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УЧРЕДИТЕЛЬ: ОБЩЕРОССИЙСКАЯ ОБЩЕСТВЕННАЯ ОРГАНИЗАЦИЯ ТРАНСПЛАНТОЛОГОВ «РОССИЙСКОЕ ТРАНСПЛАНТОЛОГИЧЕСКОЕ ОБЩЕСТВО»

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К 50-ЛЕТИЮ НМИЦ ТИО ИМЕНИ АКАДЕМИКА В.И. ШУМАКОВА И К 20-ЛЕТИЮ ЖУРНАЛА «ВЕСТНИК ТРАНСПЛАНТОЛОГИИ И ИСКУССТВЕННЫХ ОРГАНОВ»

CELEBRATING THE 50th ANNIVERSARY OF THE SHUMAKOV NATIONAL MEDICAL RESEARCH CENTER OF TRANSPLANTOLOGY AND ARTIFICIAL ORGANS AND THE 20th ANNIVERSARY OF THE RUSSIAN JOURNAL OF TRANSPLANTOLOGY AND ARTIFICIAL ORGANS

Вашему вниманию представлен третий выпуск журнала «Вестник трансплантологии и искусственных органов» за 2019 год, отмеченный знаменательным событием в отечественной трансплантологии – 50-летием Национального медицинского исследовательского центра трансплантологии и искусственных органов имени академика В.И. Шумакова. От первого, созданного в 1969 году, специализированного научно-клинического учреждения до обладающего мировой известностью лидера отечественной трансплантологии – таков путь, пройденный за полвека ведущим трансплантологическим центром нашей страны.

«Вестник трансплантологии и искусственных органов» издается с 1999 года. Таким образом, наш журнал также празднует свой юбилей – 20 лет. Впрочем, реальная история издания началась 25 лет назад – в 1994 году был инициирован выпуск журнала «Трансплантология и искусственные органы», преемником и продолжателем которого является наш «Вестник». Академик Валерий Иванович Шумаков был главным редактором журнала со дня его основания до 2008 года.

Журнал охватывает широкое информационное поле, объединяя различные аспекты клинических (кардиология, нефрология, гаст-

I am delighted to present to you the third issue of the Russian Journal of Transplantology and Artificial Organs (Vestnik transplantologii i iskusstvennyh organov) for 2019. The year 2019 marks a significant event in Russian transplantology – the 50th anniversary of the National Medical Research Center of Transplantology and Artificial Organs. This leading transplant center has transformed from the first specialized scientific and clinical institution created in 1969 into a world-famous leader in Russian transplantology. Such is the path this center has traveled over half a century.

The Russian Journal of Transplantology and Artificial Organs has been published since 1999. So, our Journal is also celebrating its 20th anniversary this year. However, the real history of publication dates back 25 years – an issue of the Transplantology and Artificial Organs was launched in 1994, which was later succeeded by our Journal. Famous Russian surgeon and transplantologist, Academician Valery Shumakov was the editor-in-chief of the Journal from its foundation till 2008.

The Journal covers a wide information field. Transplantology and the science of artificial organs combine various aspects of clinical (cardiology, nephrology, gastroenterology, surgery, etc.), biomedical (immunology, pathophysiology, etc.)

роэнтерология, хирургия и др.), медико-биологических (иммунология, патофизиология и др.) и даже медико-технических дисциплин, присущие научным работам по трансплантологии и искусственным органам, что и объясняет уникальность настоящего издания.

Сегодня журнал «Вестник трансплантологии и искусственных органов» индексируется в Scopus и размещен на платформе Web Science Core Collection: Emerging Science Citation Index. Приоритетом является качество публикуемых материалов: расширен состав редакционной коллегии, усовершенствован дизайн издания, повышены требования к порядку рецензирования, соблюдению этических принципов научных публикаций.

От имени членов редколлегии, редакционного совета, сотрудников редакции желаю авторам и читателям нашего журнала творческих успехов.

С уважением
академик РАН С.В. Готье

and even medical and technical disciplines. At the same time, none of the listed specialties can fully reflect the essence and specificity of research work on transplantology and artificial organs. That is why this journal is unique.

Today, the Russian Journal of Transplantology and Artificial Organs is a Scopus-indexed journal and it is listed in the Web of Science Core Collection: Emerging Sources Citation Index. Priority lies with the quality of published works. The composition of the editorial board has been expanded, the design of the journal has been improved, requirements for the review procedure and the ethical principles of research publications have all been raised.

On behalf of the members of the editorial board, the editorial council, and the editorial staff, I wish you, the authors and readers of our journal, some creative success.

Sincerely
Academician of the RAS, S.V. Gautier

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ORGAN DONATION AND TRANSPLANTATION IN THE RUSSIAN FEDERATION IN 2018

11TH REPORT OF THE REGISTRY OF THE RUSSIAN TRANSPLANT SOCIETY

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Aim: to monitor current trends and developments in organ donation and transplantation in the Russian Federation based on the 2018 data. **Materials and methods.** Heads of organ transplant centers were surveyed. Data obtained over years from constituent entities of the Russian Federation (also called regions) and from organ transplant centers located in these regions was analyzed and compared. **Results.** According to data retrieved from the 2018 National Registry, only 49 kidney, 28 liver and 18 heart transplant centers were functional in Russia. As of the end of 2018, there were about 6,219 people on the kidney transplant waiting list. This is about 13.8% of the total number of the 45,000 patients receiving dialysis. Donation rate in 2018 was 4.3 donors per million population, while multi-organ procurement level stood at 67.3%. An average of 2.9 organs were procured from one effective donor. In 2018, there were 9.3 kidney transplants, 3.4 liver transplants and 1.9 heart transplants per million population. In the same year, the number of transplants performed in Russia in creased by 12.3% from the year 2017. In Moscow and Moscow Oblast alone, there are 15 functioning organ transplantation centers. These centers perform half of all kidney transplants and 70% of all liver and heart transplants in the country. The number of organ transplant recipients in Russia is approaching 16,000. **Conclusion.** Russia continues to witness a long-term trend of growing number of organ transplants – 10–15% per year. The geographical presence or organ transplant centers continues to expand. The number of transplant centers and their activity is increasing. Donor programs are becoming more effective and efficient. Extrarenal transplantation technologies are being deployed in Russian regions. The number of patients on the national waiting list for organ transplantation is increasing, while their mortality is decreasing. The number of patients with transplanted organs is increasing. Shortages in donor organs in Russia is still down to human causes – poor organization. The number of organ transplants in Russian regions depends on government funding. The quality and safety of transplant programs rely on the transplant activity of centers. In order to achieve the clinical and economic benefits of organ transplantation as a treatment method, monitoring and follow-up after transplant will be required.

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

INTRODUCTION

Current trends and developments in organ donation and transplantation in Russia are monitored via the National Registry under the auspices of the relevant organ transplant commission of the Russian Ministry of Health and the Russian Transplant Society. Previous reports have been published in 2009–2018 [1–9].

In 2017, the Shumakov National Medical Research Center of Transplantology and Artificial Organs (under Russia's Ministry of Health) was included (via order No. 622 of the Ministry of Health of Russia dated September 11, 2017) in the network of national medical research centers of the Ministry of Health of Russia as a leading transplantation institution. In this status, the Research Center is authorized to manage (organizational and methodological) medical organizations engaged in donation and transplantation of human organs and tissues, as well as organizations that engage in analytical activities, including monitoring developments in organs and tissue donation and transplantation in the country (order No. 125 of the Ministry of Health of Russia dated March 13, 2019).

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; Registries of the International Society for Heart and Lung Transplantation – ISHLT Registries.

Since 2016, the national registry has been used as a tool for ensuring quality control and data integrity in

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the information system used for recording donor organs, human tissues, and information about donors and recipients. The information system is existing in accordance with order No. 355n of the Ministry of Health of Russia dated June 8, 2016.

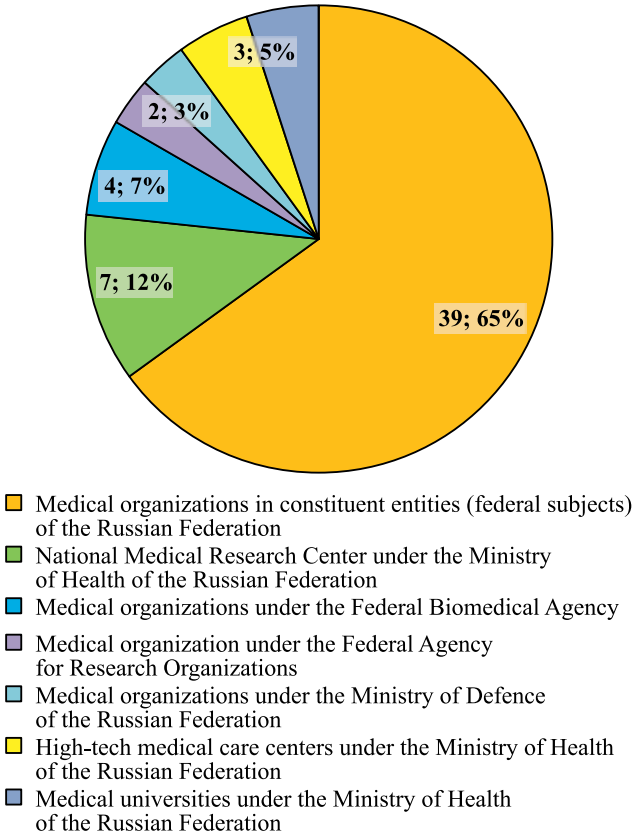

Fig. 1. Structure of the centers of organ transplantation in the Russian Federation in 2018 taking into account their departmental accessory

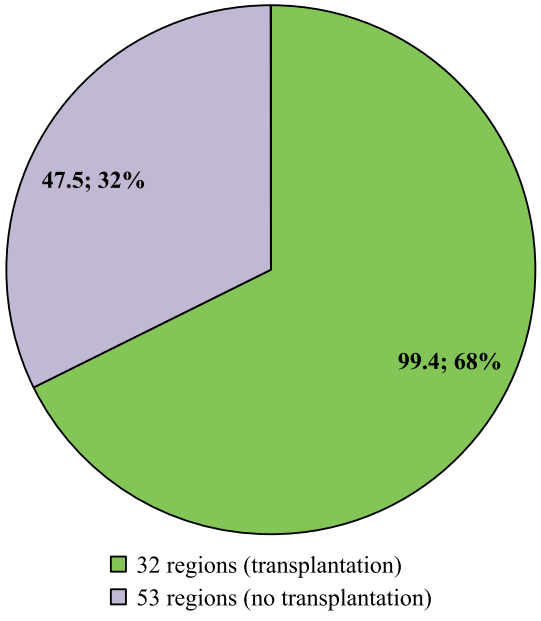

Fig. 2. Population of regions of the Russian Federation in which medical care on organ transplantation is provided (or not) in 2018

Data to be entered in the registry is collected by surveying the relevant officials at all transplant centers in Russia. Data obtained over years from Russian regions, from transplant centers located in these regions 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.

TRANSPLANT CENTERS AND WAITING LISTS

As of December 31, 2018, there were 60 organ transplantation centers functioning in Russia (52 in 2017). Kidney transplant was performed in 49 of these 60 centers, liver transplantation in 28, heart transplantation in 18, pancreatic transplantation in 6, and lung transplantation in 3.

The structure of organ transplant centers in Russia in 2018 is given in Fig. 1. Departmental affiliations of the centers were taken into account.

Of the 60 functioning organ transplant centers, 21 are federal institutions (13 institutions are under the Russian Ministry of Health, 2 institutions are under the Federal Agency for Scientific Organizations, 4 institutions are under the Federal Medical-Biological Agency and 2 institutions are under the Ministry of Defense of the Russian Federation), while 39 are institutions operating in Russia regions.

The increase in the number of organ transplant centers in the country in 2018 was mainly down to the fact that seven new transplant programs were launched at various institutions located in Russian regions, both in places where such programs were not yet available, and in areas already with functioning organ transplant programs. Another new transplant program was launched under the Russian Ministry of Defense.

Table 1 presents the number of potential recipients in organ transplant waiting lists.

The 60 transplantation centers operating in Russia are sited in 32 constituent entities of the Russian Federation. These entities are home to 99.4 million people. Of these 60 centres, 15 operate in Moscow and Moscow Oblast, while 7 centers are in St. Petersburg and Leningrad Oblast. See Fig. 2.

In 2018, some of the regions where organ transplants were performed for the first time included Ryazan Oblast (1.1 million inhabitants), Tula Oblast (1.5 million inhabitants) and Stavropol Krai (2.8 million inhabitants).

As the geographical spread of transplant programs in Russia expands, the vector of managerial decisions aimed at increasing availability and quality of transplant care for the population will likely shift from extensive replication of such programs in the constituent entities of Russia towards an increase in the efficiency of existing programs.

Moreover, 53 regions of the Russian Federation with a population of 47.5 million people do not have func-

Table 1

Transplantation centers in the Russian Federation, waiting lists

Federal district, region, population in 2018 (million people)* Kind of transplantation	**Russian Federation**	CFD					SFD			NCFD	NWFD		SbFD						UFD				VFD								FEFD
		Moscow and Moscow Oblast	Belgorod Oblast	Voronezh Oblast	Ryazan Oblast	Tula Oblast	Krasnodar Krai	Volgograd Oblast	Rostov Oblast	Stavropol Krai	St. Petersburg and Leningrad Oblast	Arkhangelsk Oblast without Nenets Autonomous Okrug	Novosibirsk Oblast	Kemerovo Oblast	Irkutsk Oblast	Omsk Oblast	Altai Krai	Krasnoyarsk Krai	Sverdlovsk Oblast	Tyumen Oblast without autonomous okrugs	Khanty-Mansi Autonomous Okrug – Yugra	Chelyabinsk Oblast	Samara Oblast	Saratov Oblast	Nizhny Novgorod Oblast	Republic of Tatarstan	Republic of Bashkortostan	Orenburg Oblast	Perm Krai	Ulyanovsk Oblast	Sakha Republic (Yakutia)
	146.9	**12.5**	**1.5**	**2.3**	**1.1**	**1.5**	**5.6**	**2.5**	**4.2**	**2.8**	**5.3**	**1.1**	**2.8**	**2.7**	**2.4**	**2.0**	**2.3**	**2.9**	**4.3**	**1.5**	**1.6**	**3.5**	**3.2**	**2.5**	**3.2**	**3.9**	**4.1**	**2.0**	**2.6**	**1.2**	**1.0**
		7.5									**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																															
Number of Transplant Centers	**49**	14	1	1	1	1	1	1	1	1	5	1	1	1	1	1	1	2	1	1	1	1	1	2	1	1	1	1	1	1	1
Number of patients waitlisted for the first time in 2018	**1728**	761	10	13	20	1	88	42	48	4	79	29	26	72	20	27	20	90	61	34	28	12	44	23	51	30	40	20	20	3	12
Total number of waitlist patients in 2018	**6219**	2229	57	119	3	1	352	131	110	4	208	64	120	173	64	70	108	165	270	119	121	148	255	118	458	243	238	104	95	3	69
Number of waitlist patients as of December 31, 2018	**4815**	1529	46	99	17	0	318	108	79	0	115	60	87	110	49	60	89	117	223	85	111	138	211	96	431	215	197	74	91	0	60
Number of waitlist patients who died in 2018	**57**	16	0	5	0	0	1	2	0	0	4	0	1	3	0	4	2	2	1	2	0	0	1	2	3	1	3	4	0	0	0
LIVER																															
Number of Transplant Centers	**26**	6	1	0	0	0	2	0	1	1	3	0	1	1	0	0	1	2	1	0	0	1	1	0	1	1	1	0	0	0	1
Number of patients waitlisted for the first time in 2018	**579**	231	5	0	0	0	32	0	51	1	60	0	23	6	0	0	17	31	32	0	0	3	10	0	39	14	10	0	0	0	14
Total number of waitlist patients in 2018	**1830**	610	73	0	0	0	85	0	123	1	248	0	87	60	0	0	39	39	123	0	0	28	10	0	177	15	90	0	0	0	22

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
Number of waitlist patients as of December 31, 2018	**1171**	208	67	0	0	0	52	0	91	0	204	0	44	54	0	0	36	20	98	0	0	25	8	0	152	14	80	0	0	0	18
Number of waitlist patients who died in 2018	**154**	55	3	0	0	0	17	0	18	0	19	0	6	3	0	0	1	5	10	0	0	1	1	0	8	0	6	0	0	0	1
HEART																															
Number of Transplant Centers	**18**	3	1	0	0	0	1	0	1	0	1	0	1	1	0	0	1	2	1	0	0	1	0	0	1	1	1	0	0	0	1
Number of patients waitlisted for the first time in 2018	**397**	254	0	0	0	0	38	0	11	0	34	0	15	27	0	0	4	20	6	0	0	3	0	0	1	9	5	0	0	0	5
Total number of waitlist patients in 2018	**823**	403	12	0	0	0	73	0	21	0	57	0	41	65	0	0	4	45	49	0	0	11	0	0	1	9	27	0	0	0	5
Number of waitlist patients as of December 31, 2018	**490**	184	10	0	0	0	58	0	16	0	36	0	30	51	0	0	2	26	36	0	0	9	0	0	0	8	20	0	0	0	4
Number of waitlist patients who died in 2018	**48**	11	1	0	0	0	4	0	0	0	5	0	4	9	0	0	0	4	6	0	0	1	0	0	0	0	3	0	0	0	0
PANCREAS																															
Number of Transplant Centers	**6**	4	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Number of patients waitlisted for the first time in 2018	**33**	25	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0
Total number of waitlist patients in 2018	**153**	111	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41	0	0	0	0	0	0
Number of waitlist patients as of December 31, 2018	**132**	96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	36	0	0	0	0	0	0
Number of waitlist patients who died in 2018	**4**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0
LUNGS																															
Number of 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
Number of patients waitlisted for the first time in 2018	**35**	33	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total number of waitlist patients in 2018	**77**	75	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Number of waitlist patients as of December 31, 2018	**34**	34	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
Number of waitlist patients who died in 2018	**15**	15	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

* http://www.gks.ru/free_doc/new_site/population/demo/Popul2018.xls

Table 2

The indicators connected with the waiting list of organ transplantation in the Russian Federation during the period from 2012 to 2018

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

tioning transplant centers, despite the existing need for organ transplantation (mostly by patients receiving renal replacement therapy) and the unused donor organ resource [10].

Thus, the potential for extensive replication of transplant programs in the constituent entities of the Russian Federation has also not yet been exhausted.

The dynamics of indicators related to the organ transplant waiting list in Russia for the period between 2012 to 2018 is presented in Table 2.

In 2018, there were 6,219 potential recipients on the kidney transplant waiting list in Russia. This is 13.8% of the total number of patients (approximately 45,000 according to data from the Russian Dialysis Society) receiving hemodialysis and peritoneal dialysis. Of these, 1,728 were included in the waiting list in 2018 for the first time. In Moscow and Moscow Oblast, 2,229 potential recipients were also on the kidney transplant waiting list (35.8% of the waiting list in the country). The kidney transplant waiting list mortality in Russia was 0.9% (57 patients) in 2018.

In 2018, the liver transplant waiting list had 1,830 potential recipients. Of this number, 579 were included in the waiting list in 2018 for the first time. In Moscow and Moscow Oblast, 610 potential recipients (33.3% of the waiting list in the country) were on the liver transplant waiting list. The liver transplant waitlist mortality in Russia was 8.4% (154 patients) in 2018.

In 2018, the heart transplant waitlist had 823 potential recipients; 397 of them were included in the waiting list in 2018 for the first time. In Moscow, the heart transplantation waiting list featured 403 potential recipients (49.0% of the waiting list in the country). The heart transplant waitlist mortality in Russia was 5.8% (48 patients).

Based on available data, in the period from 2012–2018, the number of patients in the kidney transplant waiting list increased by almost twice in Russia, the liver transplant waiting list increased by 3.75 times, while that of heart transplant increased by 2 times. At the same time, the average waiting time for organ transplantation remained unchanged. On the contrary, mortality in organ transplantation waiting list fell by 64.0% for kidney, 29.4% for liver, and 24.7% for heart transplant waitlist.

In 2018, over 2000 (2193 to be precise) organ transplants were performed in Russia for the first time. This corresponds to 14.9 per million population. Out of this number, 233 were pediatric organ transplants (in 2017, it was 1896 transplants or 12.9 per million population). See Tables 3 and 4.

Based on data obtained from the Federal Registry for high-tech medical care, 1,732 (79.0%) organ transplants were performed in 2018 using funds from the compulsory medical insurance system. These funds were allocated for provision of high-tech medical care for organ transplant (in 2017, the figure was 1443, 76.1%). See Fig. 3.

Since 2010, when funding was included in the registry as an indicator, the number of organ transplants performed using high-tech medical care funds for transplant increased 2.2 times. At the same, the share of organ transplants performed using these funds increased by 35.7%.

In 2018, 53 (88.3%) of 60 transplant centers took part in government assignment to provide high-tech medical care for organ transplant.

The statutory ratios for financial costs per unit of volume of high-tech medical care for transplant in 2018 were as follows:

880,730 rubles for transplantation of kidney, pancreas, kidney & pancreas, small intestine, lungs;

1,117,900 rubles for heart and liver transplantation;

1,596,720 rubles for heart–lung transplants.

(Decree No. 1492 of the Government of the Russian Federation dated December 8, 2017).

ORGAN DONATION

In 2018, donor programs were implemented in 29 (out of 85) regions of Russia with a population of 94.2 million people. In another 3 regions – Tula Oblast, Perm Oblast, Ulyanovsk Oblast – only kidney transplants from a living related donor were performed.

The number of effective posthumous donors in 2018 was 639 (4.3 donors per million population), which is 75 more donors than in 2017 (564). See Tables 5 and 6.

Table 3

Organ donation and transplantation in the Russian Federation in 2018

Indicator	Absolute number	Indicator per million population*
Organ donation		
Total organ donors	1003	6.8
Posthumous donors	639	4.3
Living (related) donors	364	2.5
Organ transplantation		
Total transplanted organs, *including paediatric transplants*	2193 233	14.9 1.6
Kidney, including cadaveric from a living donor, *including paediatric transplants*	1361 1161 200 89	9.3 7.9 1.4 0.6
Liver, including cadaveric from a living donor, *including paediatric transplants*	505 341 164 133	3.4 2.3 1.1 0.9
Heart, including in the “heart-lungs” complex, *including paediatric transplants*	285 3 9	1.9 0.0 0.1
Pancreas	17	0.1
Lungs, including in the “heart-lungs” complex, *including paediatric transplants*	28 3 2	0.2 0.0 0.0

* Russia’s population in 2018 was 146.9 million people
(http://www.gks.ru/free_doc/new_site/population/demo/Popul2018.xls)

Table 4

Transplantation activity in the Russian Federation in 2018

S/N	Transplant Center, Region, Federal District	Total	Kidney (total)	Kidney (cadaveric)	Kidney (related)	Liver (total)	Liver (cadaveric)	Liver (related)	Heart	Pancreas	Lungs	Heart-lungs	Small intestine
1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Central Federal District	**618**	**224**	163	61	**176**	80	96	**194**	**4**	**17**	**3**	**0**
2	Lopatkin Research Institute of Urology and Interventional Radiology, Moscow, Central Federal District	**58**	**58**	47	11	**0**	0	0	**0**	**0**	**0**	**0**	**0**
3	Russian Children’s Clinical Hospital, Moscow, Central Federal District	**33**	**33**	33	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
4	Petrovsky National Research Centre of Surgery, Moscow, Central Federal District	**50**	**39**	16	23	**9**	1	8	**0**	**2**	**0**	**0**	**0**
5	Burnazyan Federal Medical and Biophysical Center, Moscow, Central Federal District	**59**	**13**	13	0	**45**	17	28	**0**	**1**	**0**	**0**	**0**
6	Bakulev National Medical Research Center for Cardiovascular Surgery, Moscow, Central Federal District	**6**	**0**	0	0	**0**	0	0	**6**	**0**	**0**	**0**	**0**
7	National Medical Research Center for Hematology, Moscow, Central Federal District	**15**	**15**	15	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
8	National Medical Research Center for Children’s Health, Moscow, Central Federal District	**4**	**4**	0	4	**0**	0	0	**0**	**0**	**0**	**0**	**0**

Continuation of table 4

1	2	3	4	5	6	7	8	9	10	11	12	13	14
9	Morozov Children's City Clinical Hospital, Moscow, Central Federal District	**1**	**1**	0	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
10	Botkin City Clinical Hospital, Moscow, Central Federal District	**35**	**25**	25	0	**10**	10	0	**0**	**0**	**0**	**0**	**0**
11	Sklifosovsky Research Institute of Emergency Care, Moscow, Central Federal District	**296**	**187**	186	1	**90**	83	7	**5**	**8**	**6**	**0**	**0**
12	Research Institute of Emergency Pediatric Surgery and Traumatology, Moscow, Central Federal District	**1**	**1**	1	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
13	Vishnevsky 3rd Central Military Clinical Hospital, Moscow Oblast, Central Federal District	**1**	**1**	0	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
14	Vladimirsky Moscow Regional Research Clinical Institute, Moscow, Central Federal District	**76**	**59**	55	4	**17**	16	1	**0**	**0**	**0**	**0**	**0**
15	Federal Clinical Center for High Medical Technologies under the Federal Biomedical Agency (119), Moscow Oblast, Central Federal District	**25**	**25**	14	11	**0**	0	0	**0**	**0**	**0**	**0**	**0**
16	St. Joasaphus Belgorod Regional Clinical Hospital, Belgorod, Central Federal District	**12**	**8**	8	0	**3**	3	0	**1**	**0**	**0**	**0**	**0**
17	Voronezh Regional Clinical Hospital No. 1, Voronezh, Central Federal District	**15**	**15**	14	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
18	Tula Regional Clinical Hospital, Tula, Central Federal District	**1**	**1**	0	1	**0**	0	0	**0**	**0**	**0**	**0**	**0**
19	Ryazan Regional Clinical Hospital, Ryazan, Central Federal District	**3**	**3**	3	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
20	Stavropol Regional Clinical Hospital, Stavropol, North Caucasian Federal District	**5**	**4**	1	3	**1**	1	0	**0**	**0**	**0**	**0**	**0**
21	Ochapovsky Regional Clinical Hospital No. 1, Krasnodar, Southern Federal District	**57**	**33**	31	2	**13**	13	0	**11**	**0**	**0**	**0**	**0**
22	Regional Clinical Hospital No. 2, Krasnodar, Southern Federal District	**3**	**0**	0	0	**3**	3	0	**0**	**0**	**0**	**0**	**0**
23	Volzhsky Regional Urological Center, Volzhsky, Southern Federal District	**21**	**21**	16	5	**0**	0	0	**0**	**0**	**0**	**0**	**0**
24	Rostov Regional Clinical Hospital, Rostov-on-Don, Southern Federal District	**51**	**31**	31	0	**14**	12	2	**5**	**1**	**0**	**0**	**0**
25	Russian Research Center for Radiology and Surgical Technologies, St. Petersburg, Northwestern Federal District	**15**	**0**	0	0	**15**	15	0	**0**	**0**	**0**	**0**	**0**
26	Almazov National Medical Research Centre, St. Petersburg, Northwestern Federal District	**16**	**0**	0	0	**0**	0	0	**16**	**0**	**0**	**0**	**0**
27	Pavlov First St. Petersburg State Medical University, St. Petersburg, Northwestern Federal District	**50**	**43**	35	8	**5**	5	0	**0**	**0**	**2**	**0**	**0**
28	St. Petersburg Research Institute of Emergency Medicine, St. Petersburg, Northwestern Federal District	**20**	**20**	20	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
29	Leningrad Regional Clinical Hospital, St. Petersburg, Northwestern Federal District	**24**	**24**	24	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
30	Kirov Military Medical Academy, St. Petersburg, Northwestern Federal District	**6**	**1**	1	0	**5**	5	0	**0**	**0**	**0**	**0**	**0**
31	City Mariinskaya Hospital, St. Petersburg, Northwestern Federal District	**1**	**1**	1	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
32	Volosevich First City Clinical Hospital, Arkhangelsk, Northwestern Federal District	**4**	**4**	4	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
33	Republican Hospital No. 1 – National Center of Medicine, Yakutsk, Far Eastern Federal District	**12**	**9**	7	2	**2**	1	1	**1**	**0**	**0**	**0**	**0**

End of table 4

1	2	3	4	5	6	7	8	9	10	11	12	13	14
34	Meshalkin National Medical Research Center, Novosibirsk, Siberian Federal District	**8**	**0**	0	0	**1**	1	0	**7**	**0**	**0**	**0**	**0**
35	State Novosibirsk Regional Clinical Hospital, Novosibirsk, Siberian Federal District	**68**	**32**	29	3	**36**	23	13	**0**	**0**	**0**	**0**	**0**
36	Research Institute for Complex Issues of Cardiovascular Diseases, Kemerovo, Siberian Federal District	**5**	**0**	0	0	**0**	0	0	**5**	**0**	**0**	**0**	**0**
37	Belyaev Kemerovo Regional Clinical Hospital, Kemerovo, Siberian Federal District	**60**	**60**	60	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
38	Podgorbunsky City Clinical Hospital, Kemerovo, Siberian Federal District	**3**	**0**	0	0	**3**	3	0	**0**	**0**	**0**	**0**	**0**
39	Irkutsk Regional Clinical Hospital, Irkutsk, Siberian Federal District	**16**	**15**	15	0	**1**	1	0	**0**	**0**	**0**	**0**	**0**
40	Omsk City Clinical Hospital No. 1, Omsk, Siberian Federal District	**6**	**6**	6	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
41	Regional Clinical Hospital, Altai Krai (Barnaul), Siberian Federal District	**21**	**17**	17	0	**2**	2	0	**2**	**0**	**0**	**0**	**0**
42	Federal Center for Cardiovascular Surgery, Krasnoyarsk, Siberian Federal District	**4**	**0**	0	0	**0**	0	0	**4**	**0**	**0**	**0**	**0**
43	Federal Siberian Research and Clinical Center, Krasnoyarsk, Siberian Federal District	**30**	**25**	24	1	**5**	5	0	**0**	**0**	**0**	**0**	**0**
44	Regional Clinical Hospital, Krasnoyarsk, Siberian Federal District	**40**	**20**	20	0	**9**	9	0	**11**	**0**	**0**	**0**	**0**
45	Sverdlovsk Regional Clinical Hospital No. 1, Yekaterinburg, Ural Federal District	**68**	**46**	44	2	**15**	15	0	**7**	**0**	**0**	**0**	**0**
46	Chelyabinsk Regional Clinical Hospital, Chelyabinsk, Ural Federal District	**13**	**10**	8	2	**2**	2	0	**1**	**0**	**0**	**0**	**0**
47	Regional Clinical Hospital No. 1, Tyumen, Ural Federal District	**32**	**32**	26	6	**0**	0	0	**0**	**0**	**0**	**0**	**0**
48	District Clinical Hospital, Khanty-Mansiysk, Ural Federal District	**10**	**10**	7	3	**0**	0	0	**0**	**0**	**0**	**0**	**0**
49	Samara State Medical University, Samara, Volga Federal District	**44**	**43**	43	0	**1**	1	0	**0**	**0**	**0**	**0**	**0**
50	Saratov State Medical University, Saratov, Volga Federal District	**8**	**8**	0	8	**0**	0	0	**0**	**0**	**0**	**0**	**0**
51	Regional Clinical Hospital, Saratov, Volga Federal District	**12**	**12**	12	0	**0**	0	0	**0**	**0**	**0**	**0**	**0**
52	Volga Regional Medical Center, Nizhny Novgorod, Volga Federal District	**42**	**24**	21	3	**17**	10	7	**0**	**1**	**0**	**0**	**0**
53	Specialized Cardiac Surgical Clinical Hospital, Nizhny Novgorod, Volga Federal District	**1**	**0**	0	0	**0**	0	0	**1**	**0**	**0**	**0**	**0**
54	Republican Clinical Hospital, Kazan, Volga Federal District	**28**	**27**	8	19	**1**	1	0	**0**	**0**	**0**	**0**	**0**
55	Interregional Clinical Diagnostic Center, Kazan, Volga Federal District	**1**	**0**	0	0	**0**	0	0	**1**	**0**	**0**	**0**	**0**
56	Republican Clinical Hospital, Ufa, Volga Federal District	**42**	**38**	38	0	**4**	4	0	**0**	**0**	**0**	**0**	**0**
57	Republican Cardiology Clinic, Ufa, Volga Federal District	**4**	**0**	0	0	**0**	0	0	**4**	**0**	**0**	**0**	**0**
58	Perm Regional Clinical Hospital, Perm, Volga Federal District	**4**	**4**	0	4	**0**	0	0	**0**	**0**	**0**	**0**	**0**
59	Ulyanovsk Regional Clinical Center for Specialized Types of Medical Care, Ulyanovsk, Volga Federal District	**3**	**3**	0	3	**0**	0	0	**0**	**0**	**0**	**0**	**0**
60	City Clinical Hospital for Emergency Medical Care No. 1, Orenburg, Volga Federal District	**26**	**26**	19	7	**0**	0	0	**0**	**0**	**0**	**0**	**0**
Total for 2018		**2193**	**1361**	**1161**	**200**	**505**	**342**	**163**	**282**	**17**	**25**	**3**	**0**

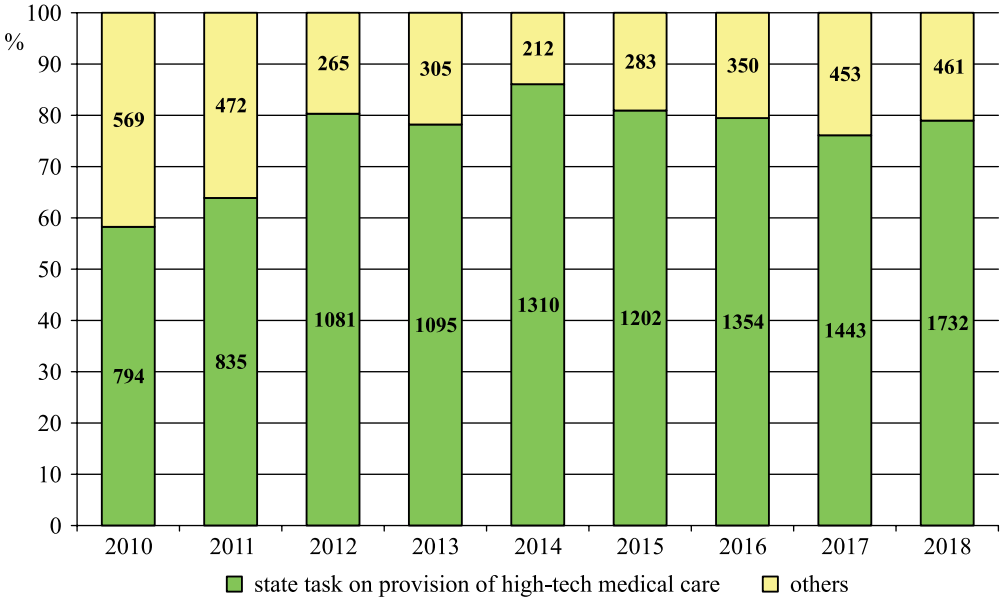

Fig. 3. Financing of transplantation in the Russian Federation in 2010–2018

In 2018, Moscow and Moscow Oblast accounted for 44.7% (286) of effective donors. The figure was 47.9% (270) in 2017.

Donor activity per population of regions implementing donor programs (94.2 million) amounted to 6.8 per million population.

Highest donor activity was recorded in Moscow (17.3), Kemerovo Oblast (11.1), Moscow Oblast (9.1), Tyumen Oblast (8.7), Leningrad Oblast (8.3), Samara Oblast (7.2), St. Petersburg (6.3), Novosibirsk Oblast (6.1), Sverdlovsk Oblast (5.6) and Krasnoyarsk Krai (5.5).

Low donor activity in 2018 was in Ryazan Oblast (1.8 at the beginning of the program), Omsk Oblast (1.6 during recession), Chelyabinsk Oblast (1.1 during recession), Republic of Tatarstan (1.0 during recession), and Stavropol Krai (0.7 at the beginning of the program).

In 2018, regional donor programs showed multidirectional dynamics. See Table 7.

In 19 regions, the number of effective donors increased in 2018 to a total of 99 donors. There was a major hike in donor activity in Moscow (+23), Krasnoyarsk Krai (+13), Tyumen Oblast (+9), Kemerovo Oblast (+8), Voronezh Oblast (+7, resumption of donor program after a collapse in 2017), Rostov Oblast (+6), Arkhangelsk Oblast and Irkutsk Oblast (+5).

In 6 regions, the number of effective donors fell in 2018 to a total of 24 (in 2017, there was a decrease in 8 regions to a total of 33 effective donors). Organ donor activity decreased considerably in Moscow Oblast (–7), Samara Oblast (–5) and Chelyabinsk Oblast (–4).

In 2018, the practice of pronouncing brain death continued to expand in Russia. The number of effective brain-dead donors was 601 (it was 516 in 2017). Their proportion in the total pool of effective donors increased to 94.0% (from 91.5% in 2017). See Fig. 4.

In 24 regions of the Russian Federation, organ donor programs worked only with donors determined to be brain dead (21 organ donor programs in 2017). For the first time, there were no organ donor programs that did not follow the protocol for diagnosing human death based on diagnosis of brain death. The protocol for determining brain death has been successfully implemented in Irkutsk Oblast: 7 effective donors (100%) in 2018.

The low proportion of brain-dead donors in the donor programs of Kemerovo Oblast (36.7%) and Saratov Oblast (62.5%) is not consistent with the modern level of technology development. Moreover, it hampers efficient use of donor resource. This therefore needs a major correction through targeted implementation and supervision of implementation of the protocol for determining brain death.

In 2018, a total of 430 multi-organ procurements were completed. This is more than the 375 recorded in 2017. The proportion of multi-organ procurements was 67.3% (66.5% in 2017).

In Moscow and Moscow Oblast, there were 239 multi-organ donors, which is 55.6% of the total number of multi-organ donors in Russia (217 donors and 57.9% in 2017).

The number of donor programs involving a high share of multi-organ procurements (more than 70%) were 13 in number – in 6 of the programs, multiple organs were procured from all (100%) the patients. They were in Voronezh, Ryazan, Rostov, Novosibirsk, Nizhny Novgorod, and the program of the Russian Ministry of Defense.

In 2018, an average of 2.9 organs were procured from one donor (2.8 in 2017). The highest number of organ procurement were, as before, in regions where extrarenal

Table 5

Donor activity in various regions of the Russian Federation in 2018

S/N	Region	Organ Donation Coordinating Center (region)	Population (million)	Number of donor bases	Effective donors (absolute, per million population)		Including with brain death diagnosis (absolute, %)		Including multi-organ donors (absolute, %)		Total harvested organs	Including harvested kidneys	Ratio of number of organs to number of donors	Percentage of harvested kidneys
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Moscow	Moscow Coordinating Center for Organ Donation, Moscow (Botkin City Clinical Hospital)	12.6	17	218	17.3	215	98.6	182	83.5	722	405	3.3	92.9
2	Moscow Oblast	Vladimirsky Moscow Regional Research Clinical Institute, Moscow	7.5	33	68	9.1	58	85.3	57	83.8	212	123	3.1	90.4
3	Belgorod Oblast	St. Joasaphus Belgorod 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	Voronezh Regional Clinical Hospital No. 1, Voronezh	2.3	10	8	3.5	8	100.0	2	25.0	19	14	2.4	87.5
5	Ryazan Oblast	Ryazan Regional Clinical Hospital, Ryazan, Central Federal District	1.1	1	2	1.8	2	100.0	2	100.0	7	4	3.5	100.0
6	Krasnodar Krai	Ochapovsky Regional Clinical Hospital No.1, Krasnodar	5.6	2	20	3.6	20	100.0	16	80.0	60	31	3.0	77.5
7	Volgograd Oblast	Volzhsky Regional Urological Center, Volzhsky	2.5	11	9	3.6	9	100.0	0	0.0	16	16	1.8	88.9
8	Rostov Oblast	Rostov Regional Clinical Hospital, Rostov-on-Don	4.2	1	19	4.5	19	100.0	19	100.0	57	37	3.0	97.4
9	Stavropol Krai	Stavropol Regional Clinical Hospital, Stavropol, North Caucasian Federal District	2.8	1	2	0.7	2	100.0	1	50.0	5	4	2.5	100.0
10	St. Petersburg	Center for Organ and Tissue Donation, St. Petersburg (St. Petersburg Research Institute of Emergency Medicine)	5.4	13	34	6.3	31	91.2	20	58.8	81	54	2.4	79.4
11	Leningrad Oblast	Leningrad Regional Clinical Hospital, St. Petersburg	1.8	1	15	8.3	15	100.0	10	66.7	47	30	3.1	100.0
12	Arkhangelsk Oblast	Volosevich First City Clinical Hospital, Arkhangelsk, Northwestern Federal District	1.1	1	5	4.5	5	100.0	3	60.0	14	9	2.8	90.0
13	Novosibirsk Oblast	State Novosibirsk Regional Clinical Hospital, Novosibirsk	2.8	10	17	6.1	17	100.0	17	100.0	54	32	3.2	94.1
14	Kemerovo Oblast	Belyaev Kemerovo Regional Clinical Hospital, Kemerovo	2.7	15	30	11.1	11	36.7	8	26.7	70	58	2.3	96.7
15	Irkutsk Oblast	Irkutsk Regional Clinical Hospital, Irkutsk	2.4	1	7	2.9	7	100.0	1	14.3	15	14	2.1	100.0
16	Omsk Oblast	Omsk City Clinical Hospital No.1, Omsk	1.9	2	3	1.6	3	100.0	2	66.7	8	6	2.7	100.0
17	Altai Krai	Regional Clinical Hospital, Barnaul	2.3	1	8	3.5	8	100.0	7	87.5	25	16	3.1	100.0
18	Krasnoyarsk Oblast	Krasnoyarsk Clinical Hospital, Krasnoyarsk	2.9	12	16	5.5	16	100.0	13	81.3	44	26	2.8	81.3
19	Sverdlovsk Oblast	Sverdlovsk Regional Clinical Hospital No. 1, Yekaterinburg	4.3	8	24	5.6	24	100.0	17	70.8	66	44	2.8	91.7
20	Chelyabinsk Oblast	Chelyabinsk Regional Clinical Hospital, Chelyabinsk	3.5	1	4	1.1	4	100.0	3	75.0	12	8	3.0	100.0

End of table 5

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
21	Tyumen Oblast	Regional Clinical Hospital No. 1, Tyumen	1.5	1	13	8.7	13	100.0	1	7.7	27	26	2.1	100.0
22	Khanty-Mansi Autonomous Okrug – Yugra	District Clinical Hospital, Khanty-Mansiysk	1.7	8	4	2.4	4	100.0	0	0.0	7	7	1.8	87.5
23	Samara Oblast	Samara State Medical University, Samara	3.2	5	23	7.2	23	100.0	4	17.4	49	43	2.1	93.5
24	Saratov Oblast	Regional Clinical Hospital, Saratov	2.4	1	8	3.3	5	62.5	0	0.0	16	16	2.0	100.0
25	Nizhny Novgorod Oblast	Volga Regional Medical Center, Nizhny Novgorod	3.2	9	12	3.8	12	100.0	12	100.0	33	21	2.8	87.5
26	Republic of Tatarstan	Republican Clinical Hospital, Kazan	3.9	1	4	1.0	4	100.0	2	50.0	10	8	2.5	100.0
27	Republic of Bashkortostan	Republican Clinical Hospital, Ufa	4.1	12	20	4.9	20	100.0	5	25.0	46	38	2.3	95.0
28	Orenburg Oblast	City Clinical Hospital for Emergency Medical Care No. 1, Orenburg	2.0	2	8	4.0	8	100.0	5	62.5	20	15	2.5	93.8
29	Sakha Republic (Yakutia)	Republican Hospital No. 1-National Center of Medicine, Yakutsk	1.0	1	4	4.0	4	100.0	0	0.0	9	7	2.3	87.5
30	Departmental program of the Federal Biomedical Agency of Russia	Burnazyan Federal Medical and Biophysical Center, Moscow	–	28	5	–	5	100.0	3	60.0	16	9	3.2	90.0
31	Departmental program of the Federal Biomedical Agency of the Russian Federation	Federal Siberian Research and Clinical Center, Krasnoyarsk	–	5	24	–	24	100.0	13	54.2	63	44	2.6	91.7
32	Departmental program of the Ministry of Defense of the Russian Federation	Kirov Military Medical Academy, St. Petersburg, Northwestern Federal District	–	1	1	–	1	100.0	1	100.0	3	2	3.0	100.0
		Total	**146.8**	**216**	**639**	**4.4**	**601**	**94.1**	**430**	**67.3**	**1845**	**1175**	**2.9**	**91.9**

Table 6

Deceased organ donors (effective donors) in 2006–2018

S/N	Region	2006	2007		2008		2009		2010		2011		2012		2013		2014		2015		2016		2017		2018	
		Number of effective donors	Number of effective donors	Absolute change for the year	Number of effective donors	Absolute change for the year	Number of effective donors	Absolute change for the year	Number of effective donors	Absolute change for the year	Number of effective donors	Absolute change for the year	Number of effective donors	Absolute change for the year	Number of effective donors	Absolute change for the year	Number of effective donors	Absolute change for the year	Number of effective donors	Absolute change for the year	Number of effective donors	Absolute change for the year	Number of effective donors	Absolute change for the year	Number of effective donors	Absolute change for the year
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
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
2	Moscow Oblast	24	45	+21	59	+14	52	–7	71	+19	82	+11	61	–21	56	–5	51	–5	44	–7	39	–5	75	+36	68	–7
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	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
5	Ryazan Oblast																								2	+2
6	Krasnodar Krai						3	+3	39	+36	52	+13	42	–10	41	–1	23	–18	25	+2	24	–1	19	–5	20	+1
7	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
8	Rostov Oblast																		1	+1	7	+6	13	+6	19	+6
9	Stavropol Krai																								2	+2
10	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
11	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
12	Arkhangelsk Oblast																								5	+5
13	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
14	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
15	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	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
17	Altai Krai														3	+3	5	+2	4	–1	4	0	8	+4	8	0
18	Krasnoyarsk Krai																3	+3	6	+3	18	+12	27	+9	16	Note
19	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
20	Chelyabinsk Oblast								6	+6	2	–4	7	+5	6	–1	10	+4	9	–1	11	+2	8	–3	4	–4
21	Tyumen Oblast																						4	+4	13	+9
22	Khanty-Mansi Autonomous Okrug – Yugra																						3	+3	4	+1
23	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

End of table 6

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
24	Saratov Oblast														4	+4	7	+3	7	0	7	0	7	0	8	+1
25	Nizhny Novgorod Oblast						7	+7	11	+4	12	+1	10	–2	8	–2	12	+4	10	–2	11	+1	10	–1	12	+2
26	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
27	Republic of Bashkortostan								2	+2	7	+5	14	+7	18	+4	19	+1	14	+5	20	+6	22	+2	20	–2
28	Orenburg Oblast																		3	+3	8	+5	9	+1	8	–1
29	Sakha Republic (Yakutia)																				2	+2	4	+2	4	0
30	Federal Biomedical Agency, Moscow														6	+6	11	+5	14	+3	16	+2	9	–7	5	–4
31	Federal Biomedical Agency, Krasnoyarsk																								24	Note
32	Russian Ministry of Defense, St. Petersburg																								1	+1
	TOTAL (in the Russian Federation)	**225**	**300**	**+75**	**364**	**+64**	**381**	**+17**	**487**	**+106**	**470**	**–17**	**412**	**–58**	**420**	**+8**	**465**	**+45**	**434**	**–31**	**481**	**+53**	**565**	**+78**	**639**	**+74**

Note. The donor activity of the Federal Siberian Research Clinical Centre (under the Federal Biomedical Agency), Krasnoyarsk, is presented as a separate program.

Table 7

Rating of regions donor activity in 2018

Constituent entities (regions) of the Russian Federation	Population in 2018 (million)	Number of effective donors per million population		Rating	
		2018	2017	2018	2017
Moscow	12.6	17.3	15.7	1	1
Kemerovo Oblast	2.7	11.1	8.1	2	5
Moscow Oblast	7.5	9.1	10.1	3	2
Tyumen Oblast	1.5	8.7	2.7	4	20
Leningrad Oblast	1.8	8.3	6.1	5	6
Samara Oblast	3.2	7.2	8.8	6	4
St. Petersburg	5.4	6.3	5.8	7	7
Novosibirsk Oblast	2.8	6.1	5.0	8	10
Sverdlovsk Oblast	4.3	5.6	5.1	9	9
Krasnoyarsk Krai	2.9	5.5	9.3	10*	3
Republic of Bashkortostan	4.1	4.9	5.4	11	8
Rostov Oblast	4.2	4.5	3.1	12	16
Arkhangelsk Oblast	1.1	4.5	0.0	13	–
Orenburg Oblast	2.0	4.0	4.5	14	11
Sakha Republic (Yakutia)	1.0	4.0	4.0	15	12
Nizhny Novgorod Oblast	3.2	3.8	3.1	16	17
Volgograd Oblast	2.5	3.6	3.6	17	13
Krasnodar Krai	5.6	3.6	3.4	18	15
Altai Krai	2.3	3.5	3.3	19	14
Voronezh Oblast	2.3	3.5	0.4	20	26
Saratov Oblast	2.4	3.3	2.8	21	18
Irkutsk Oblast	2.4	2.9	0.8	22	25
Belgorod Oblast	1.5	2.7	2.7	23	19
Khanty-Mansi Autonomous Okrug – Ugra	1.7	2.4	1.9	24	23
Ryazan Oblast	1.1	1.8	0.0	25	–
Omsk Oblast	1.9	1.6	2.0	26	22
Chelyabinsk Oblast	3.5	1.1	2.3	27	21
Republic of Tatarstan	3.9	1.0	0.8	28	24
Stavropol Krai	2.8	0.7	0.0	29	–
Russia (85 constituent entities of the Russian Federation)	146.9	4.4	3.8	–	–

Note. The donor program of the Federal Siberian Research Clinical Centre (under the Federal Biomedical Agency), Krasnoyarsk, was not included.

organs were transplanted and (or) regions where there was interregional coordination – Ryazan Oblast (3.5), Moscow (3.3), Novosibirsk Oblast (3.2), Moscow Oblast and Leningrad Oblast and Altai Krai (3.1). There were low (1.8) procurement in Volgograd Oblast and Khanty-Mansi Autonomous Okrug – Yugra.

In 2018, the rate of procurement and transplantation of donor kidneys was 91.9% (86.3% in 2017). This indicator was in the optimal range of 90–100% in 21 regions and between 80–90% in 6 regions. It was less than 80% in only 2 programs – Krasnodar Krai (77.5%) and St. Petersburg (79.4%)

In 2018, 364 organs were procured from living related donors – 36.3% of 1003 (the total number of procurements). In 2017, it was 332 procured organs or 37.0% of 896).

KIDNEY TRANSPLANTATION

In 2018, a total of 1,361 kidney transplants were performed (9.3 per million population), which is more than in previous years. See Fig. 5.

The kidney transplants took place at 49 centers.

There were 1,161 cadaveric kidney transplants in 2018, which is 187 (+19.2%) more transplants than in 2017 (974). There were 200 (201 in 2017) kidney transplants from a living related donor.

Table 8 and Fig. 6 show the kidney transplant centers where the highest number of kidney transplants was done as of the end of 2018.

The activity of kidney transplant centers in 2018 varied widely. Five centers performed over 50 transplant surgeries each, 10 centers carried out 30 to 50 surgeries per year, another 14 centers performed 15 to 29 surge-

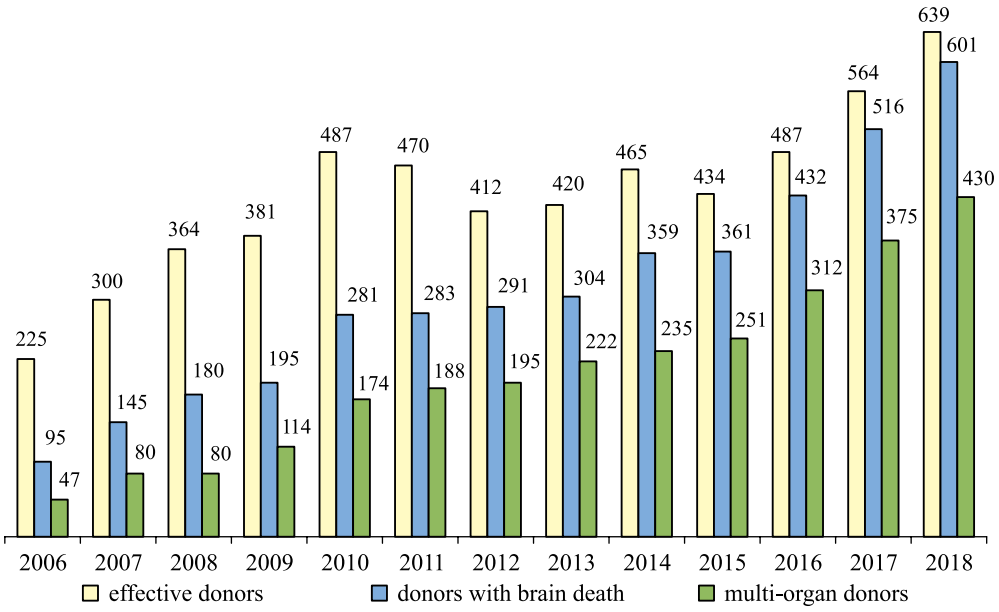

Fig. 4. Structure of effective donors in the Russian Federation in 2006–2018

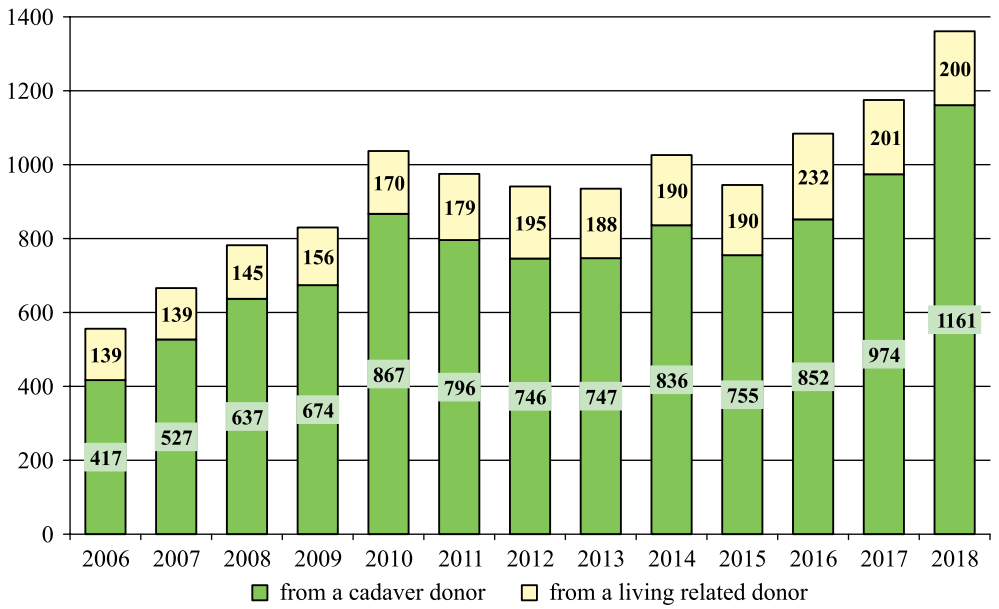

Fig. 5. Kidney transplantation in the Russian Federation in 2006–2018

ries, and the remaining 20 centers performed less than 15 kidney surgeries.

All the 14 kidney transplant centers located in Moscow and Moscow Oblast performed half – 50.3% (685) – of all kidney transplants carried out in the country (against 629 or 53.5% in 2017).

Of these, 4 centers performed more than 50 kidney transplants in a year. They are the Shumakov National Medical Research Center of Transplantology and Artificial Organs (224 kidney transplants), Sklifosovsky Research Institute of Emergency Care (187), Vladimirsky Moscow Regional Research Clinical Institute (59) and National Medical Research Radiological Center (58).

In 2018, 28 centers out of 49 in Russia performed related kidney transplant surgeries. A total of 200 transplants were performed (201 in 2017). Nine centers in Moscow and Moscow Oblast performed 117 related kidney transplants in 2018, or 58.5% of the total number of related kidney transplants performed in the country (114 and 56.5% in 2017). Two centers performed more than 20 related kidney transplants – Shumakov National Medical Research Center of Transplantology and Artificial Organs (61) and Petrovsky National Research Centre of Surgery (23). The average frequency of using intravital kidney donation in 2018 was 14.7% of the total number of kidney transplants (17.1% in 2017).

Table 8

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

Rank	Name of medical organization	Number of kidney transplants performed in 2018
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Central Federal District	224
2	Sklifosovsky Research Institute of Emergency Care, Moscow, Central Federal District	187
3	Belyaev Kemerovo Regional Clinical Hospital, Kemerovo, Siberian Federal District	60
4	Vladimirsky Moscow Regional Research Clinical Institute, Moscow, Central Federal District	59
5	National Medical Research Center for Radiology, Moscow, Central Federal District	58
6	Sverdlovsk Regional Clinical Hospital No. 1, Yekaterinburg, Ural Federal District	46
7	Pavlov First St. Petersburg State Medical University, St. Petersburg, Northwestern Federal District	43
8	Samara State Medical University, Samara, Volga Federal District	43
9	Petrovsky National Research Centre of Surgery, Moscow, Central Federal District	39
10	Kuvatov Republican Clinical Hospital, Ufa, Volga Federal District	38
	Total	797
	58.6% (1361) of the total number of kidney transplants in the Russian Federation	

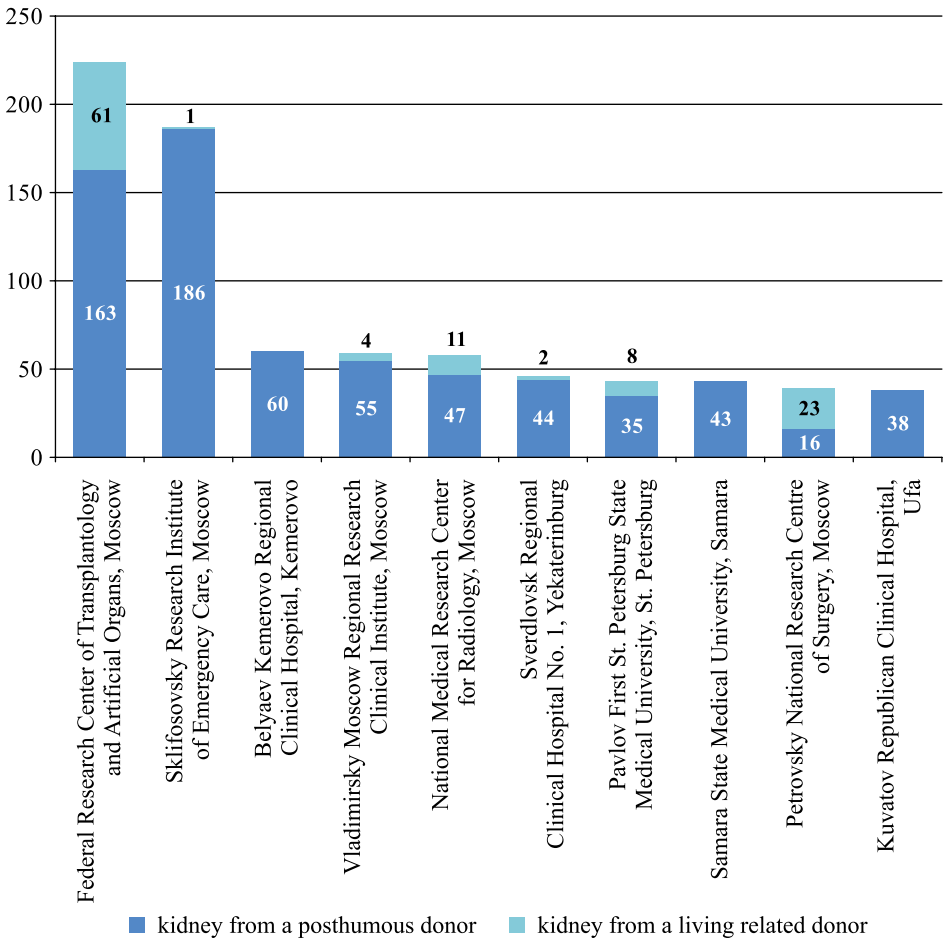

Fig. 6. The medical organizations – leaders in number of transplantations of a kidney

In 2018, nine centers performed pediatric kidney transplants. A total of 89 kidney transplants were carried out (105 in 2017); 85 (95.5%) of them in Moscow, including at Russian Children's Clinical Hospital (33), Petrovsky National Research Centre of Surgery (25) and Shumakov National Medical Research Center of Transplantology and Artificial Organs (20). See Fig. 7.

EXTRARENAL ORGAN TRANSPLANT

In 2018, 282 heart transplants were performed (1.9 per million population), of which 9 were pediatric transplants. This is more than the figure recorded in previous years, especially in 2017 (252), +11.9%.

Heart transplants were performed at 18 centers.

Two new heart transplant programs were launched in 2018: at Republican Hospital No. 1 – National Center of Medicine, Yakutsk, one heart transplant carried out;

In the Regional Clinical Hospital, Barnaul, two heart transplants were performed.

Shumakov National Medical Research Center of Transplantology and Artificial Organs (Moscow) performed 68.8% (194 heart transplants) of the total number of heart transplants done in Russia. The successful heart transplant program in this center, along with new programs, continues to direct the overall positive trend in the increase in the number of heart transplants recorded in 2009–2018 in the country.

Table 9 and Fig. 8 show thoracic organ transplant centers, where the largest number of heart and lung transplants was done as of the end of 2018.

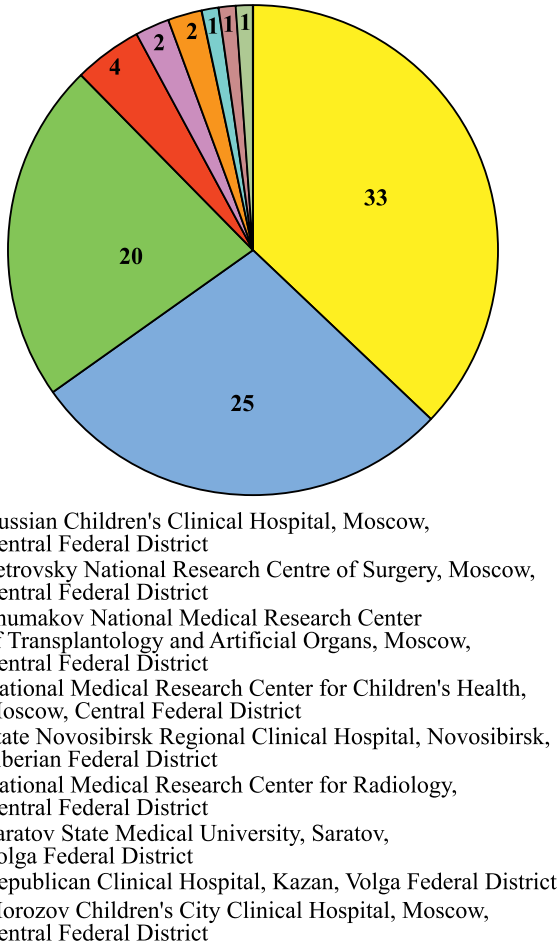

Fig. 7. Pediatric kidney transplantation in the Russian Federation in 2018

Table 9

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

Rank	Name of medical organization	Number of heart transplants performed in 2018
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Central Federal District	194
2	Almazov National Medical Research Centre, St. Petersburg, Northwestern Federal District	16
3	Ochapovsky Regional Clinical Hospital No.1, Krasnodar, Southern Federal District	12
4	Krasnoyarsk Clinical Hospital, Krasnoyarsk, Siberian Federal District	11
5	Sverdlovsk Regional Clinical Hospital No. 1, Yekaterinburg, Ural Federal District	7
6	Meshalkin National Medical Research Center, Novosibirsk, Siberian Federal District	7
7	Bakulev Scientific Center for Cardiovascular Surgery, Moscow, Central Federal District	6
8	Research Institute for Complex Problems of Cardiovascular Diseases, Kemerovo, Siberian Federal District	5
9	Rostov Regional Clinical Hospital, Rostov-on-Don, Southern Federal District	5
10	Sklifosovsky Research Institute of Emergency Care, Moscow, Central Federal District	5
	TOTAL	268
	95.0% (282) of the total number of heart transplants in the Russian Federation	

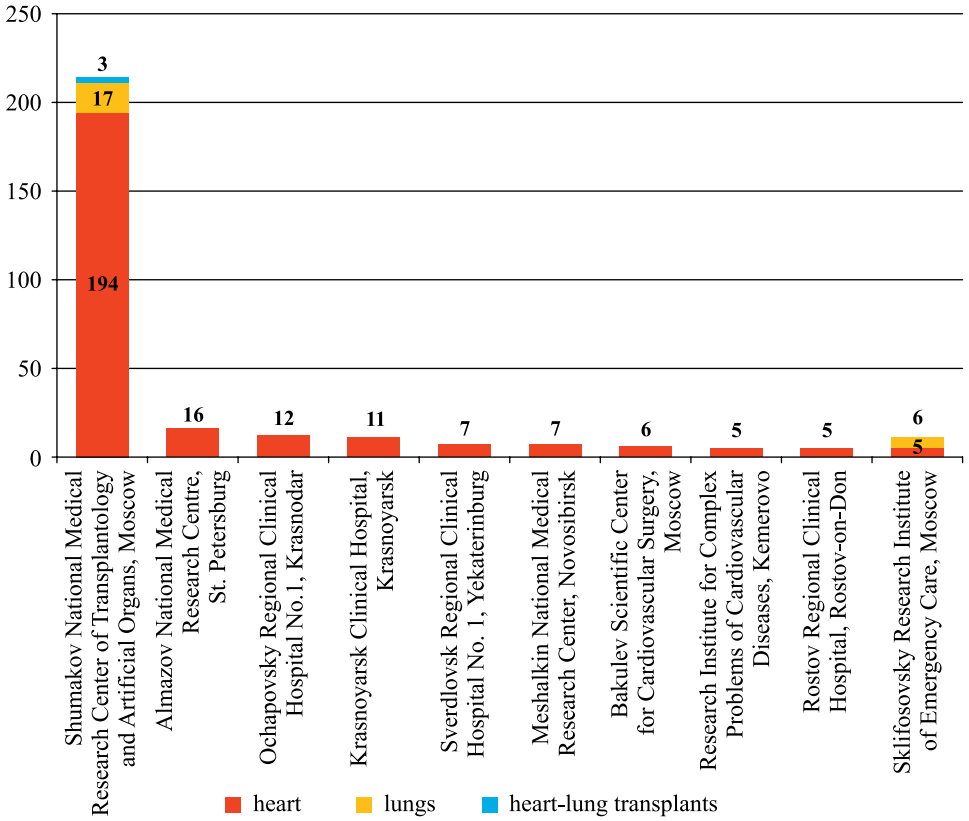

Fig. 8. The medical organizations – leaders in number of transplantations of thoracic organs

Apart from the Shumakov National Medical Research Center of Transplantology and Artificial Organs, three other transplantation centers did over 10 heart transplants in 2018 – the Almazov National Medical Research Centre in St. Petersburg (16 heart transplants), Ochapovsky Regional Clinical Hospital No. 1 in Krasnodar (11) and Regional Clinical Hospital in Krasnoyarsk (11).

In 2018, three transplantation centers performed lung transplants. A total of 25 transplants were performed (25 in 2017), of which 2 were pediatric lung transplantations; 17 lung transplants were performed at the Shumakov National Medical Research Center of Transplantology and Artificial Organs, 6 transplants at Sklifosovsky Research Institute of Emergency Care, and two at Pavlov First St. Petersburg State Medical University. In 2018, Shumakov National Medical Research Center of Transplantology and Artificial Organs also performed three heart–lung transplants.

In 2018, a total of 505 liver transplants were done – 3.4 per million population. This is more than in previous years, especially in 2017 (438), +15.3%.

Liver transplants were performed at 28 centers.

In 2018, four new liver transplant programs were launched. Specifically, Botkin City Clinical Hospital (Moscow) performed 10 transplantations from posthumous organ donors; Irkutsk Regional Clinical Hospital (Irkutsk) conducted one liver transplant from a posthumous donor.

In 2018, the five Moscow-based transplant centers retained their share in liver transplantation – 68.7% (347 transplants) – against the 68.3% (299 transplants) recorded in 2017.

Table 10 and Fig. 9 present liver transplant centers, where the highest liver transplants were done as of the end of 2018.

In 2018, four transplant centers performed over 20 liver transplants each. They are the Shumakov National Medical Research Center of Transplantology and Artificial Organs (176 liver transplant surgeries), Sklifosovsky Research Institute of Emergency Care (90), Burnazyan Federal Medical and Biophysical Center (45) and State Novosibirsk Regional Clinical Hospital (36).

Related liver transplants were performed in 9 centers. The proportion of living related donor transplantations was 164 (32.5%). In 2017, there were 11 centers that performed 131 related liver transplants (29.9%).

In 2018, a total of 133 pediatric (mostly young children) liver transplants were performed against 106 (+25.5%) in 2016. Three centers performed pediatric liver transplants: Shumakov National Medical Research Center of Transplantology and Artificial Organs (123), Petrovsky National Research Centre of Surgery (7) and State Novosibirsk Regional Clinical Hospital (3). See Fig. 10.

In 2018, six transplantation centers performed pancreas transplants. A total of 17 pancreas transplants were

done (against 6 in 2017), of which 16 were in conjunction with kidney.

There were 832 extrarenal transplants performed in 2018 or 37.9% of the total number (2193) of transplants (against 721 or 38.0% of 1896 recorded in 2017). Transplant centers located in Moscow and Moscow Oblast remain key players in extrarenal organ transplantation in the country. In 2018, the two performed 593 extrarenal

Table 10

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

Rank	Leading centers in terms of number of liver transplants performed	Number of liver transplants performed in 2018
1	Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Central Federal District	176
2	Sklifosovsky Research Institute of Emergency Care, Moscow, Central Federal District	90
3	Burnazyan Federal Medical and Biophysical Center, Moscow, Central Federal District	45
4	State Novosibirsk Regional Clinical Hospital, Novosibirsk, Siberian Federal District	36
5	Vladimirsky Moscow Regional Research Clinical Institute, Moscow, Central Federal District	17
6	Volga Regional Medical Center, Nizhny Novgorod, Volga Federal District	17
7	Russian Research Center for Radiology and Surgical Technologies, St. Petersburg, Northwestern Federal District	15
8	Sverdlovsk Regional Clinical Hospital No. 1, Yekaterinburg, Ural Federal District	15
9	Rostov Regional Clinical Hospital, Rostov-on-Don, Southern Federal District	14
10	Ochapovsky Regional Clinical Hospital No. 1, Krasnodar, Southern Federal District	13
	TOTAL	438
	86.7% (505) of the total number of liver transplants in the Russian Federation	

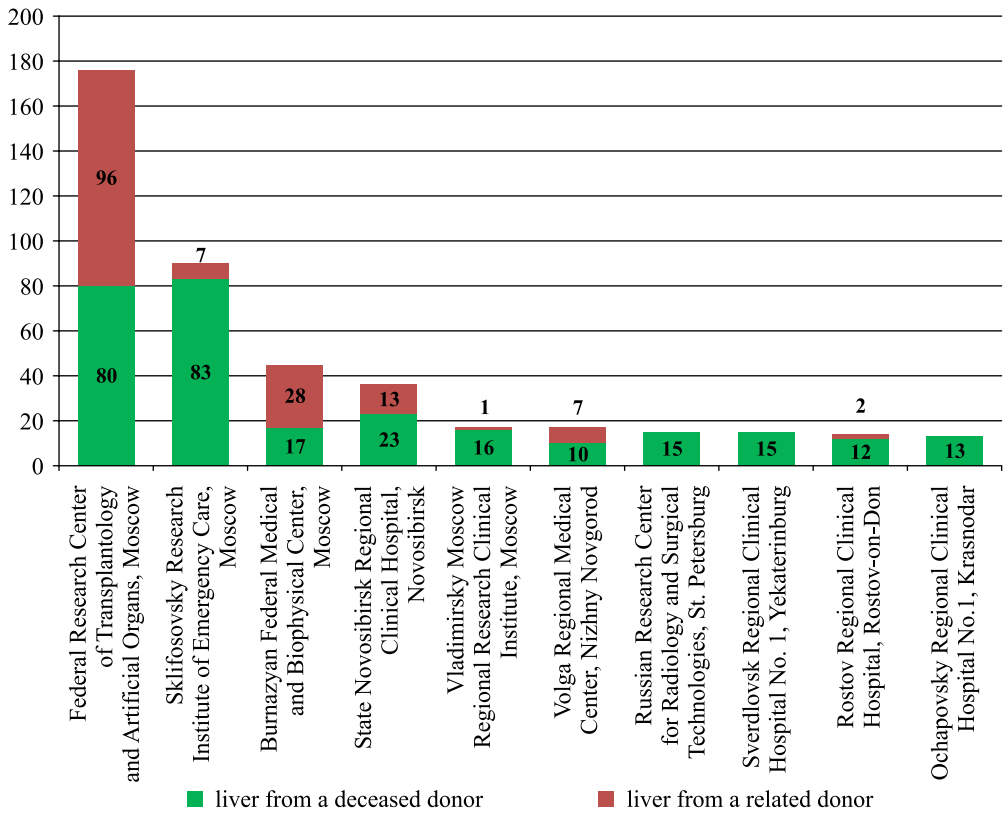

Fig. 9. The medical organizations – leaders in number of transplantations of a liver

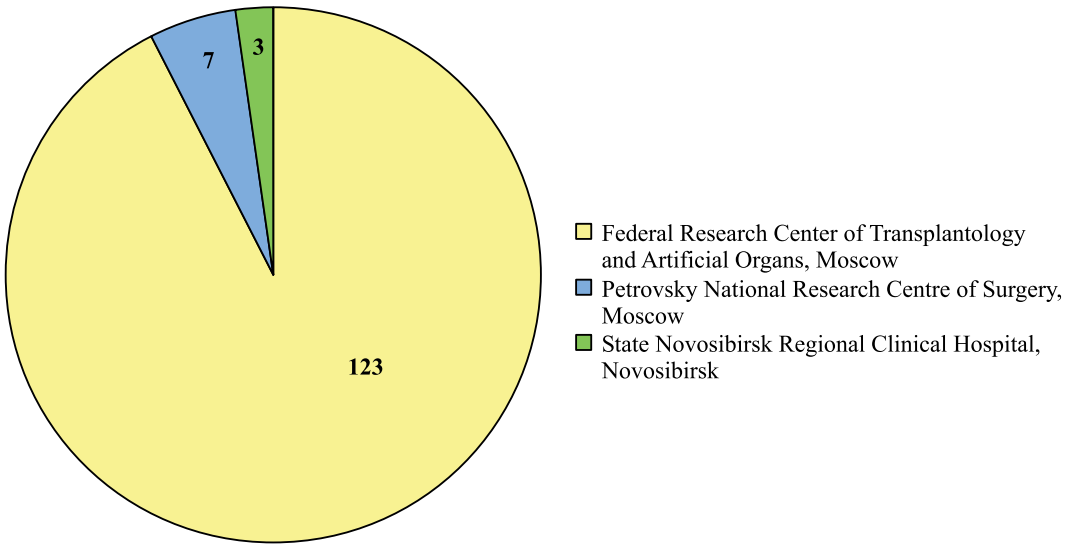

Fig. 10. Pediatric liver transplantation in the Russian Federation in 2018

organ transplantations (71.3%) against 514 (71.3%) in 2017.

Over the observation period (from 2006 to 2018), the number of extrarenal organ transplants in Russia increased by 723 (7.8 times). See Fig. 11. In the total number of transplants, the proportion of extrarenal transplantations increased by 22.0%.

Table 11 contains information on the dynamics of the number of organ transplants performed in Russia in 2006–2018.

ORGAN TRANSPLANT RECIPIENTS

Information on the number of patients in Russia with transplanted organs (from 2013 to 2018), obtained from the Federal Registry of the Ministry of Health of the Russian Federation (see Order No. 2323-r of the Government of the Russian Federation dated October 23, 2017; Decree 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 15,810 patients with transplanted organs in 2018 in Russia (107.6 per million population). Among these patients, 10,851 (73.9 per million population) had a kidney transplant, 2,632 (17.9 per million population) had liver transplant, and 1,164 (7.9 per million) had heart transplant.

Over the 6 years of observation (from 2013), organ transplant recipients in Russia has increased in number by 7,257 (84.8%).

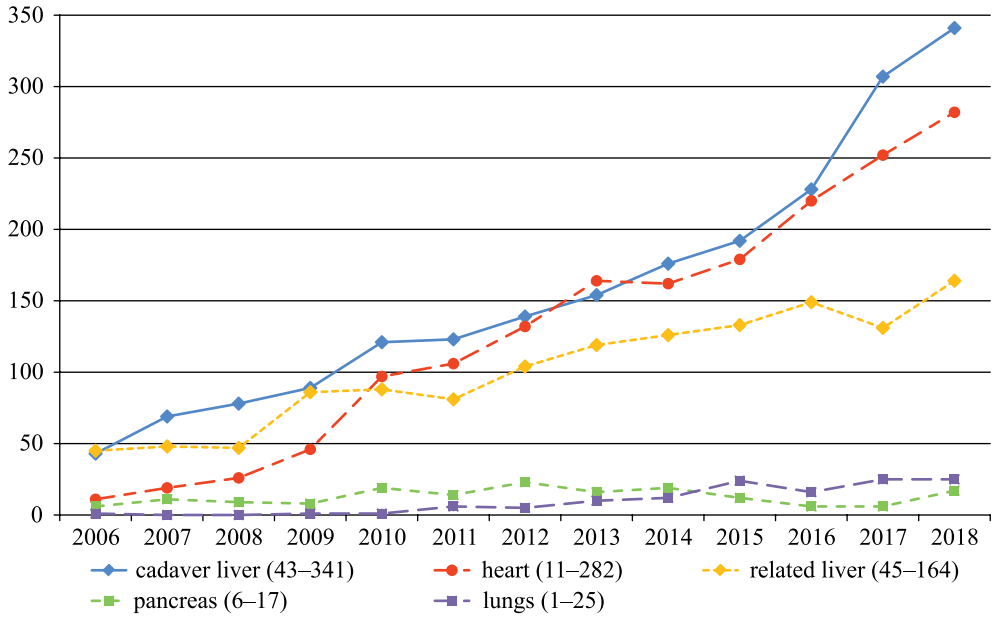

Fig. 11. Nonrenal solid organ transplantation in 2006–2018

Table 11

Organ transplantation in the Russian Federation in 2006–2018

S/N	Organ	2006	2007		2008		2009		2010		2011		2012		2013		2014		2015		2016		2017		2018	
		Absolute number	Absolute number	Change for the year	Absolute number	Change for the year	Absolute number	Change for the year	Absolute number	Change for the year	Absolute number	Change for the year	Absolute number	Change for the year	Absolute number	Change for the year	Absolute number	Change for the year	Absolute number	Change for the year	Absolute number	Change for the year	Absolute number	Change for the year	Absolute number	Change for the year
1	Kidney (total)	556	666	+110	782	+116	830	+48	1037	+207	975	–62	941	–34	935	–6	1026	+91	945	–81	1084	+139	1175	+91	1361	+186
2	including 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
3	and from a living related donor	139	139	0	145	+6	156	+11	170	+14	179	+9	195	+16	188	–7	190	+2	190	0	232	+42	201	–31	200	–1
4	Liver (total)	88	117	+29	125	+8	175	+50	209	+34	204	–5	243	+39	272	+29	302	+30	325	+23	378	+53	438	+60	505	+67
5	including 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
6	and from a living related donor	45	48	+3	47	–1	86	+39	88	+2	81	–7	104	+23	119	+15	126	+7	133	+7	149	+16	131	–18	164	+33
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
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
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
10	Heart-lung										2	+2	2	0	1	–1	0	–1	0	0	0	0	0	0	3	+3
11	Small intestine														1	+1	1	0	0	–1	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

Table 12

Number of patients with transplanted organs in the Russian Federation in 2013–2018

ICD code	Number of patients in the registry										
	2013	2014		2015		2016		2017		2018	
		Absolute	Change (%)	Absolute	Change (%)	Absolute	Change (%)	Absolute	Change (%)	Absolute	Change (%)
Z94.0 Kidney transplant status	6651	7502	12.8	8164	8.8	9063	11.0	9658	6.6	10,851	12.4
Z94.1 Heart transplant status	416	520	25.0	639	22.9	803	25.7	952	18.6	1164	22.3
Z94.2 Lung transplant status	2	3	50.0	4	33.3	5	25.0	8	60.0	28	250.0
Z94.4 Liver transplant status	1150	1406	22.3	1649	17.3	1948	18.1	2152	10.5	2632	22.3
Z94.8 Other transplanted organ and tissue status (bone marrow, intestines, pancreas)	334	467	39.8	654	40.0	808	23.5	909	12.5	1135	24.9
TOTAL	8553	9898	15.7	11,110	12.2	12,627	13.7	13,679	8.3	15,810	15.6

CONCLUSION

Results recorded in 2018 show a long-term trend – organ transplant surgeries in Russia is increasing in number (10–15% per year). Over the past year, over 2,000 (precisely 2193) organ transplants were performed in Russia for the first time.

Apart from the increasing number of organ transplants, the current trend is characterized by the following:

The geographic spread of transplant centers continues to expand,

The number of transplantation centers and their transplantation activity are increasing (21 transplants per center in 2006, and 38 in 2018),

Efficiency of donor programs (proportion of brain death diagnoses, proportion of multi-organ procurements, average number of organs procured from one donor) are all increasing,

Extrarenal transplantation technologies are being deployed in Russian regions,

Higher number of patients on organ transplant waitlists,

Lower waitlist mortality rates;

The number of organ transplant recipients in need of follow-up care and treatment is increasing.

In Russia, demand for organ transplantation still exceeds organ availability. In 2018, there were over 9,000 patients on organ transplant waitlists and the number continues to grow. Therefore, provision of adequate medical support for waitlisted patients requires special attention. This includes applying separate tariffs for such patient models in the compulsory health insurance system.

Shortages in donor organs in Russia is still down to human causes – lack of or insufficient medical organ donation activities in Russian regions. In this regard, the issue of responsibility (irresponsibility) of heads of regions, regional health care and medical organizations on organizing medical organ donation activities remains an urgent one.

Government funding, as well as the funding sources and mechanisms for bringing the funds down to organ donation and transplantation organizations remain key factors determining the extent of transplant care to the population. One of the approaches to increasing the availability of kidney transplant care could be, for example, by incorporating it into the basic compulsory health insurance program, while retaining the financial cost standards. By so doing, the government would be creating equal conditions for financing different types of renal replacement therapy. Besides, patients will be able to choose the type of treatment that they think is preferable, more effective and safer.

In 2018, transplant activity in transplantation centers varied widely. However, only those medical organizations where such high-tech surgeries are regularly performed can guarantee the quality and safety of organ transplants. The Shumakov National Medical Research Center of Transplantology and Artificial Organs recommends that regions and medical institutions should optimize their transplantation programs for certain reasons: to avoid having too many transplantation centers, to ensure that the number of such centers is dictated by demand, donor, staffing and financial level, and also to ensure that there is an acceptable level of transplantation activity (at least 30–40 organ transplants per year).

The effectiveness of organ transplantation as a treatment method will be fully recognized only when long-term survival and functioning of transplants are guaranteed. Therefore, as the number of organ transplant

recipients in Russia increases (almost 16,000 people at present), regions, chief external experts and medical organizations should focus particularly on proper organization of medical and drug support for the recipients – being guided by medical care protocols and standards, as well as national clinical recommendations for transplantation.

In 2019, the Shumakov National Medical Research Center of Transplantology and Artificial Organs continues to serve as a national medical research center in the area of transplantation – the Center performs organizational and methodological management functions, including on-site "audits" in Russian regions and remote consultations using telemedicine technologies; it also serves as a monitor, doing analytical work and training specialists.

The authors declare no conflict of interest.

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MICRORNA PROFILING IN POTENTIAL LUNG RECIPIENTS

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MicroRNAs are small RNA molecules stable in blood serum (plasma) samples. Their level of expression is associated with the severity and nature of physiological and pathological processes in the body. **Aim:** to evaluate the expression levels of five microRNAs (miR-27, miR-101, miR-142, miR-339 and miR-424) in potential lung recipients with end-stage chronic lung diseases of various etiologies. **Materials and methods.** The study included 16 patients with end-stage chronic lung diseases (potential lung recipients) aged 4 to 74 years (average 36 ± 18). Among them were two children (12.5%) – girls aged 4 and 14 years, and 14 adults aged from 21 to 74 (40 ± 16) years – 6 men (42.9%) and 8 women. The control group consisted of 12 healthy individuals. The main diseases that caused severe respiratory failure were: cystic fibrosis (n = 5), primary pulmonary hypertension (PPH; n = 4), pulmonary fibrosis of various etiologies (idiopathic pulmonary fibrosis – 1; pulmonary fibrosis associated with exogenous allergic alveolitis – 1; radiation-induced pulmonary fibrosis – 1), lymphangioleiomyomatosis (n = 2), histiocytosis (n = 1) and pulmonary emphysema (n = 1). MicroRNA expression was detected through real-time PCR. The level of microRNA expression in plasma was estimated in accordance with instructions for reagent kits (Qiagen, USA). **Results.** The levels of miR-27, miR-101 and miR-339 in potential lung recipients were significantly higher than in healthy individuals. The levels differed depending on the etiology of diseases: the levels of miR-27, miR-101, miR-142 and miR-339 were higher in patients with cystic fibrosis than in healthy individuals; in patients with other lung diseases, only miR-101 levels where higher than in healthy individuals. The miR-424 level in healthy individuals did not differ from that in potential lung recipients or in subgroups. **Conclusion.** Results obtained show the features of a number of microRNA levels (miR-27, miR-101, miR-142, and miR-339) under certain lung diseases and suggest a possibility of a diagnostic value in patients with chronic respiratory failure during pre-transplant examination.

Keywords: microRNA, biomarker, miR-27, miR-101, miR-142, miR-339, miR-424, cystic fibrosis, chronic respiratory failure.

MicroRNA is a wide range of small non-coding RNA molecules, stable in blood serum (plasma), performing regulatory functions, with the level of expression associated with the severity and nature of physiological and pathological processes in the body [1–3]. In this context, it is assumed that data on changes in the expression of certain microRNA types in recipients of solid organs may be helpful for early preclinical diagnosis of post-transplant complications [4, 5]. The change in the expression of certain types of microRNAs at the rejection of transplanted solid organs was found [6, 7].

The lung transplantation (LT) is an effective treatment for such chronic terminal lung diseases as primary pulmonary arterial hypertension (PAH), cystic fibrosis, chronic obstructive pulmonary disease, and others.

Despite significant advances in surgical technology and improvement of the immunosuppressive therapy which increased survival and improved the quality of life for recipients of solid organs, the vital task is to search for and validate biomarkers that are potentially suitable for non-invasive or minimally invasive diagnosis of post-transplant complications and prediction of long-term clinical outcomes [8–9].

Purpose of the study. Evaluation of microRNA expression level (miR-27, miR-101, miR-142, miR-339, and miR-424) in patients suffering from chronic lung diseases of different aetiology in the terminal stage (potential lung recipients).

MATERIALS AND METHODS

The study included 16 patients with terminal-stage lung diseases aged 4 to 74 (36 ± 18), among them two children (12.5%), girls aged 4 and 14 years, and 14 adult patients aged 21 to 74 (40 ± 16) years, 6 (42.9%) men and 8 women. The main diseases to cause the severe respiratory failure were cystic fibrosis (n = 5), pulmonary arterial hypertension (PAH; n = 4), pulmonary fibrosis of various etiologies (idiopathic pulmonary fibrosis – 1;

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pulmonary fibrosis as the outcome of exogenous allergic alveolitis – 1; post-radiation pulmonary fibrosis – 1), lymphangioleiomyomatosis (n = 2), histiocytosis (n = 1), and pulmonary emphysema (n = 1). The control group consisted of 12 healthy individuals. The average age and ratio of men and women in the control group did not differ from those in the test group.

Isolation of microRNA from the plasma of peripheral blood

The peripheral blood samples of patients were collected in disposable tubes with an anticoagulant ethylene diamine acetic acid (EDTA), centrifuged for 10 minutes at 3,000 rpm. Blood plasma was separated from the cell pellet and immediately frozen at –20 °C. RNA was isolated from 100 µl of blood plasma using SerumPlasma kits (Qiagen, USA) with the preliminary addition of 1.6 x 108 copies of cel-miR-39 synthetic microRNA (Qiagen) after plasma incubation with Qiazol phenolic mixture. Cel-miR-39 was used as an internal control of the efficiency of RNA isolation, synthesis of complementary DNA (cDNA), and quantitative polymerase chain reaction (PCR) in real time.

Real-time reverse transcription and quantitative PCR

MicroRNAs from each sample were converted into cDNA in a reaction mixture (20 µl) containing 1xmiScriptHiSpecBuffer buffer, 1xmiScriptNucleicsMix nucleotide mixture at t = 37 °C for 60 minutes, followed by incubation at 95 °C for 5 minutes, cooling on ice and dilution of sample volume with deionized water up to 200 µl. The synthesized cDNA (2 µl) served as the matrices in real-time PCR, using primers specific for the studied microRNAs: miR-27, miR-101, miR-142, miR-339, miR-424, cel-miR-39 (miScriptPrimerassay, Ce_miR-39_1, Qiagen), and the miScriptSYBRGreenPCRKit kit (Qiagen). PCR reaction conditions: 15 minutes at t = 95 °C followed by 40 cycles of 15 seconds at t = 94 °C, 30 seconds at t = 55 °C, and 30 seconds at t = 70 °C in the CFX 96 amplifier (Biorad). The intensity of microRNA expression was shown in relative units equivalent to $2^{-\Delta Ct}$, where ΔCt is the operating values of changes in the product obtaining cycle relative to the internal control of microRNA cel-miR-39 expression.

Statistical data processing

Statistical analysis of the results was carried out by Statistica v.13.0, StatSoftInc (USA software package). Spearman correlations and the Mann–Whitney U-test were used to compare the independent variables. The critical level of significance was taken 5% and the null hypothesis was rejected at $p < 0.05$. The obtained expression indices were checked for normal values distribution.

RESULTS AND DISCUSSION

In patients with severe respiratory failure that developed as the outcome of chronic lung diseases, microRNA expression rates varied over a wide range. The distribution of values differed from normal; therefore, in the present study, the results are provided by the values of the median and interquartile range expressed in relative units (RU). Table 1 shows the results of a comparative analysis of the expression levels of the five studied types of microRNAs (miR-27, miR-101, miR-142, miR-339, and miR-424) in men and women suffering from lung diseases, and the significance of differences between them is indicated (p).

Table 1

Comparative analysis of microRNA plasma expression levels in male and female patients

MicroRNA	Sex		Statistical significance (p)
	Male (n = 6)	Female (n = 10)	
miR-27	0.071 [0.059; 0.33]	0.030 [0.016; 0.067]	0.09
miR-101	0.057 [0.031; 0.083]	0.046 [0.022; 0.057]	0.63
miR-142	0.011 [0.007; 0.072]	0.006 [0.003; 0.011]	0.31
miR-339	0.023 [0.001; 0.130]	0.004 [0.001; 0.076]	0.43
miR-424	0.007 [0.005; 0.014]	0.002 [0.001; 0.008]	0.12

Note. Data is provided as: median [interquartile range].

An analysis of the expression levels of the studied microRNAs in potential lung recipients showed no gender differences.

Table 2 shows the results of an analysis of the relationship between microRNA expression levels and the age of patients waiting for lung transplantation.

Table 2

Correlation analysis of microRNA expression levels and the age of the patients

MicroRNA	Correlation coefficient (r)	Statistical significance (p)
miR-27	–0.50	0.04
miR-101	–0.48	0.06
miR-142	–0.41	0.12
miR-339	–0.33	0.22
miR-424	–0.06	0.83

A significant negative correlation was found between the miR-27 expression level and the age of patients (r = –0,50, p = 0,04); for other types of microRNAs, no age correlation was found.

Fig. 1 shows the results of a comparative analysis of the expression of the studied microRNAs in healthy in-

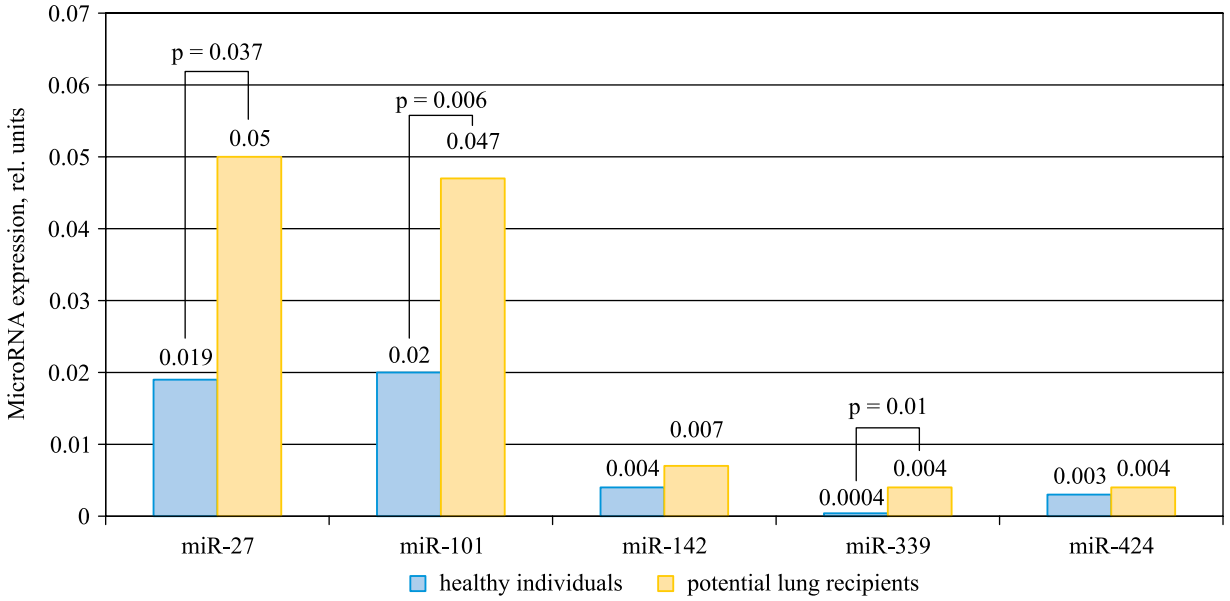

Fig. 1. Comparative analysis of microRNA levels (miR-27, miR-101, miR-142, miR-339 and miR-424) in healthy individuals and potential lung recipients

dividuals and patients suffering from respiratory failure, potential lung recipients.

In patients waiting for lung transplantation, the expression levels of three of five types of studied microRNAs were significantly higher than in healthy individuals: miR-27 ($p = 0.037$), miR-101 ($p = 0.006$), and miR-339 ($p = 0.01$)

The differences in microRNA expression were found to be associated with aetiology of the disease that caused the development of respiratory failure. In five patients, the cause of respiratory failure was an infectious mediated disease – cystic fibrosis (31.3% of the total number of patients). The remaining 11 patients suffered from the diseases with obstructive, restrictive processes as the main pathogenetic factors; vascular disorders (PAH, pulmonary fibrosis of various aetiologies, emphysema, etc.)

The analysis showed that miR-101 expression was significantly higher in patients with cystic fibrosis ($p = 0.01$) and those suffering from other lung diseases ($p = 0.03$, Fig. 2, a) compared with the group of healthy individuals.

The expression level of miR-27 in patients with cystic fibrosis was higher in comparison with both healthy individuals ($p = 0.001$, Fig. 2, b) and other patients ($p = 0.01$). It should be noted that the differences were not caused by the younger age of patients with cystic fibrosis ($p = 0.07$).

A higher level of miR-142 and miR-339 expression in comparison with healthy individuals was detected only in patients with cystic fibrosis ($p = 0.04$, Fig. 2, c; $p = 0.01$, Fig. 2, d, respectively).

The expression level of miR-424 did not differ from that in healthy individuals, neither in the group of potential recipients nor in subgroups of patients with different aetiologies of the disease (Fig. 2, e).

The important regulatory role of microRNAs in the development of pathological processes is described in many studies of recent years, which stimulates the active study of small molecules from the perspective of their potential significance as biomarkers of posttransplantational complications and use as potential targeted therapy for rejection of solid organs in recipients [10–12].

To date, a sufficiently large number of different microRNA types have been identified and described. Five of them were selected for this study, presumably playing a role in the development of diseases of the lungs and cardiovascular system and potentially significant for the diagnosis of posttransplantational complications in heart and lung recipients [10, 14].

Significant differences in the expression of miR-101, miR-142 were described in patients with heart and lung diseases [13, 14]. In patients with idiopathic pulmonary fibrosis, miR-101 has been shown to inhibit the proliferation and activation of fibroblasts, being a potential therapeutic target [15]. Data has been published on the possible involvement of miR-142 in the regulation of haematopoiesis. Besides, this type of microRNA is expressed in many tissues and performs important functions in inflammatory, immune, infectious, oncological, and fibrotic processes after transplantation of donor organs [16].

CONCLUSION

The results of this study showed that the expression profile of various types of microRNAs in patients with chronic respiratory failure waiting for lung transplantation differs from that in healthy individuals. Moreover,

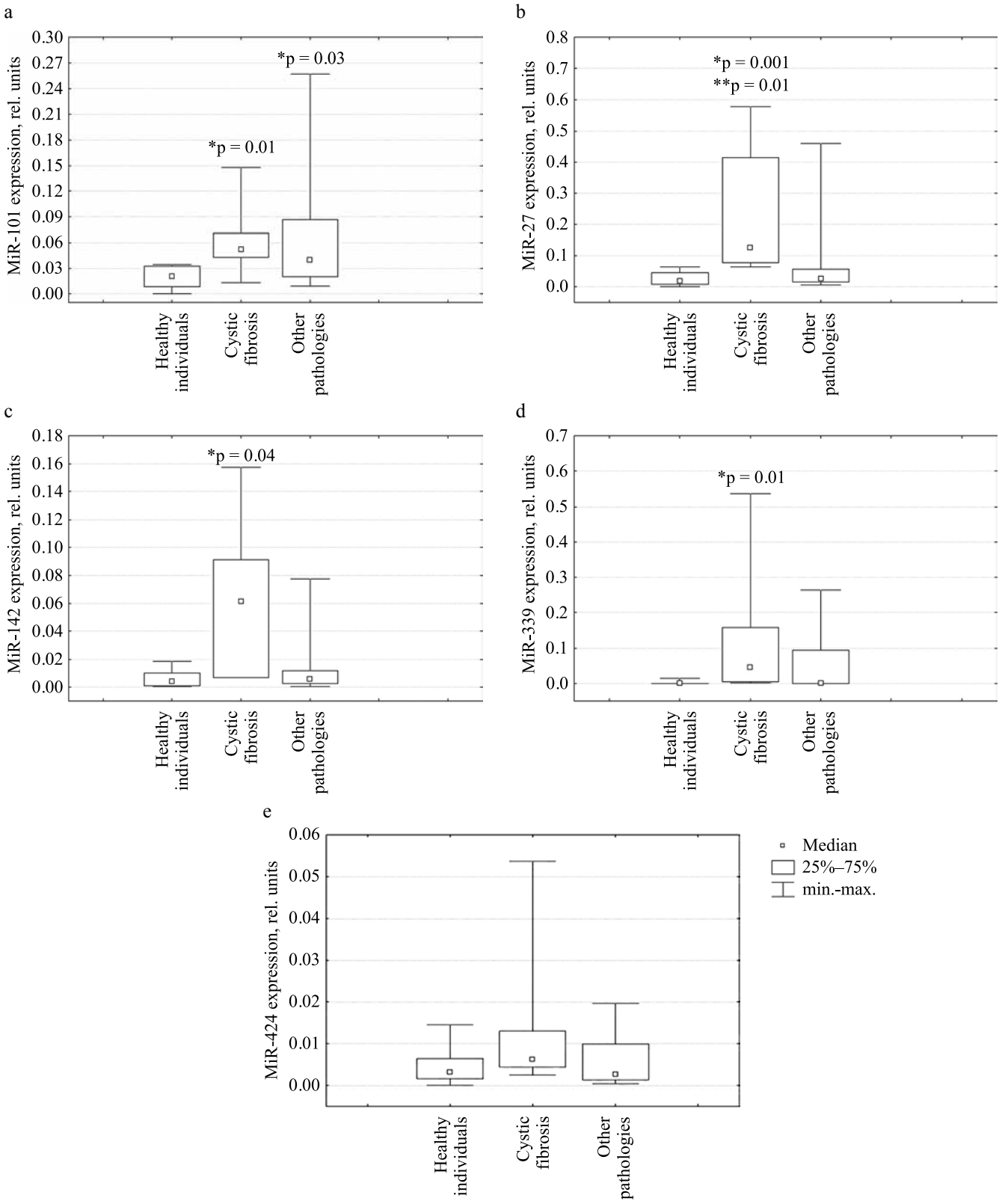

Fig. 2. Comparative analysis of miR-101 (a), miR-27 (b), miR-142 (c), miR-339 (d) and miR-424 (e) levels in healthy individuals, patients with cystic fibrosis and other lung pathologies. * – compared to healthy individuals; ** – compared to patients with other lung pathologies

the expression of certain types of microRNAs is associated with the aetiology of lung disease: in patients with cystic fibrosis, expression levels of miR-27, miR-101, miR-142, and miR-339 were significantly higher in comparison both with healthy individuals and patients with less pronounced infection mediated disorders.

The literature data, as well as the results of this study, indicate the relevance of studying the levels of microRNA expression in patients with severe pulmonary pathology, as well as in lung recipients. To clarify the possible clinical significance of the application of the expression levels of these molecules, further study of their biological functions and diagnostic efficacy in this group of patients is required.

The authors declare no conflict of interest.

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ANALYSIS OF EARLY USE OF EVEROLIMUS WITH LOW-DOSE CALCINEURIN INHIBITORS IN KIDNEY TRANSPLANT RECIPIENTS

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Aim: to evaluate the efficacy and safety of early use of everolimus in combination with a reduced dose of calcineurin inhibitors (CNI) after kidney transplantation and define approaches to the selection and management of patients on everolimus-based therapy. **Materials and methods.** Sixty-seven kidney transplant recipients were included in the study, forty of them began taking everolimus from the first day after transplantation in combination with prednisolone and CNI, and twenty-seven patients were converted from mofetil mycophenolate to everolimus 2.9 ± 2.0 months after surgery, and their dose of CNI was reduced. The duration of follow-up was 51.2 ± 35.1 months. Four-years patient and uncensored by death graft survival rate were assessed regardless of the duration of everolimus use and was compared with the data in the control group of recipients (n = 89) who did not receive everolimus. The survival rate of the method of treatment with everolimus and the event-free graft survival were also evaluated. When calculating the survival rate of method of everolimus treatment, the event that required the discontinuation of the drug was taken as the ed-point. Events such as rejection, development or progression of renal dysfunction and proteinuria have been accepted as end-points in the calculation of event – free survival rate. The number of patients discharged from their surgical hospital and taken under the supervision of a nephrologist was adopted as 100%. **Results.** Patient and graft survival rate at 4 years after transplantation in the everolimus-based and control groups did not differ ($p < 0.79$ and $p < 0.4$, respectively). The 4-year survival rate of the method of everolimus treatment was 57.2%, and the event-free graft survival rate was 47.9%. The most frequent causes of everolimus withdrawal were rejection (25.8% of all causes), proteinuria (19%), progressive graft dysfunction (16, 1%) and adverse events (16.1%). The 4-year event-free graft survival depended on the initial kidney function and was significantly decreased (up to 32%) in the group of patients having the baseline Pcr >0.13 mmol/l in comparison with 59.3% in patients with normal baseline function, $p < 0.04$. The average level of Pcr Increased during the treatment from 0.14 ± 0.04 to 0.16 ± 0.09 mmol/l ($p < 0.04$), and the daily proteinuria increased from 0.18 ± 0.12 g/day to 0.66 ± 1.31 g/day ($p < 0.004$) by the end of follow-up. **Conclusion.** Everolimus with reduced dose CNI can be start from the first days or months after kidney transplantation. However, its applicability is limited to four years in almost 43% of patients due to rejection, progressive graft dysfunction, proteinuria and adverse events.

Keywords: kidney transplantation, immunosuppression therapy, everolimus.

One of the main challenges being faced by modern transplantology, and particularly kidney transplantation, is on how to prolong the lifespan of the kidney graft as much as possible and improve the quality of life of recipients. In this respect, the highest priority is to develop new immunosuppression regimens that would both prevent transplant rejection and major renal and extrarenal complications in late post-transplant period. Widespread use of calcineurin inhibitors (CNIs), especially in combination with mycophenolate mofetil or enteric-coated salt form of mycophenolic acid, has universally shown significant improvement in early post-surgery results. The improvement was mainly attributed to a reduction in the incidence of rejection crisis [1–3]. At the same time, there has been minor increase in kidney transplant (KT) long-term survival rates over the past decades [4–6]. In support of this, 164,480 observations carried out by K.E. Lamb et al. [4] indicated that while kidney rejection decreased from 20% in 1989 to less than 8% in 2009 in the first postoperative year, in the range of 3 to 5 years and

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5 to 10 years after transplantation over the same period of time, the rejection rate practically did not change – 6–8%. Similarly, KT half-life also slightly changed to 8.3 and 8.8 in 1998 and 2005, respectively.

Calcineurin inhibitor nephrotoxicity (CNI nephrotoxicity) is admittedly one of the common causes of late KT dysfunction, along with graft failure. Some of its signs are verified through protocol biopsy specimens in more than half of recipients already one year after kidney allotransplantation (ATP) [7]. Through renal biopsy, it was found that signs of chronic CNI nephrotoxicity of varying severity were almost universal at 10 years [8]. In this regard, emergence of proliferative signal inhibitors (PSI) – immunosuppressants with no nephrotoxic effects and with cardio- and oncoprotective effect, triggered a wide resonance in the transplantological community [9, 10]. Already the first studies have shown that PSIs are most effective when used in *de novo* patients or in early conversion from CNIs (complete or partial discontinuation) to PSI-based regimen, especially if CNIs are used in combination with induction therapy [11, 12]. Large multicenter studies have shown that in both cases, early use of PSI-based regimens (sirolimus or everolimus) led to lower incidence of early rejection crises and improvement in KT function 1.2 and 3 years after surgery compared with patients who continued the traditional CNI-based immunosuppression [13–16]. Meanwhile, other studies have noted that rejection crisis after conversion to everolimus develop more often or with the same frequency as in patients continuing with traditional CNI-based therapy [17, 18].

One possible explanation for these differences was given by studies on everolimus concentrations [19–21]. Based on data obtained in clinical studies B201, B251 and B156, it was found that the use of a fixed dose of everolimus 1.5 or 3 mg/day in combination with a full dose of cyclosporine (CsA) resulted in deterioration of KT function 1 and 3 years after the start of treatment due to the synergistic action of drugs and the increased nephrotoxic effect of CNIs [13, 14, 22]. This, in further observations, allowed to reduce CsA exposure by almost 60% if the everolimus blood concentration was more than 3 ng/ml without the risk of graft rejection [15, 19, 23, 24]. According to M.I. Lorber et al. [20], graft rejection in the group of patients in whom everolimus blood concentration was maintained within the 3–8 ng/ml range, was observed almost 3.5 times less often than in recipients with lower concentrations of the drug. Similar data were obtained when evaluating the effectiveness of everolimus in combination with low-dose tacrolimus (TAC) [25].

On the other hand, analysis of the incidence of adverse events (AE) amid PSIs, showed that the latter are dose-dependent and reduce if everolimus blood concentration does not exceed 8 ng/ml. This involved such complications as delayed wound healing, proteinuria, post-transplant diabetes mellitus (PTDM), and hyperlipidemia [24, 26]. Based on the results of these studies, to date, when using PSI in combination with low-dose CNIs, the therapeutic ranges are considered as follows: 3–8 ng/ml for everolimus and 25–50 ng/ml for CsA or 3–5 ng/ml for TAC.

In addition to lower nephrotoxic effect and associated improvement in KT function, the use of everolimus as a basic therapy indicates significant reduction in the risks of cancer, cardiovascular and viral pathologies, which are recognized as leading causes of mortality after ATP [10, 15, 16, 18, 27–29]. However, the use of these drugs may be limited by development of progressive KT dysfunction or such AEs as proteinuria, peripheral edema, ulcerative stomatitis, skin reactions, pneumonitis, myelopathy, etc. [15, 24, 29–31]. In view of the above, it was of interest to evaluate the results of the use of everolimus in KT recipients in one center.

The aim of the study was to investigate the efficacy and safety of early use of everolimus in combination with low-dose CNI and to develop (on this basis) ways of selecting and managing patients taking maintenance PSI-based immunosuppressive therapy.

MATERIALS AND METHODS

Data obtained from observation of 67 kidney transplant recipients were analyzed retrospectively. In these recipients, everolimus was used to support immunosuppression in combination with prednisolone and low-dose CNI (TAC in 39 patients, CsA in 28 people). PSI was administered on 40 patients on the first day after surgery (*de novo* group), while the remaining 27 patients (conversion group) converted from mycophenolates to everolimus 2.9 ± 2.0 months (median 3.0 (1.0; 5.0) months) after ATP. The average age of the patients was 54.8 ± 11.1 g; men were 68.7% of the population. Follow-up after ATP lasted for an average of 51.2 ± 35.1 months with a median of 49.0 (32.0; 61.0) months. Baseline demographic and clinical laboratory parameters of the patients included in the study are presented in Table 1. As can be seen from Table 1, the conversion and *de novo* groups did not significantly differ statistically from the baseline, except for serum creatinine, which was higher in the conversion group.

The effect of everolimus therapy on the outcome of transplantation, regardless of duration of its use, was evaluated through a 4-year survival of recipients and KT (uncensored by death). These indicators were compared with those in the control group (n = 89) where everolimus was not used. The effect of everolimus therapy was determined based on the survival of the treatment technique, which was understood as the probable frequency of the absence of "events" that would require discontinuing the drug by a certain period after everolimus therapy had started. Along with this, the event-free and functional survival of KT was computed. In the analysis of the

Table 1

Initial demographic and clinical laboratory parameters in renal transplant recipients

Parameters	General group	*De novo* group	Conversion group	*P* between groups
Age, yr	54.8 ± 11.1	55.3 ± 11.4	54.0 ± 10.8	0.65
Male/Female, n (%)	46/21 (68.7%/ 31.3%)	27/17 (67.5%/32.5%)	19/8 (70.4/29.6)	0.51
Initial KT function, immediate/delayed, n (%)	42/ 25 (62.7%/37.3%)	28/12 (70.0%/30.0%)	14/13 (51.9%/48.1%)	0.11
Early rejection crisis, n (%)	4 (6 %)	2 (5.0%)	2 (7.4%)	0.53
Serum creatinine level at the start of everolimus therapy, mmol/l	0.14 ± 0.04	0.11 ± 0.02	0.18 ± 0.04	**0.02**
Proteinuria at the start of everolimus therapy, g/day	0.18 ± 0.12	0.18 ± 0.12	0.2 ± 0.11	0.48

event-free KT survival, "events" such as rejection, development/progression of KT dysfunction, appearance/progression of proteinuria were taken as the endpoint, while in the computation of functional survival, development of initial chronic kidney disease was taken as the endpoint. The Kaplan–Meier estimate was used to compute the survival over time. The number of patients that were placed under nephrology supervision after discharge from their surgical hospital was taken as 100%. The incidence of rejection crisis and their severity, as well as the dynamics of KT function and proteinuria level were also evaluated. The graft function was computed based on serum creatinine (pCr), which normally did not exceed 0.13 mmol/L. Urine protein level was determined based on the level of daily urinary protein excretion. A protein loss in excess of 0.3 g/day was considered pathological. When assessing pCr and proteinuria dynamics in the *de novo* group, the initial state was taken to be indicators determined by the end of 1 month after surgery, that is, by the time the graft function stabilizes. In the conversion group, indicators that were present as of the time of everolimus administration were taken as baseline data.

The cumulative frequency of end events (rejection, onset/progression of renal dysfunction, renal death, patient death, proteinuria, severe AE) was evaluated as a whole and separately, comparing them in the groups with immediate and delayed administration of everolimus.

The significance of factors affecting KT survival was analyzed using the Cox model, which included parameters such as initial graft function, rejection crisis, baseline serum creatinine and proteinuria at the time of initiation of therapy, and inadequate immunosuppression.

The adequacy of immunosuppression was analyzed based on the blood concentrations of everolimus and CNI. Immunosuppression was considered adequate if everolimus blood concentration remained in the 3–8 ng/ml range, while the CsA target level for the first 2 months after operation was in the 100–150 ng/ml range, decreased to 50–100 ng/ml from 2 to 6 months, and after 6 months remained in the 25–50 ng/ml range, which corresponded to C2 target 350–450 ng/ml. With a combination of everolimus and TAC, the concentration of the latter after operation remained in the 4–7 ng/ml range within 2 months, and in the 3–5 ng/ml range after 2 months. If these requirements were not met, therapy was considered inadequate. Biopsy was performed in all cases where KT pathology was detected. The SPSS software package (version 22) was used for statistical data processing.

RESULTS

Regardless of duration, everolimus-based therapy did not affect long-term results of transplantation. The survival rates of recipients and KT four years after ATP in the group of patients who received everolimus-based immunosuppression and in the control group were comparable (Fig. 1).

However, by the end of observation, which averaged 51.2 ± 35.1 months, everolimus therapy was discontinued in 46.3% (31 out of 67) patients (Table 2).

Survival under everolimus therapy one year after the drug was administered was 68.2%, after two years – 63.1%; after three years and by the end of four years, the likelihood of continuing treatment decreased to 57.2% (Fig. 2).

The reasons for discontinuing everolimus-based therapy in 31 (46.3%) patients are presented in Table 3.

As can be seen from Table 3, the main reason for discontinuation of everolimus therapy was graft rejection, which accounted for 25.8% of the total number of all reasons. In 19.4% of recipients in this subgroup, therapy was discontinued due to appearance/increase in proteinuria. The third most common (16.1%) was transplant dysfunction caused by acute tubular necrosis (ATN), usually accompanied by elevated blood levels of CNIs. With the same incidence, treatment was discontinued due to serious AE, which included prolonged surgical wound healing in type 2 diabetes mellitus (1 person), formation of trophic skin ulcers (1 patient), pancytopenia (2 people) and edema syndrome (1 case). Everolimus therapy was discontinued in 3 other recipients due to pregnancy planning (in 2 cases) and due to the need for specific anti-tuberculosis therapy for pulmonary tuberculosis (in 1 recipient). Four patients died. The cause of death in

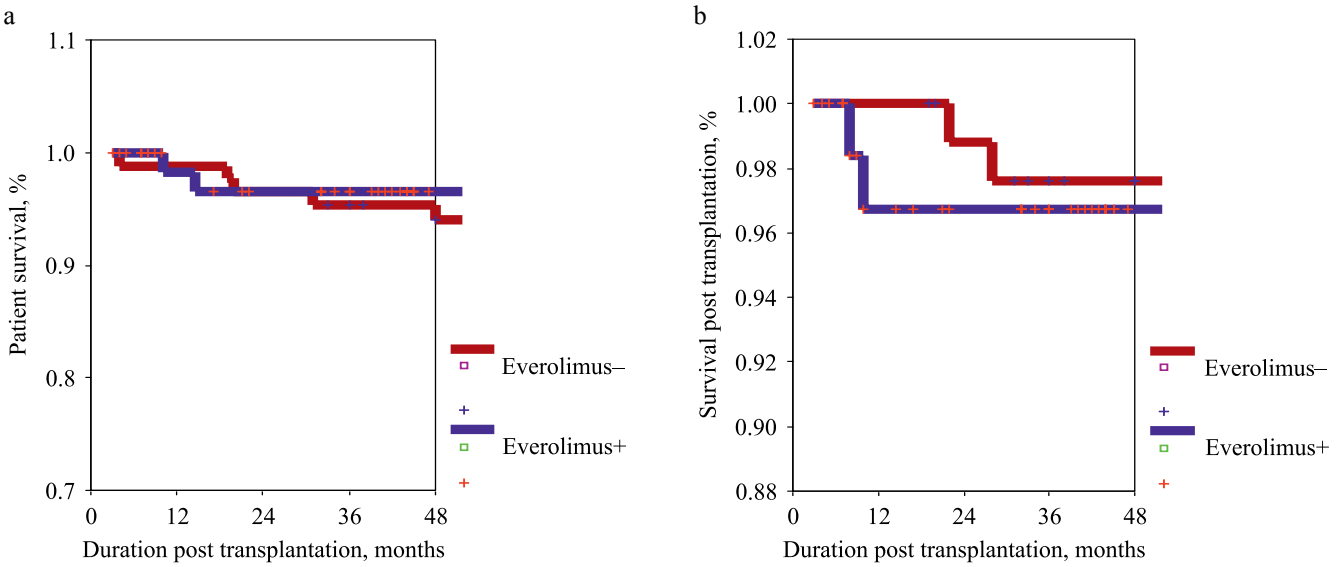

Fig. 1. Impact of everolimus therapy on the outcome of kidney transplantation: a – 4-year patient survival rate in the everolimus group (n = 67) – 96.5% *vs* 94% in the control group (n = 89), p < 0.79; b – 4-year graft survival rate (not censored for death) 96.7% *vs* 97.6% (n = 89), respectively, p < 0.4

Table 2

Results of therapy and incidence of adverse events during the use of everolimus

Event incidence indicators	Total number of patients n = 67, (100%)	*De novo* group n = 40 (100%)	Conversion group n = 27 (100%)	*P*
Duration of observation, months	51.2 ± 35.1	51.7 ± 34.6	50.5 ± 36.6	0.89
Continued everolimus therapy	36 (53.7%)	19 (47.5%)	17 (63.0%)	0.16
Discontinued treatment	31 (46.3%)	21 (52.5%)	10 (37.0%)	0.16
Cumulative incidence of adverse events	35 (52.7%)	22 (55.0%)	13 (48.1%)	0.38
Graft rejection	11 (16.4%)	5 (12.5%)	6 (22.2%)	0.29
Development/progression of dysfunctions	13 (19.4%)	8 (20%)	5 (18.5%)	0.57
AE (post-operative suture breakage, skin ulcers, pancytopenia, leukopenia)	6 (9.0%)	5 (12.5%)	1 (3.7%)	0.22
Proteinuria	29 (43.3%)	15 (37.5%)	14 (51.9%)	0.25
Renal death	4 (6.0%)	1 (2.5%)	3 (11.1%)	0.15
Patient's death	5 (7.5%)	2 (5%)	3 (11.1%)	0.32

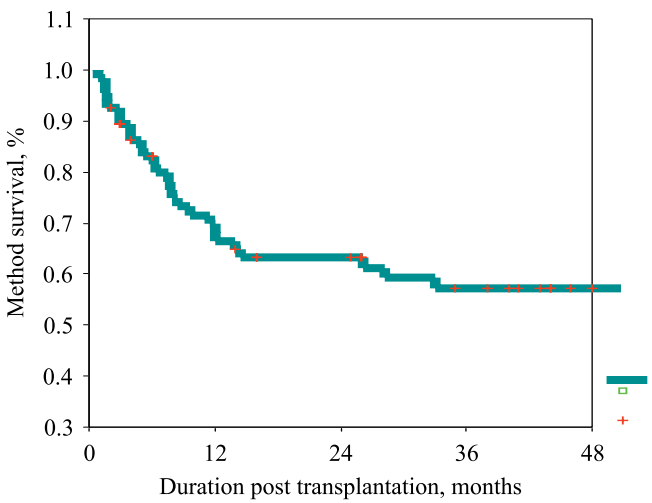

Fig. 2. The survival rate of the method of everolimus treatment: 1-year – 68.2%; 2-year – 63.1%; 3-year and 4-year – 57.2%

Table 3

Reasons for discontinuation of everolimus-based therapy

Reason for discontinuation of everolimus	Number of patients (%)
Rejection	8 (25.8%)
Proteinuria (including due to FSGS)	6 (19.4%)
KT dysfunction	5 (16.1%)
AE	5 (16.1%)
Patient's death	4 (12.9%)
Others (pregnancy planning, tuberculosis)	3 (9.7%)
Total	31 (100%)

one of them was urosepsis, which developed against the background of prostate adenoma and continuously recurrent urinary infection. The second patient died from intestinal obstruction. The cause of death could not be identified in the remaining 2 cases.

Thus, as the above data shows, in 19 (61.3%) out of 31 patients, everolimus was discontinued due to development of major graft pathology that affected the event-free survival of KT, which 1, 2, 3 and 4 years after everolimus therapy had started, was 68.2%, 62.8%, 53% and 47.9%, respectively. That is, after 4 years of everolimus therapy, only slightly less than half of the patients did not have any transplant pathology (Fig. 3).

In general, KT rejection over the entire observation period was observed in 11 (16.4%) out of the 67 patients. It appeared that after early conversion from mycophenolate mofetil (MMF) to everolimus, rejection was detected more often than in the *de novo* group (22.2% versus 12.5%). However, the differences were not of statistical significance ($p < 0.29$). In the same way, the incidence of humoral rejection did not differ (11% versus 5%, respectively, $p < 0.32$). In this regard and having in mind that there were also no differences in the compared groups in terms of cumulative incidence of adverse events (Table 2), further analysis was carried out in the combined group as a whole. In most patients (8 out of 11), rejection was diagnosed 5.0 ± 2.3 months after everolimus-based therapy had started. Humoral rejection was detected in 5 out of 11 patients with KT loss in 3 cases 8, 10 and 77 months after ATP. KT survival 4 years after surgery in the group of patients who had rejection was significantly lower than in those who did not have this complication – 66.7% versus 96.4%, respectively, $p < 0.0006$ (Fig. 4).

It also turned out that rejection was usually caused by inadequate immunosuppression. So, in cases where, for various reasons, maintaining target blood concentrations of everolimus and CNIs was not possible, the rejection

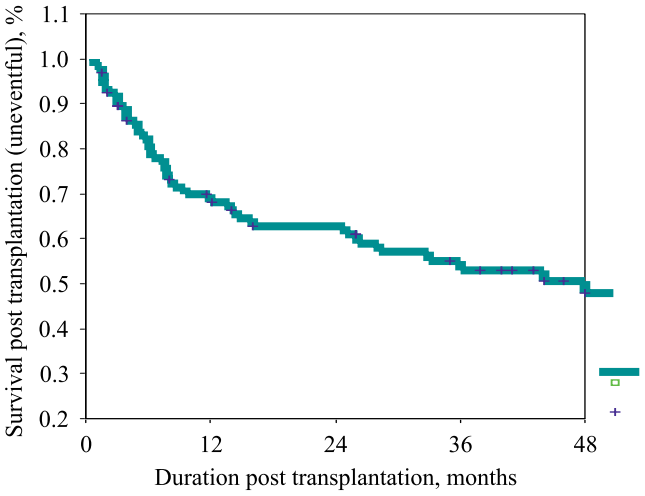

Fig. 3. The event-free survival rate: 1-year – 68.2%, 2-year – 62.8%, 3-year – 53% and 4-year – 47.9%

rate reached 29.2% (in 7 out of 24 patients), while in recipients with therapeutic blood levels of both drugs, rejection was diagnosed only in 7% (3 out of 43 people), $p < 0.015$ (Fig. 5, a). In the group of patients with inadequate immunosuppression, the functional survival of KT also decreased. The probability of absence of KT dysfunction 4 years after surgery in these patients fell to 47.1%, while in the group with adequate therapy, the survival rate within the same periods reached 66.8%, $p < 0.038$ (Fig. 5, b).

The graft function at the beginning of everolimus therapy also turned out to be one of the factors behind the unfavorable outcome of treatment. In patients with baseline serum creatinine ≤0.13 mmol/L (average 0.11 ± 0.02 mmol/L), the event-free transplant survival was significantly higher than in the group with baseline KT dysfunction (serum creatinine 0.18 ± 0.04 mmol/L) and reached 71.1%, 68.2%, 68.2% and 59.3% against 63.3%,

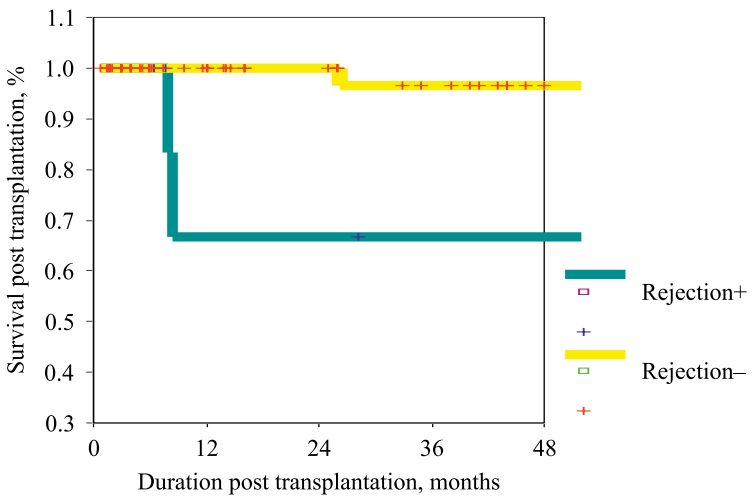

Fig. 4. The effect of rejection on 4-year graft survival: in the non-rejection group (n = 55) – 96.4% versus 66.7% in the rejection group (n = 11), $p < 0.0006$

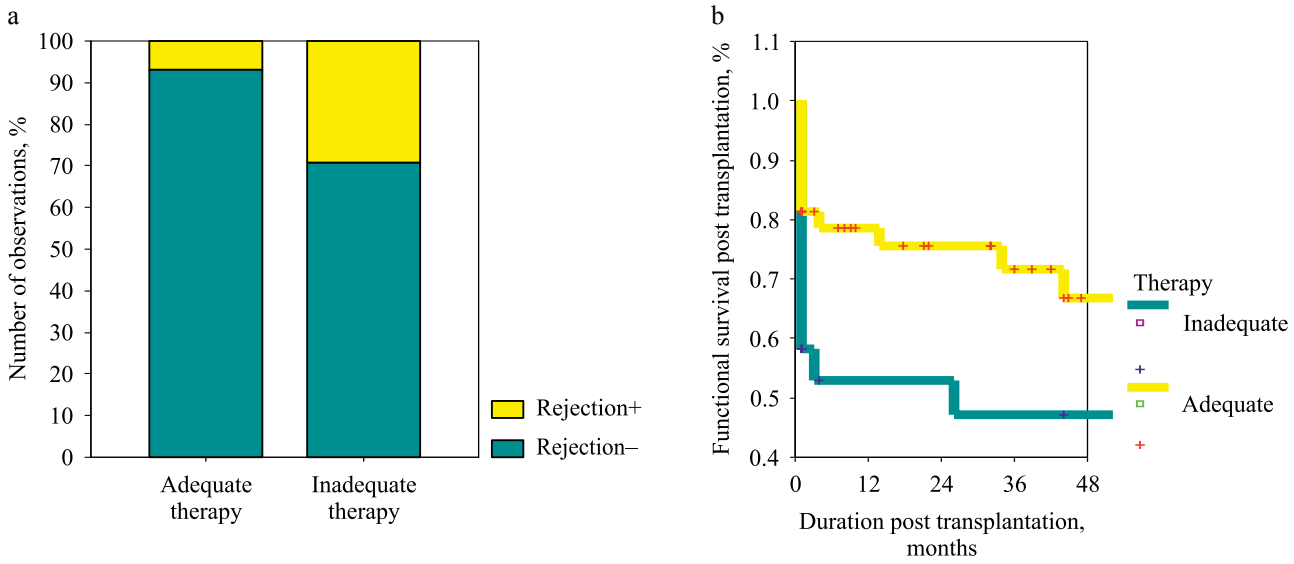

Fig. 5. The effect of adequate (n = 43) and inadequate (n = 24) immunosuppression: a – on rejection rate: 7.0% versus 29.2%, respectively, p < 0.015); b – for 4-year functional survival rate (probability of absence of renal dysfunction): 66.8% versus 47.1%, respectively, p < 0.038

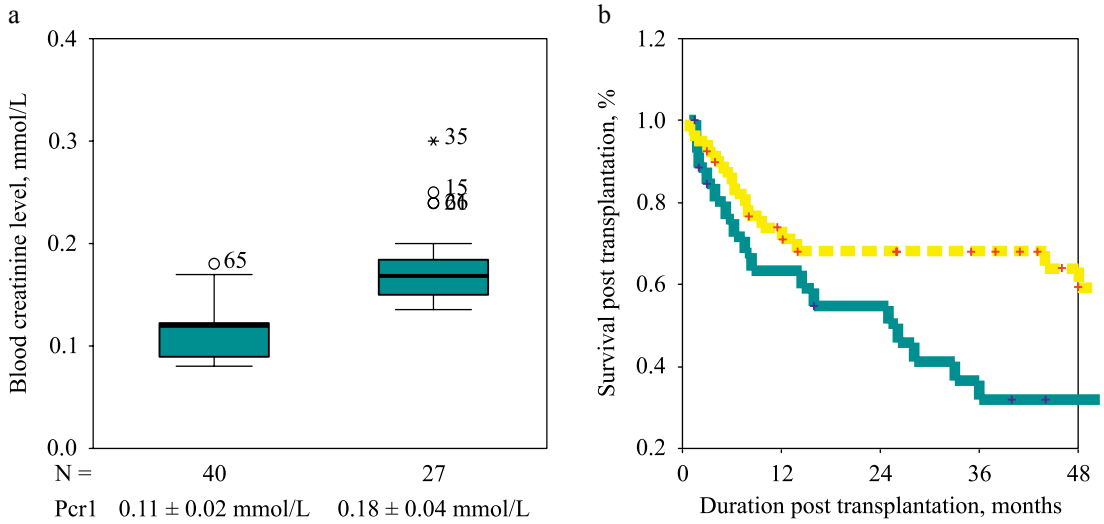

Fig. 6. Comparison of blood levels of creatinine at the beginning of everolimus therapy (Pcr1) and their influence on treatment results: a – the baseline Pcr1 in groups (n = 40) with normal (0,11 ± 0,02 mmol/l) and high (0,18 ± 0,04 mmol/l) values (n = 27), p < 0.001; b – influence of the Pcr1 on event-free graft survival: yellow indicates normal baseline Pcr1; green – high values baseline Pcr1, p < 0.04

54.9%, 32% and 32% (p < 0.04), one, two, three and four years respectively after treatment had started (Fig. 6).

Analysis of the dynamics of KT function under everolimus therapy revealed that creatinine levels in the blood slightly increased by the end of the observation in comparison with its baseline – 0.16 ± 0.09 mmol/L versus 0.14 ± 0.04 mmol/L, respectively, p < 0.04 (Fig. 7, a). At the same time, the main reasons for the fall in renal function were rejection (in 5 cases), CNI toxicity with ATN (5 people), TMA (1 person) and FSGS (2 people). Similarly, proteinuria increased during the indicated period. Its incidence rose from 17.9% to 43.3%, and daily urinary protein excretion increased from an average of 0.18 ± 0.12 g/day to 0.66 ± 1.31 g/day, p < 0.004 (Fig. 7, b).

In 12 out of 44 (27.3%) patients with proteinuria, the daily protein excretion reached subnephrotic and nephrotic levels.

In the Cox multivariate regression model, only rejection (p < 0.021) and AE (p < 0.045) turned out to be independent predictors of an adverse outcome of everolimus therapy.

Thus, regardless of its duration, everolimus-based immunosuppression therapy did not worsen the long-term results of surgery. However, by the end of a 4-year follow-up, treatment was discontinued in 42.8% of patients. The main reasons for discontinuation of everolimus were rejection, proteinuria, progressive transplant dysfunction and adverse events.

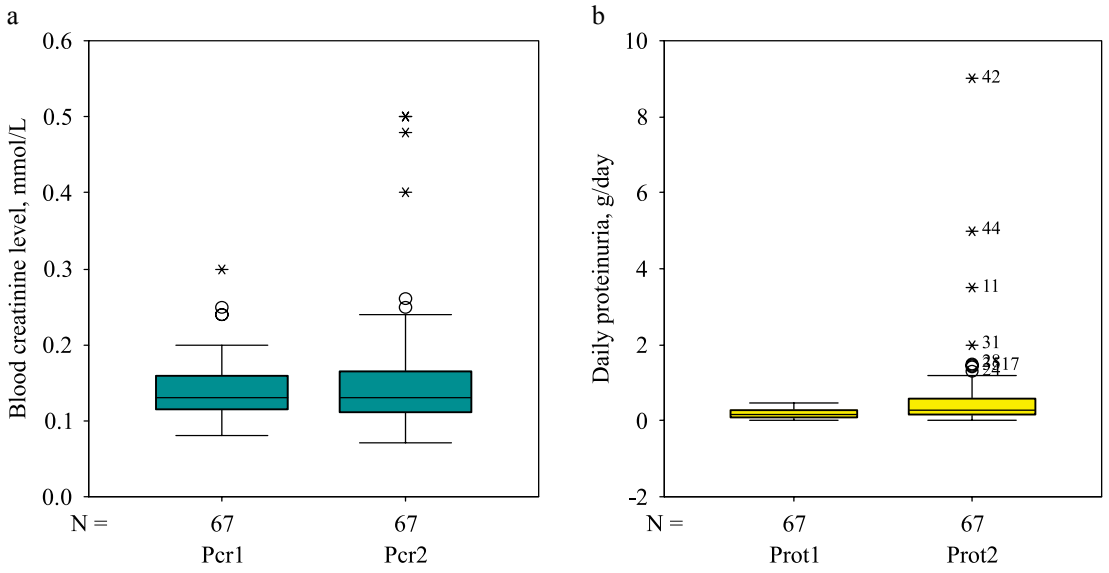

Fig. 7. Dynamics of blood level of creatinine and daily proteinuria on everolimus therapy: a – initial creatinine blood level (Pcr1) 0.14 ± 0.04 mmol/l, final (Pcr2) – 0.16 ± 0.09 mmol/l, $p < 0.04$; b – baseline proteinuria (Prot1) 0.15 (0.1; 0.29) g/day, final (Prot2) – 0.26 (0.14; 0.67) g/day, $p < 0.004$

DISCUSSION

Despite steady advancements in medical technologies and introduction of new immunosuppressive drugs in transplantation practice, used both for induction and for maintenance therapy, the problem of long-term results of kidney transplantation remains a burning issue. Although calcineurin inhibitors cyclosporine and later tacrolimus, which have become the standard drugs for various immunosuppression regimens over the past decades and have shown high and comparable efficacy, as well as indisputable advantages [5], are nephrotoxic. With prolonged use, they lead to chronic transplantation nephropathy (CTN) of which interstitial fibrosis and tubular atrophy were morphological substrate [8]. Signs of CNI-induced nephrotoxicity can be detected already in the first 6 months after ATP. They are usually associated with high blood levels of the drug ($p < 0.05$), are manifested in form of mild arteriolar hyalinosis, and are only functional in nature, being typically reversible. According to B.J. Nankivell et al. [8], the chronic phase of CNI nephrotoxicity occurs at a median onset of 3 years after surgery and does not depend on CNI blood concentrations ($p < 0.05$). It is largely irreversible and morphologically characterized by severe arteriolar hyalinosis ($p < 0.001$), progressive glomerulosclerosis ($p < 0.001$), and CsA tubulointerstitial sclerosis. Ten years after ATP, signs of CNI nephrotoxicity are detected in all patients through protocol biopsies. Moreover, in 100% of cases, arteriolar hyalinosis is detected, and in 88.0% and 79.2% – striped fibrosis and tubular microcalcification, respectively. Introduction of PSI into clinical practice has been an approach to limiting the nephrotoxic effects of CNIs. However, in the ASCERTAIN study [11], which analyzed the efficacy of late conversion from CNI to everolimus (with discontinuation of CNIs in 127 patients and dose minimization in 144 patients), the conversion did not show any advantage over traditional immunosuppression in respect of renal function compared to the control group (n = 123). Glomerular filtration rate by 24 months after randomization remained stable in the compared groups and did not differ significantly – 48.0 ± 22.0 ml/min/1.73 m^2 and 46.6 ± 21.1 ml/min/1.73 m^2 in the groups with discontinued and minimized CsA versus 46.0 ± 20.4 ml/min/1.73 m^2 in the control group. These data were confirmed later in the CONVERT trial [32], in which 6–120 months after ATP, recipients were randomized into 2 groups: a group of patients continuing traditional CNI-based immunosuppression (n = 275) and a group that converted from CNIs to sirolimus (n = 555). Analysis of the results 12 and 24 months after randomization showed no significant differences in the compared groups in graft function and in the incidence of rejection and survival of KT and recipients. Meanwhile, it was noted that KT function improved in patients who converted during the first year after ATP, in contrast to the results of late use of PSI [33]. The calculated median creatinine clearance when using sirolimus for up to 1 year after surgery rose from 30.0 (17.0, 49.7) mL/min at the time of conversion to 54.7 (35.5, 73.9) mL/min at month 12 and was 45.7 (17.0, 74.5) mL/min at 36 months after conversion. In turn, in patients that converted on a later date, the calculated creatinine clearance within the same timeframe had a distinct tendency to decrease. It was 54.8 (41.0, 70.2) mL/min at conversion, 52.6 (37.7, 69.5) mL/min at 12 months and 34.9 (10.0, 65.9) mL/min at three years after conversion [33]. In addition, in studies evaluating the outcome of late conversion, it was noted that with an initially higher level of glomeru-

lar filtration (>40–50 mL/min), graft function improved 12–24 months after conversion in comparison with cases where glomerular filtration was reduced by the time of conversion [11, 32].

These data served as a starting point for the development of an early-PSI-use strategy, applying the PSIs in *de novo* recipients (or after conversion from mycophenolate) in the first 4–6 months after ATP, with complete CNI discontinuation or minimization.

The effectiveness of early conversion to everolimus with stabilization or improvement of transplant function has been shown in several studies [17, 18, 30, 34, 35]. In one of them – multicenter study ZEUS – it was shown that when converting from CsA to everolimus 4.5 months after ATP (n = 155), KT function significantly improved by 12 months after randomization compared with continuation (n = 145) of traditional therapy (71.8 ml/min/1.73 m^2 against 61.9 ml/min/1.73 m^2, respectively) [17]. By 24 months of observations in the everolimus group, glomerular filtration was 68.9 ± 19.4 ml/min/m^2, which was higher in comparison with this indicator at the time of conversion (61.7 ± 17.4 ml/min/m^2, $p < 0.017$). At the same time, the KT function in *de novo* recipients receiving everolimus (3–8 ng/ml) in combination with reduced-exposure CsA and in patients using traditional CsA-based and mycophenolate-based therapy, did not differ after 24 months of observations in the A2309 study [18]. Whereas, according to S. Vítko et al. (2005), the blood creatinine level in the control group was lower 3 years after PSI had started [13]. In our observations, KT function fell slightly during everolimus therapy, as evidenced by the dynamics of blood levels of creatinine, which at the end of observation were significantly higher in comparison with the baseline, reaching 0.16 ± 0.09 and 0.14 ± 0.04 mmol/L, respectively, $p < 0.04$. This deterioration was associated with development of KT pathology in such cases in the form of rejection or CNI toxicity with acute tubular necrosis (ATN), thrombotic microangiopathy, or focal segmental glomerulosclerosis.

According to literature sources, with an initially relatively high level of glomerular filtration (>40–50 ml/min), KT function improves by 12–24 months after conversion compared with the cases where KT function is low at the start of PSI [11, 32]. Similarly, in our study, the initial KT function was of important prognostic significance with regards to the outcome of PSI therapy. A four-year non-event (without any renal pathology) KT survival rate at the initially normal level of serum creatinine (≤0.13 mmol/L) was significantly higher (59.3%) than in cases where blood creatinine was >0.13 mmol/L (32%, $p < 0.04$) at the start of PSI.

In 7.4% (5 out of 67 people) of cases in our observations, everolimus-based therapy was discontinued due to progressive acute transplant dysfunction, of which ATN was a morphological substrate. At the same time, one patient developed dialysis-required renal failure. The mechanism of development of acute kidney injury (AKI) is not entirely clear. Assuming that AKI could have been induced by the nephrotoxic effect of CNIs, we have unsuccessfully attempted to adjust the dose of the latter. Another explanation to ATN, as evidenced by experimental and clinical results, may be offered by enhanced apoptosis of the tubular epithelial cells caused by everolimus [36–38], which justifies the discontinuation of PSI in such cases.

There are indications in literature sources that, when CsA is eliminated amid PSI, rejection develops significantly more often than with traditional immunosuppressive therapy (31.3% versus 5.4%, respectively, $p < 0.001$) and *de novo* donor specific antibodies are also detected more often (34% versus 11.6%, respectively) [35]. In this regard, when choosing an everolimus-based supportive immunosuppression regimen, preference is still given to options with minimization, but not with CNI elimination.

On the other hand, in connection with the adverse effects of PSI, such as delayed graft function, slowing down of reparative processes in post-operative wound, and lymphocele formation, questions arise about the most acceptable timing (first day or first months) of PSI administration. The results of a 12-month CALLISTO study did not reveal the advantages of delayed (5 weeks after transplantation) administration of everolimus in combination with low-dose CNI (n = 74) over immediate use 1 day after surgery (n = 65) [12]. The incidence of primary events, including morphologically verified acute rejection, graft loss, death, delayed graft function, surgical problems associated with delayed wound healing, were comparable in the compared groups – 66.2% in patients with delayed use of PSI and 64.6% in immediate use. Our results do not contradict the data obtained in the CALLISTO study. In our observation, the cumulative incidence of all adverse events in the compared groups also turned out to be comparable (55.0% versus 48.1%, respectively, $p < 0.38$). Table 2. Most of the above complications limited the use of everolimus in such a way that by 12 months and 24 months after ATP, the number of patients who continued treatment fell to 68.2% and 63.1%, respectively, and to 57.2% by 4 years. These data are consistent with the observations of other studies, according to which, under controlled concentration of everolimus (3–8 ng/ml) after 12 months of observation, the drug was discontinued at approximately the same frequency or somewhat less frequently than in our patients – in 20–35% of cases [12, 23, 25]. According to N. Tedesco-Silva et al. [15], after a 24-month observation, PSI therapy was continued in up to 70% of patients. A somewhat more frequent discontinuation of this drug in our observations, we believe, depends on the fact that the main reason for discontinuation of everolimus in our recipients was transplant rejection (in 8 out of 31 cases, which accounted for 25.8% of the total number of reasons for therapy discontinuation). Everolimus therapy

was continued only in 3 patients after relief of acute rejection and conversion from CsA to TAC, taking into account oncological history. Therapy was completed in 8 of 11 patients with morphologically verified rejection.

According to various studies, the rejection rate in PSI by the end of a 12-month observation varies from 7.7% to 25.9% depending on the drug dosage and the choice of a low-dose CNI [15, 21, 39, 40–43]. In some studies, PSI use did not affect the rejection rate [13, 14, 33, 34, 44], which, after 36 months of observation, was 24–25% versus 26.5% in the control group [13, 14]. In the observations carried out by other authors for patients who received PSI-based immunosuppression, rejection developed more often [16, 17, 35]. So according to K. Budde, T. Becker et al., in the group of recipients, who converted from CsA to everolimus 4.5 months after surgery, the rejection rate 12 months after randomization was 10% (15 of 154 patients), while in the control group, it was only 3% (in 5 out of 146), $p = 0.036$ [16]. Further analysis by the same authors showed that similar differences persisted up to 24 months after conversion: 11% versus 4.8%, respectively [17]. Our data are somewhat consistent with the results obtained by these authors. Rejection rate in our patients during everolimus therapy was 14.9%. In the vast majority (8 out of 11 people) rejection was diagnosed during the first 12 months of PSI application. As in the study by J. Dantal et al. (2010), which showed a comparable rejection rate in groups with immediate and delayed use of PSI (20% and 20.4%, respectively) [12], our observations showed no significant differences in rejection rate in the compared recipient groups, although there was a tendency for higher rejection after early conversion from MMF to everolimus: 22.2% (6 of 27) versus 12.5% (5 of 40) in the *de novo* group ($p < 0.29$). In our opinion, the latter could be caused, firstly, by the not entirely justified use of everolimus in a surgical hospital in cases of intolerance to mycophenolates and initial transplant dysfunction, as well as after acute rejection episodes. Another reason could be problems associated with provision of adequate dosages of CNIs and PSI after conversion. The importance of having adequate dosages has been convincingly demonstrated by J.M. Kovarik et al. (2004). Based on retrospective analysis of 3355 computation of everolimus blood concentrations of 695 recipients, the group of authors revealed a relationship between the maintenance level of the drug in the blood (Cmin) and the frequency of rejection. The authors showed that while maintaining Cmin in the 1.0–3.4 ng/ml range, rejection was not detected in 68% of patients, while under blood everolimus concentrations at 3.5 to 7.7 ng/ml, the number of recipients without rejection increased to 81%–86% and, finally, reached 91% as Cmin increased to 7.8–15 ng/ml [19]. These results were later confirmed by M.I. Lorber et al. (2005) [20]. From the data obtained, it follows that everolimus blood level is optimal, which is maintained in the 3–8 ng/ml range and provides adequate immunosuppression, while minimizing the adverse effects of PSI. In this aspect, the results of our study are fully consistent with that of literature sources. In the group of patients for whom the target blood concentrations of both drugs were provided, rejection developed only in 7% (3 out of 43 people) of cases, while in the group of recipients with inadequate immunosuppression, it was diagnosed significantly more often – 29.2% (7 out of 24 people) of patients, $p < 0.015$. As a result, the 4-year functional survival of KT (without CKD) in the group with inadequate therapy decreased to 47.1% versus 66.85% in recipients who maintained the necessary target blood levels of PSI and CNIs ($p < 0.038$).

Another serious problem that complicated the use of PSI in our patients was proteinuria. Increased urinary protein excretion to some extent is observed by all researchers [14–16]. This applies to studies using everolimus in combination with both full-dose CsA (B251, B201) and low-dose CNIs (trial B2309), with varying frequency of proteinuria (13, 14, 15). In the B2309 studies, one year after everolimus use at a 1.5 mg/day dose had started, the incidence of proteinuria did not exceed 9.1% and reached 12.9% against the background of a maintenance dose of 3 mg/day [15], while in other trials, the incidence of subnephrotic proteinuria at the same time increased to 24%, which was almost 2 times higher than in patients with traditional supportive immunosuppression (CsA with MMF) [14]. These works indicate not only the associative relationship of everolimus and proteinuria, but also the dose dependence of the latter. In the B251 study on recipients receiving everolimus at a dose of 1.5 mg/day in combination with full-dose CsA, proteinuria incidence (more than 300 mg/day) was even higher – 39.2% versus 14.9% in the group receiving traditional immunosuppression, $p < 0.0001$. In the same way, the frequency of expressed proteinuria, which was 11.4% and 2.3%, respectively, $p = 0.028$ [14] also differed in these groups. In our patients, proteinuria increased with time from 0.18 ± 0.12 g/day up to 0.66 ± 1.31 g/day ($p < 0.004$), and its incidence by the end of the observation increased from 17.9% to 43.3%. In slightly less than a third of patients (27.3%), proteinuria reached subnephrotic and nephrotic levels. The appearance/increase of proteinuria after conversion from CNI to PSI can be caused, on one hand, by hemodynamic changes in the glomeruli associated with discontinuation of CNIs, and on the other, by podocytopathy. The causes of higher protein excretion in *de novo* recipients have not been fully understood. It is believed that PSI reduces the expression of basic structural proteins (nephrin, adapter protein Nck, transcription factor WT1, etc.) needed to maintain the integrity of podocytes. The latter, combined with impaired actin formation, leads to lower adhesion and mobility of podocytes and higher permeability of the slit diaphragm [45, 46]. It has also been established that, against the

background of the use of PSI (sirolimus), expression of vascular endothelial growth factor (VEGF) is enhanced, which, as shown in experimental and clinical studies (in HIV-infected patients with collapsing nephropathy), accompanies proteinuria, increasing vascular permeability [47–49]. In support of the above, Izzedine et al. (2005) conducted a comparative analysis of the patient's biopsy samples before and after conversion from conventional therapy (MMF and CsA) to sirolimus for the presence of Kaposi's sarcoma 8 years after ATP. Only cyclosporin-induced chronic arteriolopathy was morphologically verified by the start of PSI therapy, while collapsing FSGS was detected after 1 year. Glomerular pathology was accompanied by proteinuria up to 3 g/day by an increase in serum VEGF levels and an increase in its expression in collapsed glomeruli, including edematous podocytes. Based on the data obtained, the authors suggested that sirolimus induces post-transplant proteinuria, contributing to the development of collapsing FSGS associated with VEGF expression in podocytes [47]. Another mechanism of proteinuria under PSI conditions is the fall in the reabsorption capacity of tubules [50, 51], which, as demonstrated in experimental models, is due to increased apoptosis of the proximal tubule epithelial cells [36, 37]. This increased apoptosis was also confirmed in a clinical study, which compared biopsy results for patients with delayed initial graft function under sirolimus-based therapy and under traditional immunosuppression [38]. It turned out that incidence of tubular epithelial cell apoptosis under PSI was significantly higher than in recipients with traditional immunosuppression ($p < 0.001$). In addition, diffuse podocyte apoptosis was detected in 60% of patients treated with sirolimus (versus 7% in the control group, $p = 0.007$) in the absence of higher expression of activated apoptosis markers in glomeruli. This allowed the authors to suggest that accelerated death of podocytes is not associated with changes in the expression of apoptosis markers and is a consequence of the direct toxic effect of PSI [38].

FINDINGS AND CONCLUSION

We have confirmed the results obtained by other authors on the possibility of using everolimus in combination with low-dose calcineurin inhibitors (CNIs) in kidney transplant recipients early after surgery to prevent CNI-induced chronic nephrotoxicity. This immunosuppression regimen – regardless of its duration – did not worsen the long-term results of transplantation as a whole. However, the possibilities of its use were limited already 4 years after surgery in almost 43% of patients due to development of graft rejection, progressive transplant dysfunction, proteinuria and adverse events. The likelihood of rejection increased with inadequate immunosuppression, which, like reduced transplant function at the start of PSI, was a prognostically unfavorable factor for renal transplant survival. Based on the data obtained, the following conclusions are made:

1. The use of PSI in *de novo* patients and with early conversion from mycophenolate can only be recommended for recipients with low immunological risk.
2. To avoid the risk of kidney graft failure and dysfunction during PSI therapy combined with low-dose CNI, there is need to maintain the targeted blood concentrations of both drugs.
3. Early conversion from mycophenolate to PSI is not recommended for patients with reduced transplant function and/or initial proteinuria.
4. The high likelihood of proteinuria under PSI necessitates careful monitoring of daily protein excretion.
5. Given the identified reasons for discontinuation of everolimus in *de novo* patients, the use of PSI should be delayed in cases of diabetes mellitus due to the risk of a slowdown in reparative processes and limited in women of childbearing age planning a pregnancy after kidney transplantation.

The authors declare no conflict of interest.

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PRETRANSPLANT RECONSTRUCTIVE SURGERY ON DONOR HEART

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Aim: to evaluate the immediate results of reconstruction of the valve heart apparatus and the great vessels of the heart graft before implantation. **Materials and methods.** The analysis included 24 cardiac transplants with pathology of the valve apparatus and the great vessels, as well as 24 recipients who needed emergency heart transplantation and were in the clinic under UNOS status code 1A and 1B. **Results.** Before performing heart transplantation, the valve apparatus and great vessels were corrected. **Conclusion.** With a shortage of donor organs for recipients requiring emergency care, cardiovascular transplantation from "suboptimal" donors is one of the most affordable ways. Given the possibility of reconstructive operations on the valve apparatus and the great vessels of the donor heart, and evaluating satisfactory immediate results of demonstrated observations, it can be argued that the above way out would reduce urgent waitlist mortality, achieve satisfactory survival results in the early postoperative period, increase the donor resource and optimize the transplant program.

Keywords: heart transplantation, donor organ, heart failure, heart valve repair.

Despite widespread adoption of effective drugs for the heart failure treatment, the annual survival rate of patients with terminal heart failure (THF) remains extremely low and is conditioned by progressive myocardial dysfunction [1; 25]. Besides pharmacological therapy the following surgical methods are currently widely used in patients with THF: 1) myocardial revascularization, 2) resynchronization therapy, 3) partial ventriculoplasty (Batista surgery), 4) implantation of the elastic mesh stent, 5) valvular heart disease treatment. These techniques could be effective in the early stages of heart failure and stored myocardial reserves, but they have no effect at the terminal stage of the disease [2; 3].

Moreover, despite the successful development and implementation of implantable systems of long-term mechanical circulatory support (MCS), heart transplantation (HT) remains the most effective method of treatment for patients with THF characterized with more than 90% survival rate within 1 year and an average life expectancy within 10 years after the surgery. Besides, post-HT patients have no significant physical activity limitations [4]. The desire to reduce the recipients' lethality due to the decompensation in THF during the organ waiting period led to mechanical circulatory support application as a "transfer" to HT by extracorporeal membrane oxygenation (ECMO), as well as transplantation from "suboptimal donors".

During the period from 1997 to 2017 36,340 adult patients aged 18 to 64 years underwent the HT surgery in the USA [5]. Currently, the USA waiting list consists of approximately 3000 candidates waiting for heart transplantation with waiting list annual mortality rate of nearly 15% [6]. According to Eurotransplant data, as of 2017 in Europe the active line of recipients in the waiting list consists of 1141 persons. During the same period, only 548 recipients underwent the HT surgery, taking into account that only 817 heart donors have been considered [7]. For the last several years the program of heart transplantation in Russia, in particular, in the FSBU [Federal State Budgetary Institution] "V.I. Shumakov. Shumakov National Medical Research Medical Center of Transplantology and Artificial Organs" of the Ministry of Health of Russia has been characterized by active and successful development. During the period from 2006 to 2017, the number of heart transplantation centers had increased by 14 (sevenfold). The number of patients in the heart transplant waiting list has reportedly increased in the Russian Federation from 2012 to 2017, while the time of heart transplantation waiting has been gradually decreasing. The heart transplant waiting list during 2017 included 645 potential recipients, where 405 were included into the waiting list for the first time in 2017. In Moscow, 304 potential recipients were in the heart transplant waiting list (47.1% from the national waiting list). The death rate during the heart transplantation waiting period in Russia during this period was 42 patients [8–13].

The first successful heart transplantation in our country was performed by Academician V.I. Shumakov on March 12, 1987. Back then, the HT development show-

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ed that the need for heart transplantation was not accompanied by a proportional increase in the number of transplants due to the lack of donors with the standard criteria. Currently, the number of heart transplantations is increasing year after year due to the raising of new donor programs efficiency. Therefore, during the period from 2006 to 2008, in total 56 heart transplantations were performed in the Russian Federation, 35 transplantations were conducted in Shumakov National Medical Research Center of Transplantology and Artifical Organs. Furthermore, in the period from 2014 to 2017, 813 heart transplantations were performed, 492 of them took place in our center. In 2017, from a total of 252 heart transplantations conducted in the Russian Federation 63.9% (161) corresponding procedures were performed in Shumakov National Medical Research Center of Transplantology and Artificial Organs. Besides new programs, the successful program of heart transplantation in our center enables increasing the number of heart transplantations in the country. In 2018 the number of HT in Shumakov National Medical Research Center of Transplantology and Artificial Organs made 196 [8–13].

The discrepancy between the need and the availability of donor organs is the most significant limiting factor of the heart transplantation program worldwide [4].

A rather great amount of donor hearts are left unused for transplantation due to the valvular heart apparatus pathology, atherosclerotic affection of the coronary arteries, lesion of the heart transplant ascending aorta, therefore, such organs are subject to "utilization" [14–18].

In the context of the heart donors with standard criteria deficit, the issue of the relevance of HT from donors with extended criteria or the so-called "suboptimal donors" has emerged [19].

A heart donor is regarded as optimal if the next criteria are fulfilled: equivalence or compatibility of donor and recipient according to the AB0 blood type system, donor's age under 40 years old, left ventricle ejection fraction more than 50%, cardiotonic or/and angiotonic [vasopressor] support (dopamine/dobutamine minimum dose below 10 um/kg/min or norepinephrine below 100 ng/kg/min), left ventricle cardiac muscle thickness below 12 mm, absence of coronary arteries stenosis, cardiac muscle contractility function local disorders and heart valvular apparatus and great vessels pathologies, as well as presumptive transplant ischemia being less than 4 hours [19].

Recently, the significant increase of heart transplantations from donors aged 60 years and above is observed. In literary sources cases of trials on HT from elderly donors can be found, in which cases no verifiably significant difference in HT surgeries results from donors under 40 years old had been determined [Drinkwater D.C. et al., 1996; Mulvagh S. et al., 1989; Pflugfelder P.W. et al., 1991; Menkis A.H. et al., 1991; Zuckermann A., Kocher P. et al., 1997]. The great attention in the heart transplantation program is paid to HT with myocardial hypertrophy more than 1.4 cm, with an ejection fraction of the left ventricle below 50% [19]. Rarely, but there are reports regarding valvular and coronary pathology correction of the heart transplant. Observations of plastic, mitral valve prosthetics, aortic valve prosthetics, aortocoronary bypass grafting of donor hearts are also described [16–18; 20; 21; 23; 24].

Goland et al (2008) consider that in the context of donor organs deficit the heart transplantation is justified in case of strict principle "suboptimal donor – suboptimal or urgent patient" maintenance.

In the Shumakov National Medical Research Center of Transplantology and Artificial Organs, during the period from 2012 to 2019, various pretransplantational reconstructive surgeries of valvular apparatus and great vessels were performed on donor organs for 24 recipients.

This work aims at evaluating the direct results of this procedure type.

METHODS AND RESULTS

24 heart donors were included in the analysis, including 14 (58.4%) males and 10 (41.6%) females aged 27 to 63, with the mean age of 48.8 ± 7.6. Among the causes that led to brain death in a heart donor, there were: traumatic (traumatic brain injury) in 5 and hemorrhagic and ischemic strokes in 15 and 4, respectively. Donor body weight varied from 55 to 115 kg, on average: 83.5 ± 14.4 kg, body mass index ranged from 19.03 to 42.24 mg/m^2, averaging 27.93 ± 4.21 kg/m^2. Donor blood type: 0 (I) – n = 6, A (II) – n = 13, B (III) – 5. No periods of hypotension were observed during "donor conditioning". The donor "conditioning" period lasted from 1 to 4 days, on average: 1.7 ± 0.7 days.

The blood flow stagnation in the cerebral vessels leads to neurocirculatory regulation disorder and serves as a driving force for homeostasis disorder [Howlett T.A. et al., 1989]. In case of brain death, the endocrine system reaction is characterized by pronounced hormones release, which is manifested with clinical picture of the so-called "vegetative storm" [Bucker A.I., Shute Yu., 1981; Shemie S.D. et al., 2006]. Donor conditioning includes restoration and support of the blood circulation, stable arterial pressure, anemia, acidosis, hypernatremia, hypoproteinemia and polyuria correction, body temperature maintenance [Sergiyenko S.L. et al., 2010]. Hemodynamic parameters stability after donor brain death was supported using combined or isolated infusion of angiotonic and cardiotonic drugs (norepinephrine, dopamine). Due to pharmacological therapy correction at the stage of donor "conditioning", it became possible to achieve the maximum decrease of cardiotonic and angiotonic drugs dosage, in particular, of norepinephrine from 600 ng/kg/min to 80 ng/kg/min, on average: 253.4 ± 105.9 ng/kg/min; dopamine dosage ranged from 2 to 6 mg/kg/min, on average: 4.2 ± 1.4 ng/kg/min, in four cases

the drugs were used in combination, but in one case the donor "conditioning" was performed without any sympathomimetic support. Cardiovascular resuscitation was conducted to one of the donors at the observation stage on the background of the blood circulation arrest followed by effective hemodynamics restoration.

Adequate conditioning resulted in all donors being compensated by their blood electrolytes balance and having normal biochemical and clinical blood counts by the time of heart transplantation. During the clinical-instrumental examination of a potential heart donor, a transthoracic and/or transesophageal echocardiography were performed to evaluate the ejection fraction of the left ventricle, measure the size of the heart chambers and wall thickness, and the function of the valvular apparatus of the heart, assess the presence or absence of local contractility disorders, specifically hypokinesis, akinesis, dyskinesis. Among the examined heart donors, left ventricular hypertrophy (LVH) was detected in 13 patients. The thickness of the interventricular septum (IVS) ranged from 1.2 to 1.8 cm, averaging 1.29 ± 0.24 cm, the thickness of the posterior wall of the left ventricle (LVPW) was within the range of 1.2 to 1.5 centimeters, averaging 1.27 ± 0.16 cm.

During the EchoCG study in the examined group, the following was diagnosed: aneurysm of the ascending aorta above the sinotubular junction with aortic valve insufficiency in bicuspid aortic valve with stenosis revealed in one donor, complex aortic valve defect in two donors, mitral valve insufficiency in 17 donors. In one case, a combined defect of the mitral valve with insufficiency predominance was detected. Mitral valve stenosis was detected in 1 donor.

Stage 2 and 3 pulmonary hypertension was diagnosed in 6 and 1 donor, respectively.

According to electrocardiographic monitoring data aimed at detection of cardiac muscle and rhythm disorders in 9 donors arrhythmias were detected: atrial fibrillation in one donor, sinus arrhythmia with HR of 107 ± 4.8 bpm on average in 5 donorss, sinus bradycardia with HR of 53 bpm in 1 donor, stage 1 atrioventricular blockade in one donor, and in one case the rhythm was generated from atrioventricular connection. Sinus tachycardia and acute atrial fibrillation were, probably, driven by the heart donor brain death and electrolyte balance disturbance.

For the heart transplant storage, the method of cold pharmacological cardioplegia was applied using the following solutions: "Bretschneider-HTK" in two cases and "Kustodiol" in the others. The storage solution volume of 3000 ml was administered into the aortic root after the aortic clamping. During the intraoperative visual assessment of the cardiac transplant, signs of heart contusion associated with cardiac pulmonary resuscitation were observed.

After explantation, the visual and palpatory transplant examination was performed by the cardiac surgeon. During this examination, in accordance with the ultrasound findings, the mitral valve affection was revealed: cusps degeneration (n = 3), cusps thickening with calcification foci and fish-mouth mitral stenosis (concretion along commissures) (n = 2), fibrotic changes of cusps (n = 11), and mitral valve posterior cusp cleavage up to fibrous ring, anterior cups cleavage along the A2–3 margin of and posterior cusp cleavage along P2–3 margin in one patient. At the revision of aortic valve, the following lesions were visualized: bicuspid aortic valve with massive cusps calcification (n = 1), fibrous alterations of cusps calcification (n = 1). Aneurysm of ascending aorta associated with the aortic valve insufficiency was identified in two patients. The valves lesion was combined in one case: mitral valve cusps degeneration and massive calcinosis of the aortic valve cusps.

Moreover, at the revision of the heart septum, the patent foramen ovale was revealed in one donor and sutured during heart transplant processing.

24 recipients were prepared for transplantation including 17 (71%) males and 7 (29%) females aged from 16 to 64, on average 47.5 ± 11.4. At admission, the most common recipients' complaint was breathlessness at rest (n = 13) and minimal physical exertion (n = 10). Swelling in the ankles and feet were identified in 13 recipients, in particular, only feet edema in 6 and edema up to thighs in 1 recipient.

The primary disease leading to the development of terminal heart insufficiency and the need in HT conduction in 24 recipients was the DCMP [dilated cardiomyopathy] in 13 patients and cardiomyopathy as result of myocarditis in one recipient, ischemic cardiomyopathy in 7 recipients, hypertrophic cardiomyopathy in one case, postradiation anthracycline cardiomyopathy in 1 recipient and heart transplant dysfunction at 3rd year after orthotopic heart transplantation in 1 recipient.

All recipients (n = 24) required cardiotonic dopamine therapy, the dosage ranged from 3.3 to 8.4 μg/kg/min, the average dose made 4.9 ± 1.14 μg/kg/min or dobutamine, with the minimal dose of 3 μg/kg/min and the maximum dose of 10.3 μg/kg/min, the average dose made 5.3 ± 1.62 μg/kg/min, to maintain the adequate hemodynamics while waiting for HT. 6 recipients were under mechanical circulatory support during the pretransplantation period using extracorporeal membrane oxygenation with peripheral connection method lasting for 1 to 9 days (averaging 3.6 ± 2.2 days) and a productivity of 3.5 to 4.5 l/min, averaging 3.8 ± 0.26 l/min.

All recipients requiring urgent HT were under permanent intravenous infusion therapy only or/and a combination of cardiotonic drugs, hemodynamics mechanical support was used, patients' status according to the UNOS classification was 1A–B. In the study group, 1 patient was waiting for heart re-transplantation.

Average systolic BP was 95 (±6.8) mm Hg, diastolic BP = 62.6 (±5.7) mm Hg.

The results of pretransplantation EchoCG are shown in table 1.

Table 1

Pretransplantation echocardiography parameters of recipients

Parameter	Mean values
Aorta at the level of fibrous ring, cm	2.4 ± 0.37
Aorta at the level of the ascending segment, cm	3.2 ± 0.46
LV anteroposterior dimension, cm	5.2 ± 0.82
RV, cm	3.4 ± 0.65
EDD, cm	7.1 ± 1.2
ESD, cm	6.4 ± 0.96
EDV, ml	298.5 ± 101.76
ESV, ml	234.1 ± 89.96
SV, ml	70.3 ± 17.88
LVEF, %	22.8 ± 4.78
IVS thickness, cm	1.1 ± 0.22
PW thickness, cm	1.0 ± 0.14
Pulmonary artery pressure, mm Hg	51.6 ± 12.24

Note. Here and in the table 2: LV – left ventricle; RV – right ventricle; EDD – end-diastolic dimension of left ventricle; ESD – end-systolic dimension of left ventricle; EDV – end-diastolic volume of left ventricle; ESV – end-systolic volume of left ventricle; EF – ejection fraction; LVEF – left ventricle ejection fraction; IVS – interventricular septum; PW – posterior wall.

Stage 3 mitral valve regurgitation was revealed in n = 15, stage 2 in n = 8.1, stage 1 in n = 1 of recipients. Stage 3 tricuspid valve regurgitation was diagnosed in 12 recipients, stage 2 in 11 recipients and stage 4 in one observed patient.

Transplantation surgery was performed using the following methods:

1) atrial (Lower R.R., Stofer R.S., Shumway N.N., 1961) in 3 recipients;
2) cava-caval (Yacoub M., 1990; Dreyfus G., 1991) in 16 recipients;
3) complex in 1 recipient, during which the tissue "bridge" was formed from the right atrium wall with the purpose of the superior and inferior vena cava connection (Shumakov V.I., 2006) [22].

Due to the state severity of recipients in HT waiting list, the decision was made regarding heart transplantation from "suboptimal donors".

During transplantation, taking into account the valvular apparatus and great vessels changes of the heart transplant, the following surgeries were performed:

1) supracoronary ascending aorta replacement with prosthesis "Vascutec-28" and "Gelewave-28" (n = 2);
2) aortic valve replacement with mechanical valve "Medinge-25" and "Medinge-23" (n = 2);
3) mitral valve replacement with mechanical valve "Medinge-29" (n = 2);
4) mitral valve annuloplasty with supporting ring (n = 4), "Medinge-28", "Medinge-34", "Medinge-30" (n = 2);
5) mitral valve annuloplasty with supporting ring "MedtronicProfile 3D-28", suturing of MVPC and MVAC cleavage (n = 1);
6) mitral valve annuloplasty with supporting ring "Medinge-32" in combination with Alfieri mitral valve cusps plasty;
7) mitral valve annuloplasty with supporting ring with chorda replacement using PTFE fiber in 3 patients. Supporting rings "Medinge-28" (n = 2) and "Medinge-30" (n = 1) were used;
8) mitral valve annuloplasty with supporting ring "Medinge-30" with chorda replacement using PTFE fiber, MVAC cleavage suturing (n = 1);
9) mitral valve annuloplasty with supporting ring "Medinge-30" with chorda replacement using PTFE fiber, MVPC cleavage suturing and Boyd's tricuspid valve plasty;
10) mitral valve annuloplasty with supporting ring and tricuspid valve annuloplasty with supporting ring (n = 4), rings for mitral valve: "Medinge-28" (n = 3) and "Medinge-30" (n = 1), for HT "Medinge-28" (n = 2), "Medinge-30" and "Medinge-34";
11) mitral valve annuloplasty with supporting ring and deVega and Boyd tricuspid valve plasty (n = 2). Supporting rings: "Medinge-30" (n = 2);
12) mitral valve annuloplasty with supporting ring "Medinge-28" with chorda replacement using PTFE fiber and aortic valve replacement with "Medinge-21" mechanical prosthesis.

Intraoperative photos with notes are provided in Fig. 1–4.

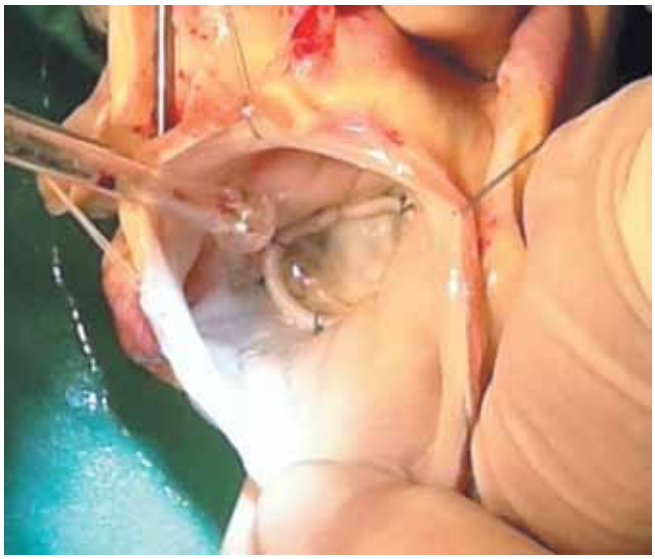

Fig. 1. Donor S., 47 years old, with brain death due to ischemic stroke with preserved pumping function of the heart, having fibrous changes in mitral valve. Hydraulic test after implantation of the support ring

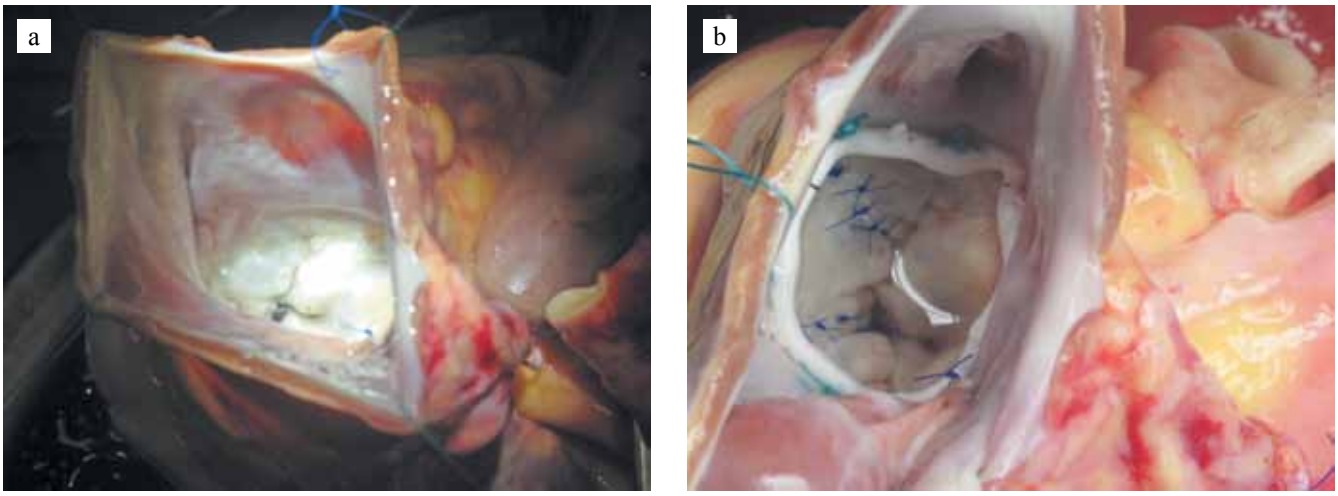

Fig. 2. Donor U., 49 years old, with brain death due to intracerebral hemorrhage, mitral valve posterior flap cleavage: a – the splitting of the mitral valve leaflets of the donor heart, unsatisfactory co-optation; b – annuloplasty of the mitral valve by the support ring and valvuloplasty of the mitral valve

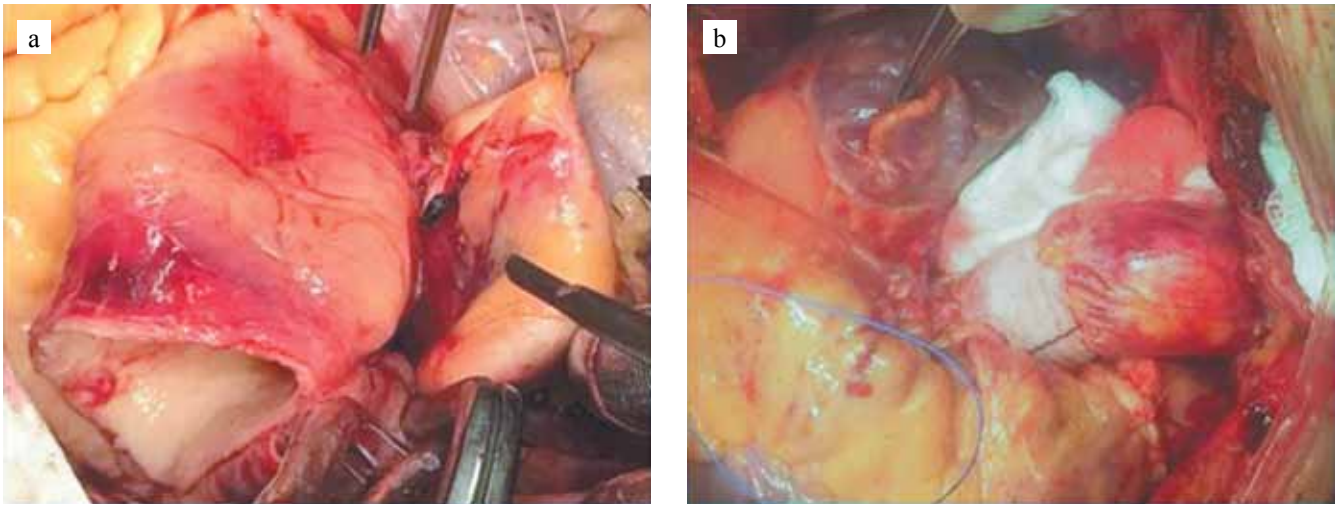

Fig. 3. Donor E., 57 years old, with brain death due to intracerebral hemorrhage, ascending aortic aneurysm and aortic valve insufficiency: a – aneurysm of the ascending part of the donor heart, mismatch of the donor and recipient aortic diameters; b – supracoronary aortic prosthetics of an implanted heart

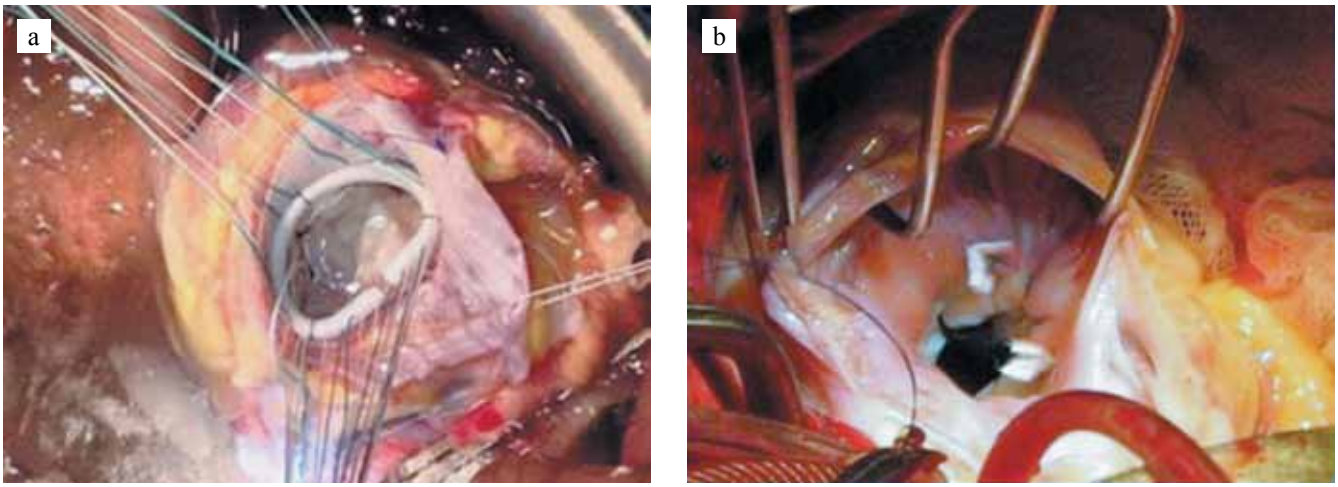

Fig. 4. Donor Z., 52 years old, with brain death due to intracerebral hemorrhage, mitral insufficiency and tricuspid valve insufficiency: a – implanted support ring in mitral position; b – Plastic tricuspid valve by De Vega

The duration of the surgery was 160 to 432 minutes, with an average of 298.23 ± 54.39 minutes. The time of artificial blood circulation ranged from 56 to 210 minutes, averaging 131 ± 30.9 minutes. Heart transplant ischemia averaged 195.1 ± 40 min. The duration of myocardial ischemia for more than 3 hours was recorded in 13 (54%) recipients. The level of hypothermia during the procedure of artificial blood circulation ranged from 35.2 to 27.9 °C, averaging 33.08 ± 10 °C.

In two (8%) patients the cardiotonic therapy was not applied in the early pre-transplantation period. In 92% of patients complex multicomponent cardiotonic support was applied.

From 6 recipients previously being under mechanical circulatory support by venoarterial ECMO [extracorporeal membrane oxygenation] before HT, the post-transplantation venoarterial peripheral ECMO was required in 5 patients and 1 recipient was disconnected from the ECMO apparatus due to the early heart transplant dysfunction. Apart from these patients, 3 recipients required post-transplantation mechanical circulatory support by veno-arterial central ECMO . The volume speed of extracorporeal blood flow was on average 2.36 ± 1 l/min. The duration of mechanical circulatory support ranged from 2 to 9 days, on average 4.5 ± 2.6 days.

In 8 patients, mechanical circulatory support was combined with sympathomimetic therapy, including epinephrine, dobutamine, and/or dopamine, the dose made on average 0.05 ± 0.03 μg/kg/min, 4.75 ± 3.5 μg/kg/min and 5.75 ± 0.37 μg/kg/min, respectively. After early transplant dysfunction regression, recipients with peripheral ECMO were disconnected from the mechanical circulatory support.

In 62.5% recipients, the functioning of the heart transplant was restored after circulatory support. In 3 patients the repeated HT was performed in three recipients with central ECMO due to irreversible heart transplant dysfunction.

In 14 recipients, not requiring mechanical circulatory support, the efficacy of pumping ability of the transplant was provided by cardiotonic therapy, including epinephrine, dobutamine and/or dopamine. Dopamine dose ranged from 2 to 15 μg/kg/min, on average – 8 ± 3.73 μg/kg/min. Dopamine dose ranged from 2 to 15 μg/kg/min, averaging 8 ± 3.73 μg/ kg/min. Dobutamine dose ranged from 3 to 8 μg/kg/min, with an average of 4.58 ± 1.5 μg/kg/min, the Adrenaline dose ranged from 0.01 to 1.2 μg/kg/min, averaging 0.41 ± 0.39 μg/kg/min.

The early postoperative period associated with renal dysfunction in 5 patients required the application of the replacement renal therapy. Antibody-related transplant failure after HT was detected in 5 recipients, therefore the immunosuppressive therapy was corrected. Furthermore, hydrothorax was among the most common postoperative period complications (n = 11).

After coronary angiography of the transplanted heart, no hemodynamically significant atherosclerotic lesions of coronary arteries were detected. Findings of the post-transplantation EchoCG are shown in table 2.

Table 2

Echocardiography findings in recipients after heart transplantation

Parameter	Mean value
Aorta at the level of fibrous ring, cm	2.52 ± 0.37
Aorta at the level of ascending segment, cm	3.09 ± 0.14
LV, cm	4.12 ± 0.91
RV, cm	2.42 ± 0.33
EDD, cm	4.35 ± 0.32
ESD, cm	2.62 ± 0.3
EDV, ml	84.31 ± 17.31
ESV, ml	26.31 ± 8.31
EF, ml	59.13 ± 11.39
LVEF, %	67.56 ± 4.6
IVS, cm	1.27 ± 0.13
LVPW, cm	1.23 ± 0.11
PA pressure (mm Hg)	36.47 ± 7.89

Stage 1 mitral valve regurgitation was detected in n = 14, stage 2 in n = 1 of recipients. Stage 3 tricuspid valve regurgitation was revealed in 1 recipient, stage 2 in 2 recipients and stage 1 in 12 of the observed patients. Despite these parameters, we have not revealed any residual insufficiency impact on the central hemodynamics parameters which were stable.

Hospital death rate after heart transplant valvular apparatus reconstructive surgeries with further HT was 12.5% (3 from 24 recipients). According to the post-mortem examination findings and results of autopsy material examination, the death of two recipients occurred on the 7th and 8th day, respectively, after the heart transplantation, due to progressive heart failure. The death of the third patient occurred on the 108th day due to multiple organ failure.

21 (87.5%) from 24 recipients with the HT from "suboptimal" donors did not require any repeated surgical interventions and were discharged. Bed days after surgery made on average 28.94 ± 14.14 days.

CONCLUSION

In the context of the donor organs global deficit, the cardiac transplantation from the so-called "suboptimal" donors is regarded as one of the most effective ways to increase availability and number of surgeries, first of all, in recipients needing urgent medical help. Hemodynamically significant defects of the valvular apparatus and great vessels of the heart served as contraindications to the selection of the cardiac transplant. Given the possibility of reconstructive operations on the heart valve apparatus, a significant number of donor hearts can be used effectively [23; 14]. Evaluating the satisfactory

direct results in the described patients, it seems fair to say that the implementation of the above project will reduce the hospital death rate of recipients, improve the immediate HT results and allow for the most efficient use of the valuable donor resource.

The authors declare no conflict of interest.

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GALECTIN-3 IN HEART TRANSPLANT REJECTION AND FIBROSIS

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Aim: to study plasma galectin-3 levels in heart recipients and to determine the potential significance of galectin-3 level in acute transplant rejection and fibrosis. **Methods.** The study included 107 heart transplant recipients, aged 16 to 70 (48 ± 13) years, of which 90 (84%) were men. Dilated cardiomyopathy was diagnosed in 57 patients prior to heart transplantation, end-stage ischemic heart disease in 50. Galectin-3 concentrations and placental growth factor (PlGF) were measured using enzyme-linked immunosorbent assay (ELISA); vascular endothelial growth factors (VEGF-D and VEGF-A), monocyte chemoattractant protein-1 (MCP-1), platelet-derived growth factors (PDGF-BB), and soluble CD40 ligand (sCD40L) were measured using multiplex technology xMAP. Acute graft rejection and myocardial fibrosis were verified through morphological examination of endomyocardial biopsy specimens. **Results.** Galectin-3 concentrations in patients with congestive heart failure (15.92 [11.80; 23.65] ng/ml) were significantly higher than in healthy individuals (11.08 [7.71; 14.47] ng/ml), p = 0.00. No correlation was found between galectin-3 levels and sex, age and pre-transplant diagnosis. A month after transplantation, plasma galectin-3 level was significantly higher than before transplantation; a year later, the levels decreased to pre-transplant levels (18.71 [13.14; 25.41] ng/ml). By the end of the first year after transplantation, the levels were significantly higher both in patients with 1-2 episodes and in the patients after 3 or more episodes of acute rejection, in contrast to recipients who were not diagnosed with rejection. By the end of the first year after heart transplantation in patients with fibrosis, plasma galectin-3 levels were significantly higher than in patients without fibrosis. By the end of the first year after heart transplantation, galectin-3 levels in the recipients were associated with the nature of myocardial fibrosis: in patients with diffuse focal fibrosis (22.52 [20.98; 26.08] ng/ml), plasma concentrations of galectin-3 were significantly higher than in patients without fibrosis (15.36 [11.95; 22.42] ng/ml, p = 0.01). **Conclusion.** Plasma levels of galectin-3 in heart recipients by the end of the first year after transplantation is associated with previous crises of acute graft rejection, irrespective of the number of rejection episodes. Elevated plasma levels of galectin-3 in heart recipients in the long term after transplantation is associated with myocardial fibrosis; galectin-3 levels are associated with the morphological characteristic of fibrosis in the transplanted heart (diffuse focal fibrosis).

Keywords: heart transplantation, biomarkers, galectin-3, myocardial fibrosis, rejection.

Despite all the recent improvements in immunosuppressive and adjuvant drug therapy and significant progress in the survival of patients with heart transplants, the recipients often develop an asymptomatic heart failure (HF) in the long-term. One of the key elements of the HF pathogenesis is fibrosis of the transplant myocardium resulting from the accumulation of the fibrillar collagen fragments. The HF development can also be caused by the rejection of a heart transplant, arterial hypertension, transplant vasculopathy, concomitant diseases, including metabolic syndrome, diabetes mellitus, impaired renal function, etc. [1–3].

Currently, a specific attention is paid to the identification of profibrogenic biological agents – biomarkers which can induce fibrosis on the one hand, and on the other, can act as indicators of the adverse events risk associated with its development. These biomarkers include the transforming growth factor β1, a marker of fibroblast activation, as well as the N-terminal region of the brain natriuretic peptide (NT-proBNP) synthesized in cardiomyocytes and fibroblasts [4–6]. The abovementioned markers are the best known and acknowledged in clinical practice. Galectin-3 is a recently described indicator of the chronic HF risk in patients, including cardiac re-

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cipients [7–9]. Galectin-3 is expressed by neutrophils, macrophages, eosinophils, osteoclasts, and myocardial fibroblasts. It is suggested that measuring Galectin-3 levels, along with other HF biomarkers and myocardial damage, may be of prognostic value for the long-term condition of the recipients after cardiac transplantation [10].

This paper studies the Galectin-3 plasma level in the heart transplant recipients and determines the potential significance of the Galectin-3 level in the acute rejection and transplantation fibrosis.

MATERIALS AND METHODS

107 patients participated in the study. From 2013 to 2016, they underwent heart transplantation (HT) at Shumakov National Medical Research Center of Transplantology and Artificial Organs of the Ministry of Health of Russia (NMRC TAO), they included 90 (84%) men; the average age of the recipients was 48 ± 13 (16 to 70) years. In 57 and 50 patients, dilated cardiomyopathy (DCM) and a coronary heart disease (CHD), respectively, caused a terminal heart failure which served as the basis for the transplantation. The maximum duration of the follow-up after HT reached 398 with a median of 347 [289; 364] days. The control group was represented by healthy adults (n = 10) with no difference in sex and age in comparison with the study group.

All the patients with HT indications went through the routine examination according to the protocol of patient management at NMRC TAO and the National Clinical Recommendations "Heart Transplantation and Mechanical Support for Blood Circulation". The routine examinations after HT included clinical assessment of the condition, full blood count and biochemical blood assay with determination of the tacrolimus concentration, daily blood pressure monitoring (for adjustment of antihypertensive therapy), echocardiographic examination, repeated myocardial biopsies, annual coronary angiography. All the recipients underwent three-component immunosuppressive therapy, which included a combination of calcineurin inhibitors (tacrolimus) and cytostatics (mycophenolate mophetyl or mycophenolic acid), as well as prednisolone orally depending on the period of time which passed from the date of surgery and frequency of rejection episodes and adjuvant drug therapy as clinically indicated [2, 3].

Acute cellular rejection of a heart transplant was diagnosed based on the results obtained from the histological, humoral – immunohistochemical test of endomyocardial biopsy samples. Endomyocardial biopsy (EMB) in cardiac recipients was conducted according to the protocol for routine clinical and laboratory examination or as indicated. The study of the biopsy sample aimed at determination of the fibrotic changes in the transplant and their nature (diffuse, focal, and diffuse focal fibrosis).

The venous plasma was used as the material for the study of biomarker concentration; a total of 233 samples were tested (1–3 samples from each patient, on average of 2.1 ± 0.6). Galectin-3 concentration was measured by enzyme immunoassay using a Human Galectin-3 Platinum ELISA reagent set (Bender MedSystems GmbH, Vienna, Austria). Placental growth factor (PlGF) was measured by enzyme immunoassay using the RandD SYSTEMS reagent sets, USA. Concentrations of vascular endothelial growth factor (VEGF-D and VEGF-A), monocyte chemoattractant protein-1 (MCP-1), platelet growth factor (PDGF-BB), and soluble ligand CD40 (sCD40L) were measured using xMAP technology with a multiplex panel based on Simplex ProcartaPlex™ reagent sets (Affymetrix, USA).

Data analysis and processing were made by the IBM SPSS STATISTICS 20 (IBM SPSS Inc., USA) scientific and engineering calculations software package. The data are given as the arithmetic mean and standard deviation (M ± SD) for parametric methods and as the median and interquartile range for nonparametric methods. Statistical processing of the obtained data engaged the methods of nonparametric statistics: when comparing dependent samples, the Wilcoxon paired test was calculated, and the Mann–Whitney U-test was used to compare the independent variables. For all the criteria, the critical significance level was assumed to be 5%, i. e. the null hypothesis was rejected at $p < 0.05$.

RESULTS AND DISCUSSION

In patients with terminal-stage HF, the range of Galectin-3 concentrations in plasma varied widely and corresponded to a nonparametric distribution. The median of Galectin-3 concentrations in patients with HF were higher than in healthy subjects (11.08 ng/ml, interquartile range – [7.71; 14.47] ng/ml) and reached 15.92 [11.80; 23.65] ng/ml, p = 0.00 (Fig. 1).

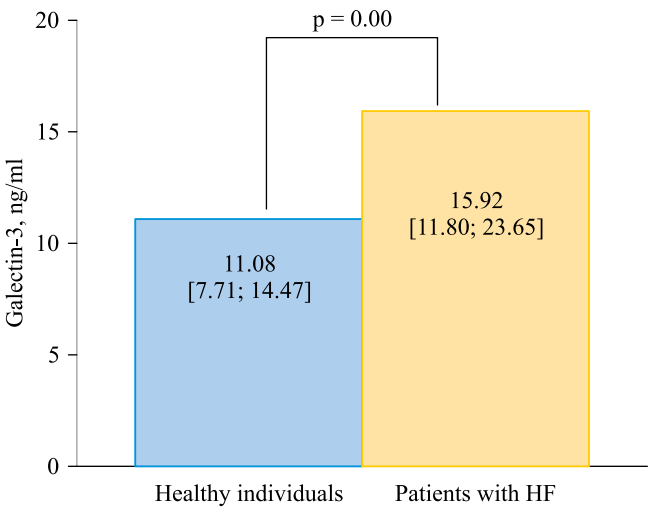
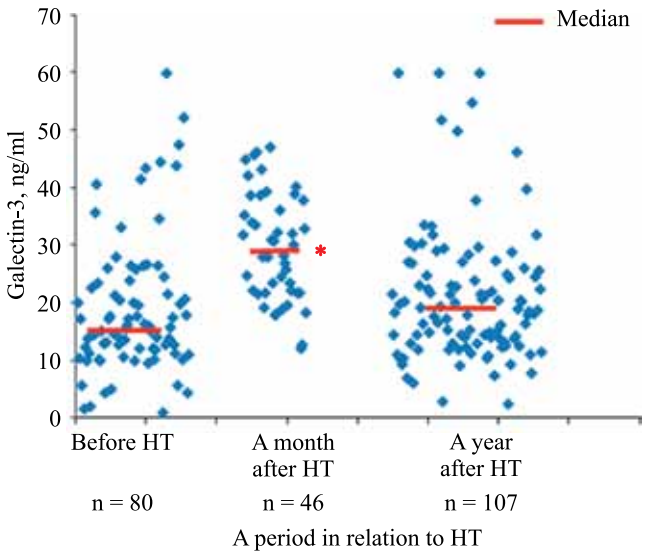


Fig. 1. Comparative analysis of galectin-3 plasma concentration of healthy individuals and patients with terminal heart failure

In men, the Galectin-3 level was 15.76 [11.80; 23.65] ng/ml and was not significantly different from that in women – 18.46 [12.46; 21.56] ng/ml, (p = 0.69). Galectin-3 levels were independent of the patient age. No differences in Galectin-3 concentrations were observed in patients with DCM 12.21 [12.12; 23.65] ng/ml and CHD 15.81 [10.92; 23.48] ng/ml (p = 0.77).

One month after HT, the Galectin-3 level in the recipients' plasma amounted to 29.21 [21.97; 37.44] ng/ml proved to be significantly higher than in patients before HT. The potential reason of higher level of Galectin-3 in the first month after HT is a complex of factors associated with surgery, early postoperative period, including systemic inflammatory response to surgery; adaptation of the recipient's organism to the transplanted organ and immunosuppressive therapy, etc.

By the end of the first year after HT, the level of Galectin-3 in patients had decreased to the pretransplantational level and amounted to 18.71 [13.14; 25.41] ng/ml (Fig. 2).



* – p < 0,05, compared with level before transplantation.

Fig. 2. The level of galectin-3 in patients before heart transplantation and recipients in early and late period after heart transplantation

Table

Correlation between the Galectin-3 plasma level in heart recipients and the level of biomarkers, potentially relevant for the diagnosis of post-transplant complications

Marker	Correlation rate, r	Confidence, p-value
VEGF-A	–0.004	0.98
VEGF-D	**–0.511**	**0.00**
PlGF	**0.293**	**0.04**
PDGF-BB	–0.208	0.15
MCP-1	**–0.285**	**0.05**
sCD40L	–0.162	0.27

The recipients' Galectin-3 plasma concentration a year after HT correlated with levels of other biomarkers potentially relevant for the diagnosis of post-transplant complications. A positive correlation of Galectin-3 concentration with the PlGF level was revealed (r = 0.293, p = 0.04) while a negative correlation was observed for VEGF-D (r = –0.511, p = 0.00) and MCP-1 (r = –0.285, p = 0.05) (Table).

The results of the analysis of the correlation between Galectin-3 plasma level in recipients and the number of episodes of acute transplant rejection are as follows.

By the end of the first month after heart transplantation, no significant difference in the median of Galectin-3 concentrations was observed in patients who suffered an acute cellular (n = 27) and humoral (n = 1) rejection and had no rejection episodes (n = 18) during the early post-transplantation period.

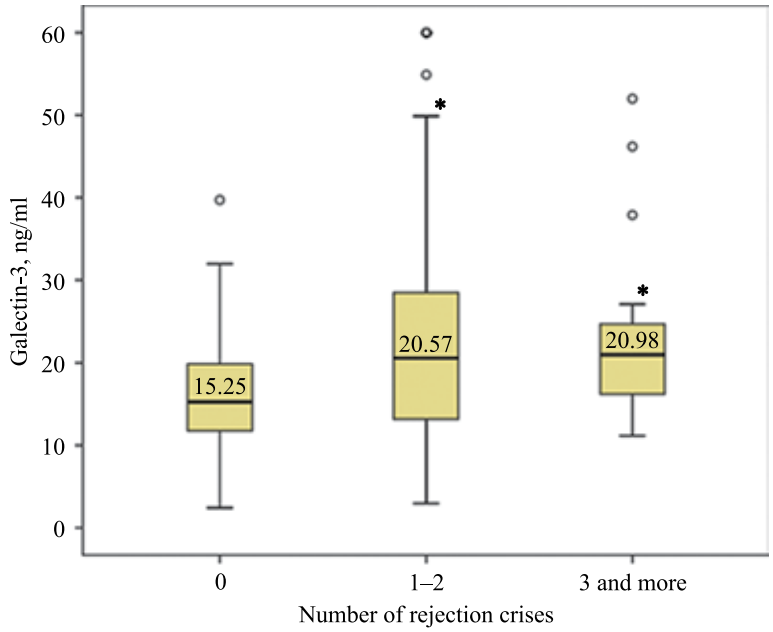
By the end of the first year after heart transplantation, 75 out of 107 participated patients had an acute rejection crises: 57 patients suffered 1–2 episodes (acute cellular rejection, n = 54 and humoral rejection, n = 3) and 18 patients had 3 and more episodes of rejection (acute cellular rejection, n = 14 and humoral rejection, n = 4). The level of Galectin-3 was significantly higher in patients with rejection crises, in contrast to the recipients not diagnosed with such crises. The Galectin-3 level did not depend on the number of crises and was higher both in patients who experienced 1–2 episodes and in those who had 3 or more rejections (Fig. 3).

Histological examination of endomyocardial biopsy samples by a qualitative imaging method was used to assess the presence, severity and nature of fibrosis as a manifestation of pathological changes in the myocardium.

A month after the HT, 32 samples with pathological changes in the myocardium, indicating fibrosis of varying severity, and 14 samples without such changes were found among the tested endomyocardial samples from 46 recipients. By the end of the first month, no reliable differences were found in the median of Galectin-3 plasma concentrations in recipients with revealed fibrosis and without it, although a higher Galectin-3 level tended to be observed in patients with fibrosis than without it (30.55 and 26.39 ng/ml, respectively, p = 0.86).

By the end of the first year after HT, fibrotic myocardial changes were revealed in 64 of the 107 studied biopsy samples. The median of Galectin-3 concentrations in recipients with the transplanted heart myocardial fibrosis reached 20.60 [14.52; 26.29] ng/ml, in recipients without fibrosis – 15.36 [11.95; 22.42] ng/ml; (p = 0.05) (Fig. 4).

All the biopsy samples with sclerotic myocardial changes underwent a qualitative assessment of the fibrosis severity. Fig. 5, a shows an example of a histological specimen of a heart with no fibrotic changes. Diffuse fibrosis, which develops in the interstitial or perivascular space and is not associated with a significant loss of functioning cells, was found in 16 recipients (Fig. 5, b).



* – p < 0,05, compared with recipients without rejection.

Fig. 3. The levels of galectin-3 concentration in recipients one year after heart transplantation, depending on the number of acute rejection episodes

Focal fibrosis with a replacement of the dead cardiomyocytes with connective tissue was found in 38 recipients (Fig. 5, c). The most severe form of fibrosis, diffuse focal fibrosis, was found in 10 recipients.

The results of the study showed that the Galectin-3 plasma level in recipients at the end of the first year after HT is associated with the nature of pathological myocardium changes. In patients with diffuse focal fibrosis, Galectin-3 level was significantly higher compared with patients without fibrosis (22.52 [20.98; 26.08] ng/ml, p = 0.01). No significant difference was found in patients with diffuse or focal fibrosis compared with no fibrosis group (18.69 [14.31; 26.14] ng/ml and 19.13 [14.36; 25.81] ng/ml, respectively. p > 0.05) (Fig. 6).

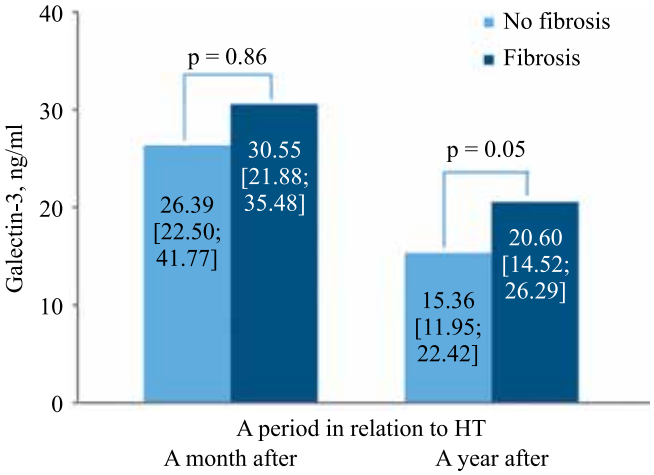


Fig. 4. Comparative analysis the concentration of galectin-3 in the cardiac recipients the early and long-term periods after transplantation with and without morphological signs of myocardial fibrosis

CONCLUSION

The results of this study showed that the Galectin-3 concentration in patients suffering from the heart failure

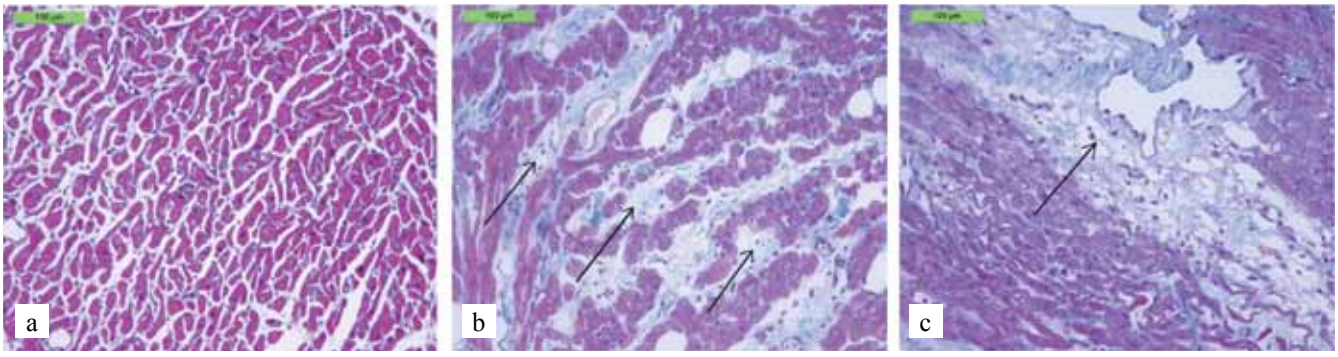
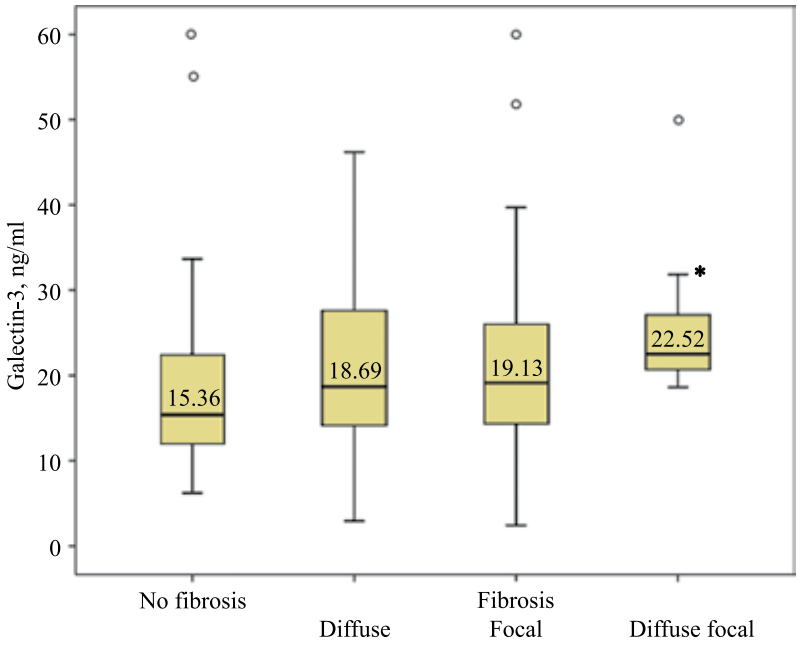


Fig. 5. Histological examination of endomyocardial biopsy specimens: a – focal protein dystrophy of cardiomyocytes, focal moderate edema of interstitium, no fibrosis; b – diffuse moderate protein dystrophy of cardiomyocytes, diffuse fibrosis; c – protein dystrophy of cardiomyocytes, focal fibrosis. Coloring according to Masson. ×200



* – p = 0,01, compared with patients without myocardial fibrosis.

Fig. 6. Comparative analysis median concentration of galectin-3 by the end of first year after transplantation in cardiac recipients with different types of fibrosis and without it

in its terminal stage (and potential heart recipients) is higher than in healthy subjects. In the early post-transplant period, Galectin-3 concentration exceeded its pretransplantational value. This fact may be associated with the factors of the early postoperative period; a year later, the average plasma Galectin-3 level in the recipients did not differ from the pretransplantational level.

By the end of the first year after transplantation, Galectin-3 level was significantly higher in the recipients who suffered acute transplant rejection crises, regardless of their number: the patients who had 1 to 2 crises and those who suffered 3 or more rejections had no difference in Galectin-3 plasma concentrations.

In the recipients with morphological signs of myocardial fibrosis, the Galectin-3 level was significantly higher compared with no fibrosis group. It was associated with the nature of pathological changes in the myocardium (with diffuse focal fibrosis).

The authors declare no conflict of interest.

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HEMODYNAMIC EVALUATION OF A NEW PULSATILE FLOW GENERATION METHOD IN CARDIOPULMONARY BYPASS SYSTEMS

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This paper proposes a new method of generating pulsatile flow using non-pulsating pumps (NPP) without modulating the rotation speed of the pump rotor. At the initial stage, this method was proposed for NPP-based cardiopulmonary bypass (CPB) systems. The method is based on parallel connection to the NPP shunt (input-output) on which a controlled valve is installed. This valve ensures periodically clamps and opens the shunt partially. A comparative evaluation of the operation of pumps with and without a pulsator was done on a hydrodynamic bench with simulation of heart failure (HF) conditions. The pump-shunt system was connected according to the "vein-artery" CPB scheme under copulsation mode. Rotaflow (Maquet Inc.) was used as the NPP. For a comparative assessment of the hemodynamic efficiency of the method, the following were used: aortic pulsatility index Ip, energy equivalent pressure (EEP) and surplus hemodynamic energy (SHE). The indices in the pulsating mode compared with the non-pulsating mode increased: Ip by 3 times, EEP index by 3.76% and SHE index increased by 4 times. Results show that the proposed method of generating a pulsating flow is effective.

Keywords: cardiopulmonary bypass, continuous flow, pulsatile flow, hydrodynamic stand, shunt, controlled valve.

INTRODUCTION

The current clinical practice mainly applies NPP systems that have certain advantages over pulsating flow pumps (PPs), especially in terms of size, reliability, durability, and ease of control [1–3]. However, the prolonged use of implantable NPP systems often causes such complications as GI bleeding, hemorrhagic strokes, and aortal insufficiency [4–12]. Besides, recent reports show the importance of the pulsating flow not only for the implanted systems but also for such short-term extracorporeal CPB systems as extracorporeal membrane oxygenation (ECMO) and CPB [13–16].

To evaluate the performance of pulsating systems, these reports use the EEP and SHE indicators which reflect the additional energy received by the blood circulatory system due to the pulsating flow [17]. Those and other observations recently raised interest in the development of control methods for the NPP that generate pulsating flow and pressure with the pump rotor speed (RS) modulation [18–22]. The main problem of the converting a non-pulsating flow to pulsating is the rotor response lag leading to limitation of the maximum flow amplitude and the phase shift relative to heartbeat phases. Besides, the RS modulation mode is featured with high shear stresses formed at the acceleration and braking of the rotor. This is confirmed by the lack of data on blood haemolysis in the mentioned publications, though in early works on the analysis of blood haemolysis in the RS modulation systems, the authors note an increased haemolysis [23]. We expect the new method of pulsating flow generation [24] to be less traumatic for blood due to the constant PP RS.

MATERIALS AND METHODS

The proposed method for pulsating flow generation is based on the parallel connecting a graft line with an adjustable solenoid valve to the input-output of the NPP pump and oxygenator. As applied to the CPB system, an option of this method shown in Fig. 1.

The proposed scheme for pulsating flow generation in the CPB systems is shown in Fig. 1. As an NNP pump, the Rotaflow centrifugal pump (Maquet Inc.) is used. The bypass line is a polyurethane tube of 6 mm outer diameter, 0.2 mm wall thickness. In the pumping (systole) phase, a voltage is applied to the valve for partial closure of the graft. At this, at the output of the pump-graft system, the flow pulse amplitude is formed, which depends on the preset constant RS and the graft closure

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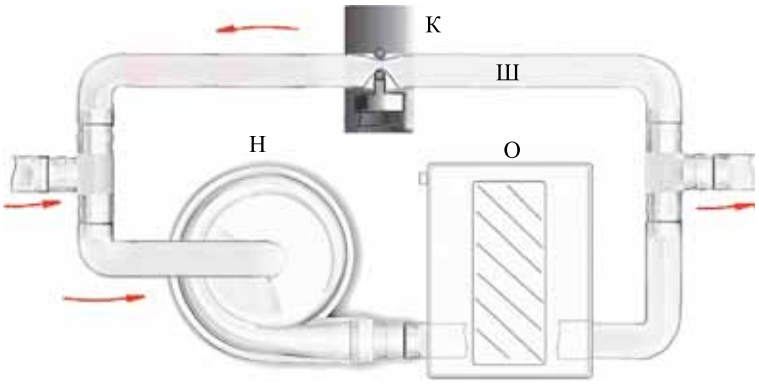

Fig. 1. Pulse generation's scheme: H – pump; K – valve; III – shunt; O – oxygenator. The flow movement show by red arrows

degree. In the next (diastole) phase, the valve opens the graft. In this case, due to bypassing the main NPP flow, the reduced flow amplitude is formed at the output of the pump-graft system, which depends more on the graft diameter. Thus, at the constant preset RS, a pulse flow is formed at the output of the pump-graft system.

The valve is a solenoid designed for controlled cross-clamping of the graft. The valve configuration (Fig. 2) includes a frame (1) with an electromagnetic coil (2) and an anchor (3) connected to the valve (4). The valve control system is based on the pulse width modulation (PWM). To trigger the anchor and switch it to the upper position, a short PWM pulse with 20 ± 10 ms duration and 15 V voltage, synchronized with the heart rate, is applied to the electromagnetic coil. At this, the anchor moves the valve to the upper stop (5), partially over-clamping the graft. Then the seal-in voltage is applied to the electromagnetic coil, determined by the pressure inside the graft. In the diastole phase, the voltage from the electromagnetic coil is removed and the graft opens due to hydrodynamic pressure. To adjust the graft cross-clamping and opening degrees, for each phase of the valve operation, the valve stops (6) and (7) are used.

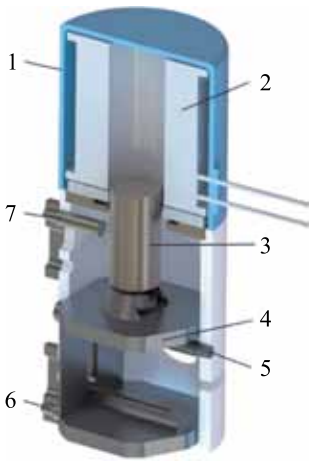

Fig. 2. AV design: 1 – shell; 2 – electromagnetic coil; 3 – anchor; 4 – valve; 5 – upper locking pin; 6 – lower stroke limiter; 7 – upper stroke limiter

Hydrodynamic bench

At the first stage of the research, the effectiveness of this pulsating flow generation method was evaluated at the hydrodynamic bench (HB) with the pump connected in the CPB mode. The overview of the bench is shown in Fig. 3. It includes the artificial heart ventricle (AHV) (1), Quadrox-i Adult (Maquet) oxygenator (2), aortal tank (3), system hydraulic resistor (4), aortal reservoir (5), venous tank (6), and pulmonary resistor (7). The NPP Rotaflow (Maquet) input (8) is connected to the AHV (1), and its output – to "aorta". The graft (9) and the valve (10) have a parallel connection with the Rotaflow. As the AHV, the Medos VFD 80 ml (Medos, Aachen) pump was used, with the SINUS-IS (MZEMA, Russia) 2-channel pneumatic drive. The flow in the aorta and at the output of the pump-graft system was recorded with the Transonic, Ithaca, NY Transonic Inc. ultrasonic flow meters (UFMs) (11, 12). The pressures in the aorta (13), the left atrium (14), and the AHV (15) were recorded by the Edwards (Edwards Life Sciences, Irvine, CA) sensors. The pressure and flow measurements were recorded with the Angioton (Biosoft-M, Moscow) multichannel pressure and flow measurement module and visualized by the Pumpax software (Biosoft-M, Moscow).

Preliminary modelling of the standard conditions was made, those set by the AHV pneumatic pressure, adjustment of the aortal tank, and peripheral resistor according to the recommendations in G.M. Pantalos et al. [25]. The cardiac insufficiency mode was set by changing the AHV pressure and peripheral resistors without changing the aortal tank capacity. The following parameters were set: average aortal flow rate – 2.5 ± 0.3 l/min, aortal pressure – 80/60 mm Hg.

The cardiac output in non-pulsating and pulsating modes was maintained the same. The flow (systole) amplitude at the output of the pump-graft system was preset by changing the pump RS and the graft cross-clamping degree. At this, the flow through the graft was 6.2 ± 0.5 l/min. To obtain the minimum flow rate at the output of the pump-graft system in the diastole phase, the graft was fully opened. The pressure in the AHV was ma-

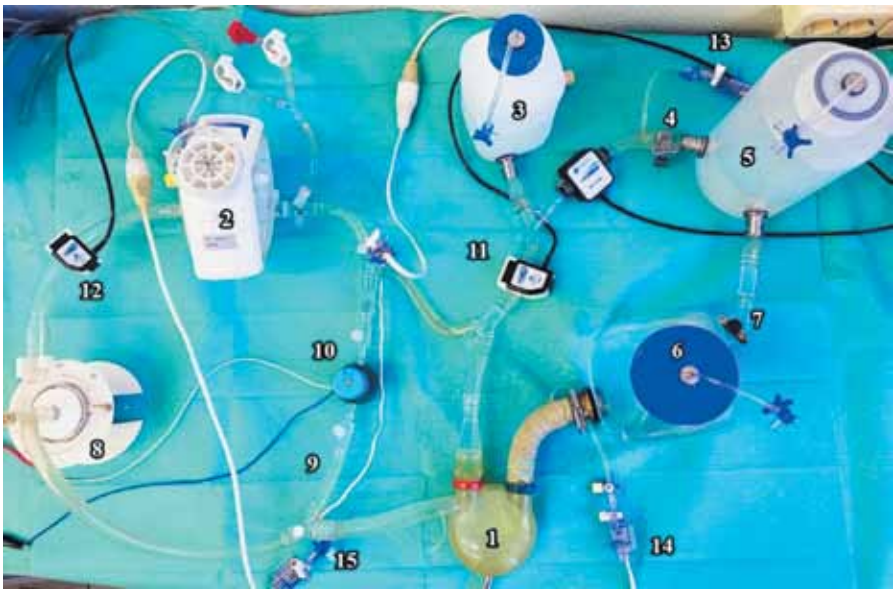

Fig. 3. Mock circulation loop: 1 – artificial left ventricle (LV); 2 – oxygenator Quadrox-i Adult (Maquet); 3 – aortic capacitance; 4 – system hydraulic resistance; 5 – aortic reservoir; 6 – venous reservoir; 7 – pulmonary resistance; 8 – Rotaflow; 9 – shunt; 10 – valve; 11 – aortic flowmeter; 12 – flowmeter pump; 13 – pressure sensor of aorta; 14 – pressure sensor of left atrium; 15 – pressure sensor of LV

nually set at 60 mm Hg with the pump operating in the non-pulsating mode, and 40 mm Hg during operation of the pump-graft system. Such pressure decrease in the left ventricle was observed in vivo, as well as on the benches, with the AHV reproducing the Frank-Starling's law. The pump-graft system was synchronized with the AHV operation by applying the pulses of the preset AHV systole duration from the "Sinus-IS" drive to the valve control unit.

The aortal pulsation obtained during the tests was determined by the pulsation index (Ip) determined by the formula [26]:

$$I_p = (P_{ao\,(max)} - P_{ao\,(min)}) / P_{ao\,(av.)}, \quad (1)$$

where $P_{ao\,(max)}$ – aortal systolic pressure, $P_{ao\,(min)}$ – aortal diastolic pressure, and $P_{ao\,(av.)}$ – average aortal pressure. Equivalent energy of pressure (EEP) was calculated by the formula [26]:

$$EEP\ (mm\ Hg) = \int_{t1}^{t2} fp\ dt\ / \int_{t1}^{t2} f\ dt, \quad (2)$$

where f(t) – aortal flow temporal curve over a fixed time, p(t) – aortal pressure temporal curve over the same time. Surplus hemodynamic energy (SHE) was calculated according to the Shepard equation [26]:

$$SHE\ (ergs/cm^3) = 1332 \cdot (EEP - P_{ao\,(av.)}). \quad (3)$$

RESULTS

Fig. 4 shows the hemodynamic parameters obtained on the assembled bench when modelling the standard cardiac insufficiency. At cardiac insufficiency, the average aortal flow rate was 2.5 ± 0.2 l/min, the aortal pressure was 80/60 mm Hg.

Fig. 5 shows the hydrodynamic parameters in non-pulsating (a) and pulsating (co-pulsation) (b) modes. The non-pulsating flow was provided by Rotaflow at RS of 2100 rpm, the pulsating flow – at RS of 2600 rpm. For both modes, the mean aortal pressure at 75 ± 2 mm Hg and average aortal flow rate at 4.8 ± 0.2 l/min were maintained. The pressure loss on the oxygenator was 40 mm Hg, which influenced the obtained RS value.

The summary of the main hydrodynamic indicators, Ip, EEP, SHE indices for the non-pulsating flow and pulsating flow modes is given in Table.

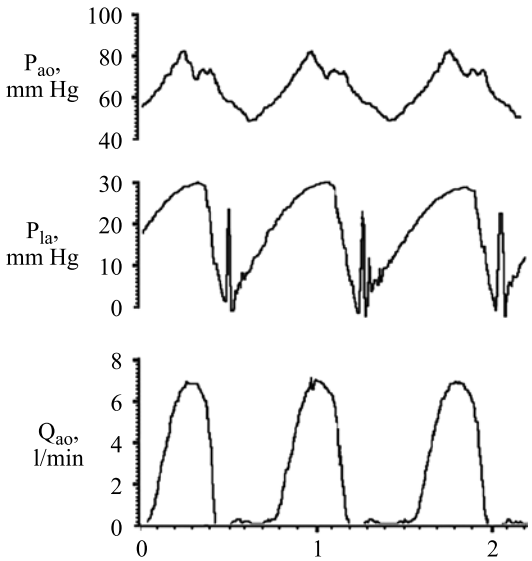

Fig. 4 The simulation results of heart failure: P_{ao} – aortic pressure; P_{la} – pressure in the left atrium; Q_{ao} – aortic flow

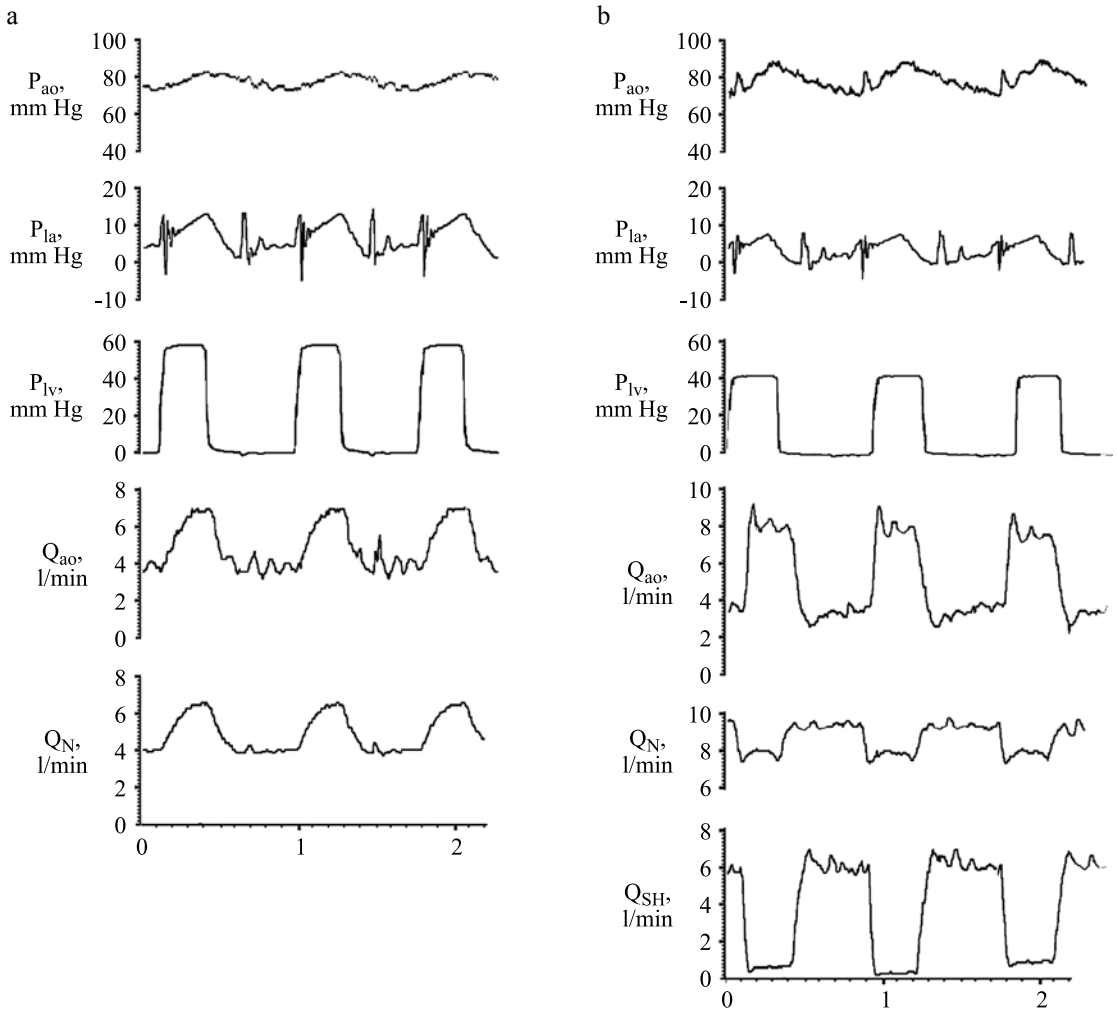

Fig. 5. Comparative results of the hydrodynamic parameters in constant speed (a) and pulsating (b) modes: P_{ao} – aortic pressure; P_{la} – pressure in the left atrium; P_{lv} – pressure in LV; Q_{ao} – aortic flow; Q_N – flow through Rotaflow; Q_{SH} – flow through shunt

Table

Hemodynamic parameters with and without graft

	P_{ao} (max / av. / min), mm Hg	Q_{ao} (max / av. / min), mm Hg	I_p	EEP, mm Hg	SHE, ergs/cm^3
Without graft	81 / 76 / 74	6.9 / 4.9 /4.1	0.092 ± 0.002	77.07 ± 1.20	1333.5 ± 1598.4
With graft	91 / 76 / 69	8.3 / 5 / 3.2	0.29 ± 0.007	79.86 ± 1.94	5141.2 ± 2584.0

According to table 1, the IP index increased by 3 times in the pulsating mode compared to the non-pulsating mode, the EEP index increased by 3.76%, and the SHE index increased by almost 4 times.

DISCUSSION

The proposed method is based on the parallel connection of graft with a controlled solenoid valve to a rotary pump. The pulsating flow is formed due to the cardiosynchronized closing and opening of the graft, which is provided by a controlled solenoid valve. The bench tests showed that in the cardiac insufficiency simulation this method shows the normalization of pulse pressure in the aorta in the co-pulsation mode. Comparison of these findings with hemodynamic parameters obtained during non-pulsating NPP operation showed that the proposed method can be more efficient in the increase of the aortal pressure and flow pulsation. Compared to the systems with RS modulation, this method is featured with smaller response lag of the system due to the rapid (15–20 ms) response of the solenoid valve which supposed to ensure more efficient operation, especially in the co-pulsation mode.

Another advantage of the proposed method for pulsating flow generation is its versatility. The method allows using any rotary pumps. The disadvantages of the method

include the difficulties of implementing the implantable version of the system. Nevertheless, this method can be used to generate a pulsating flow in the CPB systems and extracorporeal LV bypass, which has recently been of the increasing concern of many researchers [18, 20, 21].

In the present study, the first stage of research of the pulsating flow generation method for non-pulsating flow pumps without changing the pump's RS is introduced, which can be considered effective for increasing the aortal pulsation in the ECMO systems. We suggest that the pulsating flow in these systems has a positive effect on blood oxygen saturation due to blood recirculation through the oxygenator. In the future, we plan to evaluate the blood haemolysis when using this method and consider the possibilities of using the method in left ventricular, biventricular bypass, and artificial heart systems.

The authors declare no conflict of interest.

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A CASE REPORT OF SUCCESSFUL LIVER RETRANSPLANTATION IN PATIENT WITH EARLY HEPATIC ARTERY THROMBOSIS COMPLICATED BY BILE DUCTS NECROSIS AND SEPSIS

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In this present case report during liver transplantation a patient was developed dissection of hepatic artery (HA) which was noticed after arterial reconstruction step. In one hour after surgery at intervention operating room stent placement of HA was performed. At early postoperative period by hepatic angiography study indicated for a second stent placement of HA, also embolization of splenic artery to treat a steal syndrome. After 2 weeks a patient developed thrombosis of recently placed stents which was required vascular reconstruction of HA by using autovenous graft. The condition complicated by development of a cholangiogenous hepatic abscesses and sepsis despite of all used possible methods of liver graft revascularization. However, used methods of vascular correction, which combined of timely carried out intensive care and antibiotic therapy according microbiology laboratory results allows saving graft function. After treatment of septic complications and patient's somatic status stabilization and normalization of laboratory results liver retransplantation was performed.

Keywords: liver retransplantation, hepatic artery (HA) thrombosis, stent placement of HA, bile ducts necrosis, sepsis, hepatic abscesses, splenic artery steal syndrome.

INTRODUCTION

Orthotopic liver transplantation is presently the only definitive treatment option for patients with end-stage chronic liver disease. In experimental centers, the 10-year patient survival following liver transplantation is about 70% [1, 2]. However, due to the growing number of surgical interventions, despite improvements in surgical techniques, anaesthetic support and immunosuppression methods, complications such as hepatic artery thrombosis (HAT) and primary graft dysfunction occurring in the early postoperative period lead to persistent impairment of liver function. In such cases, liver retransplantation is the only alternative to death. According to data from modern world literature sources, about 10–20% of patients need retransplantation [3–5]. These surgical procedures are indisputably associated with significant technical difficulties, they are considerably expensive and carry worse results than in primary transplantation [6, 7]. Besides, the ethical issue of priority among retransplant and primary transplant candidates with regards to donor organs remains debatable. In the current situation where there is shortage of donor organs, all treatment options should be used in early complications at the appropriate time to maintain an adequately functioning graft. So, in HAT, the success of early surgical, including endovascular revascularization, reaches about 50–70% [8, 9], which may be an acceptable option for rescuing a graft or serve as a link for retransplantation when a donor appears [8].

DESCRIPTION OF OBSERVATION

Patient K., 50 years old, on April 1, 2018, underwent orthotopic liver transplantation (OLT) for chronic viral mixed hepatitis with outcome in Child-Pugh class C cirrhosis. MELD score of 17. The inferior vena cava (IVC) was reconstructed through a piggyback technique. Anastomosis of the portal veins (PV) of the donor and recipient was performed in an end-to-end fashion. Arterial anastomosis between the donor's proper hepatic artery (PHA) and the recipient's PHA (the site of the right hepatic artery and the left hepatic artery) was performed in an end-to-end way. After completing arterial reconstruction and starting blood flow through the hepatic artery, a dissection site with subintimal blood flow was detected (on the donor site of PHA, almost throughout its entire length) with a 40 ml/min volumetric blood flow (VBF) rate. An audit was performed with an umbilical catheter followed by heparinization of the HA channel. The gastroduodenal artery (GDA) was ligated, after which VBF increased to 130 ml/min. Portal vein VBF was 2500 ml/min. After end-to-end biliary reconstruction on the "lost" drainage, VBF was re-evaluated and the hepatic artery VBF was found to has reduced to 35 ml/min. Arterial

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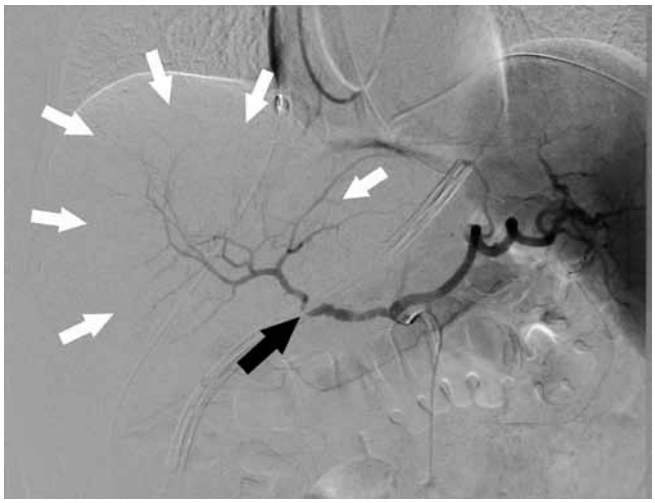
reconstruction using autologous veins or other grafts was found to be inadvisable due to the small diameter of the left hepatic artery and right hepatic artery (not more than 3 mm for each). Under X-ray operating conditions, direct celiacography (Fig. 1, a) and direct perfusion imaging of the liver (Fig. 1, b) were performed. Hemodynamically significant narrowing at the anastomosis site with pre- and post-stenotic dilatations was visualized, with subintimal dissection and turbulent blood flow. Stenting was performed on a 300 cm Boston Scientific microconductor by a stent-in-stent method. Two 4×23 mm Aneugraft stent grafts were installed. On the control angiogram, the stents were straightened, there was adequate arterial blood flow to the liver, the blood vessels was filled, there was no extravasation (Fig. 2). The decision was taken to conduct an anticoagulant therapy with heparin

a



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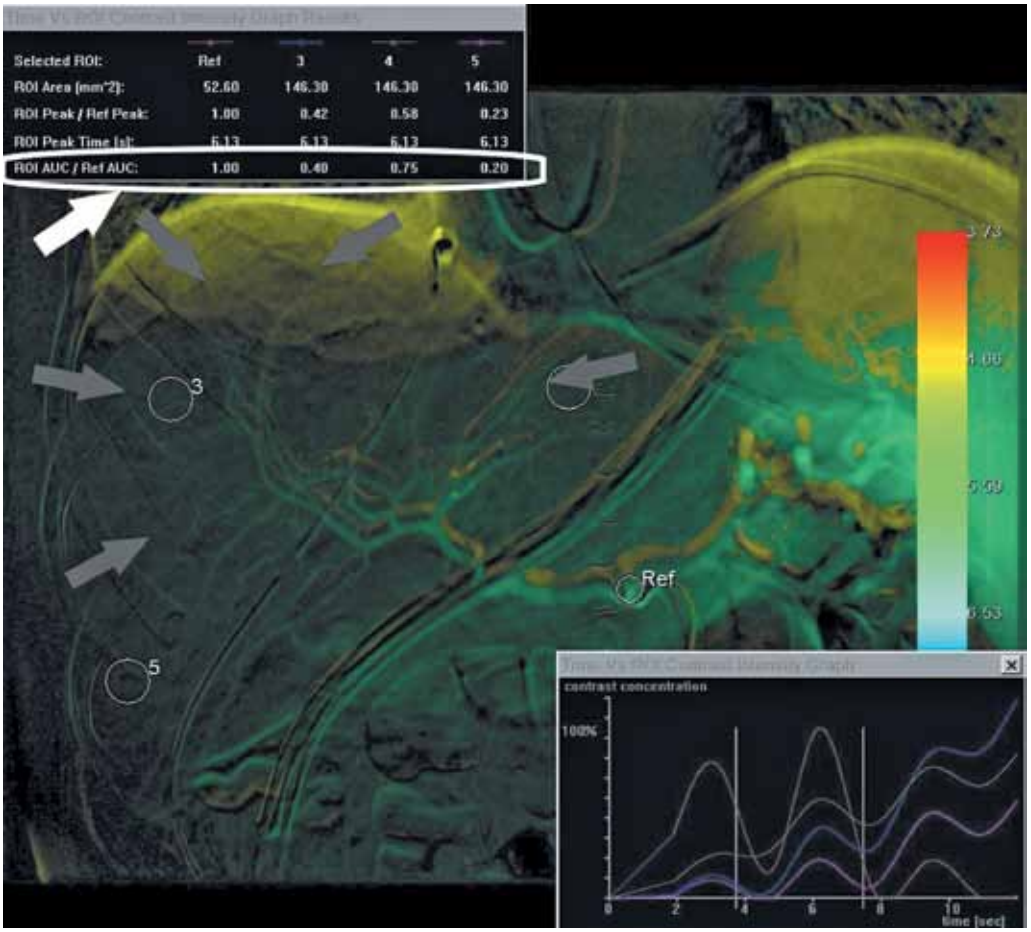

Fig. 1: a – celiacography: hemodynamically significant stenosis is visualized at the level of the common hepatic artery – a black arrow; the depletion of arterial architectonics at the segmental level – white arrows; b – perfusion study of the liver: depletion of arterial filling at the level of segments and subsegments – gray arrows; significant reduction of perfusion (ROI AUC / Ref AUC) in the projection SII, SVI, SVII – white arrow

500 units/h intravenously. On the first day after OLT and HA stenting, ultrasound examination with Doppler ultrasonography (ultrasound + DU) of the liver vessels did not detect the hepatic artery. A section of ischemia 5 cm in size was identified in the S7 liver. Repeated celiacography was performed, HA subocclusion was detected with up to 20% lumen narrowing. Patency was preserved, splenic artery steal syndrome – the splenic artery (SA) diameter was 2.5 times greater than that of the HA (Fig. 3). HA balloon angioplasty was performed with a 4×23 mm balloon, mechanical embolization of the SA was done using five Cook MReye metal coils 10 mm in diameter and one Azur Terumo 6×18 mm coils. Angiograms showed restoration of adequate HA patency and absence of blood flow through SA (Fig. 4). On the second day, control angiography showed that signs of splenic artery steal syndrome with positive dynamics remained. Repeated mechanical embolization of SA was performed with five Cook MReye metal coils 10 mm in diameter and one Azur Terumo 6×18 mm coil. After 9 days (on April 13, 2019), according to laboratory data, there was significant increase in D-dimer result 3000 ng/ml, ALT was 150 U/L, AST 270 U/L, total bilirubin 42 μmol/L, leukocytosis 11,000. Based on multispiral computed tomography (MSCT) results, signs of HA stenosis, ischemic hepatitis of S7, S8 liver were visualized. Under X-ray surgery conditions, arterihepatography was performed, HA lumen was found to have significantly narrowed and blood flow was weakened. Stenting was done with a 4×23 Aneugraft stent graft (Fig. 5) with restoration of the arterial architec-

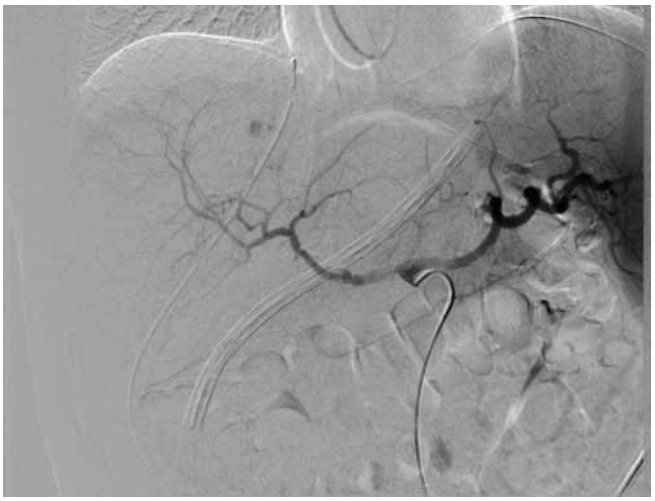

Fig. 2. Celiacography after stent placement in the subintimal dissection area

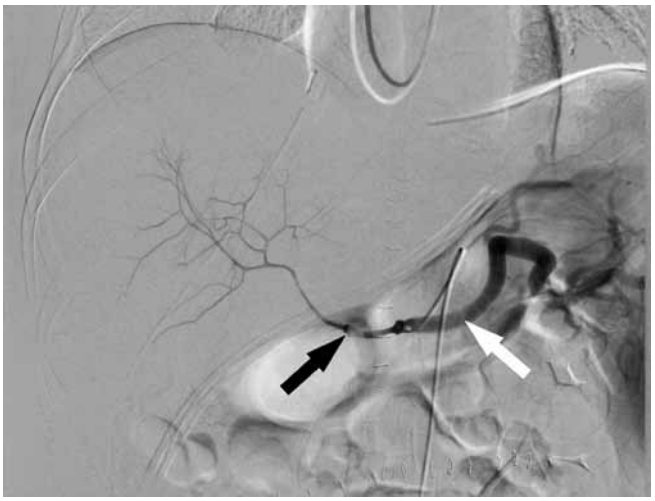

Fig. 3. Celiacography. The subocclusion of the hepatic artery in front of the previously installed stents – black arrow. Strengthening of a blood-groove and expansion of a splenic artery (splenic artery steal syndrome) – a white arrow

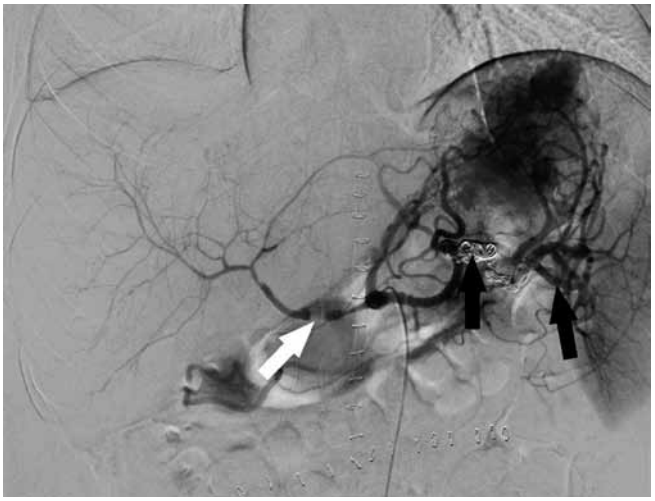

Fig. 4. Celiography after embolization of the splenic artery. Metal emboli in the trunk of the splenic artery, the trunk of the splenic artery is occluded, the arterial blood supply of the organ along the collaterals – black arrows. The area of the hepatic artery subocclusion – white arrow

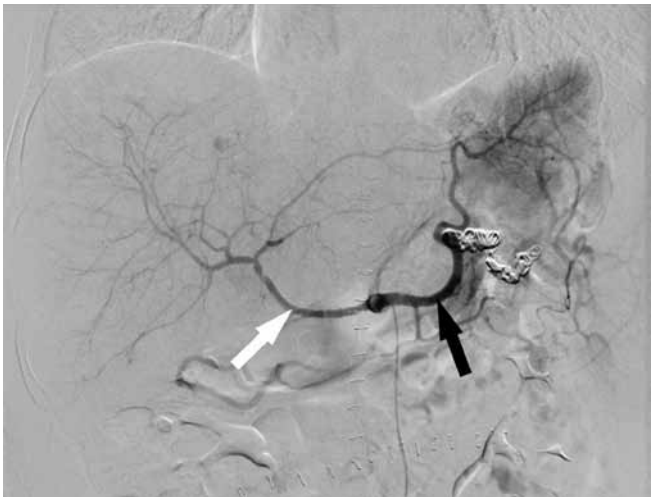

Fig. 5. Celiacography after installing the third stent "Stent in Stent". The contours and patency of the hepatic artery restored – white arrow. Splenic artery steal syndrome is eliminated – black arrow. Arterial architectonics of the liver is determined at the subsegmentary level

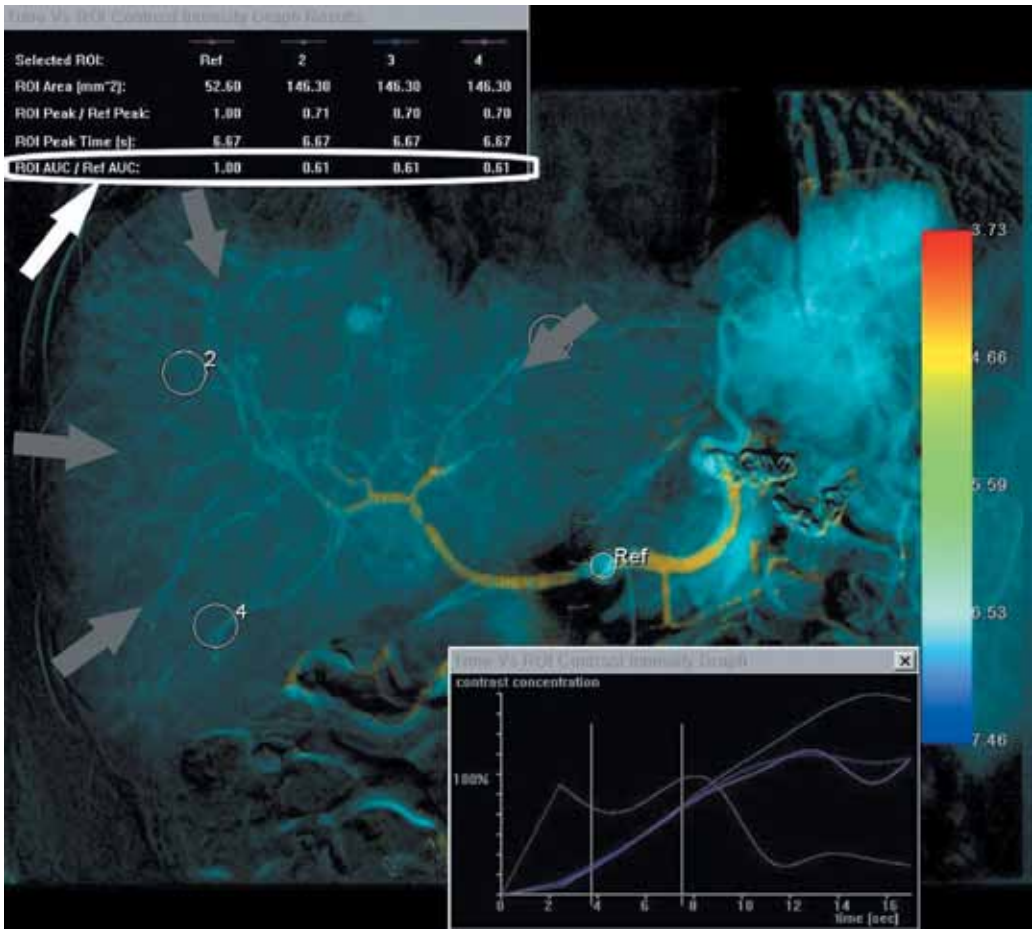

Fig. 6. Perfusion study of the liver after endovascular correction. Arterial filling at the level of segments and subsegments – gray arrows. Restoration and alignment of perfusion (ROI AUC/Ref AUC) in projection SII, SVI, SVII – white arrow

tonics of the organ and normalization of perfusion in all the liver segments (Fig. 6). According to laboratory data, April 4, 2019, D-dimer result was 3500 ng/ml, ALT was 247 U/L, AST 414 U/L, total bilirubin 87 μmol/L, leukocytosis 13 × 10^9 U/L, and procalcitonin 42 ng/ml. According to results obtained from blood culture on April 16, 2018, there was increase in Enterococcus faecium and Escherichia coli. The patient was diagnosed with sepsis. Antibiotic therapy was corrected based on the sensitivity of microorganisms. Immunosuppression was reduced. Arterial hepatography revealed thrombosis of previously installed stents. Attempts at catheterization and thromboaspiration were unsuccessful. Autogenous prosthesis installation of PHA were performed (Fig. 7). During control angiography test conducted in April 20, 2018, arterial blood supply to the liver was detected, but it was sharply weakened along the periphery. According to laboratory indicators, ALT was 508 U/L, AST 126 U/L, total bilirubin 31 μmol/L, white blood cell count 10 × 10^9 U/l. Based on CT data (April 23, 2018), the shunt was passable, but there was sharp narrowing of the hepatic arteries at a 3 mm bifurcation level, lobar arteries were less than 1 mm, there was fluid in the liver parenchyma, necrosis zone in the right lobe was 9 × 3 × 7 cm in size. On April 30, 2018, bile appeared from postoperative wound and through the drainage from the abdominal cavity. On magnetic resonance cholangiopancreatography (MRCP) performed, peripheral bile ducts were not visualized, fluid component is detected over bifurcation in the form of bile duct, lobar ducts were not visualized (Fig. 8).

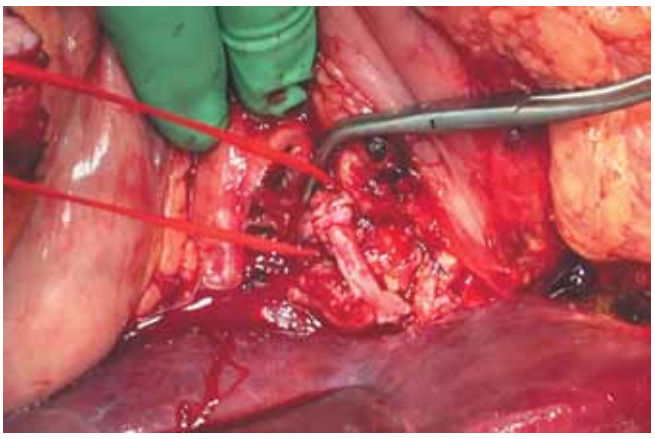

Fig. 7. Autovenous prosthetics of own hepatic artery

It was decided to perform relaparotomy. Intraoperatively: common bile duct (CBD) with signs of necrosis, but without damage to its integrity, anastomosis was

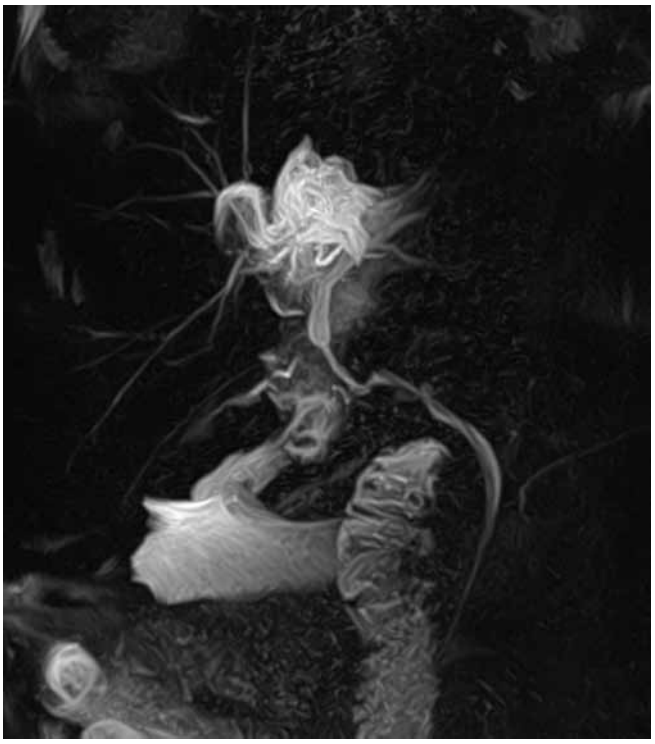

Fig. 8 The leakage of the contrast agent in the portal fissure of the liver in MRCP

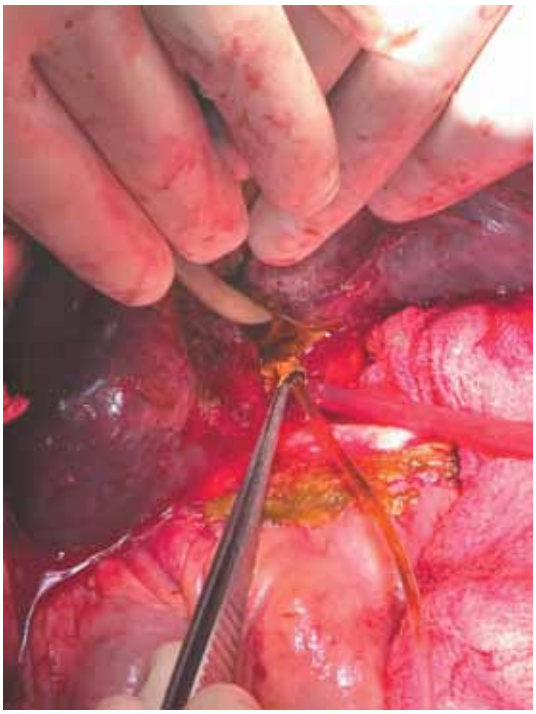

Fig. 9. Formed choledochostomy. Drainage installed in the resected abscess area S4,5

firmly done, and HA pulsation is preserved. Bile flow from the abscess of S4.5 liver was visualized. Segments 2-3, 7-8 are with signs of necrosis, without suppuration. Abscess was opened and drained in S4.5, necrotic CBD was excised, while choledochostomy and enterostomy were performed for bile reinfusion (Fig. 9).

Liver retransplantation was performed on May 10, 2018 amid improvement in the patient's somatic state, clinical and laboratory parameters Hb 110 g/L, Tr 505 × 10^9 U/L, creatinine 54 μmol/L, total bilirubin 21 μmol/L, ALT 210 U/L, AST 80 U/L, achievements in sterility of blood and bile cultures. IVC was reconstructed through a piggyback technique. PV anastomosis was performed in an end-to-end manner. Arterial anastomosis was done using a vascular graft (a donor fragment of the internal iliac artery) directly with the aorta. Biliary reconstruction – hepatitis enteroanastamosis using the Roux-en-Y jejunal limb on Voelcker's drainage. The removed liver was sent for histological examination (Fig. 10).

The postoperative period was complicated by postoperative wound suppuration, focal necrosis of S8 liver and an episode of cholangitis on the seventh day with the growth of E. Coli based on bile inoculation results. cAntibiotic therapy was carried out after determining the sensitivity of microorganisms to antibiotic and immunosuppression was reduced. This led to the disappearance of complications. The patient was discharged for outpatient treatment on the 35th day. With a 10-month follow-up period, no significant deviations in laboratory parameters and in MSCT data were detected.

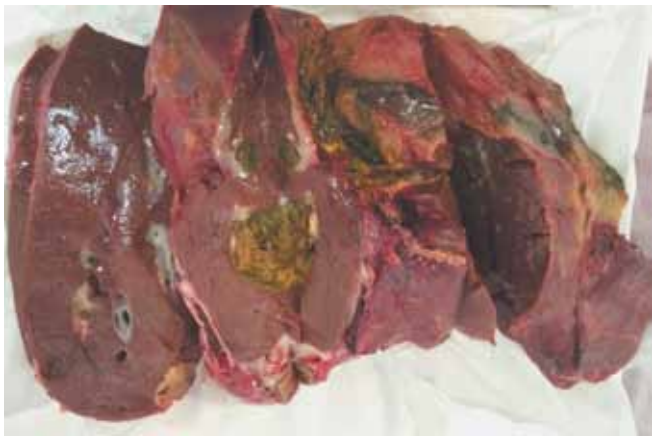

Fig. 10. Remote transplant with areas of ischemia, aseptic necrosis, biligenic abscess in S4,5

DISCUSSION

Liver transplantation is among the most complex surgical interventions in terms of both technical implementation and postoperative management of patients. Success of this operation depends on coordinated interaction among a large team of surgical and therapeutic specialists, as well as resuscitation anesthetists. The scope and nature of treatment requires the team to make right and timely decisions within the entire arsenal of opportunities that the clinic has.

According to modern world literature sources, HA thrombosis is the second main cause of liver graft loss after primary nonfunction [8]. Early diagnosis is a key point in this vascular complication. It helps to avoid

rapid graft loss. There are generally three methods for treating hepatic artery thrombosis: revascularization, retransplantation, and observation. However, the choice of any of these treatment options depends on the time of diagnosis. Retransplantation is the method of choice for most patients, showing the best results. Nevertheless, this treatment option is extremely limited due to shortage of donor organs. In this regard, emergency revascularization should be the first step in treatment, especially in cases of early diagnosis, when it is possible to assume non-critical ischemic injury to the graft [9–11]. Any perfusion disturbance is extremely sensitive for a transplanted liver since it is devoid of vascular collaterals. The etiology and risk factors for development of liver abscess are thought to be associated with the anatomy and blood supply of the biliary tree – bile ducts are nourished by their own arterial supply, the peribiliary plexus. This capillary network originates from the hepatic artery and is strictly arranged around the intrahepatic bile ducts [12]. Thus, blood supply to the biliary system mainly depends on blood flow in the hepatic artery. Therefore, with hepatic artery thrombosis, the intrahepatic ducts suffer from insufficient perfusion, which leads to formation of bilomas and biliogenic abscesses. According to several studies, infections are one of the main factors affecting the outcome of liver transplantation [13]. In our case, dissection of the intima of the hepatic artery was detected intraoperatively, stenting was performed in the first two hours. However, despite early diagnosis and revascularization, as well as in connection with the splenic artery steal syndrome that developed on the first day, significant blood flow disturbances persisted, and a fairly large area of ischemia was already visualized by ultrasound in S7.

Considering previous successful interventions in post-liver transplant vascular complications [14], in this case we also hoped for success. However, ultrasound and MSCT scans showed there was negative dynamics in the form of appearance of new ischemic foci, zones of necrosis. With a moderate increase in transaminases and bilirubin on the 14th day following transplantation, control angiography revealed thrombosis of previously installed stents. Lack of donor organs in such cases necessitates continuation of the struggle for a transplant, which was undertaken by us through autovenous prosthesis installation. Amidst immunosuppression, impaired blood supply to the liver, recurrent episodes of cholangitis, and abscess formation, the risk of developing septic complications is extremely high. Our patient also developed sepsis with the growth of Enterococcus faecium, Escherichia coli in blood cultures. Abscessing of S4.5 led to the development of delimited bile peritonitis, which necessitated relaparotomy. Choledochostomy and enterostomy were required for bile reinfusion. Many studies have shown that the best outcomes of liver retransplantation are achieved by creating optimal conditions for its implementation, stabilizing the patient's condition, normalizing laboratory parameters and kidney function, an achieving sterility of crops at the time of retransplantation [13, 15]. For almost a month before retransplantation, comprehensive intensive infusion, replacement, antibacterial therapy based on inoculation results were carried out in our patient in an intensive care unit. It is reported that renal failure is accompanied by a number of impaired cell-mediated and antibody-mediated immune responses, which predispose to postoperative sepsis and exacerbate its course [16]. Similarly, hyperbilirubinemia predisposes to endotoxemia, impaired cell-mediated immunity [17] and Kupffer cell dysfunction [18]. After there has been a decision for retransplantation, immunosuppression should be reduced so that any nephrotoxic effects of cyclosporin or tacrolimus are minimized and the effects of the patient's immune system on infection are enhanced, potentially improving the outcome [13]. Based on global trend and statistics, we reduced immunosuppression and conducted two hemodiafiltration sessions immediately before retransplantation, thereby normalizing laboratory parameters.

CONCLUSION

So, based on our own experience and that from our domestic and foreign colleagues, we believe that attempts at endovascular revascularization in cases of hepatic artery subocclusion and thrombosis in early stages are well justified. In situations requiring retransplantation, we consider it expedient to perform retransplantation only after the patient's somatic status has been stabilized, and manifestations of systemic inflammatory reaction reduced.

The authors declare no conflict of interest.

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RIGHT ATRIAL BENIGN SCHWANNOMA

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Primary schwannoma of the heart is a rare disease. It arises from vagus nerve branches and plexus. Most schwannomas are benign tumors, but sometimes they can be primary malignant neoplasms. In the MedLine database, we found only 21 publications on benign and 13 publications on primary malignant cardiac schwannoma. Moreover, according to localization in the right atrium, only eight benign schwannoma observations are described. We report a 73-year-old woman who, with echocardiography and magnetic resonance imaging of the heart, revealed a right atrial tumor with sprouting of the right atrial free wall. The tumor was radically excised through cardiopulmonary bypass and pharmaco-cold cardioplegia. Pathohistological and immunohistochemical examination of the excised tumor showed that it is benign schwannoma.

Keywords: schwannoma, cardiac tumor, malignant neoplasms.

INTRODUCTION

The heart schwannoma (also known as neurilemmoma, neurinoma) is an extremely rare tumor. It develops from Schwann cells producing myelin and located in the peripheral nerve sheaths [1]. In the heart, the schwannomas are formed from the cells of the membranes of the cardiac plexus nerves and the nervus vagus branches [2]. One of the first reports of benign schwannoma, which grew from the wall of the right atrium, was published in 1972 [3]. We have found only 21 publications on benign schwannomas of the heart and 13 publications on primary malignant ones in the MedLine database. Besides, benign schwannomas in the right atrium are described only in eight observations. The purpose of the present article is to describe such a rare pathology as a benign right atrial schwannoma which has been removed through a radical surgery in our center.

CASE STUDY

Patient K., female, aged 73, was admitted to our Center on January 30, 2019 complaining of fatigue, increased blood pressure and palpitation episodes. The medical history showed that the examination at the place of residence for hip joint pain revealed a neoplasm in the right atrium. The cardiovascular surgeon recommended an operative therapy. Concomitant and previous diseases: hypertension of the 3rd degree, stage 3, risk 4; crystalline humors replacement in both eyes (2012), appendectomy.

At the admission, the patient's condition is of moderate severity. Clear consciousness. The skin and visible mucous membranes are of normal color. Vesicular breathing is heard throughout the full lung field, no rales. BP – 140/80 mm. Hg. Heart tones are muffled, the heart rhythm is regular, the heart rate is 60 beats per minute. A slight systolic murmur is heard over the heart region. The liver is not enlarged, the spleen is non-palpable. The CVA tenderness of the lumbar region is not manifested on either side. The bowel and bladder functions show no abnormalities. No edema.

ECG: sinus rhythm, heart rate – 60 beats per minute.

The plain chest X-ray showed no focal and infiltrative lesions; the phrenicocostal sinuses are free, the heart shadow is not enlarged. Coronary angiography: the right type of the myocardium blood supply, no hemodynamically significant lesions of the coronary arteries. Echocardiography revealed a hyperechogenic neoplasm in the right atrium, fixed to the lower third of the atrial septum. MRI (30.01.2019) of the right atrium cavity revealed an additional fixed neoplasm between the inferior vena cava entry and the coronary sinus, while the results of multispiral computed tomography of the heart showed signs of a moderate accumulation of the Ultravist 370 contrast (Fig. 1). A neoplasm of 2.5 × 1.5 × 2.7 cm is attached to the lower third of the atrial septum with a base of 9 mm, deforming and narrowing the entry of the inferior vena cava and the coronary sinus (Fig. 2, 3).

Preoperative clinical diagnosis: neoplasm of the right atrium; circulatory deficiency II A; hypertension of the 3rd stage, degree 3, risk 4.

Intraoperatively, a moderate amount of clear serous fluid was found in the pericardial cavity. The ascending aorta diameter is 3.4 cm, the pulmonary trunk diameter of up to 3.2 cm. The pulmonary veins typically enter the left atrium. CPB was connected by the "aorta–vena

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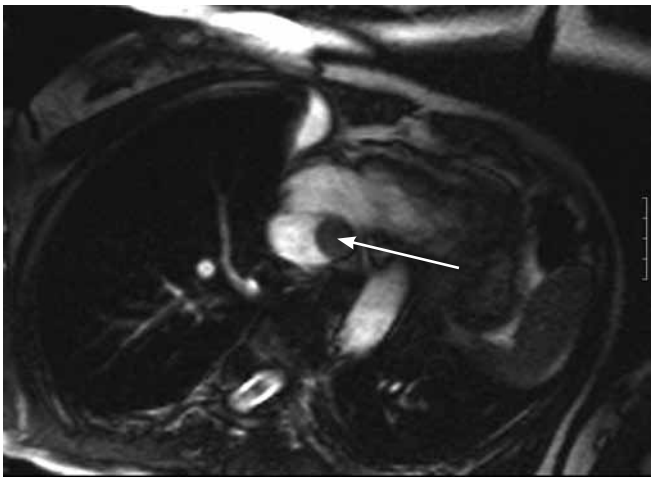

Fig. 1. Signs of moderate accumulation of a contrast agent in tumor (arrow) located in the cavity of the right atrium between the inflow of the inferior vena cava and the coronary sinus. Multislice computed heart tomography

cava" scheme. Start of artificial blood circulation. The tourniquet loops are placed on the vena cava. Spontaneous cooling to 34.6 C. Aortic clamp. Cardioplegia to the aortic root, Custodiol, 2,000 ml. The right atrium was opened with a longitudinal incision. The revision of the right atrium between the inferior vena cava entry and the coronary sinus showed a dense neoplasm, whitish, 3 × 4 cm, with a wide base of 2.5 × 3 cm, encapsulated, covered by the endocardium and tightly connected with the lateral surface of the coronary sinus, interatrial septum and the free lower wall of the right atrium. The neoplasm narrows the coronary sinus and the entry to the inferior vena cava.

Fig. 4 shows a neoplasm of the right atrium deforming the coronary sinus. It was removed through resection of the endocardium at the place of its attachment. Also the partial resection of free wall of the right atrium

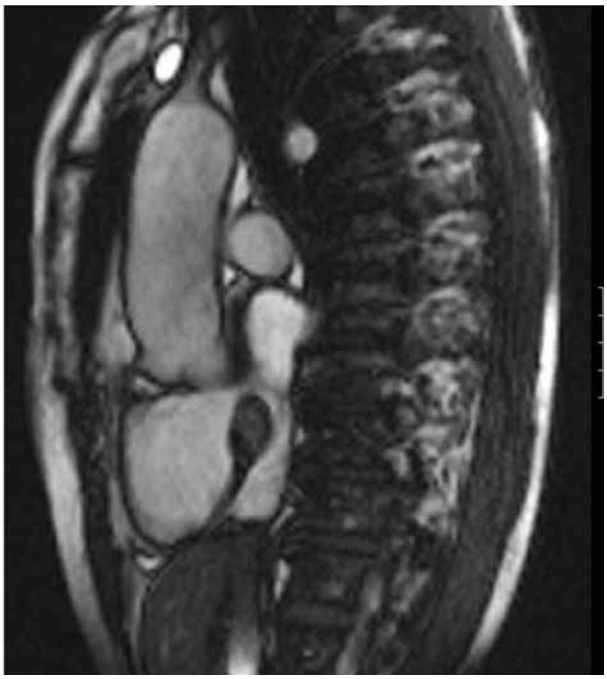

Fig. 2. Compression of the inferior vena cava by a neoplasm of the right atrium. Multislice computed heart tomography

was performed at the neoplasm extension site (Figs. 5, 6). A slit-like defect of the free lower wall of the right atrium of 3 × 1.5 mm formed. The defect is sutured with Teflon gaskets. The plastic repair of the endocardial resection area was performed with a xenopericardial patch. The incision of the right atrium wall was sutured tightly with a double-row continuous twisted suture (Prolen 4/0). The patient was warmed. Tourniquets on the vena cava were loosened. The aortic clamp was removed. Restoration of the cardiac activity with a single defibrillator discharge; sinus rhythm with a heart rate of 75 beats per minute. With the satisfactory hemodynamics parameters, artificial circulation was stopped. Decanulation of the lines in the machine for artificial blood circulation. Hemostasis.

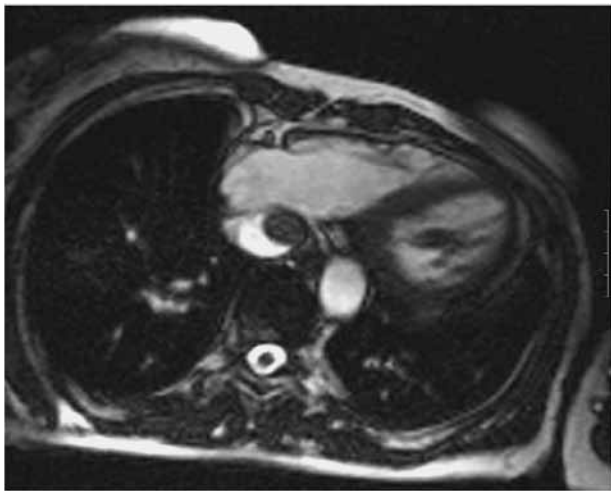

Fig. 3. Compression of the coronary sinus by a neoplasm of the right atrium. Multislice computed heart tomography

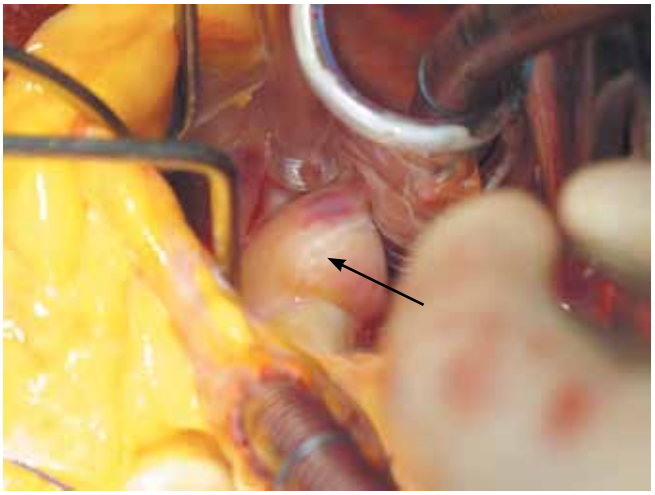

Fig. 4. Neoplasm of the right atrium fixed to the atrial septum and the free wall of the right atrium

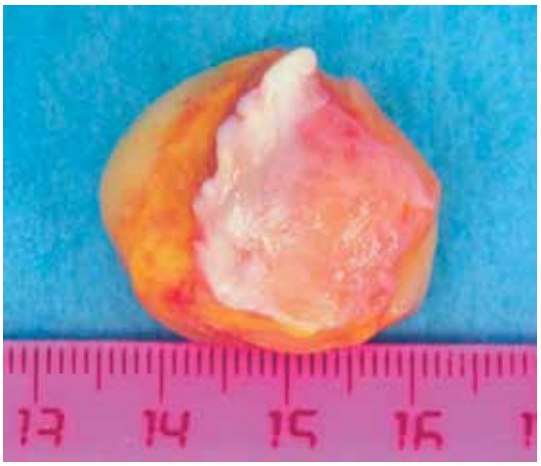

Fig. 5. Resected mass of the right atrium

Fig. 6. Neoplasm of the right atrium in the section

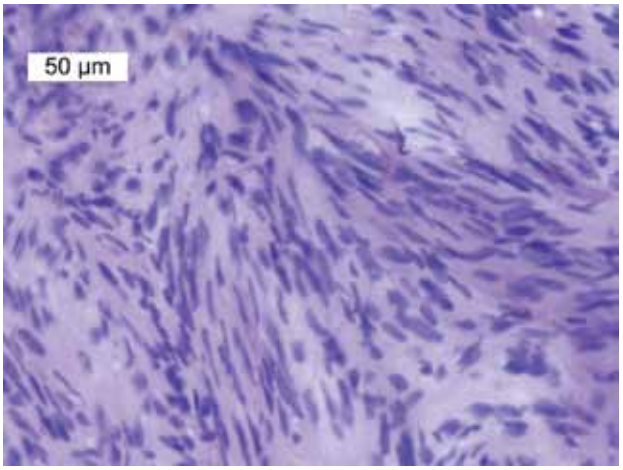

Fig. 7. Fragment of the shwannoma Antoni A (H&E stain, ×400)

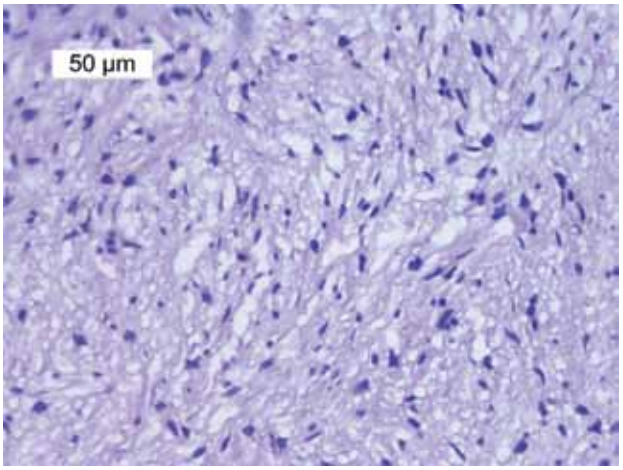

Fig. 8. Fragment of the shwannoma Antoni B (H&E stain, ×400)

The pericardium was sutured with a continuous twisted suture in the upper and middle thirds. The sternum was closed with four 8-shaped wire ligatures. Hemostasis. Layered suturing of the wound. Aseptic sticker.

The remoted neoplasm of whitish-pink color was densely elastic and sized 3 × 2 × 2 cm, having a smooth surface, in the section it was of a nodal structure and whitish-yellow color (Fig. 5, 6).

Morphological examination with histological staining (hematoxylin and eosin, trichrome Masson). The expression of S100 protein, vimentin, CD34, desmin, smooth muscle actin, MyoD1, Ki 67, GFAP was studied with the immunoperoxidase method (preparations were provided by I.B. Kaplanskaya, a leading scientist of the Pathology and Anatomy Department at P.A. Herzen Moscow Cancer Research Institute, Ph.D.).

The histological examination showed that the tumor was a benign mixed-structure schwannoma – Anthony type A and type B. In A type sections, the tumor cells were arranged in dense blocks, their nuclei were elongated and hyperchromatic (Fig. 7). In B type sections, the tumor cells were located less densely, their nuclei were polymorphic, slightly elongated, rounded, oval, and irregular in shape (Fig. 8). Multiple thin-walled

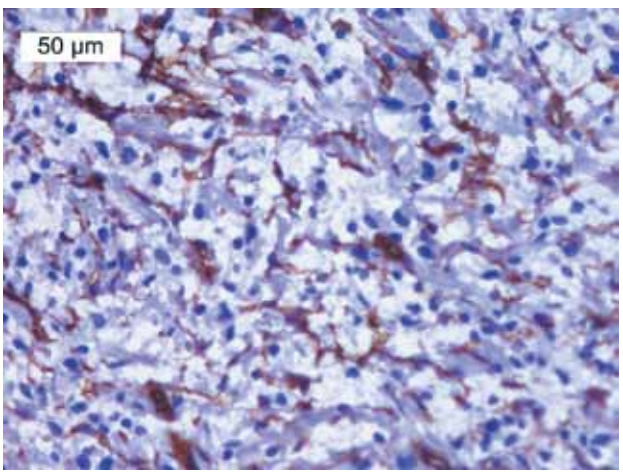

Fig. 9. The blood supply to the tumor was provided by numerous blood vessels whose visualization is well achieved by staining with the marker CD34. Immunoperoxidase method

vessels and muscle-type arteries provided for the blood supply to the tumor (Fig. 9). A nerve trunk was located deep in the tumor (Fig. 10), Schwann cells resulted in the formation of a schwannoma.

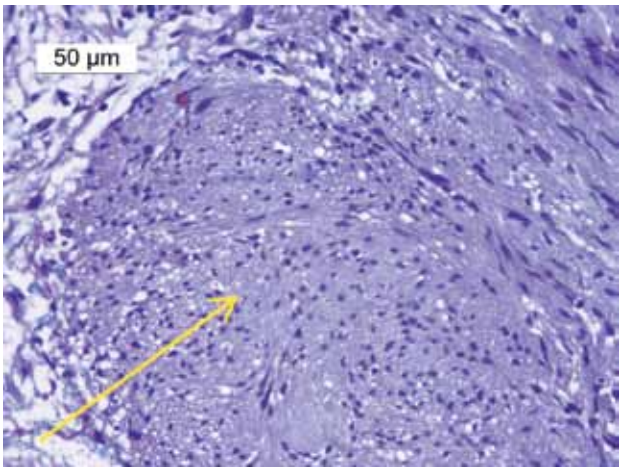

Fig. 10. Nervous trunk in schwannoma (arrow) (Masson's trichrome stain)

The immunohistochemical examination confirmed the diagnosis. The tumor manifested diffuse staining of S100 protein (Fig. 11) and vimentin (Fig. 12). There was no desmin or smooth muscle actin in tumor cells, but it produced positive staining in the arterial wall of muscle type (Fig. 13, 14). The reaction with antibodies to MyoD1, Ki 67 and GFAP (glial fibrillary acidic protein) were negative.

No complications in the postoperative period. The surgical wound was healed by the first intention. Echocardiography showed no pathological masses in the heart cavities. Normokinesis. No pathological flows on the interatrial septum. The patient was discharged in a stable condition for the follow-up by a cardiologist on the 8th day after surgery.

Final clinical diagnosis: benign right atrium schwannoma.

DISCUSSION

Schwannomas of the heart are extremely rare [4]. Therefore, the present study of a benign schwannoma

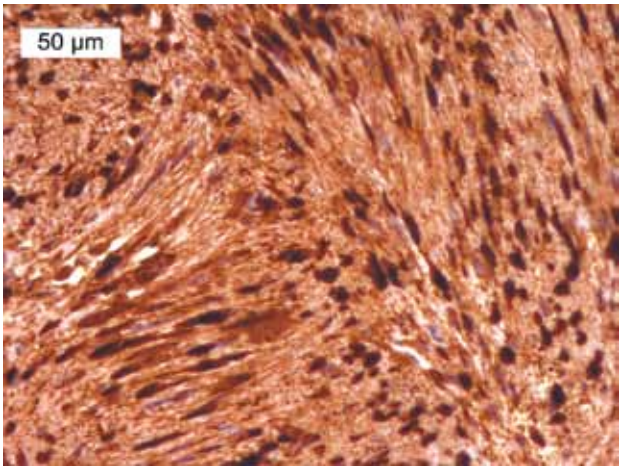

Fig. 11. Intensive positive reaction to S100 protein (×400)

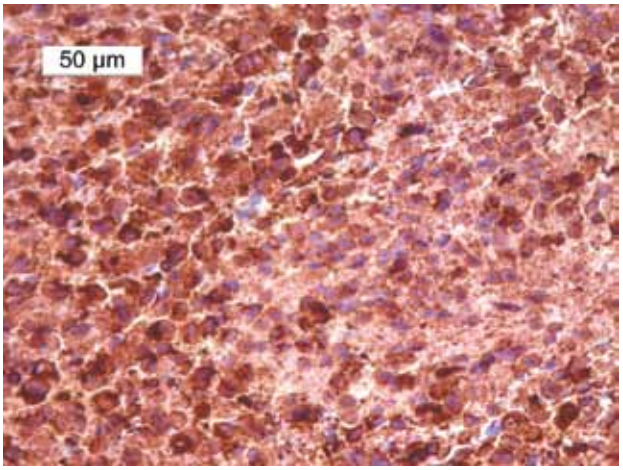

Fig. 12. Intensive positive reaction to vimentin (×400)

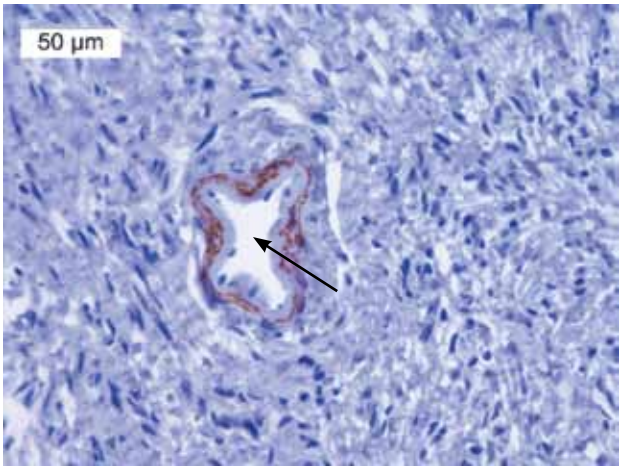

Fig. 13. Positive reaction to desmin in the muscle-type arterial wall of the schwannoma (×400)

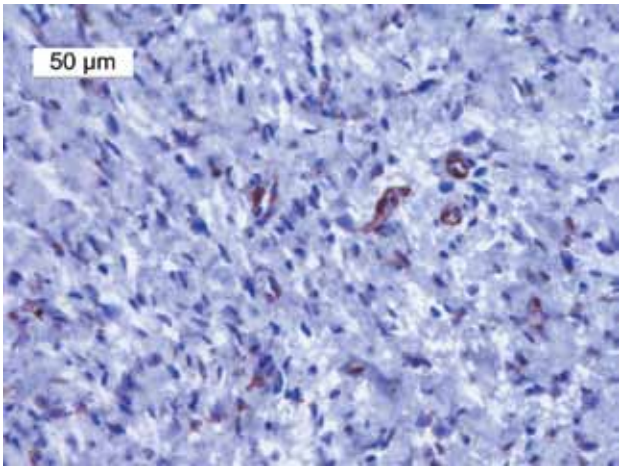

Fig. 14. Positive reaction to smooth muscle actin in the vessel wall of schwannoma (×400)

in the right atrium between the inferior vena cava entry and the coronary sinus is of great interest. The neoplasm narrowed the coronary sinus and the inferior vena cava entry, however, there were no expressed symptoms, and the tumor was discovered accidentally during the examination.

Our study of schwannomas in the right atrium is the ninth of the available sources published (Table). The right atrium is a typical location of the heart schwannomas [5]. The age of patients ranged from 33 to 72 (Table), and in our study, the patient was 73.

Table

Benign schwannomas of the right atrium

№	Author, publication date	Localization	Gender	Age
1	T.H. Gleason et al., 1972	Right atrium	–	–
2	B. Monroe et al., 1984	Right atrium	M	70
3	F. Bizzarri et al., 2001	Right atrium	M	72
4	D.S. Jassal et al., 2003	Right atrium	F	49
5	K. Nakamura et al., 2003	Right atrium	F	33
6	N.A. Stolf et al., 2006	Right atrium	F	56
7	S.A. Early et al., 2007	Right atrium	M	57
8	S. Koujanian et al., 2017	From the right atrium to the right ventricle	F	47
9	Observation	Right atrium	F	73

The size of the schwannoma in this study was small (3 × 2 × 2 cm), although such tumors can reach a giant size (12 × 8 cm) [6]. Despite its modest size, the schwannoma is visualized in ECG, CT or MRI, which may be associated with the density of its structure [7]. However, a definitive diagnosis can only be established based on histological and immunohistochemical tumor tests [8]. The intense staining of the S-100 protein confirms the diagnosis of benign schwannomas [5], and a positive response to CD56 shows the malignant nature of the tumor [9].

Radical tumor resection is necessary to prevent life-threatening complications, such as compression of vital structures, embolism, valve obstruction and sudden death [6]. In our study, the patient underwent successful complete removal of neoplasm in the right atrium within healthy tissues and plastic reconstruction of the atrial septum and the free wall of the right atrium with a patch of xenopericardium. The patient has an excellent prognosis, as schwannoma proved to be benign.

The authors declare no conflict of interest.

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CALCIFICATION OF PERIPHERAL ARTERIES AND DUAL-ENERGY X-RAY ABSORPTIOMETRY IN PATIENTS UNDERGOING RENAL REPLACEMENT THERAPY

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Vascular calcification is common in patients with chronic kidney disease and in kidney transplant recipients. It leads to increased arterial stiffness, left ventricular hypertrophy, complicates formation of arteriovenous fistula for hemodialysis, decreases coronary artery perfusion, and generally increases cardiovascular morbidity and mortality. Vascular calcification affects arteries of all sizes – starting from the intimal and medial layers of the arterial wall. In clinical practice, several non-invasive imaging techniques have been used to evaluate the location and severity of vascular calcification. There is a report positing a possibility of evaluating vascular calcification by dual-energy x-ray absorptiometry (DXA). This paper presents the experience of successful diagnosis of peripheral arterial calcification by DXA in kidney transplant recipients and end-stage renal disease patients.

Keywords: vascular calcification, dual-energy X-ray absorptiometry, chronic kidney disease, kidney transplantation.

Vascular calcification (VC) is a degenerative vascular disease affecting the main branches of the arterial vasculature. It is associated with aging. VC can occur in either the intimal (atherosclerosis) or medial (arteriosclerosis) layers of the arterial wall. First described by Virchow in 1863, intimal calcification develops at the site of an atherosclerotic plaque, in which differentiation of osteogenic cells is induced as a result of changes in lipid accumulation, pro-inflammatory cytokines and apoptosis. Intimal calcification is more common in large and medium-sized arteries, leading to a narrowing of the vessel lumen, lower perfusion and organ ischemia. The most important risk factors for its development are the traditional cardiovascular risk factors: age, male gender, hypertension, smoking, diabetes mellitus, and chronic kidney disease (CKD) [1, 2].

Medial calcification was first described by German pathologist Mönckeberg in 1903; it is characterized by injury to the muscular middle layer of the walls of arteries. It is not accompanied by a narrowing of the vessel lumen, but increases its stiffness, and can be localized in all types of arteries. Arteriosclerosis can develop in the absence of atherosclerosis, more especially in diseases that are associated with serious metabolic changes, such as diabetes mellitus and CKD. In the latter case, vascular calcification increases with decreasing renal function. Potential risk factors for its development in CKD include hyperphosphatemia, excessive calcium intake (calcium-based phosphate binders), prolonged dialysis, vitamin D deficiency, elevated fibroblast growth factor 23, inflammatory cytokines, immunosuppressive therapy in direct and indirect renal transplant recipients with direct and indirect effects, as well as inadequate inhibition of the mineralization process due to reduced fetuin-A, matrix Gla-protein, osteoprotogerin, osteopontin, and inorganic pyrophosphate [3–6]. However, most CKD patients have both types of vascular calcification due to the presence of both risk factors. However, medial calcification is the main form that affects arteries of all sizes – from small arterioles to the aorta [1, 2, 4].

VC prevalence in CKD is high both in its early stages and among dialysis patients. Studies in patients following kidney transplantation showed no signs of VC regression. Moreover, they showed that in some cases, VC continues to progress, although this process can occur at a lower rate than in dialysis therapy [3, 6–8].

The pathogenesis of medial vascular calcification in CKD patients is a complex multifactorial process, involving chronic systemic and local inflammation, metabolic disorders and genetic abnormalities that mainly affect calcium and phosphate homeostasis [4, 5, 9, 10]. Currently, VC in CKD is seen as an active or passive complex process closely resembling skeletal bone formation. One component of active vascular calcification process involves reprogramming and transdifferentiation of vascular smooth muscle cells into osteoblast-like cells. It has been shown that the main cause of phenotypic transformation of vascular smooth muscle cells is calcium and phosphate metabolism impairment, manifested by hypercalcemia and hyperphosphatemia. The resulting osteoblast-like cells produce and secrete extracellular matrix vesicles, containing phosphate and calcium minerals in the form

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of hydroxyapatite. Vesicles are released from osteoblast-like cells in response to increased intracellular calcium and serve as a focus for subsequent mineralization. In addition, hyperphosphatemia induces vascular smooth muscle cell apoptosis, since these cells cannot adapt to a hyperphosphate environment. Undergoing apoptosis, vascular smooth muscle cells secrete apoptotic bodies from their surface, which, along with matrix vesicles, act as centers for calcium and phosphate deposition. A passive process involves deposition of minerals into the vascular wall from the extracellular fluid surrounding the vascular smooth muscle cells. In addition, it is assumed that osteoblast-like cells secrete factors that reduce the number and/or activity of osteoclast-like cells in the vascular wall, which would ensure resorption of minerals [1, 2, 9].

In real clinical practice, VC (atherosclerosis and/or arteriosclerosis) in CKD patients is associated with development/progression of cardiovascular disease – acute myocardial infarction, coronary heart disease, acute cerebrovascular accident, left ventricular hypertrophy, cardiac rhythm disturbance, including fatal, circulatory failure, which are the most important cause of cardiovascular mortality, several times higher than that in other risk groups. Peripheral arterial calcification complicates formation of constant vascular access (arteriovenous fistula) for renal replacement therapy – hemodialysis. It can also create difficulty/inability to perform vascular anastomosis in kidney transplantation [1, 7, 11].

There are several non-invasive imaging methods available for topical diagnosis of VC – from the simple to the very complex. These include standard radiography (limbs, pelvis, abdominal cavity in lateral projection), electron beam and multispiral computed tomography, magnetic resonance imaging, and duplex ultrasound. Each of the above VC detection methods has a different informational value, sensitivity, accessibility, reproducibility and safety levels. However, none of these methods can clearly differentiate between intimal and medial calcification [12, 13]. There have been separate attempts to evaluate vascular calcification using dual energy X-ray absorptiometry (DXA) since the density of the calcified arterial wall corresponds to the density of bone tissue [14]. In [15], DXA was performed in end-stage kidney disease patients, who were being treated by programmed hemodialysis and continuous ambulatory peritoneal dialysis and in kidney transplant recipients. This was to measure bone mineral density (BMD) in standard skeleton sections (distal forearm, proximal femur, lumbar spine), diagnose secondary or primary osteoporosis and evaluate the effectiveness of surgical treatment of hyperparathyroidism (HPT). It turned out that in some patients, the DXA examination allows to visualize peripheral arterial calcification.

The aim of this paper is to demonstrate the possibility of detecting peripheral arterial calcification and analyzing BMD during bone DXA all at the same time.

CLINICAL OBSERVATIONS

1. Patient C. (b. 1962)

Chronic glomerulonephritis (without histological confirmation) and stage-3 CKD were diagnosed in 2005. Since chronic renal failure was progressing, the patient was placed under continuous ambulatory peritoneal dialysis in spring 2008. Cadaveric kidney transplantation was performed in fall 2010. Primary graft function. Upon discharge from the hospital (a month later), blood biochemistry analysis parameters were: creatinine 150 μmol/L (estimated glomerular filtration rate – eGFR 46 mL/min), cholesterol 6.0 mmol/L, uric acid 284 μmol/L, ionized calcium 1.0 mmol/L, phosphorus 0.82 mmol/L (2.0 mmol/L before kidney transplantation), alkaline phosphatase 219 U/L (normal 30–280 U/L). Maintenance immunosuppressive therapy (cyclosporin A level monitored in plasma, prednisone 10 mg every other day, mycophenolate mofetil 1500 mg/day), antihypertensive therapy, statins.

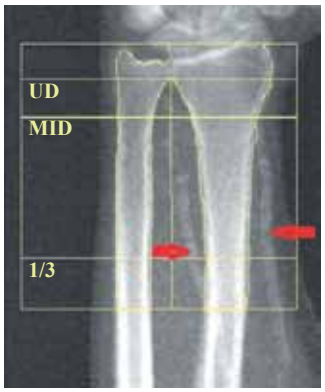
For five years, the patient's condition was satisfactory, renal graft function was stable; blood creatinine 170–200 μmol/L. In 2015, during the next ambulatory biochemical blood test: creatinine 203 μmol/L (rSCF 25 ml/min), cholesterol 4.3 mmol/L, total calcium 2.7 mmol/L, phosphorus 1.0 mmol/L, alkaline phosphatase 144 U/L (normal 60–300 U/L), parathyroid hormone (PTH) 330 pg/mL (1286 pg/mL before kidney transplantation). Anterior neck ultrasound and consultation with an endocrinologist surgeon regarding post-transplant HPT were recommended for the patient; the recommendations were not done. In 2016, type 2 diabetes was diagnosed, insulin therapy was prescribed.

Patient's condition deteriorated in fall 2017 – increasing weakness, osteoarticular pain syndrome. Was hospitalized in the kidney transplantation unit. Biochemical blood test: creatinine 206 μmol/L (eGFR 30 mL/min), total calcium 2.3 mmol/L, phosphorus 1.6 mmol/L, alkaline phosphatase 89 U/L (normal 30–120 U/L), PTH 415 pg/ml, uric acid 372 μmol/L, glucose 5.2–11.2 mmol/L, glycated hemoglobin 6.3%. Anterior neck ultrasound: in the projection of the right and left lower parathyroid glands, volumetric formations are 13 × 8 × 10 and 18 × 11 × 12 mm, respectively. DXA of standard sections of the skeleton was performed. When analyzing the BMD in the lower third of the forearm and the left proximal femur (reduced in both sections), the contours of the radial, ulnar and femoral arteries were visualized (Fig. 1). The patient was referred for a DXA of the lower third of the forearm and proximal femur on the right. This confirmed the presence of osteopenic syndrome and calcification of the radial, ulnar, and femoral arteries (Fig. 2). The

a

Name: Chizhov, Mihail Olegov
Patient ID: OHG 30206-c
DOB: 31 January 1962
Sex: Male
Ethnicity: White
Height:
Weight:
Age: 55

Referring Physician:



219 × 106

Scan Information:

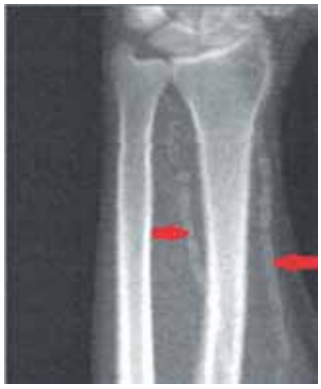
Scan Date: 05 December 2017 ID: A12051712
Scan Type: a L.Forearm
Analysis: 05 December 2017 13:58 Version 12.7
Left Forearm
Operator:
Model: Discovery A (S/N 83099)
Comment:

DXA Results Summary:

Radius	Area (cm^2)	BMC (g)	BMD (g/cm^2)	T-score	PR (%)	Z-score	AM (%)
UD	4.48	1.23	0.274	-4.6	50	-3.8	55
MID	9.72	4.99	0.510	-3.6	72	-3.2	75
1/3	3.20	2.39	0.746	-1.3	91	-0.7	95
Total	**17.45**	**8.60**	**0.493**	**-3.7**	**72**	**-3.1**	**75**

Total BMD CV 1.0%
WHO Classification: Osteoporosis

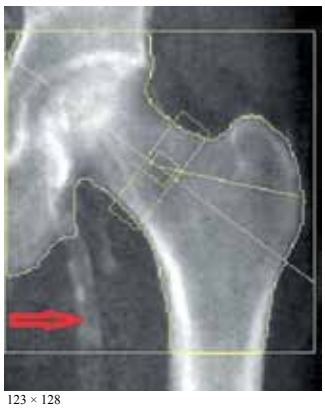
b



c

Name: Chizhov, Mihail Olegov
Patient ID: OHG 30206-c
DOB: 31 January 1962
Sex: Male
Ethnicity: White
Height:
Weight:
Age: 55

Referring Physician:



123 × 128
NECK: 49 × 15

Scan Information:

Scan Date: 05 December 2017 ID: Al 2051711
Scan Type: x Left Hip
Analysis: 05 December 2017 13:51 Version 12.7
Hip
Operator:
Model: Discovery A (S/N 83099)
Comment:

DXA Results Summary:

Region	Area (cm^2)	BMC (g)	BMD (g/cm^2)	T-score	PR (%)	Z-score	AM (%)
Neck	**5.98**	**3.56**	**0.596**	**-2.5**	**64**	**-1.6**	**73**
Total	49.03	35.28	0.719	-2.1	70	-1.7	74

d

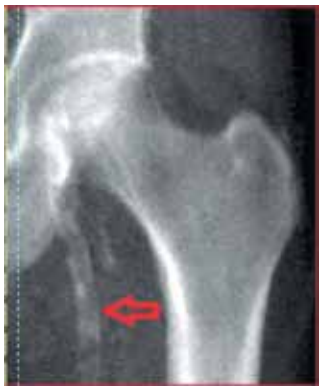


Fig. 1. DXA results for patient C.: a, b – distal bones of left forearm (arrows indicate the radial and ulnar arteries); c, d – left proximal femur (arrow indicates the femoral artery)

patient was transferred to the surgical endocrinology unit for surgical treatment of HPT.

2. Patient P. (b. 1987)

Suffers from congenital anomaly of the urinary system. Programmed hemodialysis treatment since autumn 1997 using arteriovenous fistula on the left forearm. Cadaveric kidney transplantation in 2005. In the postoperative period, delayed graft function, neo-ureteral anastomosis surgery, opening of the abscess of the anterior abdominal wall. Blood creatinine 170–180 µmol/L (eGFR 37 mL/min). Renal graft function deterioration in 2014 – proteinuria up to 4 g/day, with histological examination, glomerulosclerosis, chronic allograft nephropathy. Resumption of hemodialysis in the spring of 2015 using a permanent dialysis catheter (the formed arteriovenous fistula on the right forearm did not function). Retransplantation of cadaveric kidney on November 25, 2017. Primary graft function (Table 1).

A year later, ultrasound and CT scan of the anterior surface of the neck were performed. Two palpable abnormalities with a diameter of 10 mm were visualized in the projection of the lower poles of the thyroid gland. DXA of standard parts of the skeleton was performed, osteopenic syndrome was diagnosed in the lower third of the left forearm and the left proximal femur. The contours of the radial and ulnar arteries were visualized in both forearms, extraosseous calcified lesions on the left (in the area of aneurysmal expansion of the vascular anastomosis), femoral artery was visualized on the left thigh (Fig. 3). Consultation by an endocrinologist surgeon regarding post-transplant HPT was recommended.

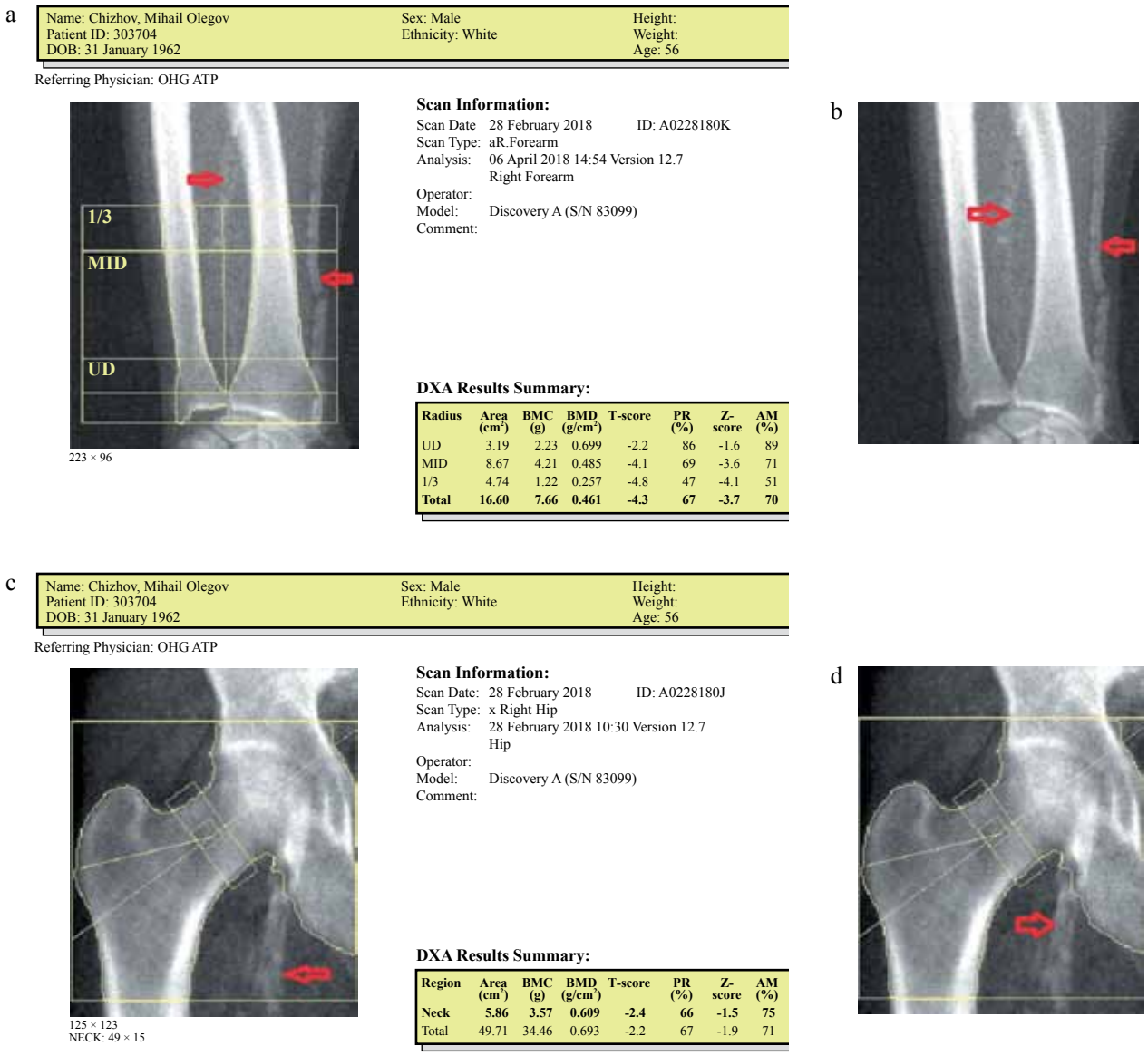


Fig. 2. DXA results for patient C.: a, b – distal bones of right forearm (arrows indicate the radial and ulnar arteries); c, d – right proximal femur (arrow indicates the femoral artery)

Table 1

Dynamic biochemical test results for patient P.

Blood indicator	Cadaver kidney retransplantation on November 25, 2017		
	Before (October 2017)	After 1 month.	After 1 year
Creatinine, μmol/L	868	80	100
Urea, mmol/L	19.6	11.6	6.6
GFR (estimated), mL/min	–	85	65
Hemoglobin, g/l	122	114	140
Total blood calcium/serum albumin, mmol/L	2.25	2.4	2.4
Phosphorus, mmol/L	2.57	1.0 (norm 0.81–1.45)	1.35 (norm 0.84–1.6)
Parathyroid hormone, pg/ml	695	180	204
Alkaline phosphatase, U/L	–	45 (norm 26–115)	113 (norm 3–258)
Cholesterol, mmol/L	–	5.2	5.7
Glycated hemoglobin, %	–	5.1	5.6

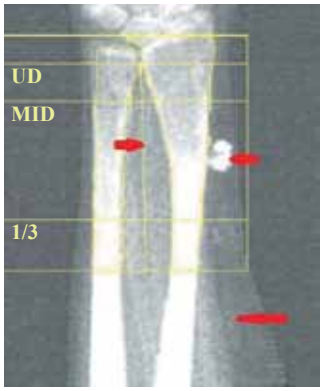
a

Name: Petrikov, Alexandr Alekseev
Patient ID: 361114
DOB: 21 July 1987

Sex: Male
Ethnicity: White

Height: 157.0 cm
Weight: 49.0 kg
Age: 31

Referring Physician: OHG ATP



194 × 94

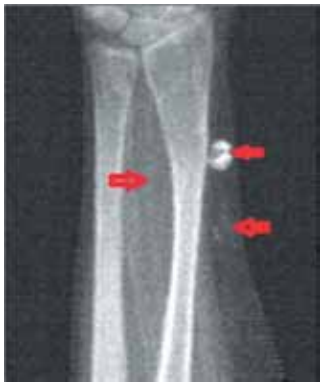
Scan Information:

ScanDate: 20 September 2018 ID: A09201809
Scan Type: a L.Forearm
Analysis: 20 September 2018 10:52 Version 12.7.4.2
Left Forearm
Operator:
Model: Discovery A (S/N 83099)
Comment:

DXA Results Summary:

Radius	Area (cm^2)	BMC (g)	BMD (g/cm^2)	T-score	PR (%)	Z-score	AM (%)
UD	3.46	0.78	0.227		41	-5.2	42
MID	6.11	2.33	0.381		54	-6.0	54
1/3	2.00	1.03	0.517		63	-5.6	64
Total	**11.56**	**4.14**	**0.358**		**52**	**-6.2**	**53**

b



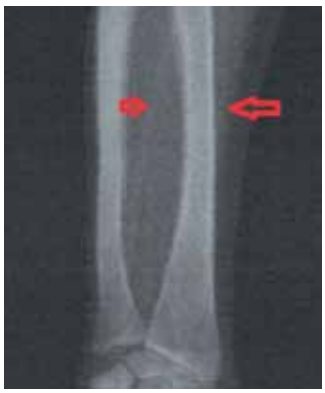
c

Name: Petrikov, Alexandr Alekseev
Patient ID: 361114
DOB: 21 July 1987

Sex: Male
Ethnicity: White

Height: 157.0 cm
Weight: 49.0 kg
Age: 31

Referring Physician: OHG ATP



Scan Information:

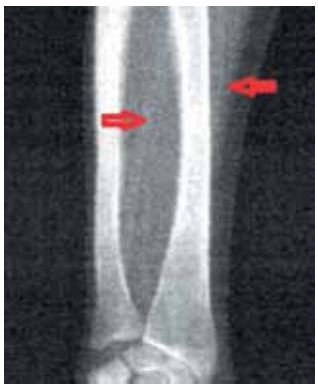
ScanDate: 20 September 2018 ID: A092Q180A
Scan Type: aR.Forearm
Analysis:

Operator:
Model: Discovery A (S/N 83099)
Comment:

DXA Results Summary:

Result data not available

d



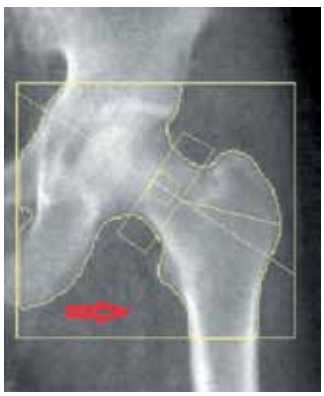
e

Name: Petrikov, Alexandr Alekseev
Patient ID: 361114
DOB: 21 July 1987

Sex: Male
Ethnicity: White

Height: 157.0 cm
Weight: 49.0 kg
Age: 31

Referring Physician: OHG ATP



111 × 102
NECK: 49 × 15

Scan Information:

ScanDate: 20 September 2018 ID: A09201807
Scan Type: x Left Hip
Analysis: 20 September 2018 10:46 Version 12.7.4.2
Hip
Operator:
Model: Discovery A (S/N 83099)
Comment:

DXA Results Summary:

Region	Area (cm^2)	BMC (g)	BMD (g/cm^2)	T-score	PR (%)	Z-score	AM (%)
Neck	**4.51**	**2.86**	**0.634**		**68**	**-2.0**	**70**
Total	31.10	20.36	0.654		63	-2.4	64

f

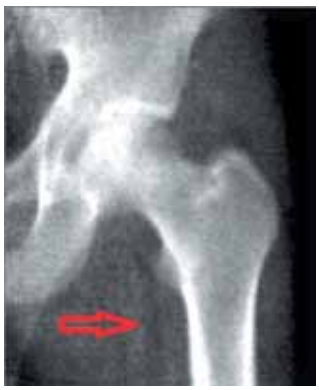


Fig. 3. DXA results for patient P.: a, b – distal part of left forearm; c, d – distal part of right forearm (arrows indicate the radial and ulnar arteries and the extraosseous calcification site); e, f – left proximal femur (the arrow indicates the femoral artery)

3. Patient O. (b. 1975)

Has been suffering from type 1 diabetes since the age of 17. Continuous ambulatory peritoneal dialysis (CAPD) since September 24, 2008 due to development of end-stage renal disease (blood urea 22.2 mmol/L, blood creatinine 538 μmol/L, eGFR 7.7 mL/min, blood hemoglobin 73 g/l). Satisfactory response to treatment, adequate CAPD program. In mid-2012, when the CAPD program became inadequate, the patient was placed under hemodialysis treatment. Repeated arteriovenous fistula formation in the lower third of the left forearm (2012), in the lower (2013) and middle third (2015) of the left shoulder using vascular prosthesis. Anemia, hypocalcemia and hyperphosphatemia were corrected (Table 2).

Persistent increase in PTH in 2012 and drug therapy were unable to normalize the function of the parathyroid gland. For two years, the patient refused surgical treatment. Parathyroidectomy was performed on June 10, 2014: three enlarged parathyroid glands were removed, the upper right gland was not found. As part of comprehensive examination, DXA of right distal forearm (arterial fistula was done on the left forearm) and the left proximal femur (Fig. 4) was performed before surgery. Osteopenic syndrome was diagnosed in both sections, while ulnar, radial and femoral arteries were visualized.

DISCUSSION

Currently, it is widely believed that there is close relationship between the bone and vascular systems in CKD patients. This is reflected in the existence of the bone-vascular axis phenomenon. It is confirmed by reports that the degree of calcification of large and medium arteries is negatively associated with BMD and positively associated with a higher rate of prevalent vertebral fractures [2, 16]. Reports also indicate that vascular calcification does not depend on the variant of renal osteodystrophy but is due to predominance of bone resorption over bone formation, and that serum phosphorus is a connecting link. In the case of adynamic bone disease, hyperphosphatemia can be the result of dietary phosphorus intake against a background of low bone turnover. With secondary HPT, on the contrary, phosphorus is released from bone due to high bone turnover [17].

Diagnosis of peripheral arterial calcification in CKD patients is very crucial in real clinical practice. When it is detected with high degree of probability, you can predict calcification of the coronary arteries, and, accordingly, fatal and non-fatal cardiovascular events [12, 18]. Experts discuss the benefits and methods of routine screening for vascular calcification as there is no convincing evidence that routine testing of this condition helps detect CKD. According to international guidelines – Kidney Disease: Improving Global Outcomes (KDIGO) – the evidence for recommendation of vascular calcification screening using lateral abdominal radiography is graded as 3C (weak and low quality of evidence) [19]. It is likely that in some dialysis patients and kidney transplant recipients, peripheral arterial calcification can be diagnosed via DXA, as our observations show.

DXA is today's established standard for measuring BMD and diagnosing primary and secondary osteoporosis. According to KDIGO guidelines, revised and updated in 2017, BMD testing is indicated for patients with CKD stages 3–5, including the dialysis population, with signs of mineral and bone disorder (MBD) and/or risk factors for osteoporosis, as there is evidence that decreased bone mineral density increases bone fracture risks [20]. HPT is one of the clinical variants of MBD in CKD and the main risk factor for formation of osteopenic syndrome (secondary osteoporosis) in these patients. It seems appropriate for all dialysis patients and kidney transplant recipients with HPT of varying severity to undergo DXA, and repeatedly for those with prolonged HPT. Measuring BMD, especially over time, will allow not only to evaluate the effectiveness of HPT therapy, but sometimes to detect peripheral arterial calcification. In patients with CKD and secondary HPT, detecting a progressive decrease in BMD and visualizing calcified arteries may be an additional argument in favor of surgical treatment of HPT. This is exactly what happened in our patients.

Table 2

Dynamic biochemical test results for patient O.

Blood parameters	Observation years									
	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017
Creatinine, μmol/L	570	500	880	840	810	680	860	720	774	902
Urea, mmol/L	11.8	9.9	11.8	12.9	13.7	16.2	20.3	7.2	10.9	10.1
Hemoglobin, g/l	97	112	107	109	97	113	132	115	121	128
Total blood calcium/serum albumin, mmol/L	2.1	2.1	2.1	2.1	2.2	2.2	2.3	2.2	2.0	2.2
Phosphorus, mmol/L	1.9	1.6	2.0	2.1	2.0	2.2	1.8	1.7	0.8	0.57
Parathyroid hormone, pg/ml	276	364	335	705	1114	1256	1754	234	190	147
Alkaline phosphatase, U/L (norm 30–120)	96	114	146	180	252	196	400	160	–	–
Cholesterol, mmol/L	5.9	6.0	5.5	6.7	6.6	4.5	4.9	4.1	–	–
Glycated hemoglobin, %	10.3	8.3	9.1	9.4	8.1	–	–	7.5	–	–

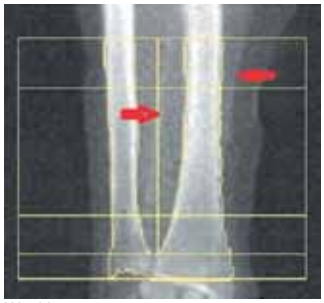
a

Name: Orlova, Natalya Anatole
Patient ID: OHG
DOB: 28 March 1975

Sex: Female
Ethnicity: White

Height:
Weight:
Age: 39

Referring Physician:



228 × 96

Scan Information:

Scan Date: 15 April 2014 ID: A04151415
Scan Type: a R.Forearm
Analysis: 12 July 2018 16:06 Version 12.7
Right Forearm
Operator:
Model Discovery A (S/N 83099)
Comment:

b

DXA Results Summary:

Radius	Area (cm^2)	BMC (g)	BMD (g/cm^2)	T-score	PR (%)	Z-score	AM (%)
UD	2.67	1.33	0.498	-3.3	72	-2.9	74
MID	6.60	2.76	0.418	-3.5	69	-3.1	71
1/3	3.26	0.90	0.275	-2.9	62	-2.7	64
Total	**12.53**	**4.99**	**0.398**	**-3.4**	**69**	**-3.1**	**71**

c

Name: Orlova, Natalya Anatole
Patient ID: OHG
DOB: 28 March 1975

Sex: Female
Ethnicity: White

Height:
Weight:
Age: 39

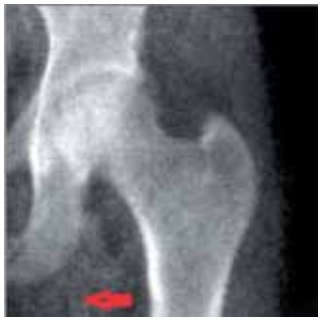
Referring Physician:

113 × 111
NECK: 48 × 15

Scan Information:

ScanDate: 15 April2014 ID: A04151414
Scan Type: x Left Hip
Analysis: 12 July 2018 16:08 Version 12.7
Hip
Operator:
Model: Discovery A (S/N 83099)
Comment:

d



DXA Results Summary:

Region	Area (cm^2)	BMC (g)	BMD (g/cm^2)	T-score	PR (%)	Z-score	AM (%)
Neck	**5.06**	**3.00**	**0.593**	**-2.3**	**70**	**-2.0**	**72**
Total	32.54	22.99	0.707	-1.9	75	-1.8	76

Fig. 4. DXA results for patient O.: a, b – right distal forearm (arrows indicate the radial and ulnar arteries); c, d – left proximal femur (the arrow indicates the femoral artery)

DXA of the distal forearm and proximal femur was performed in all patients as part of comprehensive examination before surgical treatment of severe HPT. This was to evaluate BMD. The scanning revealed not only a significant decrease in BMD in these bone sections (respectively 1/3 radius and total hip –2.2 SD and –2.1 SD according to T-criterion in the first patient, –5.6 SD and –2.4 SD according to the Z-criterion in the second patient and –2.7 SD and –1.8 SD according to the Z-criterion in the third patient). It also quite clearly visualized the radial ulnar and femoral arteries, which clearly indicates calcification. Forearm arterial calcification was what caused the unsuccessful formation of the arteriovenous fistula in the second patient and multiple formation of arteriovenous fistulas in the third patient. One of the real risk factors for vascular calcification in all patients should be the duration of CKD before dialysis, long-term renal replacement therapy (hemodialysis and kidney transplantation in the first two patients, peritoneal dialysis and hemodialysis in the third patient), as well as suboptimal renal graft function (in the first two patients) [6, 21]. In the third patient, diabetes mellitus, which was the main disease, played a major role in development of vascular calcification. Poor glycemic control (glycated hemoglobin >7.5%) observed in our patient was a serious factor in the development/progression of peripheral arterial calcification – hyperglycemia was shown to directly induce phenotypic transformation of vascular smooth muscle cells into osteoblast-like cells. It is very likely that in the first patient, post-transplant diabetes mellitus was also a serious factor for the development/progression of peripheral vascular calcification. Diagnosis 1.5 years before DXA and 6.3% glycated hemoglobin do not exclude longer existence of diabetes with episodes of decompensation. Finally, secondary HPT (hyperphosphatemia), its inadequate conservative correction and

delayed surgical treatment, played a crucial role in the development/progression of vascular calcification in all cases [8, 9]. Most likely, all patients have both vascular calcification variants – atherosclerosis and arteriosclerosis. Visualization of forearm arteries and femoral artery along the entire length in the form of fairly uniform linear cords with simultaneous presence of separate denser areas could serve as a confirmation of combined intimal and medial vascular calcification.

The clinical cases presented suggest that DXA could be used for distal forearm and proximal femur not only for measuring bone mineral density but also for detecting peripheral arterial calcification.

The authors declare no conflict of interest.

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DOI: 10.15825/1995-1191-2019-3-100-110

COMPARATIVE ANALYSIS OF REGENERATIVE ACTIVITY OF BONE MARROW CELLS AND TOTAL RNA EXTRACTED FROM THEM IN CHRONIC FIBROSING LIVER DISEASE

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Aim: to conduct a comparative assessment of the effectiveness of liver regeneration occurring after induction of chronic fibrosing liver disease (CFLD) using bone marrow mononuclear cells (BMMCs) and total RNA (tRNA) extracted from BMMCs. **Materials and methods.** The study involved 140 Wistar rats. CFLD was modeled in 100 rats, of which 25 died. The surviving 75 rats (CFLD formed by the third month) were divided into 3 groups: Group 1 – control (administered with physiological saline); Group 2 – a single injection of tRNA from BMMCs at a dose of 30 μg/100g body weight; Group 3 – a single injection of BMMCs at a dose of (30–35) × 10^6 cells. The dynamics of regenerative processes in the liver was evaluated based on the animal mortality, dynamics of restoration of biochemical markers (ALAT, ASAT, alkaline phosphatase and total protein) and morphological picture of the liver on the seventh day and after three, six and nine months. The significance of differences in the compared values was determined through Student's t-test for <0.05. **Results.** Mortality in Group 1 was 12%, in Groups 2 and 3 – 4%; In Group 1, ALAT and ASAT were restored to normal values after two months, alkaline phosphatase after 3 months, and total protein remained low for over 4 months. In Groups 2 and 3, all hepatic homeostasis markers returned to the values they were before CFLD modeling faster than in Group 1 (after two months). However, in Group 2, the regeneration rate was higher than in Group 3. It was revealed that normalization of functional liver parameters in all groups were ahead of restoration of the histological structure of the liver. Liver defibrotic processes in Group 2 were activated after 3 months, and in Groups 1 and 3 – after 6 months. The histological structure of the liver was restored in Group 2 after 6 months, and in Groups 1 and 3 after 9 months. **Conclusion.** BMMCs and tRNA extracted from them in biologically effective doses trigger liver regeneration in CFLD. However, regulatory effect from the use of tRNA appears earlier and is more effective.

Keywords: chronic liver failure, cirrhosis, bone marrow mononuclear cells, total RNA, liver regeneration.

Chronic liver failure (CLF) and liver cirrhosis are the result of major alterations in reparative regeneration processes. This creates conditions for chronically supported inflammation and progression of fibrosis [1].

At the current stage in medicine, donor liver transplantation is the only solution to irreversible liver damage in CLF patients [2, 3]. Meanwhile, steadily increasing donor organ shortage, along with continuing increase in the number of patients in need of liver transplantation limits the applicability of this method in all patients with end-stage CLF. Under these circumstances, there is need to continue the search for more accessible and effective CLF treatment methods that are based on induction of the patient's own regenerative liver reserves. The use of bone marrow stem/progenitor cells has become a new promising treatment strategy in CLF and cirrhosis.

At present, there are enough clinical and experimental observations, showing that BMMC-derived hematopoietic and stromal cells have a positive effect on the structure and function of the liver in chronic fibrosing conditions. Moreover, several studies [4–9] have even showed that there could be at least partial regression of already formed cirrhosis during stem/progenitor BMMCs transplantation. However, not all researchers recognize the fibrolytic effect of BMMCs. They even argue that BMMC use might increase fibrosis [10–12].

Finding diametrically opposite results from the use of stem/progenitor BMMCs is apparently a consequence of underestimating a number of factors: type of cells used, their initial bioregulatory potential (allogeneic cells of a healthy donor or autologous cells of a CLF patient), degree of reversibility of existing structural disorders in the liver which is reflected through severity of concomitant immune imbalance in the body, characterized by development of immunodeficiency up to immune paralysis [13, 14]. The absence or short duration of activation of fibrolytic processes in the liver during cell therapy can be caused primarily by the use of autologous BMMCs of a patient in whose body an immune imbalance has already developed, inhibiting the functional activity of BMMCs

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restored by culture and returned to the body [13]; besides, preliminary administration of G-CSF, which is used to restore reduced regulatory activity in the patient's bone marrow cells [15], also has a temporary effect, since the patient's BMMCs continue to remain under the influence of a complex of immunopathological factors paralyzing their activity. Preexisting immunopathological restructuring in a CLF patient seems to also have a paralyzing effect when using allogeneic BMMCs.

In the last decade, people started associating achievement of the regenerative potential of BMMCs with the recently discovered class of numerous protein – non-coding RNAs that are found in these cells: with the participation of microRNA molecules, long non-coding RNAs, short interfering RNAs, short nuclear RNAs, etc. [16–25], which served as the basis for extraction of total RNA from BMMCs and its use for induction of regenerative processes in the bone marrow itself when it is damaged [26–28].

Given that CLF, especially its terminal phase, proceeds amidst immune imbalance and inhibition of regulatory functions of the patient's BMMCs, which are indispensable participants in the regenerative process [14], as well as evidence that the used BMMCs (autologous and allogeneic) quickly lose their induction effect on reparative processes in the body, we have proposed that the total RNA extracted from the BMMCs of a healthy donor, will, in CLF, act as a biochemical regulator of regenerative processes in liver cells more quickly, more independently, and therefore more effectively than BMMCs.

Purpose of this work: To study ways of boosting the efficiency of liver regeneration in chronic toxic (fibrosing) liver disease, by comparatively evaluating the regulatory effect of BMMCs and total RNA from BMMCs on these processes.

MATERIALS AND METHODS

All studies using laboratory animals were carried out in strict compliance with the laws of the Russian Federation (in accordance with the Laboratory Practice Rules approved by the Ministry of Health of Russia through order No. 708 dated August 23, 2010, in accordance with standard GOST R ISO 10993-2-2009 "Medical devices. Biological evaluation of medical devices. Part 2. Animal welfare requirements") and in compliance with bioethical principles approved by the European Convention for the Protection of Vertebrate Animals (2005).

The work involved 140 male Wistar rats weighing 250–350 g. Chronic fibrosing liver disease (CFLD) was modeled in 100 of them; the remaining rats were used to obtain bone marrow mononuclear cells (BMMCs) and to extract total RNA (tRNA) from them. The animal model for CFLD was created through chronic CCl4 inoculation of animals in combination with Freund's incomplete adjuvant for 42 days according to the scheme. Of the 100 animals induced with CFLD, 25 died and by the end of the injection, 75 survived. All the surviving rats after CFLD modeling were divided into three groups: Group 1 – control (n = 25) with a single injection of physiological saline seven days after the end of injection; Group 2 – experimental (n = 25), where tRNA from the BMMCs of a healthy animal was intraperitoneally administered only once at a dose of 30 μg/100 g body weight seven days after CFLD modeling; Group 3 – experimental (n = 25), where BMMCs were intraperitoneally administered only once at a dose of (30–35) × 10^6 cells per rat seven days after CFLD modeling, which is comparable to the dose of cells used for tRNA extraction and single use. tRNA was extracted from BMMCs by a method developed by biotechnology company Evrogen (Russia) using the ExtractRNA reagent. This allowed to obtain about 148.5 ± 22.3 μg of tRNA from every 35.0 × 10^6 extracted cells.

The effectiveness of the stimulating effect of tRNA and BMMCs on reparative regeneration processes taking place in the liver after CFLD had been administered in the animals was evaluated based on animal mortality in the three groups, as well on the dynamics of restoration of hepatic homeostasis in the animal's body, and on the dynamics of elimination of structural (fibrosing) disorders in the liver tissue on the seventh day, and also after three, six and nine months. The regeneration dynamics of hepatic homeostasis in the body was evaluated by measuring the total protein and hepatic cytolysis enzymes in the blood serum: alanine aminotransferase (ALAT), aspartic aminotransferase (ASAT) and alkaline phosphatase (ALP).

Differences in the structure of liver tissue in the control and experimental groups were studied at the same time based on the severity of fibrolytic processes in the connective tissue and on the number of young newly formed hepatocytes in the hepatic lobule. To this purpose, the liver was excised within specified time frame, histological preparations were prepared from it, the sections were then stained with hematoxylin and eosin, while the connective tissue was treated with Masson trichrome stain.

The significance of differences in the markers for the compared groups was evaluated using the parametric Student's t-test for $p < 0.05$.

RESULTS AND DISCUSSION

The effectiveness of the regulatory effect of tRNA and BMMCs on regenerative processes occurring in the liver after CFLD modeling was evaluated primarily based on the animal mortality. It was found that in control Group 1, three of the 25 rats died in the first two months, which is 12%; in experimental Groups 2 and 3, the mortality rate was 4% (one rat in each group) within the entire observation period. Through a comparative

study of the restoration dynamics of biochemical markers of the functional state of the liver in the three studied groups, we were able to establish severe alterations in the markers immediately after inoculation and a gradual regeneration in all the groups. Meanwhile, the restoration rate of markers was different for each group (tables 1, 2, 3). In control Group 1 (table 1), the serum ASAT and ALAT values remained at a significantly higher level for two months than the initial values, ALP for 3 months, while the total protein content remained significantly reduced for 4 months. In Group 2 (with single tRNA injection), serum ASAT and ALAT values were restored after 1 month, serum ALP after two months, while the serum total protein content did not significantly differ from the initial values after two months (table 2). In Group 3, after a single injection of BMMCs, all the markers studied remained significantly altered from their baseline values within two months after CFLD modeling. However, when ASAT, ALAT, ALP, and total protein values were compared with each other two months after CFLD modeling, no significant differences were found in Groups 2 and 3.

Based on the results obtained from comparative study of dynamics of restoration of biochemical markers cha-

Table 1

Dynamics of changes in serum total protein and liver cell cytolysis enzymes (ALAT, ASAT and ALP) for Group 1 rats after CFLD modeling and administration of physiological saline (n = 25)

Timing after CFLD modeling	Group 1 (control group), n = 25			
	ASAT	ALAT	ALP	Total protein
Baseline	58 ± 8	40 ± 6	240 ± 24	98 ± 20
7 days	282 ± 31*	320 ± 13*	1322 ± 21*	21 ± 16*
1 month	196 ± 22*	168 ± 21*	909 ± 31*	26 ± 13*
2 months	98 ± 15*	96 ± 14*	532 ± 26*	42 ± 15*
3 months	72 ± 14	62 ± 8	426 ± 25*	48 ± 11*
4 months	62 ± 10	48 ± 7	250 ± 24	52 ± 6*
6 months	64 ± 15	44 ± 8	240 ± 20	65 ± 7

Note. * – $p < 0.05$ compared with baseline.

Table 2

Dynamics of changes in serum total protein and liver cell cytolysis enzymes (ALAT, ASAT and ALP) for Group 2 rats after CFLD modeling and administration of total RNA (n = 25)

Timing after CFLD modeling	Experimental Group 2 (tRNA), n = 25			
	ASAT	ALAT	ALP	Total protein
Baseline	58 ± 8	40 ± 6	240 ± 24	98 ± 20
7 days	303 ± 20*	278 ± 17*	1187 ± 56*	24 ± 10*
1 month	93 ± 15*	88 ± 10*	532 ± 28*	56 ± 10*
2 months	68 ± 19	68 ± 15	295 ± 14*	67 ± 14
3 months	65 ± 11	62 ± 6	275 ± 15	72 ± 9
4 months	62 ± 8	58 ± 12	247 ± 11	86 ± 7
6 months	66 ± 7	44 ± 6	230 ± 14	92 ± 12

Note. * – $p < 0.05$ compared with baseline.

Table 3

Dynamics of changes in serum total protein and liver cell cytolysis enzymes (ALAT, ASAT and ALP) for Group 3 rats after CFLD modeling and BMMCs injection (n = 25)

Timing after CFLD modeling	Experimental Group 3 (BMMCs), n = 25			
	ASAT	ALAT	ALP	Total protein
Baseline	58 ± 8	40 ± 6	240 ± 24	98 ± 20
7 days	350 ± 10*	262 ± 27*	1205 ± 47*	36 ± 16*
1 month	97 ± 16*	89 ± 16*	570 ± 35*	55 ± 10*
2 months	72 ± 15*	70 ± 13*	310 ± 18*	61 ± 8*
3 months	64 ± 12	61 ± 10	274 ± 18	68 ± 12
4 months	59 ± 12	59 ± 6	252 ± 19	78 ± 8
6 months	62 ± 9	46 ± 11	242 ± 12	88 ± 10

Note. * – $p < 0.05$ compared with baseline.

racterizing the functional state of the liver after CFLD modeling and the use of tRNA and BMMCs, we can conclude that both tRNA and BMMCs accelerates restoration of hepatic homeostasis in the body. However, the effect of tRNA was more pronounced.

Studies have shown that both in the clinic (in CLF) and in experiment (in CFLD modeling), restoration of liver biochemical markers under the influence of the therapy applied is usually not accompanied by liver regeneration at the histological level, especially in hepatic cirrhosis [29, 30]. Meanwhile, available data on the fibrolytic effect of hematopoietic and stromal stem/progenitor BMMCs in hepatic cirrhosis [4–9] compelled us to comparatively study the effectiveness of tRNA and BMMCs contained in biologically effective doses not only on biochemical but also on histology markers of the liver in CFLD modeling.

A study of the dynamics of development of morphological changes in rat liver after CFLD modeling showed that cirrhosis had formed in the liver in all the three study groups by the third month of observations. However, for these groups, the intensity of defibrotic processes in liver tissue differed with increasing observation time. One week after being injected in the pericentral zones of the liver, pronounced necrotic and dystrophic changes in hepatocytes, as well as alterations in the lobular structures of hepatic parenchyma were detected in all rats (Fig. 1, a, b).

Three months after inoculation and infusion of saline, a clear disruption in the trabecular patterns of the hepatic tissue and formation of pseudo-lobules were detected in the liver of Group 1 rats (control group); hepatocytes were without pronounced dystrophic changes; mild cell infiltration was also observed (Fig. 2, a, b).

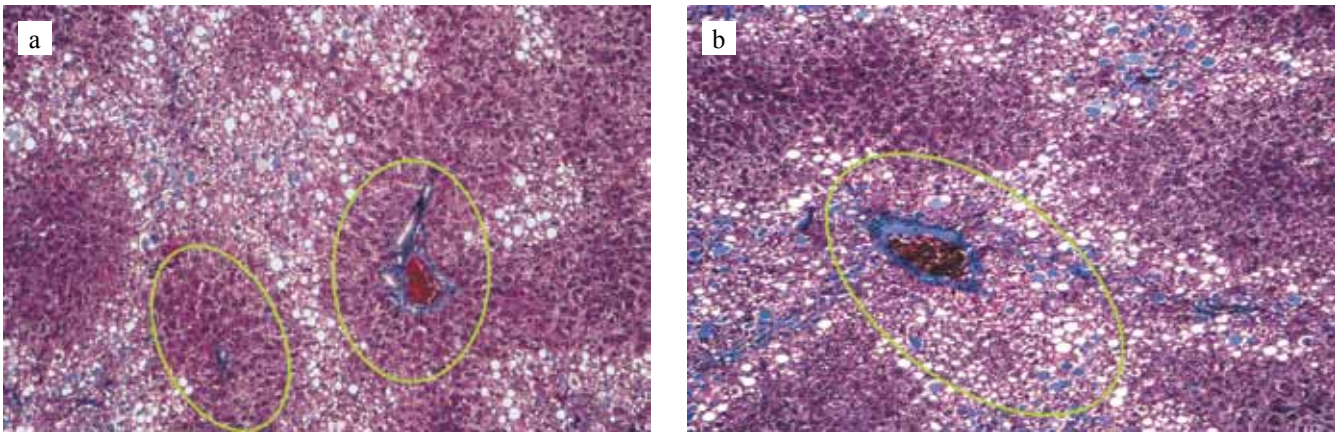

Fig. 1. Histological structure of the rat liver in 1 week after completion of CFLD modeling: a – periportal zone with the minimally expressed degenerative changes of hepatocytes; b – pericentral zone with pronounced necrotic and dystrophic changes of hepatocytes (fat and protein dystrophy); the beginning of connective tissue septa formation (marked by an oval). Masson staining. ×100

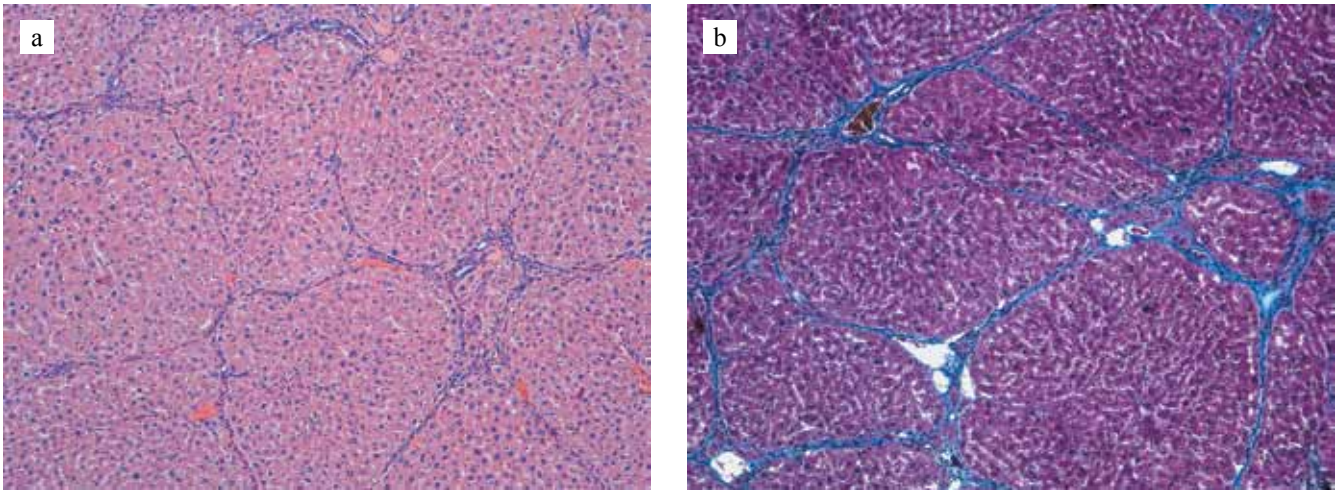

Fig. 2. Histological structure of the rat liver in 3 months after completion of CFLD modeling and administration of saline solution. Numerous false lobules (nodes) of parenchyma bounded by septa. The formed liver cirrhosis: a – hematoxilin-eosin staining; b – Masson staining. ×100

Thus, already by the third month after the end of chronic inoculation in the liver of Group 1 rats (control group), the histological picture of stage 3–4 cirrhosis was formed according to the classification by Desmet et al., 1994 [31]. Similar results of structural disorders in the liver after three months were obtained in Group 3 rats (experimental group) injected with BMMCs (Fig. 3).

Meanwhile, distinct signs of septal fibrolysis of the formed pseudo-lobules were detected at the third month in Group 2 rats (experimental group) – against the background of cirrhosis that formed. These rats were at the

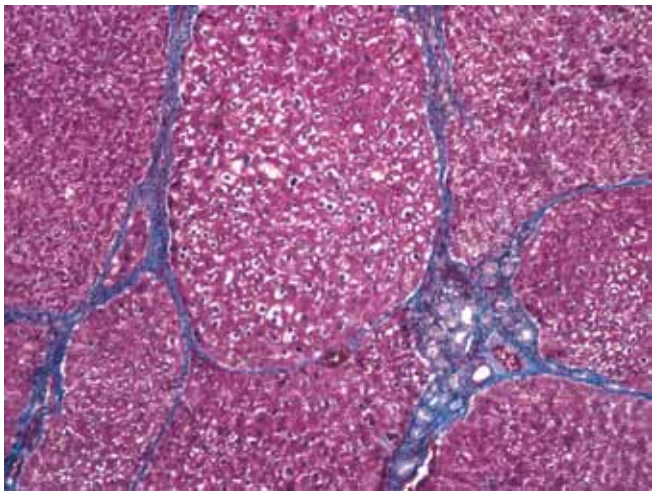

Fig. 3. Histological structure of the rat liver in 3 months after completion of CFLD modeling and administration of unsorted BMMC. The formed false lobules (nodes) of parenchyma restricted by septa . The formed liver cirrhosis. Masson staining, ×100

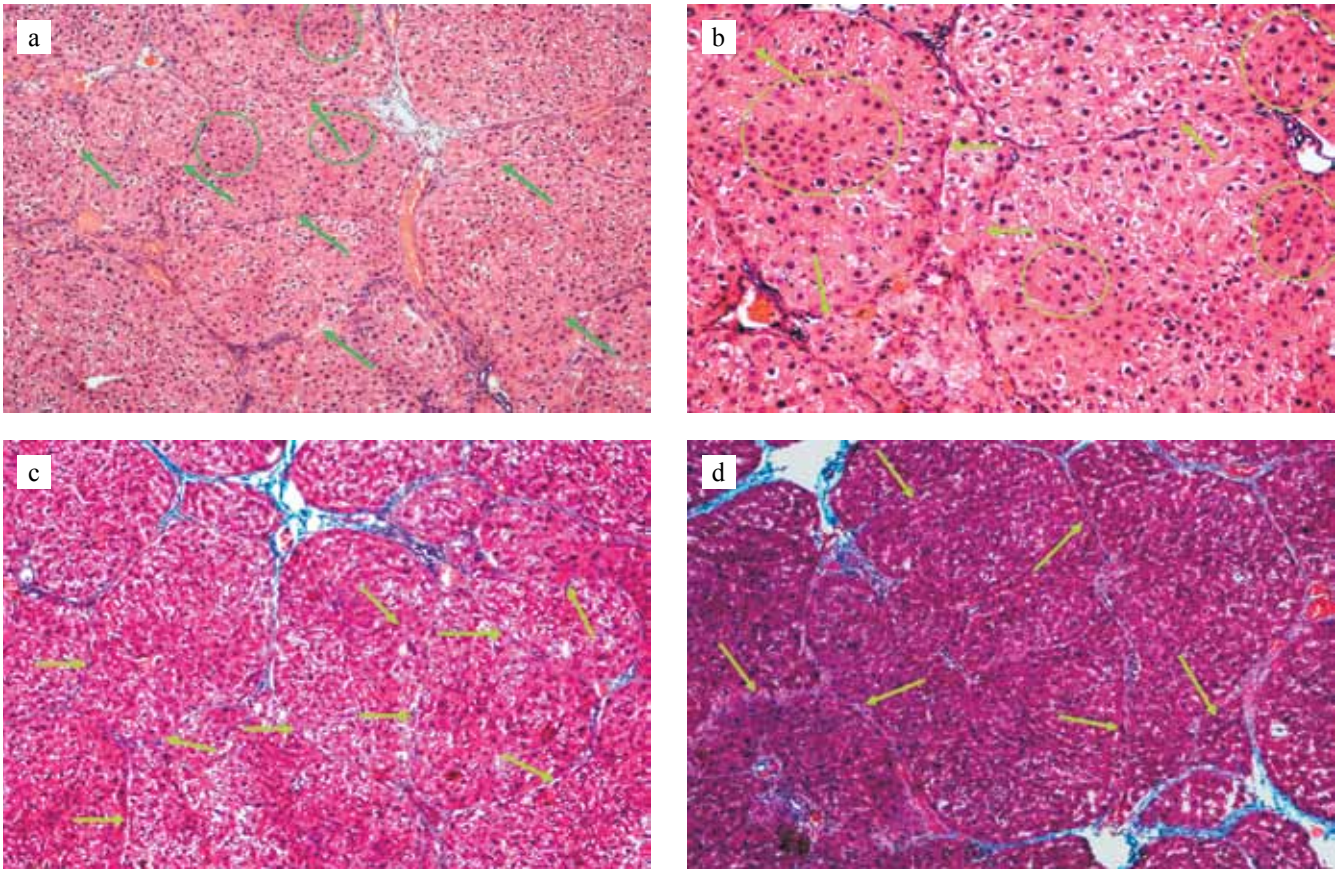

Fig. 4. Histological structure of the rat liver in 3 months after completion of CFLD modeling and infusion of the tRNA from BMMC at a dose of 30 мкг/100 g of animal weight. The septal lysis of false lobules and appearance of significant areas of young hepatocytes. The arrows indicate the zones of lysed septa; the areas of accumulation of young hepatocytes are circled by an oval; a and b – hematoxilin and eosin staining; c and d – Masson staining; a, c – ×100; b, d – ×200

end of inoculation administered once with tRNA from BMMCs. Besides, significant areas of young newly formed hepatocytes were detected in the structure of pseudo-lobules (Fig. 4, a, b, c, d). From our results, it can be argued that tRNA inhibits liver fibrogenesis by the third month unlike in the control Group 1 and experimental Group 3, where fibrotic processes remain clearly pronounced. However, at the third month, pseudo-lobules and alterations in the trabecular patterns of the liver remain in experimental Group 2, as well as in the control group (Group 1) and experimental group (Group 3).

In continuing the dynamic study of morphological changes in the liver of rats in the control and experimental groups, we noted that after six months, spontaneous lysis at the septa of the pseudo-lobules and a zone of young hepatocytes appear in the liver of rats in Group 1 (control group) and experimental Group 3 (Fig. 5, a, b, c, d).

At the same time, the histological pattern of the liver of rats in experimental Group 2 was fully restored (Fig. 6, a, b, c) by the sixth month after tRNA administration: the structure of hepatic lobules and their trabecular patterns were restored, the connective tissue septa were completely lysed in the liver tissue and no other structural alterations were detected.

Based on results obtained from comparative examination of liver tissue in the three groups, we conclude that tRNA from BMMCs accelerates the regeneration of liver tissue structure after chronic toxic fibrosing effects on it. We also state that rat liver tissue has a huge plasticity potential, since it retains the ability for spontaneous lysis of the fibrous tissue for a long time. At the same time, injection of BMMCs exerted its regulatory effect only on the functional markers of the liver. It almost did not affect the rate of restoration of structural alterations in it because defibrotic processes in the liver were activated at the same time as in the control group. Our results are fully consistent with the observations of clinicians on the use of BMMCs in chronic alcoholic liver cirrhosis [32]. By the ninth month after chronic inoculation of rats, the liver tissue structure in the control group (Group 1) and experimental Group 3 was normalized, although in the liver of Group 1 rats, sections of connective tissue septa were preserved in separate fields of view (Fig. 7, a and b).

CONCLUSION

Based on our study of the dynamics of liver regeneration after CFLD modeling and use of BMMCs and tRNA from BMMCs, we make the following conclusions.

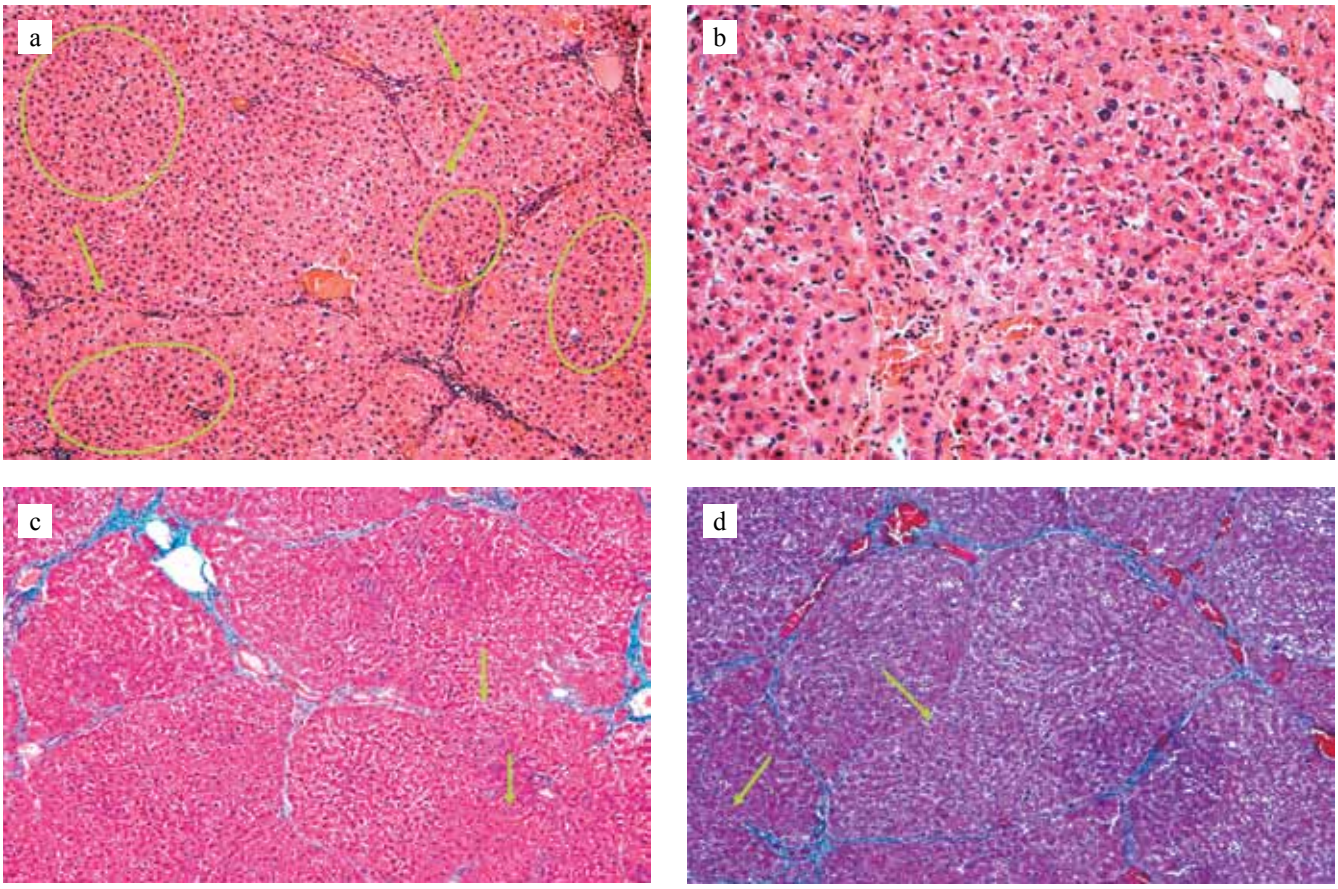

Fig. 5. Histological structure of the rat liver in 6 months. after completion of CFLD modeling and infusion of saline (a, c) and BMMC (b, d). The arrows indicate areas of septal fibrolysis of false lobules; it is seen also the areas of young (newly formed) hepatocytes (marked by an oval); a and b – hematoxilin and eosin staining; c and d – Masson staining; a, c, d – ×100, b – ×200

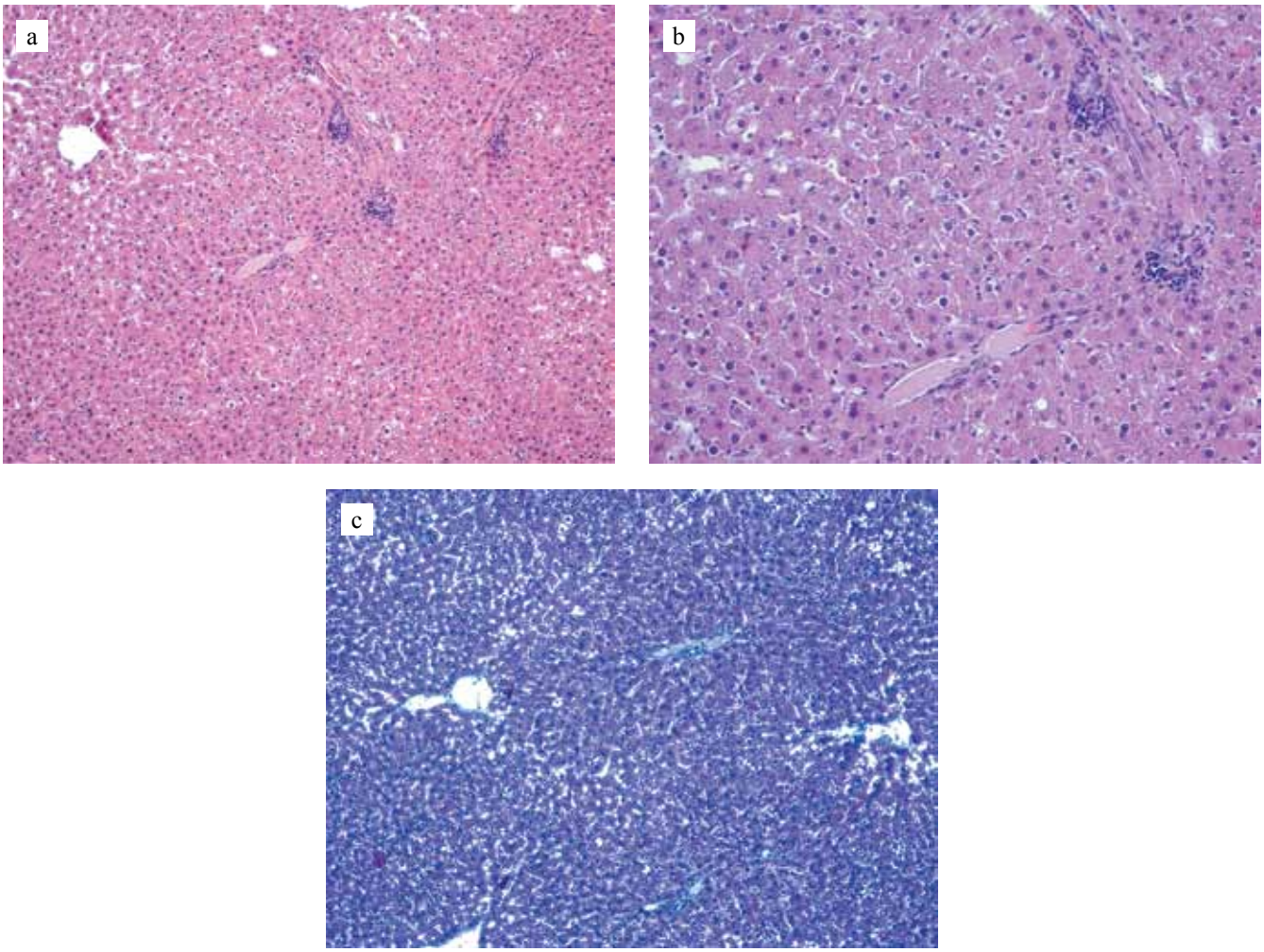

Fig. 6. Histological structure of the rat liver in 6 months. after completion of CFLD modeling and infusion of the total RNA from BMMC. The restoration of liver tissue structure. The absence of pronounced morphological signs of parenchymal fibrosis: a and b – hematoxilin and eosin staining; c – Masson staining; a and c – ×100; b – ×200

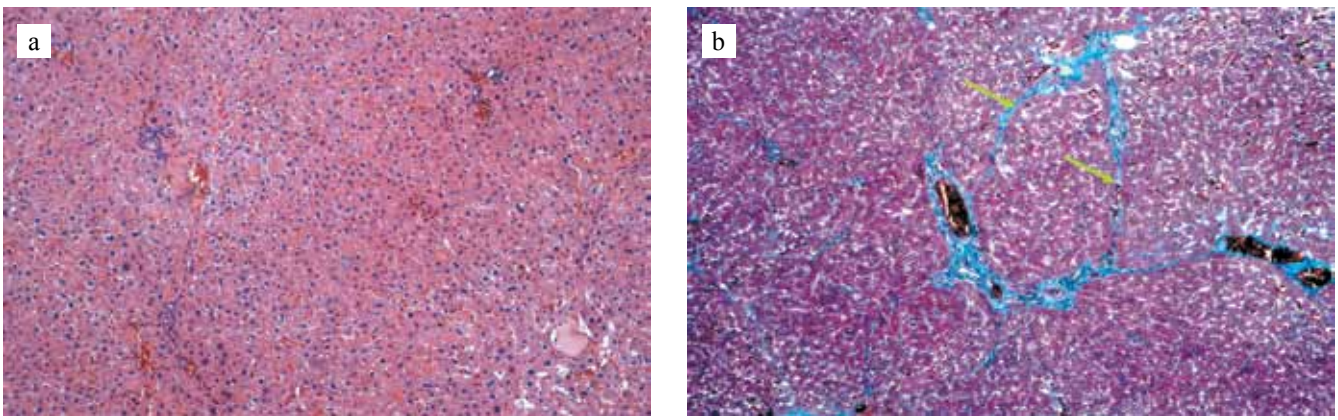

Fig. 7. The Histological structure of the rat liver in 9 months. after completion of CFLD and infusion of saline (control). The arrows indicate the remaining areas of the connective tissue septa; a – hematoxilin and eosin staining; b – Masson staining. ×100

– The liver of initially healthy rats has an extremely high plasticity potential since despite chronic injection through combined use of CCl4 and Freund's incomplete adjuvant, fibrotic processes can be reproduced in the liver within a period not exceeding six months. Six months after inoculation based on the proposed scheme, spontaneous lysis of fibrous tissue in the liver begins and progresses in time.
– Administration of BMMCs of healthy rats helps to accelerate restoration of the functional (biochemical) marker of damaged liver and does not affect the rate of liver regeneration, since disintegration of fi-

brous tissue began and zones of young hepatocytes appeared 6 months after inoculation, as in the control group.

– Administration of tRNA extracted from the BMMCs of healthy rats accelerates regenerative processes in a damaged liver. This manifests as early restoration of the functional and morphological markers of liver function unlike in the control group. After one administration of tRNA, the fibrous septa of the pseudo-lobules start disintegrating and numerous zones of young (newly formed) hepatocytes form – after 3 months; Complete liver regeneration occurs by the 6th month after tRNA administration. Regeneration of the liver tissue structure in control rats and rats injected with BMMCs occurs only by the ninth month.
– tRNA extracted from the BMMCs of a healthy donor is recommended as an alternative tool for exerting a biotechnological effect on reparative regeneration of the organ under the proposed experimental CFLD model.

The authors declare no conflict of interest.

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CULTURE OF HUMAN LABIAL MUCOSAL EPITHELIAL CELL FOR USE IN PATIENTS WITH BILATERAL LIMBAL STEM CELL DEFICIENCY

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Aim: to obtain a stable population of the human labial mucosal epithelium without feeder cells through explant culture technique and simplified formulation of the culture media. **Materials and methods.** Labial mucosa samples were obtained from 6 patients in the operating room after the patients had signed an informed consent. Samples were trimmed of the substantia propria and cut into uniformed explants. Cell culture was done using DMEM/F12 (1:1) (1.05 mM calcium) and EpiLife (0.06 mM calcium) media, supplemented with 5% fetal bovine serum, antibiotic-antimycotic, insulin (5 μg/mL), hydrocortisone (5 μg/mL) and epidermal growth factor (10 ng/mL). Primary cells were stained for stemness and proliferative markers (anti-p63), intermediate filaments (anti-vimentin), and tight junction protein-1 (anti-ZO-1). Image analysis was performed in Fiji (ImageJ). **Results.** Primary cell culture was obtained from all the samples in both media. Cellular morphology was characterized as a classic "coble-stone" phenotype. 34.7% p63-expressing cells (median, n = 3) was detected in the 1.05 mM Ca medium, while ZO-1 expression was estimated at 17.05 μm per cell (median, n = 3). In cells cultured in 0.06 mM Ca medium, positive p63 expression was 39.2% (median, n = 3), while the length of the ZO-1 expression was 5.18 μm per cell (median, n = 3). **Conclusion.** This study presents a detailed protocol on how to obtain cell culture of human labial mucosal epithelium from a small biopsy with high proliferative activity without feeder cells condition. The 1.05 mM Ca medium promoted generation of the tight junction and may be used in *in vitro* epithelium differentiation models. In contrast, the 0.06 mM Ca medium maintained reduced level of maturation in the cell culture. Thus, the media formulations, cell culture source and method described in this study, may be used for transplantation of autologous labial mucosal epithelium in patients with bilateral limbal stem cell deficiency.

Keywords: labial mucosal epithelium, primary cell culture, cell growth factors, cornea, limbal stem cell deficiency.

INTRODUCTION

Normally, the human cornea is covered with a non-keratinized stratified squamous epithelium [1], which is renewed due to the limbal epithelial stem cells (LESCs) [2] located in the limb zone of Vogt crypts and focal stromal projections [3, 4]. If LESCs are extensively damaged, limbal stem cell deficiency (LSCD) develops [5], featured by the presence of non-transparent fibrovascular pannus at the site of the anterior epithelium, which prevents the passage of light through the cornea, thus causing blindness and poor vision [6].

The bilateral LSCD can develop in such diseases as aniridia, Stevens–Johnson syndrome, and corneal burns in both eyes [7]. To rehabilitate this group of patients, surgical methods of allogenic limb transplantation in combination with prolonged immunosuppression are offered [8]. Cell therapy with cultured oral cavity cells is a promising trend of treatment for the bilateral LSCD [9]. At the beginning of the method development, the transplantation of autologous cultured buccal epithelium was performed [10, 11], as it is morphologically similar to the anterior corneal epithelium, i. e. it is non-keratinized stratified squamous epithelium and stays in contact with the environment [12].

At the same time, there are epithelial zones with identical morphology in the mouth cavity as well as in the vestibule. These are the mucosal epithelium and the lower surface of the tongue, soft palate, and mouth floor [12]. According to literature data, cheek (buccal) epithelium has long remained the major source of cells for creating a therapeutic construct [9, 13]. At this, mucosal epithelium as a source of cells for the cell epithelium construct has not been earlier studied in detail.

According to literature reports, the inscription of cultural media for selective cell growth of the oral cavity

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epithelium assumes the presence of a common mitogen, L-glutamine, decontamination components, and factors stimulating the growth of the epithelium [14]. Most of investigators used the DMEM/F12 basal medium (1:1–1:3) containing 1.05–1.425 mM of Ca to obtain the buccal epithelium culture [14]. However, according to R.Y. Freshny, the low Ca medium (0.06–0.07 mM) is preferable as it supports the proliferative activity of cultivated epithelial cells, does not cause their differentiation and contributes to the elimination of fibroblast-like cells [15]. The earlier cultivation methods also included feeder cell layers and other xenogenic components, e. g. bovine pituitary extract. It has been shown in modern studies that feeder layers can be excluded [16], and among many specific factors, only three can be used to stimulate epithelium growth; namely, insulin, hydrocortisone, and epidermal growth factor (EGF) [17], each is produced by a number of companies complying with GMP rules.

The present study is aimed at obtaining a stable population of the human labial mucosal epithelium without feeder cells using the explant method and simplified formulation of the culture media.

MATERIALS AND METHODS

Formulation of the culture media

In the present study, the following culture media with 2 mM L-glutamine were used as basal: DMEM/F12 (1:1) (D6421, Sigma Aldrich) containing 1.05 mM of Ca and phenolic red, and EpiLife (MEPICFPRF500, Gibco) with 0.06 mM of Ca without phenolic red. EpiLife medium is similar in its formulation to the medium for keratinocytes MCDB 153 [18] and, unlike such basal media as DMEM/F12, contains nickel chloride, ammonium paramolybdate and metavanadate, sodium selenite, sodium metasilicate, tin chloride, and lipoic acid [19]. As a general mitogen, fetal bovine serum (FBS) (SH30109.03, HyClone Laboratories) was used at 5% concentration. To prevent pathogenic microflora growth, 100 U/ml of penicillin, 100 μg/ml of streptomycin and 0.25 μg/ml of amphotericin B (A5955, Sigma Aldrich) were added to the culture medium. Among the specific factors used for stimulation of oral cavity epithelium growth were soluble human biosynthetic short-acting insulin, 5 μg/ml (Humulin Regular, Injectable solution, Elie Lilly & Company), 5 μg/ml hydrocortisone (injection suspension, 25 mg/ml, Pharmak), human recombinant epidermal growth factor (EGF), 10 ng/ml (FR-08000, PanEco). Prepared complete culture media were stored at +4 °C with no exposure to light.

Isolation of labial mucosal tissue

All studies were carried out in structural divisions of the S.N. Fedorov NMRC "MNTK Eye Microsurgery".

To obtain the primary cell culture of the epithelium, the remnants of free labial mucosa graft tissue were used, isolated during its transplantation at the planned surgery in the Reconstructive and Plastic Surgery Department. The study was approved by the Ethical Committee (protocol No. 88.5 of October 11, 2018). The tissue extraction was performed after the patient signed a voluntarily informed consent to the use of the tissue remnants for research purposes. The free graft was isolated according to the standard rules of aseptic and antiseptics. For this, the labial mucosa was treated with a sterile gauze sponge moistened in the Iodoftal solution (LLC "NEP MG") for one minute. Then the infiltration anesthesia of the zone of interest was performed with 2% lidocaine solution (GROTEX LLC). The labia were fixed with a holder, providing access to the mucous membrane. Then the mucous membrane was cut with the blade laterally from the frenulum to get a full-layer graft in the form of a petal. In the course of the operation, the graft required for transplanting was removed, and the remains of tissue were placed in the Borzenk–Moroz medium (LLC "NEP MG"). The edges of the formed mucous defect were covered with 5 to 7 separate nodal sutures. In the postoperative period, the patients observe hygiene of the oral cavity in accordance with the standard postoperative protocol.

Primary culture of labial mucosal epithelium

Cellular and tissue materials were processed in the Laboratory of Transplantology and Cell Biology of the Center for Fundamental and Applied Medical and Biological Problems in accordance with the established sanitary and epidemiological regime. To comply with aseptic conditions, the work with the materials was carried out in the working space of laminar boxes of II class of safety. All devices and tools were calibrated according to the standards and prepared in accordance with the aseptic requirements. The standard culturing conditions were taken to be the temperature mode of 37 °C, 5% CO_2 concentration and 100% humidity maintained in the incubator (NU5510, NuAir).

In total, 6 samples of the upper or lower labial mucosal membranes in the form of a narrow strip about 1.5–2.0 mm wide and less than 1 cm long were obtained from 6 patients. The tissue was transported and stored in the Borzenk–Moroz medium at +4 °C. The slices for the subsequent seeding were taken as follows: the tissue was transferred to the Petri dishes with the epithelial side down and, with microsurgical forceps and scissors, the submucosal part was separated until a whitish layer appeared before the epithelium layer. The resulting strip was cut into pieces with 1.0–1.5 mm transverse dimensions. In the course of the described manipulations, to prevent drying out, the Borzenk–Moroz medium was applied over the tissue and pieces. The stages of treat-

ment of the labial mucosal strip for seeding and primary cultivation of the epithelium are shown in Fig. 1.

The primary culture of the labial mucosal epithelium cells was obtained by the explant cultivation method. For this, the pieces obtained after separation of the subjacent tissue were placed with the epithelial side facing up (whitish layer downwards) on the cultural surface of Petri dishes (430165, Corning) or 4-well slide flasks (30104, SPL). Then the samples were left with an open lid for their primary "drying" for 1–2 minutes in laminar boxing conditions. Then, 40 µl of the complete culture medium was applied to the pieces, the lid of the culture dishes was closed, and the pieces were moved to standard incubator conditions for 3–4 hours. After that, 500 µl of the complete culture medium was added very slowly to the pieces cultivated under standard conditions. The cell growth was visually controlled with a phase-contrast inverted light microscope IX-81 (Olympus). The growth and morphology of cell culture were recorded. The culture medium was completely altered from the moment of visualization of the first proliferating cells and further every day. Cells in Petri dishes and slide flasks were cultivated up to 90% confluence.

Fluorescent immunocytochemistry

The cells were stained by the following protocol: the cells in cultural slide flasks were washed with phosphate buffer solution (PBS) (B-60201, PanEco) three times, 5 minutes each. Then they were fixed in 10% neutral formalin (141328, AppliChem) for 10 minutes. Permeabilization was carried out with a 0.3% solution of X-100 triton (X100, Sigma Aldrich) in PBS for 15 minutes. The blocking solution containing 5% fetal bovine serum (SH30109.03, HyClone Laboratories) and 0.1% saponin (84510, Sigma Aldrich) in PBS for 1 hour was used to block nonspecific binding sites. After that, primary antibodies diluted in the blocking solution were added to the wells to such markers as p63 (stemness, proliferation) [20] (1:300, ab124762, Abcam), vimentin (intermediate filaments) [21] (1:250, ab8978, Abcam), ZO-1 (Zonula occludens-1, dense intercellular contact protein type 1) [22] (1:100, ab216880, Abcam). Primary antibodies were not added to the control wells, and the same blocking solution volume was used. The slides were incubated for 18 hours at +4 °C. After washing in the blocking solution, secondary fluorescent antibodies Alexa Fluor 488 (1:250, ab150077, Goat Anti-Rabbit IgG, Abcam) or Alexa Fluor 594 (1:250, ab150116, Goat Anti-Mouse IgG, Abcam)

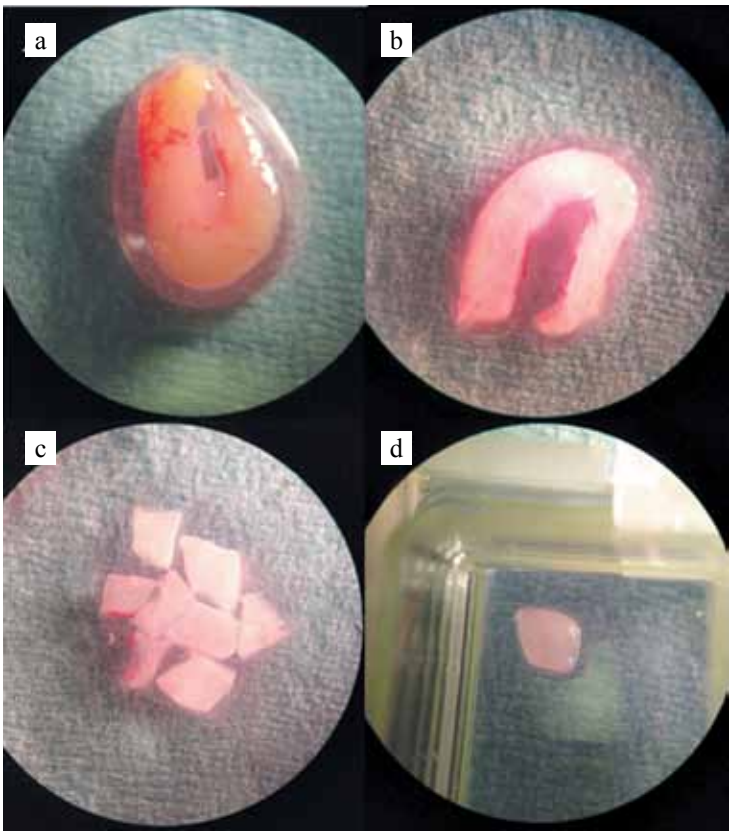

Fig. 1. Stages of processing and seeding of human labial mucosal explants, macro photography: a – overview of the strip of a labial mucosa in a drop of the Borzenk–Moroz medium, a strip of tissue facing the epithelial side down, and visible fatty submucosal layer anteriorly; b – a strip of the labial mucosa after removal of the submucosa, visible whitish layer, with underlying the epithelium; c – labial mucosa explants prepared for seeding; d – explant on the surface of the well of a glass culture slide with the epithelial side facing up

diluted in the blocking solution were added to all wells. The cells were then incubated for 1 hour at +4 °C under darkroom conditions. Secondary antibodies were then removed and stained with Hoechst (O150, PanEco) for 2 minutes in all wells. Slide flasks were then dismantled and mounted under the coverslip with the medium to enclose the histological preparations VitroGel (12-001, BioVitrum). Immunofluorescence detection was performed with the Fluoview FV10i (Olympus) confocal laser scanning microscope.

Image analysis

The images were initially prepared in the internal software environment of the Fluoview FV10i microscope. The obtained photographs were then analyzed by the Fiji (ImageJ 2.0.0.0-rc69/1.52) program [23]. Nuclei by Hoechst stain, as well as the nuclei positive for p63 marker were counted with the CellCounter plugin. Internal Set Scale, Segmented line, and Measure basic tools were used to determine the length of areas of the expressing protein ZO-1. To do this, the ratio was first set of μm to pixels in the image (Set Scale), then a line (Segmented line) was manually drawn coinciding with the ZO-1expression, and finally, the area length was calculated (Measure). The total lengths were divided by the number of nuclei in the photo.

RESULTS

Primary culture behavior *in vitro*

When observing the primary tissue attachment and the beginning of the growth of epithelial cell culture, a number of manipulations contributing to the adhesion of pieces and the growth of cell culture were empirically determined, namely drying of the samples on the cultural plastic before adding the complete culture medium provides their better attachment, the samples do not float, there is less debris and fallen off differentiated cells in the culture. At first, before the cell growth initiation, it is necessary to close the incubator doors very carefully to prevent the shaking of the pieces. A separation in the first few days slows down their readhesion. This leads to the fact that the pieces floating in the medium begin to discard the debris and cells, clogging the culture. The pieces prepared for seeding should be placed on the cultural surface with the epithelial side upwards. Otherwise, they were not attached and there was no growth. The epithelial side of the explant is distinguished by the absence of whitish stripes, by relative transparency and the presence of a smooth surface with a glare. When the first signs of growth appear, it is necessary to carefully wash the culture from the detached cells and then use the nominal volume of the complete culture medium: 1 ml for a 4-well slide flask well, and 2 ml for a 35 mm Petri dish.

In compliance with the methodological conditions described above, the primary culture of the labial mucosa epithelial cells was obtained from all donors in both groups. Cell morphology in cultures corresponded to the classical “cobblestone” type; however, variability in the cell area was noted (Fig. 2, a, b), which was more pronounced in the samples cultivated in DMEM/F12 medium. In general, the specific morphology of epithelial cells and their relative size uniformity remained for the whole period of primary cultivation.

When cultivated on a complete culture medium on the basis of DMEM/F12 with 1.05 mM Ca+++, the cells were observed to leave the slice on day 3–5. As the culture grew, the zones with cells becoming larger were formed in the culture. These zones further increased in size, and large cells began to detach, forming cavities, which then were not filled with new cells. In the process of cultivation on the specified medium, many rounded unattached cells were formed. In some cases, it was noted that fibroblasts with specific morphology were distributed from a piece. In the course of observation of the primary culture on the basis of DMEM F12, the “growth wave” phenomenon, which can be described as a wave of dividing cell conglomerate propagating from a piece of tissue, was detected. At the same time, radial cell traction was formed from a piece of tissue to a “growth wave” (Fig. 2, c, e).

When cultivated on a complete culture medium on the basis of EpiLife with 0.06 mM Ca++, a later (day 5–8) release of epithelium cells from a piece was observed. In general, cell growth was slower compared to the first group. The phenomenon of “growth wave” and the cell conglomerates traces were not noted (Fig. 2, d). However, the culture obtained on the low Ca base medium was featured by the best cell morphology. Small polygonal cells with large nuclei were mainly present. The smaller areas with larger cells and a relatively smaller nucleus were found, which is typical for ripening cells. The number of detached cells was minimal. The fibroblast growth was recorded in only one case.

Immunophenotypic characterization of cells

The fluorescent immunocytochemistry detected positive expression by p63 marker in cells, which was colocated with Hoechst nuclear stain. Namely, 34.7% of such cells were detected in cells cultivated in DMEM/F12 medium (1:1) using the median (n = 3), whereas in EpiLife medium – 39.2% (median, n = 3). In both groups of cells, a weakly positive expression of vimentin was observed with some predominance in EpiLife samples. The expression of ZO-1 protein was presented along the edge of large cells. According to the data of the program analysis, the length of ZO-1 protein expression sites in terms of the number of nuclei per cell was 17.05 μm

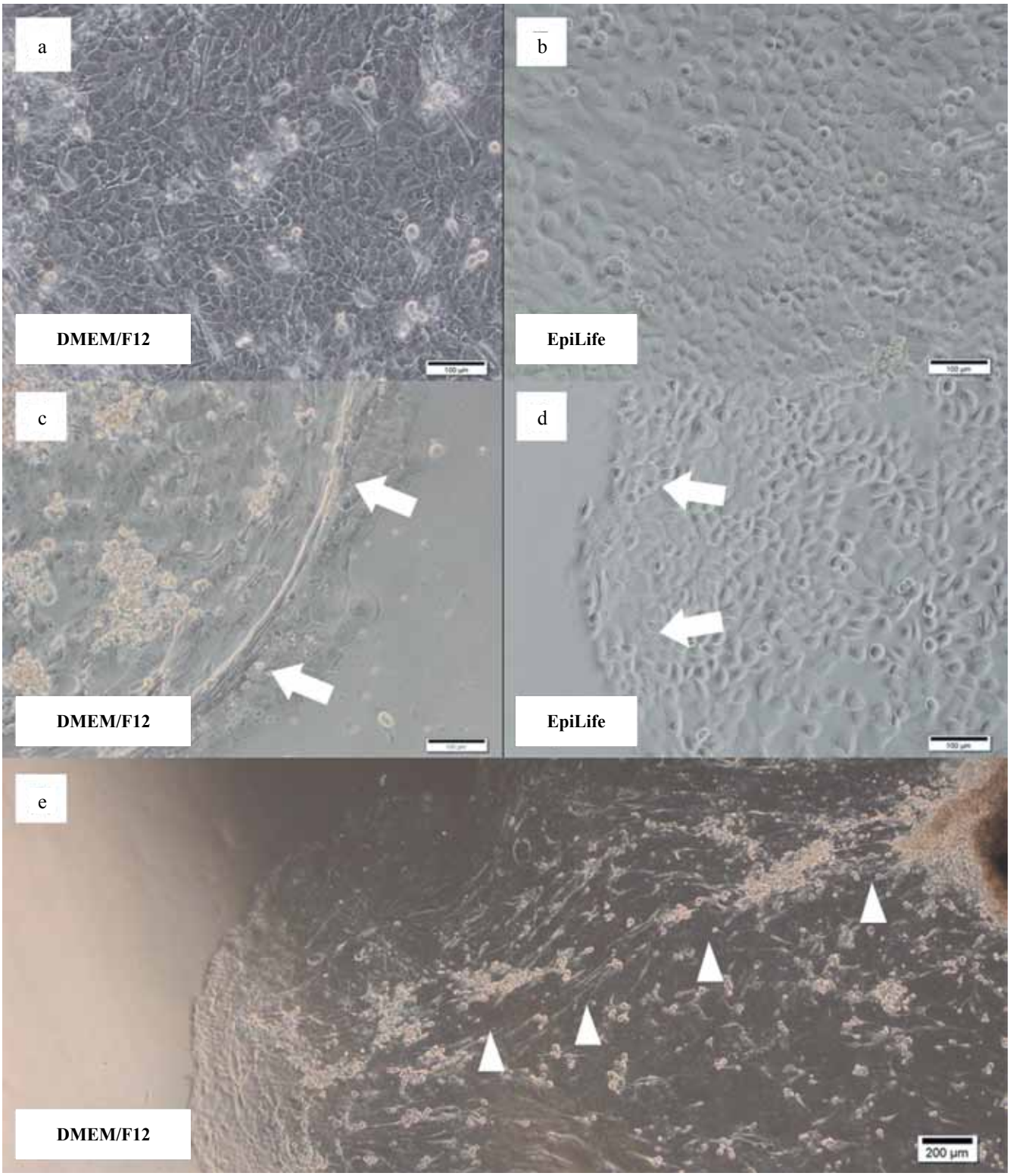

Fig. 2. Morphology of the primary culture of human labial mucosal epithelial cells obtained in DMEM/F12 (1.05 mM Ca) or EpiLife (0.06 mM Ca) media: a, b – primary cell culture had the classical "cobblestone" phenotype. Areas with large and small cells are visible; c – micrograph of the phenomenon of "growth wave" (marked by arrows); d – the absence of a "growth wave" in the epithelial cell culture on the EpiLife medium (marked by arrows); e – micrograph of the trace of conglomerates of cells from the explant (indicated by arrows); a–d – phase-contrast microscopy; e – combined photo; a–d – ×100, e – ×40

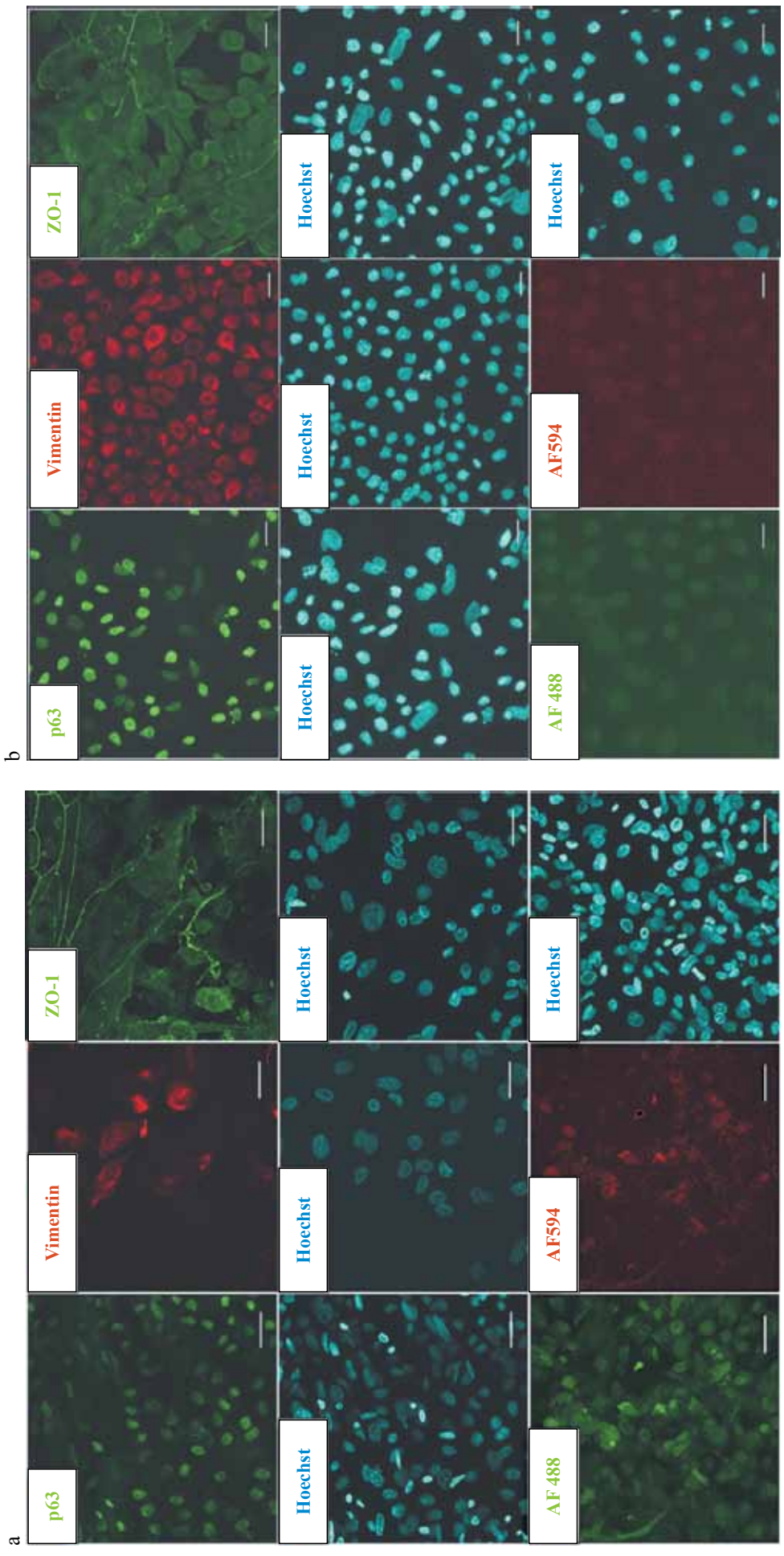

Fig. 3. Expression of immunocytochemical markers in the primary culture of human labial mucosal epithelial cells cultured in DMEM/F12 (1.05 mM Ca) (a) and EpiLife (0.06 mM Ca) media (b). The p63 marker (AlexaFluor 488), co-localized with the Hoechst nuclear dye. Vimentin (AlexaFluor 594), represented in the cytoplasm of the stained cells. ZO-1 marker (AlexaFluor 488), expressed on the edge of large cells. Control samples of fluorescent dyes Alexa Fluor 488, Alexa Fluor 594 and Hoechst (bottom row, left to right). Confocal laser scanning microscopy, ×600

per median (n = 3) for DMEM/F12 (1:1) and 5.18 μm for EpiLife.

DISCUSSION

Obtaining a stable culture of epithelial cells is a complex methodological task in general. Maintenance of epithelial cells proliferation and inhibition of fibroblast excess growth allows achieving a certain culture purity regardless of the method of cell isolation [15]. In particular, such specialized growth factors as insulin, transferrin, hydrocortisone, cholera toxin, epidermal growth factor, triiodothyronine, and Bovine Pituitary Extract can be used for the cultivation of human cheek epithelium [14]. However, to obtain a cellular construct for use in the clinic, xenobiotic components should be absent in the formulation of the medium, and the growth factors must be produced according to the rules of good clinical practice [24]. Notably, according to M. Formanek et al. [17], cholera toxin and triiodothyronine do not have a significant positive effect on the proliferation of oral cavity epithelium, in contrast to the combination of hydrocortisone, epidermal growth factor, and insulin. Therefore, these three factors were examined in the present study, and the insulin and hydrocortisone used in the formulation were released by pharmaceutical companies for use in patients.

In the present study, a low Ca (0.06 mM) medium related to selective media was used to compare the results of cultivation with a standard culture medium containing 1.05 mM Ca (DMEM/F12). This made it possible to determine the tendencies of an increase in proliferation and a decrease in the level of differentiation of cultivated epithelial cells in the low Ca medium. The feeder layers were not used in the study either, which will allow excluding feeder cells transfer during autologous cell structure transplantation to patients.

The method of cultivating explants applied in the present study has some advantages over the method of enzymatic isolation; it allows to preserve the extracellular matrix and use small tissue samples. In our opinion, the cultivation of explants in combination with a low-calcium selective medium limits the yield of resident fibroblast cells from the explant, and the presence of the best combination of specialized growth factors allows the epithelium to proliferate and occupy the cultural surface.

The results of immunofluorescent coloring in the process of labial mucosal epithelium cultivation showed high activity by p63 marker in both medium formulations. The obtained values were close to those published on the cultivation of cheek epithelial cell layers for transplantation, where it was shown that the cellular construct contained 30.7 ± 7.6% (mean ± SD) p63 positive cells [25]. The expression of vimentin, a protein of intermediate filaments, is most often associated with the mesenchymal phenotype of cultivated cells or with the phenomenon of epithelial-mesenchymal plasticity [26]. The weak expression of vimentin noted in cultures confirms that the protocol of cultivation and the formulation of media used in the work allow preserving the epithelial phenotype of cells. The expression of the dense intercellular contact protein (ZO-1) is an important marker of epithelial cell differentiation associated with the physiological process of cell attachment to each other for the formation of a flat epithelial layer [27]. In the present study, a decrease in the expression of the ZO-1 marker in the medium with 0.06 mM of calcium was noted, which is consistent with the recommendations to maintain the undifferentiated state of epithelial cells in the culture [15].

CONCLUSION

In the present study, the protocol of cultivation of human labial mucosal epithelium without feeder layer is discussed, which considerably simplifies the methodology of autologous cellular construction preparation and allows to avoid its contamination by feeder cells. The protocol allows obtaining cells with high proliferative potential using a relatively small biopsy sample. A simplified recipe of the culture medium in two variants was also tested in the work. The use of DMEM/F12 as a basic medium allows activating cell maturation, which can be used for modeling epithelium differentiation in vitro. The formulation of the medium with 0.06 mM of Ca supports a reduced level of ripening cells in the culture, and, consequently, is promising for obtaining a cellular construct and its transplantation to patients in case of corneal epithelial stem cells failure. It should be noted that these formulations of media can be improved. Thus, to completely eliminate xenogenic components, the fetal bovine serum can be replaced by autologous one, and such components as insulin, hydrocortisone, and EGF are currently produced in compliance with GMP rules.

Thus, media formulations, source, and method of cell culture described in this study may be used for the transplantation of the autologous labial mucosal epithelium in patients with bilateral limbal insufficiency.

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The authors declare no conflict of interest.

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OSTEO-REPLACEMENT PROPERTIES OF SCLERACTINIUM CORAL AQUACULTURE SKELETON (EXPERIMENTAL STUDY)

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Aim: to evaluate the osteo-replacement properties of the coral aquaculture skeleton of *P. verrucosa* and *A. abrotanoides* (CAS) on a model of a fenestral defect in the femur of rats in comparison with the natural coral skeleton of *A. cervicornis* (NCS). **Materials and methods.** CAS grown at a Russian-Vietnamese tropical research and development technology center, as well as NCS were cleaned of organic residues, crushed into 300–600 μm granules, sterilized by γ-radiation (24 kGy) and used to fill bone defects in rat femur. Three groups of animals were formed according to the number of types of coral skeleton samples. Two animals were removed from the experiment every 3, 6, 9, 12 weeks. Tissues excised from implantation zones were fixed, decalcified in EDTA, and their histological slides stained with hematoxylin-eosin were prepared. **Results.** There were no fundamental differences in the dynamics of replacement of bone defects with newly formed bone tissue after implantation of CAS and NCS. NCS, like CAS, were biocompatible and caused no inflammatory reactions in the implantation zone. In the defect area, there was good consolidation of NCS granules with the bone bed. Their bioresorption rates were also similar. Three weeks after implantation, periosteum grew over the defect zone and bone formation began by periosteal osteogenesis. By week 12, the defect area was filled with newly formed cancellous bone tissue with hematopoietic zones between the bone trabeculars. **Conclusion.** The scleractinium coral aquaculture skeleton of *P. verrucosa* and *A. abrotanoides* has osteoplastic properties similar to those of the coral skeleton of *A. cervicornis* from natural settlements.

Keywords: coral aquaculture, osteoplastic properties, marginal excision of rat femur.

INTRODUCTION

In some cases, the use of materials of natural origin to replace bone defects provides for the formation of organotypic structures in the implantation zone. For instance, good osteoplastic properties are demonstrated for coral skeleton from natural settlements (CSNS) [1–4], some chitin derivatives [5], alginates, polyoxyalkanoates [6], and silk fibroin [7]. However, the widespread adoption of some of them in clinical practice is limited by the high cost (silk fibroin), the difficulties of extraction and/or standardization of the composition (CSNS). This forces the development of alternative methods for their obtainment.

Earlier, we showed splendid bone replacement properties of CSNS *A. cervicornis* [8]. Due to the developed surface and through porosity, they were quickly populated by osteogenic predecessors, and the rate of their biodegradation corresponded to the rate of neoosteosis, which ensured organotypic replacement in the area of the bone defect. However, the limitation of CSNS production induced the study of the osteoplastic properties of their aquacultures. The employees of FSRI A.N. Severtsov Institute of Ecology and Evolution Problems of the Russian Academy of Sciences, on the basis of the Russian-Vietnam Tropical Research Technological Centre, have identified the climatic conditions for cultivation of aquaculture of some species *Pocillopora* of *Acropora*, investigated their physicochemical properties, and showed the similarity of architectonics of CSAC and CSNS, as well as significantly higher strength of CSAC [9]. We have further shown the good matrix (for cells) properties of CSAC and their biocompatibility in the subcutaneous test in small laboratory animals [10–12].

The purpose of the present stage of the investigation was to evaluate the osteo-replacing potentials of CSAC as compared with CSNS.

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MATERIALS AND METHODS

Purification of the CSAC samples (*Pocillopora verrucosa* and *Acropora abrotanoides*) and CSNS (*Acropora cervicornis*) from organic residues was carried out in several stages. At the first stage, the branches of the coral skeleton were subjected to rough mechanical cleaning using a suitable size brush with a hard synthetic bristle under running water. To remove organic residues, the skeleton was treated with a 5.0–7.5% sodium hypochlorite solution (24 h), repeatedly washed, first in running, then in distilled water in an ultrasonic cleaner ("Finnsonic", Finland, 40 kHz, 60 °C, 15 min.). Then the coral branches were mechanically crushed in a planetary ball mill ("Retch", Germany) up to a particle size of 300–600 microns. At the second stage, to clean the coral pores from coral dust, the particles were thoroughly washed in several portions of distilled water, retreated with 3% sodium hypochlorite solution (3–5 minutes) and washed again with distilled water. At the final stage of sample preparation, the coral particles were washed with ultrasound in the above-described mode, dried in a thermostat, laid out in penicillin vials, and sterilized by γ-irradiation (24 KGy).

To assess the osteoplastic potentials of CSAC and CSNS samples, a bone defect (shin bone "fenestration defect") was formed in sexually mature rat males of Wistar line weighing 180–200 g (laboratory animal breeding nursery "Andreevka branch of the Federal State Budgetary Scientific Institution Biomedical Technology Centre of the Federal Medical and Biological Agency"). The operation was performed under anaesthesia: the animals were presedated with 0.25% droperidol solution (0.5 ml, intraperitoneally), and then 0.25% ketamine solution (0.25 ml) was given intramuscularly. Further, in the position of the animal on the back, along the inner medial surface of the right thigh, about 5 mm from the knee joint, a 2–2.5 cm long skin incision was made. The skin was separated, the lower leg muscles were mobilized by moving them to the side, and the body of the shin bone was exposed. To eliminate the physiological regeneration of bone tissue, the bone was cleaned from the periosteum. Then, on the border of the upper and middle third of the bone, a "fenestration" defect was formed using drill (length – 6–8 mm, width – 1.5–2.0 mm, depth – 2.5–3.0 mm). The defect penetrated the bone canal cleaned of bone marrow. The defect area was filled with sterile CSAC or CSNS granules, and then the surgical wound was closed in layers.

Three groups of 10 animals were formed in accordance with three types of implanted materials: CSAC *P. verrucosa* and *A. abrotanoides* and CSNS *A. cervicornis*. 3, 6, 9 and 12 weeks after implantation of materials in the area of the bone defect, the sampling of material for morphological studies was carried out with taking the animals out of the experiment (under ether anaesthesia, two animals per each term). The shim bone was cut, and the bone fragment including the defect zone was removed and placed in 10% buffered formalin for fixation (7 days). Next, the material was decalcified in a 0.3 M EDTA solution (37 °C, 28–30 days). During this stage, the course of decalcification was monitored and the decalcifying fluid was replaced with a fresh portion. When the material became elastic, the residual EDTA was removed by quick rinsing in running water; samples were dehydrated and embedded into paraffin. After preparing the slides, they were stained with haematoxylin-eosin, and the light microscopy was performed using the Nicon Eclipse Ti microscope (Japan).

When evaluating the osteoconductive properties of CSAC and CSNS samples, attention was paid to presence/absence of signs of inflammation in the implantation zone, the evolution of osteoplastic material in the defect area was traced: morphological signs of its biodegradation and the appearance of de novo-formed tissue were noted, as well as the cellular composition of the regenerate, the quality of its consolidation with the parent bed, as well as the timing of formation of organotypically mature bone tissue.

Animal studies have been carried out in compliance with international bioethics rules in accordance with the requirements of the Helsinki Declaration of the World Medical Association and the rules of humane attitude to laboratory animals. During the experiments, the animals were kept in a vivarium equipped with operating and manipulation rooms, with standard food and water rations, under standard lighting and humidity conditions.

RESULTS

The results of histological studies of CSAC *P. verrucosa* and *A. abrotanoides* in comparison with CSNS *A. cervicornis* showed that there were no critical differences in the dynamics of bone defect replacement with these materials.

Thus, 3 weeks after implantation, restoration of the periosteum from dense connective tissue has occurred over the bone defect. Granules of the coral skeleton in this area (represented on the histological preparations as voids after decalcification) were walled up in the periosteum (Fig., a1, b1, c1). In some fields of vision, bone-resorbing cells were visualized at the border of the connective tissue and granules. In some places, the "tongues" of the periosteum penetrated the area of the bone defect with activation of periosteal osteogenesis. On certain preparations, it was evident that the defect areas began to be replaced by spongy bone tissue with the foci of haematopoiesis (Fig., a2, b2, c2).

In six to nine weeks after the implantation, the process of replacing a bone defect by neo-osteogenesis had been continuing: an active spongy bone formation was observed in the operating area, where the foci of bone marrow haematopoiesis were visualized between the trabeculae

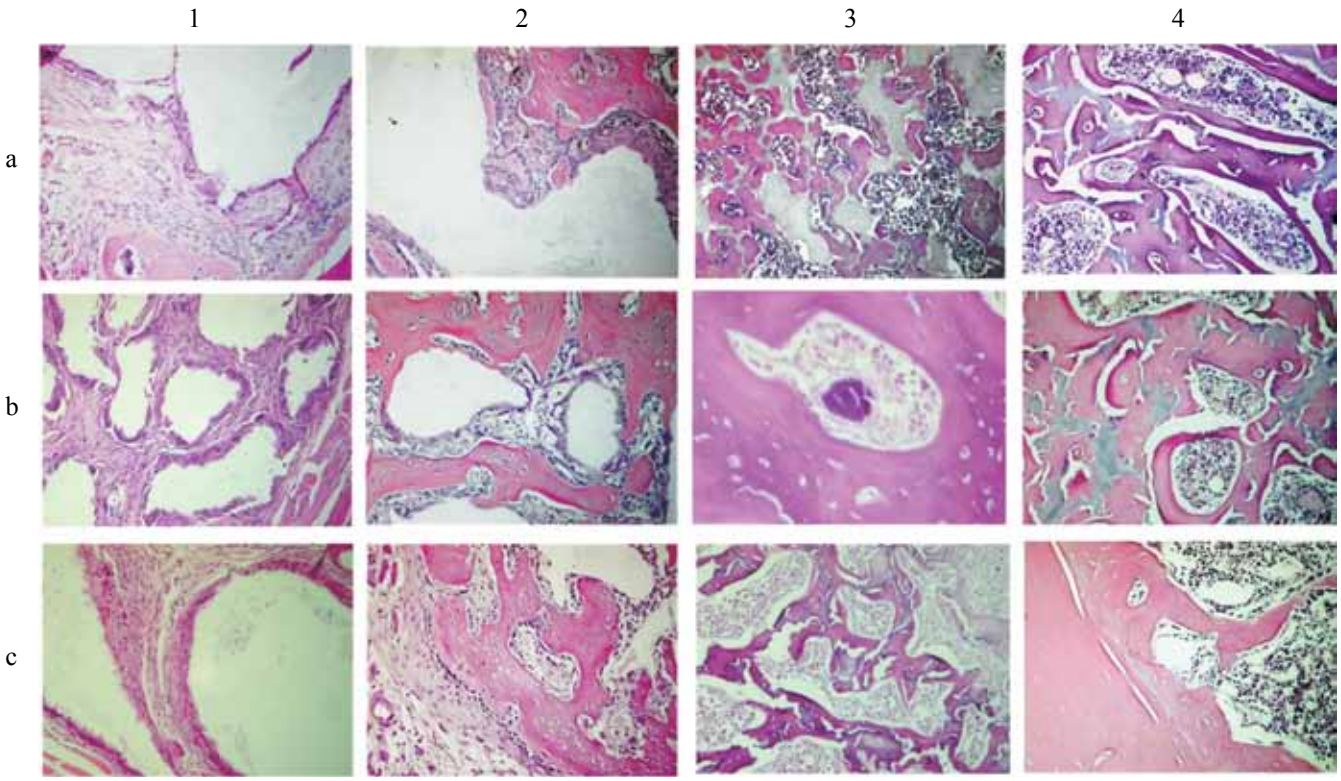

Fig. Dynamics of bone defect substitution by granules of CSAC of *P. verrucosa* (a) and *A. abrotanoides* (b) and CSNS of *A. cervicornis* (c): 1, 2 – 3 weeks; 3 – 6 weeks; 4 – 12 weeks after implantation

(Fig., a3, b3, c3). In separate fields of vision, the formation of compact bone tissue between the periosteum and the spongy bone tissue was noted. Within those timeframes, the defect area was almost completely replaced by newly formed bone tissue. However, osteogenesis cannot be considered complete due to the presence of small calcification zones in the spongy bone tissue.

In 12 weeks after the implantation, almost full completion of the osteogenesis process was observed, with organotypic replacement of the defect area with spongy bone tissue with bone marrow islets between the trabeculae of bone tissue, the primordia of osteons and the rim of compact bone tissue adjacent to the periosteum (Fig., a4, b4, c4).

DISCUSSION

The use of CSNS to replace bone defects is justified by their suitable chemical composition (calcium carbonate), strength properties superior to those of ceramic calcium-phosphate materials due to the aragonite crystal lattice [13, 14], and impurity composition close to the microelement composition of bone tissue [15]. In addition, the pronounced dependence of the rate of their passive pH degradation, as well as the ability of the bone-resorbing cells to destroy aragonite, i. e., to ensure its active biodegradation, provide the rate of utilization of the coral skeleton in the bone defect, coordinated with the rate of neo-osteogenesis. And the through porosity of corals ensures their rapid colonization by the cells and osteogenesis throughout the entire volume of the implant. It was shown that the absence of phosphorus compounds in their structure doesn't limit their use as osteo-replacing material [16–19]. ATP is probably the source of phosphorus in this case. Nowadays, some of commercial organizations offer CSNS-based products for the replacement of bone defects of various sizes and configurations ("Silorif" (Russia), "Biocoral" (France), "BoneMedik" (South Korea)). However, technogenic (tests of nuclear and other types of weapons in the oceans) and environmental (underwater volcanic eruptions) impacts can result in undesirable shifts in the microelement composition of CSNS from different water areas (an increase in the content of radioactive isotopes, sulphur, arsenic, etc). Together with the prohibition of many countries on the production of CSNS on the coastal continental shelves, this creates certain difficulties and limitations in the production of CSNS and trigger the need of elemental composition control for all the samples of this raw material.

An alternative to CSNS is the skeleton of their aquaculture used to replace bone defects in laboratory animals in the present work. Coral aquaculture was obtained on the base of Russian-Vietnam Tropical Research Technological Centre by the employees of FSRI Institute of Ecology and Evolution Problems named after A.N. Severtsov of Russian Academy of Sciences (project manager T.A. Britaev).

The cultivation of CSAC on carriers in the water column in the coastal zone, in the sector protected from waves, in the natural microenvironment, made it possible to obtain the CSAC similar to CSNS in microelement composition but superior in strength [14].

In the present study, granular CSAC samples of two families were used to replace fenestration bone defects in the femur of rats – *P. verrucosa* and *A. abrotanoides*. Previously studied CSNS *A. Cervicornis* were used as reference samples [20, 21]. In accordance with the number of sample types, 3 groups of animals were formed. We examined histological specimens from the defect area in the timeframes up to 12 weeks. No significant differences were detected in the dynamics of the replacement with newly formed bone tissue of a defect filled with CSNS and CSAC. Both CSAC and CSNS were biocompatible and did not cause inflammatory reactions in the implantation area. The speed of their bio-resorption was also similar. A good consolidation of CSAC granules with the parent bed in the area of the defect was established. The periosteum grew over the defect area and bone formation began by way of the periosteal osteosis 3 weeks after implantation. By week 12, the defect area was filled with newly formed spongy bone tissue with hematopoietic zones between the bone trabeculae.

CONCLUSION

Skeleton of the aquacultures of corals *P. verrucosa* and *A. abrotanoides* has the osteoplastic properties similar to those of the skeleton of corals *A. cervicornis* from natural settlements.

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The authors declare no conflict of interest.

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NON-ALCOHOLIC FATTY LIVER DISEASE – A RAPIDLY GROWING INDICATION FOR LIVER TRANSPLANTATION IN THE MODERN WORLD

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Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in many countries, involving about 25% of the population worldwide. This disease includes many genetic, metabolic, and environmental factors. It is closely associated with insulin resistance, metabolic syndrome, obesity, diabetes, and many other diseases. NAFLD is characterized by macrovesicular steatosis of the liver. In the natural course of NAFLD simple steatosis progresses to nonalcoholic steatohepatitis (NASH), fibrosis and ultimately, cirrhosis and hepatocellular carcinoma. Cirrhosis with Nash and hepatocellular carcinoma is an indication for liver transplantation. Obesity is a growing problem in liver transplant candidates. Cardiovascular complications related to metabolic syndrome and NASH recurrence in the transplanted liver may affect the outcome of surgery in these patients. The results after transplantation are similar to the results of liver transplantation for other indications, but cardiovascular complications are the main cause of death in patients with NASH after surgery.

Keywords: nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, liver transplantation.

INTRODUCTION

The initial description of non-alcoholic fatty liver disease (NAFLD) dates back to 1980 and belongs to J. Ludwig et al. [1]. NAFLD is defined as an abnormal accumulation of fat in the liver in the absence of other chronic liver diseases, for example, hepatitis C, or secondary steatosis with drug addiction, alcoholism, hereditary metabolic disorders, etc. Due to the ongoing persistence of obesity, NAFLD has become the most common cause of chronic liver disease worldwide, including in developing countries [2, 3]. In developing countries, up to one-fifth of the population suffer from NAFLD, and in developed countries, prevalence reaches 35% [4].

This problem became most severe first in USA and Western Europe. In Russia, NAFLD is also becoming an increasingly common disease, which has been drawing the attention of Russian researchers. Several research papers on this subject have been published in recent years [5–9]. The study of the clinical and laboratory-instrumental features of liver and biliary tract function, as well as the effectiveness of combination therapy in NAFLD, were the focus of a major study by E.V. Suchkova [10]. Clinical and metabolic features of non-alcoholic fatty liver disease in children are described in the work of E.N. Kutyreva et al. [11]. Based on epidemiological studies in Russia in 2015 under the editorship of Professor V.T. Ivashkina from the Russian Academy of Sciences, guidelines for doctors on diagnosis and treatment of non-alcoholic fatty liver disease were published [12].

In the natural course of NAFLD, the disease progresses, and simple fatty liver transforms into non-alcoholic steatohepatitis (NASH) with the development of fibrosis, which progresses to cirrhosis [13]. NAFLD is the main cause of hepatocellular carcinoma, while other chronic diseases lead much less frequently to liver cancer [14, 15].

With the development of NASH-related cirrhosis, and even more so with hepatocellular carcinoma, liver transplantation is a non-alternative treatment method [2, 4, 16–18].

EPIDEMIOLOGY OF NAFLD

Non-alcoholic fatty liver disease is highly prevalent on all continents. The prevalence of NAFLD in South America is 31%, in the Middle East – 32%, in Asia – 27%, in USA – 25.8% and in Europe – 23%. The global NAFLD prevalence is in the range of 22–29%, with an average of 25% [19]. NASH prevalence is estimated to be in the range of 1.5% to 6% [20, 21]. However, among patients whose diagnosis was based on the results obtained from liver biopsy, NASH was detected in 59.1% of cases [19].

Two major epidemiological studies of NAFLD were carried out in Russia. In the first of them (DIREG 1, 2007), it was found that the average NAFLD prevalence

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in 30,754 outpatients examined was 27%; the southern regions of the European part of Russia had the lowest prevalence (19.6%), while Siberia accounted for the highest (31.6%) [22–24].

The world's largest study, the second Russian epidemiological study (DIREG 2, 2013–2014) on NAFLD prevalence was conducted in 16 cities across Russia. It featured over 50,000 outpatients. According to the DIREG 2 study, NAFLD prevalence significantly increased by 10% to reach 37.3% against the first DIREG 1 study [24, 25]. L.K. Palgovoy et al. [24] presented the results of a study on NAFLD prevalence in the Northwest region of Russia. The incidence of NAFLD in this region was much higher than the national average: Of the 3,769 patients examined in this region, NAFLD was diagnosed in 49.1% of them.

In Mexico, a retrospective multicenter study of the etiology of cirrhosis was conducted from January 2012 to December 2017. A total of 1,210 patients were examined. The most common causes of cirrhosis were hepatitis C virus (36.2%), alcoholic liver disease (31.2%) and NASH (23.2%). The least common were hepatitis B virus (1.1%), autoimmune disorders (7.3%) and other conditions (1.0%). It was noted that in recent years, NAFLD, as an etiology of cirrhosis, increased by 100% and will soon become one of the most frequent etiological causes of cirrhosis in Mexico [26].

NAFLD was reported in Japan almost 50 years ago in genetically susceptible people with irrational (excess) nutrition. People in Asian countries are especially susceptible to NAFLD. Prevalence ranges between 20% (China), 27% (Hong Kong) and 15–45% (South Asia, Southeast Asia, Korea, Japan, and Taiwan) [27].

A recent study [28] presented a very disappointing picture obtained by predicting the progression of NAFLD prevalence in the United States until 2030. It was forecasted that prevalent NAFLD cases would increase by 21% – from 83.1 million in 2015 to 100.9 million in 2030 and NASH prevalence would increase by 63% from 16.52 million to 27.00 million. In 2030, the overall prevalence of NAFLD among the adult population aged 15 years and above is projected at 33.5% in 2030. Between 2015 and 2030, prevalent NAFLD cases among the median age will increase from 50 to 55 years. In 2015, approximately 20% of the total number of NAFLD patients suffered from NASH, and their number is projected to increase to 27% by 2030. By 2030, incidence of decompensated cirrhosis will rise by 168% to 105,430 patients, while incidence of hepatocellular carcinoma will rise by 137% to 12,240 patients. Mortality from NAFLD is expected to increase 178% to about 78,300 deaths in 2030 [28].

NAFLD is found not only in adults but also in obese children [11, 29]. The study by E.N. Kutyreva et al. [11] included 869 obese and overweight children between the ages of 3 to 17 years – an average of 12.2 ± 0.2 years. NAFLD was diagnosed in 335 (39%) patients. Based on clinical and laboratory examination, all the children were divided into two groups: Group 1 – non-alcoholic fatty liver (NAFL) (n = 228), Group 2 – NASH (n = 107). The prevalence of NASH increased side-by-side with an increase in the length of obesity and its degree.

NAFLD distribution by gender varies by age: the lowest male/female ratio (0.94) is observed among people under 30 years old, and the highest (1.31) among people aged 40–49 years [28]. The Third National Health and Nutrition Examination Survey in the United States (NHANES-III), which included 3271 people aged 60 years and above, found that NAFLD was prevalent in the elderly. NAFLD is associated with higher risk of mortality for people aged 60–74 years but was lower in those older than 74 years [30]. NAFLD prevalence is also affected by ethnic differences. In the USA, the highest rate of NAFLD is seen among Hispanics (45%), followed by whites (33%) and even lower among African Americans (24%) [31].

In different regions of the world, both similarities and differences in the epidemiology of NAFLD have been noted. For example, the condition is associated with obesity and insulin resistance in most individuals in Western countries, while the disease manifests at a lower body mass index in Asia, and many patients seem to lack insulin resistance [32].

PATHOGENESIS OF NAFLD

NAFLD is a complex and multisystem disease that has a high socio-economic impact [33]. It is believed that the pathogenesis of NAFLD is based on metabolic syndrome, insulin resistance and hyperinsulinemia [34]. These conditions are associated with the obesity epidemic in many countries of the world, especially in the United States [7, 9, 19, 35]. Most NAFLD patients are overweight (body mass index of 26.3–34.0 kg/m^2, a median of 29.4 kg/m^2) [36].

Insulin resistance is increasingly recognized as a key factor linking metabolic syndrome and NAFLD. Insulin resistance leads to insufficient inhibition of hepatic gluconeogenesis, increased lipid accumulation, and decreased glycogen synthesis [37]. Circulation of inflammation-enhancing free fatty acids is increased, overexpression of proinflammatory cytokines occurs and Kupffer cells are activated, which also promotes insulin resistance. Endoplasmic reticulum stress and inflammation, in turn, exacerbate and maintain the insulin-resistant state, forming a vicious circle [38].

An increase in the total cholesterol to high-density lipoprotein cholesterol ratio is a predictor of both cardiovascular disease and NAFLD [39]. Since in addition to insulin resistance, NAFLD signs include hypertriglyceridemia, a mandatory study of not only carbohydrate but also lipid metabolic processes in patients with liver

steatosis is necessary for the timely correction of disorders in order to reduce progression of this disease [7, 9].

Metabolic syndrome occurs with various risk factors, such as obesity, type 2 diabetes or dyslipidemia. The prevalence of this syndrome is increasing worldwide along with an increase in obesity, and there is evidence to suggest a link between NAFLD and metabolic syndrome [34, 40]. NAFLD is also considered the "hepatic manifestation" of metabolic syndrome [34]. It is important to note that most patients with NAFLD have at least one risk factor for metabolic syndrome [38].

Numerous clinical data have confirmed the existence of a bi-directional relationship between NAFLD and various components of the metabolic syndrome, especially type 2 diabetes [34, 41, 42]. A recent study [36] has shown that almost 50% of NAFLD patients suffer from diabetes. It should be borne in mind that NASH is only one of the risk factors for developing diabetes after liver transplantation. This complication is a multicausal pathology. The main reason for developing post-transplant diabetes is the use of calcineurin inhibitors as immunosuppressive agents. Pretransplant obesity and HCV infection are additional risk factors. Post-liver transplantation diabetes mellitus develops in up to 30% of liver transplant recipients [43].

Clinical evidence also suggests that NAFLD may contribute to the development of cardiovascular disease [14]. The risk of developing hypertension and atherosclerosis is parallel to NAFLD severity. A close relationship was found between NAFLD and mortality from these diseases [14, 44, 45].

Based on a study of a database of patients requiring liver transplantation (n = 138,021), type 2 diabetes, obesity, age 60 and above, female gender, and white race were found to be the strongest predictors of NASH. Type 2 diabetes was more common in patients with NASH (53%) than in patients with cryptogenic cirrhosis (29%), alcoholic cirrhosis (16%) and autoimmune hepatitis (16%). Obesity was more common in patients with NASH (65.3%) than in the other three groups (33–42%). The NASH patient group had more white people (82.3%) and fewer black, Hispanic and Asian people than in the other three groups. Hepatocellular carcinoma was more often observed with NASH (19% *vs*. 9–13% in other groups). Incidence of tumor development did not depend on obesity and type 2 diabetes [46].

NASH compared with non-alcoholic fatty liver (NAFL) is significantly more often accompanied by dyslipidemia (72%), hyperinsulinemia (37%), formation of metabolic syndrome (39%) and a low rate of fat oxidation (58.01 ± 8.02 g/day and 78.55 ± 4.85 g/day, respectively) [11].

In recent years, it has been shown that there is genetic predisposition in NAFLD [14, 29, 31, 47, 48]. Obesity enhances the genetic risk of NAFLD, which is associated with the PPNPLA3 p.I148M, TM6SF2 p.E167K and GCKR p.P446L polymorphisms [49, 50]. In an East European population, it was shown that PNPLA3 and RNF7 gene variants are associated with the risk of developing liver fibrosis and cirrhosis [51].

Genetic studies have revealed some genetic modifiers that influence the severity and progression of the disease, for example, the PNPLA3 (patatin-like phospholipase domain-containing protein 3) gene variant [48]. It was also found that epigenetics, particularly DNA methylation, increases insulin resistance and NAFLD severity [52].

Association of IL6R gene polymorphic variant rs2228145(C>A) with the development of NASH in Karelia residents has been found. The risk of developing NASH is more than 2-fold higher in carriers of CC genotype by rs2228145 polymorphic marker than in carriers of other genotypes. Plasma IL-6 levels and the content of IL6R gene transcripts in the peripheral blood leukocytes are higher in NASH patients than in healthy people. Gene IL6R polymorphic variant rs2228145(C>A) is probably involved in genetic predisposition of the Karelian population to NASH [53].

Thus, the main independent predictors of NAFLD and, by inference, potential targets for treatment are metabolic syndrome, insulin resistance, increased serum uric acid, alanine aminotransferase and serum total cholesterol [54]. Insulin resistance related to metabolic syndrome, being the main pathogenetic trigger that, combined with adverse genetic, humoral, hormonal, and lifestyle factors, accelerates development of NAFLD [14].

The pandemic of obesity and its associated complications are rapidly changing the epidemiology of many types of cancers, including hepatocellular carcinoma. NAFLD is becoming a major cause of development of hepatocellular carcinoma, with a steadily growing trend compared to viral or alcohol-induced chronic hepatitis. The higher prevalence of NAFLD in the general population and the likelihood of hepatocellular carcinoma occurring in a non-cirrhotic liver are the most disturbing aspects. Currently, systemic and hepatic molecular mechanisms involved in hepatocarcinogenesis, as well as potential early markers of hepatocellular carcinoma, are being comprehensively studied [15].

DIAGNOSIS OF NAFLD

Considering the dramatic increase in NAFLD prevalence, the urgent need to develop non-invasive, simple, reproducible and reliable methods for diagnosing this disease has long been talked about. Such methods can be useful not only in NASH diagnosis but also in evaluating response to treatment and further prognosis [14, 36]. Non-invasive serum biomarkers are a simple means of sequential observation [55]. To diagnose the progression of post-liver transplant NAFLD-related fibrosis, numerous non-invasive methods have been developed, which are described in detail in the work of Z. Galvin et

al. [36]. However, to date, despite numerous limitations, liver biopsy is the most accurate method for diagnosing fibrosis.

The final correlation when using any new test is carried out by comparing with the results of a study of liver biopsy samples. Histological analysis of liver biopsies remains the "gold standard" test against which other methods of assessment for the presence and amount of hepatic injury due to NAFLD are measured. Histological evaluation remains the sole method of distinguishing liver steatosis from advanced forms of NAFLD, i. e. NASH, assessing the degree of fibrosis and diagnosing hepatocellular carcinoma [14, 56]. Liver biopsy is both confirmation of the diagnosis and evaluation and semiquantitation of injury to the parenchyma, fibrosis, and evaluation of architectural remodeling of the liver [56].

However, liver biopsy suffers from challenges. First is that the procedure is invasive. Secondly, a small amount of biopsy. An adequate biopsy represents only 1/50,000–1/65,000 of this large organ. Therefore, puncture needles of a suitable size and sampled area should be carefully chosen. Thirdly, there may be morphological differences between the right and left lobes of the liver. Fourthly, the length of the biopsy is critical. With a ≥1.5 cm length, diagnosis is much more accurate than with a <1 cm length. Finally, experienced pathologist is important to correctly interpret liver biopsy results [56].

PATHOMORPHOLOGY OF A LIVER WITH NAFLD

In NAFLD, a wide range of histological changes occurs, ranging from non-alcoholic hepatic steatosis to severe NASH [57]. About a quarter of patients with NAFLD develop NASH [3]. Therefore, morphological changes in a liver with NAFLD depend on the stage of the disease. According to the latest definition by the American Association for the Study of Liver Diseases (AASLD), two components are necessary for diagnosis of NAFLD: 1) evidence of hepatic steatosis either by imaging or histology; and 2) lack of secondary etiologies of hepatic steatosis, including significant alcohol consumption, adverse drug effects and/or hereditary disorders [57].

Histologically, NAFLD is subdivided into NAFL and NASH.

Non-alcoholic fatty liver (NAFL). At the NAFL phase, fatty degeneration of hepatocytes takes place with accumulation of triglycerides in their cytoplasm. The presence of more than 5% of hepatocytes with macrovesicular steatosis is the minimum criterion for histological diagnosis of NAFL. Fatty liver is divided into three by severity: I (mild) – above 5% and up to 33% of hepatocyte steatosis; II (moderate) – above 33% and up to 66%; III (severe) – above 66%. Steatosis is usually macrovesicular, but may be mixed (a combination of macrovesicular with microvesicular hepatocyte obesity). An intracytoplasmic large fat droplet or a few small drops displace the core to the periphery of the hepatocyte. A distinctive feature of steatosis in adults, unlike in children, is that steatosis initially affects hepatocytes in acinar zone 3 (perivenular). As the disease progresses, it spreads to the entire acinus [56]. With NAFL, foci of lobular and/or portal inflammation and lipogranulomas may be seen. Lobular inflammation is usually mild, consisting of a mixed inflammatory infiltrate, composed of lymphocytes, a small amount of eosinophils, and, sometimes, a few neutrophils. Foci of chronic lobular inflammation, consisting mainly of lymphocytes, are rarely seen. However, lobular and portal inflammation in NAFL are very rare, and their presence indicates the progression of the disease to NASH [58]. Slight siderosis might occur in periportal hepatocytes and/or pan-acinar reticulo-endothelial cells [56].

Non-alcoholic steatohepatitis (NASH). The minimum criteria for making histological diagnosis of NASH in adult patients, in addition to steatosis, include hepatocellular injury (usually in the form of ballooning degeneration) and lobular inflammation, typically localized in acinar zone 3 [31]. With ballooning degeneration, ballooned hepatocytes are enlarged, with swollen, rarefied, pale cytoplasm and, usually, show a large, hyperchromatic nucleus. Loss of hepatocyte keratins, 8 and 18, as detected by immunostaining, might help in the objective identification of ballooned hepatocytes, in the presence of which more aggressive course of the disease and high incidence of cirrhosis and metabolic syndrome are noted. Increased hepatocyte apoptosis, as well as hepatocyte necrosis, are also morphological signs of NASH. In NASH, hepatocytes can have giant round or needle-shaped mitochondria. Giant mitochondria are more commonly observed in hepatocytes with microvesicular steatosis. Electron microscopic examination shows that in these mitochondria, there is loss of cristae, membranes and paracrystallin inclusions. NASH is characterized by the presence of large vacuolated nuclei usually observed in periportal hepatocytes. Liver biopsy specimens contain lobular microgranulomas (Kupffer cell aggregates) and lipogranulomas [56].

Fibrosis is not a necessary diagnostic feature of NASH. Nevertheless, based on a meta-analysis of the results of a punctate study of paired liver biopsies specimens performed at least 1 year apart, liver fibrosis progresses in patients with NASH [59]. Fibrosis typically begins in acinar zone 3 and has a fine mesh pattern, since collagen and other components of the extracellular matrix of connective tissue grow perisinusoidally and around hepatocytes. Perisinusoidal fibrosis in NASH, as in other chronic liver diseases, is probably the result of Kupffer cell activation. In hepatocytes in acinar zone 3, there may be eosinophilic intracytoplasmic inclusions located close to the nucleus (Mallory bodies). Portal fibrosis usually occurs after appearance of perisinusoidal and pericellular fibrosis. More severe fibrosis (F2–F4)

develops later. Cirrhosis (F4) in NASH is macronodular or mixed. Steatosis may not persist with the progression of fibrosis, especially in cirrhosis, as well as in architectural remodeling of the liver [56]. Progression of the disease to cirrhosis can lead to hepatocellular carcinoma and liver failure [31].

Histologically, NASH is often indistinguishable from liver disease caused by alcohol use. Therefore, in diagnosis of NASH, a thorough clinical and anatomical analysis is required to exclude the alcoholic nature of the disease. Unfortunately, in most patients with NASH, cirrhosis is diagnosed by chance. Although its timely diagnosis is of great clinical importance, since cirrhosis has a high probability of developing other liver diseases, including hepatocellular carcinoma [60].

TREATMENT OF NAFLD

Radical lifestyle changes aiming at normalizing body weight is the basic therapeutic intervention to manage this disease. Insulin sensitizers, antioxidants, lipid lowering drugs, incretin-based drugs, weight loss medications and bariatric surgery may be necessary for management in some cases along with lifestyle measures [61]. G.C. Farrell et al. [27] believes that public health efforts to limit excess nutrition and reduce insulin resistance are necessary to prevent and/or reduce the development of NAFLD and its adverse health effects, such as type 2 diabetes, cardiovascular disease, cirrhosis and liver cancer. Comprehensive treatment of NAFLD patients, including the use of drug therapy, balneopeloid therapy and internal intake of mineral water, provides significantly faster relief of clinical manifestations of the disease and restores the motor activity of the gallbladder, compared to drug therapy alone [62]. Conservative and surgical treatment of NAFLD is described in detail in the Guidelines for physicians published by the Russian Society for the Study of the Liver [12]. In the terminal stage of the disease, orthotopic liver transplantation is the non-alternative treatment option [14].

LIVER TRANSPLANTATION FOR NASH-RELATED CIRRHOSIS

Although in most patients, the course of NAFLD is benign, in some patients, NASH develops with subsequent cirrhosis and the risk of developing decompensation and/or hepatocellular carcinoma. Both conditions are indications for orthotopic liver transplantation [63]. Due to increased incidence of metabolic syndrome and its components, NASH-related cirrhosis and NASH-related hepatocellular carcinoma will soon become the leading indication for liver transplantation in USA and in many other countries of the world [2, 64–68]. In addition, due to increase in the incidence of NAFLD, there are more steatotic donor livers and fewer "normal" organs for transplantation [2]. Despite the increase in the number of available donor organs, waitlist mortality remains a serious concern [66].

Recently, the International Liver Transplantation Consensus Statement on End-stage Liver Disease Due to NASH have been published. The purpose of these guidelines is to highlight specific features commonly observed in NASH patients and to present strategies to optimize the evaluation of pretransplant patients and waitlist survival [63].

Patients who have NASH and are candidates for liver transplantation are usually burdened with various comorbidities [33], such as obesity, type 2 diabetes, cardiovascular disease and kidney disease [2, 63, 64]. Compared with other liver transplant recipients, recipients with NASH are more likely to have diabetes mellitus (73.5% *vs*. 20%, $P < 0.01$), metabolic syndrome (83.3% *vs*. 37.8%, $P < 0.01$), cardiovascular diseases (29.4% *vs*. 11.1%, $P < 0.01$), urogenital infections ($P = 0.03$) [67]. Comorbidities directly affect evaluation and selection of patients, waitlist morbidity and mortality, and, ultimately, posttransplant outcomes [63]. In addition, recipients with NASH are at increased risk for pre-transplant portal venous thrombosis with decompensation of the native liver [69].

Compared with other recipients, recipients with NASH are older [18]: 58.5 ± 8.0 *vs*. 53.0 ± 8.9 years; $P < 0.001$ [70]; 59.2 *vs*. 54.8 years, $P = 0.01$ [69]. They often have a high body mass index [18]: 63% *vs*. 32%; $P < 0.001$ [70]; 61.8% *vs*. 8.1%, $P < 0.01$ [67] and higher MELD [18]. Among them there are more women (47% *vs*. 29%; $P < 0.001$) and they are more likely to have hepatocellular carcinoma (12% *vs*. 19%; $P < 0.001$) [70].

The most acute liver transplant problem in end-stage NASH and hepatocellular carcinoma is in the United States. Numerous recent studies have shown that NASH-related cirrhosis is rapidly becoming the leading indication for liver transplantation in the US [17, 71, 72]. After the start of the use of safe and effective direct-acting antiviral drugs in 2015, the need for liver transplantation in hepatitis C patients decreased. Therefore, according to current trends, it is suggested that in the US, NASH will overtake hepatitis C as the most common indication for liver transplantation over the next 10 years [4].

The trend towards an increase in the number of patients who underwent liver transplantation for NASH-related cirrhosis is clearly evident from several publications listed below. In the United States, 53,738 liver transplants were performed between January 1, 1997 and October 31, 2010. Towards the end of this period, NASH became the fourth most common indication for liver transplantation. The proportion of liver transplants performed for NASH-related cirrhosis increased dramatically from 1.2% in 1997–2003 to 7.4% in 2010 [73].

Another study showed that the number of patients with NASH-related cirrhosis increased from 1.2% in 2001 to 9.7% in 2009. At that time, NASH was the third

most common indication for liver transplantation in the United States [70].

Between 2004 and 2013, in the liver transplant waitlist in the United States, the number of patients with NASH increased by 170% (from 804 to 2174), with alcoholic liver disease – by 45% (from 1400 to 2024), and with HCV – by 14% (from 2887 to 3291), whereas the number of patients with HCV infection in combination with alcoholic liver disease decreased by 9% (from 880 to 803). In 2013, NASH became the second leading etiology of liver disease after HCV among patients awaiting liver transplantation in the United States [74].

To evaluate the incidence of liver transplantation associated with NASH, a retrospective cohort study utilizing the UNOS/OPTN database for 2003–2014 was conducted [75]. Overall, 63,061 adult patients, including 8262 (13.11%) NASH patients, underwent liver transplantation during this period. The incidence of NASH surpassed alcoholic liver disease, and since 2008, has become the second leading indication for liver transplantation. In 2014, 17.38% of all liver transplants were performed due to NASH. From 2003 to 2014, the number of liver retransplantations with NASH increased by 162%, with HCV – by 33%, and with alcoholic liver disease – by 55%.

In 2016, a total of 7841 liver transplants were performed in the United States. The average waiting time for an operation is 11.3 months [66]. The number of patients on the liver transplant waiting list and the number of liver transplants for HCV decreased, but the number of patients with NAFLD increased [17].

Improvement in the diagnosis of native liver diseases has shown that patients suffering from NASH cirrhosis often "hide" under the flag of cryptogenic cirrhosis. Therefore, the number of patients diagnosed with NASH in the United States from 2002 to 2016 increased from about 1% to 16%, while cryptogenic cirrhosis decreased from 8% to 4% [76].

NASH is thought to be a rare indicator for liver transplantation in young people. However, recently published studies [72] have shown that this view is wrong. A total of 5157 young adults (54% were men, 23% obese) underwent liver transplantation and the outcomes were analyzed. The median age and body mass index were 31.6 ± 6.7 years and 26.3 ± 6.1 kg/m, respectively. The incidence of liver transplantation performed for NASH-related cirrhosis increased from 0.53% in 2002 to 4.46% in 2012. NASH was the most rapidly growing indication for liver transplantation among all other etiologies with a 14% increment per year ($P < 0.001$). The 5-year post-liver transplantation survival were comparable between NASH and non-NASH recipients. However, transplant survival was lower (63.5% *vs*. 81.4%, $P = 0.003$), and retransplantation cumulative rates were higher in NASH recipients compared with those with other metabolic liver diseases (12.7% *vs*. 4.2%, $P = 0.046$). Thus, NASH is the fastest-growing indication for liver transplantation among young adults in the US aged 18 to 40 years, and currently accounts for almost 5% of all liver transplants in this age group.

In Nordic countries, NASH is also a growing indication for liver transplantation. Of the 4,609 patients listed for liver transplantation, the number of patients with NASH increased from 2.0% in 1994–1995 to 6.2% in 2011–2015 ($P = 0.01$) and became the second fastest-growing indication [18].

The global upward trend in obesity and diabetes has made NASH one of the leading indications for orthotopic liver transplantation in Australia and New Zealand [4]. This study showed that from 1994 to 2017, of 5016 patients listed for liver transplantation, the percentage of patients with NASH increased significantly ($P < 0.001$): from 2.0% in 2003 to 10.9% in 2017. In 2017, NASH was the third leading cause of chronic liver disease among wait-list registrants behind hepatitis C virus (29.5%) and alcoholic fatty liver disease (16.1%). Similarly, there was significant increase in the percentage of patients with NASH undergoing liver transplantation [4].

POSTTRANSPLANT RECURRENCE OF NASH

Development of liver graft steatosis is a significant problem after surgery, which may happen as a recurrence of pre-existing disease or de novo NAFLD [2, 33]. Recurrent NASH following liver transplantation occurs in connection with the continuation of the action of NAFLD risk factors. Additionally, immunosuppressive therapy has influence on metabolic balance, triggering insulin resistance, diabetes mellitus, hypertension, hyperlipidemia and obesity. No statistically significant difference in steroid dosage, cholesterol and triglyceride levels and body mass index was found in patients with recurrent NASH compared with patients without a recurrence [77]. Nevertheless, many patients develop posttransplant metabolic syndrome. Considering that NAFLD is a manifestation of metabolic syndrome, it is not surprising that both recurrent and *de novo* NAFLD occur after liver transplantation [78]. Posttransplant recurrence of NASH was more likely to occur in patients who had metabolic syndrome, hypertension, or insulin-dependent diabetes mellitus [77].

Patients with recurrent NAFLD have a higher risk of cardiovascular disease and kidney dysfunction [33], which may affect the outcome of liver transplants in these patients [78].

The first study on NASH recurrence rate after liver transplantation is from W.R. Kim et al. [79]. NASH was diagnosed based on histological examination of a removed native liver. Patients with significant alcohol consumption were excluded from the study. Of 622 liver explants, 8 patients had features consistent with NASH. All patients were female with a median age of 58. Fifteen months following liver transplantation, six

patients developed persistent fatty infiltration in their graft. In two patients, transition from mild steatosis to steatohepatitis and early fibrosis was observed over one to two years. The authors concluded that patients who underwent liver transplantation for NASH may develop recurrent steatosis shortly after transplantation, with possible progression to NASH and fibrosis.

Later, a report was published about recurrence of NASH in a 58-year-old woman who drank no alcohol and who underwent a liver transplant in mid-1993. After the operation, she suffered acute rejection crisis, which was successfully stopped, and by the ninth week after surgery, liver function tests returned to normal. However, at the 66th week after surgery, with persistent moderate increase in the level of alkaline phosphatase and gamma-glutamyl transferase, transplant biopsy unexpectedly revealed NASH recurrence. A second biopsy (76 weeks after liver transplantation) revealed NASH-related cirrhosis. A third biopsy at week 87 confirmed cirrhosis. The patient did not drink alcohol. Multi-targeted polymerase chain reaction was negative for HCV [80].

In a retrospective single-center study of 46 patients with NASH and 37 patients with cryptogenic cirrhosis who underwent liver transplantation between 1996 and 2008, 20 patients showed recurrent NASH, on average, 45.7 months after surgery [77].

In one of the latest studies comprising 56 patients, on average, 75 months after a liver transplant, ultrasound elastography measurements consistent with no fibrosis (42.9%) or F1–F2 fibrosis (30.4%), advanced fibrosis (F3) was noted in 26.8%, whereas F4 (clinically compensated) was 5.4% of patients. Thirty-four patients had liver biopsy on average 47 months after liver transplantation: 41.2% were diagnosed with recurrent NASH, bridging fibrosis was noted in 20.6% of patients, but no patients had cirrhosis [65].

Bhagat et al. [77] reported recurrent NASH in 33% of patients six months following liver transplantation. Recurrence of NAFLD five years following liver transplantation, according to literature sources, occurs in a wide range from 10.0% to 100.0%, and NASH from 4.0% to 28.0%. respectively [36].

DE NOVO POSTTRANSPLANT NAFLD

De novo posttransplant NAFLD is one of the serious conditions for patients suffering from various liver diseases [2, 33]. Z. Galvin et al. [36] identifies five predictors of *de novo* NAFLD development: weight gain, high body mass index, HCV infection, and sirulimus therapy. In the absence of these factors, *de novo* NAFLD develops in only 5.4% of patients, and in the presence of all five factors, NAFLD occurs in 100% of patients. All of these factors are associated with insulin resistance, and this may be the main reason for development of *de novo* NAFLD.

On average, three years after liver transplantation, one third of biopsy specimens showed histological signs of *de novo* NAFLD [36]. The authors emphasize that these were not protocol biopsies, therefore, based on biopsy data, it is impossible to determine the actual incidence of *de novo* NAFLD. About half of the patients had simple steatosis (48.2%), and the other half had NASH (51.8%). The incidence of *de novo* NASH in this study was significantly higher than that found by other researchers. The authors attribute this to the fact that patients were older at the time of liver transplantation, among them there were more diabetic patients and patients with a higher body mass index. Histological evaluation of liver transplant biopsy samples showed significant fibrosis (F2–3) in almost 40.0% of patients with *de novo* NAFLD, and cirrhosis (F4) in more than 5.0% of patients. Moreover, the rate of transplanted liver fibrosis in *de novo* NAFLD in patients suffering from HCV or autoimmune diseases is higher than in the native liver of recipients suffering from NAFLD.

There are several reasons for accelerated progression of posttransplant fibrosis: rapid weight gain after surgery, resumption of metabolic syndrome and adverse effects associated with immunosuppressive therapy such as arterial hypertension, dyslipidemia, insulin resistance and diabetes mellitus [3]. Development of the main one – *de novo* metabolic syndrome is facilitated by high steroid dosage after liver transplantation (5.2 ± 2.4 mg/day *vs*. 7.1 ± 4.7 mg/day, P = 0.014) [81]. Within two years following liver transplantation, *de novo* metabolic syndrome affected 32.9% of 170 patients. Multivariate analysis identified glycosylated hemoglobin levels equal to or higher than 5% (P = 0.003), diabetes mellitus (P = 0.002), and arterial hypertension (P = 0.009) as independent risk factors for developing *de novo* metabolic syndrome. Incidence of metabolic syndrome correlates with high steroid dosage (5.2 ± 2.4 mg/day *vs*. 7.1 ± 4.7 mg/day, P = 0.014), with NAFLD (P = 0.001) and dyslipidemia (P = 0.013) [81].

Posttransplant graft steatosis of a part of the liver from living donors was detected in biopsy specimens in 33 patients, with NASH diagnosed in 9 of the 33 patients. Recipients with liver steatosis were younger than those without steatosis (53.4 ± 9.5 years *vs* 57.6 ± 9.9 years, respectively; P = 0.045). It should be noted that prevalence of steatosis was significantly higher among recipients who received a graft from a donor with steatosis than without (60% *vs* 23%, respectively; P = 0.001). On multivariate analysis, younger recipient age (P = 0.023) and donor steatosis (P = 0.005) were independent risk factors of liver steatosis after liver transplantation. The clinical course of steatosis is relatively benign, with only 19% developing NAFLD, and 7.6% developing severe fibrosis [82].

LIVER TRANSPLANTATION OUTCOMES FOR NAFLD

Non-alcoholic fatty liver disease increases patients' morbidity and mortality [14]. The presence of morbid obesity (BMI ≥ 40 kg/m^2) and diabetes are independent predictors of death in patients awaiting liver transplantation [83]. Evidence has suggested that elimination of risk factors for post-transplant metabolic syndrome may significantly increase the survival of patients suffering from NASH [71]. There was no significant difference in the short-term or long-term survival of patients who developed de novo NAFLD [36].

Previously, some authors had suggested that in patients with NASH, there tend to be more frequent mortality in the early postoperative period (30–90 days after liver transplantation) [64, 74] and one year after surgery [64] than inpatients suffering from other liver diseases.

In most publications, it has been noted that post-transplant mortality is comparable to or even lower for patients with NASH than patients with other diseases. For example, the University of Miami compared the results of liver transplantation for NASH-related cirrhosis and alcoholic cirrhosis. There was no significant difference in survival between the groups. Sepsis was the leading cause of posttransplant death in both groups, followed by cardiovascular conditions (P = 0.21). Recurrent steatohepatitis (33% *vs* 0%, P < 0.0001) and acute rejection (41% *vs* 23%, P < 0.023) were much more common in the NASH group than in the alcoholic cirrhosis group. However, these complications did not lead to higher rates of liver retransplantation. There was no difference in graft failure between the groups (P = 0.3973) [78].

Four international centers (in Australia, USA, UK and Italy) conducted a joint study of morbidity and mortality in 247 patients with NAFLD with progressive liver fibrosis or cirrhosis. Patients with NAFLD with progressive fibrosis or cirrhosis had lower rates of liver-related complications and hepatocellular carcinoma than corresponding patients with HCV infection, but similar overall mortality [84].

In the United States, from January 1, 1997 to October 31, 2010, the posttransplant survival of patients with NASH (n = 1810) at 1 (87.6%), 3 (82.2%) and 5 years (76.7%) was higher than the survival of patients with hepatocellular carcinoma, hepatitis C virus, alcoholic liver disease, acute hepatic necrosis, hemochromatosis, or cryptogenic liver disease. It was only lower than the survival of patients with primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune hepatitis, or hepatitis B virus [73].

X. Wang et al. [85] showed that survival and long-term outcomes of liver transplantation in NASH-related cirrhosis are similar to those in cirrhosis related to alcoholic liver disease and HCV. According to these authors, since patients with NASH have a greater risk of death from cardiovascular complications or sepsis after liver transplantation, closer attention by clinicians and more aggressive therapy for these complications are required.

In recent years, graft survival among recipients of deceased donor and living donor livers continued to improve [66]. Therefore, within this period, the overall post-transplant survival of patients with NAFLD became not only comparable to the survival of patients suffering from other diseases [18, 67, 73], but also higher than the survival of patients suffering from HCV and alcoholic liver disease (P < 0.001) [75]. On average, one-year post-transplant survival is approximately 79–90%, the three-year survival is 82–83%, and the five-year survival is 72–78% [64, 70, 75]. The liver retransplantation outcomes for NASH-related cirrhosis are significantly worse than with cirrhosis of other etiologies [76].

Previously, the cause of post-transplant death in patients with NASH was primarily infection (57.1%), which is significantly higher than in other liver diseases [64]. In more recent studies, the leading causes of mortality were cancer (25%), infectious complications (25%), and cardiovascular disease (21.9%). Only 9% of deaths were associated with post-transplant cirrhosis [65]. Patients with NASH have a high risk of death from cardiovascular complications, which, according to J. Merola et al. [71], is a leading cause of post-transplant mortality.

The authors declare no conflict of interest.

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POSSIBILITIES OF OBTAINING AND USING HYDROGEL-BASED BIOMATERIALS FOR REGENERATION OF HUMAN BONE TISSUE

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Substitution of defects in various tissues, especially bone tissues, is a major challenge in modern medicine. There is currently no universal method of filling defects which has no drawbacks. Hydrogels are one of the promising groups of alloplastic materials. At present, you can obtain materials with various biological properties like natural extracellular matrix using various methods of chemical and physical modification. These biomaterials can be used as a means of delivering stem cells and bioactive substances to the defect zone. This literature review is devoted to the various aspects of preparation and use of hydrogel-based biological materials.

Keywords: hydrogels, tissue regeneration, tissue engineering.

Replacement of bone tissue defects is an urgent problem in modern traumatology and orthopedic oncology [1–4]. Bone defects can form from open bone fractures, fractures with compressed spongy bone tissue, formation of bone cysts, as well as a result of surgical treatment (tumor removal, resection of false joints, osteomyelitis areas, and bone osteotomy) [5]. Some defects are filled independently in the process of reparative regeneration. However, filling does not occur when a defect reaches a critical value [6]. Doctors have several ways of replacing bone defects, but each has its own drawbacks. Autologous spongy bone tissue is limited in the volume of the donor zone, it does not have mechanical strength. Moreover, cosmetic and pain problems often arise in the donor region [7]. Transplantation of bone blocks on vascular pedicle is complex, requires special equipment for the operating room and staff training. It cannot be a routine and generally accessible method [8]. Distraction osteogenesis requires prolonged use of external fixation apparatus, patient and staff discipline, and may be accompanied by purulent-septic complications [9]. The use of cadaveric bone comes with a risk of infection of the patient. Besides, there are many problems involved in taking, processing, and sterilizing the material [10]. Similar problems arise when using specially treated animal bone. Synthetic materials are free from many drawbacks – they are not limited in volume, materials can be created with specified mechanical properties, there is no risk of infection transmission, and biological modification over a wide range is possible [11].

In general, ideally optimized materials for replacement of bone defects should meet the following requirements: 1) no cytotoxicity and immunogenicity in order to avoid inflammation; 2) osteoinductive properties (ability to stimulate differentiation of surrounding progenitor cells to osteoblasts); 3) osteoconductive properties (ability of a material to be a three-dimensional matrix for germination of blood vessels and tissue elements due to the corresponding pore size and associated porosity, i.e., to simulate a natural extracellular matrix to ensure cell adhesion and proliferation); 4) possible presence of osteogenic properties (ability to be a medium for placement of osteoblast progenitor cells); 5) biodegradability (possibility of decomposition by endogenous enzymes or by hydrolysis simultaneously with the substitution process to create sufficient space for formation of a new bone); 6) structural stability and mechanical strength, which can be used to correct defects in the loaded zone and prevent denaturation during sterilization [4, 12–16].

DIVERSITY AND OBTAINING VARIOUS TYPES OF HYDROGELS

Hydrogels are one of the promising groups of alloplastic materials that can meet all the above properties.

A hydrogel is a three-dimensional network of hydrophilic polymers that can swell in water and hold different amounts of water (almost 100%) or biological fluids, while maintaining its structure and properties of a solid [17].

Hydrogels were first reported in Germany at the end of the 19th century [18]. Their biomedical use was dis-

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cussed in Czechoslovakia in the late 1950s after publication of the works of professors Wichterle and Lim [19], who studied materials based on synthesized poly-2-hydroxyethyl methacrylate (polyHEMA), which was later used in the manufacture of contact lenses.

Based on origin of polymers, hydrogels can be subdivided into natural, synthetic and semi-synthetic, or mixed [15, 20].

The most commonly used natural materials include polypeptides (collagen, gelatin and fibrin) and polysaccharides (hyaluronic acid, chitosan, alginate and chondroitin sulfate) [21–23]. The main advantages of such materials are their low cytotoxicity, high biocompatibility and biodegradability, which is facilitated by *in vivo* enzymes. However, their main disadvantage is the difficulty of controlling mechanical properties and swelling.

Among synthetic substances, biodegradable polymers with controlled microstructure and mechanical properties, such as polyhydroxyethylmethacrylate (polyHEMA), polyethylene glycol (PEG) and its derivatives acrylates (PEGDA – diacrylate, PEGDMA – dimethacrylate), poly(N-isopropylacrylamide) (PNIPAm), polyvinyl alcohol (PVA), polyglycolic acid (PGA) and poly(lactic-co-glycolic acid), polyacrylic acid (PAA), polyacrylamide (PAM), etc. are more often used [15, 22, 24, 25]. Synthetic polymers have long shelf life without the risk of increase in immunogenicity. In addition, they can be produced in large volumes. In turn, the use of synthetic monomers allows you to set and control the mechanical strength and elasticity of hydrogels, biodegradation, biological and chemical behavior in the body. The main challenge in this case is the choice of biocompatible and non-toxic monomers, their polymers, as well as polymerization initiators [20].

Due to the indicated limitations of synthetic hydrogels, various combinations of natural and synthetic hydrogels with the best, according to some authors, biological and mechanical properties, such as chitosan-PEG, collagen-poly(N-isopropylacrylamide) and chitosan-poly(vinyl alcohol) are used in biotechnology [26–28].

Polymeric hydrogels are obtained via polymerization reaction, initiated by radiation (electron beam, gamma radiation, x-ray or ultraviolet radiation), changes in pH, temperature or by chemical reactions (click chemistry, disulfide crosslinking, enzyme-mediated crosslinking, Michael reaction, Schiff base cross-linking, ionic crosslinking, self-assembly) [17]. Traditional approaches to preparation of porous hydrogels include leaching of porous material, gas formation, lyophilization, and electrospinning [22, 29–31].

Despite advances in production of porous hydrogels, these methods could not provide precise control of pore size and spatial location of pores. Recently, more advanced additive technologies, such as stereolithography, 3D printing, and microfluidics, have been used to develop complex porous microarchitectures [22, 32–34].

METHODS OF MODIFYING HYDROGEL PROPERTIES

Various chemical and physical modifications are used to control the biological properties of hydrogels. They include choosing the composition of monomers, changing the degree of polymer crosslinking, constructing different architectures using 3D printing, introducing various functional groups and nanoparticles that change the properties of the whole composite.

Selection of monomers determines the production of hydrogels capable of carrying out a sol-gel phase transition when heated to body temperature. That is why hydrogels can be introduced into the body in liquid form, that is, in a minimally invasive way. Such hydrogels include, for example, poly(N-isopropylacrylamide) (PNIPAm), hydrogels that become soluble in water at a temperature below 32 °C and are reversibly converted into gel form when heated above 32 °C [35]. Thermo-sensitive injection composite materials with improved mechanical properties and biological activity can be obtained by adding to PNIPAm other functional components, such as PEG, poly(N,N-dimethylacrylamide), and poly(2-hydroxyethyl methacrylate) [36]. Currently, TSV Gel (OsteoBiol, Italy) is commercially available, which is a mixture of animal collagen and gel-forming synthetic copolymer Poloxamer 407. This drug exists in liquid form at temperatures below 8 °C, and begins to turn into a gel-like state at temperatures above 13 °C. This allows it to fill defects of complex shape [37].

The monomers selected determine the rate and conditions of degradation of hydrogels in the body. A group of scientists led by S.P. Zustiak et al. synthesized hydrolytically degradable PEG hydrogel, composed of PEG vinyl sulfone (PEG-VS) cross-linked with PEG-diester-dithiol. Degradation time and the mechanical properties of this hydrogel can be controlled by altering parameters such as distance between thiol and ester group in the cross-linker, molecular weight and polymer density [38].

Various chemical and physical methods for crosslinking polymers are important aspects of hydrogel synthesis, allowing to vary physical characteristics. Using chemical crosslinking, more stable hydrogels with enhanced mechanical properties are built through formation of strong covalent bonds [39]. A physical compound results from non-covalent interaction, such as van der Waals forces, hydrogen bonds, hydrophobic bonds and electrostatic forces [40]. Consequently, the mechanical strength of physically bonded hydrogels is relatively lower than covalently bonded ones, but they decompose more easily in the body. Chemically bound hydrogels may be less compatible with tissues due to the potential

cytotoxicity of residual polymerization initiators and organic solvents, as well as delayed degradation [41].

It should be considered that the mechanical properties of hydrogels can affect cell differentiation by various mechanotransduction pathways through the tension and integrity of actin cytoskeleton, nuclear mechanics, and integrin-mediated adhesion and signaling [42–44]. Early studies using 2D substrates suggested that rigid hydrogels promote osteogenic differentiation, while compliant hydrogels enhance neuro- and adipogenic differentiation progenitor cells [42, 45]. For example, Huebsch et al. investigated the effect of stiffness on cells grown on alginate hydrogels. The study demonstrated that osteogenic differentiation of mesenchymal stem cells was stimulated by growing cells on 3D matrices with 11–30 kPa rigidity, while adipogenic differentiation was enhanced with a gel rigidity of 2.5–5 kPa [45]. It has also been shown that rapidly relaxing hydrogels promote spreading, proliferation, and osteogenic differentiation of mesenchymal stem cells [46].

By changing photopolymerization conditions, it is possible to change such hydrogel characteristics as stiffness and viscoelastic properties. In S. Yang et al., gel stiffness and spatial organization were monitored on a photodegradable hydrogel matrix using lithographic masks and photographic coating of soft and hard regions on a micrometer scale [47]. Results showed that the cells had a large area and elongated morphologies with increasing hard areas on the hydrogel substrate. In addition, regular patterns with high stiffness enhanced osteogenic differentiation of mesenchymal stem cells compared to randomized patterns. Exact spatial control of the mechanical properties of a hydrogel can mimic the gradually varying stiffness of the interface between soft and hard tissue, such as "ligament-bone" or "tendon-bone" [48].

Free diffusion in the thickness of hydrogels is limited. So, porosity is a decisive physical factor for facilitating transport of nutrients and oxygen [49]. Absence of anastomoses and blood perfusion can delay tissue regeneration due to difficulties in cell migration and proliferation [50]. In addition, by altering the size and 3D organization of the pore system, you can create biophysical signals that regulate cellular behavior by simulating physical features at the micro and nanoscale [51].

By creating porous materials in the form of frames of various architectures, you can reach a compromise between the permeability of the material and its strength. Due to some topological optimization features, either cellular porous structures [52] or 3D periodic minimal surfaces are selected to find an optimal geometry [53, 54].

If the pore size is too large, the cells recognize their contact surface as 2D and become more susceptible to the influence of surface properties of the material, such as stiffness. When cells migrate through a smaller porous structure, the speed and efficiency of migration are more dependent on 3D geometry. Consequently, different pore sizes are required depending on the 3D geometry and properties of the frame materials and cell types [51, 55]. For example, mesenchymal stem cells in scaffolds migrated further when the pore diameter (12 μm) was relatively similar to the cell size than when the pore size was small (7 μm) or large (17 μm) [56]. Fibroblast migration rate decreases as the pore size of hydrogels increases across a range from 90 to 150 μm [57]. According to literature sources, there are various optimal pore sizes of implants for induction of regeneration of various types of tissues: 5 μm pore diameter for vascularization [58], 5–15 μm for fibroblast ingrowth [59], 20–125 μm for regeneration of adult skin [60] and 100–350 μm for bone regeneration [61].

One example of altering the properties of hydrogels by introducing functional groups is the creation of materials whose swelling depends on the pH of the environment. This is achieved by incorporating carboxyl groups into the starting monomers. Ionization/deionization of these groups induces swelling/deswelling depending on the pH of the medium [62]. In an alkaline medium, carboxyl groups are ionized and repel each other, leading to hydrogel swelling. In an acidic environment, COOH groups are protonized with charge loss and hydrogel deswelling with water release. The clinical significance of this fact is that such scaffolds can selectively deliver biomolecules to defect sites where the environment is more acidic, for example, in ischemia or inflammation. Based on this, a team of scientists led by Matsusaki prepared a pH-sensitive semi-interpenetrating polymer network like heterogels composed of γ-PGA (polyglutamic acid) and sulfonated γ-PEG [62]. Hydrogels modified in this way swell/deswell depending on pH conditions, while the sulfonic acid groups can increase proton concentration. As a result, growth factors, such as fibroblast growth factor-2 (FGF-2), are released as the surrounding acidity increases. These pH-sensitive hydrogels can be used to fill defect sites in inflammation or ischemia – these areas have comparatively acidic pH (<6.5) compared to surrounding tissues [36].

Alginate is widely used as a hydrogel crosslinked via ionic interactions due to its high biocompatibility and ease of gel formation [63, 64]. Alginate hydrogels are obtained through a combination of solutions of alginate with calcium chloride, in which Ca^{2+} ions bind to hyaluronate blocks of alginate chains. However, after crosslinking, limited release of Ca^{2+} ions from these hydrogels is accompanied by slow degradation of the material, which reduces the viability of hydrogel-encapsulated cells [65]. To solve this problem, Z. Wu et al. increased the ability of calcium-crosslinked alginate to decompose by adding sodium citrate, whose citrate ion can chelate to calcium ions in a hydrogel. By controlling the mole ratio of sodium citrate/sodium alginate, decay of 3D-printed

alginate hydrogel was regulated, which contributed to high viability and proliferation of cells introduced into the hydrogel [66].

By adding various functional groups even to natural polymers, one can significantly increase the affinity of hydrogels for water and various protein compounds. Widespread use of PEG in the medical field is based on its inherent biocompatibility and ease of control of physical and chemical properties. However, unmodified PEG hydrogels are inert and adsorb limited amount of proteins. In addition, many cell types cannot attach to PEG hydrogels or have low viability during encapsulation internally [67]. To overcome this limitation, arginyl-glycyl-aspartic acid (RGD peptide), which is a natural component of the collagen molecule, is attached to this type of hydrogels [14]. Additional substrates covalently bind to the components of the hydrogel using enzymes or factor XIII transglutaminase, which acts as a catalyst. Hydrogels modified in this way demonstrate a higher cell density than PEG hydrogels without RGD peptides due to faster penetration of mesenchymal stem cells into the material structure [68].

Another problem that could be solved by adding auxiliary substances to hydrogels is excessive shrinkage of the material when saturated with water. High degree of shrinkage can lead to a mismatch in size between the implant and tissues [69]. For example, collagen, which is the main component of connective tissue and is widely used in biomedical engineering, has low stability and can shrink severely after immersion in liquid. This limits its use for tissue regeneration [14, 70]. To address this problem, aminated bioactive glass particles were included in collagen, which formed strong chemical bonds between positively charged amine groups and negatively charged carboxyl groups of collagen. Mesenchymal stem cells, cultured in such a hydrogel, had a higher viability and a more diverse morphology than when using pure collagen [70].

The low mechanical strength of hydrogels may limit their use in regenerative engineering of supporting tissues. Since the very high density of hydrogel networks is accompanied by lower diffusion rate, the mechanical properties of hydrogels can also be modulated by including various nanomaterials [22, 71, 72]. For example, to create composite materials for bone tissue regeneration, such nanoparticles as calcium phosphates [73] and silicates [74] are introduced in hydrogels. This increases their mechanical strength and osteogenic properties. For example, introduction of hydroxyapatite particles increases the elastic modulus, ultimate deformation, and strength of the composite by up to 15% compared to empty hydrogel [75]. Studies of the physical properties of hydrogels filled with various types of calcium phosphates suggest that strength properties (compressive and tensile strength) can be highly dependent on the type of calcium phosphate used [64, 76].

Among the variety of calcium orthophosphates, hydroxyapatite is a classic and most used component in the creation of bioimplants both as the main phase [77] and as coatings or an additional bioresistive phase [14, 78, 79]. However, low resorbability of hydroxyapatite, due to the calcium/phosphorus ratio (Ca/P ratio = 1.67) [80], makes it necessary to look for a replacement for hydroxyapatite. Alternatively, calcium phosphates with a lower Ca/P ratio or their mixtures are proposed: brushite, monetite and calcium pyrophosphate (Ca/P = 1), octalcium phosphate (Ca/P = 1.33), tricalcium phosphate (Ca/P = 1.5) [23, 76, 80, 81]. These phosphates belong to the class of acid phosphates (hydrophosphates). *In vivo* studies have suggested that the biodegradation rate of alginate-based composite materials decreases depending on the type of calcium phosphate: maximum for octacalcium phosphate, less for tricalcium fascate and significantly less for carbonate hydroxyapatite [64]. Upon dissolution (resorption), they create slightly acidic pH values in the environment, which leads to partial dissolution (etching) of hydroxyapatite crystals of the surrounding bone tissue. When morphogenetic bone proteins and other bioactive factors are adsorbed on their surface [82], they can transit to a dissolved state, which locally creates higher concentration of bioactive substances and starts a chain of biological processes, as a result of which bone tissue forms in this place.

APPLICATION OF HYDROGEL-BASED BIOMATERIALS

Research on creation and use of hydrogels is still largely at the preclinical stage of development. However, there are reports on the first real applications of the above principles in creation of artificial organs.

N. Kang et al. (2016) described a method for creating cell-loaded hydrogel composites (fragments of the bones of the cranial vault and lower jaw, auricular cartilage and a fragment of the skeletal muscle) using integrated 3D printer created by the authors for printing organs and tissues. Here, multicomponent composition of the hydrogel was used. The mechanical basis of the hydrogel was polycaprolactone (PCL), whose pores were filled with a less mechanically strong (but more compatible with cells) composition of gelatin, fibrinogen, hyaluronic acid and glycerol. The cell component was lines of fibroblasts and myoblasts, chondrocytes and human amniotic stem cells. One of the features of the created materials – a microchannel system that permeates the entire structure and provides cell nutrition throughout its thickness. During implantation of the obtained materials in laboratory animals, it was shown that the cells included in the material begin to differentiate, grow and

synthesize their own surrounding matrix at the site of the absorbable hydrogel [83].

In domestic literature sources, there are examples of creation of hydrogel-based nanocomposite materials loaded with stem cells [84]. In [22], the authors describe the creation of a hydrogel matrix based on poly(L-lactide) obtained by imprint lithography, which was populated with mesenchymal stem cells. *In vitro* studies showed that stem cells differentiated along the osteogenic pathway, with good adhesion to the resulting material and high survival.

In another work [85], a preclinical *in vivo* study of a fibrin hydrogel-based composite material with tricalcium phosphate inclusions and loaded with mesenchymal stromal stem cells was performed on a model of a critical defect in the femoral epiphysis of a rabbit. This scaffold was shown to be able to transfer living stem cells while maintaining their regenerative potential and the potential for bone tissue replacement. However, the negative effect of fibrin hydrogel on the osteoconductive properties of ceramics in the composite was shown.

In *in vivo* study by Petrov et al. [86] on an animal model of a bone defect, the biological properties of biomaterial based on demineralized bovine bone collagen and hyaluronic acid and chondroitin sulfate were studied on animal model of bone defect. Histological examination showed faster and more complete replacement of ileal defect with newly formed bone tissue when using biomaterial compared with the control group.

Literature sources describe the preparation and study of alginate-based composite hydrogel materials in laboratory animals (mice and rats) with the addition of gelatin, as well as octacalcium phosphate (OCP) and tricalcium phosphate (TCP) crystals. Here, it is shown that addition of gelatin and calcium crystals helps to increase strength and porosity. It was suggested that three-component hydrogels using OCP have better osteoconductive properties and faster bone formation [2].

National literature sources describe successful clinical applications of a combination of spongy pelvic autologous bone and commercially available collagen-based hydrogels (SFERO®gel LONG, Russia) to replace critical defects of the femur and tibia in humans. At the same time, authors point to the role of hydrogel in maintaining the regenerative process launched by autologous bone [9].

CONCLUSION

This literature review shows that hydrogels are presently a very diverse class of compounds, both in chemical composition and in chemical, physical, and biological properties. Such a variety seems promising in terms of creation of biomedical materials that can effectively replace the natural structures of the body. Various methods for modifying the properties of hydrogels provide opportunities for adapting them to specific clinical situations in order to meet the needs of a customized approach in modern medicine. Despite an understanding of the general principles of creating an "ideal" tissue-engineering design for bone defect replacement, real life samples that meet all the requirements of efficiency and safety have not yet been obtained. Further research is needed to create composite materials for effective replacement of large volumes of lost bone tissue, which would allow for full and quick restoration of body functions.

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CYTOMEGALOVIRUS INFECTION AFTER KIDNEY TRANSPLANTATION: REAL PROGRESS AND PROSPECTS FOR PATHOGENESIS RESEARCH, PREVENTION AND TREATMENT

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Cytomegalovirus (CMV) infection plays an important role in clinical transplantology – it increases the risk of complications, graft failure, and patient death. The virus has both direct (direct damage to organs and tissues) and indirect immunomodulatory effects. Based on studies conducted, an international group of experts developed general principles for managing CMV infection after transplantation. This paper discusses risk factors, pathogenetic mechanisms by which CMV infection develops after kidney transplantation, the principles of diagnosis, treatment and prevention of this complication, and ways to overcome drug resistance in the virus. The prospects for the use of immunological monitoring, new antiviral drugs, as well as the possibility of using CMV vaccines, T-cell therapy, immunosuppressants (antiviral mTOR inhibitors) are discussed.

Keywords: cytomegalovirus infection, kidney transplant, graft failure, mortality, antiviral prophylaxis, mTOR inhibitors.

INTRODUCTION

Cytomegalovirus (CMV) belongs to the family of herpes viruses (*Herpesviridae*). It is the largest human herpes virus, measuring 150–200 nm in diameter [1, 2]. CMV contains at least 33 structural proteins and has a double-stranded DNA core. It is prevalent worldwide in the general population: CMV infections primarily occur in children, and the proportion of CMV seropositive adults reaches 70–90% [3, 4]. After primary infection, the virus does not undergo elimination. It rather persists throughout its lifespan in several types of cells – dendritic cells, megakaryocytes, CD14+ monocytes, CD34+ myeloid progenitor cells. This is why subpopulations of CMV-specific T lymphocytes exist in the infected body [5]. CMV does not usually cause a clinically manifest disease in immunocompetent individuals, although asymptomatic carriage of the virus may be associated with some inflammatory and age-related vascular diseases [6, 7]. Under a situation where the immune system is suppressed, for example, in HIV infection or after organ transplantation, CMV is reactivated and this is accompanied by damage to various body systems with a wide range of clinical manifestations and a real threat to the lives of patients [2, 8].

IMPORTANCE OF CMV INFECTION IN CLINICAL TRANSPLANTOLOGY. CLINICAL MANIFESTATIONS AND VIRUS REACTIVATION MECHANISMS

CMV infection can rightly be called the "number one infection" in transplantology because of its crucial role in morbidity and mortality of organ transplant recipients [2, 3, 9]. Apart from the direct effects of the virus, which is associated with its cytopathic effect, there are several "indirect effects" (general and transplant-specific) resulting from higher incidence of other types of infections, graft failure and death of recipients [10–12]. CMV infection and CMV disease are the direct effects of CMV.

According to the definition used by the International Consensus Recommendations and the American Society of Transplantation, CMV infection (asymptomatic replication of a virus, different from latent carriage of CMV) is defined as virus isolation or detection of viral proteins (antigens) or nucleic acid in any body fluid or tissue specimen [13]. CMV disease is a proven CMV infection with associated symptoms. It is further divided into viral syndrome (fever, malaise, leukopenia and/or thrombocytopenia) and tissue-invasive disease [13–15]. CMV disease can manifest itself as life-threatening pneumonitis, carditis, damage to any part of the gastrointestinal tract, pacreatitis, hepatitis, retinitis, tubulointerstitial nephritis, and less commonly as encephalitis and myeloradiculopathy [14, 16]. There have been reported separate cases of development of ureteral stenosis in renal transplant recipients in combination with tubulointerstitial nephritis caused by CMV [17].

Transplant-specific "indirect effects" of CMV are manifested in solid organ transplantation. They include chronic transplantation nephropathy and/or renal graft failure, accelerated recurrence of viral hepatitis C in liver transplant recipients, hepatic artery thrombosis in liver transplant recipients, cardiac allograft vasculopathy, and

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obliterative bronchiolitis in lung transplant recipients [11, 18–22]. A recent study confirmed that the appearance of CMV DNAemia having a viral load of ≥2000 copies/ml both in early (up to 3 months) and late onset in kidney transplant recipients is an independent risk factor for renal graft failure [23]. The general "indirect effects" of the virus consist of increased risk of bacterial and fungal infections, viral complications in general, acute rejection, post-transplant lymphoproliferative diseases, post-transplant diabetes mellitus, cardiovascular complications, accelerated aging and death [24–29].

It is well known that CMV infection is a risk factor for acute renal graft failure due to the immunomodulatory effect of the virus [30, 31]. Recent studies have shown that CMV activity may be associated with microcirculatory damage to the renal transplant due to donor-specific antibodies, i.e., with humoral rejection. In patients with CMV infection, specific γδ T lymphocytes are more frequent within glomeruli and peritubular capillaries from antibody-mediated acute rejections than within those from T cell-mediated acute rejections. In addition, a persistently increased percentage of circulating cytomegalovirus-induced γδ T cells correlated inversely with the 12-month estimated GFR only in kidney transplant recipients with donor-specific antibodies [32].

Previously, when there were no monitoring and prevention strategies yet, incidences of CMV infection/disease in kidney transplant recipients were very high: 60% for CMV infection and 30% for CMV disease [33]. Currently, the incidence of active CMV infection in renal transplant recipients has fallen considerably, but remains clinically significant. After transplantation, CMV infection can develop under two scenarios – as a primary infection (when the virus is transmitted along with the transplanted organ to a seronegative patient) or as reactivation of a recipient's latent CMV infection. In the "natural" course (without the use of prophylaxis), primary CMV infection/reactivation is clinically manifested most often in the first 3 months after kidney transplantation. However, in rare cases, a case of a transplant recipient presenting with CMV primoinfection 12 years after renal transplant has been reported [34].

Reactivation of latent CMV after transplantation is a complex, not fully understood process. However, systemic inflammatory response, mediated by several factors, such as immunosuppression, coinfection with other herpesviruses, acute graft rejection, sepsis, and even surgical intervention clearly play a key role in CMV reactivation [35]. Reactivation is associated with suppression of cellular immune response, especially CD8+ cells, as well as with the impact of several cytokines promoting transition of the virus from a latent state to an active phase. Tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) play the most important role in CMV reactivation process [36, 37]. In late CMV disease, which may develop after completion of specific prophylaxis, higher IL-10 plasma levels are predictive of this disease [38]. Experiments have shown that CMV in the replication process needs an mTOR kinase (mammalian target of rapamycin), which is part of the mTORC1 and mTORC2 complexes. Both complexes are activated during development of CMV infection in humans, while mTORC1 is involved in the production of all classes of proteins of the virus. Inactivation of the 4EBP1 protein (eukaryotic initiation factor 4E-binding protein) by the mTORC1 complex is critical for successful CMV replication. At the same time, the effect of mTOR inhibitors in the early phase of infection inhibits translation of viral proteins, which confirms the antiviral effect of this group of drugs [39, 40]. Using the model of human macrophages, it has also been shown that in the late infection phase, mTOR activation is also essential for CMV replication and synthesis of virus proteins such as pUL-44 and pp65 [41].

The risk of developing CMV infection in the post-transplant period depends on factors associated with the virus itself and factors associated with the patient's body. The former include heterogeneity of CMV (various strains), possibility of co-infection with other viruses, the effect of immune evasion, and replication dynamics. The later includes the nature of immunosuppressive therapy, including the use of high doses of calcineurin inhibitors (CIN), especially the subpopulations of CD4+, CD8+ cells, NK-cells and B-cells of the recipient, the ratio of the CMV-specific serological status of the donor and recipient, gene polymorphisms of certain cytokines and cell receptors (interleukin IL-28B, toll-like TLR9 receptors and DC-SIGN lectin receptors) involved in the antiviral immune response [35, 42, 43]. Of the drugs used to induce immunosuppression, antithymocyte globulin and alemtuzumab increases the incidence of CMV infection [44].

The CMV IgG serostatus of the donor and recipient (D/R) is of great clinical importance. The highest risk of developing CMV disease is in cases where the donor is seropositive and the recipient is seronegative (D+/R–), that is, the virus is likely to be transmitted with the donor organ to a patient who does not have CMV immunity. With the D+/R+ or D–/R+ combination, the risk is considered moderate (the risk is slightly higher for D+/R+). When both donor and recipient are seronegative (D–/R–), there is minimal risk of CMV infection in kidney transplant recipients [35]. In rare cases, CMV can be transferred to a recipient from a seronegative donor (D–) if the donor was evaluated during the "serological window", when donor infection has already occurred, but antibodies have not yet appeared [45].

DIAGNOSIS OF CYTOMEGALOVIRUS INFECTION

Evaluation aimed at preventing and detecting CMV infection can be conveniently divided into pre-transplant and post-transplant evaluation.

At the pre-transplant stage, both the organ donor and the potential recipient are evaluated, since as mentioned above, the matching/difference between the CMV serostatus (D/R) in the donor and recipient plays a major role in assessing the risk of developing CMV infection after kidney transplantation and determining the need for prevention or preemptive therapy. To assess the serostatus, detection of IgG antibodies to CMV is used. This is achieved through highly sensitive and highly specific immunological methods. In this case, detection of IgM or IgM and IgG in total should not be used, since such tests have insufficient specificity [46, 47]. If the donor and recipient are seronegative (D–/R–) during the pre-transplant evaluation, serology should be repeated at the time of kidney transplantation because the serological status may change, which affects the choice of tactics for CMV infection prevention [15].

After kidney transplantation, serological tests are not essential in diagnosis of CMV infection and CMV disease. Detection of antibodies, however, can be used in establishing current susceptibility to CMV in patients who are seronegative before transplantation and who have not yet had an active CMV infection after surgery. For example, seroconversion within three months after the end of a 100-day antiviral prophylaxis in D+/R– patients reduces the risk of late-onset CMV disease [48]. Cultivating the virus from blood is not used to identify transplant recipients with CMV infection due to the low sensitivity of the method, while cultivating it from urine and saliva cultures are not used due to low specificity [49].

The basis for diagnosis of CMV infection after transplantation consists of quantitative nucleic acid amplification testing (QNAT) methods, most often – quantitative real-time polymerase chain reaction (real-time PCR), due to its high efficiency and possibility of standardization [11]. The new International Consensus Guidelines contain consensus statements and recommendations on diagnosis and management of CMV infection. Below are the most important ones [15].

– We recommend using QNAT calibrated to the WHO standard for diagnosis, surveillance to guide preemptive antiviral treatment, and for therapeutic monitoring due to the ability to harmonize and standardize these tests. Results must be reported as IU/mL and termed as DNAemia rather than viremia. If QNAT is not available, antigenemia is a less desirable alternative (strong recommendation, high-quality evidence).
– We recommend either plasma or whole blood specimens as a biological sample for QNAT, with an appreciation for the differences in viral load values, viral kinetics and assay performance characteristics (strong recommendation, high-quality evidence). Neither the specimen type nor the assay should be changed when monitoring patients.
– Despite reporting in IU/mL, we recommend that viral load values are not directly compared across centers and/or laboratories unless identical testing reagents and procedures can be assured or equivalence has been documented (strong recommendation, high-quality evidence).
– We recommend that only changes in viral load exceeding 0.5 $\log_{10}$ IU/mL (threefold) are considered to represent clinically significant differences in DNAemia (strong recommendation, low-quality evidence).
– Although harmonization of QNAT has improved, universal thresholds for therapy or treatment endpoints have not been established and current published thresholds remain assay-specific. Accordingly, we recommend that centers establish their own thresholds and audit clinical outcomes to verify the thresholds used (strong recommendation, moderate-quality evidence).
– We do not recommend surveillance of CMV DNAemia during routine prophylaxis.
– We recommend when monitoring response to antiviral therapy, that QNAT is performed weekly (strong recommendation, moderate-quality evidence).
– With the use of highly sensitive QNAT (lower limit of quantification <200 IU/mL), we suggest discontinuing therapy after 1 result is less than the lower limit of quantification. If this approach is used, confirmatory testing should be done 1 week after discontinuing therapy. If the assay is not highly sensitive, then 2 consecutive undetectable (negative) results are needed to discontinue therapy (weak recommendation, moderate-quality evidence).
– We recommend histology coupled with immunohistochemistry for the diagnosis of tissue-invasive disease. Histopathologic examination of tissue should routinely include immunohistochemistry for CMV (strong recommendation, moderate-quality evidence).

With an appreciation for the fast dynamics of replication of the virus during development of CMV infection, the results of quantitative evaluation of CMV DNA should be available within 24–48 hours after sampling for timely clinical decisions, and this should be considered in the laboratory operating mode.

There were concerns that monitoring therapeutic response using highly sensitive DNA quantification tests to monitor the effectiveness of antiviral therapy may lead to unreasonably longer antiviral therapy. However, it turned out that in practice, the total duration of treatment was not prolonged in kidney transplant recipients with CMV

infection, although the time to reach an undetectable viral load was longer [50].

It should be emphasized that diagnosis of tissue invasive CMV disease is confirmed via detection of the virus in the tissues. The "gold standard" is to identify the cytopathic effects of CMV or virus antigens in biopsy specimens [13]. Evaluation of DNA in body tissues, for example, in biopsy specimens of the intestinal mucosa (including as a supplement to immunohistochemical studies) can be successfully used, although this method has not yet been standardized [51]. It is important that in the case of gastrointestinal CMV infection in solid organ transplant recipients and in pneumonitis in lung transplant recipients, there may be no DNAemia, or the quantity of DNA in the blood may be very small [52, 53]. The central nervous system (CNS) is rarely affected by CMV disease in solid organ transplant recipients. In the absence of data from special studies, detection of CMV DNA in cerebrospinal fluid can be considered as a confirmation of CNS viral infection. The diagnosis of CMV-retinin is based primarily on ophthalmological findings, although a positive QNAT in vitreous fluid may be helpful in guiding the diagnosis of retinitis [15].

PREVENTION OF CMV INFECTION IN KIDNEY TRANSPLANT RECIPIENTS

Since CMV infection occurs with high incidence after kidney transplantation and has a pronounced negative effect on the outcome of kidney transplantation, preventing this infection from developing is of paramount importance. The main approaches to preventing CMV infection in transplant recipients consist of preventive (preemptive) antiviral therapy and universal prophylaxis. There is a third, combined, approach – observation after completion of prophylaxis. This is also called a "hybrid approach" [11].

Universal prophylaxis involves administration of a specific antiviral drug to all patients at risk, starting from the 10th day after transplantation with continued continuous administration for a certain period (usually 3 or 6 months for kidney transplant recipients) [2, 3]. The following drugs were previously used and actively studied for prevention: acyclovir, valaciclovir, intravenous ganciclovir, oral ganciclovir (currently not available), and valganciclovir. It was further shown that ganciclovir is more effective in preventing CMV infection in kidney transplant recipients than acyclovir [54]. Valacyclovir in high doses has also been shown to be effective in preventing this complication [55], but its practical use is somewhat limited by the undesirable effects of high-dose therapy.

Currently, valganciclovir, a drug with proven effectiveness when orally administered, is most often used for universal prevention. Moreover, prevention for 6 months proved to be more effective in D+/R– kidney transplant recipients than a 3-month course [56]. It is important that introduction into clinical practice of valganciclovir prophylaxis in patients of medium risk (D+/R+ or D–/R+) was associated with considerable reduction in the incidence of significant CMV DNAemia [57]. The effectiveness of the prevention of active CMV infection with valganciclovir in the post-transplant period in kidney transplant recipients has also been confirmed by Russian and Belarusian authors [58, 59]. It is important to remember the need to select valganciclovir dose in accordance with the GFR level in a specific patient, since in patients with reduced renal function, the dose and/or frequency of administration of the drug should be lower than in normal renal function. When it comes to using a standard dose in patients with renal failure, serious adverse events may develop, primarily associated with leukopenia/neutropenia [60].

With all the positive effects of universal prevention, this approach comes with an important clinical problem – possibility of late-onset CMV disease after completion of a preemptive course. Apparently, the risk of late-onset CMV disease is associated with the absence of a virus-specific cellular immune response in patients with ongoing immunosuppression [61]. Risk factors for late-onset CMV infection/disease include certain types of transplantation (lung transplantation), high immunosuppression, graft rejection, D+/R– serostatus, GFR level less than 45 ml/min at the time of completion of prophylaxis [62–64]. This problem was what led to the emergence of a "hybrid" approach to the prevention of CMV infection. Although not all authors support this since data on the effectiveness of active surveillance after prophylaxis are somewhat contradictory [65, 66]. However, this combination approach may be applied for patients at significantly higher risk of late-onset CMV disease. In these cases, the viral load should be determined weekly for 8 to 12 weeks after the end of prophylaxis [15].

Preemptive therapy for CMV infection involves monitoring the viral load at regular intervals (blood CMV DNA must be determined at least once a week) for early detection of virus replication and conducting antiviral therapy if a predetermined DNA threshold is reached, even before clinical symptoms appear. Obviously, threshold values may vary for groups of different risks. For example, the threshold values of CMV DNAemia for D+/R– patients should be lower than for the D+/R+ group, since in the first case, viral load is doubled much faster and there might not be enough time to start preemptive therapy in the preclinical stage [67]. Preemptive therapy has some obvious advantages, which include lower incidence of late-onset CMV infection, selective treatment, reduction in the cost of therapy and the incidence of toxic effects when using antiviral drugs [2, 11, 15]. However, this tactic has obvious disadvanta-

ges: there are no common threshold values for the viral load, which serve as an indication for starting therapy (as mentioned above), logistics challenges associated with the need for weekly examination of the patient and a very fast start of treatment when the threshold viral load is reached, inconsistent or negative data on the effectiveness of preventing "indirect effects" of CMV and the impact on the survival of transplants and recipients compared with universal prophylaxis [68, 69].

Separately, the role of immunosuppressive therapy regimens in possible prevention of CMV infection should be discussed. In 2011, a combined analysis of three clinical trials of the use of various doses of everolimus in combination with cyclosporin A in *de novo* renal transplant recipients was published. A considerable decline in CMV infection/syndrome in the everolimus group versus the mycophenolate group, especially in non-prophylactic patients, was confirmed [70]. Several studies, systematic reviews, and meta-analyzes performed over the past few years have also shown a significant decrease in the incidence of CMV infection/disease in recipients treated with mTOR inhibitors. Here, this decrease was observed not only in adult kidney transplant recipients, but also in pediatric kidney transplant recipients, as well as in liver, heart and lung transplant recipients [71–77]. A meta-analysis of 28 randomized, controlled trials with 6,211 participants found that the risk of CMV infection was reduced by 46% ($p < 0.001$) in patients receiving mTOR inhibitors without CNI (calcineurin inhibitor), and 57% ($p = 0.007$) in patients taking mTOR inhibitors with reduced CNI doses compared to patients who received standard CNI doses [78]. Finally, TRANSFORM, the largest multicenter randomized study of the efficacy and safety of *de novo* everolimus-based therapy in kidney transplant recipients, which included 2,037 patients, showed that the incidence of CMV infection was significantly lower when everolimus is used in combination with reduced CNI doses than when mycophenolates and standard CNI doses are used – 3.6% versus 13.3%; incidence of BK virus infection was also lower – 4.3% versus 8% [79].

The results obtained are quite logical considering the role of mTOR kinase in CMV replication, as was mentioned above [39–41]. Besides, significant increase in CMV-specific effector-type CD8+ and CD4 T-lymphocytes was found in everolimus-treated renal transplant recipients 6 months and 24 months after surgery compared with cyclosporin A or mycophenolate treated recipients. This may also offer partial explanation for the low incidence of CMV infection with mTOR inhibitors [80].

In view of the above, one may ask: is it necessary to prevent CMV infection in patients receiving mTOR inhibitors? In a major meta-analysis published back in 2012, which showed significant reduction in the risk of CMV infection in mTOR-inhibitor treatment either alone or in combination with reduced CNI doses, it was suggested that standard mTOR inhibitor-based antiviral prophylaxis may be dispensable [81]. It was further found that the use of mTOR inhibitors protected R+ (CMV-seropositive) kidney transplant recipients from CMV even when polyclonal anti-lymphocyte globulin (high immunosuppression) was used in the absence of prophylaxis. However, early discontinuation of mTOR inhibitors increased the risk of CMV infection [82]. Apparently, in CMV-seropositive patients, the very use of *de novo* mTOR inhibitors can be a method for preventing CMV complications, so far as patients are carefully monitored. As for highest-risk D+/R– recipients, it is more advisable to adhere to the traditional approach (prophylaxis or preemptive strategy) until more complete data on the protective effect of mTOR inhibitors against CMV in this group of patients is obtained. Analysis have shown that the use of everolimus and tacrolimus in combination with induction therapy with no prophylaxis for CMV infection in renal transplant recipients provides clinical efficacy comparable to that of mycophenolates and tacrolimus (also with antibody induction), but is characterized by higher cost efficiency due to lower treatment costs [83].

Here are some of the consensus statements and recommendations of the International Consensus Guidelines regarding kidney transplantation [15].

– We recommend either universal prophylaxis or preemptive therapy We recommend either universal prophylaxis or preemptive therapy for prevention of CMV disease (strong recommendation, high-quality evidence).
– For D+/R−, we recommend the use of either prophylaxis or preemptive therapy after kidney and liver transplant. For programs or patients unable to meet the stringent logistic requirements required with a preemptive therapy strategy, prophylaxis is preferred.
– For seropositive recipients (R+) after kidney or liver transplant, we recommend either strategy (strong recommendation, high-quality evidence).
– We suggest prophylaxis may be preferred in donor and/or recipient seropositive patients whose risk for CMV may be increased, including those on recent antilymphocyte therapy, potent immunosuppression including desensitization or ABO incompatible protocols (including those on rituximab, bortezomib, eculizumab, and plasmapheresis/immunoadsorption), and those with HIV; a longer duration of prophylaxis (ie, 6 months) may be more effective (weak recommendation, moderate-quality evidence).
– For D+/R− kidney recipients, prophylaxis for 6 months is preferable (strong recommendation, high-quality evidence).
– When a prophylaxis strategy is used for prevention in R+ patients (with either D+ or D−), a majority of

the experts felt that 3 months of antiviral medication should be used for routine kidney, pancreas, liver, and heart transplant recipients (strong recommendation, high/moderate-quality evidence).
- For those receiving more potent immunosuppression (antilymphocyte antibody therapy, desensitization protocols) or vascularized composite and intestinal transplant recipients, between 3 and 6 months of prophylaxis can be used (weak recommendation, low-quality evidence).
- In CMV D−/R−, antiviral prophylaxis against other herpes infections (varicella and herpes simplex) with acyclovir, famciclovir, or valacyclovir should be considered (strong recommendation, high-quality evidence).
- To avoid transfusion-transmitted CMV, we recommend the use of leukoreduced or CMV-seronegative blood products (strong recommendation, moderate-quality evidence) especially in the highest risk group, D−/R−.
- We do not recommend the routine use of low-dose valganciclovir (weak recommendation, low-quality evidence).
- CMV seropositive recipients receiving mTOR inhibitors have a significantly lower incidence of CMV infection/disease. We suggest the use of mTOR inhibitors as a potential approach to decrease CMV infection and disease in CMV seropositive kidney transplant recipients (strong recommendation, high-quality evidence) and in liver, heart, and lung transplant recipients (strong recommendation, moderate-quality evidence). Cytomegalovirus risk is only one of the factors to consider when deciding on the optimal immunosuppression regimen. The impact of mTOR inhibitors on CMV in D+R− recipients is less clear.

TREATMENT OF CMV INFECTION IN KIDNEY TRANSPLANT RECIPIENTS

The drug of choice for treatment of CMV disease is intravenous ganciclovir. In the VICTOR study, intravenous ganciclovir and oral valganciclovir showed similar efficacy in the treatment of CMV syndrome and invasive CMV disease in adult patients after transplantation of a kidney, liver, heart, and lung [84]. However, it should be borne in mind that in instructions for valganciclovir protocols registered in Russia, there is no "treatment of infection" indication. Only "prevention of CMV infection after solid organ transplantation" is indicated. In addition, with a life-threatening infection or with a virus in the gastrointestinal tract, the use of intravenous ganciclovir is definitely indicated. Acyclovir and valaciclovir are not recommended for treatment of CMV infection. Correctly selecting a dose of intravenous ganciclovir is of fundamental importance (Table).

Table

Dosage recommendations for intravenous ganciclovir in adult patients with impaired renal function (using Cockcroft–Gault formula) [85]

Creatinine clearance, mL/min/1.73 m^2	Initial dose, mg/kg	Maintenance dose, mg/kg per day
≥70	5.0 every 12 hours	5.0
50–69	2.5 every 12 hours	2.5
25–49	2.5 per day	1.25
10–24	1.25 per day	0.625
<10	1.25 mg/kg 3 times a week after hemodialysis	0.625 mg/kg 3 times a week

Clinicians should be aware of some differences in GFR calculation when using various formulas – Cockcroft–Gault, MDRD, CKD-EPI. Suboptimal doses of ganciclovir may contribute to the development of drug resistance, and doses exceeding therapeutic doses may cause toxicity [86, 87]. During treatment, clinical blood counts should be regularly monitored to promptly detect hematological complications. In the case of leukopenia during treatment, one should not immediately discontinue ganciclovir or sharply reduce its dose. It is necessary to start by discontinuing other drugs that can suppress bone marrow hematopoiesis and introducing colony-stimulating factors.

The intensity of immunosuppressive therapy can affect the outcome of CMV infection: bicomponent immunosuppression versus ternary and lower concentrations of calcineurin inhibitors in the blood are associated with eradication of the virus after 21 days of treatment [88]. For this reason, in patients with CMV infection without concomitant graft rejection, reduction of immunosuppression is suggested in the following settings: severe CMV disease, inadequate clinical response, high viral load and/or cytopenia [15].

As already noted, the viral load during treatment should be determined weekly in order to establish the optimal duration of treatment. Plasma CMV DNA retention at the end of treatment is a significant predictor of virological recurrence [84]; therefore, therapeutic doses of ganciclovir should remain until the clinical symptoms disappear and CMV DNA is eradicated. Eradication is detected when one result of CMV DNA determination is less than the lower limit of quantification (LLOQ) with the use of highly sensitive QNAT or when two consecutive negative results are obtained by less sensitive methods [15]. The use of a therapeutic dose of ganciclovir in any case should last for at least two weeks. Routine use of intravenous immunoglobulin in the treatment of CMV infection is not recommended, although it can be considered in severe cases of the disease. Secondary prevention, i.e. the use of prophylactic doses after completion of treatment is impractical, since it usually

does not reduce the incidence of recurrence [89–91]. However, it can be used in some cases involving very high risk of recurrence.

In patients with a previous use of ganciclovir or valganciclovir lasting for over 6 weeks, or with treatment failure lasting at least two weeks, or with DNAemia during prophylaxis, drug resistance may be suspected. Drug resistance is a change in the genome of a virus, which reduces its sensitivity to one or more antiviral drugs. Among solid organ recipients, the incidence of ganciclovir resistance is on average 5–12%, and when the recipients were given D+/R prophylaxis for 100 or 200 days, ganciclovir or valganciclovir resistance incidence was less – from 0 to 3% [92–94]. Genetic testing – sequencing of the virus genome – is recommended for clarifying the causes of resistance. The database of CMV mutations associated with drug resistance is constantly growing [95, 96]. Testing should include mutation studies of the UL97 and UL54 genes. UL97 kinase gene mutations occur during initial genetic testing in 90% of cases of resistance in patients who initially received ganciclovir and disrupts drug phosphorylation required for its antiviral effect [97]. UL54 DNA polymerase gene mutations are usually detected at a later period, causing resistance to ganciclovir and often cross-resistance to cidofovir and/or foscarnet.

Unfortunately, there are currently no data from controlled trials that would allow us to choose the optimal treatment tactics for drug resistance to CMV. So, the proposed algorithms are based on the opinion of a group of experts. If laboratory testing returns no evidence supporting drug resistance, emphasis should be given to optimization of factors associated with the patient's body and drug delivery, than switching antiviral medications [15]. Immunosuppressive therapy is reduced to the minimum possible volume. Some UL97 mutations are characterized by lower levels of ganciclovir resistance, and escalating the dose of ganciclovir (up to 10 mg/kg every 12 hours) in combination with optimizing the recipient's body condition if there is no severe CMV disease might be useful [98]. This is a double standard dose, therefore, it is necessary to monitor possible bone marrow suppression and adjust the dose according to renal function.

Switching to foscarnet (which is not available in Russia) is recommended in cases where the mutation causes high-grade resistance to ganciclovir or there are combined UL97 and UL54 mutations causing high-grade ganciclovir resistance and, as a rule, cross-resistance to cidofovir. Foscarnet salvage therapy is often effective, at least initially, but metabolic disruptions and nephrotoxicity of the drug can negatively affect the final results of treatment [99–101]. There is still insufficient information on the effectiveness of salvage therapy with cidofovir in CMV infection in solid-organ transplant recipients [102, 103]. The nephrotoxicity of this drug is dose dependent. Cidofovir can be used in cases where double resistance – to ganciclovir and to foscarnet – is detected, but without cidofovir resistance. However, amidst such treatment, there have been reported cases of rapid development of virus load recurrence and appearance of new mutations that have already caused cidofovir resistance [104–106]. Apparently, the phenomenon described is associated with previously undetected subpopulations of cross-resistance mutants selected during previous ganciclovir therapy. A high dose of ganciclovir can also be used in situations where CMV is resistant to foscarnet, has no high-grade resistance to ganciclovir.

Additional treatment options for CMV infection include the use of drugs that can boost the patient's immune system or have an antiviral effect inessential for this class of drugs. Introduction of anti-cytomegalovirus intravenous immunoglobulin and infusion of CMV-specific T-lymphocytes can boost the body's antiviral defense [107, 108]. Several drugs used for other purposes, namely, everolimus, sirolimus, leflunomide, artesunate (antiprotozoal drug), have an *in vitro* anti-CMV activity and can act synergistically with antiviral drugs [109–111]. However, one should be aware that the use of leflunomide and artesunate with CMV infection has been studied in isolated cases and in small series. Besides, the use of these drugs requires special control due to possible toxic effects on the liver.

PROSPECTS FOR MONITORING, PREVENTING AND TREATING CYTOMEGALOVIRUS INFECTION IN SOLID ORGAN TRANSPLANT RECIPIENTS

In recent years, advances in clinical transplantology have been marked by significant successes in prevention and treatment of CMV infection in solid organ transplant recipients. This is associated with better immunological and molecular diagnosis of the disease, and expansion of the scope of knowledge about treatment of ganciclovir-resistant forms of CMV [112]. Nonetheless, management of CMV infection in kidney transplant recipients still has a series of unresolved problems, and there is a certain gap between scientific advances and real clinical practice. For example, no immunological monitoring method that could justify a personalized approach to prevention or preemptive therapy has been fully developed, universal DNAemia threshold for starting therapy has not been defined, the optimal duration of prevention has not been determined, and the issue of combating late-onset CMV infection has not been resolved [15]. The problem of overcoming ganciclovir resistance of the virus is also not resolved.

Immunological monitoring can be used to determine the individual risk of viral infection reactivation. *In vitro* interferon-gamma release (induced by stimulation of lymphocytes with CMV antigens) test have been de-

veloped. The commercially available QuantiFERON-CMV test is already being used and it has shown good prognostic value: the positive results of this test at the end of valganciclovir prophylaxis correlated with a low incidence of CMV disease in the future [113, 114]. More recently, the results of an interventional study of the efficacy of QuantiFERON-CMV test in patients undergoing the first episode of CMV reactivation have been published. In this study, patients who tested positive at the end of treatment for the first episode of CMV infection did not receive secondary prophylaxis, and only a single patient subsequently experienced an episode of asymptomatic DNAemia [115]. However, further research is required for widespread clinical use of this method.

As we have already noted, development of new drugs for treatment of CMV infection is extremely important due to emergence of ganciclovir resistance and high toxicity of alternative drugs – foscarnet and cidofovir. Brincidofovir, a lipid-conjugated analog of cidofovir, has higher oral bioavailability and less nephrotoxicity compared with cidofovir. However, the effectiveness of bricidofovir was low in the prevention of CMV infection in hematopoietic cell transplant recipients. Moreover, there is still extremely insufficient information on the use of the drug in solid organ recipients [116]. Maribavir is a viral UL97 kinase inhibitor. Although this drug has not been shown to be effective in preventing CMV infection in liver transplant recipients when taken 100 mg orally twice daily using it at higher doses has shown to be effective in treating resistant CMV disease in solid organ recipients [117, 118]. Maribavir ≥400 mg twice daily was quite active in the treatment of patients with refractory or resistant CMV infection in a phase 2 study, and the phase 3 study is ongoing [119]. Letermovir, a new non-nucleoside inhibitor of the CMV viral terminase complex, was approved by the FDA in 2017 for prevention of CMV infection in bone marrow transplantation. In this population, a randomized phase 3 trial showed letermovir to have superior efficacy than placebo in prevention of CMV disease. Here, myelotoxicity and nephrotoxicity were comparable to placebo [120]. Letermovir has been successfully used in a lung transplant recipient with a series of drug-resistant CMV infections; effective treatment of CMV viremia in kidney transplant recipients has also been reported [121, 122]. A clinical study comparing letermovir with valganciclovir for the prevention of CMV infection in kidney transplant recipients in a D+/R– situation is commencing (ClinicalTrials.gov ID: NCT03443869).

A promising area is the possibility of using T-cell therapy and CMV vaccines. Expansion of CMV-specific T-lymphocytes is achieved by exposing the cells to synthetic or viral CMV peptides after which T-lymphocytes are administered to the patient. This restores antiviral immunity and cures the CMV disease. T-lymphocytes can be autologous, but the process of obtaining them usually takes several weeks. That is why there is growing interest in ready-made HLA-compatible lymphocytes from cell banks. The emergence of commercially available banked CMV-specific T-lymphocytes can lead to an increase in the incidence of use of this modality of therapy in solid organ transplant recipients [123, 124]. CMV vaccines are of various types – live attenuated, recombinant/chimeric viral vectors, recombinant subunits, and DNA (gene) vaccines [125]. Generally, development of CMV vaccines has reached the phase of clinical trials in humans. However, vaccines are not yet available in real clinical practice.

Main focus should certainly be on prevention of CMV infection in kidney transplant recipients. For prevention of CMV infection, new effective antiviral drugs can be used, as well as active introduction of immunosuppressive agents with additional antiviral effects in clinical practice. In this regard, the use of *de novo* immunosuppressive protocols with an mTOR inhibitor everolimus in kidney transplant recipients, which significantly reduced the incidence of viral infections in the post-transplant period, including CMV infection, seems to be promising [79]. Since it is still far from ideal, further research is needed to optimize the prevention of CMV infection in clinical transplant practice.

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PREVENTION AND SURGICAL TREATMENT OF UROLOGICAL COMPLICATIONS IN KIDNEY TRANSPLANT RECIPIENTS

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Post-kidney transplant urological complications (failure of a newly formed anastomosis, obstructive uropathy, necrosis of graft ureter, graft ureteral stricture, development of vesicoureteral reflux in the renal graft, recurrent urinary infection) are one of the main causes of graft loss and various deaths. This literature review aims at analyzing world studies on prevention methods (routine graft ureteric stenting) and surgical techniques for treating urological complications (laparoscopic correction of supravesical urinary tract obstruction in a graft kidney) in kidney recipients.

Keywords: urological complications, kidney transplantation.

With all significant achievements and progress in treating kidney recipients, urological complications remain the main causes of long hospital stay, graft loss, and death of recipients in the earlier and later postoperation stages [1–2]. The main urological complications developing in kidney recipients are failure of the newly anastomosis (1.5–6%), obstructive uropathy (0.9–7.5%), necrosis of the graft ureter, stricture of the graft ureter (3.0–12.6%), development of vesicoureteral reflux in the renal graft (5.0–20%), and recurrent urinary tract infection. The latter is one of the biggest problems for patients on the prolonged immunosuppressive treatment and one of the leading mortality causes after kidney transplantation reaching 5 to 10% in the 1st year [2–5]. Most often, urological complications occur during the first two weeks after transplantation and are manifested by a decrease in urine output and impaired graft function [6]. The results of the treatment of urological complications in kidney recipients are associated with the time of the diagnosis. So far, the question remains open of the methods of surgical interventions indicated in the prevention and treatment of urological complications.

The urological complications rate after kidney transplantation in early studies (1970–1990) varied from 4.2 to 14.1% [7], in later studies (1990–2000) it was 3.7–6.0% [8], while at present they rate from 2 to 5% [9], which is probably reflecting of various stages of the development of transplantation, the improvement of diagnostic methods and the advance of surgical skill.

In their retrospective study, M. Whang et al. analyzed the results of 2,548 kidney transplantations and detailed the following urological complications (5.5%): reflux in the renal graft (3%), strictures of the graft ureter (1.3%), uroplania (0.9%), and urinary obstruction (0.3%). Among the factors affecting the reduction in the number of urological complications, there were single surgeon manipulations, the use of a shorter segment of the ureter by Lich-Gregoire (compared to Politano-Leadbetter) method, and routine ureter stenting [9]. The following independent risk factors for the development of the urological complications were identified: male donors, male recipients, African American recipients, Taguchi method, graft artery reconstruction, multiple renal arteries, and diabetes in recipients [10–11].

Failure of the newly anastomosis. The uroplania prevalence after kidney transplantation, which can occur shortly after transplantation or in the later postoperative period, is 1.5 to 6%, [4; 12]. In most cases, the uroplania occurs in the anastomosis area, at the bladder, ureter or kidney pelvis levels [6].

At the formation of pyeloureteral anastomosis, uroplania can occur due to improper installation of the proximal stent helix or the pelvis perforation at its installation [6; 13]; also, the uroplania may be caused by the atrophied bladder mucosa and dysfunction of the urethral catheter against the background of polyuria with the early (up to 6 weeks) removal of the urethral catheter [14].

In the first hours after surgery, the uroplania is most often manifested by an increase in the drainage volume, profuse wound blotting and delayed graft function. Most commonly, the biochemical analysis of drained fluid shows high creatinine, urea and Ca levels [15]. The indirect signs of uroplania are the concretion over the graft, genitals or thighs swelling, fever, urine output decrea-

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sed to anuria, and increased plasma creatinine [15]. The uroplania occurring in one to two weeks after surgery is caused by the ureter necrosis due to insufficient blood supply (the distal ureter is most at risk during the graft treatment) [15–16]. The delayed uroplania diagnosis can lead to inflammatory processes (abscess) in the area of the transplanted kidney and the generalized infectious process in the patient [14].

It was shown that the uroplania is less common in patients with the ureter anastomosis with the ureter stent [13].

Obstructions. These are the most diverse group of complications; they pose a serious risk of loss of transplanted kidney function and are observed in 0.9–7.5% of recipients [3]. According to J. Aurio, the incidence of obstructive uropathy is 3–4%, and the risk of their development is higher in recipients with a kidney from a donor over 65 with a graft with more than two arteries [17].

Obstructions directly related to the anastomosis formation. Ureterocysto- (pyelouretero-, ureteroanastomosis) obstruction at the sutures area, the ureter compression in the submucosal tunnel, the ureter torsion or inflection (positional obstruction).

An insufficient length of the ureter leads to an increase in the mechanical load on the anastomosis and causes a urinary fistula, while its excessive length leads to the ureter torsion and violation of the urine outflow [9]. A radical way to correct the recurrent complications is to form the pyeloureteroanastomosis with the recipient's ureter. In case of ureterocystoanastomosis obstruction (including the ureter compression in the submucosal tunnel), at the ureter torsion or inflection, the reimplantation of the ureter into the bladder is recommended [2].

Compression obstructions. The compression obstructions relate to the graft ureter compression from outside by a lymph cyst, testicular cord, abscess, neoplasm, urinoma, and hematoma.

The recurrent obstructive complications of the immediate postoperative period (to 12 weeks) include the ureter compression by a lymph cyst (found in 0.6–51% of kidney recipients) [6]. The formation of a lymph cyst is tied to an insufficiently thorough ligation of the lymphatic ducts at the identification of main blood vessels and graft treatment [18]. An increase in lymph secretion is provoked by a violation of the venous outflow, rejection episodes, and even mechanical injury to the kidney. A rational way to eliminate a lymph cyst is its marsupialization or internal drainage into the abdominal cavity, provided there is no lymph cyst pyosis [19]. In the immediate postoperative period, an external ureter compression usually develops no earlier than by the second postoperation week and can be caused by a large hematoma, urinoma, and even an abscess. Further on, the presence of a hematoma sometimes leads to the development of retroperitoneal fibrosis and ureter stenosis that usually occur in several months after kidney transplantation [13–14].

Obstruction of the ureter interior lumen. It can be obstructed by blood clots, necrotic masses, concrements, foreign bodies, and neoplasms.

The ureter necrosis is one of the adverse complications in the immediate postoperative period [20]. An excessive excision of periurethral tissue and the use of the ureter of excessive length are common causes of the ureter necrosis [16]. The correction method depends on the extent of the alterations. First, a puncture nephrostomy is performed to adequately drain the kidney collector. At maintained ureter patency, its antegrade stenting is possible [3]. At complete obliteration of the ureter, the formation of anastomosis of the graft pelvis with the recipient ureter is proposed.

The ureter obstruction by concrements is detected by the routine ultrasound examination. Due to complete graft denervation, the renal colics are absent, though a sensation of heaviness and fullness in the iliac region may be present (due to pressure on the surrounding tissues), urethrodynia, fever, arterial hypertension, urine amount decrease up to anuria (at complete obstruction) [9]. With even a moderate expansion of the pyelocaliceal system of the graft in recipients, the antegrade pyeloureterography is indicated [21].

Sclerotic obstructions. The later period is mostly featured by obstructive complications due to the development of ischemic ureter stricture, retroperitoneal fibrosis or the bladder wall sclerosis and, though much less commonly, of the ureter occlusion by calculus.

The reflux in the renal graft occurs in 1–50% of recipients, despite the use of the antireflux technique for the anastomosis [formation 3; 16; 22]. According to the literature, there is no negative reflux effect on the function of the transplanted kidney. This can be explained by the fact that the graft ureter is denervated and its length is small; therefore, when the active reflux occurs, the high hydrostatic pressure in the kidney collector, which is the damaging trigger, persists for a short time and then rapidly drops [23]. Thus, functional (and especially organic) changes in the renal graft have no time to develop. In their study, M. Margreiter et al. found that reflux does not affect such long-term outcomes as the graft survival, the recipient, the incidence of urinary tract infections, and the proteinuria severity [24].

The reflux in the renal graft is divided into active (at urination), passive (at the bladder filling), and mixed [25].

The question of the need for reflux correction is related to the degree of its influence on the graft function. An indication for surgery is the persistent vesicoureteral reflux, leading to impaired renal graft function. The remedial procedure may fail at insufficient volume and rigidity of the bladder wall, which is often in patients with chronic kidney disease after prolonged anuria. In the

presence of their own unaltered ureters, the most radical way to eliminate massive reflux in the graft is the end-to-end pyeloureteroanastomosis [9]. However, according to some surgeons, the surgery of the reflux in the renal graft could be resorted to the most extreme cases when graft function cannot be preserved by other methods. In this, less traumatic endourological transurethral correction methods are more commonly used.

The vesicoureteral anastomosis stricture is the most common urological complication after kidney transplantation. The rate of ureter stricture in kidney recipients, according to various sources, ranges from 0.9 to 34% [7; 9; 19–20; 26].

The ureter strictures are usually classified as early (<3 months) and late (>3 months) after kidney transplantation. The early ureter strictures can be caused by foldings, temporary swellings of the ureter wall, narrow anastomosis or external compression, hematoma or lymph cyst [5; 19]. The late ureter strictures are usually associated with poor ureter vascularization, leading to ischemia and the development of retroperitoneal fibrosis. The following risk factors were identified: the donor age over 65, prolonged cold ischemia, the presence of several renal arteries, delayed graft function, and a vesicoureteral anastomosis without a stent [20]. Another cause of the late ureter stenosis (2–6% of all cases) is RSV infection (poliomaviruses). Histologically, the stenotic region of the ureter looks ischemic and fibrous [26].

In a retrospective study of S. Buresley, the outcomes for 646 kidney grafts from live relative (n = 461) and deceased (n = 185) donors to patients, 81 of which were children, were analyzed. The ureter strictures (n = 15, 2.58%) were diagnosed in the later period after transplantation and was more common among children (4.23%), uroplania was observed in the early postoperative period and was more common in elderly (4.69%) patients [8].

Routine ureter stenting in patients at kidney transplantation. A lot of modern studies are aimed at assessing the role of routine ureter stenting in the development of urological complications in kidney graft recipients [27–31].

M.R. Laftavi found that the vast majority (97%) of kidney recipients without signs of bladder dysfunction who received standard kidneys without signs of ureter blood supply violation can be successfully operated without the routine use of stents [32]. It has been shown that the rate of urological complications is higher after transplantation of a kidney from a living donor, while the incidence of urinary tract infections is higher after kidney transplantation from deceased donors [33].

The routine use of stents for kidney transplantation can lead to such problems as migrated, encrusted, broken and forgotten stents, as well as pain in the lower urinary tract, hematuria and dysuria due to the small volume of the bladder [34].

Many authors state that the routine use of ureter stents favors the development of urinary tract infections, which can lead to transplanted kidney dysfunction and even death [35–37]. J. Gozdowska et al. attributed the installation of the ureter stent to risk factors for infectious complications at kidney transplantation (more often in males) (n = 34; 32%, p = 0.021) [36].

However, some studies have not found a significant difference in the incidence of urinary tract infections in kidney recipients with and without routine ureter stenting [38–41].

To solve the matter of UTI on the background of immunosuppressants in kidney recipients with stented ureters, the early stent removal is proposed [31; 37; 42–43]. Various transplantation centers report different optimal stent removal times after transplantation, ranging from 5 days to 6 weeks [41; 43–44].

Based on an analysis of kidney graft outcomes with routine ureter stenting in 48 patients, A.K. Coskun showed that early stent removal at the end of the 2nd week after kidney transplantation reduces the incidence of urinary tract infections by 2% vs. 35% (at stenting for over 2 weeks). Urological complications were not detected in any group [44]. P. Patel showed that the UTI rate was 24.6% with the stent removed after 6 weeks and 7.6% when removed on day 5 after kidney transplantation [43].

The updated Cochrane meta-analysis, which included seven randomized controlled trials, recommended the routine use of stents during kidney transplantation due to the low incidence of uroplania (1.02% vs. 5.28%; 95% CI [0.12–0.74]) and obstruction (0.51% vs. 4.40%; 95% CI [0.09–0.81]) in the group with stents [28]. However, the routine ureter stenting has been shown to increase the incidence of urinary tract infections (26.3% with a stent vs. 17.9% without a stent; OR = 1.49; 95% CI [1.04–2.14]; p = 0.03); when the stent stays for over 6 weeks, there is a risk of the stent encrusted with urinary salts [28].

A. Tavakoli et al. found that the routine use of the ureter stent at kidney transplantation reduces the risk of uroplania and urinary obstruction, while the incidence of urinary tract infections increases significantly when the stent stays longer than 30 days (p < 0.01) [29].

An intermediate analysis of a randomized prospective double-blind study showed that the ureter stent removal in 1 week reduces the UTI risk compared to the routine removal in 4 weeks (OR = 8.791; 95% CI [1.984–38.943]; p = 0.004) [45].

In their meta-analysis, J.F. Cai et al. found that early (≤7 days) removal of the ureter stents after kidney transplantation did not significantly increase the frequency of postoperative urological complications (ureter stricture, ureter obstruction, and uroplania) compared to late (≥14 days) removal (OR = 1.87, 95% CI [0.45–7.70], p > 0.05). A significant difference was observed in the UTI incidence between the early and late removal groups

with ureter stents (OR = 0.43, 95% CI [0.32–0.59], p < 0.01) [46].

Despite the high UTI risks, the current data suggest the routine use of stenting. Determining the optimal time for the ureter stents removal is important to minimize the risk of such complications as urinary tract infections associated with prolonged exposure and to prevent urological complications in patients after kidney transplantation.

Treatment of ureter strictures of a transplanted kidney. Balloon dilatation and temporary stenting of the ureter are the most common endourological procedures. As a rule, the percutaneous drainage of the pyelocaliceal graft is considered the first option, since it is simpler and has both diagnostic and therapeutic implications at hydronephrosis [47]. In the short term, endourological procedures have a high measure of efficacy (73 to 100%), which in the long term decreases to 40–55% due to the high relapse rate [48]. There are reports on the success rate of minimally invasive treatment of ureter strictures ranging from 49% to 100%, depending on the extent, stricture location, and the treatment method [47–48]. Helfand with colleagues reported on the experience of surgical treatment of ureter strictures after kidney transplantation and proposed a stricture treatment algorithm based on the stricture size (<3 cm) and the time between transplantation and epy stricture diagnosis (<3 months) [49]. In a review by Haberal et al., the recurrent balloon dilatation is recommended for resistant strictures, whereas for fibrous strictures, the temporary post-dilatation stenting is suggested. They tried to determine a treatment strategy for kidney recipients who develop ureter strictures [50]. In B. He et al., three classes of ureter strictures were determined: the 1st included hydronephrosis with ureter stenosis without strictures, the 2nd – hydronephrosis with a stricture of ≤1 cm, and the 3rd – hydronephrosis with a stricture of more than 1 cm [51].

The balloon dilatation of ureter strictures has become one of the first correction methods for patients with transplanted kidneys and showed its efficacy of 51% (44–62%) with a follow-up period of 17 to 78 months [52]. The balloon dilatation has proven effective in the treatment of ureter anastomosis with obstructive megaureter and with ureter strictures of 1 cm or less in kidney recipients [53].

In Ooms LSS retrospective study, the antegrade balloon dilatation was shown to be an effective treatment for ureter strictures after kidney transplantation, since it is minimally invasive and can prevent surgical treatment of strictures in almost 50% of cases [54].

M. Balaban et al. evaluated the efficacy of minimally invasive treatment of ureter strictures by retrograde stenting of the ureter of a transplanted kidney. Ureter strictures were found in 13 patients (1.26%) out of 1,026. The overall success rate of the introduction of a retrograde ureter stent on the first try was 75%, and the success of replacing the stent was 100%. The renal function remained stable in all patients for 41 months; no complications were detected. Thus, the method of retrograde stenting of the ureter with strictures is safe and effective in kidney recipients who are not indicated for open surgical reconstruction [55].

E.G. Yushina et al. established the benefits of preventing the failure and strictures of ureterocystoanastomosis of a transplanted kidney and of the endoscopic methods for correcting urological complications after a kidney transplantation [56].

The higher efficacy was observed with simultaneous dilatation of stricture and electro incision of the ureter wall, which is feasible with a destructor. During the follow-up period (19 months), the efficacy of the method for localization of ureter strictures in the distal region increased to 78% (60 to 100%) [21; 52].

Despite the increased potential for percutaneous obstruction correction, there remains a certain category of patients requiring surgical treatment. Surgery is indicated at the complete obliteration of the ureter in a significant area or when it is technically impossible to percutaneously remove the obstruction to the urine outflow. In some patients, using endoscopic methods and open surgery, it is not possible to restore an adequate urine passage from the graft.

J. Kwong et al. note that at the violation of the urine outflow from the graft, the most common minimally invasive correction method is the endourologic treatment, providing a successful outcome of up to 58.6% (95% CI 50.1–66.7, n = 133) [57], and up to 81% with open surgical correction methods [57–58]. The majority of the current studies show a similar rate of urological complications in the groups with ureteroneocystoanastomosis and ureteroureteroanastomosis / pyeloureteroanastomosis [59].

The balloon dilatation and laser pyelo- or ureterotomy have good clinical outcomes in patients with strictures of no more than 2 cm. If a stricture recurs, repeated dilatation and laser pyelo- or ureterotomy are not recommended [60].

D.A. Perlin et al. demonstrated the possibility to perform pyeloureteroanastomosis using the recipient's ureter (n = 2) in the treatment of urological complications after kidney transplantation with the laparoscopic method [61].

CONCLUSIONS

The review and analysis of literature data on the prevention and surgical correction of urological complications in transplanted kidney recipients make it possible to conclude that the problem is currently being comprehensively studied all over the world.

In patients after kidney transplantation, there is a risk of urological complications due to prolonged anuria before surgery, a small bladder volume, ischemia, necrosis, stenosis or compression of the graft ureter; therefore,

despite the risk of developing infectious complications, the issue of routine stenting of the graft ureter and the timing of its removal still appear relevant for the prevention of urological complications.

Analyzing the literature, we found some unresolved issues in the tactics of surgical treatment of post-transplant urological complications arising in kidney recipients in the long term. Traditionally, the correction of urological complications has been performed by open surgery, which, in turn, was a traumatic procedure for the patient, with a complicated postoperative period and slow healing of postoperative wounds against the background of immunosuppressive therapy. Currently, what comes to the fore is the implementation of minimally invasive surgical methods that reduce the risk of postoperative complications, diminish indications for open surgical interventions, and shorten the hospital stay; however, we still lack the precise and complete protocols and algorithms to treat urological complications after kidney transplantation.

The authors declare no conflict of interest.

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CONGRATULATIONS TO GEORGY PINKUSOVICH ITKIN

August 5, 2019 marked the 80th anniversary of the day Georgy Itkin, a professor of Biology, and Head of the Laboratory of Biotech Systems at the Shumakov National Medical Research Center of Transplantology and Artificial Organs, was born. He is one of the top experts when it comes to developing artificial heart and auxiliary blood circulation apparatuses and systems.

Professor Itkin has a long and thorny career, but one which is full of interesting and fruitful work. After graduating from the Moscow Aviation Institute in 1963, he worked as an engineer at Tupolev, a Soviet aerospace and defence company. In 1966, he was employed at the laboratory of artificial heart under the Scientific Research Institute of Clinical and Experimental Surgery, headed by Prof. Valery Shumakov. Till present, he continues to work at the research institute, creating mechanical circulatory support systems.

In 1975, Prof. Itkin defended his PhD dissertation, and in 1989 became a professor of biology, his project being devoted to development and comprehensive analysis of methods and means of temporary replacement of the heart. Prof. Valery Shumakov highly recognized Georgy Itkin's contributions throughout their joint activities.

Itkin has participated in the design of control systems and bench-top research under the Soviet-American intergovernmental agreement on artificial heart. At that time, many Soviet laboratory developments were ahead of their American counterparts, which was greatly credited to the celebrant. Itkin has worked closely with outstanding world-class scientists from Europe, USA, and Japan – pioneers in artificial heart and cardiopulmonary bypass devices: Valery Shumakov, Michael DeBakey, Willem Kolff, Robert Jarvik, and Tetsuzo Akutsu. He has jointly worked with Czechoslovak scientists and engineers from Jan Evangelista Purkyne University (Prof. J. Washka and Prof. Urbanek), with Austrian scientists from the University of Vienna (among whom was Prof. W. Schim), and German scientists from the Free University of Berlin (one of whom was Prof. C. Affeld).

Under Professor Itkin's leadership, an automatic control system for the artificial heart was developed using a mathematical model of the circulatory system. This innovation allowed to achieve long-term survival of artificial heart calves (Olymp lived for 102 days). In his students' projects, a model of the circulatory system was used to analyze interaction between auxiliary circulatory devices and the cardiovascular system. The name "Georgy Itkin" has been directly associated with all the achievements in experiments on long-term implantation of artificial heart for calves and its subsequent clinical testing in the USSR and Poland for a two-stage heart transplantation.

In recent years, a team of young researchers has been actively working under Professor Itkin's guidance. This team has noted the professor's inexhaustible flow of research ideas, his creative spirit and cheerfulness, which has helped them solve complex problems involved in the creation of new non-pulsatile flow systems based on centrifugal and axial pumps.

The team's major achievement is the creation of an axial flow blood pump, which has been successfully used in clinical practice since 2012. Georgy develops methods and means of medical and technical testing of pumps. The laboratory staff loves and respects their leader and is proud of him. About 9 master's theses have been completed under his guidance.

Georgy Itkin is a professor at the Department of Living Systems, Moscow Institute of Physics and Technology. He is a member of the International Society of Artificial Organs and has authored over 200 research papers. He has 64 patented inventions and has been awarded the "Merited Inventor of the USSR" award.

The editorial team of the Russian Journal of Transplantology and Artificial Organs, including the editor-in-chief Professor Sergey Gauthier, and the laboratory staff, warmly congratulate Professor Georgy P. Itkin, a world-renowned scientist, an erudite educationist, and a wonderful person, on his 80^{h} birthday. We wish him good health, good luck and energy in all his planned projects!

INSTRUCTIONS FOR AUTHORS

Submitted articles should contain original work that has not been previously published and is not under consideration for publication elsewhere. We do not charge any publication fee.

The paper size should be A4 (1 copy, 1.5 pt line spacing). The text of the body of the paper should be in Times New Roman with font size 12 pt. The paper should be presented in the form of an identical Microsoft Word file on electronic media (attached CD or via e-mail).

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Abstract

Each article must have an abstract of no more than 300 words for a literature review, and no more than 200 words for clinical observation. The abstract should be a concise summary of the entire content of the paper. It is a fully self-contained, capsule description of the paper. Avoid using abbreviations and acronyms in the abstract.

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

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- Materialsandmethods
- Results
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- Conclusion
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