard treatments (*Alofisel, Strimvelis, Zalmoxis, KYMRIAH, Yescarta*). Besides, the availability of a conditional drug registration mechanism in the EU makes it possible to use drugs with a limited safety base for quick access to treatment for patients based on risk/benefit conclusions (*Zalmoxis, Holoclar, Zynteglo*). A prerequisite is long-term CT and the creation of drug patient registries.

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REFERENCES

1. State register of drugs [Internet]. Available from: https://grls.rosminzdrav.ru/Default.aspx.
2. Summary Basis for Regulatory Action: KYMRIAH, ALL [Internet]. – Food and Drug Administration, 2017. Available from: https://www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM577221.pdf.
3. Summary basis for regulatory action – YESCARTA, DLBCL [Internet]. – Food and Drug Administration, 2017. Available from: https://www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM584335.pdf.
4. Assessment report. Kymriah (EMA/462626/2018) [Internet]. – European Medicines Agency, 2018. Available from: https://www.ema.europa.eu/en/documents/assessment-report/kymriah-epar-public-assessment-report_en.pdf.
5. Assessment report. Yescarta (EMA/CHMP/481168/2018) [Internet]. – European Medicines Agency, 2018. Available from: https://www.ema.europa.eu/en/documents/assessment-report/yescarta-epar-public-assessment-report_en.pdf.
6. Summary Basis for Regulatory Action – KYMRIAH, DLBCL [Internet]. – Food and Drug Administration; 2018. Available from: https://www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM606836.pdf.
7. *Van Den Neste E, Schmitz N, Mounier N, Gill D, Linch D, Tmeny M et al.* Outcomes of diffuse large B-cell lymphoma patients relapsing after autologous stem cell transplantation: an analysis of patients included in the CORAL study. *Bone marrow transplantation*. 2017; 52 (2): 216–221. doi: 10.1038/bmt.2016.213.
8. *Van Den Neste E, Schmitz N, Mounier N, Gill D, Linch D, Tmeny M et al.* Outcome of patients with relapsed diffuse large B-cell lymphoma who fail second-line salvage regimens in the International CORAL study. *Bone marrow transplantation*. 2016; 51 (1): 51–57. doi: 10.1038/bmt.2015.213.
9. *Locke FL, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO et al.* Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1–2 trial. *The lancet oncology*. 2019; 20 (1): 31–42. doi: 10.1016/S1470-2045(18)30864-7.
10. *Crump M, Neelapu SS, Farooq U, Van Den Neste E, Kuruvilla J, Westin J et al.* Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. *Blood*. 2017; 130 (16): 1800–1808. doi: 10.1182/blood-2017-03-769620.
11. EPAR summary for the public. Holoclar (EMA/6865/2015) [Internet]. – European Medicines Agency, 2015. Available from: https://www.ema.europa.eu/documents/overview/holoclar-epar-summary-public_en.pdf.
12. Summary of product characteristics**.** Holoclar [Internet]. – European Medicines Agency. Available from: https://www.ema.europa.eu/documents/product-information/holoclar-epar-product-information_en.pdf.
13. Assessment report. Zalmoxis (EMA/CHMP/589978/2016) [Internet]. – European Medicines Agency, 2016. Available from: https://www.ema.europa.

eu/documents/assessment-report/zalmoxis-epar-public-assessment-report_en.pdf.
14. *McBlane JW.* Preclinical safety evaluation [Internet]. – 2016. Available from: http://www.pmda.go.jp/files/000211287.pdf.
15. Summary of the risk management plan (RMP) for Holoclar (*ex vivo* expanded autologous human corneal epithelial cells containing stem cells) (EMA/26006/2015) [Internet]. – European Medicines Agency, 2015. Available from: https://www.ema.europa.eu/documents/rmp-summary/holoclar-epar-risk-management-plan-summary_en.pdf.
16. *Detela G, Lodge A*. EU regulatory pathways for ATMPs: standard, accelerated and adaptive pathways to marketing authorization. *Mol Ther Methods Clin Dev*. 2019;13: 205–232. doi: 10.1016/j.omtm.2019.01.010.
17. EPAR summary for the public. Zalmoxis (EMA/454627/2016) [Internet]. – European Medicines Agency, 2016. Available from: https://www.ema.europa.eu/documents/overview/zalmoxis-epar-summary-public_en.pdf.
18. Summary of product characteristics. Alofisel [Internet]. – European Medicines Agency. Available from: https://www.ema.europa.eu/documents/product-information/alofisel-epar-product-information_en.pdf.
19. Assessment report. Alofisel (EMA/CHMP/64055/2018) [Internet]. – European Medicines Agency, 2018. Available from: https://www.ema.europa.eu/documents/assessment-report/alofisel-epar-public-assessment-report_en.pdf.
20. EPAR summary for the public. Alofisel (darvadstrocel) (EMA/1380/2018) [Internet]. – European Medicines Agency, 2017. Available from: https://www.ema.europa.eu/documents/overview/alofisel-epar-summary-public_en.pdf.
21. Summary of product characteristics. Spherox [Internet]. – European Medicines Agency. Available from: https://www.ema.europa.eu/documents/product-information/spherox-epar-product-information_en.pdf .
22. EPAR summary for the public. Spherox (EMA/326337/2017) Spherox [Internet]. – European Medicines Agency, 2017. Available from: https://www.ema.europa.eu/documents/overview/spherox-epar-summary-public_en.pdf.
23. Summary of risk management plan for Spherox (spheroids of human autologous matrix-associated chondrocytes) [Internet]. – European Medicines Agency. Available from: https://www.ema.europa.eu/documents/rmp-summary/spherox-epar-risk-management-plan-summary_en.pdf.
24. *Hassan A, Booth C, Brightwell A, Allwood Z, Veys P, Rao K et al.* Outcome of hematopoietic stem cell transplantation for adenosine deaminase-deficient severe combined immunodeficiency. *Blood*. 2012; 120 (17): 3615–3624. doi: 10.1182/blood-2011-12-396879.
25. EPAR summary for the public. Strimvelis (EMA/CHMP/249031/2016) [Internet]. – European Medicines Agency, 2016. Available from: https://www.ema.europa.eu/documents/overview/strimvelis-epar-summary-public_en.pdf.
26. Assessment report. Strimvelis (EMA/CHMP/272303/2016 Rev 1) [Internet]. – European Medicines Agency, 2016. Available from: https://www.ema.europa.eu/documents/assessment-report/strimvelis-epar-public-assessment-report_en.pdf.
27. *Cicalese MP, Ferrua F, Castagnaro L, Pajno R, Barzaghi F, Giannelli S et al.* Update on the safety and efficacy of retroviral gene therapy for immunodeficiency due to adenosine deaminase deficiency. *Blood*. 2016; 128 (1): 45–54. doi: 10.1182/blood-2016-01-688226.
28. Assessment report. Zynteglo. [Internet]. – European Medicines Agency, 2019. Available from: https://www.ema.europa.eu/en/documents/assessment-report/zynteglo-epar-public-assessment-report_en.pdf.
29. Summary Basis for Regulatory Action. LAVIV® [Internet]. Available from: http://wayback.archive-it.org/7993/20170723023939/https:/www.fda.gov/downloads/BiologicsBloodVaccines/CellularGeneTherapyProducts/ApprovedProducts/UCM262780.pdf.
30. Fibrocell Science Inc [Internet]. Available from: http://www.annualreports.com/HostedData/AnnualReportArchive/f/NASDAQ_FCSC_2016.pdf.
31. Pharmaceutical administration and regulations in Japan [Internet]. – Japan Pharmaceutical Manufacturers Association, 2017. Available from: http://www.jpma.or.jp/english/parj/pdf/2017.pdf.
32. Report on the Deliberation Results. HeartSheet [Internet]. – 2015. Available from: http://www.pmda.go.jp/files/000215222.pdf.
33. Terumo [Internet] – Available from: http://www.terumo.com/.
34. Review Report. JACE [Internet]. – 2007. Available from: http://www.pmda.go.jp/files/000223079.pdf.
35. JACE. To all of our investors [Internet]. – 2013. Available from: http://www.jpte.co.jp/english/ir/messages/20130626.html.
36. Report on the Deliberation Results. JACE [Internet]. – 2016. Available from: http://www.pmda.go.jp/files/000223080.pdf.
37. Report on the Deliberation Results. Temcell [Internet]. – 2015. Available from: http://www.pmda.go.jp/files/000215658.pdf.
38. Osiris Therapeutics, Inc. [Internet]. Available from: http://www.osiris.com/.
39. *Lee JH, Lee JH, Lim YS, Yeon JE, Song TJ, Yu SJ et al.* Adjuvant immunotherapy with autologous cytokine-induced killer cells for hepatocellular carcinoma. *Gastroenterology*. 2015; 148 (7): 1383–1391. doi: 10.1053/j.gastro.2015.02.055.
40. *Lee JW, Lee SH, Youn YJ, Ahn MS, Kim JY, Yoo BS et al.* A randomized, open-label, multicenter trial for the safety and efficacy of adult mesenchymal stem cells after acute myocardial infarction. *J Korean med sci*. 2014; 29 (1): 23–31. doi: 10.3346/jkms.2014.29.1.23.

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LOCAL INFLAMMATORY RESPONSE TO SUTURE MATERIAL IN SURGICAL PRACTICE: EXPERIMENTAL DATA

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Objective: to study the effect of various types of suture materials, potentially suitable for cardiovascular surgery, on experimental surgical outcomes. **Materials and methods.** Polypropylene sutures (Prolene 6/0), titanium nickelide (TiNi) sutures (6/0) and absorbable polydioxanone sutures (Monoplus 6/0) were used in the study. Male Wistar rats were used for *in vivo* studies. The effect of suture materials on abdominal adhesions was studied. *In vivo* calcification process was examined, and response of blood components in contact with suture materials was also assessed *in vitro*. **Results.** There is a negative inflammatory response to suture materials. The severity of this response depended on the type of material used. Polypropylene sutures demonstrated the most severe inflammatory response provoking massive adhesion formation. In addition, large calcium deposits were found both in the suture area and in the thickness of the biomaterial, stitched with prolene and implanted subcutaneously in the rats. Titanium nickelide sutures showed high hemocompatibility and biocompatibility. The Monoplus sutures caused minimal inflammatory response and provoked calcification of the biomaterial to a lesser degree. **Conclusion.** The suture material could have significant effects on surgical outcomes and could cause postoperative complications.

Keywords: suture material, cardiovascular surgery, adhesion, calcification, hemocompatibility.

INTRODUCTION

Among unsolved challenges for cardiovascular surgery, there are complications closely related to the quality of the used suture material [1–5]. Since the tissue reaction to suture material is similar to the response to implanted foreign body, it is natural that sutures of unsatisfactory quality can result in postoperative complications. After the suture material contacts surrounding tissues, a classic pathophysiological reaction to a foreign body develops, the essence of which is an inflammatory reaction [6, 7]. The intensity of this reaction and its consequences largely depend on the chemical composition and structure of the suture material [1, 3, 6]. When suture material is used in vascular surgery, a large amount of such blood proteins as albumin, γ-globulins, and fibrinogen, are entrapped at the "prosthesis – patient artery" interface within a few minutes after the anastomosis is applied [7–9]. This is followed by the coagulation system and the complement system activation which can gradually lead to thrombus formation and aseptic inflammation [7, 8].

In Russia, the number of reparative surgeries on various vascular basins is annually increasing; in particular, there is a significant increase in arterial reconstructions. In 2016, the total number of reoperations significantly exceeded those in previous years and amounted to 71,810, which is 20% more than in 2014, and 26% more than in 2012 [9, 10]. Thrombosis of the vascular graft is the most common complication after these operations. The reasons contributing to the thrombus formation in the anastomotic zone include damage to the vessel walls, especially the intima, as well as the presence of surgical suture material protruding into the vessel lumen, only aggravating the situation [11, 12]. In this regard, special requirements are imposed on suture materials directly contacting blood: they should not negatively affect blood and its components, i.e. should be maximally hemocompatible.

Cardiovascular surgery uses absorbable and non-absorbable sutures, but the most commonly applied suture material from the polyolefin group is polypropylene, which is considered to be highly inert and strong. At the same time, some researchers report that polypropylene sutures can cause a local aseptic inflammatory reaction which can turn into a chronic inflammatory process in the vascular anastomosis area and cause neointimal hyperplasia development [4, 5].

In addition to the development of neointimal hyperplasia, there are some life-threatening complications after the cardiovascular system surgery including such processes as calcification and adhesion, which also result from chronic inflammation in the surgery site [13]. When studying the long-term results of valved biological conduits, the signs of calcification along the line of bioprostheses fixation have been noted, indicating a conceivable effect of suture material upon the mineralization process [14]. In addition to all of the above, suture material can cause a pronounced adhesive process in the mediastinum which, in turn can lead to adhesions of the heart and large great vessels with the posterior surface of

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the sternum. In cardiovascular surgery, the complication significantly increases the risk of serious adverse events in case of reoperation [15, 16].

Purpose: to perform a comparative analysis of the effect of biodegradable and non-biodegradable suture material on the development of postoperative complications in cardiovascular surgery.

MATERIALS AND METHODS

In the present study, Prolene 6/0 (Ethicon, USA) suture was used, most common in cardiovascular surgery. In comparison, the properties of absorbable suture material of Monoplus 6/0 (B. Braun, Germany) polydioxanone recommended for use in pediatric cardiovascular surgery were evaluated [2].

In vivo studies were made on Wistar subpopulation male rats, 10 animals in each group. All manipulations with laboratory animals were carried out under inhalation isoflurane (2.0%) anesthesia in a clean operating room in accordance with the Interstate Standard, Guidelines for the Care and Care of Laboratory Animals, in compliance with the Rules for the Maintenance and Care of Laboratory Rodents and Rabbits (Russian GOST 33216-2014) and the Rules for the equipment of premises and organization of procedures (Russian GOST 33215-2014).

Adhesion simulation

To study the role of suture material in the development of the adhesive process, 3–4 stitches of absorbable and non-absorbable suture materials were applied to the parietal side of animal peritoneum (200–250 g weight) under sterile conditions. The animals were taken out of the experiment after 7, 14 and 28 days. The removed "peritoneum – suture material – adhesions" complexes were examined by light microscopy with Axio Imager. A1 (Zeiss, Germany). The histological preparations were hematoxylin-eosin and Van Gieson stained.

Modeling accelerated calcification

Calcification was simulated using porcine aortic valve cusps preserved with ethylene glycol diglycidyl ether (EGDE) where several stitches of the test suture materials were applied. After that, biomaterial samples were implanted into the rat (55–65 g weight) subcutaneous pockets for 60 days. Ca amount in the removed samples was determined with Lambda-5100 (PerkinElmer, USA) atomic absorption spectrophotometer and calculated per 1 mg of dry tissue. The biomaterial structure after subcutaneous implantation was studied by light microscopy, and hematoxylin-eosin and Van Gieson staining of histological preparations.

Modeling systemic blood flow *in vitro*

The reaction of blood components upon contact with suture material was evaluated in *in vitro* test. For this, segments of the cattle internal thoracic artery preserved with EGDE were sutured with the polypropylene and polydioxanone-based material (forming a vascular anastomosis). Then, the anastomoses samples (L = 6 cm; d = 4 mm) were fixed on the pipe fittings of the 205CA multichannel peristaltic pump (Watson-Marlow, UK). The lines with fixed samples were filled with fresh citrated donor blood. The blood circulation rate was 0.04 L/min at 37 °C, with 30 min contact time. The microscopic assessment of the anastomosis area after contact with blood was studied by scanning electron microscopy (SEM) with S-3400N microscope (Hitachi, Japan). For this, a gold-palladium coating was applied to the studied sample surface by ion sputtering with the EmitechSC 7640 vacuum post (Quorum Technologies, UK).

The quantitative data were processed by conventional statistical methods with STATISTICA 6.0 data analysis and visualization program for processing medical and biological information (StatSoft Inc., USA). The nature of the distribution in the samples was assessed with Kolmogorov–Smirnov test. Distribution in the groups differed from normal ($p < 0.01$). Data are presented as mean and error of the mean. The statistical significance of the differences between two independent groups was assessed with Mann–Whitney U-test; the differences were considered significant at a significance level of $p < 0.05$.

RESULTS

Macroscopic description

7 days after surgery, adhesions were formed in the abdominal cavity of the animals, tightly clinging to the suture material. The adhesions corresponded to the phase of novice adhesions and had a loose structure. The most pronounced inflammatory reaction of the surrounding tissues was observed when polypropylene sutures were used, the adhesions had a denser structure and were separated only by sharp dissection (Fig. 1, a). A significantly less inflammatory reaction was observed with the biodegradable Monoplus suture (Fig. 1, b). The adhesion process with the polydioxanone suture was less expressed in the entire study group. The adhesions had a filmy, non-cohesive structure which could be separated by blunt dissection.

The macroscopic examination of the removed valves preserved with EGDE and stitched with Prolene and Monoplus sutures showed the presence of calcium deposits in the biomaterial thickness. Calcification was observed in all test samples, but its size varied depending on the type of suture used. In the intact samples preserved with EGDE (control), Ca was absent indicating that it was the suture material that caused the calcification of the test samples.

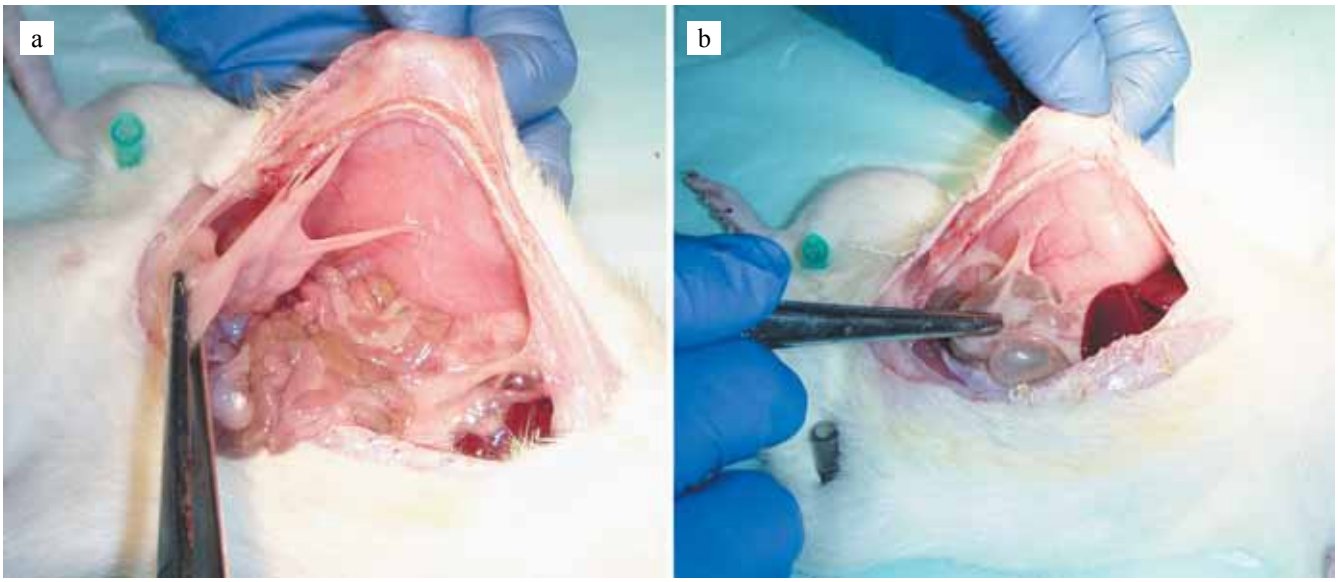

Fig. 1. The rate of adhesion formation after using a different suture material: a – Prolene; b – Monoplus

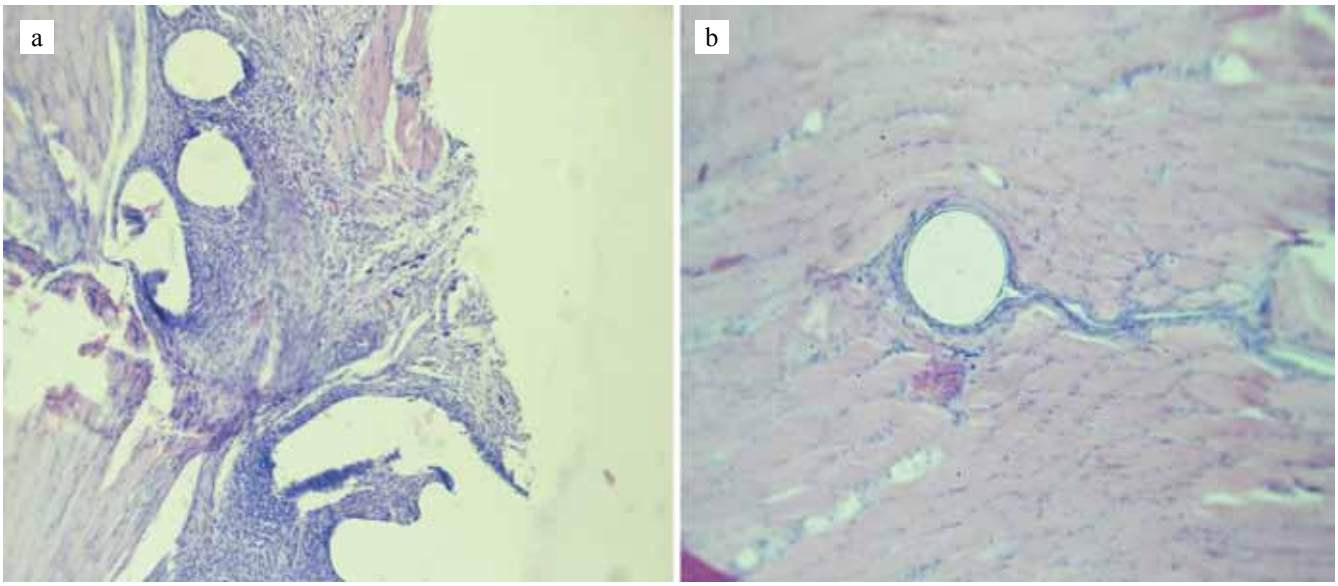

Fig. 2. Histological sections of the adhesion-peritoneum following the use of different suture material: a – Prolene; b – Monoplus. Stained with hematoxylin-eosin, ×200

Histology

Histological examination of the removed fragments showed granulation tissue formed in large amount around the sutures and massive lymphocytic infiltration with the formation of blood vessels. Smooth muscle cells were found in histological sections. This pattern is especially characteristic for Prolene sutures (Fig. 2, a). Collagen fibers were fragmented, had a loose fibrous capsule with a large number of capillaries indicating a pronounced inflammatory process around the suture material. The use of polydioxanone biodegradable sutures made it possible to minimize the destruction of collagen fibers and reduce the number of inflammatory cells in the peri-suturing area (Fig. 2, b).

Microscopic examination of the cusp tissue revealed large and small granular calcium deposits, mainly in the peri-suturing zone. Hematoxylin-eosin staining of the samples confirmed the presence of calcium phosphate. In the samples with Monoplus sutures, fine-grained calcium deposits were revealed, mainly around the suture material and in the spongy layer (Fig. 3, b). Outside the calcifications, collagen fibers retained crimp and compact arrangement. When using the Prolene suture, large calcium deposits were detected both in the suture material and in the thickness of the biomaterial (Fig. 3, a). With large calcium deposits, collagen fibers acquired a loose arrangement, fragmented in places.

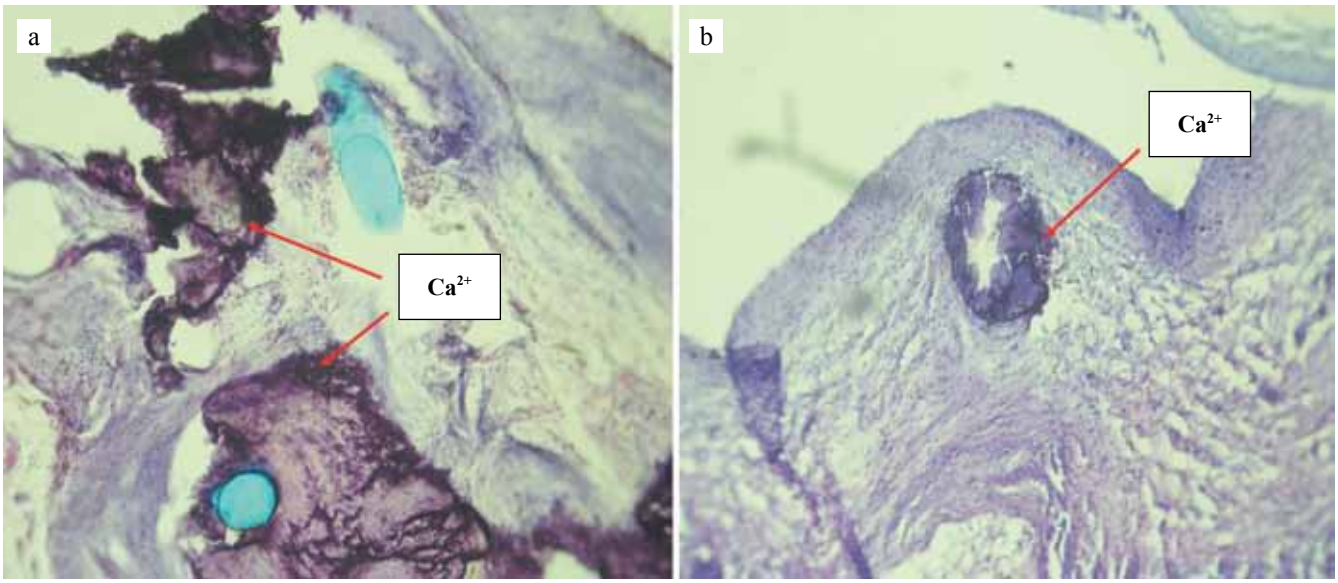

Fig. 3. Formation of calcification around retained suture material: a – Prolene; b – Monoplus. Stained with hematoxylin-eosin, ×200

Quantifying calcium level in explanted samples

2 months after implantation, the quantitative determination of the Ca level in the test samples confirmed that the use of polypropylene sutures largely provokes Ca accumulation in the biomaterial. In samples with Prolene sutures, Ca level was 151.2 ± 4.8 mg/g, while in the control samples of cusps preserved with EGDE without suture material, Ca level slightly exceeded metabolic and amounted to 2.4 ± 0.35 mg/g ($p < 0.05$). With Monoplus suture material, Ca level in the biomaterial was significantly lower than with Prolene, of up to 36.0 ± 3.1 mg/g ($p < 0.05$).

SEM

When examining by scanning electron microscopy, after their contact with blood, the anastomoses areas performed with two types of suture materials, a noticeable difference in the structure of protein deposits was found. The data obtained after 30 min of contact of the samples with blood showed that deposits of proteins with blood corpuscles appear in the anastomoses area. When using Prolene suture, the protein deposits were the most massive, with a loose and coarse structure (Fig. 4, a). Anastomosis with the polydioxanone suture had less loose protein deposits (Fig. 4, b).

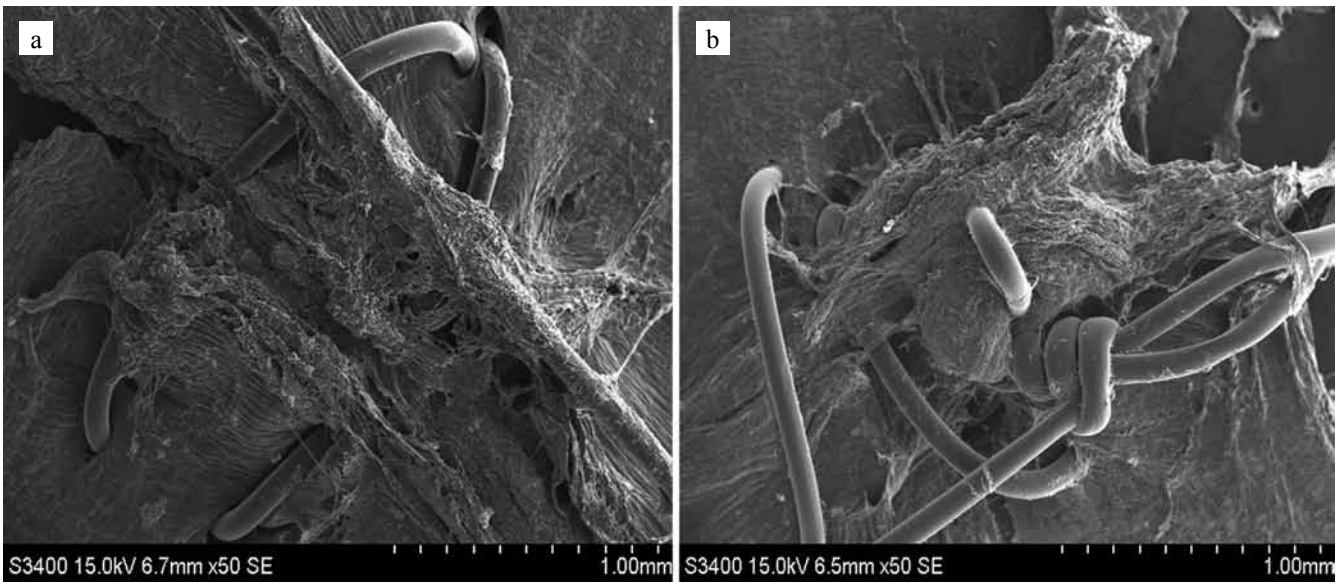

Fig. 4. Scanning electron microscopy of anastomotic sutures: a – Prolene; b – Monoplus, ×50

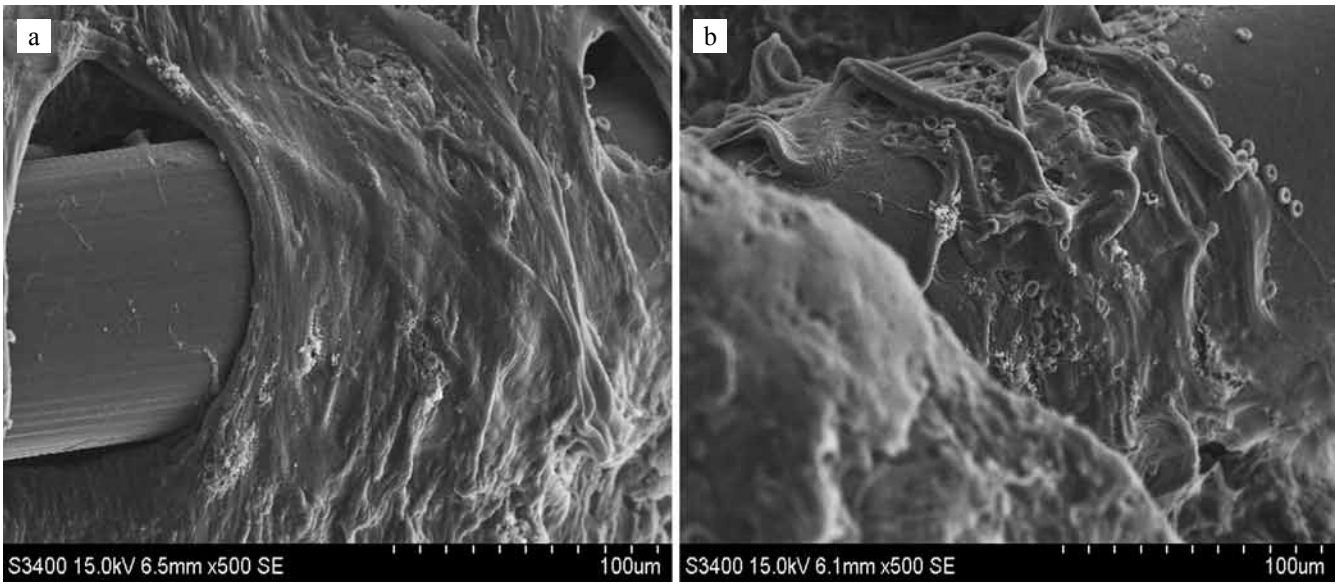

Fig. 5. Scanning electron microscopy of anastomotic sutures: a – Prolene; b – Monoplus, ×500

The differences in the reaction of the blood cell elements to the suture material are most clear at 500x (Fig. 5). The surface and surroundings of the polypropylene suture, significant fibrin and erythrocytes accumulations were seen, partially transformed into spherocytes and echinocytes (Fig. 5, a). The change of the disc-shaped form of erythrocytes into spherocytes or echinocytes is due to a negative effect, i.e. the erythrocytes reaction to a foreign body, while the transformed membrane of the altered erythrocyte tends to hemolysis [19].

The surface of the polydioxanone suture had a thin fibrin fibers network on which erythrocytes, partially transformed into echinocytes and spherocytes, were adhered (Fig. 5, b).

DISCUSSION

The comparative analysis of two types of surgical suture material showed the advantage of a biodegradable suture over polypropylene. The obtained results are consistent with the data of other studies which showed that polypropylene suture caused a more intense inflammatory response compared to biodegradable suture material [2, 13, 18]. A pronounced adhesion process, calcification, transformation of erythrocytes into echinocytes provoked by suture material based on polypropylene indicate a negative effect of the suture. The transformation of erythrocytes into echinocytes indicates low hemocompatible properties of the polypropylene suture, since such changes in erythrocytes are observed mainly during extensive surgical interventions [19] and can lead to violations of the aggregation characteristics of blood, increasing its viscosity, and as a consequence, increased risk of thrombosis of the vascular anastomosis area.

The use of a biodegradable polydioxanone suture material led to a lesser inflammatory reaction, and as a consequence, less calcification of tissues in the perisuture area. The adhesion process based on tissue injury as well as a reaction to the suture material showed a significant advantage of the biodegradable suture material over polypropylene suture. The evaluation of the effect of Monoplus suture on protein sorption in the vascular anastomosis area did not show significant advantages over polypropylene suture; nevertheless, the absence of transformed blood cells indicates higher hemocompatible properties of polydioxanone sutures.

CONCLUSION

The results of the present study show the negative reaction to the suture material and the degree of its severity depending on the type of material used. The most striking inflammatory response was demonstrated by polypropylene-based suture material, while it is polypropylene suture that is widely used in cardiovascular surgery. The polypropylene suture also significantly enhances the inflammatory response, adhesion, and calcification of the surrounding tissues. The biodegradable polydioxanone-based suture material has demonstrated significant advantages over polypropylene sutures. Monoplus suture causes less inflammation, calcification, and adhesion.

The authors declare no conflict of interest.

REFERENCES

1. *Holzheimer RG.* Adverse events of sutures: possible interactions of biomaterials? Eur *J Med Res*. 2005; 10 (12): 521–526.
2. *Gasbarro V, Traina L, Mascoli F, Coscia V, Buffone G, Grande R et al.* Absorbable suture material in carotid sur-

gery. *Vasa*. 2015; 44 (6): 451–457. doi: 10.1024/0301-1526/a000468.
3. *Lock AM, Gao R, Naot D, Coleman B, Cornish J, Musson DS.* Induction of immune gene expression and inflammatory mediator release by commonly used surgical suture materials: an experimental *in vitro* study. *Patient Saf Surg*. 2017; 11: 16. doi: 10.1186/s13037-017-0132-2.
4. *Beloyarcev DF, Talybly OL, Volkov SK.* Sostoyanie bifurkacii sonnoj arterii posle operacii s primeneniem rassasyvayushchegosya shovnogo materiala. *Angiologiya i sosudistaya hirurgiya*. 2015; 21 (4): 192–197.
5. *Bae S, DiBalsi MJ, Meilinger N, Zhang C, Beal E, Korneva G. et al.* Heparin-eluting Electrospun Nanofiber Yarns for Antithrombotic Vascular Sutures. *ACS Applied Materials a Interfaces*. 2018; 10 (10): 8426–8435. doi: 10.1021/acsami.7b14888.
6. *Selvi F, Cakarer S, Can T, Kirli Topcu Sİ, Palancioglu A, Keskin B et al.* Effects of different suture materials on tissue healing. *J Istanb Univ Fac Dent*. 2016; 50 (1): 35–42. doi: 10.17096/jiufd.79438.
7. *Anderson JM, Rodriguez A, Chang DT.* Foreign body reaction to biomaterials. *Semin Immunol*. 2008; 20 (2): 86–100. doi: 10.1016/j.smim.2007.11.004.
8. *Moore LB, Kyriakides TR.* Molecular Characterization of Macrophage-Biomaterial Interactions. *Adv Exp Med Biol*. 2015; 865: 109–122. doi: 10.1007/978-3-319-18603-0_7.
9. *Kudryavceva YuA, Zinchenko SS, Zhuravleva IYu, Ivanov SV, Barbarash LS.* Sorbcionnye i kontaktno-aktivacionnye svojstva zony anastomoza “bioprotez–arteriya”: vliyanie shovnogo materiala (soobshchenie II). *Angiologiya i sosudistaya hirurgiya*. 2008; 14 (1): 113–117.
10. *Pokrovskij AV, Ivandaev AS.* Sostoyanie sosudistoj hirurgii v Rossii v 2016 godu. *Rossijskoe obshchestvo angiologov i sosudistyh hirurgov*. 2017: 5.
11. *Thevenot P, Hu W, Tang L.* Surface chemistry influences implant biocompatibility. *Curr Top Med Chem*. 2008; 8 (4): 270–280. doi: 10.2174/156802608783790901.
12. *Kur’yanov PS, Razuvaev AS, Vavilov VN.* Giperplaziya intimy v zone sosudistogo anastomoza. *Angiologiya i sosudistaya hirurgiya*. 2008; 14 (4): 146–151.
13. *Kastellorizios M, Tipnis N, Burgess DJ.* Foreign Body Reaction to Subcutaneous Implants. *Adv Exp Med Biol*. 2015; 865: 93–108. doi: 10.1007/978-3-319-18603-0_6.
14. *Latypov AK.* Kliniko-funkcional’naya ocenka biologicheskih klapanosoderzhashchih konduitov v posleoperacionnom periode u detej. [Dissertation]. Novosibirsk, 2005, 23.
15. *Krane M, Bauernschmitt R, Hiebinger A, Wottke M, Voss B, Badiu CC et al.* Cardiac reoperation in patients aged 80 years and older. *Ann Thorac Surg*. 2009; 87 (5): 1379–1385. doi: 10.1016/j.athoracsur.2009.01.045.
16. *Svobodov AA, Zelenikin MA.* Sposob bezopasnoj resternotomii. *Grudnaya i serdechno-sosudistaya hirurgiya*. 2008; 1: 66–67.
17. *Gyunter VEh, Hodorenko VN, Chekalkin TL, Olesova VN, Dambaev GC, Sysolyatin PG i dr.* Medicinskie materialy s pamyat’yu formy. Medicinskie materialy i implantaty s pamyat’yu formy. T. 1. Tomsk, 2011, 534.
18. *Ye X, Wang Z, Zhang X, Zhou M, Cai L.* Hemocompatibility research on the micro-structure surface of a bionic heart valve. *Biomed Mater Eng*. 2014; 24 (6): 2361–2369. doi: 10.3233/BME-141049.
19. *Shen X, Su F, Dong J, Fan Z, Duan Y, Li S. In vitro* biocompatibility evaluation of bioresorbable copolymers prepared from L-lactide, 1, 3-trimethylene carbonate, and glycolide for cardiovascular applications. *J Biomater Sci Polym Ed*. 2015; 26 (8): 497–514. doi: 10.1080/09205063.2015.1030992.

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USE OF PEROXIREDOXIN FOR PRECONDITIONING OF HETEROTOPIC HEART TRANSPLANTATION IN A RAT

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Peroxiredoxin 6 (Prdx6) is an antioxidant enzyme in the human body that performs a number of important functions in the cell. Prdx6 restores a wide range of peroxide substrates, thus playing a leading role in maintaining redox homeostasis in mammalian cells. In addition to peroxidase activity, Prdx6 has an activity of phospholipase A2, thus taking part in membrane phospholipid metabolism. Due to its peroxidase and phospholipase activity, Prdx6 participates in intracellular and intercellular signal transmission, thereby facilitating the initiation of regenerative processes in the cell, suppression of apoptosis and activation of cell proliferation. Given the functions performed, Prdx6 can effectively deal with oxidative stress caused by various factors, including ischemia-reperfusion injury. On an animal model of rat heterotopic heart transplantation, we showed the cardioprotective potential of exogenous recombinant Prdx6, introduced before transplantation and subsequent reperfusion injury of the heart. It has been demonstrated that exogenous Prdx6 effectively alleviates the severity of ischemia-reperfusion injury of the heart by 2–3 times, providing normalization of its structural and functional state during heterotopic transplantation. The use of recombinant Prdx6 can be an effective approach in preventing/alleviating ischemia-reperfusion injury of the heart, as well as in maintaining an isolated heart during transplantation.

Keywords: ischemia-reperfusion injury, peroxiredoxin, heterotopic heart transplantation.

INTRODUCTION

One of the key problems of cardiac surgery and transplantology is ischemia-reperfusion myocardial injury [13, 14, 16, 18]. Disruption of normal blood flow and the inadequacy of oxygen demand and delivery to tissues trigger a cascade of pathological ischemic processes leading to the formation of reactive oxygen species (ROS) and disruption of the structural and functional integrity of metabolic active tissues. Restoration of oxygenated blood flow (reperfusion) to ischemic tissues leads to an even greater ROS increase, the development of oxidative stress and aggravates the damage to myocardial tissues [16, 17]. This formidable complication occurs almost always, and only the level of damage can vary. Today, the number of proven and effective approaches to reducing the damaging effect of reperfusion is extremely small [12, 15].

Since the pathogenesis of ischemia-reperfusion injury (IRI) is associated with oxidative stress, the main direction in therapy may lie in reducing ROS concentration in the affected tissues using antioxidant drugs [1, 4].

Among many antioxidant enzymes, the most attractive is the family of peroxiredoxins (Prx) [5]. Prx play an important role in maintaining redox homeostasis in mammals. As a rule, their level increases with oxidative stress contributing to the normalization of ROS level in ischemic tissues. Among the family of peroxiredoxins, Prx6 is featured by the widest range of neutralized peroxide substrates of organic and inorganic nature including alkyl hydroperoxides, phospholipid peroxides, long-lived protein radicals, peroxynitrite, etc. [7]. Given the role of Prx6 in tissue protection against adverse effects, the use of Prx6 in transplantation should be explored to improve the safety of donor organs.

Purpose. To assess the possibility of using peroxiredoxin (Prx6) as a means to increase the resistance (preconditioning) of the myocardium to IRI.

TASKS

With a biological model of heterotopic rat heart transplantation, to compare the degree of damage to the donor heart in terms of troponin I concentration, rhythm disturbances and myocardial contractility, and to assess myocardial morphology in the group of animals receiving Prx6 and without Prx6.

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MATERIALS AND METHODS

The experiments used diclinous Wistar rats (250 g weight). The experimental program was approved by the Committee on Biological Safety and Bioethics. The experiments were performed in compliance with the rules of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes and Directive 2010/63/EU.

All rats were divided into 2 groups of 20 animals each. In group 1 (control group), 20 hearts of donor animals were transplanted into recipient animals by heterotopic transplantation. In group 2, the recipient animals underwent heterotopic heart transplantation from 20 donor animals with Prx6 introduced at the stage of reperfusion.

Recombinant Prx6 was obtained at the laboratory of reception mechanisms of the Institute of Cell Biophysics of the Russian Academy of Sciences by the previously described technique [19].

The model of a heterotopic rat heart transplant comprised the stages of donor anesthesia, heart explantation, heart storage in Custodiol solution, anesthesia of the recipient, transplantation of the donor heart to the recipient's abdominal aorta, wound closure, and the recipient eduction. The operated animals were placed in a vivarium for 24 hours under observation. The rats were kept in standard cages with a heating pad and *adlibitum* water supply under 12-hour day/night cycle. 24 hours after the operation, the animals were euthanized for histological examination of the state of myocardial tissues.

Heart explantation stage. After treating the surgical site with an antiseptic solution, a complete midline laparotomy was performed, the inferior vena cava (IVC) and abdominal aorta were isolated from the surrounding tissues below the renal arteries. Heparin solution (20 U) was injected into the IVC, then a micro-clip was applied to the injection site to prevent bleeding. Next, the aorta was cannulated with a 22G catheter, and continuous perfusion with 100 ml Custodiol cardioplegic solution was initiated through the infusion pump for 7 min. To decompress the right and left sections, the IVC and left pulmonary veins were transected. After the onset of cardioplegia, a median sternotomy was performed, and the edges of the wound were diluted with a dilator. The donor's heart was covered with ice. At the end of cardioplegia, cardiac explantation was started. To do this, step by step, the inferior and superior vena cava, then the aorta and the pulmonary trunk were isolated and ligated. The aorta was transected at the level of the origin of the brachiocephalic trunk, and the pulmonary trunk at the level of the bifurcation. The pulmonary veins were ligated in a single block. The inferior and superior vena cava were ligated separately.

After explantation, the donor heart was placed in a sterile container with a Custodiol solution. The container was covered with ice and stored at +4 °C for 4 hours until implantation. The total ischemia time was 5 hours.

The recipient was *anesthetized* in the same way as the donor.

Transplantation of a donor heart into the recipient's abdominal aorta. After anesthesia, a complete midline laparotomy was performed, the small intestine loops were removed to the left in relation to the operating wound and covered with a damp gauze wad to prevent drying. The wound edges were parted with a retractor, and the aorta and inferior vena cava were exposed in the infrarenal section. The lumbar branches, 3–4 permanent branches on average, were sutured. After lumbar vein ligation and vascular mobilization, 20 U heparin solution was injected into the inferior vena cava, a micro clip was applied to the injection site. A few minutes later, a vascular clamp was applied to the inferior vena cava and aorta in the proximal and distal directions. The recipient's aorta was cut longitudinally, and the aortic lumen was washed with heparinized saline to remove blood from the lumen. The heart graft was placed into the abdominal cavity, an end-to-side anastomosis was applied with a 10/0 atraumatic needle on a piercing needle. Then the recipient vena cava was opened longitudinally, and an end-to-side anastomosis was applied between the recipient's inferior vena cava and the donor pulmonary artery with a similar suture material. After performing the anastomoses, Antegrade Prx6 injection was performed at a calculated dose of 3 mg, the distal clamp was removed, filling the donor heart with blood. The donor's aorta was punctured with a 10/0 needle to prevent air embolism. Then the proximal forceps were removed. The restoration of cardiac activity occurred spontaneously. In case of disturbances in the implanted heart rhythm, electrical stimulation (ES) was used with an ЭКСН-4М pacemaker with a heart rate of 110 bpm and 6 mA amplitude. After controlling hemostasis, the loops of the small intestine were returned to the abdominal cavity, and 6/0 silk sutures were placed on the anterior abdominal wall (separately on the aponeurosis). The skin was sutured with a continuous 5/0 PET suture and antiseptic-treated. After the cessation of the inhalation anesthetic, the animal was taken out of anesthesia for 5 minutes. Then the recipient was placed in a standard cage with a heating pad and access to water.

To assess the effectiveness of Prx6 as a means for increasing myocardial resistance to IRI, the following parameters were analyzed:

– time to spontaneous restoration of the rhythm, intensity of cardiac activity, and myocardial kinetics;
– TnI concentration in blood.

A histological examination was also performed including Masson's and hematoxylin/eosin staining of myocardial preparations.

The spontaneous recovery of the heart rhythm was assessed in terms of the time from the moment of removal

of the proximal and distal clamps and the start of blood flow at the site of implantation of the donor heart to the appearance of electrical activity of the heart and visual signs of myocardial contraction, as well as the duration of the required temporary pacemaker. To confirm the data, 1-lead ECG was performed. Four electrodes were applied on the surrounding tissue around the graft. In addition to objective methods of control, the intensity of cardiac activity and myocardial kinetics were determined by intraoperative palpation assessment of the strength of heart contractions on the left ventricle (high/low) and the total filling of the graft chambers (high/low).

TnI concentration was determined in 60 minutes after the start of reperfusion and 24 hours after transplantation and restoration of blood circulation. TnI concentration was recorded by i-Stat System (Abbott Point of Care, USA) analyzer with TnI assay cartridges (Abbott Point of Care, USA). The initial TnI concentration did not exceed 0.01 ng/ml which corresponds to the norm.

The graft was retrieved for histology in 24 hours after the transplantation. For histological studies, myocardial samples were fixed in 10% formaldehyde solution. Images of histological sections were obtained using a Carl Zeiss Axio lab A1 microscope.

Table

Baseline characteristic of procedure and intraoperative parameters

	Group 1, n = 20	Group 2, n = 20	p
Animal weight, g	250 ± 7	250 ± 8	1
Duration of surgery, min	62.4 ± 5.2	71.4 ± 5	0.003
Duration of explantation, min	13.8 ± 1.5	14.3 ± 1.7	0.5
Duration of graft ischemia, min	305.3 ± 3.6	304.9 ± 2.7	0.8
Duration of graft perfusion with Custodiol, min	7	7	1
Custodiol volume for graft perfusion, ml	100	100	–
Myocardial contractile activity, % high low низкая	 0 30 70	 90 0 10	 0.0001

Note. Group 1 – rats after heterotopic heart transplantation, group 2 – rats after heterotopic heart transplantation with Prx6 administration 3 mg during reperfusion. There is standard deviation after "±".

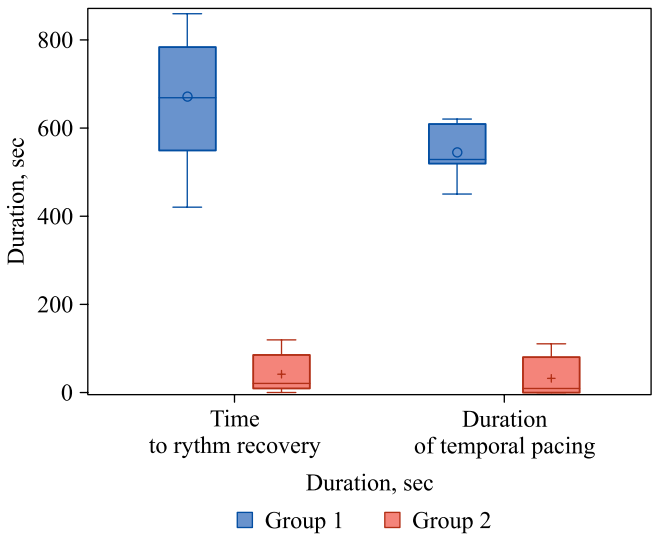

Fig. 1. Time to rhythm recovery of transplant and duration of temporal transplant pacing. Group 1 – rats after heterotopic heart transplantation, group 2 – rats after heterotopic heart transplantation with Prx6 administration 3 mg during reperfusion. There are statistically significant differences between groups: time to rhythm recovery of, p = 0,002; duration of temporal transplant pacing, p = 0,0001

Statistical data processing

All studied parameters were checked for normality of distribution [11]. In the case of a normal distribution, the t-test was used to compare the groups; when it differed from normal, Mann–Whitney test was used [10]. Qualitative indicators were compared with exact chi-squared test. Statistical data processing and plotting were performed with the SAS Enterprise Guide 6.1 licensed software.

RESULTS

Baseline characteristic of procedure and intraoperative parameters are given in Table.

Contractive activity of the transplanted heart

The contractile activity and the parameters of the electrical activity of the myocardium were better in group 2 (Table, Fig. 1).

Biochemical analysis of myocardial damage level

Myocardial injury in group 2 was significantly less than in group 1 (Fig. 2).

Histology of transplanted heart

Histology of myocardial fragments in group 2 showed the greater tissue structure preservation compared with the control group (Fig. 3, 4).

DISCUSSION

To date, 6 types of Prx have been identified in mammals, which, according to the number of conserved cysteine residues in the active site and mechanisms of catalysis, are subdivided into typical 2-Cys (Prx1–4), atypical 2-Cys (Prx5) and 1-Cys (Prx6). Besides the ability to neutralize a wide range of ROS, Prx have a number

of other important functions, among them the initiation of regenerative processes in cells due to chaperone and signal-regulatory activities [2, 8]. Of particular interest among mammalian peroxiredoxins is Prx6 capable of neutralizing a wide range of peroxide substrates of both inorganic and organic nature, including alkyl hydroperoxides, phospholipid peroxides, long-lived protein radicals, peroxynitrite, etc. [7]. Besides its peroxidase activity, Prx6 exhibits the activity of Ca2+-independent phospholipase A2 (aiPLA2), which normally manifests itself only under acidic conditions (at pH 4–5) is important in the metabolism of phospholipids and the transmission of intracellular and intercellular signals [3]. Prx6-knockout animals are featured by increased sensitivity to oxidative stress [3, 9]. Exogenous Prx6 has been shown to realize its signal-regulatory function through the TLR4/NF-kB pathway [7]. Thus, Prx6 is a multifunctional enzyme that participates in many cell processes and plays an important key role in antioxidant protection. It should be noted that the amount of intrinsic endogenous Prx6 synthesized in ischemic tissues is insufficient to suppress the development of oxidative stress. At the same time, the introduction of exogenous recombinant human Prx6 changes the situation. Thus, a high therapeutic activity of exogenous Prx6 has been demonstrated in laboratory rats in vivo [6]. At the same time, no toxic effects were observed when high doses of recombinant Prx6 were introduced into the body of laboratory animals. The introduction of Prx6 before or after an adverse effect contributes to the preservation or rapid restoration of the morphofunctional state of tissues, which may indicate a high therapeutic efficiency of the protein [3].

Due to these features, Prx6 can be considered a potential agent in perfusion solutions for the preservation and subsequent transplantation of isolated organs.

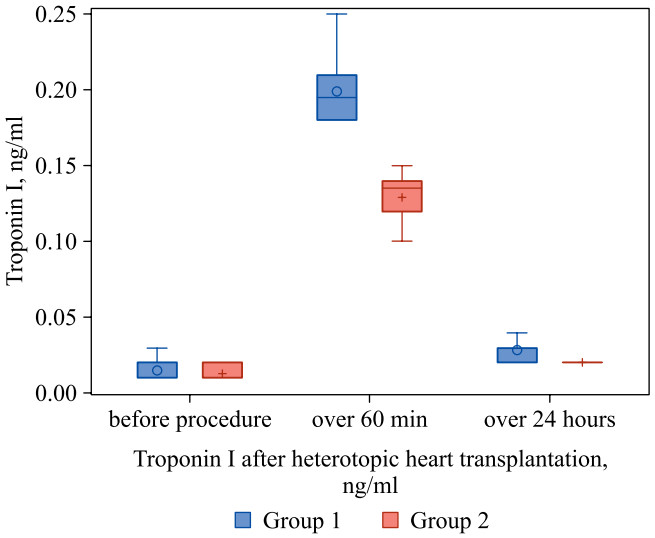

Fig. 2. Dynamic of troponin I level after heterotopic heart transplantation. Group 1 – rats after heterotopic heart transplantation, group 2 – rats after heterotopic heart transplantation with Prx6 administration 3 mg during reperfusion. Upper reference limit of troponin I is 0,05 ng/ml

Based on the data obtained, it can be assumed that Prx6 mitigates myocardial reperfusion injury after prolonged ischemia. This is supported by a lower increase in troponin I concentration, as well as better myocardial contractility in group 2. However, it should be noted that along with objective methods for assessing myocardial damage in the work performed, myocardial contractility was assessed subjectively, by a manual method, which is a limitation of the study. It is possible that the study of Prx6 in experiments on an isolated heart using a Langendorf device will help clarify the prospects for using Prx6 in transplantation.

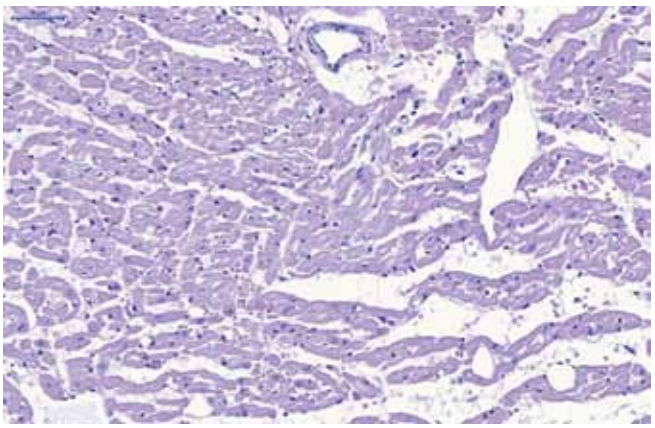

Fig. 3. Morphology of transplant with Prx6 cardioprotection. There are light myocardial sclerosis, activated endothelium and edema as well. Mild myocardial ischemic-reperfusion injury is presented

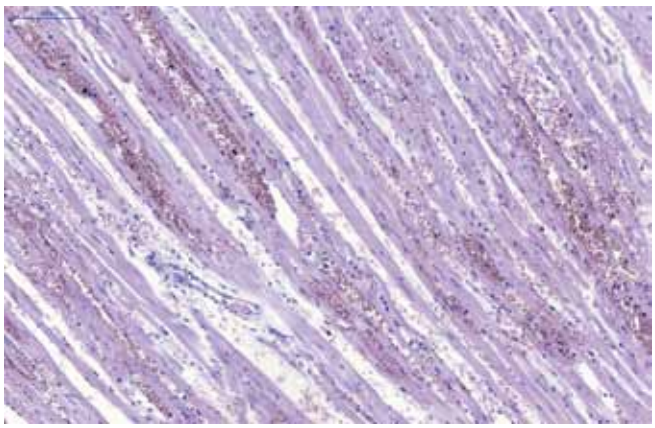

Fig. 4. Morphology of transplant in control group. There are hemorrhage with fibrin debris, activated endothelium and necrosis of cardiomyocytes with edema. Sever myocardial ischemic-reperfusion injury is presented

CONCLUSION

The experimental studies have shown that Prx6 can be considered a potential agent in perfusion solutions to protect the explanted organs. However, further experimental studies are required to clarify and quantify the protective effect of Prx6.

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The authors declare no conflict of interest.

REFERENCES

1. *Alam SR, Lewis SC, Zamvar V, Pessotto R, Dweck MR, Krishan A et al.* Perioperative elafin for ischaemia-reperfusion injury during coronary artery bypass graft surgery: a randomised-controlled trial. *Heart*. 2015; 101 (20): 1639–1645. doi: 10.1136/heartjnl-2015-307745.
2. *Conroy F, Yewdall NA*. Peroxiredoxin Proteins as Building Blocks for Nanotechnology. *Methods Mol Biol.* 2020; 2073: 39–54. doi: 10.1007/978-1-4939-9869-2_3.
3. *Fisher AB.* The phospholipase A2 activity of peroxiredoxin 6. *J Lipid Res*. 2018; 59 (7): 1132–1147. doi: 10.1194/jlr.R082578.
4. *Novoselov VI, Ravin VK, Sharapov MG.* Modified peroxiredoxins as prototypes of drugs with powerful antioxidant action. *BIOPHYSICS*. 2011; 56: 836–842. [In Russ].
5. *Rhee SG*. Overview on Peroxiredoxin. *Mol Cells.* 2016 Jan; 39 (1): 1–5.
6. *Sharapov MG, Novoselov VI, Fesenko EE, Bruskov VI, Gudkov SV.* The role of peroxiredoxin 6 in neutralization of X-ray mediated oxidative stress: effects on gene expression, preservation of radiosensitive tissues and postradiation survival of animals. *Free Radic Res*. 2017; 51 (2): 148–166. doi: 10.1080/10715762.2017.1289377.
7. *Sharapov MG, Novoselov VI, Gudkov SV.* Radioprotective Role of Peroxiredoxin 6. *Antioxidants* (Basel). 2019; 8 (1) doi: 10.3390/antiox8010015.
8. *Sharapov MG, Novoselov VI, Ravin VK*. Construction of a Fusion Enzyme Exhibiting Superoxide Dismutase and Peroxidase Activity. *Biochemistry* (Mosc). 2016; 81 (4): 420–427.
9. W*ang X, Phelan SA, Forsman-Semb K, Taylor EF, Petros C, Brown A et al.* Mice with targeted mutation of peroxiredoxin 6 develop normally but are susceptible to oxidative stress. *J Biol Chem*. 2003; 4 (278, 27): 25179–25190.
10. *Bunenkov NS, Bunenkova GF, Komok VV, Grinenko OA, Nemkov AS*. SAS Enterprise Guide 6.1 for physicians: multiple comparisons. *Medical academic journal*. 2019; 19 (4): 33–40. doi: 10.17816/MAJ17736. [In Russ.].
11. *Bunenkov NS, Bunenkova GF, Beliy SA, Komok VV, Grinenko OA, Nemkov AS*. SAS Enterprise Guide 6.1 for physicians: getting started. *Medical academic journal*. 2019; 19 (3): 27–36. doi: 10.17816/MAJ19327-36. [In Russ.].
12. *Galagudza MM, Sonin DL, Pochkaeva EI.* The no-reflow phenomenon: mechanisms and therapeutic targets. *Regional blood circulation and microcirculation*. 2018; 17 (1): 5–12. doi: 10.24884/1682-6655-2018-17-1-5-12. [In Russ.].
13. *Galagudza MM, Minasian SM, Dmitriev YuV, Poleschenko YaI, Shubina PYu, Protsak ES et al.* Comparison of hemodynamic and infarct-limiting effects of preservation solution based on Krebs–Henseleit buffer and HTK solution in the rat model of heterotopic heart transplantation. *"Arterial'naya Gipertenziya" ("Arterial Hypertension")*. 2019; 25 (1): 84–89. [In Russ.] doi: 10.18705/1607-419X-2019-25-1-84-89.
14. *Gautier SV.* 1000 heart transplantations in one center. *Russian Journal of Transplantology and Artificial Organs.* 2018; 20 (2): 5. [In Russ].
15. *Minasian SM, Galagudza MM, Dmitriev YuV, Karpov AA, Bobrova EA, Krasichkov AS et al.* Donor heart preservation: history and current status in terms of translational medicine. *Regional blood circulation and microcirculation.* 2014; 13 (3): 4–16. [In Russ.]. doi: 10.24884/1682-6655-2014-13-3-4-16.
16. *Molchan NS, Polushin YuS, Zhloba AA, Kobak AE, Khryapa SS.* Is it possible to augment myocardial protection during cardiopulmonary bypass by administration of inhalational anesthetics? *Almanac of Clinical Medicine.* 2019; 47 (3): 221–227. [In Russ.]. doi: 10.18786/2072-0505-2019-47-036.
17. *Sokolov AV, Kostevich VA, Gorbunov NV, Grigorieva DV, Gorudko IV, Vasilyev VB et al.* A link between active myeloperoxidase and chlorinated ceruloplasmin in blood plasma of patients with cardiovascular diseases. *Medical Immunology (Russia)*. 2018; 20 (5): 699–710. [In Russ.]. doi: 10.15789/1563-0625-2018-5-699-710.
18. *Khubulava GG, Shishkevich AN, Mihajlov SS, Bessonov EYu*. Sindrom reperfuzii miokarda: patogenez, klinika, diagnostika. *Vestnik Rossijskoj voenno-medicinskoj akademii*. 2020; 1 (69): 196–200. [In Russ.].
19. S*harapov MG, Novoselov VI, Ravin VK*. Klonirovanie, ekspressiya kDNK i sravnitel'nyj analiz peroksiredoksina 6 razlichnyh organizmov. *Molekulyarnaya biologiya*. 2009; 43 (3): 505–511. [In Russ.].

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MONITORING TACROLIMUS WHOLE BLOOD CONCENTRATIONS

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Tacrolimus (*TAC*) is the primary drug for most immunosuppressive therapy regimens. It has a narrow therapeutic index, meaning that insufficient dose can lead to graft and tissue rejection, while overdose can lead to increased risk of infections, toxicity, and cancerous tumors in organ transplant recipients. *TAC* belongs to a group of calcineurin inhibitors inhibiting T-cell activation. The use of *TAC* requires regular clinical observation of recipients and laboratory monitoring of the drug concentrations in the blood. This is to ensure correct dosage of the drug and to limit the potential risk of harmful side effects. The review presents data on some clinical, genetic factors affecting the bioavailability and concentration of *TAC* in the blood. We also present data on the methodological aspects of *TAC* laboratory control.

Keywords: tacrolimus, P450 gene polymorphism, prescription protocols, drug monitoring, organ transplantation.

Combined immunosuppressive therapy is an important aspect of treating the patients after solid organ transplantation. Basic immunosuppressive drugs in recipients of solid organs include calcineurin inhibitors (tacrolimus, cyclosporin A), proliferative signal inhibitors (sirolimus, everolimus), corticosteroids, mycophenolic acid, etc.

The factors that play a role in the pharmacokinetic variability of tacrolimus (*TAC*) include patient characteristics (age or weight), polymorphism of genes encoding enzyme proteins involved in *TAC* metabolism [1]. The clear benefits of TAC must be balanced against its side effects. Besides, multiple drug interactions with inducers and inhibitors of cytochrome P450 3A (CYP3A) CYP3A4/A5 isoforms increase the risk of insufficient or excessive *TAC* effects.

The present review examines the clinical aspects of the pharmacodynamics of TAC, pharmacogenetic factors influencing the results of allotransplantation, laboratory monitoring of the drug concentration.

Tacrolimus known as FK-506 is a macrolide immunosuppressant isolated from *Streptomyces tsukubaensis*. The drug was obtained in 1984 by Japanese researchers [2]. *TAC* is a sustained release drug that inhibits calcineurin, a protein phosphatase necessary for the activation of T lymphocytes. Compared with cyclosporin A, *TAC* has a more pronounced antiproliferative effect and better tolerance. The current use of TAS exceeds that of cyclosporin A; its powerful immunosuppressive effect is 100 times stronger than that of cyclosporin A. Due to the fact that *TAC* is metabolized through the cytochrome P-450 system, its concentration in the blood can alter at the simultaneous administration of drugs using the same metabolic pathways [3, 4].

DRUG PHARMACODYNAMICS: ACTION MECHANISM

TAC has become one of the most commonly prescribed immunosuppressants after solid organs – heart, lungs, kidneys, and pancreas – transplants. TAS binds to FKBP-12, an immunophilin (FKBP12 – FK506 complex) responsible for signal transduction and forms a pentameric complex with Ca2+ calmodulin and calcineurin. The resulting formation inhibits the action of the nuclear factor activated T cells (NFAT). Expression of NFAT is required for the production of interleukin-2 (IL-2) to initiate the activation of T lymphocytes. *TAC* was found to not only inhibits the activation of T cells, but also reduces the production of IL-10, which prompts B cells to produce large amounts of antibodies. *TAC* can inhibit the release of inflammatory mediators and molecules from basophils and mast cells. The main mechanism of TAS action is to inhibit the redistribution of calcineurin in the slit diaphragm [5, 6].

DRUG PHARMACOKINETICS AND PHARMACOGENETICS

TAC is absorbed mainly in the small intestine, with food significantly affecting the relative bioavailability of the drug. Whereas the highest absorption occurs in fasting state, a diet high in fats and carbohydrates lowers the mean area under the curve (AUC) and maximum *TAC* concentrations in blood. *TAC* concentration in blood

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reaches its peak (Cmax) in about 1–3 hours; bioavailability varies in solid organ recipients from 4 to 93%, with 25% on average. *TAC* is primarily redistributed in erythrocytes. Concentrations in whole blood are 10–30 times higher than drug concentrations in plasma; therefore, the measurement of *TAC* in whole blood is most widely used in clinical practice. TAS is 99% bound to plasma proteins: albumin, α-1 acid glycoprotein (orosomucoid), lipoprotein, and globulins [7]. The pharmacokinetics of TAS are influenced by such factors as age, the patient ethnicity, the donor organ condition, comorbidities, medications, diet, and polymorphism of the drug metabolizing enzyme and carrier protein. *TAC* is almost completely metabolized by isoenzymes CYP3A4 and CYP3A5 in the liver and is a substrate of P-glycoprotein (P-gp) encoded by the multidrug resistance gene 1 (ABCB1) [8, 9]. Studies have shown that CYP3A5 is the predominant enzyme in *TAC* metabolism. Polymorphism of the CYP3A5 gene is the main cause of the toxic effect when taking *TAC*. Replacement of the A6986G nucleotide in the CYP3A5 gene (CYP3A5* 3 allele) leads to the lack of functional activity of CYP3A5 in liver tissue (CYP3A5 are not expressors). For patients with this phenotype, lower doses of *TAC* are required. Heterozygous or homozygous carriers of the wild-type CYP3A5* 1 allele, designated * 1 / * 1 and * 1 / * 3, produce high levels of CYP3A5 mRNA and protein (CYP3A5 expressors). With these phenotypes, higher doses of the drug may be required for patients taking *TAC* [10, 11].

IMMUNOSUPPRESSION IN CHILDREN

At immunosuppressive therapy in children, it is necessary to consider the features of children's immunity. The difference between the immune system of young children is the immaturity of T and B lymphocytes. Immature B cells are featured with producing only class M immunoglobulins. Maturation of B cells goes on during the first year of life and is reflected in the sequential appearance of different classes of immunoglobulins in the blood serum. IgA synthesis, especially its secretory form, is completely absent in newborns and appears after the 3rd month of life, which gives reason to speak about the insufficiency of the local immunity system in the first years of life. The suppressor function of the immune system of infants in the first year of life to the mother's lymphocytes is physiological and is aimed at preventing severe immunocomplex pathology which is possible upon contact with a large number of antigens [12]. In children, the half-life of *TAC* is two times shorter than in adults, the drug clearance rate is 2–4 times higher, and the volume of distribution is 1.8 times higher in the early post-transplant period [13]. When *TAC* is administered orally, some children require a longer period for drug accumulation, while others, on the contrary, quickly achieve the required therapeutic level. It has also been shown in children that the CYP3A5 polymorphism has a significant effect on the pharmacokinetic variability of *TAC*. Children with the CYP3A5* 1 allele have higher *TAC* dose requirements than CYP3A5 non-expressors. For children and adolescents with at least one CYP3A5* 1 allele, an increase of 1.5–2 times in dose is similar to the recommendation for adults. Although CYP3A5 may explain up to 45% of *TAC* pharmacokinetic variability between individuals, other factors can also influence *TAC*: differences in gastric emptying rate or *TAC* inability to dissolve in gastric contents. The effect of immunosuppressants on the growth and development of children, especially on the course of infectious processes and higher rates of post-transplant lymphoproliferative morbidity should be considered [14, 15].

TOOLS AND SCHEMES FOR IMMUNOSUPPRESSION SELECTION

The problem of selecting an immunosuppression regimen after organ transplantation is relevant due to the fact that recipients, on the one hand, when prescribing high doses of immunosuppressants, have a high risk of developing infectious complications, malignant neoplasms, and on the other hand, when prescribing minimal doses of immunosuppressants, transplant rejection and dysfunction may develop.

Initial immunosuppression is selected empirically based on the body weight of the recipient and the scheme of twice a day administration; further, biochemical parameters and the level of the drug in blood are considered. *TAC* can be administered in a variety of forms and schemes: intravenously, per os, twice daily with immediate release, once daily with modified release, and dose requirements change over time. This makes it necessary to develop various programs for selecting the drug dose based on population models of pharmacokinetics.

There are a number of electronic resources for routine selection and dose adjustment of various drugs including *TAC*, considering the therapeutic value prescribed by the attending physician [16]. The ISBA website (www.pharmaco.chu-limoges.fr) allows for individual dose adjustment of immunosuppressants. The user fills out a form in which a number of parameters are indicated, the type of transplanted organ, time between the administration of the drug and the measurement of the concentration, concomitant medications, etc. The request is confirmed within 24 hours by a qualified pharmacologist providing individual recommendations to achieve the therapeutic goals.

DoseMe (www.doseme.com.au) is another available tool suitable for dose adjustment of TAS and other drugs using previously published population pharmacokinetic models [17]. It is available as a website with a user interface. The program handles a variety of immunosuppression regimens: twice daily or once daily, based on the population model by Woillard et al. [18]. Other resources (MWPharm and BestDose) are computer software, all actions are performed online, and the user only has to

provide input data, which is automatically checked to exclude erroneous values and interpret the report.

Some medical centers are developing their own personalized treatment regimens based on immunosuppressive drugs for the patients with solid organ transplantation.

LABORATORY MONITORING IMMUNOSUPPRESSANTS

The severity of potential adverse events necessitates regular monitoring of the scheme of administering such basic immunosuppressive drugs as *TAC*, cyclosporin A, everolimus, etc., and their blood concentration in recipients of these drugs. Drugs are monitored in many laboratories using one of the methods, immunochemical analysis, or liquid chromatography with tandem mass spectrometry (LC-MS/MS) [19, 20].

As with many drugs, *TAC* levels tend to vary greatly among patients, depending on many factors, so there are no established reference values for its concentration. Test results should only be interpreted by a physician and used in conjunction with other diagnostics. In some cases, *TAC* can be used in combination with other immunosuppressants to reduce the effects of harmful side effects. In some cases, the patient needs to be kept at low *TAC* concentrations (3–7 ng/ml); for this, laboratories must use techniques with low sensitivity limits, from 1 ng/ml [21].

The study by the International Association Of Therapeutic Drug Monitoring And Clinical Toxicology (IATDMCT) identified LC-MS/MS as the "gold" drug monitoring technique due to its high specificity in 53% of the laboratories surveyed, 76 in 14 countries. This method allows the optimal separation of molecules into fragments [22].

Mass spectrometry can also be applied to analyze dried blood samples. Its advantage is that the samples are collected from the patients at home and then sent to the laboratory for measurement, thus reducing transport costs and saving time for the patient (the technique has not been registered in Russia). However, high hematocrit has been shown to affect paper permeability. Patient samples with increased cell volumes have lower paper permeability, creating a smaller stain affecting accurate results [23, 24].

TAC quantitative assessment at the immunochemical analysis is one of the advantages of the technique. Similar to LC-MS/MS, sample measurement in an immunoassay is carried out after a pretreatment step, an example is sample preparation for analysis with ARCHITECT i2000SR device (Abbott Diagnostics) by chemiluminescence which uses methanol/zinc sulfate to precipitate protein and extract *TAC* from a whole blood sample with ethylenediaminetetraacetic acid (EDTA). To assess *TAC* concentration in the blood, COBAS (Roche) and Dimension (Siemens Healthcare Diagnostics) analyzers can be used which imply the techniques of enzyme immunoassay and antibody-conjugated magnetic immunoassay.

The main disadvantage observed in many non-chromatographic systems is the potential cross-reactivity between the parent drug and its metabolites which can lead to falsely elevated blood drug concentrations [19].

Advances in immunochemical assay include automatic sample pretreatment, improved reagent stability to reduce potential matrix effects, and new anti-*TAC* antibodies that provide greater sensitivity and proximity to target concentration. Immunoassay is used in many laboratories because of its ease of use and reduced costs associated with services, the manufacturer often provides training, support, and service for these systems. LC-MS/MS testing requires high technical skills and extensive training. This technique also presumes a high initial cost and full validation to use [25].

ISSUES OF DRUG INTERACTIONS

The lifelong use of immunosuppressive drugs, on the one hand, improves the survival of recipients, and on the other hand, it leads to the problem of toxic side effects on the kidneys, heart and other organs against the background of long-term drugs administration. In patients with a low of risk, the dose of immunosuppressive drugs may be reduced for 1–2 years. Reducing the effect of nephrotoxicity usually means reducing the dose of calcineurin inhibitors and corticosteroids.

The concomitant diseases in the recipient (arterial hypertension, infectious complications, renal failure, etc.) is accompanied by the appointment of additional medications, which increases the risk of unwanted interactions. Additional prescription of drugs should be performed considering their potential effect (increase or decrease) on *TAC* concentration [3, 26].

CONCLUSION

The use of inhibitors of calcineurin, cyclosporin A and tacrolimus improved graft and patient survival rates, significantly reducing the incidence of acute and chronic rejection. However, long-term use of these drugs leads to the development of nephrotoxicity, metabolic and cosmetic side effects, as well as other possible complications (systemic arterial hypertension, neurotoxicity, an increased risk of developing infectious complications, the occurrence of post-transplant lymphoproliferative disorders).

However, the issues of developing approaches to the individualization of immunosuppressive therapy through studies of the pharmacokinetics of tacrolimus, including genetic aspects, as well as issues of drug interactions in recipients with comorbid pathology, remain relevant.

TAC laboratory monitoring is an important part of the post-transplant management of recipients and can be performed by two different methods, LC-MS/MS or immunochemical analysis. To ensure accurate and accu-

rate results, the selected blood TAC monitoring platform must be certified, standardized, and well supported.

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REFERENCES

1. Immunosupressiya pri transplantatsii solidnykh organov / Pod red. S.V. Gautier. M.–Tver': Triada, 2011. 472 s.: il.
2. *Bowman LJ, Brennan DC*. The role of tacrolimus in renal transplantation. *Expert Opin Pharmacother*. 2008; 9 (4): 635–643.
3. *Gautier SV, Shevchenko AO, Popcov VN*. Pacient s transplantirovannym serdcem. Rukovodstvo dlja vrachej po vedeniju pacientov, perenesshih transplantaciju serdca. M.–Tver': Triada, 2014. 144.
4. *Gao L, Liu J, Zhang Y et al*. Low incidence of acute graft-versus-host disease with short-term tacrolimus in haploidentical hematopoietic stem cell transplantation. *Leuk Res*. 2017; 57: 27–36.
5. *Maguire O, Tornatore KM, O'Loughlin KL et al.* Nuclear translocation of nuclear factor of activated T cells (NFAT) as a quantitative pharmacodynamic parameter for tacrolimus. *Cytometry A*. 2013; 83: 1096–1104.
6. *Nassereddine S, Rafei H, Elbahesh E, Tabbara I.* Acute graft versus host disease: a comprehensive review. *Anticancer Res*. 2017; 37 (4): 1547–1555.
7. *Yu M, Liu M, Zhang W, Ming Y.* Pharmacokinetics, pharmacodynamics and Pharmacogenetics of Tacrolimus in Kidney Transplantation. *Curr Drug Metab*. 2018; 19 (6): 513–522.
8. *Vanhove T, Annaert P, Kuypers DRJ*. Clinical determinants of calcineurin inhibitor disposition: a mechanistic review. *Drug Metab Rev*. 2016; 48 (1): 88–112.
9. *Tang JT et al*. Pharmacogenetic aspects of the use of tacrolimus in renal transplantation: recent developments and ethnic considerations. *Expert Opin Drug Metab Toxicol*. 2016; 12 (5): 555–565.
10. *Shuker N et al*. A randomized controlled trial comparing the efficacy ofCyp3a5 genotype-based with body-weight-based tacrolimus dosing after living donor kidney transplantation. *Am J Transplant*. 2016; 16 (7): 2085–2096.
11. *Birdwell KA et al*. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for CYP3A5 Genotype and tacrolimus dosing. *Clin Pharmacol Ther*. 98 X. Zhang et al. Biomedicine & Pharmacotherapy. 2018; 102: 107–114. 2015; 1: 19–24.
12. *Tsirulnikova OM, Lurie YuE, Tsirulnikova IE*. Features of immunosupressive therapy in children. Immunosupressive therapy in children at liver transplantation. Immunosupression at solid organ transplantation. M.–Tver: Triada; 2011: 273–337. [In Russian].
13. *Agarwal A, Pescovitz MD*. Immunosupression in pediatric solid organ transplantation. *Semin Pediatr Surg*. 2006 Aug; 15 (3): 142–152.
14. *Turmelle YP, Nadler ML, Anderson CD et al*. Towards minimizing immunosuppression in pediatric liver transplant recipients. *Pediatr Transplantation*. 2009; 13: 553–559.
15. *Hao GX, Song LL, Zhang DF et al.* Off-label use of tacrolimus in children with glomerular disease: Effectiveness, safety and pharmacokinetics. *Br J Clin Pharmacol*. 2020 Feb; 86 (2): 274–284. doi: 10.1111/bcp.14174. Epub 2020 Jan 14.
16. *Fuchs A, Csajka C, Thoma Y, Buclin T, Widmer N*. Benchmarking therapeutic drug monitoring software: a review of available computer tools. *Clin Pharmacokinet*. 2013; 52: 9–22. http://dx.doi.org/10.1007/s40262-012-0020-y.
17. *Burton ME, Brater DC, Chen PS, Day RB, Huber PJ, Vasko MR*. A Bayesian feedback method of aminoglycoside dosing. *Clin Pharmacol Ther*. 1985; 37: 349–357.
18. *Woillard J-B, de Winter BCM, Kamar N, Marquet P, Rostaing L, Rousseau A*. Population pharmacokinetic model and Bayesian estimator for two tacrolimus formulations – twice daily Prograf and once daily Advagraf. *Br J Clin Pharmacol*. 2011; 71: 391–402, http://dx.doi.org/10.1111/j.1365-2125.2010.03837.x.
19. *Gounden V, Soldin SJ*. Tacrolimus measurement: building a better immunoassay. *Clin Chem*. 2014; 60 (4): 575–576.
20. *McShane AJ, Bunch DR, Wang S.* Therapeutic drug monitoring of immunosuppressants by liquid chromatography – mass spectrometry. *Clin Chim Acta*. 2016; 454: 1–5.
21. *Bargnoux AS, Sutra T, Badiou S et al.* Evaluation of the new Siemens tacrolimus assay on the Dimension EXL integrated chemistry system analyzer: comparison with an ultra-performance liquid chromatography-tandem mass spectrometry method. *Ther Drug Monit*. 2016; 38 (6): 808–812.
22. *Polledri E, Mercadante R, Ferraris Fusarini C, Maiavacca R, Fustinoni S.* Immunosuppressive drugs in whole blood: validation of a commercially available liquid chromatography/tandem mass spectrometry kit andcomparison with immunochemical assays. *Rapid Commun Mass Spectrom*. 2017; 31 (13): 1111–1120.
23. *Koster RA, Veenhof H, Botma R et al.* Dried blood spot validation of five immunosuppressants, without hematocrit correction, on two LC-MS/MS systems. *Bioanalysis*. 2017; 9 (7): 553–563.
24. *Koster RA, Alffenaar JW, Greijdanus B, Uges DR.* Fast LC-MS/MS analysis of tacrolimus, sirolimus, everolimus and cyclosporine A in dried blood spots and the influence of the hematocrit and immunosuppressant concentration on recovery. *Talanta*. 2013; 115: 47–54.
25. *McShane AJ, Bunch DR, Wang S.* Therapeutic drug monitoring of immunosuppressants by liquid chromatography – mass spectrometry. *Clin Chim Acta*. 2016; 454: 1–5.
26. *Shevchenko AO, Nikitina EA, Koloskova NN, Shevchenko OP, Gautier SV.* Kontroliruemaja arterial'naja gipertenzija i vyzhivaemost' bez nezhelatel'nyh sobytij u recipientov serdca. *Kardiovaskuljarnaja terapija i profilaktika*. 2018; 17 (4): 4–11. [In Russ, English abstract].

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GUIDELINES ON IMPROVING HEALTH CARE QUALITY IN TRANSPLANTATION SERVICES IN THE FEDERAL SUBJECTS OF RUSSIA

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Objective: to provide guidelines on how to improve the quality of health care in transplantation services for Russian regions; the recommendations were prepared by the Shumakov National Medical Research Center of Transplantology and Artificial Organs (Shumakov Center) based on field trips made by Shumakov Center from 2018 to 2019 in order to assess the status and development prospects of organ donation and organ transplantation in various regions of the Russian Federation. **Materials and methods.** Based on the over 40 field trips made by the Center, analytical reports, as well as recommendations were prepared by the Ministry of Health of Russia for regional health authorities and transplant centers. Guidelines were selected from these recommendations to address the most significant and common gaps and errors inherent in organ donation and organ transplantation in the regions. On the basis of the analytical reports, guidelines for the roadmap (plan) for organ donation and transplantation in the region were prepared. **Results.** A methodological framework for solving a wide range of managerial challenges on organ donation and organ transplantation is proposed for heads and experts at health-care institutions and medical organizations. **Conclusion.** Organ donation and organ transplantation should be developed for the regions using the roadmap (plan) prepared and approved by the regional health authority. This approach brings stability and consistency to the development process, creates a resource base for development, and provides coordination and oversight.

Keywords: organ donation, organ transplantation, Schumakov center of transplantology, transplant register, transplant recommendations.

INTRODUCTION

In 2018–2019, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russia (the Center), guided by the order of the Ministry of Health of Russia of September 11, 2017 No. 622, by the order of the Ministry of Health of Russia of March 13, 2019 No. 125, within the framework of its authority as the national medical research center for transplantation, carried out field trips to the subjects of the Russian Federation to assess the organization of medical care for transplantation and organization of medical activities related to organ donation. In total, the specialists of the Center made over 40 visits in this period.

At this, the workshops were held with participating top managers of health administration, chief doctors of hospitals, chief external expert, where topical regional problems and tasks for the development of transplantation were discussed. In medical institutions of the 3rd level providing or planning to provide medical care for organ transplantation, scientific and practical conferences, joint visits to structural units were held, medical documentation was reviewed, and discussions with medical staff were held. By the results of each field trip, the Center prepared an analytical report for the Russian Ministry of Health.

Analyzes of the material collected in the regions showed certain resemblance of the problems and tasks facing the regional medical institutions as well as common faults in the development of organ donation and transplantation.

Based on this, the Center prepared recommendations for improving the organization of medical care for transplantation in the subjects of the Russian Federation which are presented in this article.

The recommendations were prepared using long-term data from the register of the Russian Transplant Society [1–10].

This material has been prepared for managers and experts of health authorities and medical institutions as a methodological basis for solving a wide range of management tasks in the field of organizing organ donation and transplantation. The recommendations are planned to be regularly updated and supplemented; the study of the methodological aspects of organizing medical care

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for organ transplantation and medical activities related to organ donation for transplantation is planned to continue in the regions of the Russian Federation.

RECOMMENDATIONS FOR ROADMAP (PLAN) CONTENT FOR DEVELOPMENT OF ORGAN DONATION AND TRANSPLANTATION IN THE REGION

A common drawback in organizing organ donation and transplantation in the subjects of the Russian Federation revealed during the field trips of the Center experts is the lack of the subjects' coherent strategy for the development of this field of high-tech medical care which is confirmed by the absence of an appropriate methodological or regulatory legal act.

In this regard, the Center, within the framework of the organizational and methodological guidelines, recommends that regional health authorities guided by Federal Law No. 323-ФЗ of November 21, 2011 (Article 16), should obligatorily prepare and approve such a document.

As a methodological support, the Center proposed recommendations for the content of the road map, a plan for the development of organ donation and transplantation in the region (hereinafter, the road map).

Such a roadmap should include an assessment of the current state of organization of medical care for organ transplantation, medical activities related to organ donation for transplantation. As a general rule, such an analytical report is prepared by the chief external transplant expert of the regional health authority.

The satisfaction of the region's population with the availability and quality of medical care for organ transplantation must be necessarily assessed.

The costs of the regional budgetary and non-budgetary funds for renal replacement therapy by dialysis and kidney transplantation must be compared. It is known that kidney transplantation as a method of renal replacement therapy is significantly more cost effective than hemodialysis. Savings on medical support for the kidney transplant patients, including drug therapy in comparison with those on dialysis averages RUR 1.2 million per year, starting from the 2nd year after kidney transplantation.

It is necessary to describe the planned (desired) state of the organization of medical care for organ transplantation, medical activities related to organ donation for transplantation purposes. The number of kidney transplants in the region should be such as to effectively use the existing and unique donor resource, and most importantly, contain the growth of the number of patients on dialysis as much as possible.

The road map should provide for measures to form a network of medical institutions involved in the provision of medical care for organ transplantation, in medical activities related to organ donation for transplantation (licensing, listing, charter).

It is necessary to legally consolidate, distribute the rights and responsibilities between medical institutions in the organization and provision of medical care for organ transplantation, medical activities related to organ donation for transplantation. The same applies to the rights and responsibilities of the experts of medical institutions in the organization and provision of medical care for organ transplantation, medical activities related to organ donation for transplantation.

It is necessary to distribute the rights and responsibilities between the chief external experts of the region in the organization and provision of medical care for organ transplantation, medical activities related to organ donation for transplantation.

The road map should include measures for the material and technical support of medical institutions involved in the provision of medical care for organ transplantation; in medical activities related to organ donation for transplantation in accordance with regulatory requirements. This should be guided by the procedures for the provision of medical care, and consideration of the standards, clinical and methodological recommendations for transplantation.

Mandatory for development are measures for staffing medical institutions involved in the provision of medical care for organ transplantation, in medical activities related to organ donation for transplantation in accordance with regulatory requirements. In compliance with the requirements of the Procedure for the "surgery (transplantation of human organs and (or) human tissues)" profile of October 31, 2012 No. 567н, experts involved in the provision of medical care for organ transplantation must undergo advanced training on relevant issues.

The road map should provide measures for the technological support of medical institutions involved in the provision of medical care for organ transplantation, in medical activities related to organ donation for transplantation. For example, kidney removal from live relative donors at the present stage is advisable to be performed laparoscopically; accordingly, this technique should be introduced in transplant centers where related kidney transplantations are performed.

Medical institutions involved in the provision of medical care for organ transplantation, in medical activities related to organ donation for transplantation, must be methodically provided. For this, in particular, it is necessary to work out measures for the implementation of clinical guidelines and protocols for transplantation.

The road map should include measures for drug provision of medical institutions involved in the provision of medical care for organ transplantation, in medical activities related to organ donation for transplantation, for patients before and after organ transplantation.

It is necessary to provide grounds for the timely identification and follow-up of patients in need of organ transplantation (waiting list) in accordance with clinical guidelines.

It is necessary to work out measures to ensure dynamic monitoring of patients with transplanted organs in accordance with clinical guidelines.

It is necessary to provide conditions for registering donor organs, donors and recipients in accordance with regulatory requirements.

The roadmap should include measures to ensure the timely identification of potential organ donors, to introduce the diagnosis of brain death in accordance with the methodological recommendations.

Mandatory for development are measures to ensure the quality and safety of medical care for organ transplantation and medical activities related to organ donation for transplantation.

The basic condition for the development of organ donation and transplantation in the region is the proper legal support for the organization of medical care for organ transplantation, medical activities related to organ donation for transplantation in the region. In this regard, measures to improve legal support are also mandatory for the road map.

The roadmap should include activities to inform and educate the population, including the medical community, on organ donation and transplantation, and work with the media.

It is also advisable that the road map includes measures for interaction with the national medical centers for transplantation profile including remote consultation of patients using telemedicine technologies.

The road map should Obviously provide for measures of the financial support for the organization of medical care for organ transplantation and medical activities related to organ donation for transplantation in the region.

Considering the judicial practice in cases in the field of health care in a number of regions, it may be useful to include in the road map measures for interaction with law enforcement agencies to inform about organ donation and transplantation and prevent offenses.

The layout template of the roadmap is presented in the Table.

STANDARD SOLUTIONS FOR IMPROVING THE ORGANIZATION OF MEDICAL CARE FOR TRANSPLANTATION

The Center has prepared standard solutions for regional health authorities and medical institutions to improve the organization of medical care for transplantation in the subjects of the Russian Federation. It is advisable to work out these recommendations, specificate and include them in the road map (plan) for the development of organ donation and transplantation in the region.

At all levels of regional health care, the persons responsible for the development of organ donation and transplantation should be appointed; in the regional health authority, it is the head or one of the deputy heads and the deputy of the profile unit; at the transplantation center, the chief physician or one of the deputy chief physicians, the head or deputy of the profile department (responsible for the transplantation program), the head or deputy of the profile department (responsible for the donor program); at the donation center, the chief physician or one of the deputy chief physicians, the head or deputy of the profile department (responsible for the donor program).

The regional health authority's chief external transplant expert should be appointed. The chief external transplant expert should ensure that an annual analysis of the state and prospects for the development of medical care for organ transplantation in the region is performed.

It is advisable to form a coordination council under the regional health authority for the development of organ donation and transplantation, to approve its rules and regulations, composition and working plan.

It is recommended that the regional health authority approve a road map (plan) for the development of organ donation and transplantation in the region; in the state regional program on the development of regional health care, include measures for the development of organ donation and transplantation in compliance with the order of the Ministry of Health of Russia of June 4, 2019 No. 365; develop and/or update the regulatory legal act of the regional health authority regulating the organization of medical care for organ transplantation, organization of medical activities related to organ do-

Table

Road map (plan) for the development of organ donation and transplantation in the region

Nos.	List of activities from recommendations of the Center	Content (essence) of events	Target indicator	Target indicator value	Deadlines	Responsible person	Legal support	Financing
1	2	3	4	5	6	7	8	9

nation for transplantation in the region; plan and assist medical institutions providing medical care for organ transplantation, carrying out medical activities related to organ donation for transplantation, in their licensing and inclusion in the Lists (Order of the Ministry of Health of Russia and the Russian Academy of Sciences of February 20, 2019 No. 73н/2); conduct an audit and, if necessary, equip these medical institutions in accordance with the requirements of the Procedure for the provision of medical care in the field of surgery (transplantation of organs and/or human tissues) by the Order of the Ministry of Health of Russia dated October 31, 2012 No. 567н; conduct an audit and, if necessary, equip medical institutions carrying out medical activities related to organ donation for the purpose of transplantation, in accordance with the requirements of the Procedure for the provision of medical care to the adult population in the profile for anesthesiology and resuscitation by the Order of the Ministry of Health of the Russian Federation of November 15, 2012 No. 919н.

Medical institutions are to ensure compliance with the qualification requirements for experts involved in the provision of medical care for organ transplantation and/or the implementation of medical activities related to organ donation for transplantation; ensure compliance with qualification requirements in accordance with the Procedure for the provision of medical care for surgery (transplantation of organs and/or human tissues by the order of the Ministry of Health of Russia dated October 31, 2012 No. 567н; ensure compliance with regulatory requirements for additional training of experts involved in the provision of medical care for organ transplantation and/or the implementation of medical activities related to organ donation for transplantation; ensure compliance with the requirements for additional training of experts in accordance with the Procedure for the provision of medical care for surgery (transplantation of organs and/or human tissues) by the Order of the Ministry of Health of Russia of October 31, 2012 No. 567н. For example, a pathologist of a medical organization providing medical assistance for organ transplantation needs to be additionally trained on pathological anatomy in patients after organ transplantation for at least 72 hours.

For the effective implementation of organ transplantation, donor activities in medical institutions, it is recommended, in addition to creating specialized clinical departments (in accordance with the requirements of the above Procedure), to form functional medical teams responsible for organ (kidney, liver, heart) transplantation programs and for donation coordination.

Organ donation and transplantation in medical institutions are recommended to be organized in such a way that different experts are responsible for the provision of medical care for organ transplantation and implementation of medical activities related to organ donation for transplantation (order to prevent delinquency and conflict of interest). It is not recommended to combine organ donation and transplantation services in one center.

The regulatory legal act of the regional health authority is recommended, to organize a regional center for coordinating organ donation on the basis of a subordinate medical organization licensed to work (provide services) for the removal, storage of human organs and/or tissues for transplantation, as well as for their transportation. It is advisable to provide interaction between the regional coordinating center for organ donation. the regional vascular center, and the regional trauma center with the possibility of obtaining real-time data on potential donors. It is recommended to organize, on the basis of the regional coordination center of organ donation, regular training for resuscitators of medical institutions involved in medical activities related to organ donation for transplantation. It is recommended to register hospital mortality in intensive care units of medical institutions involved in medical activities related to organ donation for transplantation with regard to the patients aged 18–65 with isolated vascular and/or traumatic brain damage who die within 7 days after admission to the intensive care unit. It is necessary to introduce into the practice of medical institutions involved in medical activities related to organ donation for transplantation, the ascertaining of a person's death based on the diagnosis of brain death by the Order of the Ministry of Health of Russia of December 25, 2014 No. 908н). It is recommended to provide conditions (experts, medical equipment) for performing the procedure for diagnosing brain death on a 24/7 basis.

The Center recommends that regional health authorities approve, as one of the performance indicators of the chief physicians of subordinate medical institutions involved in medical activities related to organ donation for transplantation purposes, the indicator of the number of identified potential organ donors.

It is necessary to introduce into the practice of medical institutions providing medical care for organ transplantation, carrying out medical activities related to organ donation, standardized protocols for the diagnosis, treatment and prevention of patients (donors and recipients) in transplantation, based on the clinical guidelines of the Russian Transplant Society.

Medical institutions need to ensure internal quality control of medical care for organ transplantation, including quality control of medical records. It is recommended to check all medical records of recipients and live organ donors without exception. It is recommended to ensure the pathological and anatomical autopsies of patients who die in the hospital and can be effective donors (to exclude death in connection with medical and diagnostic measures, paragraph 5 of Appendix No. 1 to the Order of the Ministry of Health of Russia of June 6, 2013 No. 354н). It is advisable to provide histological control of organ transplants aimed at disposal.

The Center recommends that medical institutions organize the maintenance of the Patient Log on the Organ Transplant Waiting List, the Organ Transplant Patient Log, the Organ Transplant Medical Care Record Book / Transplant Coordinators.

Medical institutions providing medical assistance for organ transplantation, carrying out medical activities related to organ donation for transplantation should organize their work as to ensure compliance with patient confidentiality in terms of data on the donor and recipient (Law of the Russian Federation of December 22, 1992 No. 4180-I, Article 14.

If there is a commission in the region for the selection of patients for renal replacement therapy, it is recommended to include a chief external transplant expert.

In medical institutions providing medical care for kidney transplantation, it is recommended to Provide conditions for HLA typing of all kidney donors and recipients by three loci (A, B, Dr). A direct cross test for compatibility between the donor and the potential recipient (crossmatch reaction) should be performed by lymphocytotoxic test (serological method) or by flow cytometry. To perform the lymphocytotoxic test, the presence of an inverted microscope in the medical institution providing medical assistance for organ transplantation must be ensured.

It is advisable to provide informing and rational referral of patients needing organ transplantation to federal medical centers of the Ministry of Health of Russia, considering their territorial location, specialization by types of transplantation, and the volume of high-tech medical care for transplantation.

The regional health authority should ensure funding medical care for organ transplantation including work related to organ donation from the regional budget. Such authorities are provisioned by parts 2, 9.1, 9.2 of Article 83 of the Federal Law of the Russian Federation of November 21, 2011 No. 323-ФЗ On the basics of protecting the health of citizens in the Russian Federation. In particular, targeted spending of funds allocated for financial support of work related to organ donation must be foreseen.

On the basis of the approved standards of medical care and clinical guidelines, it is necessary to calculate and approve the rates of compulsory medical insurance for the provision of medical care to patients under dynamic observation in the waiting list for organ transplantation as well as their dynamic observation after organ transplantation.

It is necessary to provide an acceptable level of donor and transplant activity. The target value of the indicator of the number of effective organ donors at planning the development of organ donation in the region for a period of up to 5 years should not be less than 15 per 1 million population. The target value of the indicator of the number of kidney transplants at planning the development of organ transplantation in the region for a period of up to 5 years should not be less than 30 per 1 million population. It is necessary to work out a strategy and/or an action plan for the introduction in the region (in a medical institution) of high-tech methods of treating diseases with transplantation of extrarenal organs (liver, heart, lungs, pancreas). It is necessary to develop a strategy and/or an action plan for the rational use of organs from cadaver donors suitable for transplantation but not in demand in the region (in a medical institution).

It is necessary to ensure the introduction of a methodology for laparoscopic removal of a donor kidney from a live donor in case of related kidney transplantation in a medical institution.

It is necessary to ensure monitoring the blood immunosuppressants concentration in patients after organ transplantation in accordance with the standards of medical care at the expense of compulsory medical insurance funds (the Order of the Ministry of Health of Russia of December 28, 2012 No. 1575н). It is necessary to exclude the addition of doses of drugs of different trade names within one international nonproprietary name in patients with transplanted organs under guidance of the clinical recommendations of the Russian Transplant Society, Drug monitoring and interchangeability of original and generic immunosuppressants with a narrow therapeutic index.

It is necessary to provide remote consultations with the Center in accordance with the approved list of diseases (conditions) in the amount of at least 10% of profile patients, to include remote consultations in the system of monitoring the effectiveness, safety and quality of pharmacotherapy.

It is necessary to regulate and ensure control by the regional health authority of cases of transfer of donor organs unclaimed but suitable for transplantation to medical institutions located in other subjects of the Russian Federation, to regulate and ensure control by the regional health authority of cases of disposal of donor organs unclaimed but suitable for transplantation.

It is advisable to publish on the regional health authority, subordinate medical organizations official websites a link to the official resource of the Russian Ministry of Health on organ donation and transplantation at donorstvo.org.

If there is a state medical university in the region, it is advisable to organize an educational course on Fundamentals of donation and transplantation of human organs for medical students (6^{th} year). It is recommended to use the guidance paper of the Department of Transplantology and Artificial Organs of the I.M. Sechenov First Moscow State Medical University of the Ministry of Health of Russia.

It is recommended to ensure cooperation with public organizations in the region and in the Russian Federation representing the interests of patients in need of organ

transplantation as well as patients after organ transplantation, to ensure collaboration with public organizations representing the interests of the professional medical community in the field of organ donation and transplantation in the region and in the Russian Federation.

CONCLUSION

When solving the problem of organizing or developing a program for organ donation and transplantation in a subject of the Russian Federation, top managers and experts of health authorities and medical institutions face a wide range of management issues that require resource and legal support, monitoring and administration.

In this regard, Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation, recommends solving these problems through a roadmap (plan) for the development of organ donation and transplantation in the region, allowing for ensured stability and consistent development, planning a resource base for advance and ensuring coordination and control.

The results of the analysis of materials collected at the field trips in the regions and summary reports made by Shumakov National Medical Research Center of Transplantology and Artificial Organs, the Ministry of Health of Russia prepared standard solutions for regional health authorities and medical institutions to improve the organization of medical care for transplantation in the subjects of the Russian Federation. With this, a methodological basis for the development of road maps (plans) for the development of organ donation and transplantation in the regions has been proposed.

Shumakov National Medical Research Center of Transplantology and Artificial Organs will further continue to provide methodological assistance to top managers and experts of regional health authorities and medical institutions on preparation and implementation of road maps (plans) for development within the framework of implementation of its authorities as the national medical research center for transplantation.

REFERENCES

1. Organ donation and transplantation in Russian Federation in 2009. *Transplantology 2009: results and prospects*. Vol. I / Ed. by S.V. Gautier. M.–Tver: Triada, 2010: 408, 8–20.
2. Organ donation and transplantation in Russian Federation in 2006–2010. *Transplantology: results and prospects*. Vol. II. 2010 / Ed. by S.V. Gautier. M.–Tver: Triada, 2011: 464, 18–32.
3. Organ donation and transplantation in Russian Federation in 2011 (IV report of National Registry). *Transplantology: results and prospects*. Vol. III. 2011 / Ed. by S.V. Gautier. M.–Tver: Triada, 2012: 416, 14–37.
4. Organ donation and transplantation in Russian Federation in 2012. (V report of National Registry). *Transplantology: results and prospects*. Vol. IV. 2012 / Ed. by S.V. Gautier. M.–Tver: Triada, 2013: 304, 8–28.
5. Organ donation and transplantation in Russian Federation in 2013 (VI report of National Registry). *Transplantology: results and prospects*. Vol. V. 2013 / Ed. by S.V. Gautier. M.–Tver: Triada, 2014: 352, 32–57.
6. Organ donation and transplantation in Russian Federation in 2014 (VII report of National Registry). *Transplantology: results and prospects*. Vol. VI. 2014 / Ed. by S.V. Gautier. M.–Tver: Triada, 2015: 488, 44–75.
7. Organ donation and transplantation in Russian Federation in 2015 (VIII report of National Registry). *Transplantology: results and prospects*. Vol. VII. 2015 / Ed. by S.V. Gautier. M.–Tver: Triada, 2016: 488, 38–71.
8. Organ donation and transplantation in Russian Federation in 2016 (IX report of National Registry). *Transplantology: results and prospects*. Vol. VIII. 2016 / Ed. by S.V. Gautier. M.–Tver: Triada, 2017: 368, 33–66.
9. Organ donation and transplantation in Russian Federation in 2017 (X report of National Registry). *Transplantology: results and prospects*. Vol. IX. 2017 / Ed. by S.V. Gautier. M.–Tver: Triada, 2018: 392, 26–63.
10. Organ donation and transplantation in Russian Federation in 2018 (XI report of National Registry). *Transplantology: results and prospects*. Vol. X. 2018 / Ed. by S.V. Gautier. M.–Tver: Triada, 2019: 504, 46–85.

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