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Глубокоуважаемые коллеги!

25 сентября 2023 года в ФГБУ «НМИЦ трансплантологии и искусственных органов им. ак. В.И. Шумакова» Минздрава РФ состоялось выездное заседание Бюро секции клинической медицины Отделения медицинских наук Российской академии наук «Современная трансплантология: сложные хирургические решения и векторы научного поиска». Заседание явилось частью VI Российского национального конгресса с международным участием «Трансплантация и донорство органов».

Министр здравоохранения РФ Михаил Мурашко отметил, что трансплантология в нашей стране активно развивается, открываются новые трансплантологические центры: в 37 регионах нашей страны функционируют 67 учреждений, оказывающих трансплантологическую помощь, качество и результаты которой соответствуют мировому уровню. Современная трансплантология представляет собой яркий пример сочетания достижений высокотехнологичной медицины и прорывных научных результатов. И все это в кратчайшие сроки транслируется в практику.

Руководитель секции клинической медицины Отделения медицинских наук РАН академик И.В. Решетов подчеркнул, что заседание является частью общей плановой деятельности научного совета по хирургическим наукам Отделения медицинских наук РАН. Более того, проведение этого мероприятия в формате программы конгресса трансплантологов является весьма эффективным, так как позволило привлечь к участию и обсуждению наиболее широкий круг специалистов, ученых и практиков, – более 500 человек.

Академик РАН С.В. Готье выступил с докладом «Трансплантационная хирургия: высший пилотаж vs ящик пандоры?» и подчеркнул, что даже блестящее хирургическое решение является только началом большого пути сохранения жизни и работоспособ-

TRANSPLANT SURGERY: AEROBATICS VS PANDORA'S BOX?

Dear colleagues,

On September 25, 2023, the Shumakov National Medical Research Center of Transplantology and Artificial Organs hosted a retreat by the Bureau of the Clinical Medicine Section of the Department of Medical Sciences, Russian Academy of Sciences. The retreat, titled "Modern Transplantology: Complex Surgical Solutions and Vectors of Scientific Inquiry", was held as part of the 6th Russian National Congress "Transplantation and Organ Donation", featuring international participants.

Russia's Minister of Health, Mikhail Murashko, noted that transplantology is rapidly developing and flourishing in Russia and that new transplant centers are coming up: there are 67 transplant care institutions in 37 federal subjects across the country; the quality and outcomes of this care are consistent with highest global standards. Modern transplantology represents a striking example of a blend between advances in high-tech medicine and scientific breakthroughs. And all this is translated into practice in a short time frame.

Head of the Clinical Medicine Section of the Department of Medical Sciences, Russian Academy of Sciences, Academician Igor Reshetov, emphasized that the session is a part of the general planned activity of the Research Council on Surgical Sciences of the Department of Medical Sciences, Russian Academy of Sciences. Moreover, holding this event as a program under the Congress of Transplantologists is very effective, as it enabled a wide range of specialists – researchers and practitioners, over 500 people – to participate and discuss.

Sergey Gautier, a fellow of the Russian Academy of Sciences, presented a report titled "Transplant surgery: aerobatics vs pandora's box?". In the presentation, he emphasized that even a brilliant surgical solution is only the beginning of a long journey to preserving a patient's

ности пациента. Трансплантационная хирургия должна создать условия для адекватного функционирования трансплантата в организме реципиента. Успех базируется не только на безупречной хирургии, но и на понимании и прогнозировании физиологических и патофизиологических процессов, происходящих как в самом донорском органе, так и в организме реципиента сразу после перфузии донорского органа. Тут и открывается тот самый ящик Пандоры, содержащий множество диагностических, лекарственных и хирургических протоколов, применяемых для мониторинга и своевременной коррекции функций биосистемы «донорский орган – реципиент».

Анализ проблем, достижений и перспектив в этой мультидисциплинарной области включал широкий комплекс вопросов, касающихся трансплантации и донорства органов («Высокотехнологичная двухэтапная мультиорганная эксплантация у доноров: от нефрэктомии до перфузии органов ex vivo», А.В. Шабунин и М.Г. Минина); осложнений трансплантационной хирургии («Ранние и поздние хирургические осложнения у реципиентов печени: визуализация и стратегия малоинвазивной коррекции», Д.А. Гранов и И.И. Тилеубергенов); патофизиологии поддержания жизни реципиента при трансплантации (В.Н. Попцов), трансплантации печени (А.Р. Монахов); хирургических и перфузионных технологий при трансплантации легких (И.В. Пашков); хирургии трансплантации сердца и механической поддержки кровообращения (Т.А. Халилулин). Анализ наиболее значимых научных результатов, имеющих перспективы практической реализации, представил член-корреспондент РАН А.О. Шевченко в докладе «Активное долголетие реципиентов донорских органов: от хирургии до протеомики».

В обсуждении приняли участие действительные члены Российской академии наук А.Ш. Ревишвили, М.Ш. Хубутия, член-корреспондент РАН А.М. Чернявский, академик НАН Республики Беларусь Ю.П. Островский.

Заседание бюро секции клинической медицины Отделения медицинских наук, посвященное проблемам трансплантологии, явилось значимым и резонансным событием для медицинской науки и всей отрасли здравоохранения. Оно показало высокий уровень научных исследований в этой области, их направленность на достижение практического результата, на создание отечественного высокотехнологичного оборудования, в частности для перфузии донорских органов; систем механической поддержки кровообращения для укрепления технологического суверенитета нашей страны.

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

life and keeping his or her fit. Transplant surgery must create the necessary conditions for a graft to function adequately in the recipient's body. Success is based not only on a perfect surgery, but also on understanding and predicting the physiological and pathophysiological processes occurring both in the donor organ itself and in the recipient's body immediately after organ perfusion. This is where the Pandora's box opens, bringing along a variety of diagnostic, drug and surgical protocols used for monitoring and early correction of the functions of the "donor organ/recipient" biosystem.

Analysis of problems, achievements and prospects in this multidisciplinary field included a wide range of issues relating to organ transplantation and donation ("High-tech two-stage multi-organ explantation in donors: from nephrectomy to ex vivo organ perfusion", A.V. Shabunin and M.G. Minina); complications of transplant surgery ("Early and late surgical complications in liver recipients: visualization and minimally invasive intervention strategy", D.A. Granov and I.I. Tileubergenov); pathophysiology of life support for transplant recipients (V.N. Poptsov), liver transplantation (A.R. Monakhov); surgical and perfusion techniques in lung transplantation (I.V. Pashkov); heart transplant surgery and mechanical circulatory support (T.A. Khalilulin). An analysis of the most significant scientific achievements with prospects for practical implementation was presented by an associate member of the Russian Academy of Sciences, A.O. Shevchenko, in his report titled "Active Longevity of Organ Recipients: from Surgery to Proteomics".

Full members of the Russian Academy of Sciences A.S. Revishvili and M.S. Khubutia, associate member of the Russian Academy of Sciences A.M. Chernyavsky, and a fellow of the National Academy of Sciences of the Republic of Belarus Y.P. Ostrovsky, all participated in the discussion.

The meeting by the Bureau of the Clinical Medicine Section of the Department of Medical Sciences, which was devoted to the challenges of transplantology, was a significant and high-profile event for medical science and the entire healthcare industry. It showed the high level of scientific research in this field, its focus on achieving practical results, on creating Russian high-tech equipment, particularly for donor organ perfusion, mechanical circulatory support systems, to strengthen our country's technological sovereignty.

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

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SUCCESSFUL TWO-STAGE TRANSPLANT HEPATECTOMY USING THE ALPPS PROCEDURE FOR ADVANCED HEPATOCELLULAR CANCER

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In the presented case, after liver transplantation (LT) for hepatocellular cancer (HCC), the disease progressed in the graft, left lung and bronchopulmonary lymph nodes after 16 months, according to the Milan criteria. Against the background of combined treatment – hepatic artery chemoembolization (HAC), systemic targeted therapy and stereotactic radiotherapy for metastatic node of the left lung – HCC in the extrahepatic foci was stabilized. In this situation, we considered resection of the liver transplant as the only therapeutic option that provides a chance for significant prolongation of the patient's life. However, extensive resection of the right liver lobe seemed unsafe due to a number of limiting factors – borderline functional residual capacity of the remaining liver: future liver remnant (FLR), 599 cm^3 (32%); plasma disappearance rate (PDR), 12.3%/min; tumor invasion of the middle hepatic vein basin. In this case, right portal vein branch (RPVB) embolization could promote vicarious hypertrophy of the remaining part of the liver, but the waiting period usually exceeds three to four weeks, and the RPVB was already partially blocked by the tumor at that time. The only option for surgical intervention was, in our opinion, two-stage hepatectomy according to the Associated Liver Partition and Portal Vein Ligation for Staged hepatectomy (ALPPS) procedure, despite the absence of literature data on the performance of such operations on a liver transplant. On postoperative day 5 from the first stage, a 799 cm^3 FLR hypertrophy was achieved, which allowed to perform the second stage of intervention relatively safely. Competent tactics regarding medication in the intensive care unit (ICU) and renal replacement therapy allowed to cope with sepsis and acute renal failure – the prevailing postoperative complications.

Keywords: hepatocellular cancer, liver transplantation, Milan criteria, transplant hepatectomy, Associated Liver Partition and Portal Vein Ligation for Staged hepatectomy (ALPPS).

INTRODUCTION

Currently, LT is the most effective treatment for HCC patients on the background of liver cirrhosis. Strict selection of recipients according to modern criteria allows achieving acceptable outcomes. Commonly known and most widespread in clinical practice, the Milan criteria, demonstrate a 5-year overall survival of about 70–80% according to different sources [1, 2]. Available literature data shows that the indications for LT in HCC can be expanded. The use of the California and "up to seven" criteria slightly worsens the long-term outcomes: overall survival 75% and 71%, respectively [2]. Despite satisfactory survival rates, tumor aggressiveness and postoperative immunosuppression lead to recurrence of the disease in 15–20% of cases within two years [3]. In the current realities, there is a wide arsenal of treatment options for relapse in the form of systemic antitumor targeting therapies, locoregional therapy methods: hepatic arterial chemoembolization (HAC), radiofrequency ablation (RFA) and transplant hepatectomy. According to several available reports, active radical surgical tactics, if technically feasible, demonstrate the best survival rates after recurrence. Inability to perform hepatectomy has been shown to be an independent predictor of poor prognosis [4]. Median survival after tumor recurrence is 65 months in patients with HCC amenable to surgery, compared to 5 months in patients not suitable for surgery [5]. In a single-center retrospective study evaluating 106 patients developing posttransplant HCC recurrence, it was demonstrated that patients receiving surgical therapy had significantly longer survival (27.8 months) than those receiving nonsurgical therapy (3.7 months) [6]. Available data on transplant hepatectomy are extremely scarce (less than 2000 operations) due to the objective complexity of technical execution and the risks of developing post-resection liver failure. Operation – two-stage hepatectomy – ALPPS is a variant of aggressive approach in case of insufficient liver reserve. In the sources we studied, the most common extent of graft resection was bisegmentectomy. Extensive hepatectomies were rarely performed. There were no mentions in the literature about two-stage hepatectomy according to the ALPPS technique in patients with recurrent HCC in the graft.

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DESCRIPTION OF CASE

Male patient, 45 years old, diagnosed with HCC, T2N0M0 stage II. G3. BCLC A. on the background of Child–Pugh B cirrhosis, MELD 23. Chronic hepatitis C since 2014 (Complete eradication after antiviral therapy in 2015). Chronic hepatitis B.

According to abdominal contrast-enhanced computed tomography (CT) scan carried out on June 10, 2019: HCC nodule in the 7th segment of the liver 25 × 21 mm, cirrhosis. Alpha-fetoprotein (AFP) level as of June 15, 2019, was 3.5 IU/mL. In July 2019, he was put on the waiting list for orthotopic LT at Russian Research Center of Radiology and Surgical Technologies in St. Petersburg. In neoadjuvant mode, HAC was performed on July 23, 2019. According to the control abdominal CT scan on April 23, 2020 (9 months after HAC): complete response according to mRECIST. The HCC node completely contains embolisate, is avascular, measuring 18 × 14 mm. Abdominal CT scan on September 09, 2020 (14 months after HAC): progression of disease (PD) according to mRECIST. HCC node is vascularized and has dynamically increased in size – 41 × 31 mm. An increase in AFP over time was also noted – September 09, 2020 – 35 IU/mL. The patient underwent another HAC on September 30, 2020.

Subsequently, LT from a deceased donor was performed using the piggy-back technique on October 01, 2020. The waiting list period was 13 months. At the time of LT, the tumor process in the liver was within the Milan criteria. In the early postoperative period, on October 7, 2020, mechanical splenic artery embolization was performed to correct the splenic artery steal syndrome. In the postoperative period, the patient received a standard triple-drug immunosuppression treatment consisting of tacrolimus, mycophenolic acid, prednisolone. At the outpatient stage of treatment, immunosuppression was corrected: everolimus, tacrolimus. The underlying disease progressed 16 months after LT. A CT scan conducted on February 01, 2022 revealed a 52 × 48 mm HCC node in the 8th segment of the liver, the para-aortic and bronchopulmonary lymph nodes were enlarged, and there was a metastatic focus in the third segment of the left lung 17 mm. The focus in the lung was histologically verified. Lenvatinib therapy was administered from March 2022. In September 2022, according to CT scan, the foci continued to grow in the graft, left lung, and lymph nodes. In September 2022, HAC was performed and a complete response to treatment was achieved according to mRECIST criteria. In February 2023, stereotactic radiation therapy was performed on the metastasis area in the left lung and bronchopulmonary lymph nodes on the left side. Stabilization of the process was considered as the treatment outcome. The AFP level as of March 2023 was 9 IU/mL.

Given the stabilization of the tumor process in the lungs and slow growth in the liver, it became clear that the only method that can give a chance for a meaningful prolongation of life could be the removal of the tumor from the liver transplant.

However, under the existing conditions, extensive resection of the right lobe of the liver was impossible due to insufficient functional reserve of the remaining part of the liver: FLR was 599 cm^3 (32%) (Fig. 1); PDR of indocyanine green (ICG) was 12.3%.

In this situation, theoretically, right portal vein (RPV) embolization could promote vicarious hypertrophy of the remaining part of the liver, but the waiting time usually exceeds three to four weeks. At the same time, there was no understanding of how the graft would behave. Besides, the RPV was already blocked by the tumor at that moment (Fig. 2), i.e. hypertrophy had already taken place, but it was obviously insufficient.

In this situation, the only option for surgical intervention was, in our opinion, two-stage hepatectomy using the ALPPS procedure. It is known that the positive side of this technique is that it achieves vicarious hypertrophy

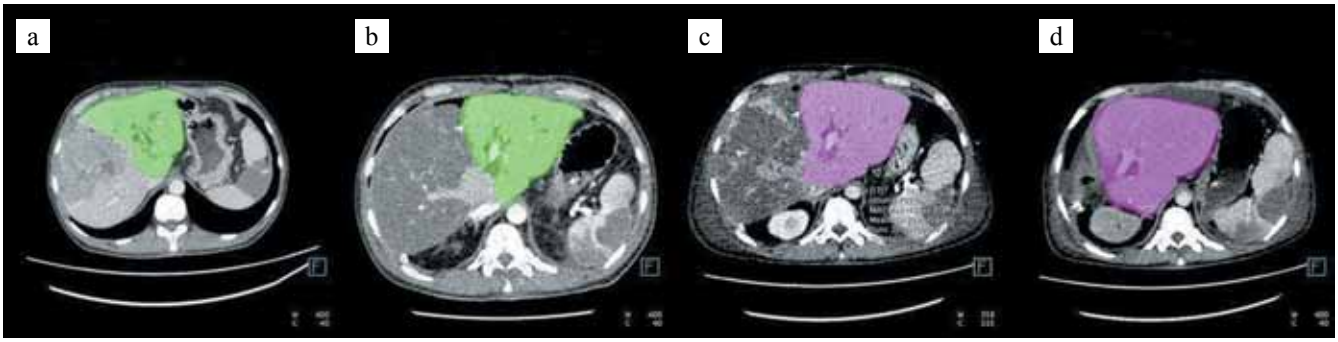

Fig. 1. Perioperative CT liver volumetry with assessment of FLR, highlighted in green/purple: a, preoperative CT volumetry on April 11, 2023; FLR volume is 599 cm^3 (32%), highlighted in green; b, CT liver volumetry on April 19, 2023 (postoperative day 1 from the first stage of two-stage transplant hepatectomy); FLR volume is 649 cm^3 (35%), highlighted in green; c, CT liver volumetry on April 23, 2023 (postoperative day 5 from the first stage of two-stage transplant hepatectomy); FLR volume is 799 cm^3 (43%), highlighted in purple; d, postoperative CT liver volumetry on May 02, 2023 (postoperative day 9 from the second stage of two-stage transplant hepatectomy); FLR volume is 1244 cm^3, highlighted in purple

in the remaining part of the liver in a fairly short period of time – up to two weeks – and allows for optimizing the time of the second stage.

The first stage was performed on April 18, 2023. Considering that such graft resections have not been previously described in the available literature, we consider it necessary to describe in detail the course of the surgical intervention.

Laparotomy was performed with excision of the old postoperative scar using a Rio Branco type approach. No ascites or carcinomatosis were detected in the abdominal cavity. There was a pronounced fibrotic process after the previous LT. With significant technical difficulties, the right and left liver lobes were isolated from the adhesions. The right lobe of the liver was mobilized before the donor conduit of the right inferior vena cava (IVC) and piggy-back caval anastomosis were visualized. The mouth of the right hepatic vein of the liver graft was visualized at its caval hilum. In the right lobe of the liver, occupying practically all its segments, with the largest volume mainly in segment 8 with partial extension to segment 4a, there was a multinodular neoplasm measuring up to 15 cm of dense consistency (Fig. 3). There was also a pronounced fibrous-adhesive process in the hepatic-duodenal ligament. When dissecting its elements, the first step was to isolate the hepatic artery in the zone of division into right and left. The right lateral wall of the portal vein and the bile duct were differentiated with technical difficulties. Between the bile duct and the portal vein on its anterior wall, there was a lymph node measuring up to 3 cm in size, suspicious for tumor. With pronounced technical difficulties, the bile duct was isolated, taken on a "holder", which allowed to perform lymphadenectomy from the anterior wall of the portal vein. The portal vein was isolated up to the fork into the right and left lobar veins. Its right branch (Fig. 4) was ligated. The appearance of demarcation along the Cantlie line was noted. Intraoperative Doppler ultrasound was performed. The study noted that the tumor node spread to the siva and was located in the basins of the right and

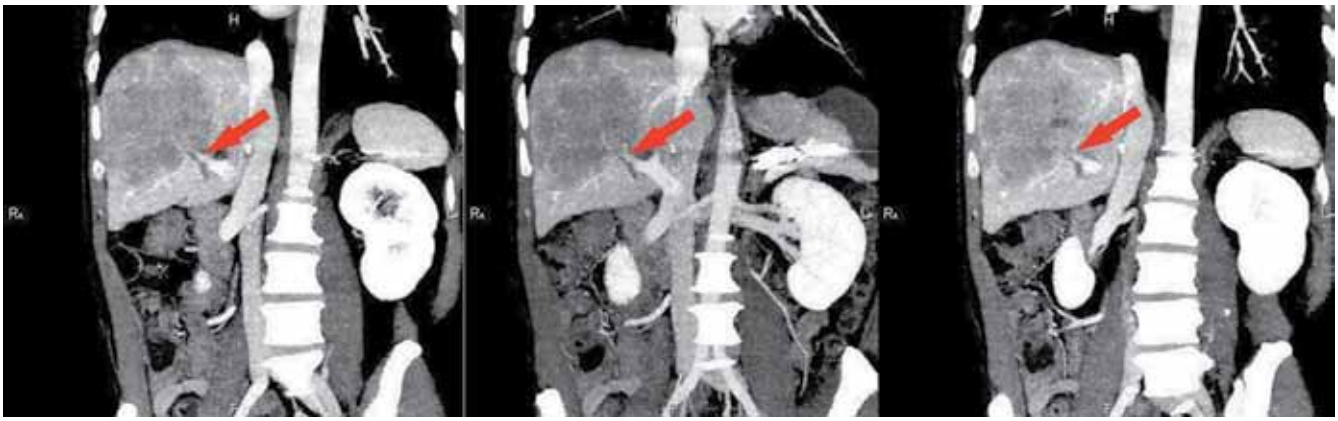

Fig. 2. Contrast-enhanced abdominal CT scan on April 11, 2023 (frontal slices). The right branch of the portal vein is blocked by a tumor, indicated by red arrow

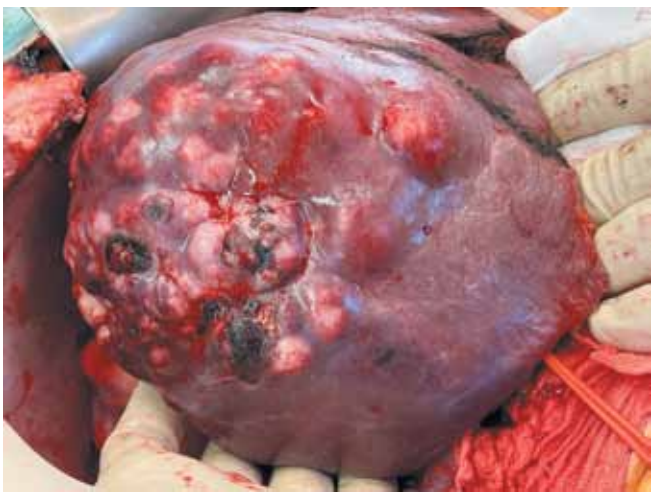

Fig. 3. Intraoperative photo (first stage of two-stage transplant hepatectomy). Multinodular neoplasm (hepatocellular carcinoma) of the right lobe of the liver graft

middle hepatic veins, while the mouth of the middle hepatic vein was free of the tumor process. A decision was made to dissect the parenchyma 1 cm to the right of the trunk of the middle hepatic vein, while preserving its orifice. Using monopolar and bipolar coagulators, water jet dissector with alternate ligation and suturing of significant vascular-secretory elements, the liver parenchyma was dissected (Fig. 5) up to the fibrous plate of the portal vein. Hemostasis was performed by argon-plasma and bipolar coagulation, using hemostatic agent Surgicel. The operation was completed by abdominal drainage and layer-by-layer suturing of the postoperative wound. The first stage lasted for 340 minutes, blood loss was 500 mL.

The postoperative period was characterized by extremely pronounced cytolytic syndrome, increasing markers of systemic inflammatory reaction (C-reactive

protein, procalcitonin), as well as significant renal failure (Table).

Fig. 4. Intraoperative photo (first stage of two-stage transplant hepatectomy). The right lobular branch of the portal vein on a black ligature. The right lobular bile duct is on a yellow rubber band

Taking into account the pronounced progression of encephalopathy, respiratory failure, with the need for mechanical ventilation, anuria and the need for renal replacement therapy (RRT), increasing dosages of vasopressor drugs on day 5, indications for the second stage were set. At the same time, based on the results of control CT liver volumetry conducted on April 23, 2023, the volume of the remaining part of the liver increased from 599 ml to 799 ml (Fig. 1).

The second stage was performed on April 23, 2023. Revision of the abdominal cavity revealed up to 500 mL of clear ascitic discharge without signs of infection, and small volume of blood clots (up to 100 ml). Moderate adhesions (Fig. 6). The removed liver parenchyma was bluish in color and had a soft-elastic consistency. The remnant liver parenchyma had visually physiological color and consistency, somewhat edematous, increased in volume (hypertrophy) in comparison with that of April 18, 2023. Adhesiolysis was performed with technical difficulties. The right hepatic artery was crossed between ligatures. The right branch of the portal vein was re-ligated and crossed. The portal fibrous plate was crossed between two clamps and the remaining part was sutured. The right hepatic vein was isolated with application of a vascular clamp on its orifice. The drug was removed.

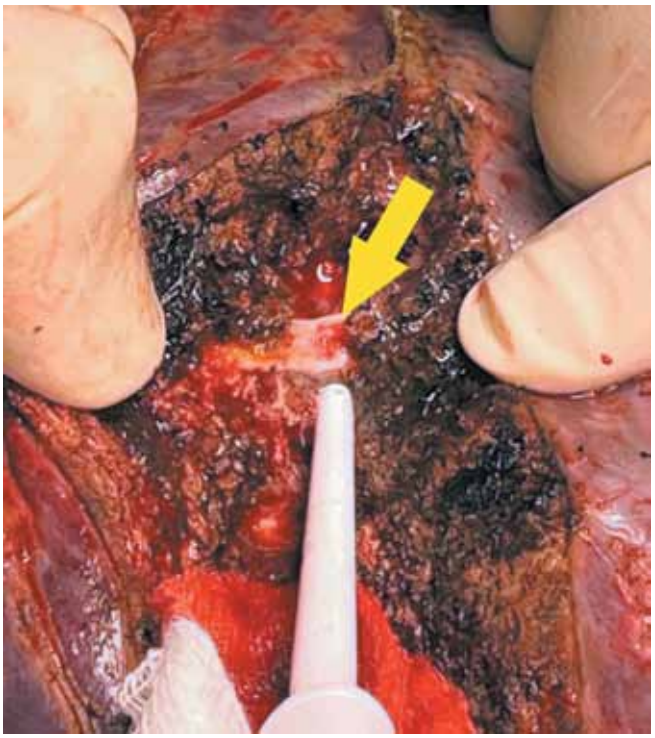

Fig. 5. Intraoperative photo (first stage of two-stage transplant hepatectomy). Dissection of the liver graft parenchyma with a water-jet dissector along the branch of the middle hepatic vein (indicated by yellow arrow)

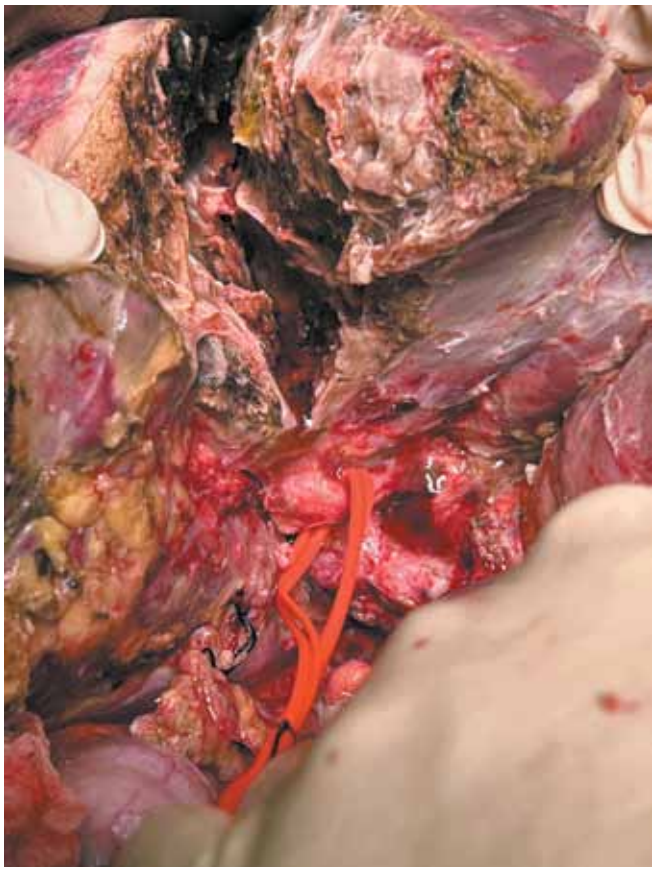

Fig. 6. Intraoperative photo (second stage of two-stage transplant hepatectomy). Moderately pronounced abdominal adhesions. The right hepatic artery is on a red rubber band

The resection plane was treated with argon-plasma and bipolar coagulation (Fig. 7), Surgicel plates were installed. The mouth of the right hepatic vein was sutured. The operation was completed with abdominal drainage and layer-by-layer suturing of the postoperative wound.

The postoperative period at the end of the second stage was complicated by renal failure requiring continuation of RRT (Table), with its subsequent transfer to intermittent mode and periodic episodes of encephalopathy managed conservatively.

The patient was discharged for outpatient treatment 45 days after the first stage of surgical intervention.

The follow-up period so far has reached three months, there is no data on tumor progression.

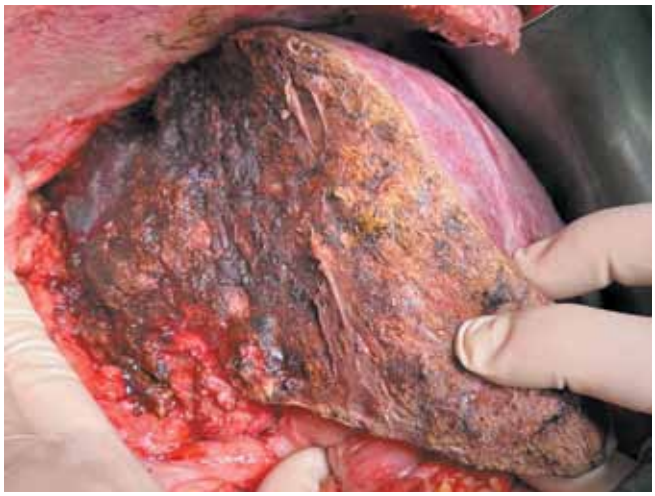

Fig. 7. Intraoperative photo (second stage of two-stage transplant hepatectomy). The resection plane of the liver graft after tumor removal and completion of hemostasis (argon plasma coagulation, Surgical plate installed)

DISCUSSION

Despite strictly being within the Milan criteria, HCC recurrence rate was 8% to 20% and usually occurs in the

Table

Dynamics of main laboratory parameters, future liver remnant volume, plasma disappearance rate of indocyanine green and invasive and extracorporeal methods of organ systems support in a 45-year-old patient in the perioperative period during a two-stage transplant hepatectomy by ALPPS technique with hepatocellular cancer progression

	ALPPS stage 1				ALPPS stage 2													
Postoperative day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	21	23
ALT (U/L)	**5462**	**4238**	**2387**	1401	822	508	396	272	206	118	103	68	55	50	57	38	39	35
AST (U/L)	**11,175**	**4650**	**1686**	494	223	373	232	117	87	58	65	53	46	55	73	69	56	29
Bilirubin (μmol/l)	20	21	14	24	25	26	18	13	12	12	13	11	12	9	12	9	8	7
Albumin (g/L)	23	21	20	23	26	28	23	22	22	25	24	20	22	20	21	20	19	17
Quick prothrombin (%)	47	45	60	65	69	51	65	79	73	76	81	78	82	79	75	72	70	63
INR	1.7	1.7	1.4	1.3	1.2	1.6	1.3	1.2	1.2	1.2	1.1	1.2	1.1	1.2	1.2	1.2	1.3	1.4
Creatinine (μmol/L)	162	**370**	**497**	**606**	292	232	254	273	267	258	276	285	451	252	179	220	399	401
Urea (mmol/L)	5	13	19	25	14	11	13	15	15	16	15	17	27	17	13	16	33	42
C-reactive protein (mg/L)	77	**211**	**222**	**213**	**210**	112	110	78	65	60	99	92	104	89	97	107	107	104
Procalcitonin (ng/mL)	–	–	–	**67**	**73**	**63**	–	–	–	–	–	10	–	–	–	–	–	–
Spontaneous diuresis	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	+	+	+
Renal replacement therapy	–	–	–	+	+	+	+	+	+	+	+	+	–	+	+	–	–	–
Ventilator	–	–	–	+	+	+	–	–	+	+	+	+	+	+	+	–	–	–
Future liver remnant volume (mL^3)	599				799									1244				
ICG-PDR (%)	12.3				16.4									21.3				

Note. ALT, alanine transaminase; AST, aspartate transaminase; INR, international normalized ratio; ICG-PDR, indocyanine green plasma disappearance rate.

first 12–16 months after LT [7]. In most cases when a recurrence develops, the prognosis is extremely unfavorable and the median survival from the time of recurrence is between 7 and 16 months [8]. Optimal treatment strategies for post-LT recurrent HCC have not been defined and therapeutic options are limited. In most patients, the disease continues to progress despite therapy. There are rather scarce data in literature on the use of regional therapies for recurrence. In 11 patients with tumor recurrence in the graft after microwave ablation performed, the two-year survival rate was 15%, and the average survival was 17 months [9].

The efficacy of conventional transcatheter arterial chemoembolization (TACE) in 28 patients with recurrent HCC after LT from a living donor was evaluated by Ko et al. [10]. After HCC, the targeted tumor reduced in size by ≥25% in 19 of the 28 study patients (67.9%). However, intrahepatic recurrence or extrahepatic metastasis occurred in 92.9% of patients during the 6-month period following TACE. Moreover, long-term prognosis was extremely poor, with 1-, 3-, and 5-year survival rates of 47.9%, 6.0%, and 0%, respectively, and a mean survival of 9 months [10].

The median overall survival for the sorafenib/regorafenib sequence (counting from the start of sorafenib use) was 28.8 months. All patients receiving regorafenib experienced side effects, but adverse events (above grade 3) were severe in 14 patients (38.9%) [11].

Despite the promising results of immunotherapy, the ability to safely utilize checkpoint inhibitors in the post-transplant setting remains a current challenge. In parenchymal organ transplant recipients, the PD-1/PD-L1 pathway is fundamental in the regulation of alloimmunity and graft tolerance [12]. Thus, using these drugs after transplantation may expose these patients to the risk of graft rejection and graft loss, and in more severe cases this may lead to death [13, 14].

At the same time, it is well established that hepatectomy, when potentially feasible, has a much better outcome compared with palliative methods [4–6]. An Italian multicenter study reported a significantly better 4-year survival rate in patients with resectable recurrences compared to patients with unresectable disease: 57% *vs.* 14% [15]. Undoubtedly, such a radical surgical tactic carries objective difficulties and requires a balanced decision based on assessment and acceptance of possible risks and complications, especially in the absence of wide international experience and clear clinical guidelines. Technical nuances include pronounced adhesions after previously performed LT, undesirability of performing the Pringle maneuver due to the high risk of ischemic cholangiopathy, i.e. resection with preserved blood flow, which requires extreme precision and delicacy of the manipulations performed. Foreign colleagues confirm the difficulties of performing such interventions and postoperative management of such patients, and describe an in-hospital mortality rate of 21.4% [16]. In addition to refined surgical skills, post-LT hepatectomy requires highly skilled patient management in the intensive care unit due to compromised immune status and renal function resulting from immunosuppression with the possibility of adequate antimicrobial therapy and RRT. Nevertheless, most authors agree that such operations are feasible at specialized centers, and one of the main criteria for success is an adequate volume of FLR. And if in planning a primary hepatectomy, the necessary safe FLR is widely known: more than 25–30% of parenchyma without cirrhosis and more than 40–45% with cirrhotic transformation [17], then in the case of post-LT hepatectomy, this figure is not strictly regulated due to the lack of understanding of the regenerative capabilities of the graft. In available reports, statistical calculations are not divided into cohorts based on the volume of hepatectomies performed after LT; therefore, it is extremely difficult to predict the risks of post-hepatectomy liver failure in extended liver resection [4, 6]. In our case, FLR was 32%, which, in our opinion, was an extremely borderline value. For additional risk stratification, liver function was assessed by PDR of indocyanine green (ICG). The PDR was 16.7%/min, which also does not reliably guarantee a safe hepatectomy. We excluded the option of embolization of the right branch of the portal vein due to tumor blocking of the vessel and long waiting time for hypertrophy. In this situation, the only possible option, in our opinion, was a two-stage transplant hepatectomy using the ALPPS procedure. It should be emphasized that in the available literature, there are no cases of such operations performed after LT. An additional factor in favor of ALPPS was the close proximity of the tumor to the middle hepatic vein and the likelihood of extending the resection volume to the 4th segment. Based on our experience, we consider it necessary to use a water-jet dissector when dissecting the parenchyma with preserved blood flow, which ensures accurate and precise manipulations.

An important aspect in terms of perioperative management of the patient, in our opinion, was the complete withdrawal of immunosuppression three days before and after the intervention, given the high risks of septic complications and renal failure. Even so, these complications could not be avoided, but regular monitoring of the flora with antibacterial therapy according to the sensitivity spectrum and RRT according to indications, ensured final success.

CONCLUSION

The prognosis for the recipient's life after LT for HCC is determined not only by the known selection criteria and immediate success of the operation, but also by the effective treatment of recurrence. In large, specialized centers with tremendous experience in LT and hepatecto-

my, active surgical tactics should definitely be considered in case of recurrence in the graft.

We are certainly aware of the fact that the chosen tactics is an operation of desperation. However, if the fundamental principles of liver surgery, anesthetic and resuscitation therapy are observed, this tactic can be successful. The authors are far from thinking that the operation will completely save the patient from further progression of the oncologic process. However, they hope that, considering the addition of drug therapy, it can improve the prognosis for the patient's survival.

The authors declare no conflict of interest.

REFERENCES

1. *Kardashian A, Florman SS, Haydel B, Ruiz RM, Klintmalm GB, Lee DD et al.* Liver Transplantation Outcomes in a U.S. Multicenter Cohort of 789 Patients With Hepatocellular Carcinoma Presenting Beyond Milan Criteria. *Hepatology*. 2020 Dec; 72 (6): 2014–2028. doi: 10.1002/hep.31210. PMID: 32124453.
2. *Ince V, Sahin TT, Akbulut S, Yilmaz S.* Liver transplantation for hepatocellular carcinoma: Historical evolution of transplantation criteria. *World J Clin Cases*. 2022 Oct 16; 10 (29): 10413–10427. doi: 10.12998/wjcc.v10.i29.10413. PMID: 36312504; PMCID: PMC9602233.
3. *Hoffman D, Mehta N.* Recurrence of hepatocellular carcinoma following liver transplantation. *Expert Rev Gastroenterol Hepatol*. 2021 Jan; 15 (1): 91–102. doi: 10.1080/17474124.2021.1823213. Epub 2020 Sep 23. PMID: 32933351.
4. *Sapisochin G, Goldaracena N, Astete S, Laurence JM, Davidson D, Rafael E et al.* Benefit of Treating Hepatocellular Carcinoma Recurrence after Liver Transplantation and Analysis of Prognostic Factors for Survival in a Large Euro-American Series. *Ann Surg Oncol*. 2015 Jul; 22 (7): 2286–2294. doi: 10.1245/s10434-014-4273-6. Epub 2014 Dec 4. PMID: 25472651.
5. *Kornberg A, Küpper B, Tannapfel A, Katenkamp K, Thrum K, Habrecht O et al.* Long-term survival after recurrent hepatocellular carcinoma in liver transplant patients: clinical patterns and outcome variables. *Eur J Surg Oncol*. 2010 Mar; 36 (3): 275–280. doi: 10.1016/j.ejso.2009.10.001. Epub 2009 Oct 25. PMID: 19857941.
6. *Bodzin AS, Lunsford KE, Markovic D, Harlander-Locke MP, Busuttil RW, Agopian VG.* Predicting Mortality in Patients Developing Recurrent Hepatocellular Carcinoma After Liver Transplantation: Impact of Treatment Modality and Recurrence Characteristics. *Ann Surg*. 2017 Jul; 266 (1): 118–125. doi: 10.1097/SLA.0000000000001894. PMID: 27433914.
7. *Pelizzaro F, Gambato M, Gringeri E, Vitale A, Cillo U, Farinati F et al.* Management of Hepatocellular Carcinoma Recurrence after Liver Transplantation. *Cancers (Basel)*. 2021 Sep 29; 13 (19): 4882. doi: 10.3390/cancers13194882. PMID: 34638365; PMCID: PMC8508053.
8. *Filgueira NA.* Hepatocellular carcinoma recurrence after liver transplantation: Risk factors, screening and clinical presentation. *World J Hepatol*. 2019 Mar 27; 11 (3): 261–272. doi: 10.4254/wjh.v11.i3.261. PMID: 30967904; PMCID: PMC6447422.
9. *Zhai H, Liang P, Yu XL, Cheng Z, Han ZY, Liu F et al.* Microwave ablation in treating intrahepatic recurrence of hepatocellular carcinoma after liver transplantation: An analysis of 11 cases. *Int J Hyperthermia*. 2015; 31 (8): 863–868. doi: 10.3109/02656736.2015.1091953. Epub 2015 Nov 25. PMID: 26608701.
10. *Ko HK, Ko GY, Yoon HK, Sung KB*. Tumor response to transcatheter arterial chemoembolization in recurrent hepatocellular carcinoma after living donor liver transplantation. *Korean J Radiol*. 2007 Jul-Aug; 8 (4): 320–327. doi: 10.3348/kjr.2007.8.4.320. PMID: 17673843; PMCID: PMC2627154.
11. *Iavarone M, Invernizzi F, Ivanics T, Mazza S, Zavaglia C, Sanduzzi-Zamparelli M et al*. Regorafenib Efficacy After Sorafenib in Patients With Recurrent Hepatocellular Carcinoma After Liver Transplantation: A Retrospective Study. *Liver Transpl*. 2021 Dec; 27 (12): 1767–1778. doi: 10.1002/lt.26264. Epub 2021 Sep 16. PMID: 34388851.
12. *Kittai AS, Oldham H, Cetnar J, Taylor M.* Immune Checkpoint Inhibitors in Organ Transplant Patients. *J Immunother*. 2017 Sep; 40 (7): 277–281. doi: 10.1097/CJI.0000000000000180. PMID: 28719552.
13. *Finn RS, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY et al.* Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. *N Engl J Med*. 2020 May 14; 382 (20): 1894–1905. doi: 10.1056/NEJMoa1915745. PMID: 32402160.
14. *Rimassa L, Personeni N, Czauderna C, Foerster F, Galle P.* Systemic treatment of HCC in special populations. *J Hepatol*. 2021 Apr; 74 (4): 931–943. doi: 10.1016/j.jhep.2020.11.026. Epub 2020 Nov 26. PMID: 33248171.
15. *Regalia E, Fassati LR, Valente U, Pulvirenti A, Damilano I, Dardano G et al.* Pattern and management of recurrent hepatocellular carcinoma after liver transplantation. *J Hepatobiliary Pancreat Surg*. 1998; 5 (1): 29–34. doi: 10.1007/pl00009947. PMID: 9683751.
16. *Pohl JMO, Raschzok N, Eurich D, Pflüger M, Wiering L, Daneshgar A et al.* Outcomes of Liver Resections after Liver Transplantation at a High-Volume Hepatobiliary Center. *J Clin Med*. 2020 Nov 17; 9 (11): 3685. doi: 10.3390/jcm9113685. PMID: 33212913; PMCID: PMC7698397.
17. *Asencio JM, García Sabrido JL, Olmedilla L.* How to expand the safe limits in hepatic resections? *J Hepatobiliary Pancreat Sci*. 2014 Jun; 21 (6): 399–404. doi: 10.1002/jhbp.97. Epub 2014 Feb 13. PMID: 24532454.

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CHANGES IN COMMON CAROTID ARTERY ELASTICITY IN SOLID ORGAN RECIPIENTS

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Background. Cardiovascular diseases are very common among solid organ recipients. They are associated with worsening transplant outcomes. Arterial vascular wall elasticity is an important prognostic indicator and a risk marker for cardiovascular events. Noninvasive measurement of common carotid artery (CCA) elasticity may be useful in assessing cardiovascular risk in solid organ recipients. **Objective:** To conduct a comparative analysis of indicators of CCA elasticity in solid organ recipients and to study their relationship with factors that potentially have a negative impact on the risk of adverse events. **Materials and methods.** The study included 154 patients aged 10 to 75 years, including heart (n = 77), liver (n = 9), and kidney (n = 35) recipients, as well as 33 patients with end-stage heart failure waitlisted for heart transplantation (HT). In all participants, carotid artery ultrasound was performed, pulse wave velocity was measured, and CCA elasticity was calculated. **Results.** CCA elasticity was found to be strongly inversely correlated with age, body mass index, systolic blood pressure, renal tubular filtration rate, CCA intima media thickness, and aortic pulse wave velocity. In heart recipients, CCA elasticity was significantly lower than in liver and kidney recipients (p = 0,002) and it inversely correlated with the length of time elapsed after transplantation, which is probably associated with cardiac denervation. **Conclusion.** CCA elasticity calculated via noninvasive ultrasound reflects the degree of adverse effects of pathological factors on the main arteries in solid organ recipients.

Keywords: vascular wall elasticity, common carotid artery, heart, kidney, and liver recipients.

INTRODUCTION

Cardiovascular diseases are common in solid organ recipients and are associated with worse transplant outcomes [1]. Cardiovascular risk assessment is clinically important because preventive interventions that are initiated early may reduce the incidence of complications in solid organ recipients. At the same time, inappropriate interventions can be a burden on both the health care system and patients. In this regard, various cardiovascular risk assessment scales are actively used in clinical practice, but their limited accuracy constitutes a significant disadvantage [2]. It is necessary to identify new and more effective ways of assessing cardiovascular risk in solid organ recipients. Assessment of the common carotid artery elasticity is promising among them.

Arterial vascular wall elasticity (AVWE) is an important prognostic indicator and a risk marker for cardiovascular events at the preclinical stage and cardiovascular mortality in the population [3]. Noninvasive measurement of this index on carotid arteries may be a useful method in assessing risk in solid organ recipients.

The AVWE in renal recipients is associated with clinical risk factors and independently predicts mortality, cardiovascular events, and graft dysfunction [4–6]. Data on arterial elasticity in recipients of other solid organs are few and mostly obtained in pediatric recipients.

The **objective** of the study was to comparatively analyze the indicators of CCA elasticity in solid organ recipients and to investigate their association with factors that potentially have a negative impact on the risk of adverse events.

MATERIALS AND METHODS

The examination and treatment of heart, liver and kidney recipients, as well as persons included in the HT waitlist, who are under supervision at Shumakov National Medical Research Center of Transplantology and Artificial Organs, from February 2022 to June 2023, were carried out in accordance with the clinical guidelines of the Russian Transplant Society and protocols adopted at Shumakov National Medical Research Center of Transplantology and Artificial Organs.

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The study included 154 patients aged 10 to 75 years, including 113 males and 41 females. Patient mean age was 47.0 ± 2.2 years.

Among the patients examined were:

– Patients with end-stage heart failure (ESHF) listed for HT (n = 33);
– Heart recipients (n = 77);
– Liver recipients (n = 9);
– Kidney recipients (n = 35).

Inclusion criteria were: 10–75 years of age; a history of heart, liver, or kidney transplantation or the presence of ESHF with inclusion on the HT waiting list.

All patients received mono-, bi-, or triple-drug immunosuppressive therapy after transplantation, including tacrolimus, everolimus, mycophenolic acid drugs, methylprednisolone, cyclosporine, and azathioprine.

Exclusion criteria: over 75 years of age or younger than 10 years.

In addition to routine examinations, carotid artery ultrasound and pulse wave velocity measurement were performed in all participants. The CCA elasticity index was calculated as the ratio of the difference in the cross-sectional area of the CCA in systole and diastole to the systole-diastolic BP difference. The study of functional parameters of the CCA wall was performed using the Vivid S70N ultrasound system with a 9 MHz linear multifrequency transducer by ultrasound scanning method with measurement of the vessel lumen diameter and assessment of hemodynamic parameters using spectral Doppler mode (Fig.).

Statistical analysis followed the International Committee of Medical Journal Editors (ICMJE) recommendations and the Statistical analyses and methods in the Published Literature (SAMPL) guidelines. Statistical significance was determined by the value of 95% confidence interval and number of degrees of freedom. In all cases, $p < 0.05$ was considered statistically significant. WizardPro software was used for mathematical processing.

RESULTS AND DISCUSSION

There were no significant differences in age, sex, and body mass index (BMI) in the presented subgroups of recipients and heart failure patients.

In the patients included in the study, CCA elasticity index ranged from 0.0013 to 0.0137 $m^2 \cdot kPa^{-1}$; on average, this index was 0.0040 ± 0.0001 $m^2 \cdot kPa^{-1}$. In all subgroups, CCA elasticity index was independent of tacrolimus trough levels.

In ESHF patients, CCA elasticity index (ss = 0.007 ± 0.001 $m^2 \cdot kPa^{-1}$) was significantly higher ($p = 0.04$ in both cases) than in liver and kidney recipients (0.005 ± 0.003 and 0.005 ± 0.002 $m^2 \cdot kPa^{-1}$, respectively); in the latter it was significantly higher ($p = 0.02$) than in the subgroup of heart recipients (ss = 0.0040 ± 0.0001 $m^2 \cdot kPa^{-1}$). We did not find similar data for comparison in the available scientific literature.

Statistical analysis revealed that CCA elasticity index inversely correlated with age, BMI, systolic BP levels, glomerular filtration rate (GFR), CCA intima-media thickness index and aortic pulse wave velocity.

Similar data can be found in the work of Cheddani et al. [7]. The authors note that high aortic stiffness is associated with lower GFR, older age, diabetic status (and diabetic nephropathy), smoking and hypertension.

It should be noted that in foreign studies, a measure of blood vessel wall elasticity is usually arterial stiffness [8, 9], which is assessed by the carotid-femoral pulse wave velocity (PWV).

Studies assessing the progression of arterial stiffness over time in patients after transplantation are conflicting [5]. According to published studies, arterial stiffness measured by PWV, is markedly reduced after kidney transplantation [4]. Some studies have shown that this improvement applies primarily to recipients of kidneys from young donors (17–41 years) [11], and was also more pronounced in cases of transplantation from living donors [11]. However, in a 5-year follow-up by Alatič J. et al. [13], which included 48 kidney recipients, PWV did not significantly change during the follow-up period ($p = 0.137$) [12]. No differences in the annual evolution of PWV were also found in the work by Bachelet-Rousseau et al. [13].

Previously, we have shown that the CCA stiffness index does not change significantly over time in heart transplant patients. However, the CCA stiffness index increases during rejection and then decreases during therapy [14]. Thus, the assessment of CCA stiffness index

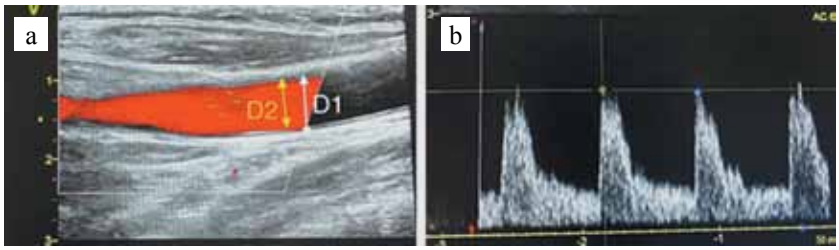

Fig. Technique for investigating CCA conditions. a, measurement of CCA intraluminal diameter; b, measurement of Doppler indicators; Vmax, peak systolic velocity (Vs); D1 and D2, minimum and maximum diameters of the CCA, respectively

can be used to identify heart recipients at high risk of rejection.

In our present study, the CCA elasticity index in cardiac recipients, in contrast to other subgroups, negatively correlated with the length of time elapsed after transplantation. This is probably associated with an additional factor – cardiac denervation. Reduced elasticity of the wall of the main arteries may explain the phenomenon of arterial hypertension that develops in most heart recipients [15] and aggravate injury to target organs (kidneys, brain) against the background of other pathological factors; it suggests the prescription of adjuvant drug therapy for prophylactic purposes.

Arterial stiffness is independently associated with several risk factors (age, blood pressure control, diabetes mellitus, smoking, previous cardiovascular events, total cholesterol, creatinine and triglyceride levels, and GFR) and in liver recipients [16].

In addition, according to Szewc et al. [17], BMI is associated with arterial stiffness in liver recipients. In their study, the PWV level was 7.62 m/s in patients after liver transplantation, whose BMI value was within the normal range, and 8.58 m/s in overweight and obese patients ($p < 0.05$).

Arterial stiffness is also an independent predictor of cardiovascular events and all-cause mortality in cardiac recipients. De Souza-Neto et al. [18] found that arterial hypertension was more than four times more common in patients after heart transplantation with increased arterial stiffness. Major risk factors for arterial stiffness included arterial hypertension, diabetes mellitus, dyslipidemia, and chronic kidney disease.

To date, it is unknown whether therapeutic interventions aimed at increasing arterial wall elasticity will improve outcomes in solid organ recipients.

CONCLUSION

The CCA wall elasticity index, determined by noninvasive ultrasound, reflects the degree of adverse effects of pathological factors on the main arteries; the index is significantly lower in solid organ recipients than in patients on the HT waitlist; it is significantly lower in heart recipients than in liver and kidney recipients and, unlike them, depend on the time elapsed after transplantation.

Studies on the association between CCA elasticity index and other important risk factors for cardiovascular events, as well as assessment of long-term prognostic value allows to answer the question of whether arterial wall elasticity may represent an additional therapeutic target for improving survival among solid organ recipients.

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REFERENCES

1. *Hernández D, Vázquez T, Armas-Padrón AM, Alonso-Titos J, Casas C, Gutiérrez E et al.* Peripheral Vascular Disease and Kidney Transplant Outcomes: Rethinking an Important Ongoing Complication. *Transplantation.* 2020; 105 (6): 1188–1202. http://dx.doi.org/10.1097/tp.0000000000003518.
2. *Di Maira T, Rubin A, Puchades L, Aguilera V, Vinaixa C, Garcia M et al.* Framingham score, renal dysfunction, and cardiovascular risk in liver transplant patients. *Liver Transplantation.* 2015; 21 (6): 812–822. http://dx.doi.org/10.1002/lt.24128.
3. *Messas E, Pernot M, Couade M.* Arterial wall elasticity: State of the art and future prospects. *Diagnostic and Interventional Imaging.* 2013; 94 (5): 561–569. http://dx.doi.org/10.1016/j.diii.2013.01.025.
4. *Korogiannou M, Xagas E, Marinaki S, Sarafidis P, Boletis JN.* Arterial Stiffness in Patients With Renal Transplantation; Associations With Co-morbid Conditions, Evolution, and Prognostic Importance for Cardiovascular and Renal Outcomes. *Frontiers in Cardiovascular Medicine.* 2019; 6: 67. http://dx.doi.org/10.3389/fcvm.2019.00067.
5. *Junarta J, Hojs N, Ramphul R, Lowe-Jones R, Kaski JC, Banerjee D.* Progression of endothelial dysfunction, atherosclerosis, and arterial stiffness in stable kidney transplant patients: a pilot study. *BMC Cardiovascular Disorders.* 2020; 20 (1): 6. http://dx.doi.org/10.1186/s12872-019-01309-y.
6. *Salib M, Simon A, Girerd N, Kearney-Schwartz A, Rossignol P, Benetos A et al.* Association between Long-Term Change in Arterial Stiffness and Cardiovascular Outcomes in Kidney Transplant Recipients: Insights from the TRANSARTE Study. *Journal of Clinical Medicine.* 2022; 11 (5): 1410. http://dx.doi.org/10.3390/jcm11051410.
7. *Cheddani L, Haymann JP, Liabeuf S, Tabibzadeh N, Boffa J-J, Letavernier E et al.* Less arterial stiffness in kidney transplant recipients than chronic kidney disease patients matched for renal function. *Clinical Kidney Journal.* 2020; 14 (4): 1244–1254. http://dx.doi.org/10.1093/ckj/sfaa120.
8. *Angoff R, Mosarla RC, Tsao CW.* Aortic Stiffness: Epidemiology, Risk Factors, and Relevant Biomarkers. *Frontiers in Cardiovascular Medicine.* 2021; 8: 709396. http://dx.doi.org/10.3389/fcvm.2021.709396.
9. *Ikonomidis I, Thymis J.* The vicious circle of arterial elasticity, blood pressure, glycemia, and renal function. *Hypertension Research.* 2023; 46 (6): 1599–602. http://dx.doi.org/10.1038/s41440-023-01262-6.
10. *Delahousse M, Chaignon M, Mesnard L, Boutouyrie P, Safar ME, Lebret T et al.* Aortic Stiffness of Kidney Transplant Recipients Correlates with Donor Age. *Journal of the American Society of Nephrology.* 2008; 19 (4): 798–805. http://dx.doi.org/10.1681/asn.2007060634.
11. *Karras A, Boutouyrie P, Briet M, Bozec E, Haymann J, Legendre C et al.* Reversal of Arterial Stiffness and Maladaptative Arterial Remodeling After Kidney Transplantation. *Journal of the American Heart Associati-*

on. 2017; 6 (9): e006078. http://dx.doi.org/10.1161/jaha.117.006078.
12. *Alatič J, Lindič J, Godnov U, Kovač D.* Arterial Stiffness in Renal Transplant Recipients: 5-Year Follow-up. *Transplantation Proceedings.* 2021; 53 (10): 2907–2912. http://dx.doi.org/10.1016/j.transproceed.2021.09.032.
13. *Bachelet-Rousseau C, Kearney-Schwartz A, Frimat L, Fay R, Kessler M, Benetos A.* Evolution of arterial stiffness after kidney transplantation. *Nephrology Dialysis Transplantation.* 2011; 26 (10): 3386–3391. http://dx.doi.org/10.1093/ndt/gfr058.
14. *Shevchenko AO, Tyunyaeva IYu, Nasyrova AA, Mozheiko NP, Gautier SV*. Dynamics of the stiffness index of the common carotid artery wall during treatment of transplanted heart rejection. *Bulletin of transplantology and artificial organs*. 2015; 17 (3): 8–13 (In Russ). https://doi.org/10.15825/1995-1191-2015-3-8-13.
15. *Shevchenko AO, Nikitina EA, Koloskova NN, Shevchenko OP, Gautier SV*. Kontroliruemaja arterial'naja gipertenzija i vyzhivaemost' bez nezhelatel'nyh sobytij u recipientov serdca. *Kardiovaskuljarnaja terapija i profilaktika*. 2018; 17 (4): 4–11 [In Russ, English abstract].
16. *Sastre L, García R, Gándara J-G, Fernández-Llama P, Amor AJ, Sierra C et al.* The Role of Arterial Stiffness in the Estimation of Cardiovascular Risk in Liver Transplant Recipients. *Transplantation Direct.* 2021; 8 (1): e1272. http://dx.doi.org/10.1097/txd.0000000000001272.
17. *Szewc UZ, Czyżewski L, Wyzgal J, Szarpak L.* Assessment of Arterial Stiffness and Body Composition in Stable Liver Transplant Recipients. *Transplantation Proceedings.* 2018; 50 (7): 2009–2013. http://dx.doi.org/10.1016/j.transproceed.2018.05.004.
18. *De Souza-Neto JD, de Oliveira ÍM, Lima-Rocha HA, Oliveira-Lima JW, Bacal F.* Hypertension and arterial stiffness in heart transplantation patients. *Clinics.* 2016; 71 (9): 494–499. http://dx.doi.org/10.6061/clinics/2016(09)02.

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TREATMENT OF VASCULAR COMPLICATIONS FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION. THE EXPERIENCE OF A REGIONAL CENTER

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Objective: vascular complications (VCs) following liver transplantation (LT) can pose a significant threat to the recipient's life – as the risk of graft loss increases significantly when blood flow in the graft is impaired. Diagnosis and early treatment of VCs seems to be a pressing issue in transplantology. The aim of this study is to evaluate the incidence, treatment and outcome of VCs in patients after orthotopic LT at the Center for Surgery and Donor Coordination, Rostov Regional Clinical Hospital. **Materials and methods.** Between July 2015 and April 2023, 100 orthotopic LTs were performed. VCs were retrospectively identified and analyzed. **Results.** The overall incidence of VCs was 24% (n = 24): hepatic artery stenosis, 5% (n = 5); intra-abdominal bleeding, 6% (n = 6); hepatic artery dissection, 2% (n = 2); intrahepatic venous thrombosis Budd–Chiari syndrome), 2% (n = 2); portal vein thrombosis, 1% (n = 1); inferior vena cava thrombosis/iliofemoral deep vein thrombosis, 2% (n = 2); inferior vena cava stenosis, 1% (n = 1); hepatic vein stenosis, 1% (n = 1); recurrent hepatic artery stenosis/thrombosis, 2% (n = 2); mesenteric vein thrombosis, 2% (n = 2). **Conclusion.** Most VCs following orthotopic LT occur in the early postoperative period and can lead to a high risk of graft dysfunction and patient death. Early recognition, diagnosis, and treatment of post-LT complications are critical to successful short- and long-term graft function and patient survival, even in patients with asymptomatic complications. Treatment options typically include surgical revascularization, percutaneous thrombolysis, percutaneous angioplasty, retransplantation, or, less commonly, a conservative approach.

Keywords: liver transplantation, vascular complications.

INTRODUCTION

Orthotopic LT (OLT) is the most effective treatment modality for end-stage liver disease. Significant advances in surgical techniques for organ retrieval, introduction of potent immunosuppressive drugs, and improved peri- and postoperative patient care have resulted in increased patient survival after OLT to >90% [1]. However, VCs have life-threatening consequences for the patient as they impair blood flow in the graft. Most of these complications occur within the first month following OLT and require early detection, diagnosis, and immediate treatment [2]. Bleeding, stenosis and thrombosis can occur in any of the vascular anastomoses, as well as pseudoaneurysm in the arterial anastomosis [3, 4], with an overall reported incidence of 7.2% to 15% in adults (mainly arterial 5–10%, followed by portal 1–3% and caval <2%) [5–8]. However, the rate can be as high as 25% for hepatic artery thrombosis (HAT) and hepatic artery stenosis (HAS) [7].

Hepatic artery thrombosis (HAT) following OLT occurs in 1.9% to 16.6% of cases [8]. It is the most frequent and severe vascular complication following OLT. It accounts for more than 50% of all arterial complications and usually leads to graft loss [2, 9–14]. HAT is the main cause of biliary necrosis and massive liver necrosis accompanied by uncontrolled sepsis under immunosuppression, inevitably leading to patient death [15]. HAT occurring in the first month after LT is accompanied by mortality in 55% of cases; later stages of the thrombosis lead to death in 15% of cases [16]. In general, there are three treatment options for HAT: revascularization, retransplantation, and observation. The choice of any of these methods depends on the time of diagnosis [17]. Retransplantation rates are high in patients with untreated HAT (25–83%) compared to patients who receive graft revascularization (28%–35%) [9, 18, 19] and it provides the best survival outcomes. However, this option is subject to donor shortage and patient condition [9, 10, 20, 22].

Hepatic artery stenosis (HAS) is usually defined as a narrowing of the transverse diameter of the hepatic artery resulting in graft ischemia [9, 23–28]. HAS incidence

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is 2–13% according to different sources [9, 13, 23, 24, 26–28]. Percutaneous angioplasty is an alternative to surgical treatment for HAS [26]. If thrombectomy and angioplasty are ineffective, only LT can save the patient's life [17, 15].

Hepatic artery pseudoaneurysm (HAP) is a rare but life-threatening complication following OLT, occurring in approximately 0.27% to 3% of cases [13, 18, 31]. Ruptured extrahepatic aneurysm can lead to massive intraperitoneal bleeding and, as a consequence, to hemorrhagic shock. Color spectral Doppler, computed tomography, and selective angiography are useful diagnostic tests for differentiating complications [32].

Venous complications, compared with arterial complications, are less common, with an estimated overall incidence of less than 3% [5–7, 20, 35].

Portal vein thrombosis (PVT) and **portal vein stenosis (PVS)** complicate the postoperative period following OLT in 1–3% of cases [5–7, 20, 35]. The main methods in the early stages of treatment of PVT are thrombectomy and portal vein reconstruction combined with thrombolytic and anticoagulant drugs. If these methods are ineffective, urgent re-transplantation is the only method in most cases [36]. The treatment of choice for PVS is balloon angioplasty or stenting.

Inferior vena cava or hepatic vein stenosis are relatively rare complications arising after LT, with an incidence of less than 3% [2]. Clinical manifestations range from lower limb edema, hepatomegaly, ascites, pleural effusion, Budd–Chiari syndrome, hepatic and renal failure to low blood pressure leading to allograft loss and multiple organ failure [20, 36, 37]. Treatment of PVT includes systemic anticoagulant therapy, thrombolytic therapy by radiologic intervention with stent placement, portosystemic shunt, and re-transplantation in cases that are refractory to treatment [38].

MATERIALS AND METHODS

During this study, the medical documentation of patients who underwent LT at the Center for Surgery and Donor Coordination, Rostov Regional Clinical Hospital, from July 2015 to April 2023, was reviewed. Analyzed were data on gender, age, and concomitant diseases of recipients; operations previously undergone by recipients; indications for LT; number and type of VCs; method used to diagnose complications; clinical course and treatment of patients; date and time of the operation. MS Office and Statistica software packages were used to analyze the data.

RESULTS

A total of 100 (n = 100) OLTs were performed. The average age of the recipients was 43.5 ± 15.8 years. The indication for surgery was liver cirrhosis of various etiologies, the most common cause of end-stage liver disease in patients was viral hepatitis, 33% of cases (Fig. 1).

For 10 recipients, their close relatives were the liver donors, and 90 recipients received an organ from brain-dead donors. For two patients, the donor organ was obtained by splitting the liver into two lobes using the split in situ technique. LT in all patients was performed in accordance with ethical and legal standards.

The average duration of the surgical intervention was 5.14 ± 1.92 hours. Intraoperative blood loss did not exceed 1400 mL (1076.1 ± 191.8 mL). Using the reinfusion system, it was possible to return up to 93% of lost blood (996.5 ± 177.5 mL of blood on average), and the additional volume of erythrocyte mass transfused in 48.1%

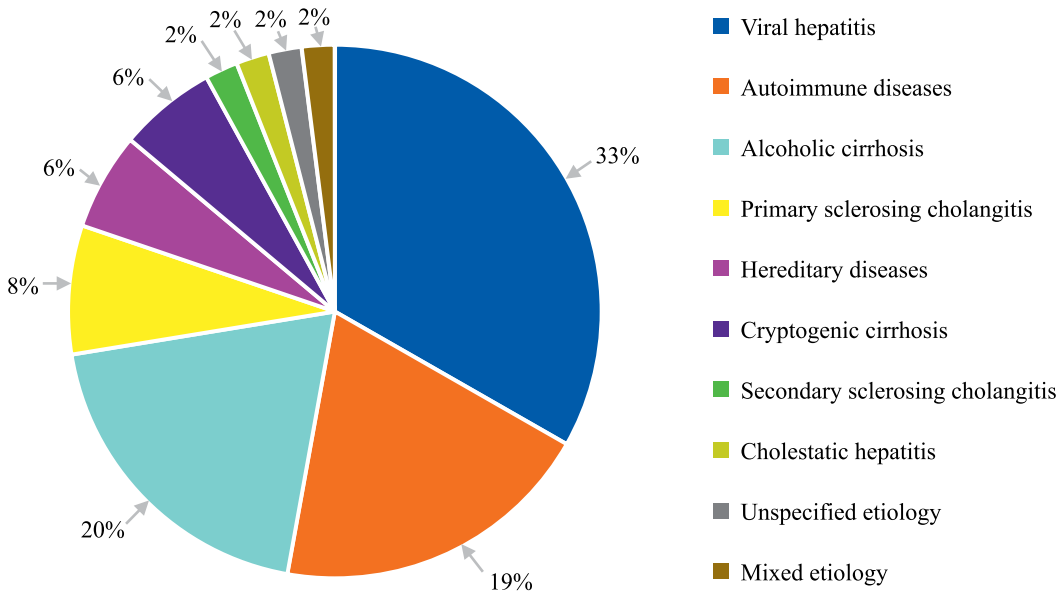

Fig. 1. Distribution of liver recipients by disease etiology

of patients during the operation and in the immediate postoperative hours was 238.7 ± 133.1 mL on average. In all cases, fresh frozen plasma was transfused with an average transfusion volume of 1394.7 ± 303.1 mL.

Vascular complications

In the early postoperative period, VCs following OLT were diagnosed in 21/100 patients (21%), which is comparable to world literature reports [5–8, 42], while some patients had several complications at once, so the total number of VCs was 24 cases: hepatic artery stenosis and hepatic artery dissection (7%, n = 7), intra-abdominal bleeding (6%, n = 6), intrahepatic venous thrombosis (Budd–Chiari syndrome) (2%, n = 2), PVT (1%, n = 1), inferior vena cava/iliofemoral thrombosis (2%, n = 2), inferior vena cava stenosis (1%, n = 1), hepatic vein stenosis (1%, n = 1), recurrent HAS/HAT (2%, n = 2), mesenteric vein thrombosis (2%, n = 2) (Table).

Characteristics of recipients who developed VCs after OLT:

- Recipient's gender: 16 males (69.6%) and 7 females (30.4%).
- Mean age at the time of surgery was (M ± SD) 46.46 ± 11.33 years.
- Mean body weight, (M ± SD) 75.52 ± 18.55.
- Mean body mass index (BMI), (M ± SD) 25.96 ± 4.16.
- 20 (95.2%) patients had comorbidities – arterial hypertension, thrombocytopenia, coagulopathy, chronic heart failure and morbid obesity.
- Child–Pugh classification: class B (Child B) 7–9 points in 8 cases (38.1%) and class C (Child C) 10–15 points in 12 cases (61.9%).
- MELD score and 3-month mortality:
  - in the range 30–39 points (52.6% mortality) in 3 cases (14.3%);
  - in the range 20–29 points (19.6% mortality) in 14 cases (66.7%);
  - in the range 10–19 points (6.0% mortality) in 3 cases (14.3%);
  - in the range <9 (1.9% mortality) in 1 case (4.8%).
- UNOS criteria:
  - status 1 (acute liver failure), 19 cases (90.5%);
  - status 2B (decompensated chronic liver disease), 2 cases (9.5%).
- METAVIR scale, F4 (Cirrhosis. Irreversible changes), 21 cases (100%).
- ABO blood group compatibility was the same in 21 cases (100%).

Hepatic artery stenosis with or without hepatic artery thrombosis occurred predominantly early after surgical intervention and during the patient's hospital stay. It occurred in 18/100 cases (18%). The diagnosis was established by Doppler ultrasound and angiography combined with laboratory investigation methods. Treatment priority was given to minimally invasive methods through endovascular technique, without exposing the patient to risks associated with thrombectomy and open reconstruction of the arterial anastomosis. From the right transradial access, catheterization of the celiac trunk was performed with a diagnostic angiographic catheter; selective catheterization of the common hepatic

Table

Number of vascular complications following orthotopic liver transplantation at Rostov Regional Clinical Hospital and according to global reports

Vascular complication	Data from Rostov Regional Clinical Hospital			Data from world literature
	Number of cases	Percentage of all vascular complications (n = 24)	Percentage of total number of patients who underwent orthotopic liver transplantation (n = 100)	
Hepatic artery stenosis and hepatic artery dissections	7	29.2	7	2–13% [9, 13, 23, 24, 26–28]
Intra-abdominal bleeding	6	25.0	6	7.2–15% [5–8]
Intrahepatic venous thrombosis (Budd–Chiari syndrome)	2	8.3	2	The incidence is 1 per 100,000 population [40]
Portal vein thrombosis	1	4.2	1	1–3% [5–7, 20, 35]
Inferior vena cava thrombosis/ iliofemoral deep vein thrombosis	2	8.3	2	<3% [2]
Inferior vena cava stenosis	1	4.2	1	<3% [2]
Hepatic vein stenosis	1	4.2	1	<3% [2]
Recurrent hepatic artery stenosis/ thrombosis	2	8.3	2	No data
Mesenteric vein thrombosis	2	8.3	2	11% of all forms of visceral deep vein thrombosis [41, 42]

artery was performed; a coronary conduit was placed in its distal parts through the thrombus and the stenosis zone of the arterial anastomosis (Fig. 2); 15 mg of the drug Actilyse was injected through a microcatheter into the thrombosis zone using an infusion pump at a rate of 1 mg/min; a 20-minute exposure was carried out, after which a coronary stent, coated with the drug Everolimus, was implanted in the area of the stenosed anastomosis of the common hepatic artery with the donor liver artery with a length exceeding the length of the stenosis, and angiographic control was performed in the stenting zone (Fig. 3) [15].

Treatment failed in 3/100 cases (3%), accompanied by progression of liver failure, which required repeated hepatic artery angioplasty and stenting; subsequently, one patient underwent organ retransplantation; one patient died against the background of multiple organ and intra-abdominal bleeding.

Portal vein thrombosis developed in 1/100 patients (1%) during a 5-day hospital stay. The diagnosis was established by ultrasound and then confirmed by angiography. Treatment was performed by open thrombectomy from the portal vein, but the patient died on the background of graft failure.

Hepatic vein thrombosis occurred in 2/100 cases (2%) within 4–10 months after hospital discharge. The pathology was diagnosed by Doppler ultrasound followed by spiral CT phlebography. One patient underwent partial splenic vein embolization and was subsequently listed for liver retransplantation. One patient underwent organ retransplantation 9 months after primary transplantation (5 months after hepatic vein thrombosis).

Hepatic vein stenosis was noted in 1 patient. The diagnosis was established by ultrasound and then confirmed by direct cavography. The hepatic vein was stented. Subsequently, against the background of graft failure, the patient died.

Stenosis of the inferior vena cava in the anastomosis zone, extending in the cranial direction, was noted in 1 patient; balloon angioplasty of the artery was performed 24 hours later. Pressure gradient in the area of stenosis was measured before and after angioplasty to confirm patency. Partial thrombosis of the inferior vena cava was noted in one patient, conservative therapy was performed. Iliofemoral deep vein thrombosis complicated by pulmonary embolism with subsequent placement of a cava filter was noted in 1 patient.

All patients with stenotic and thrombotic vascular complications were treated with anticoagulant therapy (heparin or low molecular weight heparin) at the calculated dosage in combination with selective thrombolysis in patients with X-ray image-guided endovascular reconstructions. Heparin infusion up to 180–200 IU/kg/day, adjusted according to the activated partial thromboplastin time (target levels, 50–70 seconds).

Bleeding episodes were reported in 5 patients after OLT, of which 2 episodes occurred after hepatic artery stenting and selective thrombolysis.

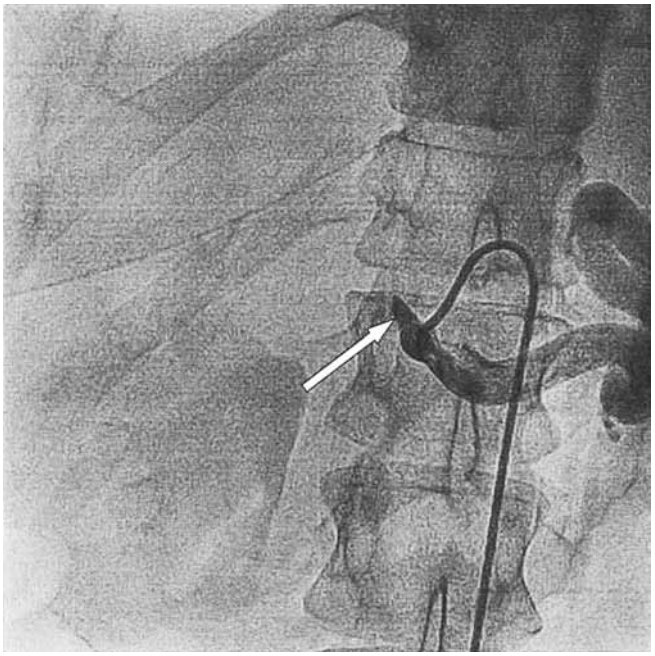

Fig. 2. Selective angiography of the common hepatic artery after liver transplantation. Thrombotic prestenotic occlusion in the proximal part – contrast break, arterial anastomosis stenosis

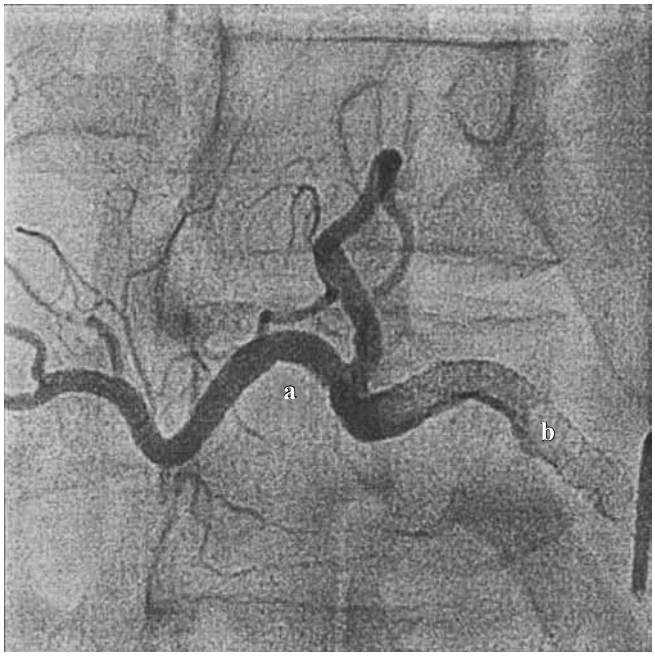

Fig. 3. Selective angiography of the common hepatic artery after liver transplantation: a, restoration of blood flow in the liver after selective thrombolysis; b, the hepatic artery anastomosis area is stented

DISCUSSION

The frequency of vascular complications described in the literature varies greatly according to world and Russian literature. Despite technological advances, VCs are still an important factor in allograft loss, increasing postoperative morbidity and mortality.

Arterial complications are more common, occur in the early postoperative period, and are associated with a high incidence of graft loss and patient mortality. Conversely, venous complications are less frequent, occur in the late postoperative period and do not have a significant impact on graft loss or mortality.

The most common risk factors of arterial complications in LT are technical difficulties and errors at the stage of arterial anastomosis. This is because of the anatomical features of the graft, as well as previous reconstructions at the back table stage, atherosclerotic lesion of the organ artery in expanded criteria donors, significant difference in the diameter of the anastomosed arterial stumps, arterial spasm, and previous surgical interventions on abdominal organs in the recipient. The most common risk factors for venous complications in LT are previous thrombosis in the portal vein system, splenectomy, a significant difference in the diameter of the portal vein sections being sutured, a high MELD score, and the etiologic nature of the recipient's liver damage. Unfortunately, the impact of most of the described factors can only be prevented by improving surgical technique and working with the selection of patients on the waiting list. Identification of the above risk factors, prevention of technical complications and early diagnosis of VCs can reduce mortality, morbidity and the need for repeat transplantation.

Current trends have shown an increasing use of endovascular interventions initially to treat VCs after LT with good outcomes.

When endovascular procedures fail, open surgical repair still plays a role.

CONCLUSION

Most VCs following OLT occur in the early postoperative period (up to 1 month) and can lead to a high risk of graft dysfunction and patient death.

LT outcome depends on the competence and skills of the specialists.

Early detection, diagnosis, and treatment of post-OLT complications are critical for successful short- and long-term graft function and patient survival, even in asymptomatic patients.

The leading methods for detecting and diagnosing VCs at early and late stages of treatment after OLT, included in most study protocols, are Doppler ultrasound and CT/selective angiography, which allows for early detection of VCs.

Treatment options typically include surgical revascularization, percutaneous thrombolysis, percutaneous angioplasty, retransplantation, or, less commonly, a conservative approach.

A combined approach to resolving post-OLT VCs can achieve a positive outcome with minimal surgical trauma to the patient.

VCs following OLT are a formidable and potentially dangerous complication. Therefore, further accumulation and systematization of experience in the diagnosis and treatment of this condition is important.

The authors declare no conflict of interest.

REFERENCES

1. *Stange BJ, Glanemann M, Nuessler NC, Settmacher U, Steinmüller T, Neuhaus P.* Hepatic artery thrombosis after adult liver transplantation. *Liver Transpl*. 2003 Jun; 9 (6): 612–620. Text: electronic. URL: https://aasldpubs.onlinelibrary.wiley.com/doi/epdf/10.1053/jlts.2003.50098 (date of access: 11.07.2022). doi: 10.1053/jlts.2003.50098.
2. *Piardi T, Lhuaire M, Bruno O, Memeo R, Pessaux P, Kianmanesh R, Sommacale D.* Vascular complications following liver transplantation: A literature review of advances in 2015. *World J Hepatol*. 2016 Jan 8; 8 (1): 36–57. – Text: electronic. URL: https://www.wjgnet.com/1948–5182/full/v8/i1/36.htm. doi: 10.4254/wjh.v8.i1.36. PMID 26783420.
3. *Bonnet S, Sauvanet A, Bruno O, Sommacale D, Francoz C, Dondero F, Durand F, Belghiti J et al*. Long-term survival after portal vein arterialization for portal vein thrombosis in orthotopic liver transplantation. *Gastroenterol Clin Biol*. 2010 Jan; 34 (1): 23–28. Text: electronic. URL: https://www.sciencedirect.com/science/article/abs/pii/S0399832009002395?via%3Dihub (date of access: 11.11.2022). doi: 10.1016/j.gcb.2009.05.013. PMID: 19643558.
4. *Schwope RB, Margolis DJ, Raman SS, Kadell BM.* Portal vein aneurysms: a case series with literature review. *J Radiol Case Rep*. 2010; 4 (6): 28–38. Text: electronic. URL: https://www.radiologycases.com/index.php/radiologycases/article/view/431. doi: 10.3941/jrcr.v4i6.431. PMID: 22470738.
5. *Duffy JP, Hong JC, Farmer DG, Ghobrial RM, Yersiz H, Hiatt JR, Busuttil RW.* Vascular complications of orthotopic liver transplantation: experience in more than 4,200 patients. *J Am Coll Surg.* 2009 May; 208 (5): 896–903. Text: electronic. URL: https://pubmed.ncbi.nlm.nih.gov/19476857/ (date of access: 19.07.2022). Access mode: paid. doi: 10.1016/j.jamcollsurg.2008.12.032. PMID 19476857.
6. *Pérez-Saborido B, Pacheco-Sánchez D, Barrera-Rebollo A, Asensio-Díaz E, Pinto-Fuentes P, Sarmentero-Prieto JC et al.* Incidence, management, and results of vascular complications after liver transplantation. *Transplant Proc.* 2011 Apr; 43 (3): 749–750. Text: electronic. URL:

https://www.sciencedirect.com/science/article/abs/pii/S0041134511001886?via%3Dihub. Publication date: 12.04.2011. Access mode: paid. doi: 10.1016/j.transproceed.2011.01.104.

7. *Steinbrück K, Enne M, Fernandes R, Martinho JM, Balbi E, Agoglia L et al.* Vascular complications after living donor liver transplantation: a Brazilian, single-center experience. *Transplant Proc.* 2011 Jan-Feb; 43 (1): 196–198. Text: electronic. URL: https://www.sciencedirect.com/science/article/pii/S0041134510019093?via%3Dihub. Publication date: 16.02.2011. doi: 10.1016/j.transproceed.2010.12.007.
8. *Hejazi Kenari SK, Zimmerman A, Eslami M, F Saidi R.* Current state of art management for vascular complications after liver transplantation. *Middle East J Dig Dis.* 2014 Jul; 6 (3): 121–130. Text: electronic. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4119668/. PMID: 25093059; PMCID: PMC4119668.
9. *Saad WE, Davies MG, Saad NE, Westesson KE, Patel NC, Sahler LG et al.* Catheter thrombolysis of thrombosed hepatic arteries in liver transplant recipients: predictors of success and role of thrombolysis. *Vasc Endovascular Surg.* 2007 Feb-Mar; 41 (1): 19–26. Text: electronic. URL: https://journals.sagepub.com/doi/10.1177/1538574406296210?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed (access mode: by subscription). doi: 10.1177/1538574406296210. PMID: 17277239.
10. *Singhal A, Stokes K, Sebastian A, Wright HI, Kohli V.* Endovascular treatment of hepatic artery thrombosis following liver transplantation. *Transpl Int.* 2010 Mar 1; 23 (3): 245–256. Text: electronic. URL: https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1432–2277.2009.01037.x (date of access: 16.07.2022). doi: 10.1111/j.1432–2277.2009.01037.x.
11. *Bekker J, Ploem S, de Jong KP.* Early hepatic artery thrombosis after liver transplantation: a systematic review of the incidence, outcome and risk factors. *Am J Transplant.* 2009 Apr; 9 (4): 746–757. Text: electronic. URL: https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1600–6143.2008.02541.x (date of access: 09.07.2022). doi: 10.1111/j.1600–6143.2008.02541.x.
12. *Tzakis AG, Gordon RD, Shaw BW Jr, Iwatsuki S, Starzl TE.* Clinical presentation of hepatic artery thrombosis after liver transplantation in the cyclosporine era. *Transplantation.* 1985 Dec; 40 (6): 667–671. Text: electronic. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2952489/. doi: 10.1097/00007890-198512000-00019. PMID: 3907040; PMCID: PMC2952489.
13. *Langnas AN, Marujo W, Stratta RJ, Wood RP, Shaw BW Jr.* Vascular complications after orthotopic liver transplantation. *Am J Surg.* 1991 Jan;161(1):76-82. Text: electronic. URL: https://www.americanjournalofsurgery.com/article/0002-9610(91)90364-J/pdf. doi: 10.1016/0002-9610(91)90364-j. PMID: 1987861.
14. *Drazan K, Shaked A, Olthoff KM, Imagawa D, Jurim O, Kiai K et al.* Etiology and management of symptomatic adult hepatic artery thrombosis after orthotopic liver transplantation (OLT). *Am Surg.* 1996 Mar 01; 62 (3): 237–240. Text: electronic. URL: https://europepmc.org/article/med/8607585 (date of access: 17.07.2022). PMID 8607585.
15. Patent № 2736905 Rossiyskaya Federatsiya, MPK A61B17/00 (2006–01–01). Sposob lecheniya tromboza i stenoza arterial'nogo anastomoza posle transplantatsii pecheni: № 2020118245: zayavl. 2020.05.25: opubl. 2020.11.23 / Korobka V.L., Shapovalov A.M., Kostrykin M.Yu., Malevannyy M.V., Dabliz R.O., Kotov O.V.; zayavitel' Korobka V.L. URL: https://patenton.ru/patent/RU2736905C1 (data obrashcheniya: 15.08.2021). Tekst: elektronnyy.
16. *Khubutia MSh, Zhao AV, Shadrin KB.* Postoperative complications in liver transplant recipients: modern cconsiderations of pathogenesis and main areas of prophylaxis and treatment. *Russian Journal of Transplantology and Artificial Organs*. 2009; 11 (2): 60–66. Text: electronic. URL: https://journal.transpl.ru/vtio/article/view/234/177 (date of access: 30.07.2021). https://doi.org/10.15825/1995-1191-2009-2-60-66.
17. *Granov DA, Polikarpov AA, Tileubergenov II, Zhuikov VN, Moiseenko AV, Sheraliev AR, Kardanova IG.* A case report of successful liver retransplantation in patient with early hepatic artery thrombosis complicated by bile ducts necrosis and sepsis. *Russian Journal of Transplantology and Artificial Organs*. 2019; 21 (3): 76–83. Text: electronic. URL: https://journal.transpl.ru/vtio/article/view/1066/825 (date of access: 16.07.2022). https://doi.org/10.15825/1995-1191-2019-3-76-83.
18. *Lui SK, Garcia CR, Mei X, Gedaly R.* Re-transplantation for Hepatic Artery Thrombosis: A National Perspective. *World J Surg*. 2018 Oct; 42 (10): 3357–3363. Text: electronic. URL: https://onlinelibrary.wiley.com/doi/10.1007/s00268-018-4609-7 (date of access: 11.07.2022). doi: 10.1007/s00268-018-4609-7. PMID: 29616318.
19. *Novruzbekov MS, Olisov OD.* Vascular complications after orthotopic liver transplantation. *Transplantologiya*. 2017; 9 (1): 35–50. Text: electronic. URL: https://cyberleninka.ru/article/n/sosudistye-oslozhneniya-posle-ortotopicheskoy-transplantatsii-pecheni (date of access: 16.07.2022). doi: 10.23873/2074-0506-2017-9-1-35-50.
20. *Pawlak J, Wróblewski T, Małkowski P, Nyckowski P, Zieniewicz K, Grzelak I et al.* Vascular complications related to liver transplantation. *Transplant Proc.* 2000 Sep; 32 (6): 1426–1428. Text: electronic. URL: https://www.sciencedirect.com/science/article/pii/S0041134500012811?via%3Dihub. Publication date: 14.09.2000. doi: 10.1016/s0041–1345(00)01281-1.
21. *Silva MA, Jambulingam PS, Gunson BK, Mayer D, Buckels JA, Mirza DF, Bramhall SR.* Hepatic artery thrombosis following orthotopic liver transplantation: a 10-year experience from a single centre in the United Kingdom. *Liver Transpl.* 2006 Jan; 12 (1): 146–151. Text: electronic. URL: https://aasldpubs.onlinelibrary.wiley.com/doi/10.1002/lt.20566 (date of access: 17.07.2022). doi: 10.1002/lt.20566.

22. *Pareja E, Cortes M, Navarro R, Sanjuan F, López R, Mir J.* Vascular complications after orthotopic liver transplantation: hepatic artery thrombosis. *Transplant Proc.* 2010 Oct; 42 (8): 2970–2972. Text: electronic. URL: https://www.sciencedirect.com/science/article/pii/S0041134510011085?via%3Dihub. Publication date: 21.10.2010. doi: 10.1016/j.transproceed.2010.07.063. PMID 20970585.
23. *Mattera J, Ciardullo M, Pekolj J, Stork G, Quiñonez E, Palavecino M et al.* Vascular complications after liver transplantation. *Transplantation*. 2004 July 27; 78 (2): 696. Text: electronic. URL: https://journals.lww.com/transplantjournal/Fulltext/2004/07271/VASCULAR-COMPLICATIONS_AFTER_LIVER.1904.aspx.
24. *Saad WE, Davies MG, Sahler L, Lee DE, Patel NC, Kitanosono T et al.* Hepatic artery stenosis in liver transplant recipients: primary treatment with percutaneous transluminal angioplasty. *J Vasc Interv Radiol.* 2005 Jun; 16 (6): 795–805. Text: electronic. URL: https://www.sciencedirect.com/science/article/pii/S1051044307606848. Publication date: 30.03.2007. Access mode: paid. doi: 10.1097/01.RVI.0000156441.12230.13.
25. *Hamby BA, Ramirez DE, Loss GE, Bazan HA, Smith TA, Bluth E, Sternbergh WC 3rd.* Endovascular treatment of hepatic artery stenosis after liver transplantation. *J Vasc Surg.* 2013 Apr; 57 (4): 1067–1072. Text: electronic. URL: https://reader.elsevier.com/reader/sd/pii/S0741521412023075?token=2EAB3BDDAC8D24B6D1846EE7BEC59AD46054D05133457AA22AF46376AD639F7A3256301E6B6C38F2AE477F8B2ACEAE5A&originRegion=eu-west-1&originCreation=20220730161353. Publication date: 18.01.2013. doi: 10.1016/j.jvs.2012.10.086.
26. *Rostambeigi N, Hunter D, Duval S, Chinnakotla S, Golzarian J.* Stent placement versus angioplasty for hepatic artery stenosis after liver transplant: a meta-analysis of case series. *Eur Radiol.* 2013 May; 23 (5): 1323–1334. Text: electronic. URL: https://link.springer.com/article/10.1007/s00330–012–2730–9. Publication date: 13.12.2012. Access mode: paid. doi: 10.1007/s00330-012-2730-9.
27. *Sommacale D, Aoyagi T, Dondero F, Sibert A, Bruno O, Fteriche S et al.* Repeat endovascular treatment of recurring hepatic artery stenoses in orthotopic liver transplantation. *Transpl Int.* 2013 Jun; 26 (6): 608–615. Text: electronic. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/tri.12089 (date of access: 18.07.2022). doi: 10.1111/tri.12089.
28. *Denys AL, Qanadli SD, Durand F, Vilgrain V, Farges O, Belghiti J et al.* Feasibility and effectiveness of using coronary stents in the treatment of hepatic artery stenoses after orthotopic liver transplantation: preliminary report. *AJR Am J Roentgenol.* 2002 May; 178 (5): 1175–1179. Text: electronic. URL: https://www.ajronline.org/doi/full/10.2214/ajr.178.5.1781175 (date of access: 18.07.2022). doi: 10.2214/ajr.178.5.1781175.
29. *Holbert BL, Baron RL, Dodd GD 3rd.* Hepatic infarction caused by arterial insufficiency: spectrum and evolution of CT findings. *AJR Am J Roentgenol.* 1996 Apr; 166 (4): 815–820. Text: electronic. URL: https://www.ajronline.org/doi/epdf/10.2214/ajr.166.4.8610556 (date of access: 15.08.2021). doi: 10.2214/ajr.166.4.8610556.
30. *Dodd GD 3rd, Memel DS, Zajko AB, Baron RL, Santaguida LA.* Hepatic artery stenosis and thrombosis in transplant recipients: Doppler diagnosis with resistive index and systolic acceleration time. *Radiology.* 1994 Sep; 192 (3): 657–661. Text: electronic. URL: https://pubs.rsna.org/doi/epdf/10.1148/radiology.192.3.8058930. Publication date: 01.09.1994. Access mode: paid. doi: 10.1148/radiology.192.3.8058930. PMID 8058930.
31. *Leelaudomlipi S, Bramhall SR, Gunson BK, Candinas D, Buckels JA, McMaster P et al.* Hepatic-artery aneurysm in adult liver transplantation. *Transpl Int.* 2003 Apr; 16 (4): 257–261. Text: electronic. URL: https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1432–2277.2003.tb00296.x. Publication date: 15.02.2003. doi: 10.1111/j.1432–2277.2003.tb00296.x.
32. *Crossin JD, Muradali D, Wilson SR.* US of liver transplants: normal and abnormal. *Radiographics.* 2003 Sep-Oct; 23 (5): 1093–1114. Text: electronic. URL: https://pubs.rsna.org/doi/10.1148/rg.235035031. Publication date: 01.09.2003. Access mode: paid. doi: 10.1148/rg.235035031.
33. *Marshall MM, Muiesan P, Srinivasan P, Kane PA, Rela M, Heaton ND et al.* Hepatic artery pseudoaneurysms following liver transplantation: incidence, presenting features and management. *Clin Radiol.* 2001 Jul; 56 (7): 579–587. Text: electronic. URL: https://www.clinicalradiologyonline.net/article/S0009-9260(01)90650-6/pdf. Access mode: paid. doi: 10.1053/crad.2001.0650.
34. *Volpin E, Pessaux P, Sauvanet A, Sibert A, Kianmanesh R, Durand F et al.* Preservation of the arterial vascularisation after hepatic artery pseudoaneurysm following orthotopic liver transplantation: long-term results. *Ann Transplant.* 2014 Jul 18; 19: 346–352. Text: electronic. URL: https://pubmed.ncbi.nlm.nih.gov/25034853/. Publication date: 18.07.2014. doi: 10.12659/AOT.890473. PMID 25034853.
35. *Woo DH, Laberge JM, Gordon RL, Wilson MW, Kerlan RK Jr.* Management of portal venous complications after liver transplantation. *Tech Vasc Interv Radiol.* 2007 Sep; 10 (3): 233–239. Text: electronic. URL: https://www.techvir.com/article/S1089–2516(07)00095–9/fulltext (date of access: 18.07.2022). Access mode: paid. doi: 10.1053/j.tvir.2007.09.017. PMID 18086428.
36. *Pawlak J, Grodzicki M, Leowska E, Małkowski P, Michałowicz B, Nyckowski P et al.* Vascular complications after liver transplantation. *Transplant Proc.* 2003 Sep; 35 (6): 2313–2315. Text: electronic. URL: https://www.mendeley.com/search/?page=1&query=10.1016%2Fs0041-1345%2803%2900836-4&sortBy=relevance (date of access: 19.07.2022). Access mode: paid. doi: 10.1016/s0041-1345(03)00836-4. PMID 14529925.
37. *Navarro F, Le Moine MC, Fabre JM, Belghiti J, Cherqui D, Adam R et al.* Specific vascular complications of orthotopic liver transplantation with preservation of the

retrohepatic vena cava: review of 1361 cases. *Transplantation.* 1999 Sep 15; 68 (5): 646–650. Text: electronic. URL: https://journals.lww.com/transplantjournal/Fulltext/1999/09150/SPECIFIC_VASCULAR_COMPLICATIONS_OF_ORTHOTOPIC.9.aspx. (date of access: 19.07.2022). doi: 10.1097/00007890-199909150-00009.

38. *Borsa JJ, Daly CP, Fontaine AB, Patel NH, Althaus SJ, Hoffer EK et al.* Treatment of inferior vena cava anastomotic stenoses with the Wallstent endoprosthesis after orthotopic liver transplantation. *J Vasc Interv Radiol.* 1999 Jan; 10 (1): 17–22. Text: electronic. URL: https://www.academia.edu/4591349/Treatment_of_Inferior_Vena_Cava_Anastomotic_Stenoses_with_the_Wallstent_Endoprosthesis_after_Orthotopic_Liver_Transplantation (access mode: by subscription). doi: 10.1016/s1051-0443(99)70003-5. PMID: 10872484.
39. *Korobka VL, Kostrykin MYu, Pak ES, Dabliz RO, Kotov OV, Shapovalov AM*. A five-year liver transplant experience in Rostov Oblast. *Russian Journal of Transplantology and Artificial Organs*. 2020; 22 (2): 35–43. Text: electronic. URL: https://journal.transpl.ru/vtio/article/view/1182/928 (date of access: 01.06.2022). doi: https://doi.org/10.15825/1995-1191-2020-2-35-43.
40. *Rajani R, Melin T, Björnsson E, Broomé U, Sangfelt P, Danielsson Å et al.* Budd–Chiari syndrome in Sweden: epidemiology, clinical characteristics and survival – an 18-year experience. *Transpl Int*. 2009; 29 (2): 253–259. Text: electronic. URL: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1478-3231.2008.01838.x (date of access: 16.04.2022). Access mode: paid. doi: 0.1111/j.1478-3231.2008.01838.x. PMID 18694401.
41. *Khripun AI, Shurygin SN, Mironkov AB, Pryamikov AD*. Venous acute disturbance of mesenteric circulation: diagnosis and treatment. *Pirogov Russian Journal of Surgery = Khirurgiya. Zurnal im. N.I. Pirogova.* 2017; (12): 95–102. Text: electronic. URL: https://www.mediasphera.ru/issues/khirurgiya-zhurnal-im-n-i-pirogova/2017/12/downloads/ru/1002312072017121095 (date of access: 16.04.2022). doi: 10.17116/hirurgia20171295-102.
42. *Filonik DYu, Sauchuk MM, Scherba AE, Rummo OO*. Vascular complications development after orthotopic liver transplantation. *Medical Journal*. 2014; 2 (48): 34–39. Text: electronic. URL: https://www.elibrary.ru/item.asp?id=21476932 (date of access: 20.07.2022). Access mode: Scientific Electronic Library eLibrary.

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EVALUATION OF HEMOSTASIS PARAMETERS IN RECIPIENTS AFTER RELATED RIGHT LOBE LIVER TRANSPLANTATION

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Objective: to study the dynamics of hemostasis parameters in the early postoperative period and to identify the timing of restoration of the level of procoagulants and anticoagulants synthesized by the liver (received from a living related donor) in liver lobe recipients. **Materials and methods.** Under observation were 31 recipients and 31 related donors of liver lobe. They were treated at the Republican Specialized Scientific and Practical Medical Center for Surgery in Tashkent, Uzbekistan, from August 2022 to August 2023. Hemostasis parameters were determined in recipients, whose postoperative period was uneventful. **Results.** It was revealed that compensation in the hemostasis system occurs even at low levels of coagulation factors on day 10 after liver transplantation (LT). In recipients, a decrease in anticoagulants was more pronounced than that of procoagulants. In general, the hemostasis system was in an unstable equilibrium, which, under the influence of external and internal factors, can easily shift both towards hypercoagulable and hypocoagulable state. Activity of the fibrinolytic system and fibrinogen level are significant influencing factors. Gradual recovery of fibrinogen levels by the end of day 1 after surgery is the result of activation of the synthetic function of the liver. After LT, there were signs of endothelium activation, but not endothelial damage, which regress and normalize by postoperative day 10. At the same time, in the initial status, recipients had an increase in both the amount and activity of von Willebrand factor, which indicates endothelial damage and dysfunction. The low level of homocysteine in recipients is probably a protective factor against the development of thrombotic complications, and homocysteine dynamics reflects the gradual restoration of the functional activity of the liver, adaptation of the donor liver to functioning. **Conclusion.** Monitoring of hemostasis system in recipients after liver transplantation allows to prevent thrombohemorrhagic complications in time but also to assess the dynamic equilibrium of procoagulants and anticoagulants, the timing of restoration of the activity of the main hemostasis factors and, according to this, to vary the administration regimes of anticoagulants, antiplatelet medications, and fibrinolysis inhibitors, to carry out replacement therapy and to realize the concept of hemostasis management.

Keywords: liver transplantation, thrombohemorrhagic complications, endothelial dysfunction, coagulation factors, hemostasis.

INTRODUCTION

Thrombohemorrhagic complications in LT represent a major problem in the perioperative period [1–3]. Compromised initial status in patients with end-stage liver disease (ESLD), unbalanced hemostasis system, as well as the anatomical features of angioarchitecture of the donor liver, the direct technical peculiarities of all stages of LT, including processing of donor liver on the back table, create prerequisites for the development of these complications [4, 5]. Various reports have shown that the incidence of bleeding and thrombosis varies greatly, ranging from 0.02 to 25%, and it is impossible to predict the risk of thrombohemorrhagic complications solely based on preoperative hemostasiogram parameters [6, 7]. These difficulties are down to the fact that each patient has individual peculiarities of the reserve of compensatory capabilities of the hemostasis system, the degree of endothelial dysfunction and endotoxemia.

The initial metabolic status in ESLD patients is characterized by different degrees of dysproteinemia due the reduced protein-synthetic function of the liver (hypoalbuminemia on the background of hyperglobulinemia), manifestations of cholestasis and cytolysis syndrome, as well as hypocholesterolemia and hyperglycemia resulting from decreased glycogen and lipoprotein metabolism in the liver [8, 9]. Due to impaired synthetic function of hepatocytes, the production of protein coagulation factors and natural anticoagulants is also reduced, which is a prerequisite for the development of both thrombotic and hemorrhagic complications with a shift in the delicate dynamic balance of hemostasis factors [10]. It is known that almost all coagulation hemostasis factors (II, V, VII, IX, X, XI, XII, XIII), coagulation inhibitor factors (antithrombin, heparin cofactor II, protein C, protein S, tissue factor pathway inhibitor), fibrinolytic system components (plasminogen, alpha-2-antitrypsin, plasmin inhibitor) are synthesized in the liver [10, 11]. At the

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same time, such factors as urokinase-type plasminogen activator, thrombomodulin are synthesized outside the liver and their level depends on the degree of tissue and endothelial damage [12]. A significant aggravating factor in the development of thrombohemorrhagic complications is portal hypertension, varicose veins, hypertrophied diffuse collateral venous blood flow, which causes platelet sequestration in the spleen [11]. Portopulmonary hypertension is observed in 3–8% of cases and causes procoagulant shifts in the pulmonary endothelium, which may be the basis of the pathogenesis of this condition; intrapulmonary microthrombi at autopsy in patients with portopulmonary syndrome confirm this [13]. Borst et al. (2018) reported that bleeding and thrombotic events in LT developed in 20.7% and 25% of cases, respectively, and 50% of liver retransplantations were for thrombotic complications [14].

MATERIAL AND METHODS

There were 31 recipients and 31 related donors of liver lobe treated at the Republican Specialized Scientific and Practical Medical Center for Surgery in Tashkent, from August 2022 to August 2023. This group of recipients was selected at the second stage of mastering the LT technique at our Center, i.e. 4 years after the first LT in 2018.

The recipients were predominantly male (22 *vs.* 9 females); mean age was 40.15 (95% CI: 38.7–45.6) years, the etiology of cirrhosis was dominated by chronic hepatitis B and delta in 26 (83.9%) cases; mean score on the Model of End-Stage Liver Disease (MELD) scale was 14.4 (95% CI: 12.8–15.9); fibroscan was 33.6 (95% CI: 29.1–38.0). The baseline risk of thrombohemorrhagic complications according to the Wells probability scale was high, as 28 (90.3%) had varicose veins (2.8 (95% CI: 2.61–3.14) trunk) and a history of bleeding in 10 (32.2%) patients (1 to 5 episodes). All recipients had an uncomplicated postoperative period.

Hemostatic factors were studied on an automatic coagulometer ACL-TOP (USA) using standard kits: activated partial thromboplastin time (aPTT), thrombin time (TT), prothrombin complex including prothrombin time (PT) and international normalized ratio (INR), antithrombin III (AT III) were determined, plasminogen, D-dimer (highly sensitive by immunoturbidimetric method), von Willebrand factor (vWF), vWF activity, homocysteine, natural plasma anticoagulants – protein C and protein S. The parameters were evaluated preoperatively, immediately postoperatively (p/o), 12 hours, 24 hours after LT, as well as on days 5–7 and day 10 of the postoperative period.

RESULTS

The average graft weight in our study was 627.2 ± 45.8 grams. Both at baseline and at all follow-up periods, the parameters of prothrombin complex – PT, INR – characterizing the level of factor II, were significantly lower than in donors, they were also below the lower limit of the reference interval. Within 1 day (p/o, 12h and 24h after LT), PT was prolonged 1.6–1.63 times ($p < 0.05$), INR increased 1.6–1.65 times ($p < 0.05$), and a gradual increase in factor II synthesis during the first week after LT still did not lead to achievement of target values. On days 5–7, PT and INR had positive dynamics, but were lower than in donors by 1.35 and 1.3 times ($p < 0.05$), respectively, remaining at this level until day 10 of follow-up. On day 10 of follow-up, INR was 1.3 times lower than donors' values, PT was 1.33 times prolonged ($p < 0.05$) (Fig. 1). The peak of PT prolongation and INR increase occurred at the end of day 1 after surgery, indicating a deficiency of coagulation factors in this period.

Partial restoration of prothrombin levels by day 10 was sufficient for balance in the hemostatic system, which is reflected by TT and aPTT levels. On day 10, TT and aPTT were within the reference interval. It should be noted that the peak of TT and aPTT prolongation occurred in the period immediately after the end of the

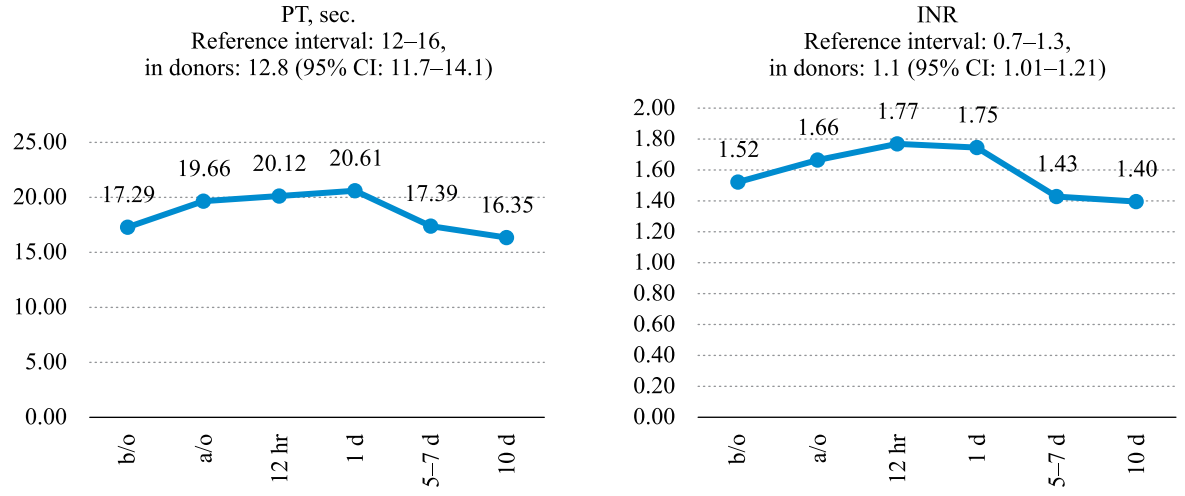

Fig. 1. Dynamics of Prothrombin Time and International Normalized Ratio in liver lobe recipients

operation, which is connected with the peculiarities of back-table preparation of the donor liver, application of washing and preserving solutions, as well as management of the graft reperfusion period during its implantation. Back-table preparation of the liver lasted for 93.3 ± 17.6 min. In the period from 12 hours to 5 days after LT, the TT parameter was at a steady-state level and prolonged relative to donors by 1.3–1.35 times, and aPTT had a second peak of lengthening to 59.8 seconds (95% CI: 29.5–77.6) by the end of day 1 of follow-up (Fig. 2).

The wide variability of aPTT was remarkable, which was due to individual characteristics of each recipient, heparin dose and AT III levels, since aPTT is an indicator of heparin therapy efficacy. On days 5–7, aPTT was at the target level of 41.1 seconds (95% CI: 33.7–52.6), and by day 10, it was 33.7 seconds (95% CI: 20.5–47.6), which is within the reference interval. At the same time, there was a deficiency of AT III, as well as natural anticoagulants synthesized by the liver – proteins C and S.

AT III levels in liver recipients remained low up to day 10 of the postoperative period, although the level tended to increase gradually starting from day 5 (Fig. 3).

We emphasize that low AT III levels reduces the ineffectiveness of heparin therapy, because heparin is not active in the absence of its cofactor, which is AT III. AT III levels in healthy donors is 100.3% with a reference interval of 83–128%, and in liver recipients it was reduced relative to this level by 3.2; 3.0; 3.6; 2.3 and 1.7 times during the observation stages ($p < 0.05$). The peak of AT III decrease occurred on day 1 after the operation, after that AT III tended to increase; by day 5, it increased 1.6 times relative to the previous period and increased in dynamics by day 10, but still remained below the reference interval and 1.7 times lower than in donors. The low level of AT III during anticoagulant therapy may serve as an indicator in favor of choosing alternative anticoagulants (Xa inhibitors, etc.) up to day 5 after surgery due to the possible ineffectiveness of unfractionated heparin under AT III deficiency.

The level of proteins C and S, which were initially reduced relative to the level of healthy donors by 1.9 ($p < 0.05$) and 1.1 ($p > 0.05$) times, respectively, were partially restored on day 10 of follow-up. By day 10 of follow-up, protein C was restored only to 57%, which is 1.5 times lower than the target level ($p < 0.05$), and protein S was restored to 52.9%, which is 1.6 ($p < 0.05$) times lower than the target level. Moreover, the peak of protein C and S decrease was 12 hours after LT, when protein C was reduced 2.8-fold and protein S was reduced 2.3-fold relative to donor levels ($p < 0.05$) (Fig. 4).

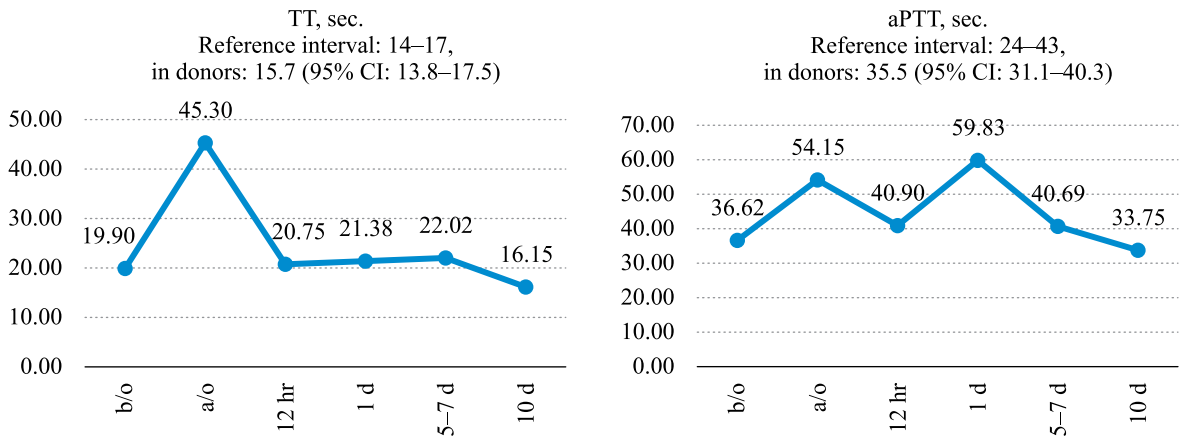

Fig. 2. Dynamics of Thrombin Time and Activated Partial Thromboplastin Time in liver lobe recipients

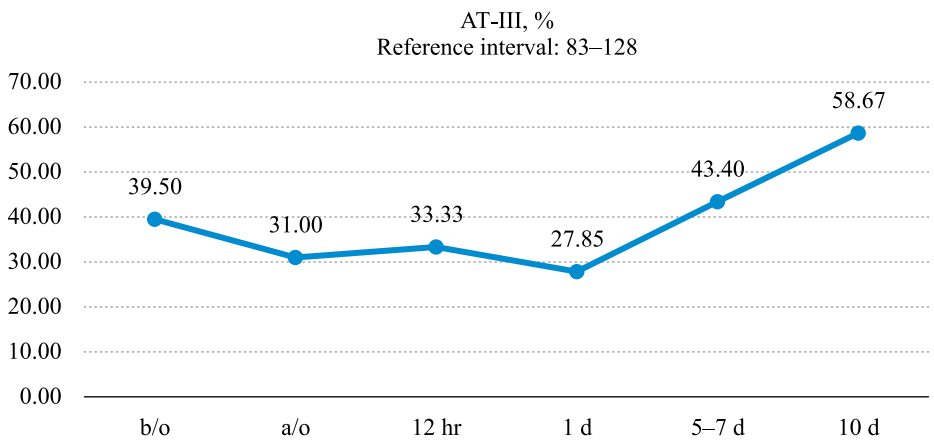

Fig. 3. Dynamics of antithrombin III in liver lobe recipients

Thus, the results show that compensation in the hemostatic system occurs even at low levels of coagulation factors on day 10 of LT, when TT and aPTT are within the reference interval, while INR is prolonged 1.3 times, AT III is reduced 1.7 times, protein C and S are reduced 1.5 and 1.6 times relative to donors, respectively.

As can be seen, the decrease in anticoagulants is more pronounced than in procoagulants, and in general the hemostatic system is in unstable equilibrium, which, under the influence of external and internal factors, can easily shift both towards a hyper- and hypocoagulable state. A significant influencing factor in this case is fibrinolytic activity and fibrinogen levels (Fig. 5).

Thus, plasminogen activity was reduced during the first week after LT, being 2.2 and 2.5 times lower ($p < 0.05$) relative to donors 12 hours and 24 hours after LT. By day 5, plasminogen activity slightly increased, and by day 10 it was within the reference interval, exceeding that of donors by 1.2 times. Fibrinogen levels were decreased to a greater extent immediately after LT and gradually increased over 1 week, reaching the reference-interval level on day 10 after LT.

Assessment of vWF amount and vWF activity showed that both parameters decreased in the recipients by day 10 after LT (Fig. 6).

Immediately after the end of the operation, there was increased vWF activity (vWF Act) without an increase in vWF amount (vWF Ag), and subsequently there was a clear tendency to a decrease in vWF amount, but without a decrease in its activity up to day 5 of follow-up. On day 10, the quantitative content of vWF Ag normalized and vWF Ag activity decreased to the level of the reference interval, but both indices were higher than in donors by 2.1 and 1.5 times for vWF Act and vWF Ag, respectively ($p < 0.05$).

Platelet count in recipients decreased 4.6 times relative to the donor level on day 1 and remained at this level until day 5 after LT, and by day 10 increased 2.1 times relative to the previous period, although it did not reach the level of the lower limit of the reference interval and the donor level (Fig. 7). It should be noted that on day 1 after LT, thrombocytopenia is probably compensated by an increase in their aggregation properties under increased vWF activity, and by day 5, vWF activity synchronously decreases and platelet count increases, providing the balance of anti- and pro-aggregation properties. At the same time, platelet aggregation did not increase and there was no initiation of the blood coagulation cascade with the development of thrombotic (both arterial and venous) complications. D-dimer levels were elevated

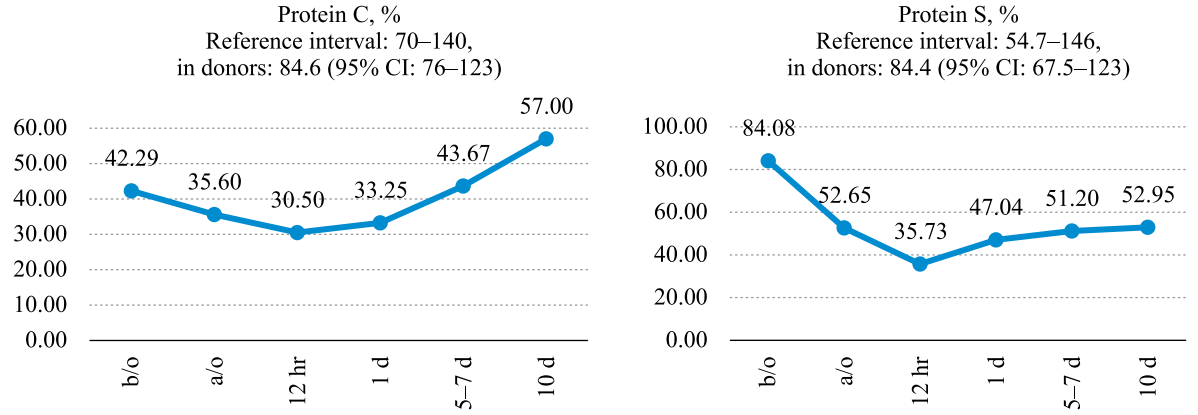

Fig. 4. Dynamics of proteins C and S in liver lobe recipients

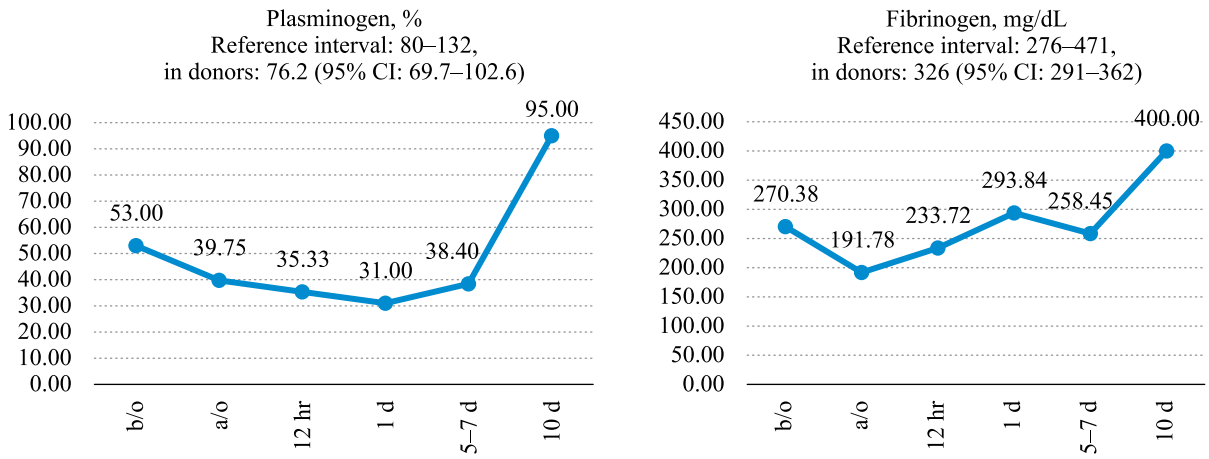

Fig. 5. Dynamics of plasminogen and fibrinogen levels in liver lobe recipients

in all periods, averaging 962–3869 ng/mL, reflecting multidirectional shifts in this index.

The study of homocysteine levels in the recipients showed that it decreased 2.5 times relative to the donor level immediately after surgery, increased 2 times from this level after 12 hours p/o, a plateau up to 24 hours p/o and gradually increased to the level of donors and reference interval, which was noted already on day 5 after LT (Fig. 8).

This indicates the restoration of amino acid metabolism reactions in the liver, in particular transmethylation reactions involving methionine and homocysteine,

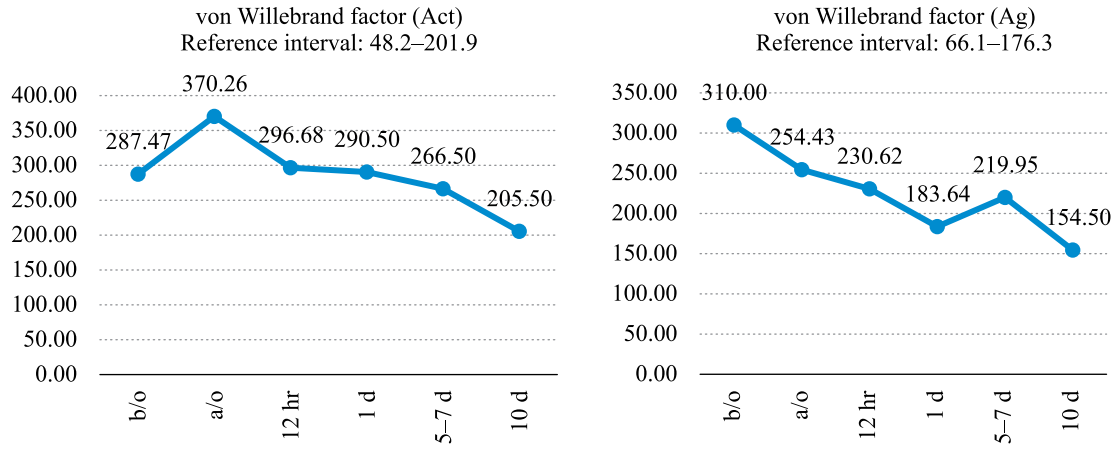

Fig. 6. Dynamics of Willebrand factor levels in liver lobe recipients

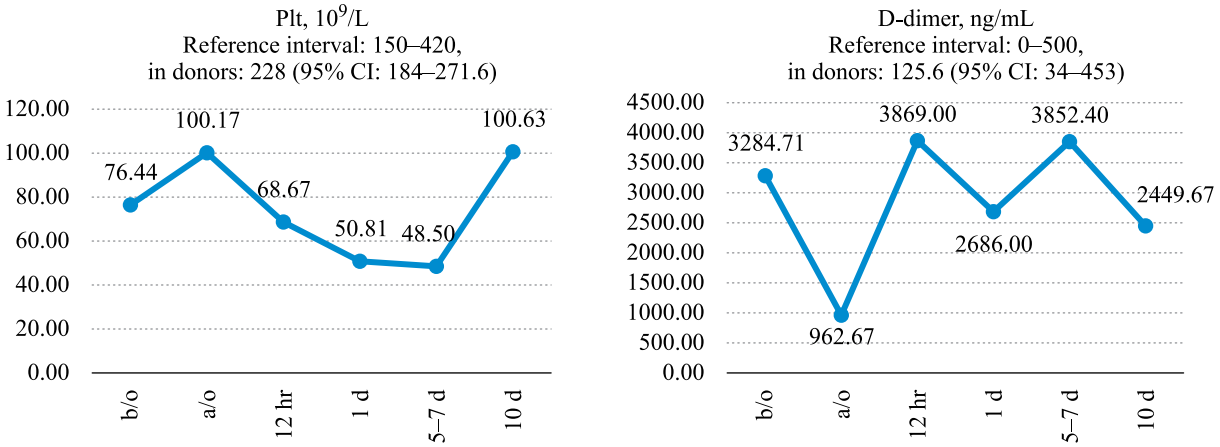

Fig. 7. Dynamics of platelet (Plt) and D-dimer levels in liver lobe recipients

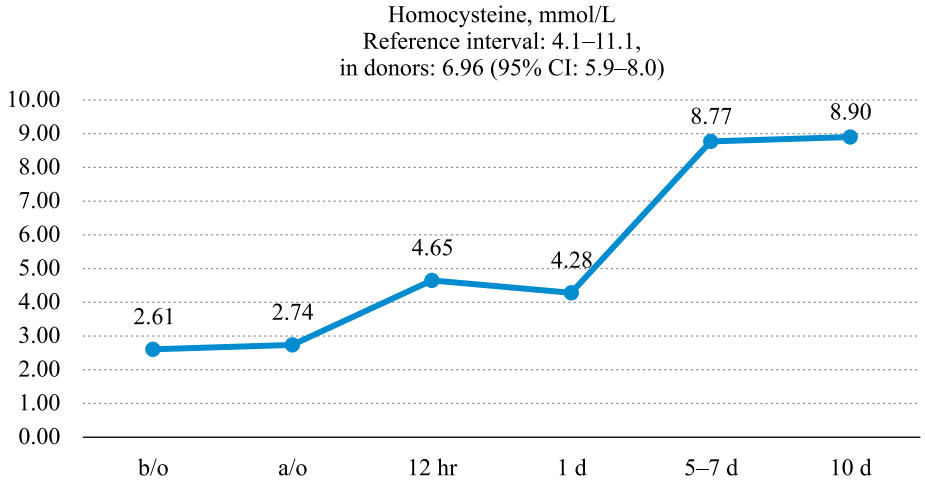

Fig. 8. Dynamics of homocysteine levels in liver lobe recipients

as well as the general amino acid catabolic pathways through transamination, decarboxylation and deamination. Note that homocysteine, when its level is elevated, is an independent factor in the development of stroke and thrombosis, and is also associated with the development of brain disorders (depression, Alzheimer's disease, chronic fatigue syndrome), and osteoporosis. Low level of homocysteine in liver recipients is probably a protective factor against the development of thrombotic complications, and homocysteine dynamics reflects the gradual recovery of the functional activity of the liver, and adaptation of the donor liver to function in the recipient's body.

DISCUSSION

LT from a living related donor differs from LT from a deceased donor in that the recipient does not receive the whole organ, but only a part of it. Consequently, the timing of liver function restoration will be different, which is related to the amount of functioning liver parenchyma. According to reports, in recipients of whole liver from a cadaveric donor, recovery of synthetic liver function and normalization of hemostasis factors occur on days 1–2 [8, 11]. We found that the liver function to synthesize enough coagulation factors and anticoagulants in liver lobe recipients recovered slowly, because the target values were partially reached only on day 5–7. The synthesis of natural anticoagulants lagged behind the synthesis of coagulation factors, and fibrinolytic activity depended on the course of the postoperative period. It is known that plasminogen activation level depends on the degree of tissue damage with the release of tissue factors (plasminogen activators, PAI-1 plasminogen activator inhibitors, the degree of endotoxemia, the severity of endothelial dysfunction and the intensity of deactivation of bioactive molecules, including liver involvement) [11]. Progression of a decrease in natural plasma anticoagulants 12 hours after LT, which we identified, may be a risk factor for thrombotic complications during this period, especially under AT III deficiency, and requires mandatory monitoring every 12 hours during the first 3 days after LT.

Low levels of protein C with normal levels of protein S, its cofactor, also indicates a predisposition to thrombotic complications. It is known that protein C deficiency leads to impaired inactivation of Y and YII factors, which increases the procoagulant potential of blood plasma, because active thrombin (IIa) not only catalyzes fibrinogen conversion into fibrin, but also activates the anti-clotting mechanism through protein C activation. When protein C is sufficient, the following cascade is triggered: IIa interacts with thrombomodulin, calcium, prothrombin, and further this complex activates protein C, and it interacts with cofactor S and calcium ions, and the complex consisting of protein C, protein S, and calcium destroys active factors V and VII, thus inhibiting coagulation hemostasis through both intrinsic and extrinsic pathways [11, 13]. The deficiency of natural anticoagulants synthesized by the liver is partially balanced by the deficiency of procoagulants; however, this dynamic equilibrium is unstable.

The decrease in fibrinogen immediately after LT is probably due to the fact that fibrinogen is the factor that first responds to hemodilution and massive blood loss. The mean volume of blood loss in our study was 1388.9 ± 198.9 mL for the entire period of surgery, which lasted, on average, 596.7 ± 29.0 min. Gradual recovery of fibrinogen levels by the end of day 1 is the result of the inclusion of the synthetic function of the liver.

Von Willebrand factor is an informative marker of the endothelium state. Our results show that recipients after LT have signs of endothelial activation, but not endothelial damage, which regress and normalize by day 10 after LT. At the same time, in the initial status, recipients have an increase in both vWF amount and vWF activity, which indicates endothelial damage and endothelial dysfunction.

D-dimer levels in recipients was elevated in all follow-up periods, which was expected given the end stage of the liver disease at baseline and surgical trauma resulting from LT. Taking into account the low specificity of D-dimer for prognosis of thrombotic complications in the postoperative period, D-dimer elevation should be considered as typical, and monitoring of the dynamics of this index should be performed as indicated, if clinically necessary. We also note that all recipients received anticoagulant therapy, we did not observe arterial and venous thromboembolic complications in any case. The low level of homocysteine, which we observed in our patients, may be protective against these formidable complications.

Taking into account the above mentioned, monitoring the hemostatic system in liver lobe recipients allows not only to timely diagnose thrombohemorrhagic complications, but also to ascertain the dynamic balance of pro- and anticoagulants, the timing of restoration of the activity of the main factors of the hemostatic system and, according to this, to vary the regimes of anticoagulants, antiplatelet agents, fibrinolysis inhibitors administration and to carry out replacement therapy, i.e. to implement the concept of hemostasis management.

CONCLUSION

The results we obtained have allowed us to draw the following conclusions.

1. In liver lobe recipients in the initial status, there is a balanced decrease in coagulation factors (IIa), as well as anticoagulants (proteins C, S, AT III) against the background of significantly ($p < 0.05$) increased vWF Ag and vWF Act relative to donors.
2. During the first day after LT, INR was reduced by 1.6–1.63 times; TT was prolonged 1.3 times, AAT III was decreased 3.0–3.6 times, protein C 2.8–2.5 times; protein S 2.3–1.4 times, plasminogen 2.2–2.5 times, while there is a clear tendency of prolongation of TT and PT, and decrease in proteins C and S by 12 hours

of the postoperative period against the background of sharply reduced AT III level. This allows us to consider this period as a critical one, when the synthetic and elimination function of the liver is insufficient.

3. By day 5–7 after LT, there is no complete recovery of the level of hemostatic factors, although INR, AT III, protein C, and plasminogen levels were significantly higher relative to day 1 of follow-up, however, lower than the donor level by 1.3, 2.3, 1.9 times ($p < 0.05$), respectively.
4. Compensation in the hemostatic system occurs even at low level of coagulation factors on day 10 of LT, when TT and aPTT are within the reference interval, and INR is prolonged 1.3 times, AT III is reduced 1.7 times, protein C and S are reduced 1.5 and 1.6 times relative to donors, respectively ($p < 0.05$).
5. Immediately after the end of the operation, there was increased vWF activity (vWF Act) without increased vWF amount (vWF Ag), and subsequently there was a clear tendency to a decrease in vWF quantitatively, but without a decrease in vWF activity up to 5 days of follow-up; on day 10, the quantitative content of vWF Ag normalized and its activity decreased to the reference interval level. However, both indices were higher than in donors by 2.1 and 1.5 times for vWF Act and vWF Ag, respectively ($p < 0.05$), reflecting first endothelial activation and then its regression.
6. The synthesis of natural anticoagulants lags behind the synthesis of coagulation factors, and fibrinolytic activity depends on the course of the postoperative period; in recipients after LT there are signs of endothelial activation, but not endothelial damage, as evidenced by the level of vWF Ag, vWF Act, when vWF Ag decreases while vWF Act remains high, and subsequently there is a tendency towards normalization of these indices to the reference interval level by day 10 after LT.
7. Low level of homocysteine in liver recipients is probably a protective factor against the development of thrombotic complications, and the dynamics in the increase in homocysteine levels by day 10 after LT reflects the gradual recovery of the functional activity of the liver, adaptation of the donor liver to function in the recipient's body.

The authors declare no conflict of interest.

REFERENCES

1. *Gautier S, Monakhov A, Gallyamov E, Tsirulnikova O, Zagaynov E, Dzhanbekov T.* Laparoscopic left lateral section procurement in living liver donors: A single center propensity score-matched study. *The Journal of Clinical and Translational Research*. 2018 Sep; 32 (9): e13374. doi: 10.1111/ctr.13374.
2. *Moysyuk YaG, Malinovskaya YuO, Bogomolov PO.* Transplantatsiya pecheni. Sovremenniye dostijeniya i problemi. *Vestnik MEDSI.* 2017; 29.
3. *Buros C, Dave AA, Furlan A.* Immediate and Late Complications After Liver Transplantation. *Radiologic Clinics of North America*. 2023 Sep; 61 (5): 785–795. doi. org/10.1016/j.rcl.2023.04.002.
4. *Bezinover D, Molly FD, Dalal PG, Moores RP, Stine JG, Wang M et al.* Perioperative thrombotic complications associated with pediatric liver transplantation: a UNOS database evaluation. *HPB*. 2019; 21 (3): 370–378. doi: 10.1016/j.hpb.2018.08.014.
5. *Feltracco P, Barbieri S, Cillo U, Zanus G, Senzolo M, Ori C.* Perioperative thrombotic complications in liver transplantation. *World J Gastroenterol.* 2015 July 14; 21 (26): 8004–8013. doi: 10.3748/wjg.v21.i26.8004.
6. *Khubutia MSh, Zhao AV, Shadrin KB*. Postoperative complications in liver transplant recipients: modern cconsiderations of pathogenesis and main areas of prophylaxis and treatment. *Russian Journal of Transplantology and Artificial Organs*. 2009; 11 (2): 60–66. (In Russ.). https://doi.org/10.15825/1995-1191-2009-2-60-66.
7. *Soltys K, Lemoine C, Zhang X, Bhat R, Bucuvalas J, Rasmussen S et al*. Factors associated with thrombotic and hemorrhagic complications in pediatric liver transplant: A multi-center analysis from the Starzl Network for Excellence in Pediatric Transplantation. *Pediatr Transplant*. 2023 Jun; 27 (4): e14521. doi: 10.1111/PETR.14521.
8. *Medeubekov USh, Kuandikov TK, Mutagirov VV.* Otsenka gemostaza i yevo korrektsiya u patsientov posle transplantatsii pecheni. *Byulleten khirurgii Kazakhstana*. 2016; 3: 30–33.
9. *Lo CM.* Complications and long-term outcome of living liver donors: a survey of 1,508 cases in five Asian centers. *Transplantation*. 2003 Feb 15; 75 (3 Suppl): S12–S15. doi: 10.1097/01.TP.0000046534.45645.47. PMID: 12589131.
10. *Podoplelova NA, Nechipurenko DY, Ignatova AA, Sveshnikova AN, Panteleev MA.* Procoagulant platelets: Mechanisms of generation and action. *Hämostaseologie*. 2021 Apr; 41 (2): 146–153. doi: 10.1055/a-1401-2706.
11. *Lisman T, Hernandez-Gea V, Magnusson M, Roberts L, Stanworth S, Thachil J et al.* The concept of rebalanced hemostasis in patients with liver disease: Communication from the ISTH SSC working group on hemostatic management of patients with liver disease. *Journal of Thrombosis and Haemostasis.* 2021; 19 (4): 1116–1122. doi.org/10.1111/jth.15239.
12. *Grigoreva KN, Bitsadze VO, Khizroeva DKh, Tsibizova VI, Tretyakova MV, Blinov DV et al*. Prognostic value of von Willebrand factor in clinical practice. *Obstetrics, Gynecology and Reproduction*. 2022; 16 (5): 588–599. doi: 10.17749/2313-7347/ob.gyn.rep.2022.363.
13. *Hartmann M, Szalai C, Saner FH*. Hemostasis in liver transplantation: Pathophysiology, monitoring, and treatment. *World J Gastroenterol*. 2016 Jan 28; 22 (4): 1541–1550. doi: 10.3748/wjg.v22.i4.1541. PMID: 26819521; PMCID: PMC4721987.
14. *Borst AJ, Sudan DL, Wang LA, Neuss MJ, Rothman JA et al.* Bleeding and thrombotic complications of pediatric liver transplant. *Pediatr Blood Cancer*. 2018; 65 (5): e26955. doi: 10.1002/pbc.26955.

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THULIUM FIBER LASER USE IN INTERVENTIONAL BRONCHOSCOPY IN LUNG RECIPIENTS

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Bronchial stenosis is a major cause of severe postoperative period in lung recipients. One of the methods to restore airway patency is recanalization using laser. This technique is popular due to the combination of cutting and coagulation effects. In this article, we demonstrate the possibility of intraluminal use of a thulium fiber laser (TFL) to recanalize bronchial stenosis in lung recipients.

Keywords: lung transplantation, bronchial complications, bronchial stenosis, thulium laser.

Lung transplantation (LT) is a universally recognized, radical method of surgical treatment for chronic lung diseases in the stage of decompensated respiratory failure that is resistant to other existing methods of conservative treatment. It has been over 60 years since the first LT was performed by James Hardy. Since then, LT has become a routine and accessible type of medical care. The increased number of lung recipients, as well as their longer life expectancy, naturally leads to an increase in the number of bronchial complications detected at different periods after the operation.

One of the main types of bronchial complications – bronchial stenosis (BS) – is one of the reasons for severe long-term postoperative period in donor lung recipients. Airway patency is restored using interventional bronchoscopy methods, including the use of TFL.

Interventional bronchoscopy is an important component of a multidisciplinary approach to the postoperative management of lung recipients.

INTRODUCTION

Post-LT bronchial stenosis is a persistent, respiratory-independent narrowing of the lumen, mainly due to a scar or granulation tissue.

BS is one of the main causes of severe long-term postoperative period in donor lung recipients [1–3]. Despite the relatively low incidence of severe bronchial complications, stenoses significantly worsen graft function and quality of life of the recipient, progressing and leading to life-threatening conditions [4].

The main techniques of endoscopic treatment of bronchial stenoses in lung recipients include:

– Balloon dilatation [5];
– Argon plasma coagulation (APC) [6, 7];
– Laser use;
– Cryoablation [8];
– Application or injection of mitomycin into the scar area [9, 10];
– Glucocorticoid injection into the scar area [11];
– Brachytherapy [12];
– Stenting [13–17].

The use of laser in restoring airway patency has advantages due to the combination of cutting and coagulating effects on tissues [18, 19]. The penetrating ability of laser-induced coagulation varies within 1–2 mm, instead of uncontrolled deep tissue coagulation when using APC [20].

In order to ensure optimal exposure during manipulation, the laser is used under rigid bronchoscopy [21]. The main complications are bleeding, perforation, and bronchial fistula formation. In the work of Cavaliere et al., 119 out of 5049 patients developed serious complications after airway lumen was restored using laser (2.4%), and mortality was 0.3% [22].

Also, one of the complications characteristic of laser use is the risk of airway fire [23]. For the purpose of prevention, it is recommended to reduce FiO_2 below 40% or, if the clinical situation allows, to work under apnea [24].

MATERIALS AND METHODS

109 lung transplants were performed at Shumakov National Medical Research Center of Transplantology and Artificial Organs from 2014 to May 2023; these transplant surgeries included heart-lung transplantation.

Spirometry, multislice chest CT scan, and observational video-assisted bronchoscopy were used as diagnostic methods to detect and determine the degree of stenosis.

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Bronchial complications, in particular bronchial stenosis, were classified according to the International Society for Heart and Lung Transplantation (ISHLT) guidelines (Table) [14].

Therapeutic approaches to bronchial tree stenosis in lung transplants are characterized by the stage of application and increasing degree of invasiveness of recanalization methods in cases where this complication is reoccuring.

The criteria for selecting recipients for laser recanalization were grade 3–4 stenosis according to the classification given above (Fig. 1).

A TFL (FIBERLASE U3) was used in all cases. The fiber diameter was 365 μm and the wavelength was 1.94 μm. Pulse energy was set at minimum parameters 0.025 J, and the frequency 240 Hz. Where it was necessary to increase the intensity of exposure, first of all, the radiation frequency was increased rather than the energy.

Surgical interventions were performed in the operating room under general anesthesia, under rigid bronchoscopy and high-frequency artificial ventilation. It should be noted that, in order to prevent combustion, the stages of surgical intervention associated with laser exposure were performed under apnea conditions.

A catheter was used to perform continuous irrigation and aspiration of 0.9% sodium chloride solution (Fig. 2).

RESULTS

Bronchial stenosis requiring endoscopic interventions occurred in 21 lung recipients (19.3%) during this period. Nine patients had multifocal stenosis.

On average, each lung recipient with recurrent bronchial stenosis underwent from 1 to 6 endoscopic interventions of varying degrees of invasiveness to restore bronchial patency. Persistent remission lasting at least 6 months was achieved in 15 cases (71.4%). We used TFL for the first time in our practice in June 2021. In total, laser recanalization was applied in 14 cases in 11 recipients. It should be noted that in all cases of application, the laser was used in combination with balloon dilatation, cryotherapy and stenting.

Table

ISHLT classification of bronchial complications

Ischemia and Necrosis	Location	a. Perianastomotic – within 1 cm of anastomosis
		b. Extending >1 cm from anastomosis to major airways (bronchus intermedius and distal left main-stem)
		c. Extending >1 cm from anastomosis into lobar or segmental airways
	Extent	a. <50% circumferential ischemia
		b. >50% to 100% circumferential ischemia
		c. <50% circumferential necrosis
		d. >50% to 100% circumferential necrosis
Dehiscence	Location	a. Cartilaginous
		b. Membranous
		c. Both
	Extent	a. 0% to 25% of circumference
		b. >25% to 50% of circumference
		c. >50% to 75% of circumference
		d. >75% of circumference
Stenosis	Location	a. Anastomotic
		b. Anastomotic plus lobar/segmental
		c. Lobar/segmental only
	Extent	a. 0% to 25% reduction in cross-sectional area
		b. >25% to 50% reduction in cross-sectional area
		c. >50% but <100% reduction in cross-sectional area
		d. 100% obstruction
Malacia	Location	a. Perianastomotic – within 1 cm of anastomosis
		b. Diffuse – involving anastomosis and extending beyond 1 cm

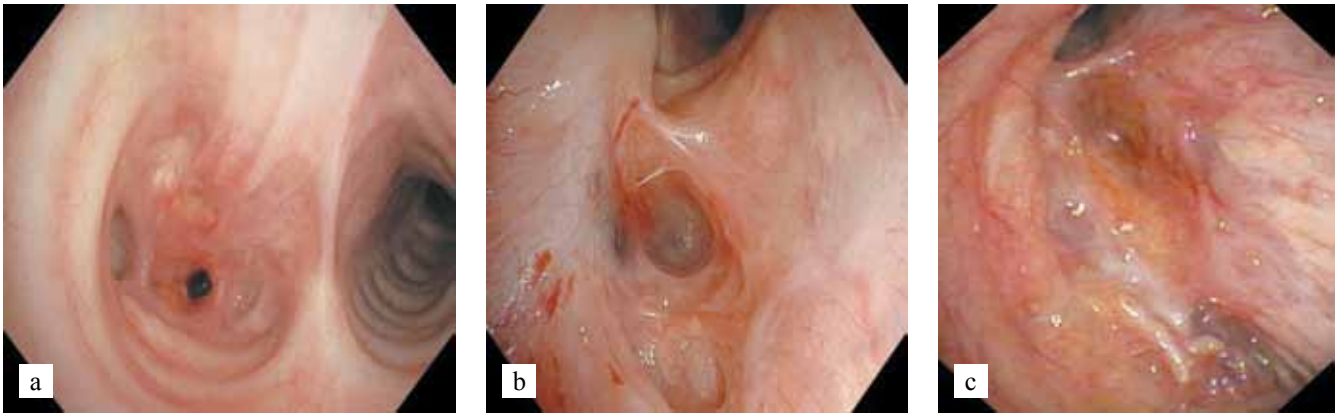

Fig. 1. Bronchial stenosis: a, b, grade III bronchial stenosis; c, grade IV bronchial stenosis

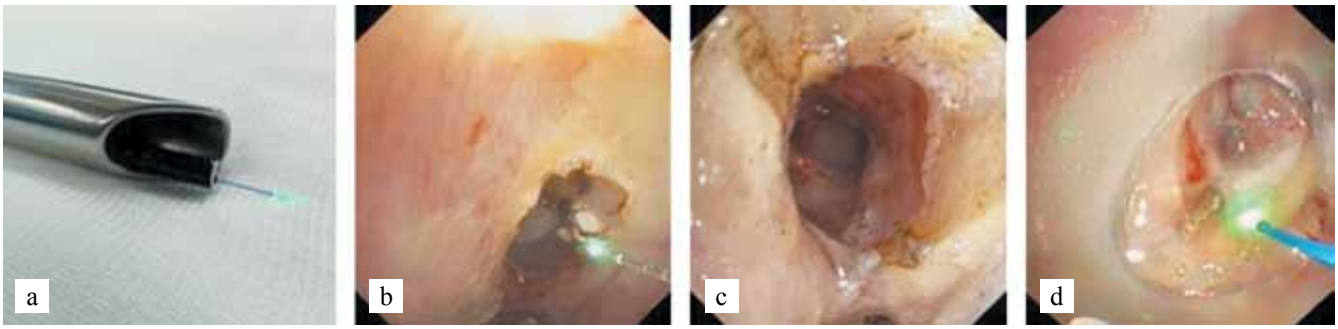

Fig. 2. Stages of bronchial stenosis recanalization: a, flexible bronchoscope is located in the lumen of a stiff bronchoscope with a thulium laser fiber; b, c, endoscopic picture at the moment of recanalization with thulium laser; d, laser application in aqueous medium using a distal bulb with irrigation with sodium chloride 0.9%

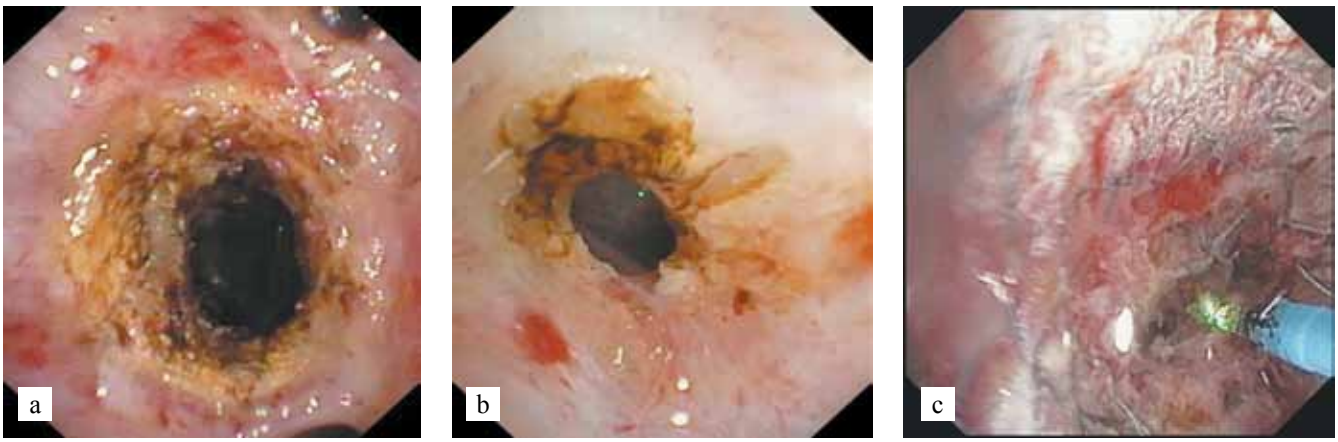

Fig. 3. Extent of carbonization during bronchial recanalization: a, exposure to argon plasma coagulation; b, exposure to thulium laser in air medium; c, exposure to thulium laser in aqueous medium, with an implanted stent in the bronchial lumen

Less scab formation (carbonization) was observed when using the laser in aqueous medium compared to the use of the laser in air or with argon plasma coagulation (Fig. 3). This pattern is associated with a greater vaporization effect in aqueous medium.

In our practice, no serious TFL-associated complications have been noted.

CONCLUSIONS

- The use of TFL in interventional bronchoscopy in lung recipients is an effective and safe method of bronchial recanalization;
- The use of laser in an aqueous environment significantly reduces the formation of rough scab in the coagulation area, and also prevents oxygen combustion in the airways. However, it should be noted that due to the peculiarities of the bronchial tree, it is not always possible to recreate an aqueous environment;
- The most effective use of TFL is its use in combination with other methods of bronchial stenosis recanalization, in combination with balloon dilatation, cryoablation and/or stenting.
- The existing methods of high-intensity intraluminal interventions within the framework of interventional bronchoscopy can solve a wide range of tasks aimed at improving the long-term outcomes of donor lung transplantation.

The authors declare no conflict of interest.

REFERENCES

1. *Mohanka M, Banga A*. Alterations in Pulmonary Physiology with Lung Transplantation. *Comprehensive Physiology*. 2023; 13 (1): 4269–4293. doi: 10.1002/cphy.c220008.
2. *Lequia L, Frisch A, Sinniah RS, Girgis RE, Egan J, Sathiyamoorthy G et al*. Post-transplant bronchial stenosis: A single center retrospective study. *Chest*. 2022; 162 (4): 2582–2583. doi: 10.1016/j.chest.2022.08.2111.
3. *Kim HH, Jo KW, Shim TS, Ji W, Ahn JH, Oh DK et al*. Incidence, risk factors, and clinical characteristics of airway complications after lung transplantation. *Scientific Reports*. 2023; 13 (1): 1–10. doi: 10.1038/s41598-023-27864-1.
4. *Delbove A, Senage T, Gazengel P, Tissot A, Lacoste P, Cellerin L et al*. Incidence and risk factors of anastomotic complications after lung transplantation. *Therapeutic Advances in Respiratory Disease*. 2022; 16: 1–10. doi: 10.1177/17534666221110354.

5. *De Gracia J, Culebras M, Alvarez A, Catalán E, De la Rosa D, Maestre J et al*. Bronchoscopic balloon dilatation in the management of bronchial stenosis following lung transplantation. *Respiratory medicine*. 2007; 101 (1): 27–33. doi: 10.1016/j.rmed.2006.04.019.
6. *Keller CA, Hinerman R, Singh A, Alvarez F*. The use of endoscopic argon plasma coagulation in airway complications after solid organ transplantation. *Chest*. 2001; 119 (6): 1968–1975. doi: 10.1378/chest.119.6.1968.
7. *Bergler W, Hönig M, Götte K, Petroianu G, Hörmann K*. Treatment of recurrent respiratory papillomatosis with argon plasma coagulation. *The Journal of Laryngology & Otology*. 1997; 111 (4): 381–384. doi: 10.1017/S0022215100137387.
8. *Maiwand MO, Zehr KJ, Dyke CM, Peralta M, Tadjkarim, S, Khagani A, Yacoub MH*. The role of cryotherapy for airway complications after lung and heart-lung transplantation. *European journal of cardio-thoracic surgery*. 1997; 12 (4): 549–554. doi: 10.1016/S1010-7940(97)00208-X.
9. *Cosano-Povedano J, Muñoz-Cabrera L, Jurado-Gámez B, del Carmen Fernández-Marín M, Cobos-Ceballos MJ, Cosano-Povedano A*. Topical mitomycin C for recurrent bronchial stenosis after lung transplantation: a report of 2 cases. *Journal of Bronchology & Interventional Pulmonology*. 2008; 15 (4): 281–283. doi: 10.1097/LBR.0b013e3181879e3a.
10. *Davidson KR, Elmasri M, Wahidi MM, Shofer SL, Cheng GZ, Mahmood K*. Management of lung transplant bronchial stenosis with mitomycin C. *Journal of Bronchology & Interventional Pulmonology*. 2019; 26 (2): 124–128. doi: 10.1097/LBR.0000000000000540.
11. *Tremblay A, Coulter TD, Mehta AC*. Modification of a mucosal-sparing technique using electrocautery and balloon dilatation in the endoscopic management of web-like benign airway stenosis. *Journal of Bronchology & Interventional Pulmonology*. 2003; 10 (4): 268–271.
12. *Warszawski-Baumann A, Baumann R, Karstens J, Christiansen H, Gottlieb J, Welte T*. HDR brachytherapy: an option for preventing nonmalignant obstruction in patients after lung transplantation. *Strahlentherapie und Onkologie*. 2012; 188 (12): 1085.
13. *Machuzak M, Santacruz JF, Gildea T, Murthy SC*. Airway complications after lung transplantation. *Thoracic surgery clinics*.2015; 25 (1): 55–75. doi: 10.1016/j.thorsurg.2014.09.008.
14. *Crespo MM, McCarthy DP, Hopkins PM, Clark SC, Budev M, Bermudez CA et al*. ISHLT Consensus Statement on adult and pediatric airway complications after lung transplantation: Definitions, grading system, and therapeutics. *The Journal of Heart and Lung Transplantation*. 2018; 37 (5): 548–563. doi: 10.1016/j.healun.2018.01.1309.
15. *Choong CK, Sweet SC, Zoole JB, Guthrie TJ, Mendeloff EN, Haddad FJ et al*. Bronchial airway anastomotic complications after pediatric lung transplantation: incidence, cause, management, and outcome. *The Journal of thoracic and cardiovascular surgery*. 2006; 131 (1): 198–203. doi: 10.1016/j.jtcvs.2005.06.053.
16. *Chhajed PN, Tamm M, Glanville AR*. Role of flexible bronchoscopy in lung transplantation. *Seminars in respiratory and critical care medicine*. 2004; 25 (04): 413–423. doi: 10.1055/s-2004-832714.
17. *Murthy SC, Blackstone EH, Gildea TR, Gonzalez-Stawinski GV, Feng J, Budev M et al*. Impact of anastomotic airway complications after lung transplantation. *The Annals of thoracic surgery*. 2007; 84 (2): 401–409. doi: 10.1016/j.athoracsur.2007.05.018.
18. *Colt HG*. Bronchoscopic laser in the management of airway disease in adults. MediLib c2020 [updated 202 august 11; cited 2023 March]. Available frome: https://www.medilib.ir/.
19. *Jeong BH, Um SW, Suh GY, Chung MP, Kwon OJ, Kim H, Kim J*. Results of interventional bronchoscopy in the management of postoperative tracheobronchial stenosis. *The Journal of Thoracic and Cardiovascular Surgery*. 2012; 144 (1): 217–222. doi: 10.1016/j.jtcvs.2012.03.077.
20. *Gesierich W, Reichenberger F, Fertl A, Haeussinger K, Sroka R*. Endobronchial therapy with a thulium fiber laser (1940 nm). The Journal of Thoracic and Cardiovascular Surgery. 2014; doi: 147 (6): 1827–1832.
21. *Perrin G, Colt HG, Martin C, Mak MA, Dumon JF, Gouin F*. Safety of interventional rigid bronchoscopy using intravenous anesthesia and spontaneous assisted ventilation: a prospective study. *Chest*. 1992; 102 (5): 1526–1530. doi: 10.1378/chest.102.5.1526.
22. *Cavaliere S, Dumon JF*. Laser bronchoscopy. In Interventional bronchoscopy. *Karger Publishers*. 2000; 41 (1): 108–119. doi: 10.1159/000162111.
23. *Mahmood K, Wahidi MM*. Ablative therapies for central airway obstruction. *Seminars in respiratory and critical care medicine*. 2014; 35 (06): 681–692. doi: 10.1055/s-0034-1395501.
24. *Caplan RA, Barker SJ, Connis RT, Cowles C, de Richemond AL, Nickinovich DG et al*. Practice advisory for the prevention and management of operating room fires: an updated report by the American Society of Anesthesiologists Task Force on Operating Room Fires. *Anesthesiology*. 2013; 118 (2): 271–290. doi: 10.1097/ALN.0b013e31827773d2.

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VASCULAR COMPLICATIONS AFTER LIVER TRANSPLANTATION: CONTEMPORARY APPROACHES TO DETECTION AND TREATMENT. A LITERATURE REVIEW

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Vascular complications (VCs) after liver transplantation (LT) are rare but are one of the most dreaded conditions that can potentially lead to graft loss and recipient death. This paper has analyzed the international experience in the early diagnosis of various VCs that can develop following LT, as well as the optimal timing and methods of treatment of these complications.

Keywords: liver transplant, vascular complications.

INTRODUCTION

LT is a very complex and comprehensive method of treating end-stage liver disease, and it has proven to be the only method that can significantly prolong the life of incurable patients [1]. However, this surgical procedure is associated with significant risks, such as VCs [2].

The overall incidence of VCs varies in different world centers, with a cumulative incidence of about 7% in orthotopic LT from deceased donors and about 13% in liver fragment transplantation from living donors in both adult and pediatric cohorts of recipients [3–7].

Since VCs carry the greatest threat of graft loss, their diagnosis and treatment are a serious aspect in terms of graft and recipient survival. This explains why many transplant teams nowadays closely monitor all vascular anastomoses using Doppler ultrasonography for early detection and treatment of these complications before the liver graft is irretrievably lost [8–10].

Indeed, VCs can suddenly interrupt blood supply to the liver with a high probability of graft loss.

Generally, regardless of the type of complication, treatment measures include:
– conservative therapy;
– endovascular plasty/stenting/thrombolysis;
– percutaneous transhepatic therapies;
– open surgical recanalization;
– retransplantation.

Although open surgery has been considered the primary choice for graft revascularization, advances in endovascular surgery have enabled less invasive and very effective revascularization. In recent decades, huge advances in interventional radiology have radically changed diagnostic and therapeutic approaches to the management of patients with post-LT vascular complications [1, 5, 11–18].

In fact, percutaneous endovascular interventions (i.e. thrombolytic procedures, balloon angioplasty and stenting) performed by experienced endovascular surgeons have become increasingly used and are gradually replacing open surgery, becoming the surgical method of choice in the treatment of VCs following liver transplantation [18–20].

Further, during the narrative, we will dwell on each VC in detail and consider optimal methods of detecting the complication and its treatment based on data from world literature sources.

1. HYPERCOAGULABLE STATES IN PATIENTS WITH CIRRHOSIS

Recent studies have shown that bleeding is not the only risk in LT for cirrhosis of various etiologies. Several risk factors must be considered in the setting of liver surgery, such as vascular constriction, veno-venous bypass, presence of central venous catheters, use of antifibrinolytic drugs, tissue ischemic injury, venous stasis, etiology of liver disease, endothelial damage, and ischemia time. All these factors may increase the likelihood of thrombotic complications [21–23].

The end stage of liver disease is itself a risk factor for thrombosis. In liver disease and liver surgery, conventional coagulation tests often fail or provide incorrect information about the status of hemostasis. For example, prothrombin time (PT), activated partial thromboplastin time (aPTT), and international normalized ratio (INR) are only indicative of procoagulant factors and are insensitive to plasma levels of anticoagulant factors. So, these tests are not always reliable for describing the hemostatic status of patients with terminal liver disease.

A marked decrease in both procoagulant factors (factors II, V, VII, IX, X, XI, XII) and anticoagulant factors

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(antithrombin III, protein C, and protein S), an increase in von Willebrand factor (vWF), and ADAMTS13, a protease that cleaves on vWF, are characteristic features of the course of cirrhosis and lead the patient to a new hemostatic balance [11]. vWF fulfills its hemostatic functions by binding to factor VIII and connective tissue components, and also promotes platelet adhesion to endothelial surfaces and platelet aggregation [24].

Thrombocytopenia resulting from hypersplenism in patients with portal hypertension, abnormal thrombopoietin metabolism, increased platelet destruction mediated by antiaggregant antibodies, and alcohol-induced bone marrow suppression, antiviral and immunosuppressive therapy, is another condition that develops in cirrhosis [25]. Unless the platelet count is very low ($<50 \times 10^9$/L), thrombocytopenia does not pose an increased risk of intraoperative bleeding. Such a platelet count is usually sufficient to guarantee normal thrombin formation, and the low platelet count is compensated for by a higher level of vWF, which is responsible for platelet adhesion [26, 27]. Hyperfibrinolysis is another described sign of end-stage liver disease, but its role in the coagulopathy of cirrhosis is still debated [28]. Elevated levels of tissue plasminogen activator and deficiency of thrombin-activated fibrinolysis inhibitor have been associated with laboratory changes that are typical of hyperfibrinolysis and increased risk of bleeding [29]. However, liver cirrhosis is also associated with decreased fibrinolysis, as evidenced by decreased plasminogen levels and increased plasminogen activator inhibitor levels. These contrasting results explain the ongoing debate regarding the absence or presence of a hyperfibrinolytic state in patients with liver disease, even though the balance of fibrinolysis is probably restored by parallel changes in profibrinolytic and antifibrinolytic factors (see Table 1) [30].

The above factors affecting the imbalance of the coagulation and anticoagulation systems may have a direct impact on the risks of post-liver transplant VCs. 95% CI:

2. ARTERIAL COMPLICATIONS

Arterial complications remain the most formidable and leading causes of morbidity and mortality after orthotopic LT [20, 34, 173]. Generally, the liver graft is supplied by the portal vein, hepatic artery (or several hepatic arteries). The hepatic artery (HA) plays an extremely important role as it supplies blood to both the liver parenchyma and the biliary tree. Absence of or reduction in arterial blood flow often leads to biliary complications due to ischemic processes, with the formation of biliary necrosis, liver abscesses, which leads to graft dysfunction, septic complications and graft loss, leading recipient death [20, 34, 174]. That is why extremely rapid detection of this problem and early treatment are very important.

The main arterial complications following liver transplantation are:

- HA thrombosis (1.9%–16.6% incidence rate) [10];
- stricture of the arterial anastomosis (0.8–9.3%) [175];
- splenic artery steal syndrome (≤10.1%) [26–31];
- HA pseudoaneurysm (0.1%–3%) [18];
- HA rupture (arterial bleeding, incidence <1%) [176].

Based on timing, these complications are divided into early (complications occurring in the first month after LT), and late complications (complications developing after one month following LT).

Special attention should be paid to early complications because they are associated with graft loss and high mortality. The timing of early and late complications continues to be debated in different studies. Most authors have defined late complications as those that occurred within the first four weeks of transplantation and others within the first six months [13, 32–34]. However, according to the most recent international consensus, early complications are defined as those that occur within the first month after LT [8, 13, 18, 33–34].

2.1. Hepatic artery thrombosis

Transplant hepatic artery thrombosis (HAT) occurs when a blood clot forms in the HA that provides blood flow to the liver. According to the classification of VCs described above, there are early and late HAT [8, 13, 18, 32–34].

HAT is the most common (about 50% of all VCs) and the most severe arterial complication that can develop

Table 1

Balance of antihemostatic and prohemostatic factors in cirrhosis

	Antihemostatic factors	Prohemostatic factors
Primary hemostasis	Platelet dysfunction	Increased von Willebrand factor
	Thrombocytopenia	Decreased ADAMTS 13
	Reduced thrombopoietin synthesis	Platelet reactivation
Coagulation	Reduced synthesis of factors II, V, VII, IX, X, XI	Increased factor VIII
	Vitamin K deficiency	Decreased anticoagulant protein C, protein S, and antithrombin III
	Hypodysfibrinogenemia	Procoagulant changes in fibrin structure
Fibrinolysis	Low levels of alpha 2-antiplasmin and factor XIII, decreased thrombin activatable fibrinolysis inhibitor	Low plasminogen
		Increased plasminogen activator inhibitor 1

after LT. This complication is one of the main causes of primary graft dysfunction, which can lead to graft loss and patient death in the early postoperative period [37]. Arterial thrombosis is more common in younger recipients [7, 8, 16, 17, 34–37].

If not diagnosed in time, the chances of graft loss are extremely high. The only method of treatment in this situation is liver re-transplantation. Indeed, the frequency of re-transplantation is very high in case of late graft revascularization and, according to reports, it is 25%–83%. Methods of early revascularization with the help of endovascular intervention have been actively introduced in recent years [5, 8, 13, 16, 17, 36, 38–45].

The true incidence of early HAT is unknown, but it varies widely (0% to 12%) [7, 32, 36, 43, 46, 47, 175]. Becker et al. in 2009 reported that based on an analysis of 21,822 patients who underwent orthotopic LT, there were 843 cases (3.9%, adults and children) of early HAT [34]. Also, this analysis showed that the number of VCs decreased slightly as new surgical techniques emerged year after year. Among other things, this report shows that the number of HAT cases remains approximately the same worldwide, regardless of the clinics. The mean time to detection (development) of HAT ranged from 1 to 18 days from the time of transplantation. Late complications occurred on average six months after LT [34].

There is no clear evidence in the literature on whether the incidence of HAT depends on whether the transplantation was performed from a deceased donor or from a living donor. Many studies show conflicting results. A meta-analysis of data from several major transplant centers found no significant difference in the development of thrombosis (3.1% in related transplants, 4.6% in liver transplants from a postmortem donor) [3, 17, 34, 47, 60, 175]. In addition, it has been reported that when surgical microscopes were used during arterial anastomosis, the incidence of HAT remained the same [17, 34, 47, 102, 175].

Risk factors

There are several factors that increase the risk of developing HAT.

The risk factors for early HAT include surgical problems, namely [7, 8, 17, 19, 32–34, 40, 48, 49]:
- difficulties with arterial reconstruction;
- small HA diameter;
- large HA tortuosity;
- arterial dissection to create a site for arterial anastomosis;
- multiple arteries feeding the graft;
- arterial anomalies requiring complex arterial reconstructions, including the use of vascular grafts;
- poor quality of donor/recipient vessels.

It has been shown that the more experienced the operating team is, the lower the risk of early HAT. So, surgical causes are probably not a major risk factor for early HAT [17, 34, 35, 37, 43]. Also, minimally invasive techniques in related liver donation have been developed recently. For example, laparoscopy-assisted liver graft harvesting is widely used in related liver transplantation. Evidence has been analyzed, showing that the graft harvesting technique does not affect the incidence of early arterial complications [50–53].

Also, the transarterial chemoembolization procedure in hepatocellular liver cancer can be attributed to risk factors. For instance, Panaro in 2014 showed that patients with a history of selective transarterial HA chemoembolization are prone to HA intima damage, which, in turn, may lead to HAT in the early postoperative period [54].

Factors influencing the development of late HAT include [33, 40, 49, 55]:
- cytomegalovirus infection;
- hepatitis C;
- female donors;
- male recipients;
- tobacco smoking;
- retransplantation.

Also, many authors believe that a hypercoagulable state may be the main cause of HAT [7, 9, 17, 35, 49, 55].

Clinical presentation

The clinical presentation of HAT ranges from mild elevation of cytolytic enzymes and bilirubin in peripheral blood serum to acute liver failure. Elevation of cytolytic enzymes (AST and ALT) occurs in 75% of patients with HAT; development of biliary complications on the background of HAT occurs in about 15% of cases. Fever and sepsis develop in 6% of HAT cases. Acute graft dysfunction or liver failure occurs in 4% of cases [7].

The severity of clinical manifestations depends on the time HAT develops, as well as on how developed the hepatic arterial collaterals are [7, 32, 33].

Biliary complications, such as bile duct strictures or bile leakage, sometimes leading to liver abscesses, are more often, but not exclusively, associated with late HAT, while early graft dysfunction (liver failure) is most often associated with early HAT [17, 33, 46].

Also, the severity of clinical manifestations depends on the presence of arterial collaterals, which can develop already within two weeks after LT. Therefore, the following are the two main forms of HAT [32]:
1. Early HAT characterized by a severe clinical course;
2. Late HAT, characterized by a milder clinical course.

In almost every case, early HAT is clinically manifested by fever, leukocytosis, and elevated liver enzymes [20, 34, 173]. Ischemia of bile ducts and hepatocytes with subsequent necrotization often develops, which leads to multiple liver abscesses, followed by uncontrolled septic shock (against the background of immunosuppressive therapy) and patient death [32–34, 37, 41, 43].

If HAT develops late after LT, clinical manifestations are usually associated with biliary complications [8, 19,

33, 41]. In 50% of cases, late HAT manifests asymptomatically, only a biochemical test can detect a slight increase in cytolysis markers. Subsequently, patients suffer from recurrent cholangitis, some of them develop bile duct strictures or bile leakage. Also, intrahepatic bile duct necrosis with formation of liver abscesses develops. As a rule, late HAT symptoms and signs are insidious and require special attention by physicians [17, 33, 34, 41, 43, 56].

Diagnosis of hepatic artery thrombosis

Early diagnosis of HAT is critical because of the high risk of graft loss. Diagnostic procedures include biochemical tests (increase in cytolysis enzymes) and Doppler flowmetry. If necessary, bolus contrast-enhanced multislice computed tomography (MSCT) is performed to assess blood flow through the arterial bed of the graft, or angiography is carried out [17, 173].

Ultrasound Doppler flowmetry is a non-invasive method and is the gold standard for diagnosis. Doppler flowmetry detects decreased arterial blood flow and increased resistive index (Ri). Ultrasound should be used as a screening method for early diagnosis of HAT and it should be performed at least once a day in all liver transplant patients [17, 32, 43]. That said, in some transplant centers, routine ultrasound examination is performed every 6 hours after transplantation for 7 days (acute period) for early detection of occlusion and initiation of immediate treatment [57, 173]. The protocol used in the Russian Federation and Central Asia is presented in Fig. 1. This protocol is also used in the pediatric liver transplantation program [173].

If the patient has elevated hepatic enzymes (ALT and AST) and changes in HA indices, it makes sense to perform bolus contrast-enhanced MSCT and/or selective angiography of the celiac trunk (celiacography) [43, 173].

Treatment of hepatic artery thrombosis

There are several classic treatments for HAT:

– administration of anticoagulants/antiplatelet agents and dynamic monitoring;
– revascularization (surgical or endovascular);
– retransplantation;

At present, the most effective treatment approach remains contentious, and the choice of any of these treatments depends on the timing of diagnosis. Early diag-

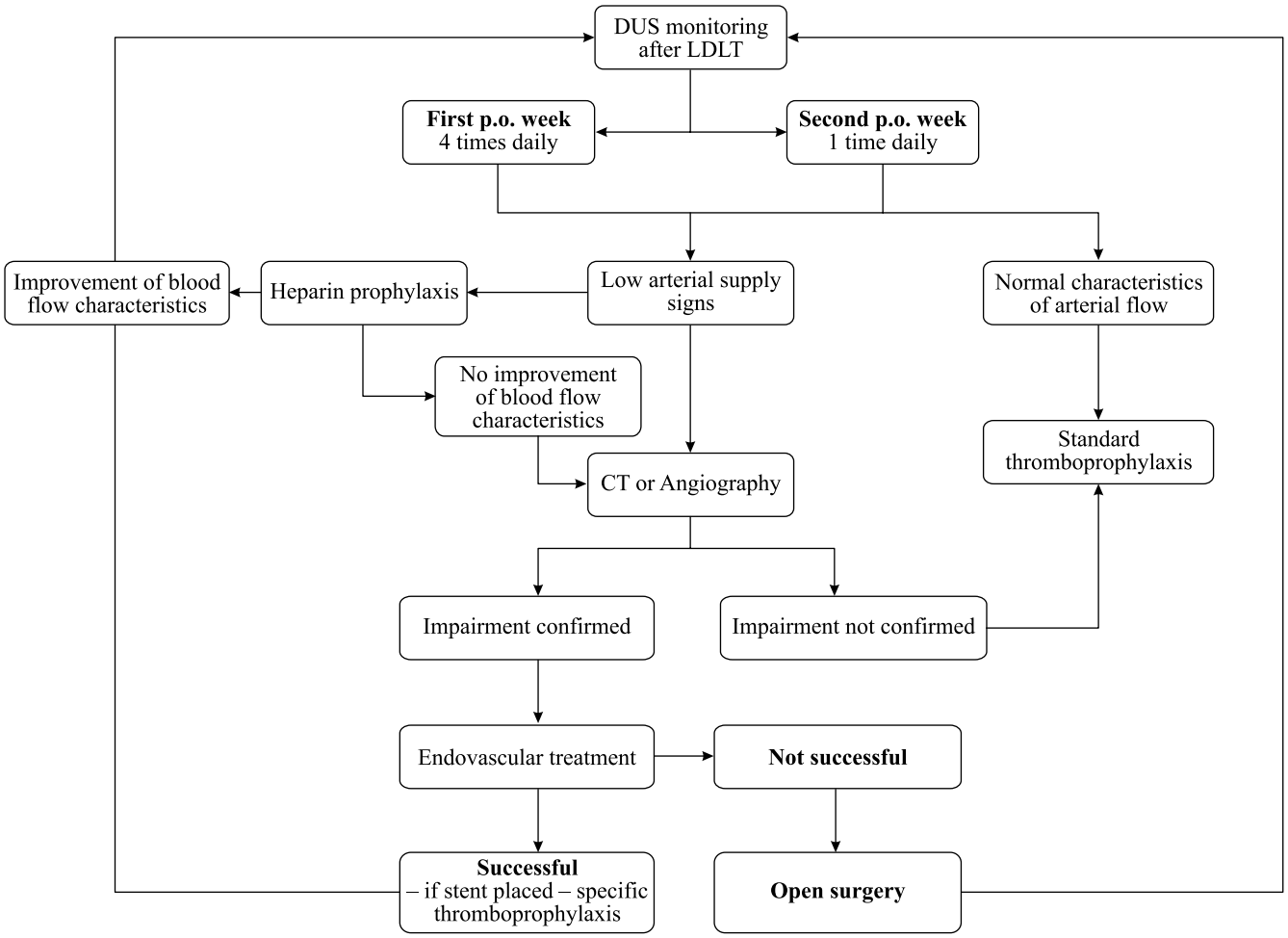

Fig. 1. Ultrasound monitoring protocol for screening of vascular complications after liver transplantation and plan of action in case of complications detection [57]

nosis, conservative therapy, surgical revascularization or retransplantation are considered the only solution to save patients with HAT [2, 8, 10, 17, 20, 173].

Some patients receive anticoagulant/antiplatelet, thrombolytic therapy for the treatment/prevention of thrombosis. Acetylsalicylic acid, clopidogrel, rivaroxaban, apixaban, urokinase, streptokinase, alteplase, calcium nadroparin, and heparin are used [17, 20, 58]. A reliably better protocol is not yet known, and there are currently no specific guidelines for the use of thrombolytic therapy in these patients. Nevertheless, when thrombosis is reliably detected, thrombolytic therapy is actively used in many surgical centers despite the high risk of postoperative bleeding [2, 17, 20, 57–59]. Indeed, bleeding is the most frequent side effect of thrombolytic therapy and occurs in approximately 20% of patients. Bleeding manifests itself in a variety of ways – from a mild hemorrhagic discharge through safety drains to intra-abdominal hemorrhage, which can be fatal in some cases. Bleeding on the background of thrombolytic therapy typically occurs in the early postoperative period [16, 20].

Thrombosis prevention protocols at different transplant centers are presented in Table 2.

There is selective endovascular thrombolysis (drugs are injected directly into the HA). This method offers several advantages, such as lower thrombolytic dose, high local concentration of drugs and relatively small effect on systemic coagulation (Fig. 2) [11, 20, 162].

As reported from various sources, combined therapy has a good effect: endovascular balloon correction, blood flow correction (with or without artery stenting), together with the administration of anticoagulants/antiplatelet agents.

In some centers, early after transplantation, permanent heparinization under aPTT control is used if arterial thrombosis is suspected based on ultrasound diagnostics. The authors note that if this method is ineffective, it

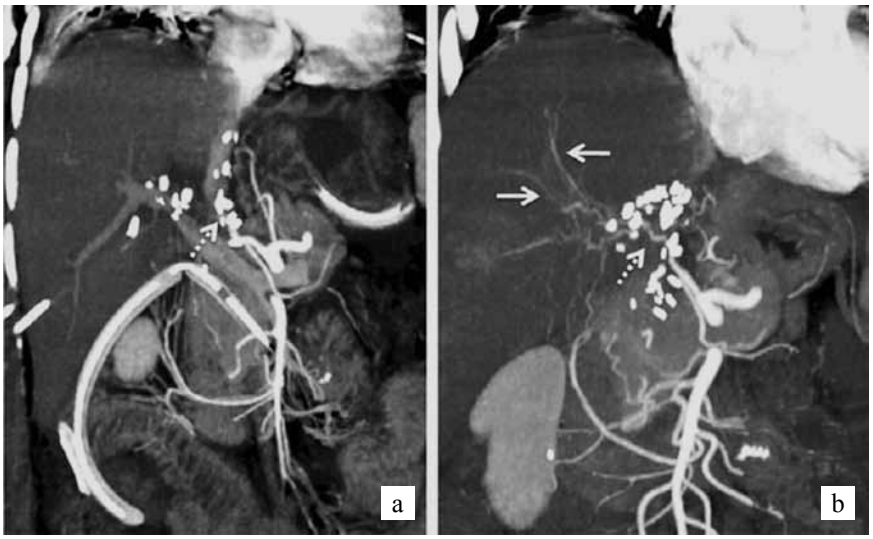

Fig. 2. Frontal projection when performing celiacography. A, The dotted arrow indicates the site of hepatic artery thrombosis after liver transplantation. B, The white arrows indicate the presence of arterial blood supply to the graft after thrombolytic therapy [162]

Table 2

Comparison of thromboprophylaxis protocols among transplant centers

Study	Thromboprophylaxis protocol	Number of cases	Vascular complications	Bleeding
Gautier, Monakhov et al. 2021 [173]	Prostaglandin E1, intraoperatively for 7 days; Enoxaparin, on day 1 after surgery in the absence of thrombocytopenia $<70 \times 10^9$/L within 14 days; Acetylsalicylic acid, with the start of oral nutrition or on postoperative day 4 for 3 months. If thrombosis/stenosis of afferent vessels is suspected, heparin is administered, targeting an aPTT of 60–80 seconds	416 patients, children, transplantation of various liver fragments from a living related donor and split liver transplantation	Arterial thrombosis (17; 4%) Portal vein thrombosis (no information)	No information

End of table 2

Study	Thromboprophylaxis protocol	Number of cases	Vascular complications	Bleeding
Blasi et al. 2016 [168]	Enoxaparin or not routinely administered unless the patient has had an intraoperative thrombectomy or the patient was on anticoagulant treatment prior to liver transplantation. No thromboprophylaxis if platelet count is below 30×10^9/L	328 patients, adults, cadaveric liver transplantation	Portal vein thrombosis (8; 2.4%) Arterial thrombosis/ stenosis (no information)	No information
Kaneko et al. 2005 [169]	Administration of dalteparin, target activated coagulation time (ACT) is 130–160 seconds	128 patients, adults, right lobe liver transplantation from a living donor	Arterial thrombosis (2; 1.5%) Portal vein thrombosis (1; 0.78%) Arterial thrombosis + portal vein thrombosis (1; 0.78%)	11 (8.5%) surgical revisions and 8 (6.25%) patients with hemorrhagic complications were treated conservatively
Gad et al. 2016 [170]	Heparin infusion up to 180–200 U/kg/day, adjusted depending on ACT (target levels, 180–200 seconds) and/or APTT (target levels, 50–70 seconds)	186 patients, transplantation of various liver fragments from a living donor	Arterial thrombosis (4; 1.8%) Portal vein thrombosis (5; 2.3%) Arterial thrombosis + portal vein thrombosis (4; 1.8%)	4 (1.8%)
Semash et al. 2023 [57]	Prostaglandin E1, intraoperatively for 5 days; Enoxaparin, on postoperative day 1 in the absence of thrombocytopenia $<50 \times 10^9$/L for 14 days; Acetylsalicylic acid, with the start of oral nutrition or on postoperative day 4 for 3 months. If thrombosis/stenosis of afferent vessels is suspected, heparin is administered, targeting an aPTT of 60–80 seconds	30 patients, adults, right lobe liver transplantation from a living donor	Arterial thrombosis (0) Portal vein thrombosis (no information	No information
Sugawara et al. 2002 [171]	Enoxaparin, Prostaglandin E1 (0.01 g/kg/hour) immediately after transplantation, administration of protease inhibitors	172 patients, adults, right lobe liver transplantation from a living donor	Arterial thrombosis (7; 4%) Portal vein thrombosis (4; 2.3%)	No information
Mori et al. 2017 [172]	Heparin infusion at a dose of 5 U/kg/h during the first week after liver transplantation	282 patients, adults, right lobe liver transplantation from living donor, 48 patients with portal vein thrombosis	Arterial thrombosis/ stenosis (no information) Portal vein thrombosis (8; 17%)	No information
Yip et al. 2016 [183]	Heparin injection, 5000 units subcutaneously every 8 hours	999 patients, adults, cadaveric liver transplantation	No information	No information
Vivarelli et al. 2007 [184]	100 mg aspirin orally	838 patients, adults, cadaveric liver transplantation (236 received thromboprophylaxis and 592 did not receive thromboprophylaxis)	Arterial thrombosis (1; 0.4%) in the thromboprophylaxis group and 13 (2.2%) in the comparison group. Portal vein thrombosis (no information	0%
Uchikawa et al. 2009[185]	Continuous infusion of dalteparin administered in the non-hepatic phase to maintain ACT at 140 to 150 seconds (Group A) versus continuous intravenous infusion of dalteparin administered immediately after surgery and adjusted according to clinical data (Group B)	42 patients, adults, cadaveric liver transplantation. Group A, 10 patients; Group B, 32 patients	Arterial thrombosis: 5 (15.6%) in group A, 0 in group B. Portal vein thrombosis: 5 (15.6%) in group A, 0 in group B	1 (3.1%) in group A and 0% in group B

is necessary to perform emergency revascularization. Moreover, if the thrombosis was successfully resolved against the background of permanent heparinization, which was confirmed by ultrasound and/or contrast-enhanced MSCT, revascularization was not performed, these patients were subsequently prescribed a prophylactic course of antiplatelet drugs [57, 173].

Historically, retransplantation in patients with post-LT occlusive HAT has been shown to have the best patient survival outcomes [7, 16]. On the other hand, percutaneous liver biopsy techniques for blood flow correction have been strongly developed recently and show decent outcomes. Currently, balloon angioplasty and/or stenting of graft artery, with subsequent administration of antiplatelet and anticoagulant therapy, are being actively performed. According to evidence from recent studies, good graft survival rate was achieved using the above-described method [2, 59, 173].

There are also cases when patients do not undergo any intervention, and the graft survives due to the arterial collaterals developing in it. The percentage of such cases is extremely small [17, 20, 43, 44, 46]. However, open surgical revascularization or retransplantation may also be ineffective. Despite the encouraging outcomes of endovascular interventions, these treatments also have a downside, as complications may occur during endovascular procedures. Moreover, after failed attempts at endovascular revascularization, either open interventions or retransplantation are required. Thus, urgent revascularization with endovascular interventions as a primary option may offer a chance to avoid retransplantation, but it is not successful in all cases [8, 10, 17, 20].

Below are the complications of endovascular correction of HA thrombosis:
– recurrent thrombosis;
– extravasation (minor damage to the artery);
– HA rupture with subsequent bleeding.

Any of these may require open revascularization or liver retransplantation [17, 20, 59, 173].

Open surgical revascularization for HAT is another type of treatment for graft salvage. Open surgical revascularization can be performed in different ways depending on the length and integrity of the artery. Fogarty catheter thrombectomy is used, and hepatic arterial anastomosis is transposed [16].

Meta-analysis of treatment methods has shown that liver retransplantation for early HAT shows the best patient survival compared to conservative therapy and revascularization in different variants. At the same time, some patients with late HAT survive without revascularization or retransplantation due to collateral circulation in the graft [7, 8].

Prognosis

With revascularization, patient survival in HAT is 40%. Survival reaches 85% when revascularization is performed with combined use of antiplatelet/anticoagulant/thrombolytic therapy. There have been different reports on an overall mortality reaching 23%–33% in patients with early HAT. The risk of graft loss in HAT according to some studies may be as high as 53.1%. The most effective prognosis of graft survival depends on the time of HAT detection and the speed of revascularization [17, 33, 34].

HAT develops quite rarely but represents the most common vascular complication after liver transplantation. A definitive diagnosis is established by angiography, during which therapeutic manipulations can also be performed using endovascular procedures, such as balloon angioplasty and/or arterial stenting.

Currently, it seems advisable to perform endovascular treatment first, mainly because of organ shortage and the high mortality rate associated with retransplantation. However, it has been proven that patients with early HAT with severe graft dysfunction require liver retransplantation.

2.2. Transplant hepatic artery stenosis

Transplant hepatic artery stenosis (HAS) is a narrowing of the lumen of a liver transplant artery leading to reduced arterial blood flow and partial ischemization of the graft. Significant HAS is a narrowing of the lumen of the graft artery by more than 50%. HAS along with HAT, are the most common arterial complications with high morbidity and mortality [4, 16, 36, 61, 63–66].

According to various reports, HAS develops in 2% to 13% after LT [4, 16, 36, 61–67]. There are cases where HAS is in turn complicated by thrombosis [4].

Similar to HAT, HAS is divided into early (developing within the first 30 days of LT) and late (developing after 30 days of LT).

Based on a meta-analysis, early HAS is statistically less common than late HAS (40% *vs.* 60%, respectively). The mean time to diagnosis of HAS is 94–160 days after LT (1–1220 days) [68].

The anastomotic portion of the liver graft artery has been shown to be the most common site for HAS within three months after LT [69].

Also, transplant HA kinking is considered to be a narrowing of the liver transplant artery [17]. In turn, arterial kinking can lead to HAT [7, 17].

Risk factors

The risk factors associated with HAS are not really known and seem to have a multifactorial origin [68]. The authors believe that technical factors such as arterial injury (during clamping, intima separation, improper anastomotic sutures), anatomical features of the donor and recipient arteries (excessive length, arterial kinking, diameter difference between donor and recipient arteries), impaired vascular blood supply to the artery, coagulation injury to the vessels, etc. may increase the risks

of arterial occlusive complications, including transplant hepatic artery stenosis, etc [66].

Clinical presentation

The clinical presentation of HAS varies from asymptomatic disease to graft dysfunction associated with ischemia and necrosis. Moreover, HAS can lead to graft dysfunction both early and late postoperatively. Many patients with asymptomatic course may demonstrate minor deviations from normal blood biochemistry (cytolysis, cholestasis) [16, 61, 66–68, 70, 71]. Mostly, in patients with asymptomatic HAS, the diagnosis is established incidentally, during Doppler ultrasound (DU) screening. That is why regular DU screening at early and late periods after LT is so important [57, 173].

The risk of biliary complications is less common in HAS than in HAT. Ideally, HAS should be diagnosed before biliary complications occur because, according to reports, they develop in 67% of HAS cases [70, 71].

Diagnosis of hepatic artery stenosis

Ultrasound is a well-established noninvasive and inexpensive method of assessing liver graft arterial patency; it has been reported in many studies to be effective in the early diagnosis of HAS [57, 61, 66, 173]. Velocity of blood flow through the artery is assessed in combination with resistive index (Ri) – in arterial stenosis, it decreases <0.5, delayed systole and "rounding" of the systolic peak occur. Sometimes, on the contrary, when peripheral resistance increases, Ri increases, the diagnostic criterion is Ri >0.85 [173]. Some authors describe turbulent blood flow through the HA – an increase in velocity >100 cm/sec (Fig. 3) [163]. Many transplant teams also use contrast-enhanced MSCT and direct angiography to confirm the diagnosis, which is the gold standard for diagnosing HAS [8, 72, 73].

Treatment of hepatic artery stenosis

As with HAT, treatment of HAS includes:

– revascularization (surgical or endovascular);
– retransplantation.

In patients with asymptomatic HAS, endovascular angioplasty (balloon vasodilatation and/or stenting of the graft artery) is performed [59, 62, 67, 71, 74, 173, 177]. A positive effect was achieved in 87% of patients [61]. In 7% of patients who underwent endovascular angioplasty, complications developed, including arterial rupture, requiring open revision. Some authors have noted the development of HAT after endovascular correction of arterial stenoses. This is typically associated with inadequate postoperative management of patients (inappropriately selected antiplatelet/anticoagulant therapy after arterial stenting) [61, 66]. According to a meta-analysis report, open revascularization with excision of the anastomotic narrowing, with the use of vascular grafts in some cases, showed a 100% restoration of blood flow through the HA [61].

Despite this, a case series meta-analysis published in 2015 showed that interventional techniques for correcting arterial blood flow are highly effective for early HAS, they do not differ in complication rates compared to open arterial reconstructions, and they help to reduce the number of liver retransplantations [75].

With timely early diagnosis of HAS by DU screening and regular laboratory monitoring of blood biochemical parameters (liver panel) and early revascularization by either method, the risk of graft loss and retransplantation is reduced significantly.

When endovascular intervention fails to restore blood flow through the HA, surgical revascularization should be undertaken before considering retransplantation, which has a lower postoperative survival rate, given that HAS is associated with subsequent biliary complications.

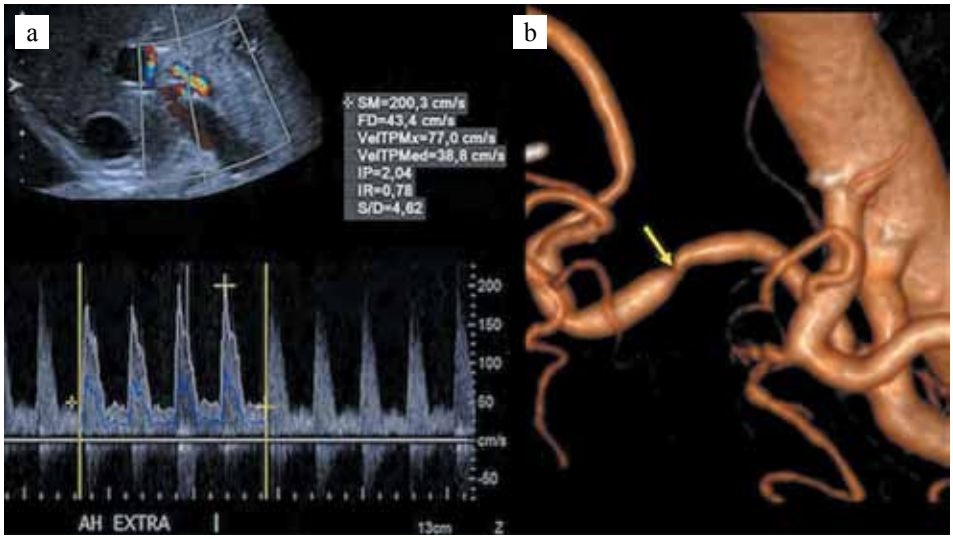

Fig. 3. Visualization of liver graft artery stenosis: a, triplex ultrasound of the liver graft, turbulent arterial blood flow in flowmetry; b, volume rendered image from a CT angiography. The arrow indicates the site of hepatic artery stenosis [163]

Carefully performed arterial anastomosis during transplantation appears to prevent arterial stenosis.

2.3. Splenic artery steal syndrome

Splenic artery steal syndrome (SASS) is another cause of graft hypoxia or ischemia. SASS can be described as a decrease in blood flow into the HA in the absence of HAT or HAS. This condition is associated with increased arterial inflow through the enlarged splenic artery, since the liver and spleen in most cases are supplied from the same basin. According to world reports, SASS remained without attention from surgeons for a long time because the actual surgical problem in the arterial anastomosis was not revealed. But it was shown that SASS can reliably lead to graft hypoxia/ischemia against the background of hepatic hypoperfusion and is a threatening complication, which, in turn, can lead to irreversible consequences, up to graft loss and patient death [57, 76–80].

Risk factors

A complex combination of factors, including HA hypoperfusion and portal hyperperfusion, can lead to SASS. The first and the main risk factor is portal hypertension, against which the volume of the spleen and vessels feeding it increases. Some authors cite the following pattern: a difference between splenic and HA diameters of 1.5 times or more in favor of the splenic artery is a risk factor for SASS. Some authors consider the splenic artery diameter of more than 5 mm as a risk factor regardless of the difference in the diameter of the liver and spleen vessels [57, 81, 177]. There are also works that determine an increased risk of SASS when the graft-to-recipient weight ratio (GRWR) is less than 0.9% [57, 82, 83].

Clinical presentation

Similar to HAS, the clinical presentation of SASS can be diverse. This condition is often asymptomatic, but hepatic failure, graft ischemia and necrosis may develop if diagnosis is delayed. Patients with asymptomatic SASS may show minor deviations from normal blood biochemistry parameters (cytolysis, increased bilirubin, alkaline phosphatase, and gamma-glutamyl transferase) [76–80, 84].

Diagnosis of splenic artery steal syndrome

Most often, diagnosis is made early after transplantation and is detected through routine use of ultrasonography. Ultrasound signs of SASS include difficulties in visualizing the HA at the anastomosis level and in the graft parenchyma, and decreased total blood flow velocity of less than 15 cm/sec, while blood flow indices may be normal. If SASS is suspected, contrast-enhanced MSCT or selective angiography of the celiac trunk (celiacography) should be performed [25]. During angiography, there will be increased discharge of contrast agent into the enlarged splenic artery, while the inflow into the liver graft artery will be reduced [57, 84, 173, 181].

Prevention of splenic artery steal syndrome

Currently, there are several SASS prevention techniques. According to numerous reports, endovascular methods of prophylaxis are used, for example, selective splenic artery embolization before LT [182]. At the stage of examining the recipient in preparation for transplantation, if signs of hypersplenism and enlarged splenic artery are detected, the above procedure is performed. According to world reports, this method reduces the risk of SASS [79]. However, the procedure is not always effective. Cases have been described where after splenic artery embolization, a powerful collateral blood flow developed in the spleen, and patients experienced SASS after transplantation [57].

Intraoperative splenic artery ligation is another described method for preventing SASS. During transplantation, the celiac trunk and its branches are skeletonized and the splenic artery is ligated. According to reports from studies describing this technique, there was not a single case of SASS developing after splenic artery ligation. At the same time, ischemic disorders in the spleen were practically not described and they were asymptomatic [57, 81, 178–180].

Treatment of splenic artery steal syndrome

The mainstay of treatment for SASS is currently endovascular selective embolization of the splenic artery (Fig. 4) [25, 57, 76–80, 83–85, 173, 181]. Embolization is performed either with emboli or coils. At the same time, cases have been described where, in the early stages after LT during angiography, it was technically impossible to perform cannulation of the splenic artery for embolization; in such cases, relaparotomy and open ligation of the splenic artery were performed to restore adequate arterial perfusion of the liver graft [57]. Also, there were cases where during embolization, the guidewire migrated into the HA and caused dissection of the arterial anastomosis, which made it necessary to perform open surgery to stop bleeding and open splenic artery ligation [57].

With early diagnosis of SASS using ultrasound methods and timely selective splenic artery embolization, the risk of graft loss and retransplantation is significantly reduced.

It is recommended to use prophylactic methods (splenic artery embolization before transplantation, splenic artery ligation during transplantation) to prevent the risks of SASS.

2.4. Hepatic artery pseudoaneurysm

Hepatic artery pseudoaneurysm is a formation resulting from a breach of the integrity of the arterial wall and

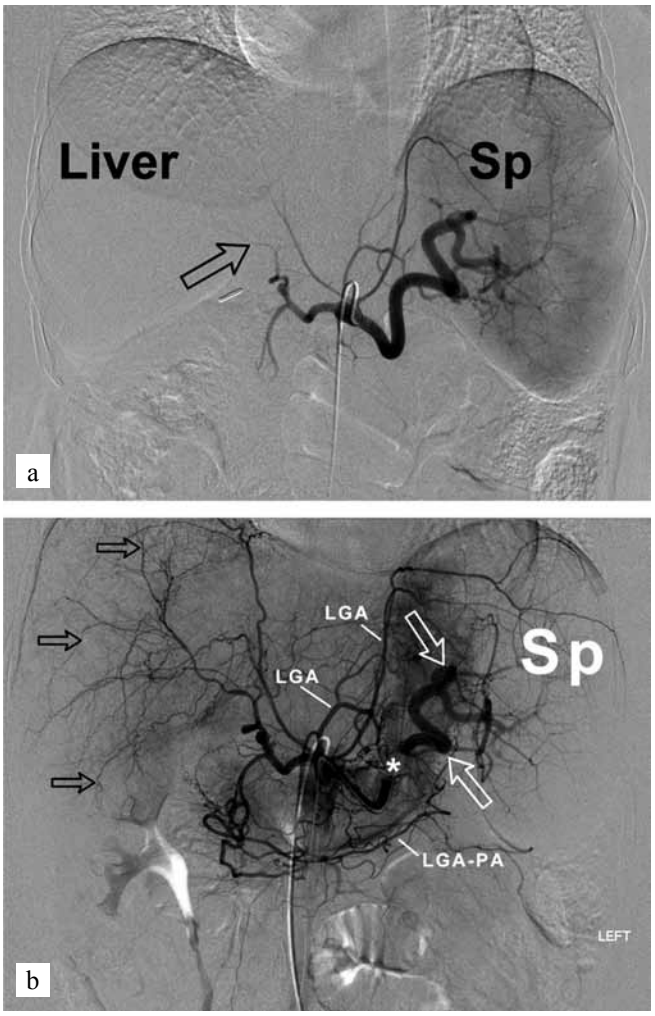

Fig. 4. Celiacography in splenic artery steal syndrome: a, no evidence of hepatic artery stenosis/thrombosis, with reduced blood flow to the liver (indicated by the black arrow), the splenic artery is enlarged in diameter; b, white asterisk marks the place of spiral placement in the splenic artery. Black arrows indicate active filling of the graft along the arterial channel after splenic artery embolization [177]

ongoing bleeding. The spilled blood accumulates in the tissues around the artery forming a tumor-like formation. As a rule, pseudoaneurysms in a liver graft are iatrogenic. Their incidence according to different data varies from 0.27% to 3% [36, 86–96, 176].

A retrospective meta-analysis by Volpin et al. on 787 liver transplants performed between January 1990 and December 31, 2005 reported an incidence of 2.5%, evenly distributed over a 16-year period. The authors showed that this complication did not significantly affect any specific laboratory findings in patients after LT [96]. In 16 patients, the anatomical location of the pseudoaneurysms was extrahepatic and developed early after liver transplantation. In fact, most pseudoaneurysms developed in the early postoperative period within an average of one month after transplantation: 69% were diagnosed within 20 days and 81% within 35 days after LT. The average time for the development of pseudoaneurysms was 13 days [36, 93, 96, 176].

Risk factors

Several predisposing factors for the development of HA pseudoaneurysms have been suggested, including peritoneal infections, technical difficulties in arterial anastomosis, and bile duct leaks [36, 97–101, 176]. In patients with extrahepatic localization of pseudoaneurysms, the rate of detection of bacteria and fungi in the culture of abdominal contents was very high: according to various literature sources from 81% to 100% bacterial or fungal growth was detected [96].

It has also been reported that some patients with bile duct leaks who underwent biliodigestive anastomosis during LT developed HA pseudoaneurysms [36, 96–101, 176].

Clinical presentation

The clinical presentation of pseudoaneurysms ranges from asymptomatic course and incidental diagnosis on DU, MSCT, or angiography to abdominal pain combined with fever, gastrointestinal bleeding (25% of cases), massive intra-abdominal bleeding in the early postoperative period (31% of cases), and acute hemorrhagic shock (81% of cases) [96].

Diagnosis of hepatic artery pseudoaneurysm

The diagnosis of pseudoaneurysm is established based on instrumental diagnostic methods (Fig. 5) [96]:
- DU;
- Bolus contrast-enhanced MSCT;
- MRI;
- Angiography.

Treatment of hepatic artery pseudoaneurysm

Open surgery or endovascular correction are the main methods of treating pseudoaneurysms [90, 93, 96, 100, 161, 176]. Thus, according to literature data, HA ligation was performed as a surgical benefit for some patients. Postoperative mortality in such patients reached 85%, and those patients who survived developed biliary complications and liver abscesses, which eventually led to the need for retransplantation [176]. Another group of patients underwent excision of the arterial defect with subsequent arterial reconstruction, including the use of shunt grafts. Postoperative mortality in this group was 28%; 66% of patients did not develop any postoperative complications. The remaining 6% developed biliary complications. Two patients underwent endovascular intervention. One was embolization of the pseudoaneurysm. The second one was placement of a covered stent. Both patients have been alive for more than 10 years and have not had any complications after the endovascular intervention [96].

Prognosis

In the literature, HA pseudoaneurysm is associated with a high mortality rate of 69% to 100% [36, 96–101, 176].

It should be noted that early detection of HA pseudoaneurysm in high-risk patients (patient with peritoneal infection, bacteremia, biliary leakage, biliodigestive anastomosis) is crucial for diagnostic evaluation and subsequent treatment with endovascular correction methods.

Open surgery should be followed by immediate revascularization even in the infected area if endovascular treatment fails. Detection of the pseudoaneurysm before it ruptures should ensure a successful outcome in 100% of cases.

It is worth keeping in mind that pseudoaneurysms are usually asymptomatic until they rupture, most commonly in the first five weeks after LT.

DU is not a highly effective method of diagnosing a HA pseudoaneurysm. Contrast-enhanced MSCT, MRI, or angiography should be performed.

2.5. Hepatic arterial rupture

Hepatic arterial rupture (HAR) is defined as the development of bleeding from the hepatic artery trunk. This is a rather severe complication leading to both impaired blood supply to the graft and the risk of patient death from bleeding [176].

HAR is a rare complication (0.64%); in most cases, it typically develops against the background of graft arterial pseudoaneurysm accompanied by infection, or occurs iatrogenically after endovascular interventions on the liver graft artery [88, 93, 96]. A ruptured hepatic artery leads to high patient mortality; therefore, it requires emergency surgical treatment [103].

Clinical presentation and diagnosis

The clinical presentation has always been accompanied by sudden bleeding: hemoperitoneum (58.8%), gastrointestinal bleeding (29.4%), hematoma (5.9%) and hemobilia in one patient (5.7%). The presence of fungal infection in the arterial wall was confirmed in 35% of patients. Bile leakage occurred in 41% of patients [176].

Treatment of hepatic arterial rupture

Since a ruptured hepatic artery is accompanied by acute bleeding, many surgical treatment options are available. In case of graft artery rupture, the following are performed: endovascular correction with embolization, arterial stenting, open arterial reconstruction, aorto-hepatic bypass, graft artery ligation, and retransplantation [176].

To date, the mortality rate in ruptured hepatic artery remains high, so there is no definite consensus on the choice of specific surgical treatment tactics.

Early postoperative mortality in such patients is 35%, the main causes being recurrent bleeding or sepsis [176].

A retrospective analysis has shown that revascularization in hepatic artery ruptures is not always indicated because one of the main causes of rupture is infection, and such patients subsequently die of sepsis. A study by Boleslawski et al. (2013) showed that HA ligation is efficient. Their study showed that 83% of patients who underwent revascularization died within 90 days of revascularization, with all patients who underwent ligation surviving 90 days after HA ligation. The one-year and three-year survival rates of patients after HA ligation were 100% and 80%, respectively, while the survival rates of patients who underwent revascularization were 14% and 14%, respectively [176].

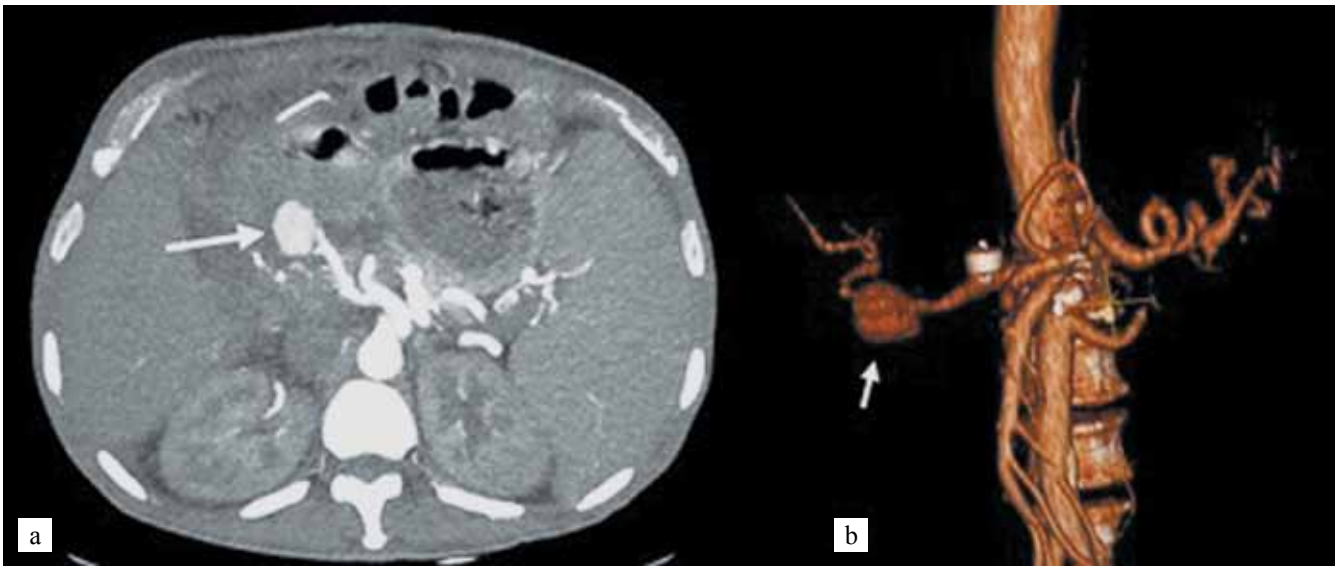

Fig. 5. Contrast-enhanced multislice computed tomography. The arrow indicates the site where hepatic artery pseudoaneurysm developed after liver transplantation. a, coronal projection; b, volume rendering [161]

3. VENOUS COMPLICATIONS

Compared to arterial complications, venous complications are less common with an established overall incidence of about 3%. They can be potentially dangerous, can lead to liver graft dysfunction and therefore represent an important source of post-LT morbidity and mortality, especially if they occur in the early postoperative period [6, 7, 10, 104–106, 175]. Numerous scientific studies have shown that the incidence of venous complications in pediatric transplants is higher than in adult patients [2, 72, 105, 107, 107, 108, 175]. Venous complications include portal vein complications and complications of the inferior vena cava and hepatic veins [7, 10, 109, 175].

3.1. Portal vein complications

The incidence of portal vein complications after liver transplantation is low (1%–3% of patients). These complications are more common after split transplantation, as well as in liver fragment transplantation from living donors, and in pediatric recipients [6, 7, 10, 109–112, 175].

3.1.1. Portal vein thrombosis

The incidence of portal vein thrombosis (PVT) after liver transplantation ranges from 0.3% to 2.6% [3, 111]. However, PVT incidence in patients who received a graft from a living donor is approximately 4%. This is due to technically more complicated venous reconstruction of the portal vein during transplantation, as well as to the fact that it is not always possible to take a liver fragment with a long section of the portal vein from a living related donor, especially when transplanting the right lobe of the liver [113]. PVT occurs more often in the early postoperative period, with 73% of all PVTs after liver transplantation occur in the first three months after surgery [113].

Risk factors

The most common causes of PVT are technical errors associated with an excessively long portal vein and its kinks and/or stenosis of the portal anastomosis [111].

Other risk factors are [36, 111–117]:

– previous surgical interventions on the portal vein;
– portal vein thrombosis before liver transplantation, requiring thrombectomy during surgery,
– small portal vein diameter (<5 mm);
– history of splenectomy;
– pepatic microvascular dysplasia;
– portosystemic shunts;
– use of vascular grafts for portal vein reconstruction.

Additional risk factors in patients who received a transplant from a living donor:

– Small size of the portal vein (length and/or diameter);
– Spatial position of the liver graft in the abdominal cavity.

Clinical presentation

The clinical presentation depends on the timing of thrombosis [36]. When PVT occurs early postoperatively, acute graft dysfunction predominates. If thrombosis occurs late, the clinical symptoms depend on the degree of collateral venous circulation [36, 111, 112].

The most important clinical manifestations of late PVT are manifestations of portal hypertension, including ascites, splenomegaly, cytopenia, and gastrointestinal bleeding from esophageal varices [36, 111].

Diagnosis of portal vein thrombosis after liver transplantation

Ultrasound monitoring should be performed regularly after LT to assess portal vein patency. Ultrasound is the easiest way to assess the patency of the portal vein of the graft, the speed of its blood flow, as well as the presence of blood clots in the portal system. Doppler flowmetry is used intraoperatively, as well as methods for measuring volumetric blood flow to exclude portal vein thrombosis, as well as to determine indications for modulation of portal blood flow [165]. Portal pressure is measured by direct cannulation of the portal vein or its tributaries, such as the inferior mesenteric vein or other mesenteric veins. It should be noted that high central venous pressure can affect portal vein pressure (PVP), and the PVP values in this case may be erroneous [165–167]. Most postoperative ultrasound monitoring protocols vary worldwide, with the belief that graft vessel DU monitoring should be performed daily for the first 5–7 days after LT [2, 59, 118–120]. If PVT is suspected, it is advisable to perform contrast-enhanced MSCT (Fig. 6) [121, 164]. Some authors have suggested the use of contrast-enhanced magnetic resonance imaging (MRI) of the liver with the administration of gadolinium, an MRI contrast agent. Also, there are protocols for the use of high-contrast ultrasound [119, 121].

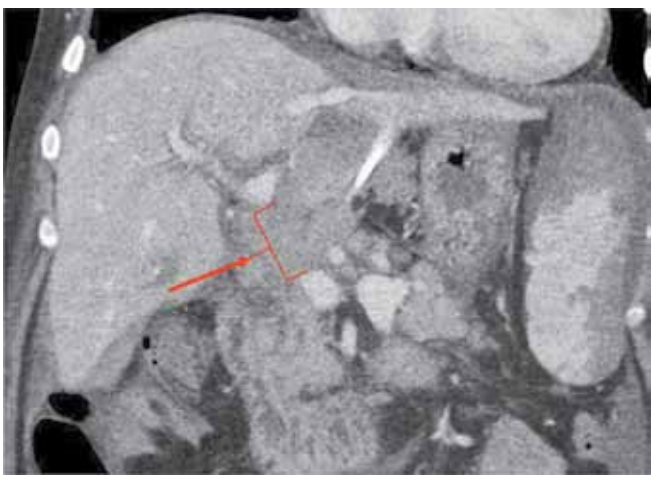

Fig. 6. Contrast-enhanced multispiral computed tomography. The arrow indicates the site of portal vein thrombosis in the patient after liver transplantation [164]

Treatment of portal vein thrombosis after liver transplantation

Treatment protocols include various methods of treatment: from anticoagulant administration to open portal reconstruction surgery. Currently, percutaneous transhepatic correction of portal vein blood flow is actively used. Interventional techniques include balloon angioplasty, portal vein stenting, anticoagulant administration into the portal vein system via a transjugular intrahepatic portosystemic shunt (TIPS) [122–125, 129, 130].

Among other things, the approach to PVT treatment differs depending on how long after LT it develops. Thus, in early PVT (in the first 72 hours after LT), accompanied by acute graft dysfunction, open revision of the portal anastomosis, thrombectomy, and reconstruction of the portal anastomosis must be performed. In PVT developing from postoperative day 3 to 30, treatment is initiated with the use of systemic anticoagulants. Endovascular correction of portal blood flow is also performed [122, 124, 126–128].

In late PVT, in the late period after LT, the main manifestations of portal vein thrombosis will be the development of portal hypertension syndrome (splenomegaly, ascites, esophageal varices, formation of collateral shunts, with the development of gastrointestinal bleeding) [36, 111]. As a rule, late after transplantation, endovascular techniques are used first, followed by various variants of portosystemic shunting, including TIPS, or open surgical reconstruction. In addition, treatment may require endoscopic hemostasis for bleeding from esophageal varices [122–125, 128–131].

It is worth noting that when TIPS is performed or when portal vein stenting is performed, different thrombolytic protocols (antiplatelet/anticoagulant therapy) are administered to patients [124].

Prognosis

PVT is fraught with graft loss and patient death. However, when PVT is diagnosed and treated early, real-world data show a good outcome with a survival rate >89% [139].

PVT is a rare but serious complication, especially when it develops in the early postoperative period. The physician's goal is to detect PVT as early as possible using ultrasound screening protocols. Open thrombectomy is required for PVT detected early posttransplant, but percutaneous interventions are gradually becoming the best therapeutic option with good outcomes and safety.

3.1.2. Portal vein stenosis

The true incidence of portal vein stenosis (PVS) after LT is not reliably known. The few data reported in the literature suggest an incidence of <3% [109].

Risk factors

Like PVT, the main risk factors are surgical technical errors during LT. Stenosis most often develops in portal anastomosis that is technically difficult to perform. Most often, such difficulties occur when there is a difference between the diameters of the portal vein of the donor and the recipient. This is often the case when transplanting liver fragments to children [109, 133, 134].

Clinical presentation

PVS is characterized by the clinical picture of portal hypertension syndrome and/or graft dysfunction [105, 106, 133, 135]. In practice, most patients with PVS have no complaints, and the diagnosis of stenosis is an incidental finding discovered during routine screening ultrasound [109, 133].

If patients develop a clinical picture, it is usually consistent with that of portal hypertension. These patients may develop gastrointestinal bleeding, ascites, and splenomegaly. Laboratory changes in the biochemical panel are not consistent and, therefore, are not specifically significant for PVS diagnosis [105, 106, 133, 135].

Diagnosis of portal vein stenosis after liver transplantation

The main methods of diagnosis and screening also include ultrasound (DU). The criteria for diagnosis by ultrasonography include [118]:

– the presence of a narrowing site in the portal vein;
– normal or reduced blood flow velocity through the portal vein to the narrowing site;
– post-stenotic portal vein dilatation;
– increased blood flow velocity (turbulent blood flow) after the portal vein narrowing site.

At the same time, according to some scientific papers, ultrasound has been considered as a sensitive method of investigation in relation to PVS, but not specific. In view of this, ultrasound criteria for PVS after LT have been calculated [118]:

1. Ratio of portal vein diameters before narrowing and after narrowing ≥50%;
2. Blood flow velocity is greater after the narrowing site than before the site >3:1.

If both criteria are present, contrast-enhanced MSCT is indicated for additional diagnosis and confirmation of PVS [118, 134, 136].

Treatment of portal vein stenosis

Surgical treatment, including anastomotic revision or retransplantation, is usually performed when PVS develops early after LT [137]. In case of asymptomatic PVS, patients with normal graft function should be followed up (systematic DU screening) without any intervention [114].

In case of clinical manifestations, the method of choice is percutaneous transhepatic methods of blood

flow correction (Fig. 7) [105, 106, 115–117, 133–135, 138–141]. Both balloon angioplasty and portal vein stenting, followed by antiplatelet therapy are performed. A disadvantage of stenting may be stent thrombosis, which may subsequently lead to the need for retransplantation. However, it has been shown that the risk of stent thrombosis can be significantly reduced with anticoagulants/antiplatelet agents [133, 134, 138–141].

Also, percutaneous interventions may pose a risk of complications, such as bleeding due to liver vessel injury, hemobilia due to ductal injury [137, 139].

PVS is a rather rare venous complication following LT. It develops most commonly after pediatric liver fragment transplantation or after transplantation of liver fragments from living donors. Ultrasound is an important diagnostic tool to assist the clinician because most asymptomatic cases may progress until PVS is clinically manifested by signs of portal hypertension. This in turn will adversely affect the prognosis of graft survival and ultimately patient survival.

Percutaneous intervention with stent placement has been shown to be the preferred treatment modality with a high success rate and low recurrence and/or complication rate.

3.2. Complications of the inferior vena cava and hepatic veins

Currently, impaired blood outflow from the liver graft by kinking, stenosis or thrombosis of the inferior vena cava (IVC) or hepatic veins, especially in transplants from living donors, are rare post-LT complications with a reported incidence of less than 3% [142, 143].

Clinical presentation and diagnosis

The clinical manifestations are diverse: lower limb edema, hepatomegaly, ascites, hydrothorax, Budd–Chiari syndrome, polyserositis, liver dysfunction, multiple organ failure, which may eventually lead to graft loss and patient death [6, 110, 144].

The main risk factor leading to caval complications is technical errors committed while performing caval anastomosis, which lead to kinking or thrombosis in the early postoperative period. Many authors have developed technical intraoperative techniques to prevent these complications. Thus, the piggyback technique and a modified version of the piggyback, i.e., hepatectomy techniques with preservation of the recipient inferior vena cava and formation of caval anastomosis directly with the recipient's hepatic veins, have been developed [145–151].

In the late postoperative period, chronic stenosis at the caval anastomosis site is the result of fibrosis, hyperplasia and/or external compression due to liver graft enlargement [6, 110, 144].

The diagnosis is made on the basis of DU, contrast-enhanced CT scan (Fig. 8) and with the help of transjugular cavography, which enables therapeutic manipulations during the procedure [152].

Treatment of caval complications

The treatment methods depend on the extent to which complications related to impaired blood flow from the transplant have developed in the long-term period. In case of severe graft dysfunction or if multiple organ failure develops, retransplantation is always indicated [132, 152].

In addition, minimally invasive endovascular interventions are the treatment of choice because the mortality rate after minimally invasive surgery is 11.1% compared with 41.6% for retransplantation.

Angioplasty by balloon-assisted transjugular intrahepatic portosystemic shunt placement can restore anasto-

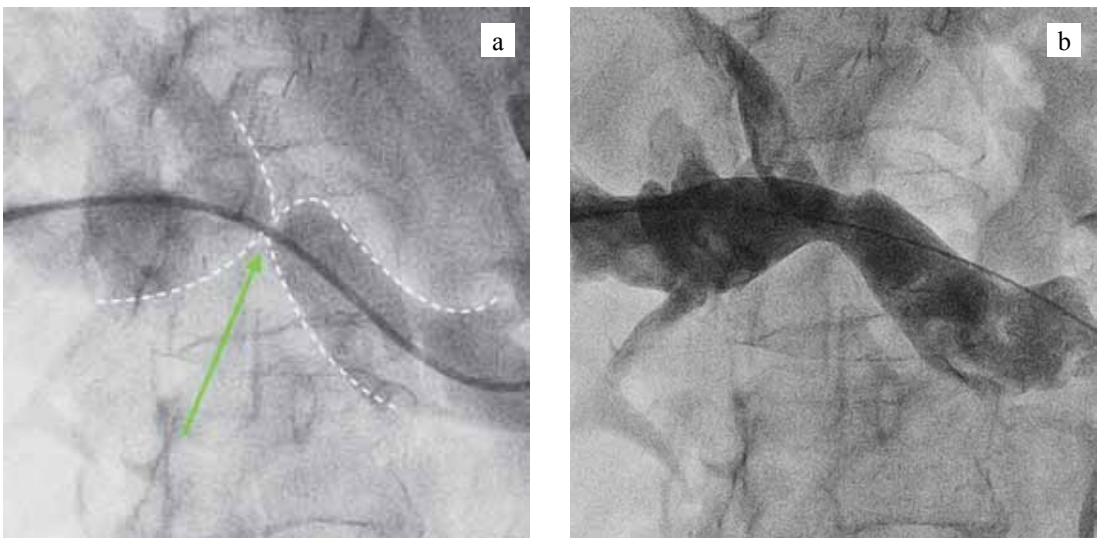

Fig. 7. Percutaneous retrograde portography: a, the arrow indicates the site of portal vein stenosis in percutaneous transhepatic portography; b, effect after balloon angioplasty of the site of portal vein stenosis [106]

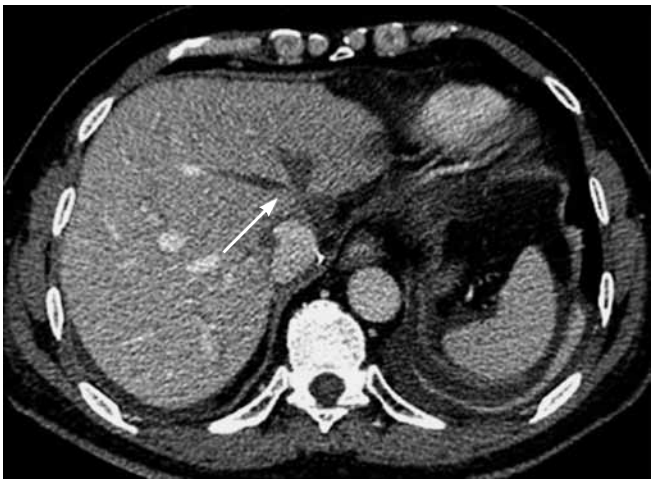

Fig. 8. Contrast-enhanced multislice computed tomography. The arrow indicates the site of hepatic vein thrombosis in the area of caval anastomosis [75]

motic patency in almost 100% of cases, but recurrence of stenosis is quite common and repeated angioplasties may be required [152].

Reports suggest that stenting of the caval anastomosis may be the best treatment option with a high success rate ranging from 73% to 100%. This method is safe and shows good long-term outcomes [132, 139, 152–160].

4. CONCLUSION

Vascular complications remain a major problem after liver transplantation. They are associated with a high mortality rate, especially if they manifest in the early postoperative period (first month after transplantation) and if they are not diagnosed in time.

The only solution to reduce the severity of VCs is to prevent them by controlling risk factors and, if this is not possible, early diagnosis is necessary, even in asymptomatic patients.

Many transplant centers around the world advocate the use of routine screening investigations such as ultrasound (DU) and, if in doubt, to perform contrast-enhanced CT scan or angiography, which is the standard.

If a VC is identified and the patient does not have severe liver graft dysfunction, it is more appropriate to attempt to resolve the complication by endovascular blood flow correction, as this method has demonstrated effective and safe outcomes.

Conversely, if there are serious consequences for the liver graft, the most effective therapeutic procedure is emergency retransplantation, which shows better outcomes in terms of efficacy and survival. However, organ shortage severely limits this treatment option.

The authors declare no conflict of interest.

REFERENCES

1. *Gautier SV, Moysyuk YG, Poptsov VN, Kornilov MN, Tsirulnikova OM, Yaroshenko EB et al*. One hundred deceased donor liver transplantations at a single center. *Russian Journal of Transplantology and Artificial Organs*. 2012; 14 (1): 6–14. https://doi.org/10.15825/1995-1191-2012-1-6-14.
2. *Gautier SV, Voskanov MA, Monakhov AR, Semash KO*. The role of endovascular and endobiliary methods in the treatment of post-liver transplant complications. *Russian Journal of Transplantology and Artificial Organs*. 2020; 22 (4): 140–148. https://doi.org/10.15825/1995-1191-2020-4-140-148.
3. *Khalaf H.* Vascular complications after deceased and living donor liver transplantation: a single-center experience. *Transplant Proc*. 2010; 42: 865–870. https://doi.org/10.1016/j.transproceed.2010.02.037.
4. *Wozney P, Zajko AB, Bron KM, Point S, Starzl TE.* Vascular complications after liver transplantation: a 5-year experience. *AJR Am J Roentgenol*. 1986; 147: 657–663. https://doi.org/10.2214/ajr.147.4.657.
5. *Karatzas T, Lykaki-Karatzas E, Webb M, Nery J, Tsaroucha A, Demirbas A et al.* Vascular complications, treatment, and outcome following orthotopic liver transplantation. *Transplant Proc*. 1997; 29: 2853–2855. https://doi.org/10.1016/s0041-1345(97)00706-9.
6. *Pawlak J, Grodzicki M, Leowska E, Małkowski P, Michałowicz B, Nyckowski P et al.* Vascular complications after liver transplantation. *Transplant Proc*. 2003; 35 (6): 2313–2315. https://doi.org/10.1016/S0041-1345(03)00836-4.
7. *Duffy JP, Hong JC, Farmer DG, Ghobrial RM, Yersiz H, Hiatt JR, Busuttil RW.* Vascular complications of orthotopic liver transplantation: experience in more than 4,200 patients. *J Am Coll Surg*. 2009; 208: 896–903; discussion 903–905. https://doi.org/10.1016/j.jamcollsurg.2008.12.032.
8. *Hejazi Kenari SK, Zimmerman A, Eslami M, F Saidi R.* Current state of art management for vascular complications after liver transplantation. *Middle East J Dig Dis*. 2014; 6: 121–130. PMCID: PMC4119668.
9. *Boyvat F, Aytekin C, Firat A, Harman A, Karakayali H, Haberal M.* Diagnostic and therapeutic management of hepatic artery thrombosis and stenosis after orthotopic and heterotopic liver transplantation. *Transplant Proc*. 2003; 35: 2791–2795. https://doi.org/10.1016/j.transproceed.2003.09.086.
10. *Pérez-Saborido B, Pacheco-Sánchez D, Barrera-Rebollo A, Asensio-Díaz E, Pinto-Fuentes P, Sarmentero-Prieto JC et al.* Incidence, management, and results of vascular complications after liver transplantation. *Transplant Proc.* 2011; 43: 749–750. https://doi.org/10.1016/j.transproceed.2011.01.104.
11. *Figueras J, Busquets J, Dominguez J, Sancho C, Casanovas-Taltavull T, Rafecas A et al*. Intra-arterial thrombolysis in the treatment of acute hepatic artery thrombosis after liver transplantation. *Transplantation.* 1995; 59: 1356–1357. PMID: 7762074.

12. *Pawlak J, Wróblewski T, Małkowski P, Nyckowski P, Zieniewicz K, Grzelak I et al*. Vascular complications related to liver transplantation. *Transplant Proc*. 2000; 32: 1426–1428.
13. *Stange BJ, Glanemann M, Nuessler NC, Settmacher U, Steinmüller T, Neuhaus P.* Hepatic artery thrombosis after adult liver transplantation. *Liver Transpl.* 2003; 9: 612–620. https://doi.org/10.1053/jlts.2003.50098.
14. *Zhou J, Fan J, Wang JH, Wu ZQ, Qiu SJ, Shen YH et al.* Continuous transcatheter arterial thrombolysis for early hepatic artery thrombosis after liver transplantation. *Transplant Proc.* 2005; 37: 4426–4429. https://doi.org/10.1016/j.transproceed.2005.10.113.
15. *Li ZW, Wang MQ, Zhou NX, Liu Z, Huang ZQ.* Interventional treatment of acute hepatic artery occlusion after liver transplantation. *Hepatobiliary Pancreat Dis Int.* 2007; 6: 474–478. PMID: 17897908.
16. *Saad WE, Davies MG, Saad NE, Westesson KE, Patel NC, Sahler LG et al*. Catheter thrombolysis of thrombosed hepatic arteries in liver transplant recipients: predictors of success and role of thrombolysis. *Vasc Endovascular Surg.* 2007; 41: 19–26. https://doi.org/10.1177/1538574406296210.
17. *Singhal A, Stokes K, Sebastian A, Wright HI, Kohli V.* Endovascular treatment of hepatic artery thrombosis following liver transplantation. *Transpl Int.* 2010; 23: 245–256. https://doi.org/10.1111/j.1432-2277.2009.01037.x.
18. *Chen J, Weinstein J, Black S, Spain J, Brady PS, Dowell JD.* Surgical and endovascular treatment of hepatic arterial complications following liver transplant. *Clin Transplant.* 2014; 28: 1305–1312. https://doi.org/10.1111/ctr.12431.
19. *Porrett PM, Hsu J, Shaked A.* Late surgical complications following liver transplantation. *Liver Transpl.* 2009; 15 Suppl 2: S12–S18. https://doi.org/10.1002/lt.21893.
20. *Abdelaziz O, Hosny K, Amin A, Emadeldin S, Uemoto S, Mostafa M.* Endovascular management of early hepatic artery thrombosis after living donor liver transplantation. *Transpl Int.* 2012; 25: 847–856. https://doi.org/10.1111/j.1432-2277.2012.01509.x.
21. *De Pietri L, Montalti R, Nicolini D, Troisi RI, Moccheggiani F, Vivarelli M.* Perioperative thromboprophylaxis in liver transplant patients. *World J Gastroenterol*. 2018 Jul 21; 24 (27): 2931–2948. doi: 10.3748/wjg.v24.i27.2931. PMID: 30038462; PMCID: PMC6054944.
22. *Ambrosino P, Tarantino L, Di Minno G, Paternoster M, Graziano V, Petitto M et al*. The risk of venous thromboembolism in patients with cirrhosis. A systematic review and meta-analysis. *Thromb Haemost*. 2017; 117: 139–148.
23. *Stine JG, Pelletier SJ, Schmitt TM, Porte RJ, Northup PG.* Pre-transplant portal vein thrombosis is an independent risk factor for graft loss due to hepatic artery thrombosis in liver transplant recipients. *HPB (Oxford)*. 2016; 18: 279–286. [PMC free article] [PubMed] [Google Scholar] [Ref list].
24. *Tripodi A.* Hemostasis abnormalities in cirrhosis. *Curr Opin Hematol*. 2015; 22: 406–412. https://doi.org/10.1097/moh.0000000000000164.
25. *Violi F, Basili S, Raparelli V, Chowdary P, Gatt A, Burroughs AK.* Patients with liver cirrhosis suffer from primary haemostatic defects? Fact or fiction? *J Hepatol*. 2011; 55: 1415–1427. https://pubmed.ncbi.nlm.nih.gov/21718668.
26. *Lisman T, Bongers TN, Adelmeijer J, Janssen HL, de Maat MP, de Groot PG, Leebeek FW.* Elevated levels of von Willebrand Factor in cirrhosis support platelet adhesion despite reduced functional capacity. *Hepatology*. 2006; 44: 53–61.
27. *Tripodi A, Primignani M, Chantarangkul V, Clerici M, Dell'Era A, Fabris F et al*. Thrombin generation in patients with cirrhosis: the role of platelets. *Hepatology*. 2006; 44: 440–445. https://doi.org/10.1002/hep.21266.
28. *Leebeek FW, Rijken DC.* The Fibrinolytic Status in Liver Diseases. *Semin Thromb Hemost*. 2015; 41: 474–480. https://doi.org/10.1055/s-0035-1550437.
29. *Caldwell SH, Hoffman M, Lisman T, Macik BG, Northup PG, Reddy KR et al*. Coagulation in Liver Disease Group. Coagulation disorders and hemostasis in liver disease: pathophysiology and critical assessment of current management. *Hepatology*. 2006; 44: 1039–1046. https://doi.org/10.1002/hep.21303.
30. *Colucci M, Binetti BM, Branca MG, Clerici C, Morelli A, Semeraro N, Gresele P.* Deficiency of thrombin activatable fibrinolysis inhibitor in cirrhosis is associated with increased plasma fibrinolysis. *Hepatology*. 2003; 38: 230–237. https://doi.org/10.1053/jhep.2003.50277.
31. *Quintini C, Hirose K, Hashimoto K, Diago T, Aucejo F, Eghtesad B et al.* "Splenic artery steal syndrome" is a misnomer: the cause is portal hyperperfusion, not arterial siphon. *Liver Transpl*. 2008; 14: 374–379. https://doi.org/10.1002/lt.21386.
32. *Pastacaldi S, Teixeira R, Montalto P, Rolles K, Burroughs AK.* Hepatic artery thrombosis after orthotopic liver transplantation: a review of nonsurgical causes. *Liver Transpl.* 2001; 7: 75–81. https://doi.org/10.1053/jlts.2001.22040.
33. *Silva MA, Jambulingam PS, Gunson BK, Mayer D, Buckels JA, Mirza DF, Bramhall SR.* Hepatic artery thrombosis following orthotopic liver transplantation: a 10-year experience from a single centre in the United Kingdom. *Liver Transpl.* 2006; 12: 146–151. https://doi.org/10.1002/lt.20566.
34. *Bekker J, Ploem S, de Jong KP.* Early hepatic artery thrombosis after liver transplantation: a systematic review of the incidence, outcome and risk factors. *Am J Transplant.* 2009; 9: 746–757. https://doi.org/10.1111/j.1600-6143.2008.02541.x.
35. *Tzakis AG, Gordon RD, Shaw BW, Iwatsuki S, Starzl TE.* Clinical presentation of hepatic artery thrombosis after liver transplantation in the cyclosporine era. *Transplantation.* 1985; 40: 667–671. https://doi.org/10.1097%2F00007890-198512000-00019.
36. *Langnas AN, Marujo W, Stratta RJ, Wood RP, Shaw BW.* Vascular complications after orthotopic liver transplantation. *Am J Surg.* 1991; 161: 76–82; discussion 82–83. https://doi.org/10.1016/0002-9610(91)90364-j.
37. *Drazan K, Shaked A, Olthoff KM, Imagawa D, Jurim O, Kiai K et al*. Etiology and management of symptoma-

tic adult hepatic artery thrombosis after orthotopic liver transplantation (OLT). *Am Surg.* 1996; 62: 237–240. PMID: 8607585.

38. *Pinna AD, Smith CV, Furukawa H, Starzl TE, Fung JJ.* Urgent revascularization of liver allografts after early hepatic artery thrombosis. *Transplantation.* 1996; 62: 1584–1587. https://doi.org/10.1097%2F00007890-199612150-00010.
39. *Sheiner PA, Varma CV, Guarrera JV, Cooper J, Garatti M, Emre S et al*. Selective revascularization of hepatic artery thromboses after liver transplantation improves patient and graft survival. *Transplantation.* 1997; 64: 1295–1299. https://doi.org/10.1097/00007890-199711150-00011.
40. *Torras J, Lladó L, Figueras J, Ramos E, Lama C, Fabregat J et al.* Diagnostic and therapeutic management of hepatic artery thrombosis after liver transplantation. *Transplant Proc.* 1999; 31: 2405. https://doi.org/10.1016/s0041-1345(99)00403-0.
41. *Bhattacharjya S, Gunson BK, Mirza DF, Mayer DA, Buckels JA, McMaster P, Neuberger JM*. Delayed hepatic artery thrombosis in adult orthotopic liver transplantation – a 12-year experience. *Transplantation.* 2001; 71: 1592–1596. https://doi.org/10.1097/00007890-200106150-00018.
42. *Jain A, Costa G, Marsh W, Fontes P, Devera M, Mazariegos G et al.* Thrombotic and nonthrombotic hepatic artery complications in adults and children following primary liver transplantation with long-term follow-up in 1000 consecutive patients. *Transpl Int.* 2006; 19: 27–37. https://doi.org/10.1111/j.1432-2277.2005.00224.x.
43. *Pareja E, Cortes M, Navarro R, Sanjuan F, López R, Mir J.* Vascular complications after orthotopic liver transplantation: hepatic artery thrombosis. *Transplant Proc.* 2010; 42: 2970–2972. https://doi.org/10.1016/j.transproceed.2010.07.063.
44. *Fouzas I, Sklavos A, Bismpa K, Paxiadakis I, Antoniadis N, Giakoustidis D et al.* Hepatic artery thrombosis after orthotopic liver transplantation: 3 patients with collateral formation and conservative treatment. *Transplant Proc.* 2012; 44: 2741–2744. https://doi.org/10.1016/j.transproceed.2012.09.002.
45. *Marín-Gómez LM, Bernal-Bellido C, Alamo-Martínez JM, Porras-López FM, Suárez-Artacho G, Serrano-Diaz-Canedo J et al*. Intraoperative hepatic artery blood flow predicts early hepatic artery thrombosis after liver transplantation. *Transplant Proc.* 2012; 44: 2078–2081. https://doi.org/10.1016/j.transproceed.2012.07.077.
46. *Panaro F, Gallix B, Bouyabrine H, Ramos J, Addeo P, Testa G et al*. Liver transplantation and spontaneous neovascularization after arterial thrombosis: "the neovascularized liver". *Transpl Int.* 2011; 24: 949–957. https://doi.org/10.1111/j.1432-2277.2011.01293.x.
47. *Unal B, Gonultas F, Aydin C, Otan E, Kayaalp C, Yilmaz S.* Hepatic artery thrombosis-related risk factors after living donor liver transplantation: single-center experience from Turkey. *Transplant Proc.* 2013; 45: 974–977. https://doi.org/10.1016/j.transproceed.2013.02.070.
48. *Sakamoto Y, Harihara Y, Nakatsuka T, Kawarasaki H, Takayama T, Kubota K et al.* Rescue of liver grafts from hepatic artery occlusion in living-related liver transplantation. *Br J Surg.* 1999; 86: 886–889. https://doi.org/10.1046/j.1365-2168.1999.01166.x.
49. *Oh CK, Pelletier SJ, Sawyer RG, Dacus AR, McCullough CS, Pruett TL, Sanfey HA.* Uni- and multi-variate analysis of risk factors for early and late hepatic artery thrombosis after liver transplantation. *Transplantation.* 2001; 71: 767–772. https://doi.org/10.1097/00007890-200103270-00014.
50. *Gautier S, Monakhov A, Gallyamov E, Tsirulnikova O, Zagaynov E, Dzhanbekov T et al.* Laparoscopic left lateral section procurement in living liver donors: A single center propensity score-matched study. *Clin Transplant*. 2018; 32: e13374. https://doi.org/10.1111/ctr.13374.
51. *Monakhov A, Gautier S, Tsiroulnikova O, Semash K, Latypov R, Dzhanbekov T et al*. Gallamov. Living donor left lateral sectionectomy: Should the procedure still be performed open? *Journal of Liver Transplantation.* 2021; 1: 100001. ISSN 2666-9676. https://doi.org/10.1016/j.liver.2020.100001.
52. *Gautier SV, Monakhov AR, Gallyamov EA, Zagaynov EV, Tsirulnikova OM, Semash KO et al*. Laparoscopic Approach in Liver Harvesting from Living Donors for Transplantation in Children. *Annaly khirurgicheskoy gepatologii = Annals of HPB Surgery*. 2018; 23 (1): 13–18. (In Russ.). https://doi.org/10.16931/1995-5464.2018-1-13-18.
53. *Semash KO.* Laparoskopicheskoe iz"yatie levogo lateral'nogo sektora u prizhiznennogo donora: dis. … kand. med. nauk. M., 2020; 113.
54. *Panaro F, Ramos J, Gallix B, Mercier G, Herrero A, Niampa H et al*. Hepatic artery complications following liver transplantation. Does preoperative chemoembolization impact the postoperative course? *Clin Transplant.* 2014; 28: 598–605. https://doi.org/10.1111/ctr.12358.
55. *Pungpapong S, Manzarbeitia C, Ortiz J, Reich DJ, Araya V, Rothstein KD, Muñoz SJ.* Cigarette smoking is associated with an increased incidence of vascular complications after liver transplantation. *Liver Transpl.* 2002; 8: 582–587. https://doi.org/10.1053/jlts.2002.34150.
56. *Margarit C, Hidalgo E, Lázaro JL, Murio E, Charco R, Balsells J.* Biliary complications secondary to late hepatic artery thrombosis in adult liver transplant patients. *Transpl Int.* 1998; 11 Suppl 1: S251–S254. https://doi.org/10.1007/s001470050472.
57. *Semash K, Dzhanbekov T, Akbarov M, Gaybullaev T.* Prevention of the splenic artery steal syndrome in patients after living donor liver transplant. *Actual questions of mini-invasive surgery.* 2023: 62–63.
58. *Raschke RA, Reilly BM, Guidry JR, Fontana JR, Srinivas S.* The weight-based heparin dosing nomogram compared with a "standard care" nomogram: A randomized controlled trial. *Ann Intern Med*. 1993; 119 (9): 874–881. https://doi.org/10.7326/0003-4819-119-9-199311010-00002.
59. *Voskanov MA.* Interventsionnye metody korrektsii sosudistykh oslozhneniy i biliodigestivnykh striktur posle

transplantatsii pecheni u detey: dis. … kand. med. nauk. M., 2020; 109.

60. *Gautier SV, Monakhov AR, Tsiroulnikova OM, Latypov RA, Dzhanbekov TA, Mescheryakov SV et al.* Split liver transplantation: a single center experience. *Almanac of Clinical Medicine*. 2020. 48 (3): 162–170. doi: 10.18786/2072-0505-2020-48-031. URL: https://cyberleninka.ru/article/n/split-transplantatsiya-pecheni-opyt-odnogo-tsentra.
61. *Saad WE, Davies MG, Sahler L, Lee DE, Patel NC, Kitanosono T et al*. Hepatic artery stenosis in liver transplant recipients: primary treatment with percutaneous transluminal angioplasty. *J Vasc Interv Radiol*. 2005; 16: 795–805. https://doi.org/10.1097/01.rvi.0000156441.12230.13.
62. *Da Silva RF, Raphe R, Felício HC, Rocha MF, Duca WJ, Arroyo PC et al.* Prevalence, treatment, and outcomes of the hepatic artery stenosis after liver transplantation. *Transplant Proc.* 2008; 40: 805–807. https://doi.org/10.1016/j.transproceed.2008.02.041.
63. *Hamby BA, Ramirez DE, Loss GE, Bazan HA, Smith TA, Bluth E, Sternbergh WC*. Endovascular treatment of hepatic artery stenosis after liver transplantation. *J Vasc Surg*. 2013; 57: 1067–1072. https://doi.org/10.1016/j.jvs.2012.10.086.
64. *Rostambeigi N, Hunter D, Duval S, Chinnakotla S, Golzarian J.* Stent placement versus angioplasty for hepatic artery stenosis after liver transplant: a meta-analysis of case series. *Eur Radiol.* 2013; 23: 1323–1334. https://doi.org/10.1007/s00330-012-2730-9.
65. *Sommacale D, Aoyagi T, Dondero F, Sibert A, Bruno O, Fteriche S et al*. Repeat endovascular treatment of recurring hepatic artery stenoses in orthotopic liver transplantation. *Transpl Int.* 2013; 26: 608–615. https://doi.org/10.1111/tri.12089.
66. *Abbasoglu O, Levy MF, Vodapally MS, Goldstein RM, Husberg BS, Gonwa TA, Klintmalm GB*. Hepatic artery stenosis after liver transplantation – incidence, presentation, treatment, and long term outcome. *Transplantation.* 1997; 63: 250–255. https://doi.org/10.1097/00007890-199701270-00013.
67. *Sabri SS, Saad WE, Schmitt TM, Turba UC, Kumer SC, Park AW et al*. Endovascular therapy for hepatic artery stenosis and thrombosis following liver transplantation. *Vasc Endovascular Surg.* 2011; 45: 447–452. https://doi.org/10.1177/1538574411407088.
68. *Denys AL, Qanadli SD, Durand F, Vilgrain V, Farges O, Belghiti J et al*. Feasibility and effectiveness of using coronary stents in the treatment of hepatic artery stenoses after orthotopic liver transplantation: preliminary report. *AJR Am J Roentgenol*. 2002; 178: 1175–1179. https://doi.org/10.2214/ajr.178.5.1781175.
69. *Chen GH, Wang GY, Yang Y, Li H, Lu MQ, Cai CJ et al*. Single-center experience of therapeutic management of hepatic artery stenosis after orthotopic liver transplantation. Report of 20 cases. *Eur Surg Res.* 2009; 42: 21–27. https://doi.org/10.1159/000166601.
70. *Orons PD, Sheng R, Zajko AB*. Hepatic artery stenosis in liver transplant recipients: prevalence and cholangiographic appearance of associated biliary complications. *AJR Am J Roentgenol*. 1995; 165: 1145–1149. https://doi.org/10.2214/ajr.165.5.7572493.
71. *Orons PD, Zajko AB, Bron KM, Trecha GT, Selby RR, Fung JJ.* Hepatic artery angioplasty after liver transplantation: experience in 21 allografts. *J Vasc Interv Radiol*. 1995; 6: 523–529. https://doi.org/10.1016/s1051-0443(95)71128-9.
72. *Uller W, Knoppke B, Schreyer AG, Heiss P, Schlitt HJ, Melter M et al*. Interventional radiological treatment of perihepatic vascular stenosis or occlusion in pediatric patients after liver transplantation. *Cardiovasc Intervent Radiol*. 2013; 36: 1562–1571. https://doi.org/10.1007/s00270-013-0595-1.
73. *Frongillo F, Grossi U, Lirosi MC, Nure E, Sganga G, Avolio AW et al*. Incidence, management, and results of hepatic artery stenosis after liver transplantation in the era of donor to recipient match. *Transplant Proc*. 2013; 45: 2722–2725. https://doi.org/10.1016/j.transproceed.2013.08.007.
74. *Boyvat F, Aytekin C, Harman A, Sevmiş S, Karakayali H, Haberal M.* Endovascular stent placement in patients with hepatic artery stenoses or thromboses after liver transplant. *Transplant Proc*. 2008; 40: 22–26. https://doi.org/10.1016/j.transproceed.2007.12.027.
75. *Piardi T, Lhuaire M, Bruno O, Memeo R, Pessaux P, Kianmanesh R, Sommacale D.* Vascular complications following liver transplantation: A literature review of advances in 2015. *World J Hepatol*. 2016 Jan 8; 8 (1): 36–57. https://doi.org/10.4254%2Fwjh.v8.i1.36.
76. *Uflacker R, Selby JB, Chavin K, Rogers J, Baliga P*. Transcatheter splenic artery occlusion for treatment of splenic artery steal syndrome after orthotopic liver transplantation. *Cardiovasc Intervent Radiol*. 2002 Aug; 25 (4): 300–306. https://doi.org/10.1007/s00270-002-2614-5.
77. *Nüssler NC, Settmacher U, Haase R, Stange B, Heise M, Neuhaus P*. Diagnosis and treatment of arterial steal syndromes in liver transplant recipients. *Liver Transpl*. 2003 Jun; 9 (6): 596–602. https://doi.org/10.1053/jlts.2003.50080.
78. *Fleckenstein FN, Luedemann WM, Kücükkaya A, Auer TA, Plewe J, Hamm B et al.* Splenic artery steal syndrome in patients with orthotopic liver transplant: Where to embolize the splenic artery? *PLoS One*. 2022; 17 (3): e0263832. https://doi.org/10.1371/journal.pone.0263832.
79. *Dokmak S, Aussilhou B, Belghiti J.* Liver transplantation and splenic artery steal syndrome: the diagnosis should be established preoperatively. *Liver Transpl*. 2013 Jun; 19 (6): 667–668.
80. *Madoff DC, Denys A, Wallace MJ, Murthy R, Gupta S, Pillsbury EP et al*. Splenic arterial interventions: anatomy, indications, technical considerations, and potential complications. *Radiographics*. 2005; 25 Suppl 1: S191–S211. https://doi.org/10.1148/rg.25si055504.
81. *Song JY, Shi BY, Zhu ZD, Zheng DH, Li G, Feng LK et al*. New strategies for prevention and treatment of splenic artery steal syndrome after liver transplantation. *World J Gastroenterol*. 2014 Nov 7; 20 (41): 15367–15373. https://doi.org/10.3748%2Fwjg.v20.i41.15367.

82. *Domingues L, Diogo D, Donato P, Pereira Da Silva F, Martins R, Oliveira P et al*. Splenic Artery Syndrome After Liver Transplantation – Predictive Factors: Experience Of A Center. *Revista Portuguesa De Cirurgia.* 2021; (50): 43–49. https://doi.org/10.34635/rpc.896.
83. *Wong TC, Fung JYY, Cui TYS, Sin SL, Ma KW, She BWH et al*. The Risk of Going Small: Lowering GRWR and Overcoming Small-For-Size Syndrome in Adult Living Donor Liver Transplantation. *Ann Surg*. 2021 Dec 1; 274 (6): e1260–e1268. http://dx.doi.org/10.1097/SLA.0000000000003824.
84. *Matsuda H, Yagi T, Sadamori H, Matsukawa H, Shinoura S, Murata H et al.* Complications of arterial reconstruction in living donor liver transplantation: A single-center experience. *Surg Today*. 2006; 36 (3): 245–251. https://doi.org/10.1007/s00595-005-3131-3.
85. *Gunsar F, Rolando N, Pastacaldi S, Patch D, Raimondo ML, Davidson B et al.* Late hepatic artery thrombosis after orthotopic liver transplantation. *Liver Transpl.* 2003; 9: 605–611. https://doi.org/10.1053/jlts.2003.50057.
86. *Houssin D, Ortega D, Richardson A, Ozier Y, Stephan H, Soffer M, Chapuis Y.* Mycotic aneurysm of the hepatic artery complicating human liver transplantation. *Transplantation*. 1988; 46: 469–472. https://doi.org/10.1097/00007890-198809000-00033.
87. *Lerut J, Gordon RD, Iwatsuki S, Starzl TE.* Surgical complications in human orthotopic liver transplantation. *Acta Chir Belg*. 1987; 87: 193–204. PMID: 3303776; PMCID: PMC2987662.
88. *Madariaga J, Tzakis A, Zajko AB, Tzoracoleftherakis E, Tepetes K, Gordon R et al*. Hepatic artery pseudoaneurysm ligation after orthotopic liver transplantation – a report of 7 cases. *Transplantation*. 1992; 54: 824–828. https://doi.org/10.1097%2F00007890-199211000-00011.
89. *Bonham CA, Kapur S, Geller D, Fung JJ, Pinna A.* Excision and immediate revascularization for hepatic artery pseudoaneurysm following liver transplantation. *Transplant Proc*. 1999; 31: 443. https://doi.org/10.1016/s0041-1345(98)01698-4.
90. *Lowell JA, Coopersmith CM, Shenoy S, Howard TK.* Unusual presentations of nonmycotic hepatic artery pseudoaneurysms after liver transplantation. *Liver Transpl Surg*. 1999; 5: 200–203. https://doi.org/10.1002/lt.500050306.
91. *Stange B, Settmacher U, Glanemann M, Nuessler NC, Bechstein WO, Neuhaus P.* Aneurysms of the hepatic artery after liver transplantation. *Transplant Proc*. 2000; 32: 533–534. https://doi.org/10.1016/s0041-1345(00)00877-0.
92. *Leonardi LS, Soares C, Boin IF, Oliveira VC.* Hemobilia after mycotic hepatic artery pseudoaneurysm after liver transplantation. *Transplant Proc*. 2001; 33: 2580–2582. https://doi.org/10.1016/s0041-1345(01)02103-0.
93. *Marshall MM, Muiesan P, Srinivasan P, Kane PA, Rela M, Heaton ND et al.* Hepatic artery pseudoaneurysms following liver transplantation: incidence, presenting features and management. *Clin Radiol*. 2001; 56: 579–587. https://doi.org/10.1053/crad.2001.0650.
94. *Turrión VS, Alvira LG, Jimenez M, Lucena JL, Ardaiz J.* Incidence and results of arterial complications in liver transplantation: experience in a series of 400 transplants. *Transplant Proc*. 2002; 34: 292–293. https://doi.org/10.1016/s0041-1345(01)02767-1.
95. *Leelaudomlipi S, Bramhall SR, Gunson BK, Candinas D, Buckels JA, McMaster P et al*. Hepatic-artery aneurysm in adult liver transplantation. *Transpl Int*. 2003; 16: 257–261. https://doi.org/10.1007/s00147-003-0551-0.
96. *Volpin E, Pessaux P, Sauvanet A, Sibert A, Kianmanesh R, Durand F et al*. Preservation of the arterial vascularisation after hepatic artery pseudoaneurysm following orthotopic liver transplantation: long-term results. *Ann Transplant*. 2014; 19: 346–352. https://doi.org/10.12659/aot.890473.
97. *Jarzembowski TM, Sankary HN, Bogetti D, Manzelli A, Ong E, Oberholzer J et al*. Living donor liver graft salvage after rupture of hepatic artery pseudoaneurysm. *Int Surg*. 2008; 93: 300–303. PMID: 19943434.
98. *Panaro F, Miggino M, Bouyabrine H, Carabalona JP, Berthet JP, Canaud L et al*. Reversed saphenous bypass for hepatic artery pseudoaneurysm after liver transplantation. *Ann Vasc Surg*. 2013; 27: 1088–1097. https://doi.org/10.1016/j.avsg.2013.01.007.
99. *Sellers MT, Haustein SV, McGuire BM, Jones C, Bynon JS, Diethelm AG, Eckhoff DE*. Use of preserved vascular homografts in liver transplantation: hepatic artery aneurysms and other complications. *Am J Transplant*. 2002; 2: 471–475. https://doi.org/10.1034/j.1600-6143.2002.20513.x.
100. *Patel JV, Weston MJ, Kessel DO, Prasad R, Toogood GJ, Robertson I.* Hepatic artery pseudoaneurysm after liver transplantation: treatment with percutaneous thrombin injection. *Transplantation*. 2003; 75: 1755–1757. https://doi.org/10.1097/01.tp.0000063936.94587.10.
101. *Kim HJ, Kim KW, Kim AY, Kim TK, Byun JH, Won HJ et al.* Hepatic artery pseudoaneurysms in adult living-donor liver transplantation: efficacy of CT and Doppler sonography. *AJR Am J Roentgenol*. 2005; 184: 1549–1555. https://doi.org/10.2214/ajr.184.5.01841549.
102. *Semash K, Janbekov T, Akbarov M, Usmonov A, Gaibullaev T.* Stages of preparation and examination of related liver donors and their perioperative management. *Coloproctology and Endoscopic Surgery in Uzbekistan*. 2023; (1): 41–54. https://doi.org/10.56121/2181-4260-2023-1-41-54. https://www.coloproc.uz/index.php/journal/article/view/12.
103. *Golse N, Spina A, Abdelaal A, Mennesson N, Feugier P, Dumortier J et al*. Extra-anatomical hepatic artery reconstruction following post-embolization iatrogenic dissection and arterial anastomotic rupture in two liver transplant recipients. *Gastroenterol Clin Biol*. 2010; 34: 111–114. https://doi.org/10.1016/j.gcb.2009.11.003.
104. *Li W, Bokkers RPH, Dierckx RAJO, Verkade HJ, Sanders DH, de Kleine R, van der Doef HPJ*. Treatment strategies for hepatic artery complications after pediatric liver transplantation: A systematic review. *Liver Transpl*. 2023 Sep 13. doi: 10.1097/LVT.0000000000000257. Online ahead of print. PMID: 37698924.

105. *Monakhov AR, Mironkov BL, Dzhanbekov TA, Semash KO, Khizroev KM, Gautier SV*. Correction of extrahepatic portal hypertension in pediatric patient after liver transplantation. *Russian Journal of Transplantology and Artificial Organs*. 2017; 19 (1): 47–51. (In Russ.). https://doi.org/10.15825/1995-1191-2017-1-47-51.
106. *Semash K, Djanbekov T, Akbarov M, Usmonov A, Shermatov M, Gaybullaev T.* Interventional correction of extrahepatic portal hypertension in patient after liver transplant. The first case report in Uzbekistan. *Central Asian Journal of Medicine*. 2023; (1): 87–96. Retrieved from https://journals.tma.uz/index.php/cajm/article/view/556.
107. *Yilmaz A, Arikan C, Tumgor G, Kilic M, Aydogdu S.* Vascular complications in living-related and deceased donation pediatric liver transplantation: single center's experience from Turkey. *Pediatr Transplant*. 2007; 11: 160–164. https://doi.org/10.1111/j.1399-3046.2006.00601.x.
108. *Orlandini M, Feier FH, Jaeger B, Kieling C, Vieira SG, Zanotelli ML.* Frequency of and factors associated with vascular complications after pediatric liver transplantation. *J Pediatr (Rio J)* 2014; 90: 169–175. https://doi.org/10.1016/j.jped.2013.08.010.
109. *Woo DH, Laberge JM, Gordon RL, Wilson MW, Kerlan RK*. Management of portal venous complications after liver transplantation. *Tech Vasc Interv Radiol*. 2007; 10: 233–239. https://doi.org/10.1053/j.tvir.2007.09.017.
110. *Parrilla P, Sánchez-Bueno F, Figueras J, Jaurrieta E, Mir J, Margarit C et al.* Analysis of the complications of the piggy-back technique in 1,112 liver transplants. *Transplantation*. 1999; 67: 1214–1217. https://doi.org/10.1097/00007890-199905150-00003.
111. *Sánchez-Bueno F, Hernández Q, Ramírez P, Robles R, Acosta F, Rodríguez JM, Parrilla P.* Vascular complications in a series of 300 orthotopic liver transplants. *Transplant Proc*. 1999; 31: 2409–2410. https://doi.org/10.1016/s0041-1345(99)00406-6.
112. *Lerut J, Tzakis AG, Bron K, Gordon RD, Iwatsuki S, Esquivel CO et al*. Complications of venous reconstruction in human orthotopic liver transplantation. *Ann Surg*. 1987; 205: 404–414. https://doi.org/10.1097%2F00000658-198704000-00011.
113. *Kyoden Y, Tamura S, Sugawara Y, Matsui Y, Togashi J, Kaneko J et al*. Portal vein complications after adult-to-adult living donor liver transplantation. *Transpl Int.* 2008; 21: 1136–1144. https://doi.org/10.1111/j.1432-2277.2008.00752.x.
114. *Kaneko J, Sugawara Y, Ohkubo T, Matsui Y, Kokudo N, Makuuchi M.* Successful conservative therapy for portal vein thrombosis after living donor liver transplantation. *Abdom Imaging*. 2003; 28: 58–59. https://doi.org/10.1007/s00261-001-0151-3.
115. *Cheng YF, Ou HY, Tsang LL, Yu CY, Huang TL, Chen TY et al.* Vascular stents in the management of portal venous complications in living donor liver transplantation. *Am J Transplant*. 2010; 10: 1276–1283. https://doi.org/10.1111/j.1600-6143.2010.03076.x.
116. *Azzam AZ, Tanaka K.* Management of vascular complications after living donor liver transplantation. *Hepatogastroenterology*. 2012; 59: 182–186. https://doi.org/10.5754/hge10453.
117. *Abdelaziz O, Hosny K, Elmalt O, Emad-Eldin S, Hosny A.* Intra-operative Ultrasound-guided Thrombectomy and Thrombolysis for Post-operative Portal Vein Thrombosis in Living Liver Donors. *Int J Organ Transplant Med*. 2015; 6: 33–40. PMID: 25737775; PMCID: PMC4346461.
118. *Huang TL, Cheng YF, Chen TY, Tsang LL, Ou HY, Yu CY et al.* Doppler ultrasound evaluation of postoperative portal vein stenosis in adult living donor liver transplantation. *Transplant Proc*. 2010; 42: 879–881. https://doi.org/10.1016/j.transproceed.2010.02.036.
119. *Lee SJ, Kim KW, Kim SY, Park YS, Lee J, Kim HJ et al*. Contrast-enhanced sonography for screening of vascular complication in recipients following living donor liver transplantation. *J Clin Ultrasound*. 2013; 41: 305–312. https://doi.org/10.1002/jcu.22044.
120. *Lee H, Lim CW, Yoo SH, Koo CH, Kwon WI, Suh KS, Ryu HG.* The effect of Doppler ultrasound on early vascular interventions and clinical outcomes after liver transplantation. *World J Surg*. 2014; 38: 3202–3209. https://doi.org/10.1007/s00268-014-2721-x.
121. *Ma L, Lu Q, Luo Y.* Vascular complications after adult living donor liver transplantation: Evaluation with ultrasonography. *World J Gastroenterol*. 2016 Jan 28; 22 (4): 1617–1626. https://doi.org/10.3748/wjg.v22.i4.1617.
122. *Saad WE.* Portal interventions in liver transplant recipients. *Semin Intervent Radiol.* 2012 Jun; 29 (2): 99–104. doi: 10.1055/s-0032-1312570.
123. *Durham JD, LaBerge JM, Altman S, Kam I, Everson GT, Gordon RL, Kumpe DA*. Portal vein thrombolysis and closure of competitive shunts following liver transplantation. *J Vasc Interv Radiol*. 1994; 5: 611–615; discussion 616–618. https://doi.org/10.1016/s1051-0443(94)71562-1.
124. *Cherukuri R, Haskal ZJ, Naji A, Shaked A*. Percutaneous thrombolysis and stent placement for the treatment of portal vein thrombosis after liver transplantation: long-term follow-up. *Transplantation*. 1998; 65: 1124–1126. https://doi.org/10.1097/00007890-199804270-00018.
125. *Kensinger CD, Sexton KW, Baron CM, Lipnik AJ, Meranze SG, Gorden DL.* Management of portal vein thrombosis after liver transplantation with a combined open and endovascular approach. *Liver Transpl*. 2015; 21: 132–134. https://doi.org/10.1002/lt.24011.
126. *Haskal ZJ, Naji A.* Treatment of portal vein thrombosis after liver transplantation with percutaneous thrombolysis and stent placement. *J Vasc Interv Radiol*. 1993; 4: 789–792. https://doi.org/10.1016/s1051-0443(93)71974-0.
127. *Bhattacharjya T, Olliff SP, Bhattacharjya S, Mirza DF, McMaster P.* Percutaneous portal vein thrombolysis and endovascular stent for management of posttransplant portal venous conduit thrombosis. *Transplantation*. 2000; 69: 2195–2198. https://doi.org/10.1097/00007890-200005270-00042.
128. *Baccarani U, Gasparini D, Risaliti A, Vianello V, Adani GL, Sainz M et al.* Percutaneous mechanical fragmentation and stent placement for the treatment

of early posttransplantation portal vein thrombosis. *Transplantation*. 2001; 72: 1572–1582. https://doi.org/10.1097/00007890-200111150-00016.

129. *Lerut JP, Goffette P, Molle G, Roggen FM, Puttemans T, Brenard R et al*. Transjugular intrahepatic portosystemic shunt after adult liver transplantation: experience in eight patients. *Transplantation*. 1999; 68: 379–384. https://doi.org/10.1097/00007890-199908150-00009.
130. *Ciccarelli O, Goffette P, Laterre PF, Danse E, Wittebolle X, Lerut J.* Transjugular intrahepatic portosystemic shunt approach and local thrombolysis for treatment of early posttransplant portal vein thrombosis. *Transplantation*. 2001; 72: 159–161. https://doi.org/10.1097/00007890-200107150-00030.
131. *López-Benítez R, Barragán-Campos HM, Richter GM, Sauer P, Mehrabi A, Fonouni H et al*. Interventional radiologic procedures in the treatment of complications after liver transplantation. *Clin Transplant*. 2009; 23 Suppl 21: 92–101. https://doi.org/10.1111/j.1399-0012.2009.01115.x.
132. *Cavallari A, Vivarelli M, Bellusci R, Jovine E, Mazziotti A, Rossi C.* Treatment of vascular complications following liver transplantation: multidisciplinary approach. *Hepatogastroenterology*. 2001; 48: 179–183. PMID: 11268960.
133. *Schneider N, Scanga A, Stokes L, Perri R.* Portal vein stenosis: a rare yet clinically important cause of delayed-onset ascites after adult deceased donor liver transplantation: two case reports. *Transplant Proc*. 2011; 43: 3829–3834. https://doi.org/10.1016/j.transproceed.2011.09.068.
134. *Wei BJ, Zhai RY, Wang JF, Dai DK, Yu P.* Percutaneous portal venoplasty and stenting for anastomotic stenosis after liver transplantation. *World J Gastroenterol*. 2009; 15: 1880–1885. https://doi.org/10.3748%2Fwjg.15.1880.
135. *Semash KO, Dzhanbekov TA, Akbarov MM, Usmonov AA, Shermatov MM, Yigitaliev SKh, Gaibullaev TZ.* Interventional correction of extrahepatic portal hypertension in a patient after liver transplantation. The first clinical observation in the Republic of Uzbekistan. *Vestnik Tashkentskoy meditsinskoy akademii*. 2023; (4): 157–162.
136. *Shibata T, Itoh K, Kubo T, Maetani Y, Shibata T, Togashi K, Tanaka K.* Percutaneous transhepatic balloon dilation of portal venous stenosis in patients with living donor liver transplantation. *Radiology*. 2005; 235: 1078–1083. https://doi.org/10.1148/radiol.2353040489.
137. *Ko GY, Sung KB, Yoon HK, Lee S.* Early posttransplantation portal vein stenosis following living donor liver transplantation: percutaneous transhepatic primary stent placement. *Liver Transpl*. 2007; 13: 530–536. https://doi.org/10.1002/lt.21068.
138. *Olcott EW, Ring EJ, Roberts JP, Ascher NL, Lake JR, Gordon RL.* Percutaneous transhepatic portal vein angioplasty and stent placement after liver transplantation: early experience. *J Vasc Interv Radiol*. 1990; 1: 17–22. https://doi.org/10.1016/s1051-0443(90)72496-7.
139. *Zajko AB, Sheng R, Bron K, Reyes J, Nour B, Tzakis A.* Percutaneous transluminal angioplasty of venous anastomotic stenoses complicating liver transplantation: intermediate-term results. *J Vasc Interv Radiol*. 1994; 5: 121–126. https://doi.org/10.1016/s1051-0443(94)71467-6.
140. *Park KB, Choo SW, Do YS, Shin SW, Cho SG, Choo IW.* Percutaneous angioplasty of portal vein stenosis that complicates liver transplantation: the mid-term therapeutic results. *Korean J Radiol*. 2005; 6: 161–166. https://doi.org/10.3348%2Fkjr.2005.6.3.161.
141. *Shiba H, Sadaoka S, Wakiyama S, Ishida Y, Misawa T, Yanaga K.* Successful treatment by balloon angioplasty under portography for late-onset stenosis of portal vein after cadaveric liver transplantation. *Int Surg.* 2013; 98: 466–468. https://doi.org/10.9738%2FINTSURG-D-12-00031.1.
142. *Audet M, Piardi T, Panaro F, Cag M, Habibeh H, Gheza F et al*. Four hundred and twenty-three consecutive adults piggy-back liver transplantations with the three suprahepatic veins: was the portal systemic shunt required? *J Gastroenterol Hepatol*. 2010; 25: 591–596. https://doi.org/10.1111/j.1440-1746.2009.06084.x.
143. *Schmitz V, Schoening W, Jelkmann I, Globke B, Pascher A, Bahra M et al*. Different cava reconstruction techniques in liver transplantation: piggyback versus cava resection. *Hepatobiliary Pancreat Dis Int*. 2014; 13: 242–249. https://doi.org/10.1016/s1499-3872(14)60250-2.
144. *Navarro F, Le Moine MC, Fabre JM, Belghiti J, Cherqui D, Adam R et al*. Specific vascular complications of orthotopic liver transplantation with preservation of the retrohepatic vena cava: review of 1361 cases. *Transplantation*. 1999; 68: 646–650. https://doi.org/10.1097/00007890-199909150-00009.
145. *Starzl TE, Groth CG, Brettschneider L, Penn I, Fulginiti VA, Moon JB et al*. Orthotopic homotransplantation of the human liver. *Ann Surg.* 1968; 168: 392–415. https://doi.org/10.1097%2F00000658-196809000-00009.
146. *Calne RY, Williams R.* Liver transplantation in man. I. Observations on technique and organization in five cases. *Br Med J*. 1968; 4: 535–540. https://doi.org/10.1136%2Fbmj.4.5630.535.
147. *Tzakis A, Todo S, Starzl TE.* Orthotopic liver transplantation with preservation of the inferior vena cava. *Ann Surg*. 1989; 210: 649–652. https://doi.org/10.1097%2F00000658-198911000-00013.
148. *Belghiti J, Panis Y, Sauvanet A, Gayet B, Fékété F.* A new technique of side to side caval anastomosis during orthotopic hepatic transplantation without inferior vena caval occlusion. *Surg Gynecol Obstet.* 1992; 175: 270–272. PMID: 1514163.
149. *Bismuth H, Castaing D, Sherlock DJ.* Liver transplantation by "face-à-face" venacavaplasty. *Surgery*. 1992; 111: 151–155. PMID: 1736384.
150. *Cherqui D, Lauzet JY, Rotman N, Duvoux C, Dhumeaux D, Julien M, Fagniez PL.* Orthotopic liver transplantation with preservation of the caval and portal flows. Technique and results in 62 cases. *Transplantation*. 1994; 58: 793–796. PMID: 7940712.
151. *Kishi Y, Sugawara Y, Matsui Y, Akamatsu N, Makuuchi M.* Late onset portal vein thrombosis and its risk

factors. *Hepatogastroenterology*. 2008; 55: 1008–1009. PMID: 18705318.

152. *Darcy MD.* Management of venous outflow complications after liver transplantation. *Tech Vasc Interv Radiol*. 2007; 10: 240–245. https://doi.org/10.1053/j.tvir.2007.09.018.
153. *Weeks SM, Gerber DA, Jaques PF, Sandhu J, Johnson MW, Fair JH, Mauro MA.* Primary Gianturco stent placement for inferior vena cava abnormalities following liver transplantation. *J Vasc Interv Radiol*. 2000; 11: 177–187. https://doi.org/10.1016/s1051-0443(07)61462-6.
154. *Yamagiwa K, Yokoi H, Isaji S, Tabata M, Mizuno S, Hori T et al.* Intrahepatic hepatic vein stenosis after living-related liver transplantation treated by insertion of an expandable metallic stent. *Am J Transplant*. 2004; 4: 1006–1009. https://doi.org/10.1111/j.1600-6143.2004.00440.x.
155. *Wang SL, Sze DY, Busque S, Razavi MK, Kee ST, Frisoli JK, Dake MD.* Treatment of hepatic venous outflow obstruction after piggyback liver transplantation. *Radiology*. 2005; 236: 352–359. https://doi.org/10.1148/radiol.2361040327.
156. *Liu XL, Li FQ, Li X, Li B, Yan LN, Wei YG.* Treatment of hepatic venous outflow stenosis after living donor liver transplantation by insertion of an expandable metallic stent. *Hepatobiliary Pancreat Dis Int*. 2009; 8: 424–427. PMID: 19666414.
157. *Ikeda O, Tamura Y, Nakasone Y, Yamashita Y, Okajima H, Asonuma K, Inomata Y.* Percutaneous transluminal venoplasty after venous pressure measurement in patients with hepatic venous outflow obstruction after living donor liver transplantation. *Jpn J Radiol*. 2010; 28: 520–526. https://doi.org/10.1007/s11604-010-0463-8.
158. *Lee JM, Ko GY, Sung KB, Gwon DI, Yoon HK, Lee SG.* Long-term efficacy of stent placement for treating inferior vena cava stenosis following liver transplantation. *Liver Transpl.* 2010; 16: 513–519. https://doi.org/10.1002/lt.22021.
159. *Ferro C, Andorno E, Guastavino A, Rossi UG, Seitun S, Bovio G, Valente U.* Endovascular treatment with primary stenting of inferior cava vein torsion following orthotopic liver transplantation with modified piggyback technique. *Radiol Med*. 2014; 119: 183–188. https://doi.org/10.1007/s11547-013-0325-4.
160. *Lorenz JM, van Beek D, Funaki B, Van Ha TG, Zangan S, Navuluri R, Leef JA.* Long-term outcomes of percutaneous venoplasty and Gianturco stent placement to treat obstruction of the inferior vena cava complicating liver transplantation. *Cardiovasc Intervent Radiol*. 2014; 37: 114–124. https://doi.org/10.1007/s00270-013-0643-x.
161. *Campos NMF, Donato P.* Hepatic Artery Pseudoaneurysm after Hepatic Transplant – Endovascular Treatment with Graft Stenting. *J Angiol Vasc Surg.* 2022; 7: 089. http://dx.doi.org/10.24966/AVS-7397/100091.
162. *Sureka B, Bansal K, Rajesh S, Mukund A, Pamecha V, Arora A.* Imaging panorama in postoperative complications after liver transplantation. *Gastroenterology Report*. 2016 May; 4 (2): 96–106. https://doi.org/10.1093/gastro/gov057.
163. *Kimura Y, Tapia Sosa R, Soto-Trujillo D, Kimura Sandoval Y, Casian C.* Liver Transplant Complications Radiologist Can't Miss. *Cureus.* 2020 Jun 5; 12 (6): e8465. https://doi.org/10.7759%2Fcureus.8465.
164. *Couri T, Harmath C, Baker T, Pillai A.* Acute portal vein thrombosis after liver transplant presenting with subtle ultrasound abnormalities: A case report and literature review. *World J Hepatol*. 2019 Feb 27; 11 (2): 234–241. https://doi.org/10.4254%2Fwjh.v11.i2.234.
165. *Osman AM, Hosny AA, El-Shazli MA, Uemoto S, Abdelaziz O, Helmy AS.* A portal pressure cut-off of 15 versus a cut-off of 20 for prevention of small-for-size syndrome in liver transplantation: a comparative study. *Hepatol Res*. 2017; 47 (4): 293–302. doi: 10.1111/hepr.12727.
166. *Hori T, Ogura Y, Yagi S, Iida T, Taniguchi K, El Moghazy WM et al.* How do transplant surgeons accomplish optimal portal venous flow during living-donor liver transplantation? Noninvasive measurement of indocyanine green elimination rate. *Surg Innov*. 2014; 21 (1): 43–51. doi: 10.1177/1553350613487803.
167. *Ogura Y, Hori T, El Moghazy WM, Yoshizawa A, Oike F, Mori A et al.* Portal pressure <15 mm Hg is a key for successful adult living donor liver transplantation utilizing smaller grafts than before. *Liver Transpl*. 2010; 16 (6): 718–728. doi: 10.1002/lt.22059.
168. *Blasi A, Molina V, Sanchez-Cabús S, Balust J, Garcia-Valdecasas JC, Taura P.* Prediction of thromboembolic complications after liver resection for cholangiocarcinoma: is there a place for thromboelastometry? *Blood Coagul Fibrinolysis*. 2018; 29: 61–66. https://doi.org/10.1097/mbc.0000000000000672.
169. *Kaneko J, Sugawara Y, Tamura S, Togashi J, Matsui Y, Akamatsu N et al.* Coagulation and fibrinolytic profiles and appropriate use of heparin after living-donor liver transplantation. *Clin Transplant*. 2005; 19: 804–809. https://pubmed.ncbi.nlm.nih.gov/16313329.
170. *Gad EH, Abdelsamee MA, Kamel Y.* Hepatic arterial and portal venous complications after adult and pediatric living donor liver transplantation, risk factors, management and outcome (A retrospective cohort study). *Ann Med Surg (Lond)*. 2016; 8: 28–39. https://doi.org/10.1016%2Fj.amsu.2016.04.021.
171. *Sugawara Y, Kaneko J, Akamatsu N, Imamura H, Kokudo N, Makuuchi M.* Anticoagulant therapy against hepatic artery thrombosis in living donor liver transplantation. *Transplant Proc*. 2002; 34: 3325–3326. https://doi.org/10.1016/s0041-1345(02)03576-5.
172. *Mori A, Iida T, Iwasaki J, Ogawa K, Fujimoto Y, Uemura T et al.* Portal vein reconstruction in adult living donor liver transplantation for patients with portal vein thrombosis in single center experience. *J Hepatobiliary Pancreat Sci*. 2015; 22: 467–474. https://doi.org/10.1002/jhbp.235.
173. *Gautier S, Monakhov A, Tsiroulnikova O, Mironkov B, Voskanov M, Dzhanbekov T et al.* Time is of the essence: A single-center experience of hepatic arterial supply impairment management in pediatric liver transplant reci-

pients. *Pediatr Transplant*. 2021; 25: e13934. https://doi.org/10.1111/petr.13934.

174. *Moore FA, Moore EE, Seagraves A.* Nonresectional management of major hepatic trauma. An evolving concept. *Am J Surg.* 1985; 150: 725–729. https://doi.org/10.1016/0002-9610(85)90417-9.
175. *Steinbrück K, Enne M, Fernandes R, Martinho JM, Balbi E, Agoglia L et al*. Vascular complications after living donor liver transplantation: a Brazilian, single-center experience. *Transplant Proc.* 2011 Jan-Feb; 43 (1): 196–198. https://doi.org/10.1016/j.transproceed.2010.12.007.
176. *Boleslawski E, Bouras AF, Truant S, Liddo G, Herrero A, Badic B et al.* Hepatic artery ligation for arterial rupture following liver transplantation: a reasonable option. *Am J Transplant.* 2013; 13: 1055–1062. https://doi.org/10.1111/ajt.12135.
177. *Saad WE.* Nonocclusive hepatic artery hypoperfusion syndrome (splenic steal syndrome) in liver transplant recipients. *Semin Intervent Radiol*. 2012; 29: 140–146. https://doi.org/10.1055%2Fs-0032-1312576.
178. *Geissler I, Lamesch P, Witzigmann H, Jost U, Hauss J, Fangmann J.* Splenohepatic arterial steal syndrome in liver transplantation: clinical features and management. *Transpl Int.* 2002; 15: 139–141. https://doi.org/10.1007/s00147-002-0386-0.
179. *Sevmis S, Boyvat F, Aytekin C, Gorur SK, Karakayali H, Moray G, Haberal M.* Arterial steal syndrome after orthotopic liver transplantation. *Transplant Proc*. 2006; 38: 3651–3655. https://doi.org/10.1016/j.transproceed.2006.10.145.
180. *Kirbas I, Ulu EM, Ozturk A, Coskun M, Harman A, Ogus E, Haberal M.* Multidetector computed tomographic angiography findings of splenic artery steal syndrome in liver transplantation. *Transplant Proc*. 2007; 39: 1178–1180. https://doi.org/10.1016/j.transproceed.2007.02.024.
181. *Mogl MT, Nüssler NC, Presser SJ, Podrabsky P, Denecke T, Grieser C et al*. Evolving experience with prevention and treatment of splenic artery syndrome after orthotopic liver transplantation. *Transpl Int.* 2010 Aug; 23: 831–841. https://doi.org/10.1111/j.1432-2277.2010.01062.x.
182. *Grieser C, Denecke T, Steffen IG, Avgenaki M, Fröhling V, Mogl M et al.* Multidetector computed tomography for preoperative assessment of hepatic vasculature and prediction of splenic artery steal syndrome in patients with liver cirrhosis before transplantation. *Eur Radiol*. 2010; 20: 108–117. https://doi.org/10.1007/s00330-009-1535-y.
183. *Yip J, Bruno DA, Burmeister C, Kazimi M, Yoshida A, Abouljoud MS, Schnickel GT.* Deep Vein Thrombosis and Pulmonary Embolism in Liver Transplant Patients: Risks and Prevention. *Transplant Direct*. 2016; 2: e68. https://doi.org/10.1097/txd.0000000000000578.
184. *Vivarelli M, La Barba G, Cucchetti A, Lauro A, Del Gaudio M, Ravaioli M et al*. Can antiplatelet prophylaxis reduce the incidence of hepatic artery thrombosis after liver transplantation? *Liver Transpl*. 2007; 13: 651–654. https://doi.org/10.1002/lt.21028.
185. *Uchikawa Y, Ikegami T, Masuda Y, Ohno Y, Mita A, Urata K et al*. Administration of dalteparin based on the activated clotting time for prophylaxis of hepatic vessel thrombosis in living donor liver transplantation. Transplant Proc. 2009; 41: 3784–3790. https://doi.org/10.1016/j.transproceed.2009.04.011.

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CASE REPORT ON PROLONGED KIDNEY GRAFT SURVIVAL WITHOUT IMMUNOSUPPRESSIVE THERAPY AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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Background. The possibility of inducing immunological tolerance in allogeneic organ transplant recipients is a research goal of the transplantology community, as it will ensure the likelihood of complete engraftment of a foreign organ. However, such a task presently remains difficult to accomplish. **Objective:** to demonstrate long-term kidney graft survival without signs of acute rejection and without immunosuppressive therapy in a patient who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) from a haploidentical donor for post-transplant lymphoproliferative disorder (PTLD). **Methods and materials.** Recipient's graft function was assessed using clinical, laboratory, instrumental and pathomorphological examination methods. **Results.** With no immunosuppressive therapy for more than four years, the kidney recipient showed stable, satisfactory graft function. **Conclusion.** The described clinical case demonstrates the development of immunological tolerance to a kidney graft in a recipient of allogeneic hematopoietic stem cells (HSCs).

Keywords: immunological tolerance, kidney transplantation, chimerism.

INTRODUCTION

The main challenge of organ transplantation is the body's immune response, manifested by acute or chronic rejection reactions. Immunosuppressive therapy (IST) in most cases suppresses the immune system and allows transplanted organs to function for a limited time. Currently, all known immunosuppressants have more or less significant side effects. This has encouraged researchers to develop new ways to suppress the immune system [1]. It is optimal to develop immune tolerance in the recipient, allowing the graft to function as long and efficiently as possible. Immune tolerance is understood as the absence of a specific response to certain foreign antigens, while retaining the ability to develop a full-fledged response to other antigens. The coexistence of cells of more than one genotype in the same individual is called a biological chimera. Chimerism is categorized into full and mixed chimerism. Full chimerism exists when all hematopoietic cells are of donor origin, while mixed chimerism is the coexistence of both donor and recipient cells in different proportions [2]. Mixed hematopoietic chimerism was first demonstrated by Ray Owen in 1945. He showed that bovine fraternal twins with common placental circulation are chimeric and tolerant to each other [3]. In 1953, Billingham, Brent, Medawar et al. described a state of "actively acquired tolerance" to skin allografts that developed after transplantation of viable allogeneic cells into embryos or newborn mice [4]. Studies of mixed chimerism and immunological tolerance in animals have led to the use of HSCs transplantation as a method of inducing tolerance in solid organ transplantation in humans [5–7].

Tolerance formation is a determining condition for long-term functioning of transplanted organs and tissues. Central and peripheral tolerance differ in terms of mechanism of development. Central tolerance is aimed at preventing the appearance of autoreactive T and B lymphocytes in the process of their maturation and occurs in the central organs of immunogenesis in the thymus and bone marrow [8, 9]. In this case, elimination of potentially dangerous T lymphocytes reacting to their own antigens is effected by inducing programmed cell death (apoptosis). This mechanism is called clonal deletion or negative selection. For B lymphocytes, another mechanism is also possible – receptor editing. With receptor editing, receptors can no longer bind to their own antigens. In allo-HSCT, central tolerance is a key mechanism and is determined by the presence and selection of donor immune cells in the thymus [10]. In

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allo-HSCT, immature T cells that are newly formed in the bone marrow for further maturation and proliferation, populate the thymus, where they actively proliferate and undergo positive selection by binding to short fragments of proteins on the major histocompatibility complex (MHC) molecules, which in humans are designated as HLA (human leukocyte antigens) classes I and II. As a result, the process of positive selection leads to the survival of mature CD8+ and CD4+ T cells that can recognize HLA molecules [11]. If T cell receptors bind too strongly to HLA molecules in the thymus, intracellular signaling is so strong that it ends in apoptotic cell death, thereby destroying cells with a high probability of autoreactivity (negative selection).

Peripheral (postthymic) tolerance is aimed at identifying and controlling autoreactive cell clones that have escaped central tolerance mechanisms. Peripheral tolerance is ensured by various mechanisms (1) by ignoring the antigen when there is insufficient or excess quantity of it, and when antigen presentation is impaired, T cell anergy occurs due to insufficient expression of T cell receptor or coreceptor molecules; (2) there is negative activation of lymphocytes leading them to apoptosis; (3) under the action of regulatory T cells. Given the toxicity of IST, the search for approaches that are aimed at tolerance induction after solid organ transplantation continues. These approaches include costimulatory blockade, lymphodepletion, induction of regulatory T cell formation, and mixed chimerism [12]. Despite the progress made in research on induction of immune tolerance in animal models, transferring the proposed strategies to humans is a challenging task.

Attempts to avoid or minimize IST in patients after kidney transplantation lead to rapid graft rejection and graft loss [13]. In this clinical case report, we can speak about the formation of tolerance to a transplanted organ in the patient.

Objective: to demonstrate long-term kidney graft survival without signs of acute rejection and without IST in a patient who underwent allo-HSCT from a haploidentical donor for PTLD.

CLINICAL CASE REPORT

Patient B., female, born in 1985, was diagnosed with mesangial proliferative glomerulonephritis in adolescence after suffering from pharyngitis, and received pathogenetic therapy involving prednisolone and mycophenolates. By adulthood, the girl had progression of chronic kidney disease to the end stage. As a result, long-term hemodialysis therapy was initiated. In 2011, at the age of 25, she underwent kidney allotransplantation from a deceased donor at Sklifosovsky Research Institute of Emergency Care. Mismatches in MHC antigens in the donor-recipient pair consisted of four antigens: A3, B7, 35, and Dr10. The patient received standard IST: tacrolimus, mycophenolates, prednisolone and basiliximab induction on postoperative days 0 and 4. Graft function was delayed, diuresis was restored from postoperative day 38, blood creatinine levels (121 μmol/l) normalized on day 57. In 2013, she suffered acute graft pyelonephritis, then a graft biopsy was performed, which revealed grade 1 chronic allograft nephropathy. In 2016, due to a planned pregnancy, mycophenolates were converted to azathioprine. She was in a satisfactory condition until April 2017; blood nitrogen metabolism parameters were creatinine 110–130 μmol/L and urea 7–8 mmol/L.

In May 2017, her condition deteriorated, which was clinically manifested by food-borne toxicoinfection, blood creatinine levels increased to 170 μmol/l. From June to August 2017, her condition worsened: weakness, fever episodes, stomach heaviness and increase in stomach size, dry cough; she did not seek medical help. In August 2017, she was hospitalized at the Transplanted Kidney Pathology ward of the Municipal Clinical Hospital No. 52 with leukopenia (2.8×10^9), proteinuria (2.3 g/day), elevation of C-reactive protein (to 115 mg/dL) and blood creatinine level (214 μmol/L). Azathioprine was discontinued due to leukopenia. PTLD was suspected; trepanobiopsy and sternal puncture were performed, and paraproteinemia tests were taken. On August 23, 2017, she was transferred to the National Medical Research Center for Hematology, where the final diagnosis was established: diffuse large B cell lymphoma, DLBCL (post-transplant lymphoproliferative disorder, PTLD) with liver, spleen, stomach and bone marrow involvement, probably associated with IST. Given the severity of the condition, sepsis and immunodeficiency, the remaining IST (tacrolimus and prednisolone) was discontinued. For the treatment of DLBCL, the patient received 6 cycles of CHOD (total doses: cyclophosphamide 1200 mg, doxorubicin 75 mg, vincristine 2 mg, dexamethasone 80 mg) from August 26, 2017 to January 11, 2018, in combination with rituximab (600 mg); the first cycle was performed with a prephase dexamethasone and cyclophosphamide. After that, the patient was in remission of the lymphoproliferative disorder for a year.

After completion of chemotherapy (6 courses), due to the risk of kidney graft rejection, tacrolimus-based IST (2 mg/day) was resumed from April 2018.

In January 2019, PTLD-DLBCL reoccurred and 3 polychemotherapy courses (cytarabine (Σ = 4 g) + etoposide (Σ = 400 mg) combined with lenalidomide (Σ = 30 mg)) were administered. The first course was given with a prephase cyclophosphamide and dexamethasone. A second PET-negative remission of PTLD-DBCCL was achieved. There was still a need to resume IST to prevent graft rejection, which would entail another relapse of the disease. Therefore, on May 14, 2019, the patient underwent HSCs transplantation from a related haploidentical donor (mother) using the TCRαβ+/CD19+ graft depletion technique. The choice of this transplantation approach was based on the fact that it does not invol-

ve long-term (6 months) IST. Graft-versus-host disease prophylaxis was performed according to the scheme: rituximab + bortezomib + tocilizumab + abatacept, and it was completed 1 month after haploidentical allo-HSCT. It was assumed that the donor immune system would "accept" the kidney graft as a host tissue and that tolerance to the graft would also be formed.

Due to not taking immunosuppressive drugs within three months from the moment of haploidentical allo-HSCT, the patient was examined at Sklifosovsky Research Institute of Emergency Care in August 2019 to assess the state of the kidney graft. No evidence of kidney allograft (KA) rejection was found – blood creatinine 120 μmol/L, glomerular filtration rate (GFR) 34 ml/min, no anti-HLA antibodies were detected. Considering the non-standard clinical situation and the patient's informed consent, a kidney transplant biopsy was carried out. The biopsy revealed no signs of rejection; moderate focal global glomerulosclerosis, arteriosclerosis, interstitial fibrosis and tubular atrophy were observed (Fig. 1).

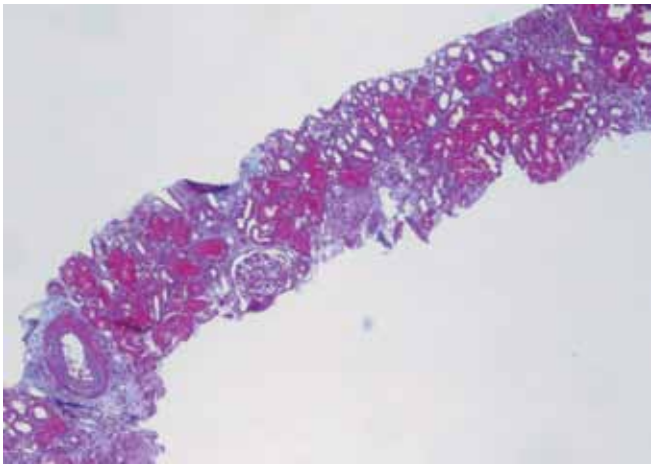

Fig. 1. Micrograph. Kidney transplant specimen: focal global glomerulosclerosis, arteriolosclerosis, interstitial fibrosis and tubular atrophy grade 2-3

Transplantologists and hematologists jointly decided to continue monitoring the patient without prescribing IST due to the high risk of recurrent DLBCL, as well as due to the high probability of developing immunological tolerance as a result of the functioning of the new donor immune system.

At patient B.'s repeated follow-up hospitalization in April 202: the patient's condition was satisfactory and stable. During hospitalization, blood creatinine levels fluctuated in the 162–178 μmol/L range, GFR was 54 ml/min, daily proteinuria 1 g. Ultrasound examination found no evidence of graft dysfunction: graft dimensions were within normal values, 112 × 49 mm, there was arterial blood flow in the entire kidney up to the capsule, resistivity indices were 0.56–0.66 (Fig. 2).

Given the moderate increase in blood creatinine levels and proteinuria, kidney graft biopsy was performed. The histologic findings showed chronic inactive rejection, moderate interstitial fibrosis and tubular atrophy (Fig. 3). Deposition of complement component C4d in the renal graft tissues was not detected by immunofluorescence. When blood plasma was examined for the presence of anti-HLA antibodies, including donor-specific antibodies, the test returned negative.

Given the absence of active kidney graft rejection, no anti-crisis therapy was administered to the patient. In the hospital, conservative treatment was performed to improve microcirculation in the KA, and blood creatinine decreased to 150 μmol/L. The patient was discharged with recommendations to continue follow-up without taking immunosuppressants.

During follow-up examination in March 2022, patient B. remained without IST, was in good health, quality of life was high, and was socially adapted – she worked as a programmer. Laboratory tests: blood creatinine was

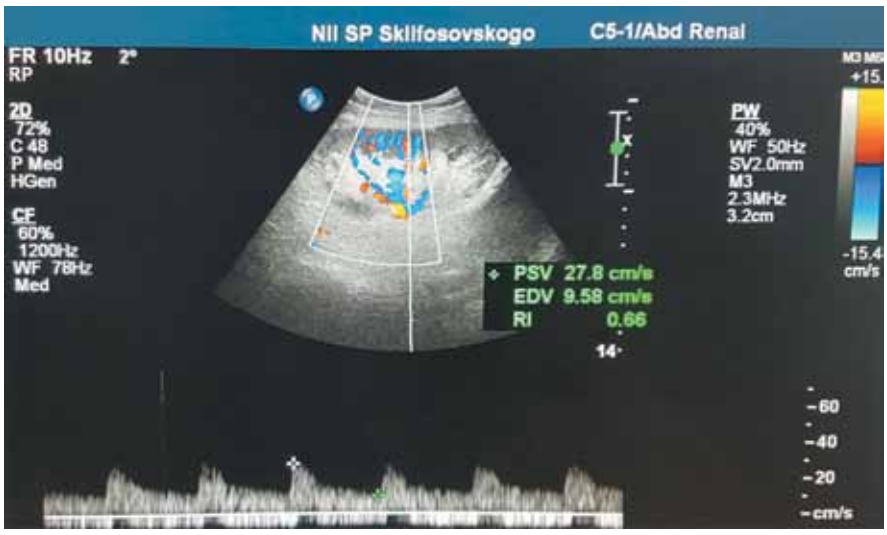

Fig. 2. Ultrasonogram of the kidney graft of patient B. April 2021

168 μmol/L, urea 13.6 mmol/L, GFR 44 l/min, and daily proteinuria 0.86 g/day. Ultrasound examination found no evidence of graft dysfunction. The doctors decided not to perform a biopsy due to stable graft function.

In January 2023, the patient was called for a routine checkup. She did not take immunosuppressive drugs. Her health was satisfactory, and she continued to work. Her blood creatinine level was 154 μmol/L, urea was 17 mmol/L, GFR was 44 ml/min, and daily proteinuria 0.87 g. No anti-MHC antigen antibodies were detected in the blood. Ultrasound examination found no signs of KA dysfunction (Fig. 4).

Thus, evidence obtained suggests a stable satisfactory renal graft function in the patient who has not received IST for more than 4 years. At the time of writing this paper, graft function lasted for 12 years. Control examinations showed that PET-negative remission of PTLD-DLBCL, on the background of 100% donor chimerism, with no signs of graft-versus-host reaction, persisted within 4 years after haploidentical allo-HSCT.

DISCUSSION

The presented clinical case is a unique example of the development of tolerance to a transplanted solid organ against the background of formation of full hematopoietic donor chimerism due to transplantation of HSCs. It is worth noting that the approach in which immunological tolerance was observed when using allo-HSCT in solid organ transplantation, is considered the most promising direction, making IST unnecessary. Thus, the results of three pilot studies on induction of tolerance to renal allografts from living donors, performed at Stanford, Northwestern, and Massachusetts General Hospitals have been published [14, 15]. Patients underwent combined kidney and bone marrow transplantation from an HLA-mismatched donor. All subjects received non-

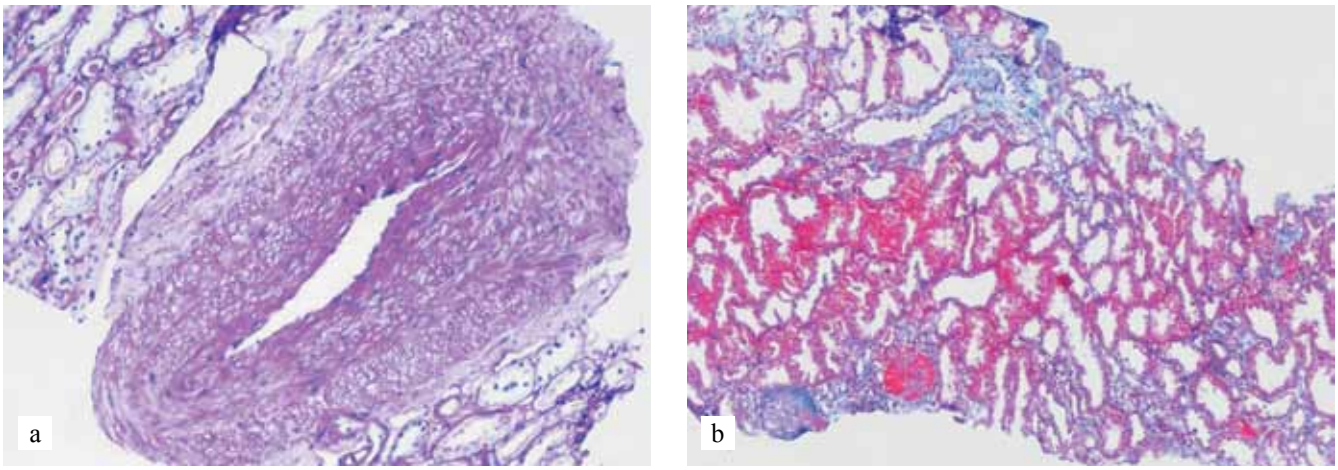

Fig. 3. Micrograph. Kidney graft specimens: a, transplant vasculopathy (arterial wall thickening due to myointimal proliferation and intimal fibrosis, inflammatory cells in the intima thickness); b, focal global glomerulosclerosis, interstitial fibrosis and tubular atrophy grade 2

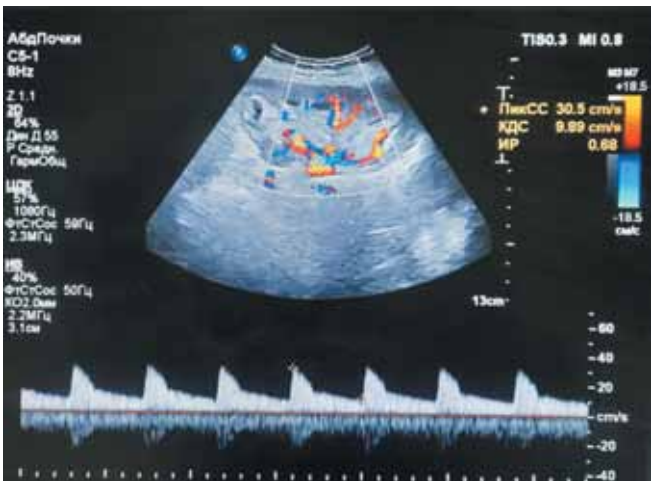

Fig. 4. Ultrasonogram of the kidney graft (12 years after kidney transplantation). Resistive index in the segmental artery of the renal graft is within normal range

myeloablative conditioning at the stages of preparation for transplantation. Patients achieved stable chimerism in 38.5% of cases and transient chimerism in 26% of cases, which allowed complete withdrawal of IST in 63% of cases. Despite good renal graft engraftment, this approach is extremely limited due to the high risk of graft-versus-host disease [12, 14, 15].

The uniqueness of this case of tolerance to a kidney allograft is due to a number of major differences from the strategies of induction of hematopoietic chimerism described in the literature. An aggressive B-cell lymphoma emerging six years after kidney transplantation was a complication of long-term IST in the patient. PTLD treatment included the first and repeated stages of long-term chemotherapy, and subsequently, transplantation of allogeneic HSCs from a related haploidentical donor. We can assume that the formation of immunological

tolerance to the kidney allograft is most likely due to the fact that the cells of the donor immune system, during the process of engraftment and expansion, saw the kidney graft as a host tissue via a universal mechanism. Since all antigens in the recipient's body are foreign to the donor immune system, the donor alloreactive T cells were restricted against the kidney allograft as well.

Thus, because after allo-HSCT, immunological tolerance was induced not only to all host tissues but also to the tissue functioning in the kidney recipient's body from another donor, there was no need for lifelong immunosuppressants.

CONCLUSION

The clinical case described represents the only successful case of formation of immunological tolerance in our practice, which allows a kidney transplant recipient to get along without IST for more than 4 years.

The authors declare no conflict of interest.

REFERENCES

1. *Willekens B, Wens I, Wouters K, Cras P, Cools N*. Safety and immunological proof-of-concept following treatment with tolerance-inducing cell products in patients with autoimmune diseases or receiving organ transplantation: A systematic review and meta-analysis of clinical trials. *Autoimmun Rev*. 2021 Aug; 20 (8): 102873. doi: 10.1016/j.autrev.2021.102873. Epub 2021 Jun 11. PMID: 34119672.
2. *Sykes M, Sachs DH*. Mixed chimerism. *Philos Trans R Soc Lond B Biol Sci*. 2001 May 29; 356 (1409): 707–726. doi: 10.1098/rstb.2001.0853. PMID: 11375074; PMCID: PMC1088458.
3. *Owen RD*. Immunogenetic Consequences of Vascular Anastomoses Between Bovine Twins. *Science*. 1945 Oct 19; 102 (2651): 400–401. doi: 10.1126/science.102.2651.400. PMID: 17755278.
4. *Billingham RE, Brent L, Medawar PB*. Actively acquired tolerance of foreign cells. *Nature*. 1953 Oct 3; 172 (4379): 603–606. doi: 10.1038/172603a0. PMID: 13099277.
5. *Spitzer TR, Delmonico F, Tolkoff-Rubin N, McAfee S, Sackstein R, Saidman S et al*. Combined histocompatibility leukocyte antigen-matched donor bone marrow and renal transplantation for multiple myeloma with end stage renal disease: the induction of allograft tolerance through mixed lymphohematopoietic chimerism. *Transplantation*. 1999 Aug 27; 68 (4): 480–484. doi: 10.1097/00007890-199908270-00006. PMID: 10480403.
6. *Kawai T, Cosimi AB, Spitzer TR, Tolkoff-Rubin N, Suthanthiran M, Saidman SL et al*. HLA-mismatched renal transplantation without maintenance immunosuppression. *N Engl J Med*. 2008 Jan 24; 358 (4): 353–361. doi: 10.1056/NEJMoa071074. PMID: 18216355; PMCID: PMC2819046.
7. *Ciancio G, Burke GW, Garcia-Morales R, Suzart K, Rosen A, Ricordi C et al*. Effect of living-related donor bone marrow infusion on chimerism and in vitro immunoregulatory activity in kidney transplant recipients. *Transplantation*. 2002 Aug 27; 74 (4): 488–496. doi: 10.1097/00007890-200208270-00010. PMID: 12352907.
8. *Khubutiya MSh, Gulyaev VA, Khvatov VB, Lemenev VL, Kabanova SA, Novruzbekov MS et al*. Immunological tolerance in organ transplantation. *Transplantologiya. The Russian Journal of Transplantation*. 2017; 9 (3): 211–225. (in Russ). doi: 10.23873/2074-0506-2017-9-3-211-225.
9. *Vatazin AV, Kil'dyushevskiy AV, Fedulkina VA, Fayenko AP*. Mekhanizmy ottorzheniya pochechnogo allotransplantata i immunologicheskaya tolerantnost'. *Nefrologiya*. 2016; 20 (6): 33–41. (in Russ).
10. *Manilay JO, Pearson DA, Sergio JJ, Swenson KG, Sykes M*. Intrathymic deletion of alloreactive T cells in mixed bone marrow chimeras prepared with a nonmyeloablative conditioning regimen. *Transplantation*. 1998 Jul 15; 66 (1): 96–102. doi: 10.1097/00007890-199807150-00015. PMID: 9679828.
11. *Bluestone JA, Anderson M*. Tolerance in the Age of Immunotherapy. *N Engl J Med*. 2020 Sep 17; 383 (12): 1156–1166. doi: 10.1056/NEJMra1911109. PMID: 32937048; PMCID: PMC7534289.
12. *Ezekian B, Schroder PM, Freischlag K, Yoon J, Kwun J, Knechtle SJ*. Contemporary Strategies and Barriers to Transplantation Tolerance. *Transplantation*. 2018 Aug; 102 (8): 1213–1222. doi: 10.1097/TP.0000000000002242. PMID: 29757903; PMCID: PMC6059978.
13. *Davis S, Cooper JE*. Acute antibody-mediated rejection in kidney transplant recipients. *Transplant Rev (Orlando)*. 2017 Jan; 31 (1): 47–54. doi: 10.1016/j.trre.2016.10.004. Epub 2016 Oct 10. PMID: 28126347.
14. *Kawai T, Sachs DH, Sprangers B, Spitzer TR, Saidman SL, Zorn E et al*. Long-term results in recipients of combined HLA-mismatched kidney and bone marrow transplantation without maintenance immunosuppression. *Am J Transplant*. 2014 Jul; 14 (7): 1599–1611. doi: 10.1111/ajt.12731. Epub 2014 Jun 5. PMID: 24903438; PMCID: PMC4228952.
15. *Oura T, Cosimi AB, Kawai T*. Chimerism-based tolerance in organ transplantation: preclinical and clinical studies. *Clin Exp Immunol*. 2017 Aug; 189 (2): 190–196. doi: 10.1111/cei.12969. Epub 2017 Apr 20. PMID: 28369830; PMCID: PMC5508349.

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CLINICAL CASE OF RECURRENT aHUS AFTER ALLOGENEIC CADAVERIC KIDNEY TRANSPLANTATION

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Introduction. Atypical hemolytic uremic syndrome (aHUS) is a systemic orphan disease that reproduces as an uncontrolled activation of the alternative pathway of the complement system and is expressed as systemic thrombotic microangiopathy (TMA). The classical triad of aHUS symptoms are hemolytic anemia, thrombocytopenia, and acute kidney injury (AKI). Currently, diagnosis of aHUS is a diagnosis of exclusion and has no pathognomonic features. It is established based on the clinical presentation of the disease after excluding other forms of TMA, both primary and secondary. **Objective:** to increase physicians' awareness of this rare disease, the diagnosis and treatment of aHUS using a clinical case study. **Conclusion.** Early diagnosis of aHUS is extremely important, as timely targeted therapy can significantly improve or completely restore the functions of the affected organ.

Keywords: atypical hemolytic uremic syndrome, thrombotic microangiopathy, kidney transplantation, eculizumab, hemolytic anemia, thrombocytopenia.

INTRODUCTION

Atypical hemolytic uremic syndrome (aHUS) is a chronic systemic disease, the basis of which is a defect in the complement system activation leading to massive thrombus formation in the microvasculature (complement-mediated thrombotic microangiopathy (TMA)) [1–3]. Prevalence of the disease in Russia has not been precisely established, but it is comparable to that in Europe (1.5–1.8 cases per 1 million population) and the USA (about 2 cases per 1 million). The incidence is estimated at 0.2–0.5 cases per million population per year [5]. The clinical course of the disease is characterized by significant polymorphism of symptoms. However, the classical triad of aHUS symptoms are non-immune microangiopathic hemolytic anemia, thrombocytopenia and AKI [1, 3]. The generalized nature of TMA in aHUS determines the development of extrarenal signs of the disease, associated with damage to the microvasculature of various organs and systems, including the brain, heart, lungs, and gastrointestinal tract. Extrarenal manifestations of aHUS are observed in 20% of patients, of which almost two thirds have more than one extrarenal sign [1, 3].

Most patients with aHUS have an underlying hereditary and/or acquired complement abnormality, which leads to dysregulation of the activity of its alternative pathway on the endothelial surface. However, exposure to complement-activating factors (triggers) is necessary for the syndrome to develop in predisposed individuals. The most common of them are infections, autoimmune diseases, malignant tumors, pregnancy and childbirth, bone marrow and solid organ transplantation, and some drugs [1–4, 6]. Below is a clinical case of the development of clinical and laboratory manifestations of TMA after allogeneic cadaveric kidney transplantation.

CLINICAL CASE

Patient I., 27 years old, female, outpatient card No. 126734. Since 2016, proteinuria up to 1 g/day with "empty" urinary sediment, and episodes of increased blood pressure have been recorded. It was interpreted as chronic glomerulonephritis. The patient categorically refused to perform kidney biopsy and received no pathogenetic therapy. Kidney function progressively decreased, anemia appeared and gradually increased. The malignant course of arterial hypertension, resistant to multidrug antihypertensive therapy, drew attention. Platelet count remained within the reference values throughout the entire follow-up period, and anemia was considered as a manifestation of chronic kidney disease. In January 2022, due to critically high azotemia and development

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of anasarca, renal replacement therapy (hemodialysis) was initiated urgently.

During follow-up at the dialysis center, target hemoglobin levels were achieved, platelet count remained within reference values, and the malignant nature of arterial hypertension persisted against the background of multi-drug antihypertensive therapy and an adequate ultrafiltration volume.

On July 26, 2022, a kidney allotransplantation operation from a deceased immunocompatible donor was performed. Perioperative blood loss was estimated at 700 mL. Graft function was delayed. The early postoperative period was complicated by pyelonephritis reflux of the graft associated with the growth of Pseudomonas aeroginosae, as well as by hematoma accumulation in the graft bed. Laboratory tests revealed severe anemia, Hb 59 g/L Ht 19%; thrombocytopenia up to 108 × 10^9/L; leukocytosis 31 × 10^9/L; CRP 71 mg/L, procalcitonin 14 ng/mL; azotemia (creatinine) 433 μmol/L, azotemia (urea) 42 mmol/L. The graft ureteral stent was removed, and antibiotic therapy was initiated according to sensitivity. In order to reduce immunosuppression, the mycophenolate mofetil dose was reduced to 1 g/day, serum tacrolimus level was reduced to the minimum permissible, 6 ng/mL, glucocorticoid (prednisolone) dose was gradually reduced to 5 mg per day.

Against the background of the therapy, there was a positive clinical and laboratory effect – normalized body temperature, decreased proinflammatory laboratory markers, increased platelet count to 330 × 10^9/L, decreased azotemia (creatinine) to 260 μmol/L, azotemia (urea) to 10 mmol/L. Ultrasound showed that hematoma remains in the graft bed in the same volume, organized according to the timing. On September 30, 2022, the patient was discharged for outpatient follow-up, immunosuppressive therapy in the following volume: tacrolimus, with target serum level of 8–12 ng/mL, prednisolone 10 mg per day, mycophenolate mofetil 1500 mg per day.

On October 3, 2022, a scheduled laboratory monitoring was performed: hemoglobin 86 g/L, platelets 462 × 10^9/l, azotemia (creatinine) 485 μmol/l, azotemia (urea) 19 mmol/L. The patient's condition was considered as acute graft rejection. Pulse therapy with prednisolone ex juvantibus was initiated in order to relieve acute rejection. On October 4, 2022, a diagnostic graft biopsy was performed.

On October 6, 2023, febrile fever up to 39 °C, laboratory tests showed an increase in pro-inflammatory markers, bacteria culture test conducted on October 6, 2022 showed a growth in Klebsiela pneumonia, Pseudomonas aeruginosa, and polyresistant strains. Pelvic MRI scan: on the posterior surface of the kidney, with spread to the lower anterior surface of the kidney, a subcapsular chronic hematoma, heterogeneous structure, ~ dimensions (vertical x anterior x sagittal) 10.9 × 7.4 × 3.4 cm (previous dimensions 11.7 × 8.2 × 5.0 cm dated August 6, 2022, 9.4 × 8.81 × 5.72 cm dated August 3, 2022); in the projection of the upper pole of the kidney on the lateral surface, there is a wedge-shaped zone ~ measuring 3.2 × 2.8 cm; a similar zone is traced in the lower pole of the kidney ~ measuring 1.5 × 0.8 cm – more likely the infarction zone; along the left iliac vessels, chronic hematomas remain in the left parts of the small pelvis, with a maximum size of 8.0 × 2.0 cm. The patient's condition was considered as sepsis on the background of immunosuppressive therapy, a subcapsular hematoma in the graft was considered as the source of infection. On October 7, 2022, a transplantectomy, revision and sanitation of the graft bed were performed, immunosuppressive therapy was canceled, combined antibacterial therapy was prescribed. On the background of the treatment, the patient's condition showed positive dynamics – decreased level of proinflammatory markers (c-reactive protein, procalcitonin), no growth in bacterial blood test dated October 11, 2022, however, episodes of febrile fever persisted, severe anemia, pancytopenia attracted attention. Antibacterial therapy was continued, red blood cell suspension transfusions were performed.

On October 10, 2022, the result of histologic report on kidney graft biopsy material was received:

A standard examination of renal graft biopsy was performed: by light-optical method on paraffin sections using hematoxylin and eosin staining, PAS reaction, Masson's trichrome stain, Jones silver salts impregnation; immunofluorescence method on fresh frozen sections using FITC-conjugated antibodies to IgA, IgG, IgM, C3, C1q, fibrin, kappa and lambda free light chains; immunohistochemical immunoperoxidase procedure using antibodies to C4d component of the complement system and Polyoma-SV40. Diffuse severe tubulitis with acute tubular necrosis and dense interstitial infiltration represented by lymphocytes, plasma cells, neutrophilic leukocytes and single eosinophilic leukocytes were revealed (Fig. 1, a). In a slice of the only medium-caliber artery presented, weak endarteritis was determined in the form of slight subendothelial edema and few subendothelial lymphocytes (Fig. 1, b). There was a focal sharp thickening of the walls of some arterioles and small-caliber arteries due to pronounced subendothelial edema, with subtotal obturation of their lumen (Fig. 2, a, b). The glomeruli were without pathology. There were no signs of chronicity – namely, glomerulosclerosis, tubulointerstitial renal fibrosis and arteriolosclerosis. Immunofluorescence and immunohistochemistry revealed no specific expression.

According to study results, the following histological report about combined damage to the graft tissue was made:

1) *Acute T-cell mediated rejection, Banff type IIA, with minor endarteritis (v1), severe tubulitis (t3), severe*

interstitial infiltration (i3); acute tubular necrosis; no evidence of chronicity.

2) *Focal acute occlusive graft microangiopathy (focal "TMA") with focal acute subendothelial edema and subtotal obturation of the lumen of individual arterioles.*

A pathologist commented to the histological report with a diagnostic judgment that given the unverified primary kidney disease with loss of function at a young age, history of high blood pressure, and histological pattern of focal "TMA", a primary disease from the aHUS group and its recurrence in the graft is possible.

Given the histological report and the pathologist's comments suggesting aHUS as the main cause of loss of kidney function, as well as the fact that aHUS is essentially a diagnosis of exclusion, the patient was further examined as part of the differential diagnosis of TMA. A slight elevation in lactate dehydrogenase (LDH) level to 308 U/L was noted, the erythrocyte population structure was examined, and 1–2% schizocytosis was detected. Indirect Coombs test was negative. An ELISA test for a shiga-toxin test was performed – negative.A diagnosis of antiphospholipid syndrome (APS) was conducted, the results of tests for antinuclear factor, antibodies to beta-2 glycoprotein, and antibodies to cardiolipin IgM/IgG came out negative, which reduces the probability of primary and secondary APS. A study of the complement system was conducted – C3 level was slightly below reference values, C4 was within reference values; ADAMTS-13 was diagnosed in 75.7% plasma, which excludes the diagnosis of thrombotic thrombocytopenic purpura; antibodies to factor H 2.6 U/mL (N < 32), reducing the likelihood of acquired (antibody variant) aHUS.

In November 2022, neurological symptoms came to the forefront in the clinical picture, against the back-

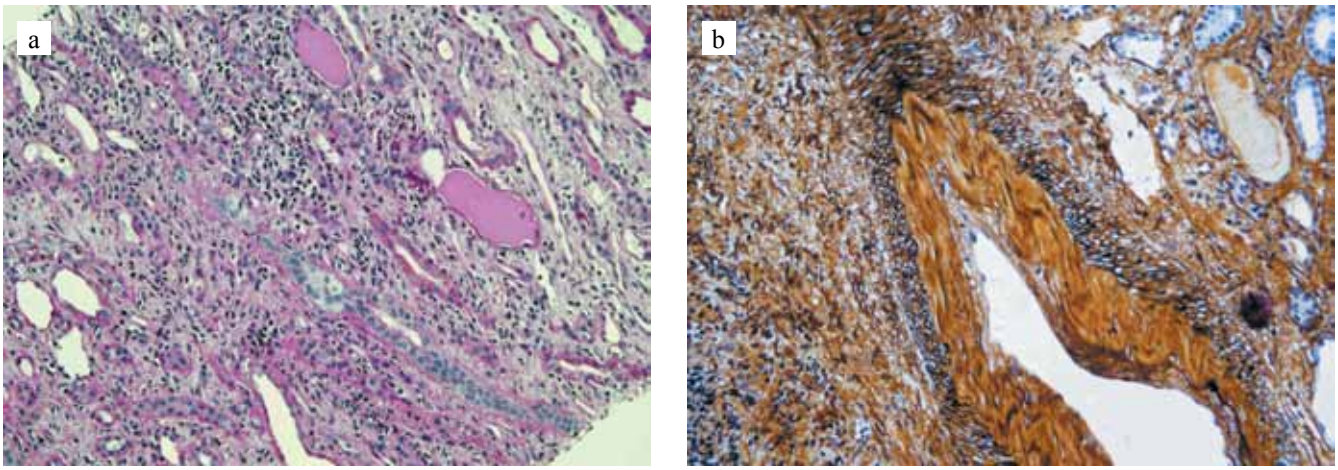

Fig. 1. Light microscopy. Acute T cell-mediated rejection: a, severe tubulitis (t3) and pronounced interstitial infiltration (i3); H&E stain, magnification 200×; b, mild endarteritis with single subendothelial lymphocytes (v1); Jones' methenamine silver stain, magnification 200×

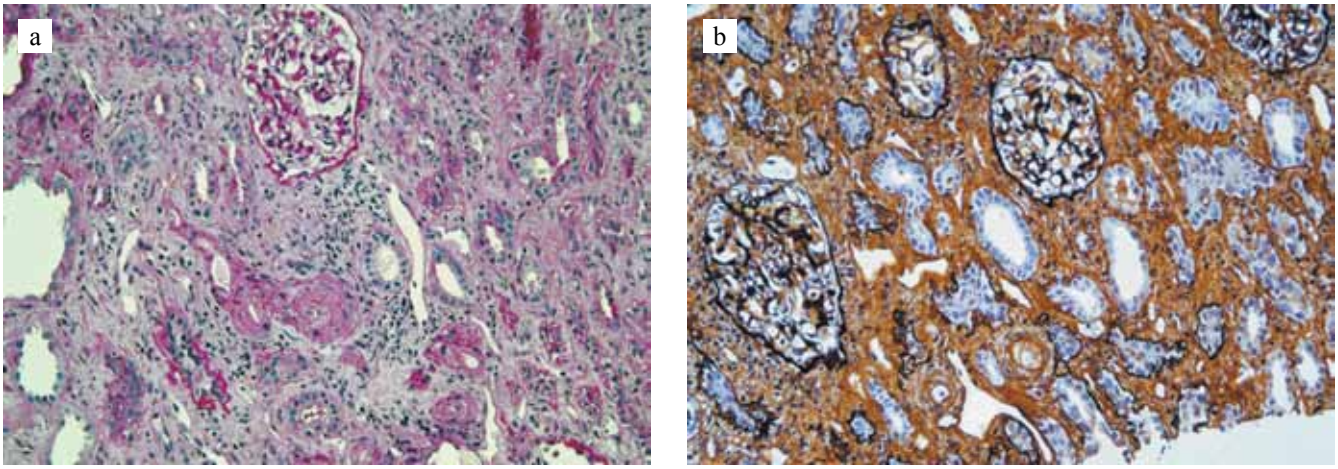

Fig. 2. Light microscopy. Focal acute occlusive microangiopathy: a, the slice shows three arterioles: two of them with severe subendothelial edema of the wall and lumen narrowing; the third arteriole is intact; PAS reaction, magnification 200×; b, the slice shows two arterioles: one of them with pronounced subendothelial edema and severe lumen narrowing; the second one is intact; Jones' methenamine silver stain, magnification 200×

ground of relative well-being, episodes of generalized seizures appeared.

On November 2, 2022, the patient underwent brain MRI, the picture is as follows:

- *subacute subdural hematoma in the left parietal region against the background of subarachnoid hemorrhage. The inclusion of inflammatory changes such as meningoencephalitis cannot be reliably ruled out;*
- *right-sided medial dislocation.*

A lumbar puncture was performed: 4 ml of turbid pink-colored cerebrospinal fluid was obtained. The liquor flowed out under increased pressure of 90 drops per minute. Cytological examination of the liquor: pink in color, turbid, cytosis 4.7 × 10^6/l, red blood cells in large quantities, cerebrospinal fluid protein 0.3 g/l. An infectious genesis of the seizure syndrome was ruled out.

Anticonvulsant therapy with levitiracetam was initiated at a dose of 1000 mg per day with a gradual increase to 1500 mg per day. On the background of monotherapy, convulsive episodes persisted up to 10 times in 24 hours; phenobarbital 0.3 g per day was added to the therapy. When epileptic seizures recurred, sodium thiopental solution was administered for relief.

Neurological symptoms prevailed clinically for a long time – a series of convulsive seizures daily, up to 6–7 episodes per day, against the background of baseline anticonvulsant therapy. They were controlled by administration of sodium thiopental solution in large doses.

Laboratory examination revealed persistent anemia, coagulopathy, thrombocytopenia. Hemocomponent therapy was carried out – transfusion of red blood cell suspension, cryoprecipitate, and fresh frozen plasma.

Given the medical history of the disease (unverified glomerulonephritis, malignant arterial hypertension, TMA triad after kidney transplantation), exclusion of possible other primary (thrombotic thrombocytopenic purpura, STEC hemolytic uremic syndrome) and secondary TMA, the patient's condition was considered as recurrent aHUS after kidney transplantation.

Due to the fact that the expected benefit of aHUS targeted therapy outweighed the risk of possible side effects, it was decided to initiate treatment with Elisaria (Eculizumab) according to the following regimen: 900 mg IV for 4 weeks, 1200 mg at week 5, thereafter 1200 mg IV once every 2 weeks, against the background of antibacterial prophylaxis for meningococcal infection, until the possibility of vaccination.

Targeted therapy began on November 23, 2022. During therapy, after the first administration, there was already a positive clinical and laboratory dynamics: improved general condition, regressed generalized convulsive seizures – tonic and clonic seizures were completely stopped against a reduction in the dose of baseline anticonvulsant therapy, decreased severity of arterial hypertension; increased levels of platelets (to 286 × 10^9/L) and hemoglobin (to 93 g/L), and decreased LDH level (to 196 U/L).

The patient was discharged on December 27, 2022 to continue targeted therapy and renal replacement therapy sessions on an outpatient basis at a dialysis center. Genetic screening of the disease panel "Atypical hemolytic uremic syndrome" is planned to determine the necessary duration of targeted therapy and prognosis of the disease.

DISCUSSION

The presented observation has a history of malignant arterial hypertension and unverified disease leading to end-stage chronic kidney disease. In the early postoperative period, the patient developed symptoms typical for aHUS – anemia, decreased platelet count and acute kidney graft injury. However, nonspecific symptoms were considered to be the consequences of significant blood loss during surgery, with subsequent formation of a graft bed hematoma and delayed graft function. The discussion of TMA diagnosis became possible, first of all, due to the results of a morphological examination of the graft tissue and was complicated against the background of postoperative complications and current septic condition. This observation illustrates the complexity involved in diagnosing aHUS, as well as the probability of a favorable outcome provided that the diagnosis is made in a timely manner and adequate therapy is initiated promptly. A special feature of Eculizumab-based targeted therapy is the possibility of improving organ function or complete regression of organ lesions, in this example, the brain.

CONCLUSION

In recent years, the issue of diagnosis and treatment of aHUS has been actively discussed in the medical community and is widely disseminated in the specialized literature. However, despite its simple and most common clinical features – thrombocytopenia and hemolytic anemia – diagnosis of aHUS still seems difficult due to the lack of pathognomonic signs and is a diagnosis of exclusion.

The authors declare no conflict of interest.

REFERENCES

1. *Ariceta G, Besbas N, Johnson S, Karpman D, Landau D, Licht C et al.* Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. *Pediatr Nephrol*. 2009 Apr; 24 (4): 687–696. doi: 10.1007/s00467-008-0964-1.
2. *Barbour T, Johnson S, Cohney S, Hughes P.* Thrombotic microangiopathy and associated renal disorders. *Nephrol Dial Transplant*. 2012 Jul; 27 (7): 2673–2685. doi: 10.1093/ndt/gfs279.

3. *Fakhouri F, Zuber J, Fromeaux-Bacchi V, Loirat C*. Haemolytic uraemic syndrome. *Lancet*. 2017 Aug 12; 390 (10095): 681–696. doi: 10.1016/S0140-6736(17)30062-4.
4. *Sullivan M, Erlic Z, Hoffmann MM, Arbeiter K, Patzer L, Budde K et al*. Epidemiological Approach to Identifying Genetic Predispositions for Atypical Hemolytic Uremic Syndrome. *Human Genetics*. 2010 Jan; 74 (1): 17–26. https://doi.org/10.1111/j.1469-1809.2009.00554.x.
5. *Fakhouri F, Delmas Y, Provot F, Barbet C, Karras A, Makdassi R et al*. Insights from the use in clinical practice of eculizumab in adult patients with atypical hemolytic uremic syndrome affecting the native kidneys: an analysis of 19 cases. *Am J Kidney Dis*. 2014 Jan; 63 (1): 40–48. doi: 10.1053/j.ajkd.2013.07.011.
6. *Stea ED, Skerka C, Accetturo M, Pesce F, Wiech T, Hartman A et al*. Case report: Novel FHR2 variants in atypical Hemolytic Uremic Syndrome: A case study of a translational medicine approach in renal transplantation. *Front Immunol*. 2022 Nov 14; 13: 1008294. doi: 10.3389/fimmu.2022.1008294.
7. *Tang ZC, Hui H, Shi C, Chen X*. New findings in preventing recurrence and improving renal function in AHUS patients after renal transplantation treated with eculizumab: a systemic review and meta-analyses. *Ren Fail*. 2023 Dec; 45 (1): 2231264. doi: 10.1080/0886022X.2023.2231264.

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DONOR-DERIVED MYELOID SARCOMA IN A KIDNEY TRANSPLANT RECIPIENT: CLINICAL CASE STUDY AND RELEVANCE OF A MULTIDISCIPLINARY APPROACH IN THERAPY AND DIAGNOSIS

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Background. Malignant tumors are one of the main causes of unfavorable outcomes in solid organ transplant recipients in the long term after transplantation. Localization of these tumors in a transplanted organ may cause loss of graft function. After chronic graft dysfunction and infections, malignant neoplasms come next as one of the leading causes of late kidney graft loss. The incidence of different types of malignancies varies according to the transplanted organ. Knowledge of etiology, pathogenesis, peculiarities of diagnosis and treatment of malignant tumors in solid organ transplant recipients is a significant part of screening at any stage of post-transplant period. Late diagnosis of malignancies in a transplanted kidney amidst disconnected stages of treatment and follow-up leads not only to graft loss, but also jeopardizes the life of recipients. **Clinical case description.** The patient is a 29-year-old female. History: IgA nephropathy with nephrosclerosis. Renal replacement therapy (RRT) with long-term hemodialysis since March 2019. Kidney transplantation from a deceased donor to the right external iliac vessels on March 13, 2019. Graft function is immediate. In October 2020, a tumor in the transplanted kidney was detected for the first time. In November 2021, an emergency graft nephrectomy was performed for health reasons. Antibacterial, antifungal therapy was carried out. Results of morphological study of the removed renal graft with immunohistochemistry (IHC) were obtained. The structure and phenotype of the tumor are consistent with myeloid sarcoma. Trephine biopsy: normocellular bone marrow. **Conclusion.** The 29-year-old patient was diagnosed with donor-derived myeloid sarcoma in her kidney transplant with the development of paraneoplastic syndrome and multi-organ failure. Currently, the patient is receiving RRT by long-term scheduled hemodialysis. Organ recipients need to be managed by a multidisciplinary team of specialized and highly specialized specialists, taking into account comorbid status and features of the course of the underlying disease.

Keywords: myeloid sarcoma, kidney transplantation, chronic kidney disease, immunohistochemistry, pathomorphology, oncohematology.

INTRODUCTION

After chronic graft dysfunction and infections, malignant tumors come next as one of the leading causes of late kidney graft loss, accounting for about 10% [1]. Most often, these are post-transplant lymphoproliferative disorders and kidney cancer, but, of course, other tumor localizations are not ruled out [2]. The risk of carcinogenesis in transplant recipients is significantly higher than in the general population due to the loss of immunological supervision over the appearance and proliferation of atypical cells against the background of immunosuppressants. Posttransplant malignancies are thought to develop by three mechanisms: *de novo* development, donor-related transmission, and recurrence of a recipient's pretransplant malignancy. Although nonmelanoma skin cancer, Kaposi sarcoma, posttransplant lymphoproliferative disorder, anogenital cancer, and lung cancer are malignancies that are thought to arise *de novo*, malignant melanoma and cancers that arise in the renal allograft are frequently donor related [3]. Nonmelanoma skin cancer, lip cancer, post-transplant lymphoproliferative disorders and anal cancer have the highest incidence in the organ recipient population. The incidence of different types of malignancies varies depending on the organ transplanted [4].

The incidence of myeloid sarcoma in patients after kidney transplantation is very rare, with only a few cases described in the world literature [5–10].

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Myeloid sarcoma (extramedullary myeloid tumor, granulocytic sarcoma, chloroma) is a tumor composed of myeloid progenitor cells arising anywhere other than the bone marrow (most commonly in the skin, lymph nodes, gastrointestinal tract, bones, soft tissues, and testes). Myeloid sarcoma may develop *de novo*, preceding acute myeloid leukemia (AML), parallel to the development of AML, or manifest as a blast transformation of myelodysplastic syndrome, a myeloproliferative disorder. Diagnosis is based on tumor biopsy and further use of cytochemical and immunohistochemistry (IHC) methods. Sarcoma in the general population occurs in 2.5–9.11% of AML patients [11]. Isolated myelosarcoma, without bone marrow involvement, is extremely rare (less than 1% of cases) [12]. The misdiagnosis rate is 75%, and 25–47% when IHC is used [13–15].

It is worth noting that traditional cytogenetic analysis in myeloid sarcoma is rarely performed because it is often mistaken for a solid tumor at the time of diagnosis, and samples suitable for cytogenetic analysis are not collected. Most data on genomic abnormalities are derived from individual case reports and karyotyping of the corresponding bone marrow, whereas myeloid sarcoma samples have been tested for targeted abnormalities using fluorescence in situ hybridization (FISH) [16]. Complete concordance between FISH and conventional cytogenetic results has been reported in only 71% of evaluable patients [17]. This suggests that conventional cytogenetic studies of both bone marrow and peripheral blood blasts (if available) and FISH analysis of myeloid sarcoma cells are complementary and should be performed in a clinical setting. In isolated myeloid sarcoma, FISH or conventional cytogenetic analysis of freshly obtained sarcoma cells is recommended [18].

CLINICAL OBSERVATION

A 29-year-old female patient with a long history of IgA nephropathy with outcome in nephrosclerosis, which required the start of renal replacement therapy (RRT) with long-term hemodialysis from March 2019.

Kidney transplantation from a deceased donor to the right external iliac vessels was performed at the transplant center of the Siberian Federal District on March 13, 2019. Graft function was immediate. Induction therapy included basiliximab and methylprednisolone. In the postoperative period, immunosuppressive therapy with cyclosporine, mycophenolic acid, methylprednisolone was used. The patient was discharged to the outpatient stage at her place of residence, in the Far Eastern Federal District, where she was observed by a nephrologist. The frequency of examinations and their volume at the place of residence are unknown. The patient did not keep in touch with the specialists of the center where the transplantation was performed. The outpatient follow-up data are not presented in full. Based on the provided documentation, it is known that in October 2020, the patient complained of pain in the graft area, liquid stools, and general weakness. An ultrasound that was performed at the patient's place of residence, detected, for the first time, a hypoechoic mass in the lower third of the transplanted kidney, measuring 23 × 29 mm in size (not classified according to Bosniak by the specialists at the place of residence). Taking into account laboratory examination and MRI data (the protocol was not provided), the detected changes were interpreted by the supervising nephrologist as graft pyelonephritis. Ertapenem-based antibiotic therapy was administered with a pronounced positive effect. Given the stable laboratory parameters, dynamic follow-up tactics were chosen. At subsequent irregular ultrasound screenings, a mass in the transplanted kidney was considered by specialists as a cyst.

In September 2021, MRI detected in the lower pole an enlarged mass of irregular round shape with unclear, uneven contours, a heterogeneous structure (due to the presence of areas of necrosis and cystic inclusions), 63 × 66 mm in size with heterogeneous accumulation of contrast agent. The level of azotemia during this period was unknown.

Following this, a biopsy of the transplanted kidney mass was performed at the patient's place of residence on October 11, 2021. Based on the results of histological examination of the biopsy specimen, the presence of a lymphoproliferative disorder was ruled out. IHC was not performed due to insufficient amount of material.

For further examination and treatment, in November 2021 the patient on her own came to Petrov National Medical Research Center for Oncology, St. Petersburg. A revision of morphologic preparations identified the pattern and immunophenotype of infiltrate from hematopoietic cells. Within the available biopsy, taking into account clinical data and negativity to many linear markers, one cannot categorically differentiate reactive inflammatory infiltrate and lymphoproliferative disorder. A more accurate differential diagnosis can be made on a more voluminous material.

At the same time, the patient felt worse, complaining of decreased diuresis. There was increased azotemia at the pre-hospital stage, which was detected during consultations at the Petrov National Medical Research Center for Oncology. On November 2, 2021, the patient was urgently hospitalized in a non-core hospital in St. Petersburg, where percutaneous nephropyelostomy of the transplanted kidney was performed due to graft hydronephrosis. Post-renal acute kidney injury (AKI) was diagnosed. Positive clinical and laboratory dynamics were achieved against the background of treatment. During this hospitalization, it was decided to perform repeat fine-needle percutaneous renal biopsy of the graft mass. The postoperative period was complicated by the formation of abdominal wall hematoma. Antibacterial

therapy and red blood cell transfusions were performed. The patient was discharged from the hospital at her own request on November 11, 2021.

On the same day, due to progressive deterioration in her condition, the patient, on her own, came to the kidney transplant department of Pavlov University.

The results of repeat biopsy of the kidney transplant mass (on November 8, 2021) were in progress at the time of admission.

On examination: the patient's condition was severe, hemodynamics was stable, breathing was independent, against the background of oxygen insufflation at a rate of 5 l/min, saturation 90%, body temperature 38 °C, volume of diuresis is reduced to 750 ml per day.

At the time of admission, the patient continued to receive basic immunosuppressive therapy. Mycophenolic acid was discontinued.

Chest X-ray showed signs of hyperhydration: fluid was detected in the left pleural cavity up to the level of the 8th rib. No infiltrative changes were detected in the visible parts of the lung tissue. Pulmonary pattern was diffusely enhanced due to the interstitial component.

According to laboratory investigations, we paid attention to significant leukocytosis up to 32.29 × 10^9/L, increased C-reactive protein (CRP) up to 352.3 mg/L, decreased hemoglobin level to 85 g/L, thrombocytopenia 26 × 10^9/L, high level of azotemia – creatinine 643 μmol/L, urea 27.4 mmol/L, in the general urine analysis: proteinuria 2.3 g/L, significant leukocyturia and bacteriuria, unchanged red blood cells 20–24–29 cells in the field of view.

Ultrasound examination: kidney transplant was located in the right iliac region, its contour is unclear in the lower third, approximate dimensions were 176 × 87 mm, the thickness of the parenchyma was 24 mm, increased echogenicity of II degree. Elements of the pyelocaliceal system: the cups of the upper group are expanded to 20 mm; a hyperechoic tubular structure is located in the projection of the upper cups – nephrostomy. In Doppler colour flow mapping (DCFM) mode, blood flow in the "vascular tree" of the graft was depleted at all levels. The resistive index at the level of the superior segmental artery was satisfactory. The middle and lower third of the graft were made of a hypoechoic mass with an indistinct contour and approximate dimensions of 79 × 67 × 73 mm; in the DCFM mode, vascular elements were identified in the structure of the mass.

Considering the above, the severity of the patient's condition was determined by the presence of graft neoplasm with impaired urodynamics and development of secondary pyelonephritis. In the somatic status, a differential diagnosis was made between urosepsis, paraneoplastic syndrome, and graft rejection. Ceftriaxone-based antibacterial therapy, disinfection therapy, and oxygen insufflation were empirically prescribed.

During the observation period, the patient's condition worsened, there were increased laboratory markers of inflammatory reaction.

According to radiography and chest computed tomography (CT) scan, negative dynamics were noted: marked increase in interstitial pulmonary edema (compared to the chest X-ray of November 11, 2021), clinically accompanied by hemoptysis (Fig. 1). In order to reduce systemic hyperhydration, an RRT session (long-term hemodialysis) through a central two-way venous catheter was performed.

Due to the growing phenomena of multiple organ failure, according to vital indications, urgent transplantectomy was performed (Fig. 2) on November 14, 2021.

Macroscopic description: the kidney was decapsulated, its dimensions were 140 × 110 × 90 mm. In the lower pole, there was a round tumor nodule without clear boundaries, about 70 mm in diameter, dense, yellowish-gray with whitish interlayers, homogeneous structure. The border of the cortical and medulla layers is not traceable, multiple confluent hemorrhages.

In the early postoperative period, transfusions of fresh frozen plasma, platelet concentrate, red blood cell mass were carried out, RRT (long-term hemodialysis) was resumed. Against the background of triple-drug antibacterial therapy (linezolid, meropenem, gentamicin), manifestations of systemic inflammatory reaction decreased: decreased CRP (114.10 mg/L) and

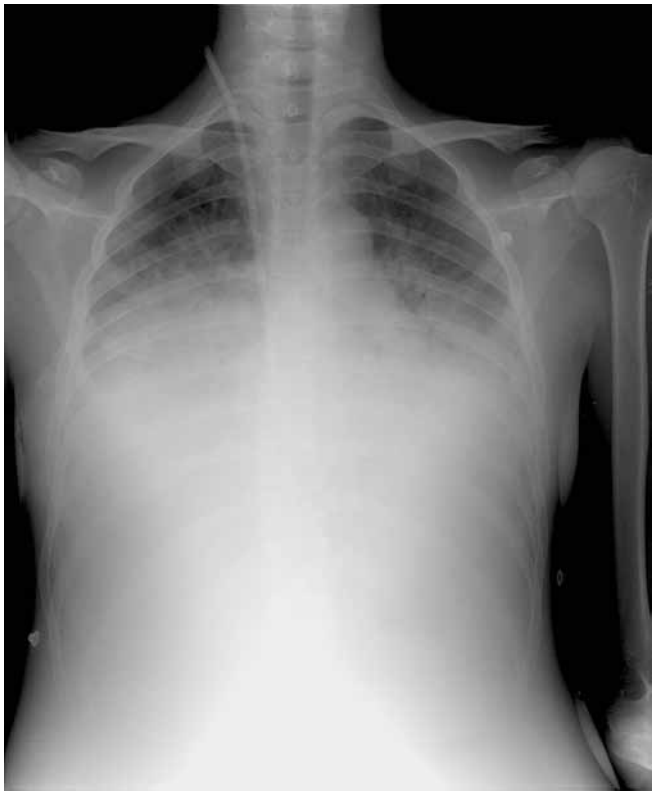

Fig. 1. Chest X-ray dated November 13, 2021

leukocytosis (14.34 × 10^9/L); high level of procalcitonin (68.4300 mcg/L) remained.

According to the results of urine culture conducted on November 16, 2021: Klebsiella pneumoniae ssp pneumoniae 1 × 10^6 cfu/mL, sensitivity to amikacin, gentamicin, and sulfamethoxazole / trimethoprim). Culture of wound discharge: Klebsiella pneumoniae ssp pneumoniae moderate growth, cross-sensitivity to the above-mentioned drugs was revealed. Blood culture – no microbial growth detected.

In the postoperative period, ultrasound detected no fluid accumulation in the graft bed.

On day 4 after the surgical intervention, the patient's condition worsened – in the form of febrile fever up to 39 °C, increase in leukocytosis up to 30.52 × 10^9/L, CRP up to 214 mg/l, despite ongoing antibacterial therapy. Abdominal and chest CT scans were conducted: CT signs of polyserositis (ascites, bilateral hydrothorax), small amount of blood in the pouch of Douglas, infiltration of the anterior abdominal wall in the hypogastric region, infiltration in the surgical intervention area – most likely, postoperative changes with the accession of inflammatory process. CT picture of alveolar-interstitial pulmonary edema.

In order to sanitize the focus of infection, for vital indications, emergency surgical intervention was performed – revision of the graft bed, laparotomy, sanitation and drainage of the abdominal cavity. As a result of revision in the graft bed, a liquid formation – an organized hematoma with signs of infection – was detected, serous peritonitis was detected in the retroperitoneal space. Cultures of the wound discharge: Klebsiella pneumoniae ssp pneumoniae abundant growth, same sensitivity.

In the postoperative period, fever regressed, leukocytosis reduced to 11.97 × 10^9/L, CRP to 101.9 mg/L. A mycological study of bronchoalveolar lavage (BAL) was carried out to determine sensitivity to antimycotics: Candida albicans 1 × 10^5 cfu/mL, sensitive to fluconazole, voriconazole. Therapy was adjusted according to the culture.

On the control chest X-ray conducted on November 22, 2021, compared to the X-ray conducted on November 17, 2021, there was still a decrease in the airiness of the lower parts of the lungs (no dynamics). Against this background, in the middle lung sections on both sides and in the lower lung sections on the right side, bilateral infiltration of lung tissue was determined, there was increasing severity of infiltration on the right side (negative dynamics).

On November 29, 2021, a morphological study of the removed kidney transplant was obtained: the tumor is represented by an infiltrate consisting mainly of small and medium-sized cells with irregularly shaped nuclei and light eosinophilic cytoplasm, a moderate amount of plasma cells (Fig. 3).

IHC study was performed to determine the immunophenotype of the tumor: atypical cells strongly expressed CD45, CD38, CD43, CD33, myeloperoxidase (Fig. 4). There was no expression of CD34, CD117, CD3, CD7, CD20, Pax-5, MuM.1, EBER, cytokeratins (pan-AE1/AE3) in the tumor cells (Fig. 5).

Reactions with antibodies to the light chains of kappa- and lambda immunoglobulins were performed, and

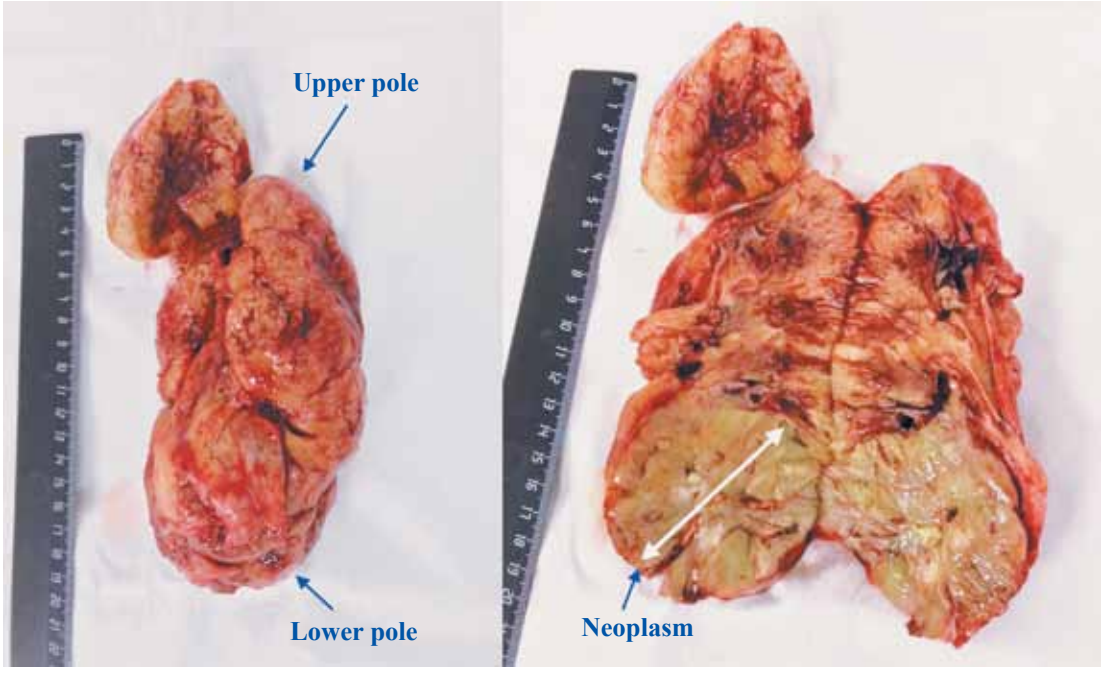

Fig. 2. Removed kidney graft with neoplasm

background staining was obtained. Ki-67 was not less than 60% (Fig. 6).

Thus, the tumor structure and phenotype are consistent with myeloid sarcoma (CD45+, CD38+, CD43+, CD33+, myeloperoxidase+, Ki-67 >60%).

Chest CT scan conducted on December 6, 2021: CT picture of increasing right-sided pleural effusion, pericardial effusion, minimal decrease in the severity of bilateral interstitial-alveolar changes, their extent is the same. The changes may correspond to manifestations of pulmonary interstitial-alveolar edema, manifestations of lymphoproliferative disorder; against this background, infection (bacterial/fungal) cannot be ruled out (Fig. 7).

Given the presence of bilateral infiltrative changes in the lungs, not regressing for a long time on the background of multidrug antibacterial, antifungal therapy, bronchoscopy was performed on December 7, 2021: diffuse catarrhal moderate endobronchitis. BAL examination was performed: bacteriological examination with determination of sensitivity to antibiotics – no growth was detected; determination of galactomannan antigen – the sample was positive (positivity index 4.974); cytolo-

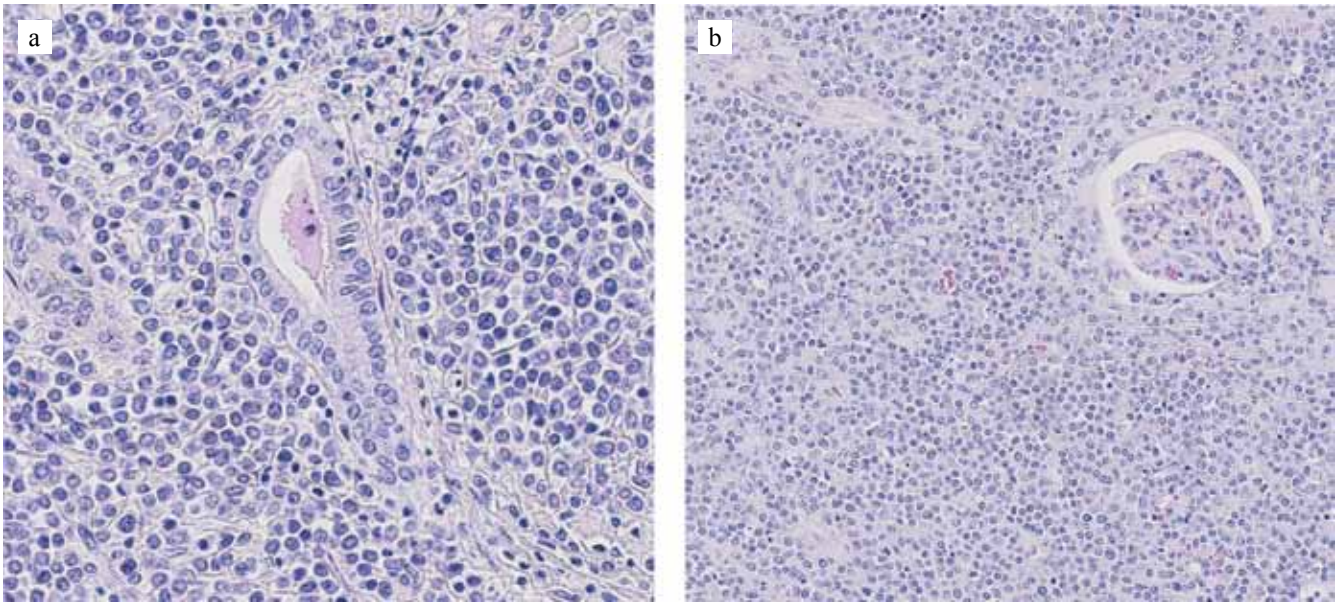

Fig. 3. Morphological picture of post-transplant lymphoproliferative disorder. H&E stain, magnification 630× (a), 400× (b)

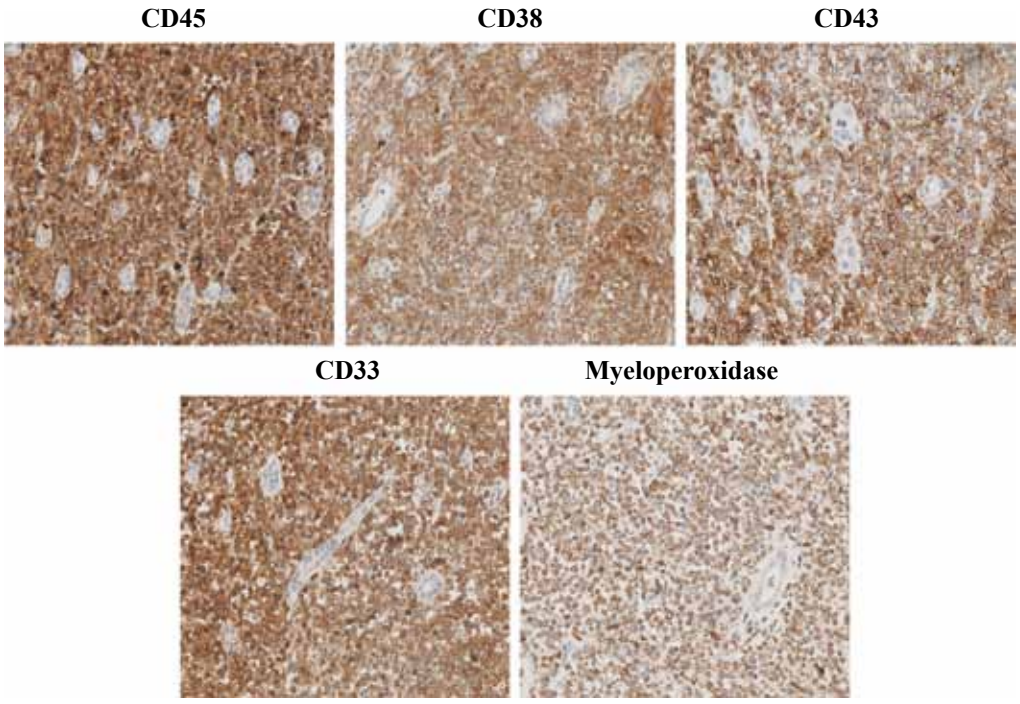

Fig. 4. IHC: atypical cells strongly express CD45, CD38, CD43, CD33, myeloperoxidase

gical examination of upper respiratory tract swabs – no evidence of malignant growth; microscopic examination for fungi – no fungal elements were detected during microscopy. Antifungal and antibacterial therapy was continued at the same level.

Chest CT scan of December 14, 2021: CT picture of pronounced positive dynamics of changes – resolved bilateral pleural effusion, regressed severity of pulmonary interstitial-alveolar edema. CT picture of bilateral peribronchial interstitial changes.

The hemogram test showed persistent anemia – decrease in hemoglobin to 64 g/L, normalization of platelet 344 × 10^9/L and white blood cell levels 7 × 10^9/L, no blasts.

Bone marrow trephine biopsy was performed: normocellular bone marrow, features of dysplasia in the granulocyte lineage (Fig. 8).

Myelogram: the presented bone marrow punctate preparations were normocellular, stromal fragments were filled with hematopoietic elements, without bone marrow

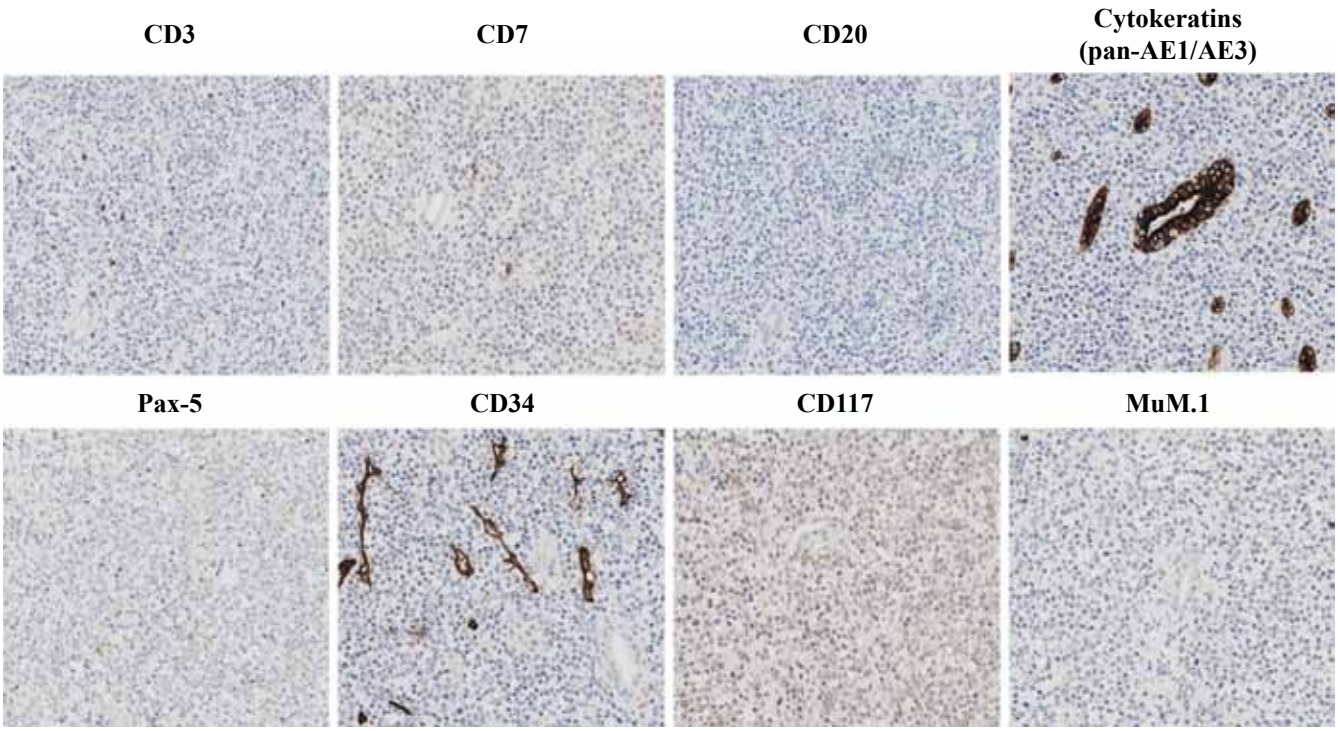

Fig. 5. IHC: atypical cells do not express CD34, CD117, CD3, CD7, CD20, Pax-5, MuM.1, cytokeratins (pan-AE1/AE3)

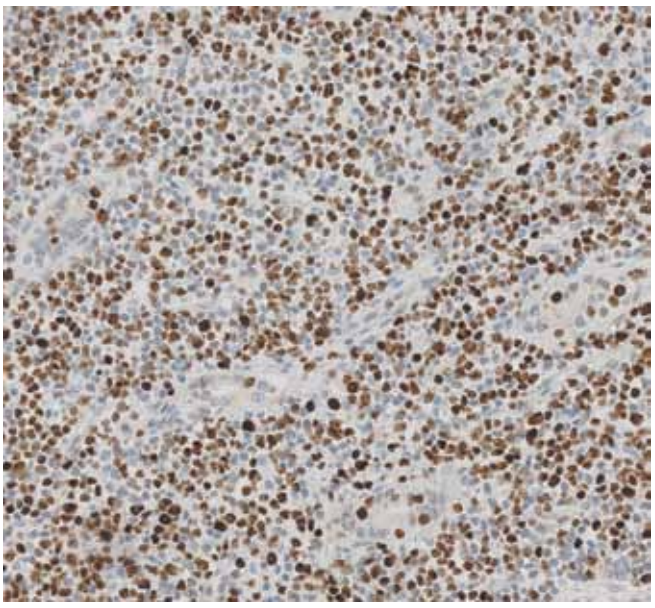

Fig. 6. Ki-67 >60%

lesions in myeloid sarcoma. Immunophenotyping of bone marrow cells – no pathology was detected. Karyotyping of bone marrow cells – no chromosomal pathology was detected. Molecular genetic markers of acute leukemia – no pathology was detected.

FISH analysis of X and Y chromosomes was not performed due to the donor's gender.

The patient was discharged from the transplant department of the Pavlov University on December 27, 2021 in a satisfactory condition, with positive dynamics in the form of improved well-being, normalized body temperature, increased hemoglobin level up to 100 g/L, decreased CRP up to 20.7 mg/L, pronounced positive dynamics in the form of resolved bilateral pleural effusion and regressed severity of pulmonary interstitial-alveolar edema. At present, the patient is receiving RRT (long-term hemodialysis) as planned. Guidelines for follow-up by an oncohematologist at the place of residence were given.

DISCUSSION

Given the complexity of screening for this pathology and the consequent high incidence of misdiagnosis, the diagnosis is often made in the later stages of the disease. Verification at early stages probably ensures a less severe course of this pathology.

From this clinical case, it is not possible to determine whether the disease is *de novo* or donor-derived, but the likelihood of this disease occurring should be considered at the donor stage.

If a hematopoietic tumor (CD45+) is suspected and T- and B-cell markers are absent, CD43 and myeloid markers (myeloperoxidase) should be included in the diagnostic panel.

Knowledge of epidemiological causes, pathogenesis, imaging features and treatment of malignant tumors in solid organ transplant recipients is a significant part of diagnostic screening at any stage of the post-transplant period.

CONCLUSION

From this clinical case, it is not possible to establish the nature of the disease – *de novo* or donor-derived. A 29-year-old patient was diagnosed with myeloid sarcoma of a kidney transplant with the development of paraneoplastic syndrome (anemia, leukemoid reaction, thrombocytopenic purpura, hypocoagulation), secondary pyelonephritis, sepsis, bilateral fungal pneumonia, increasing azotemia, and hyperhydration phenomena, which required emergency graft nephrectomy and resumption of long-term hemodialysis.

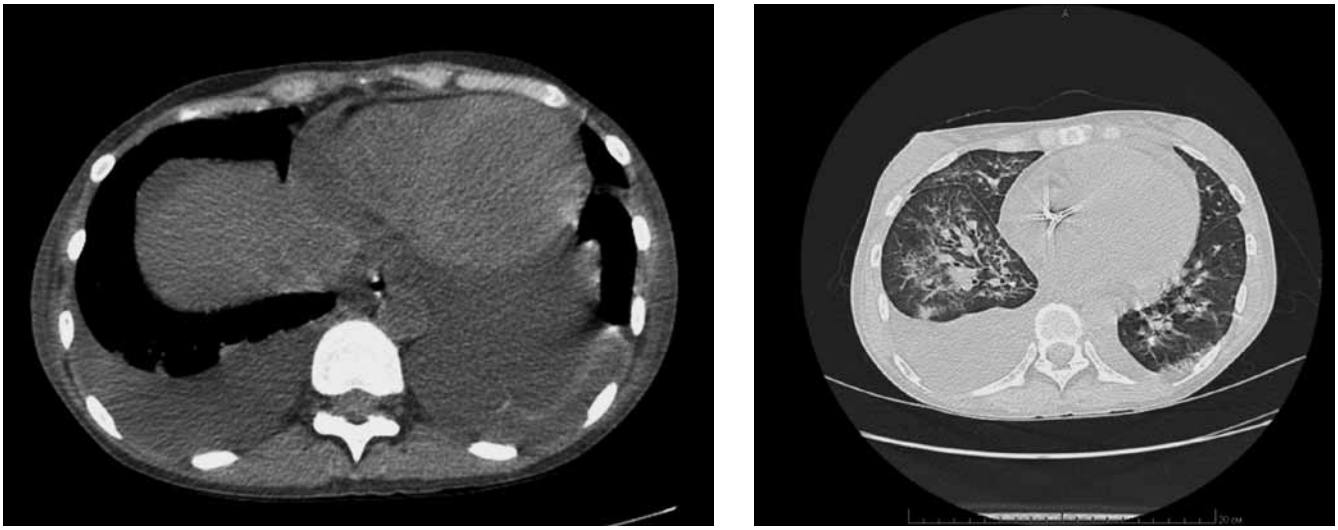

Fig. 7. Chest CT scan dated December 6, 2021

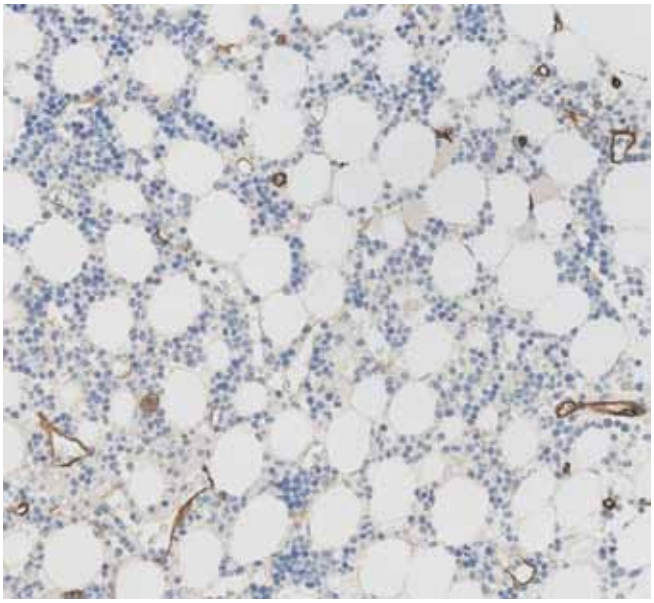

Fig. 8. Bone marrow trephine biopsy. The proportion of CD34+ cells is less than 2% of all karyocyte cells

An essential prerequisite for a successful kidney transplant outcome is communication between the center where the transplant was performed and the patients who underwent the operation. Late diagnosis of malignant tumors in a transplanted kidney in conditions where the stages of treatment and follow-up are disconnected, leads not only to graft loss but also jeopardizes the life of recipients.

The key to early detection of kidney transplant disease and provision of medical care in due time is prompt hospitalization in a specialized hospital. Successful management of such patients involves the work of a transplant multidisciplinary team.

The authors declare no conflict of interest.

REFERENCES

1. *Danovich GM.* Kidney transplantation / Trans. from english edited by Ya.G. Moisyuk. M.: GEOTAR-Media, 2014; 414.
2. *Turshudzhyan A.* Post-renal transplant malignancies: Opportunities for prevention and early screening. *Cancer Treat Res Commun*. 2021; 26: 100283. doi: 10.1016/j.ctarc.2020.100283. Epub 2020 Dec 11. PMID: 33338850.
3. *Katabathina VS, Menias CO, Tammisetti VS, Lubner MG, Kielar A, Shaaban A et al*. Malignancy after Solid Organ Transplantation: Comprehensive Imaging Review. *Radiographics*. 2016 Sep-Oct; 36 (5): 1390–1407. doi: 10.1148/rg.2016150175. PMID: 27618321.
4. *Collett D, Mumford L, Banner NR, Neuberger J, Watson C.* Comparison of the incidence of malignancy in recipients of different types of organ: a UK Registry audit. *Am J Transplant*. 2010 Aug; 10 (8): 1889–1896. doi: 10.1111/j.1600-6143.2010.03181.x. PMID: 20659094.
5. *Palanisamy A, Persad P, Koty PP, Douglas LL, Stratta RJ, Rogers J et al*. Donor-derived myeloid sarcoma in two kidney transplant recipients from a single donor. *Case Rep Nephrol*. 2015; 2015: 821346. doi: 10.1155/2015/821346. Epub 2015 Apr 21. PMID: 25977825; PMCID: PMC4419238.
6. *Wong RL, Ketcham M, Irwin T, Akilesh S, Zhang TY, Reyes JD et al*. Donor-derived acute promyelocytic leukemia presenting as myeloid sarcoma in a transplanted kidney. *Leukemia*. 2020 Oct; 34 (10): 2776–2779. doi: 10.1038/s41375-020-0903-0. Epub 2020 Jun 10. PMID: 32523036; PMCID: PMC7515823.
7. *AlGhunaim M, AlSuwaida A, Zaidi SN, Kfoury H*. First case report of acute myeloid sarcoma post renal transplant. *Transplant Proc*. 2014 Sep; 46 (7): 2411–2413. doi: 10.1016/j.transproceed.2014.02.007. Epub 2014 Aug 20. PMID: 25150606.
8. *Wu D, Lu X, Yan X, Gao R*. Case report: Rare myeloid sarcoma development following renal transplantation with KRAS and DNMT3A gene mutations. *Diagn Pathol*. 2021 Aug 31; 16 (1): 82. doi: 10.1186/s13000-021-01141-z. PMID: 34465355; PMCID: PMC8406562.
9. *Shen J, Du X, Diao X, Zhao L, Wang W, Yu Y, Luo H.* Myeloid Sarcoma Presenting With Multiple Skin and Subcutaneous Mass Without Leukemic Manifestations After Renal Transplantation. *Transplant Proc*. 2015 Sep; 47 (7): 2227–2232. doi: 10.1016/j.transproceed.2015.06.005. PMID: 26361685.
10. *Williams T, Aljitawi OS, Moussa R, McHugh S, Dusing R, Abraha J, Yarlagadda SG.* First case of donor transmitted non-leukemic promyelocytic sarcoma. *Leuk Lymphoma*. 2012 Dec; 53 (12): 2530–2534. doi: 10.3109/10428194.2012.695360. Epub 2012 Jul 9. PMID: 22616573.
11. *Savchenko VG, Parovichnikova EN*. Acute leukemia. *Clinical oncohematology: a guide for doctors* / Ed. M.A. Volkova. M.: Medicine, 2007: 409–502.
12. *Solh M, Solomon S, Morris L, Holland K, Bashey A.* Extramedullary acute myelogenous leukemia. *Blood Rev*. 2016 Sep; 30 (5): 333–339. doi: 10.1016/j.blre.2016.04.001. Epub 2016 Apr 8. PMID: 27094614.
13. *Meis JM, Butler JJ, Osborne BM, Manning JT.* Granulocytic sarcoma in nonleukemic patients. *Cancer*. 1986 Dec 15; 58 (12): 2697–2709. doi: 10.1002/1097-0142(19861215)58:12<2697::aid-cncr2820581225>3.0.co;2-r. PMID: 3465429.
14. *Antic D, Elezovic I, Milic N, Suvajdzic N, Vidovic A, Perunicic M et al*. Is there a "gold" standard treatment for patients with isolated myeloid sarcoma? *Biomed Pharmacother*. 2013 Feb; 67 (1): 72–77. doi: 10.1016/j.biopha.2012.10.014. Epub 2012 Nov 19. PMID: 23218987.
15. *Yamauchi K, Yasuda M*. Comparison in treatments of nonleukemic granulocytic sarcoma: report of two cases and a review of 72 cases in the literature. *Cancer*. 2002 Mar 15; 94 (6): 1739–1746. doi: 10.1002/cncr.10399. PMID: 11920536.
16. *Mirza MK, Sukhanova M, Stölzel F, Onel K, Larson RA, Stock W et al*. Genomic aberrations in myeloid sarcoma without blood or bone marrow involvement: characterization of formalin-fixed paraffin-embedded samples by chromosomal microarrays. *Leuk Res*. 2014 Sep; 38 (9): 1091–1096. doi: 10.1016/j.leukres.2014.05.004. Epub 2014 May 21. PMID: 25088808; PMCID: PMC4157130.
17. *Pileri SA, Ascani S, Cox MC, Campidelli C, Bacci F, Piccioli M et al*. Myeloid sarcoma: clinico-pathologic, phenotypic and cytogenetic analysis of 92 adult patients. *Leukemia*. 2007 Feb; 21 (2): 340–350. doi: 10.1038/sj.leu.2404491. Epub 2006 Dec 14. PMID: 17170724.
18. *Avni B, Koren-Michowitz M.* Myeloid sarcoma: current approach and therapeutic options. *Ther Adv Hematol*. 2011 Oct; 2 (5): 309–316. doi: 10.1177/2040620711410774. PMID: 23556098; PMCID: PMC3573418.

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A 5-YEAR SINGLE-CENTER EXPERIENCE IN HEART TRANSPLANTATION IN ROSTOV OBLAST

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Objective: to evaluate the outcomes of heart transplants performed at Rostov Regional Clinical Hospital within five years. **Materials and methods.** Between 2017 and 2022, 29 orthotopic heart transplants (HT) were performed in our clinic. Heart failure was caused by postinfarction cardiosclerosis (21 cases, 72.4%) and dilated cardiomyopathy (8 cases, 27.6%). Among the recipients, 27 (93.1%) were men and 2 (6.9%) were women. Mean age was 53.14 ± 8.7 years (34 to 67 years). All patients received quadruple-drug immunosuppressive therapy, including induction with monoclonal antibodies; calcineurin inhibitor, mycophenolic acid, and corticosteroid were used after HT. **Results.** In-hospital mortality was 10.34% (n = 3). The causes of death were multiple organ failure and infectious and septic complications. After discharge, 4 (13.8%) recipients died over 5 years. Rejection reaction with the development of graft dysfunction (3 recipients, 75%), infectious and septic complications (1 recipient, 25%) were the causes of death in the long-term period. The survival rate was analyzed according to the Kaplan–Meier estimate. One-year survival was 80.9%. Three-year survival rate corresponded to the 5-year survival rate – 70.56%. Five-year survival of patients surviving the first year after HT was 86.1%. Maximum follow-up period was 64 months. **Conclusion.** HT continues to be the gold standard for patients with end-stage heart failure. Five-year HT experience in our center has shown a survival rate that is comparable to that of the International Society for Heart and Lung Transplantation (ISHLT).

Keywords: heart transplantation, heart failure, immunosuppressive therapy.

INTRODUCTION

Despite the significant evolution in the treatment of heart failure (HF), in some cases, even if optimal therapy is selected, the patient's condition remains severe, quality of life is low, and prognosis is disappointing [1].

Over the last 20 years, the number of patients with functional class (FC) III–IV chronic heart failure (CHF) in the Russian Federation increased by 1.3% (from 1.8% to 3.1%), reaching 4.5 million people. Prognosis of such patients is unfavorable: the median survival time for FC III–IV CHF is 3.8 years. Annual mortality in this group, even when treated in a specialized hospital, is 10.2% [2]. In the group of patients with refractory CHF, mortality within a year can reach 50% [3].

To date, HT is the only effective method of treatment for end-stage CHF, which reliably increases survival, improves exercise tolerance and quality of life. In addition, in most cases, HT allows patients to return to active activity [4].

Currently, more than 5000 HT are performed annually worldwide [5]. Continuous development of surgical techniques and technologies, improvement and emergence of new immunosuppressive therapy regimens, have significantly improved prognosis after HT [6]. According to ISHLT reports, the survival rate of patients after transplantation has improved considerably over the last decades and today the median survival rate exceeds 12 years [5].

National healthcare has come a long way over the past decades. Due to the active development of transplantology, the number of HT has increased 10-fold in the past 14 years. For instance, the number of HTs in Russia increased from 0.2 per million population in 2008 to 2.0 in 2022. And the total number of HTs performed over 35 years was 2200 [7].

MATERIALS AND METHODS

The HT program in Rostov Oblast was started in 2017. Over five years, 29 orthotopic HTs have been performed at the cardiac surgical center of Rostov Regional Clinical Hospital. The outcomes were analyzed retrospectively.

From November 2017 to November 2022, there were 54 patients on the HT waiting list. Of these, transplantation was performed in 29 (53.7%) patients; 10 (18%)

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patients on the waitlist died from CHF progression in the absence of technical possibility to perform HT or use mechanical circulatory support as a bridge to HT. Two (4%) patients were withdrawn from the waitlist for various reasons. One patient had remission of the disease on the background of selected therapy, his hemodynamic parameters improved, ejection fraction increased, in view of which he was delisted. Thirteen (24%) patients remained in the HT waitlist at the moment of writing this paper.

Those with refractory end-stage congestive heart failure (CHF) and a prognosis of 1-year transplant-free survival <50% were selected for inclusion on the HT waiting list. Objective criteria for this prognosis include:

– <20% left ventricular ejection fraction;
– >20 mmHg pulmonary wedge pressure;
– <12 mL/kg/min decrease in peak oxygen consumption (peak VO_2) in patients not receiving beta-blockers and peak <14 mL/kg/min VO_2 against the background of maximum tolerated dose of beta-blockers;
– signs of severe myocardial ischemia in patients with coronary heart disease, which significantly limit daily activities when revascularization by coronary artery bypass grafting or percutaneous coronary angioplasty is impossible;
– recurrent life-threatening rhythm disturbances refractory to drug therapy, as well as to electrophysiological methods of treatment (catheter ablation and(or) implantation of cardioverter-defibrillator) [8].

The main contraindication for transplant listing was the detection of high vascular resistance of the small pulmonary circulation (>5 Wood's units), with no effect on inhaled vasodilators.

Among the operated patients, the etiology of end-stage heart failure was postinfarction cardiosclerosis in 21 cases (72.4%) and dilated cardiomyopathy in 8 cases (27.6%); 93.1% (n = 27) of recipients were male, 6.9% (n = 2) were female. The mean age was 53.14 ± 8.7 years (34 to 67 years).

To assess the degree of HF, a 6-minute walk test was performed, which averaged 257 ± 83.4 m: 16 (55.2) recipients had the New York Heart Association (NYHA) functional class (FC) IV, 13 (44.8%) had NYHA FC III.

Echocardiography revealed a marked decrease in left ventricular (LV) myocardial contractility – LV EF 22.11 ± 8 (10–47%), cardiomegaly (LV end-diastolic diameter 71.54 ± 8.7 mm (55–87 mm), LV end-diastolic volume 291.6 ± 79.8 ml (160–550 mL), moderate pulmonary hypertension (mean pulmonary artery pressure 32.7 ± 11.24 mmHg).

The results of right heart catheterization showed moderately elevated pulmonary artery pressure of 29.37 ± 13.28 mmHg (10 to 63), elevated pulmonary vascular resistance in Wood units of 1.58 ± 0.83 (0 to 3.1), and low cardiac index of 1.85 ± 0.58 L/min/m^2 (0.46 to 3.2).

The bicaval HT technique was used in all cases.

The time parameters were as follows: CPB 178.9 ± 38.77 min (123–273), donor heart anoxia time 144.5 ± 32.87 min (78–220), and operation time 296.39 ± 61.5 min (218–450). The mean length of stay at the intensive care unit was 7.36 ± 3.1 days.

In the postperfusion period, all patients received dopamine-based inotropic support, combined with adrenaline (91% of cases), and with vasopressor support with norepinephrine (in 79% of cases). Right ventricular failure (RVF) was managed using levosimendan, sildenafil, nitric oxide inhalation and ilprost. All the drugs were administered at moderate therapeutic doses.

After HT, all patients received triple-drug immunosuppressive therapy, which was selected to minimize the toxic effects of the drugs on the recipient. Histological evaluation of biopsy specimens was performed according to the ISHLT-2004 classification. The average length of stay at the hospital by the recipient was 36.2 ± 12.4 days.

RESULTS

All cases of orthotopic HT performed were analyzed. During the follow-up period, 24% (n = 7) of recipients died. In-hospital mortality was 10.3% (n = 3). The 30-day mortality was 3.44% (n = 1). During the follow-up period of up to 3 months, 13.8% (n = 4) of recipients died.

The main causes of early mortality were multiple organ failure (MOF) and infectious septic complications, which amounted to 100% (n = 4) in the structure of early mortality (Fig. 1). The cause of in-hospital mortality in 2 cases was MOF developing against the background of acute RVF, and 1 patient died due to a combination of sepsis and MOF. One recipient was discharged in satisfactory condition, but a month later he was re-hospitalized with double pneumonia and died of infectious septic complications.

Among non-fatal complications (Fig. 2) in the early postoperative period, RVF occurred in 18 (69.2%) patients. To correct RVF, levosimendan was used in all cases, sildenafil was used in 18 (62%) patients, nitric oxide inhalation was used in 5 (17.2%) patients, and 2 (6.9%)

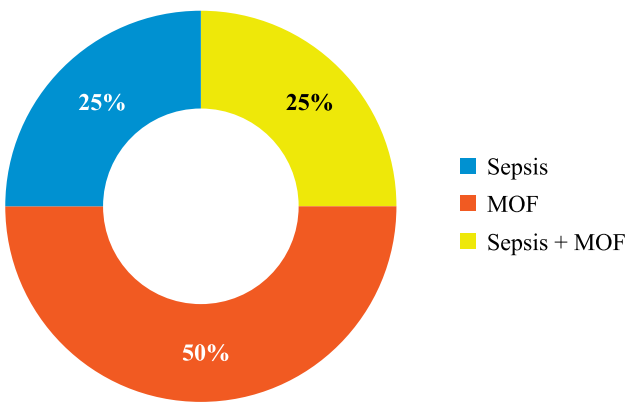

Fig. 1. Causes of early post-HT mortality

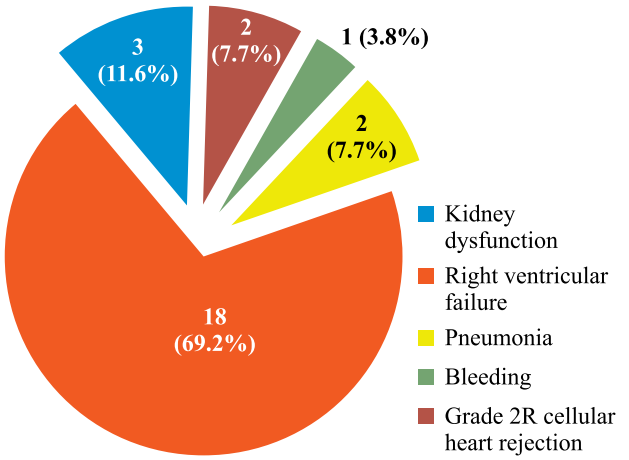

Fig. 2. Structure of non-fatal in-hospital complications

recipients required triple-drug therapy: sildenafil, nitric oxide inhalation, and ilprost.

Postoperative renal dysfunction requiring renal replacement therapy (RRT) was noted in 3 (11.6%) patients.

Infectious complications during the hospital period were represented by bacterial pneumonia in 1 (3.85%) patient.

In one case, a patient developed bleeding on day 10 after HT, which was successfully controlled. The subsequent postoperative period was uneventful, and the patient was discharged from the hospital on day 32.

One patient, operated on in 2021, was transferred to an infectious diseases hospital on day 2 after testing positive for SARS-CoV-2. The postoperative period was uneventful, therapy was monitored and coordinated remotely. After 13 days, she was transferred to the cardiology department. After the necessary instrumental examinations, including endomyocardial biopsies, and after achieving the target laboratory parameters, she was discharged on day 15.

Acute rejection reaction during the hospital period was diagnosed in 2 (7.7%) recipients.

Biopsy results were analyzed according to the ISHLT-WF 2004 (International Society for Heart and Lung Transplantation – working formulation, 2004) recommended classification of acute rejection. To manage the acute rejection crisis, pulse therapy with methylprednisolone (1.0 g, 3 times a day), plasma filtration, and immunoglobulin therapy were implemented.

At control examination at different periods after HT, coronary artery disease was detected in the transplanted heart of 5 recipients (17.2%), which in two cases required stenting of the affected coronary artery segments, and in cases where stenoses were hemodynamically insignificant, adjustment of lipid-lowering therapy.

Three recipients died in the long-term period. The cause of death in all cases was graft rejection reaction combined with other complications. So, one patient died in 7 months due to infectious septic complications on the background of acute antibody-mediated rejection (AMR). One patient died in 14 months from pulmonary thromboembolism on the background of grade 2R acute cellular rejection, acute AMR. One patient died in 24 months from myocardial infarction on the background of acute AMR.

During the entire follow-up period, grade 1R cellular rejection was diagnosed in 13 (44.8%) recipients, and grade 2R in 4 (13.8%). Grade 1 AMR was diagnosed in 3 (10.3%) recipients, all these cases were fatal.

DISCUSSION

Currently, there are two most common HT techniques: biatrial (developed in 1921 by R. Lower and N. Shumway) and bicaval (proposed in 1990 by M. Yacoub and D. Sievers) [9, 10]. Considering the advantages of the bicaval technique – maintaining the normal geometry of the right atrium, reducing the frequency of sinus node dysfunction and tricuspid regurgitation – we consider this technique to be the most optimal and use it in our center. All patients had sinus rhythm at the time of discharge.

Evaluating our HT outcomes, we see that the main cause of early postoperative mortality in patients is associated with MOF and septic complications on the background of immunosuppression.

Immunosuppressive therapy regimens used today can, in most cases, achieve a balance by adequately suppressing rejection reactions and preventing excessive immunosuppression. And the use of induction therapy can reduce the risk of acute rejection and delay the administration of nephrotoxic calcineurin inhibitors.

In our case, immunosuppression was induced via oral administration of mycophenolic acid (Mayfortic) 360–720 mg before the operation, infusion of monoclonal antibodies (basiliximab) 20 mg before aortic clamping and administration of methylprednisolone before blood flow was activated. On day 4 after HT, basiliximab was re-injected. Subsequently, the patients received a triple-drug immunosuppressive therapy: calcineurin inhibitor (tacrolimus), sodium mycophenolate (Mayfortic) and corticosteroid (prednisolone). Calcineurin inhibitors were administered from day 2–3 after surgery under the control of renal function, gradually increasing the dose of the drug to the required level. In accordance with the scheme proposed by specialists at Shumakov National Medical Research Center of Transplantology and Artificial Organs, the target tacrolimus blood trough level at year 1 after HT was considered to be a decreasing level from 15 to 5 ng/mL, and subsequently the level was maintained at 5 ng/mL [11, 12].

In our practice, there were 2 cases of seizure syndrome developing against the background of tacrolimus administration, and blood levels were within the target values, which required replacing the drug with cyclosporine.

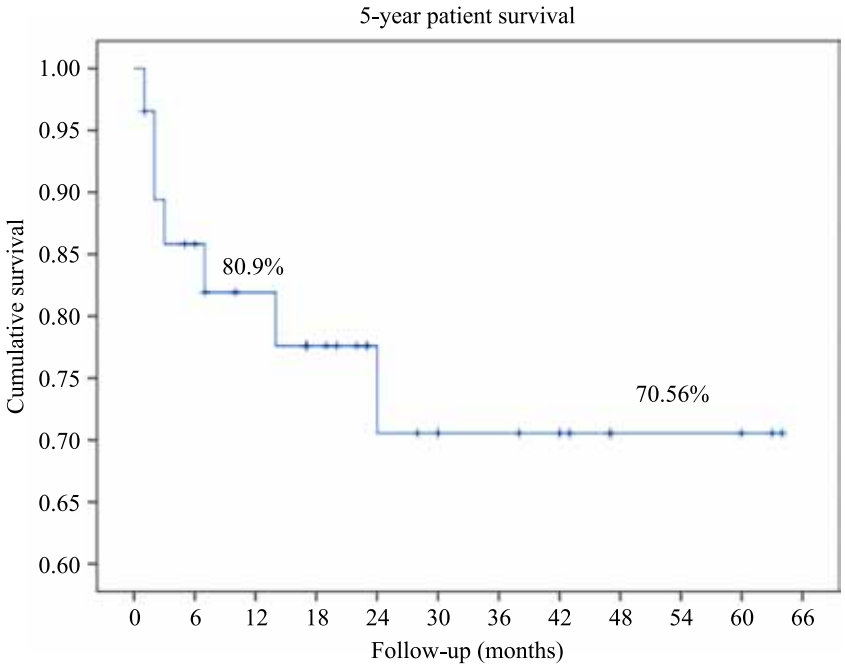

Fig. 3. Kaplan–Meier patient survival

Despite constant improvement in immunosuppressive therapy and the emergence of new drugs, there is no ideal immunosuppression regimen, and some patients require individual selection of therapy.

CONCLUSION

Summarizing the 5-year experience in our center, we can say that HT is the most effective method of treatment for end-stage CHF; it prolongs the life of patients and also improves their quality of life, and in most cases return them to active activity.

The highest rate of fatal complications is still characteristic of the first months of HT, which indicates the need for further improvement in immunosuppressive therapy regimens, to avoid both acute rejection and infectious complications. Optimization of the waiting list and the activities of district medical institutions on early detection and routing of patients with severe/end-stage CHF will reduce the proportion of MOF in the structure of early mortality and increase the survival rate of patients after surgery.

The best outcomes are achieved with careful selection of patients and strict compliance with all medical guidelines in the postoperative period.

The 1-year Kaplan–Meier survival rate in our study was 80.9% (Fig. 3). The overall 5-year survival was 70.56%. Five-year survival of patients surviving the first year after HT was 86.1%, which is comparable to ISHLT data.

The authors declare no conflict of interest.

REFERENCES

1. *Truby LK, Rogers JG.* Advanced heart failure: epidemiology, diagnosis, and therapeutic approaches. *JACC Heart Fail*. 2020; 8 (7): 523–536.
2. *Polyakov DS, Fomin IV, Belenkov YuN, Mareev VYu, Ageev FT, Artemjeva EG et al*. Chronic heart failure in the Russian Federation: what has changed over 20 years of follow-up? Results of the EPOCH-CHF study. *Kardiologiia*. 2021; 61 (4): 4–14. https://doi.org/10.18087/cardio.2021.4.n1628.
3. *Khush KK, Cherikh WS, Chambers DC, Goldfarb S, Hayes D Jr,, Kucheryavaya AY et al.* The international thoracic organ transplant registry of the international society for heart and lung transplantation: thirty-fifth adult heart transplantation report – 2018; focus theme: multiorgan transplantation. *J Heart Lung Transplant*. 2018; 37 (10): 1155–1168.
4. *Stehlik J, Kobashigawa J, Hunt SA, Reichenspurner H, Kirklin JK.* Honoring 50 years of clinical heart transplantation in circulation: in-depth state-ofthe-art review. *Circulation*. 2018; 137 (1): 71–87.
5. *Penninga L, Møller C, Gustafsson F, Steinbrüchel D, Gluud C*. Tacrolimus versus cyclosporine as primary immunosuppression after heart transplantation: systematic review with meta-analyses and trial sequential analyses of randomised trials. *Eur J Clin Pharmacol*. 2010; 66: 1177–1187.
6. *Kirk R, Edwards LB, Aurora P, Taylor DO, Christie J, Dobbels F et al.* Registry of the International society for heart and lung transplantation: Eleventh official lung and heart/lung transplantation report – 2008. *J Heart Lung Transplant*. 2008; 27 (9): 978–983.
7. *Gautier SV, Khomyakov SM*. Organ donation and transplantation in the Russian Federation in 2021. 14th Report from the Registry of the Russian Transplant Soci-

ety. *Russian Journal of Transplantology and Artificial Organs*. 2022; 24 (3): 8–31. (In Russ.). https://doi.org/10.15825/1995-1191-2022-3-8-31.

8. Klinicheskie rekomendatsii MZ RF "Transplantatsiya serdtsa, nalichie transplantirovannogo serdtsa, otmiranie i ottorzhenie transplantata serdtsa", 2020 g. https://transpl.ru/upload/medialibrary/d27/d273c88a64b6b5197bf6ff-2ca177765a.pdf.
9. *Lower RR, Shumway NE.* Studies on the orthotopic homotransplantation of the canine heart. *Surg Forum*. 1960; 11: 18.
10. *Sarsam MA, Campbell CS, Yonasn NA, Deiraniya AK, Rahman AN.* An alternative surgical technique in orthotopic cardiac transplatation. *J Card Surg*. 1993; 8: 344–349.
11. *Gautier SV, Moysyuk YaG.* Transplantology. Pharmacotherapy without errors. A guide for doctors. M., 2014; 432.
12. *Khubutiya MS, Sokolov VV, Redkoborodyy AV, Kozlov IA, Timerbaev VK, Khutsishvili LG et al*. The experience of 70 heart transplants in a multidisciplinary medical care facility. *Transplantologiya. The Russian Journal of Transplantation*. 2018; 10 (3): 197–206. https://doi.org/10.23873/2074-0506-2018-10-3-197-206.

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A CASE OF SUCCESSFUL ORTHOTOPIC HEART RETRANSPLANTATION IN AN 11-YEAR-OLD CHILD

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Objective: to present a clinical case of an 11-year-old child who underwent repeat heart transplantation (HT) at Almazov National Medical Research Centre in St. Petersburg, Russia. **Materials and methods.** A case of successful heart retransplantation in an 11-year-old child with cardiac allograft vasculopathy (CAV) is presented. **Results.** The postoperative period after heart retransplantation had no significant differences with the postoperative period of primary heart recipients. The complexity of the intraoperative stage was determined by pronounced adhesions. As part of preoperative preparation, the patient underwent chest CT scan, which, in our experience, allows us to evaluate the heart syntopy and, in turn, is an important preparatory stage in planning repeat interventions. **Conclusion.** Our first experience of cardiac retransplantation in pediatric patients suggests that repeat HT is the most optimal treatment for pediatric patients with CAV and requires more thorough preoperative preparation.

Keywords: heart retransplantation, orthotopic heart retransplantation, artificial circulation.

INTRODUCTION

HT is the gold standard for the treatment of American College of Cardiology/American Heart Association (ACC/AHA) stage D heart failure (HF). For HF, 35,703 adult HTs were performed worldwide from 2009 to 2017, and about 5800 heart transplants are performed annually [1]. Between 2009 and 2022, 2224 HTs were performed in the Russian Federation. The median survival after adult HT exceeds 12 years, the survival rate depends on the 1-year survival rate, which exceeds 50% after 14 years of follow-up [1], and the unadjusted 1-year survival rate after HT is 85% [2]. Post-transplant complications include graft failure, rejection, and CAV.

According to world reports, the number of HTs performed is increasing annually and averages 100 to 120 per year worldwide or 2% to 4% of all HTs in adults. In 2010, a repeat HT was performed for the first time in the Russian Federation. Today, the number of such operations remains sporadic.

INCIDENCE AND EPIDEMIOLOGY OF REPEAT HEART TRANSPLANTATION

Compared to primary HT recipients, retransplant recipients are on average younger, more sensitized, and tend to be more acutely ill with worsening renal function, increased likelihood of hospitalization, dialysis, intubation, inotropic support, or extracorporeal membrane oxygenation (ECMO) [3]. Between 2006 and 2013, 51.6% of adult heart transplant recipients were hospitalized at the time of retransplantation, 48% received inotropic therapy, 6.7% were implanted with left ventricular assist device, 4.6% with right ventricular assist device, 7.2% with intra-aortic balloon pump counterpulsation, 8% with mechanical ventilation, 2% with total artificial heart, and 5.8% with ECMO [3].

The three main indications for heart retransplantation (RTx) are acute rejection, early graft failure, and CAV [4].

Although RTx accounts for no more than 5% of all HTs, it was important to review the outcomes of repeat HTs in critically ill patients. There were marked clinical differences between patients who required cardiac RTx with and without CAV. However, RTx with CAV should be considered as the optimal treatment option in this group of patients. Various options for mechanical circulatory support in patients scheduled for surgical treatment should be considered as a way to stabilize the patient as well as a bridge to RTx. There has been an annual improvement in survival in patients undergoing RTx for CAV [4].

A study by N.K. Chou et al. that was conducted from March 1995 to May 2005, featured 8 patients with cardiac allograft failure, of whom 6 (75%) had CAV and 2 (25%) had acute rejection. The mean interval to RTx was 32 to 84 months. CAV was diagnosed on the basis of any localized coronary artery anomalies or diffuse coronary artery narrowing. A left main trunk lesion ≥70% of the primary vessels with stenosis ≥70%, or isolated branch stenosis ≥70% in all three systems was classified as a severe NYHA class III to IV lesion for which re-transplantation should be considered. These patients underwent heart RTx. As a control for acute graft rejec-

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tion, endomyocardial biopsy was performed weekly for the first month, then every 3 months for the first year, and then annually thereafter. Coronary angiography was performed 1 month after RTx. Acute rejection was defined as acute humoral or cellular rejection grade 3A or higher using the International Society for Heart and Lung Transplantation (ISHLT) classification criteria [4].

Of 628 HTs in 606 patients in the Russian Federation, operated on between 1986 and early 2016, 22 patients (3.63%) required repeat heart transplantation. The mean age of recipients for the RTx was 45.59 ± 14.66 years. In 8 (36.4%), the reason for performing RTx was chronic persistent graft rejection with hemodynamic disorders; in 10 (45.6%) recipients, RTx was performed early due to primary graft dysfunction. Two recipients (9.0%) had secondary graft dysfunction resulting from heart transplant coronary artery disease (TxCAD), and two had acute graft myocarditis (9.0%). The interval between the primary and the repeat HT was 734.59 ± 1235.4 days. All recipients received triple-drug immunosuppressive therapy, including tacrolimus or cyclosporine, mycophenolate mofetil and methylprednisolone [5].

By 2018, the experience of performing heart RTx at Shumakov National Medical Research Center of Transplantology and Artificial Organs had reached 27 surgeries in patients for periods from 1 day to 15 years after primary transplantation [5].

The most common indications for RTx are CAV and allograft rejection. While primary graft failure is the most common cause in the first month after HT, CAV is the most common cause after the first year. Other causes include graft rejection or heart valve defect [6]. A group of scientists led by Syed-Saif Abbas Rizvi in 2017 conducted a systematic review of 11 studies that included 7,791 patients, of which 7,446 patients underwent primary HT, whereas 345 required RTx. Indications for RTx were CAV (60.2%), acute rejection (20.7%), and early graft failure (19.1%) [7].

An earlier analysis of the ISHLT/UNOS registry for all cardiac retransplants performed in the United States from 1987 to 1998 showed that the time from primary transplant to RTx ranged from 1 day to 15.5 years, with 56% undergoing RTx for chronic rejection or CAV, 18% for primary or nonspecific graft failure, 9% for acute rejection, and 3% due to hyperacute rejection. Most of these patients (60%) were in the intensive care unit at the time of RTx, and 40% were on some form of life support (e.g., ventricular assist device, inotropic therapy) [8].

The presence of severe CAV (left coronary artery stenosis ≥50% or stenosis of two or more primary vessels ≥70% or branch stenosis ≥70% in all 3 systems) is associated with poor 1-year survival and high mortality. Such patients should be considered for RTx [9, 10].

The aim of the study was to present a clinical observation of a patient who underwent repeat heart transplantation at Almazov National Medical Research Centre.

MATERIALS AND METHODS

An 11-year-old child, on April 12, 2021, was urgently hospitalized at Almazov National Medical Research Centre due to increasing chronic heart failure on the background of dilated cardiomyopathy, CAV. From the anamnesis: supraventricular extrasystoles were detected from the age of five after an acute respiratory viral infection, left ventricular ejection fraction (LVEF) 54%. In April 2015, he was hospitalized at Children's Republican Clinical Hospital in Saransk for decompensated chronic heart failure (DCHF), as well as rhythm disturbances such as supraventricular extrasystoles and unstable paroxysmal supraventricular tachycardia. He was discharged on the background of improved diagnosis: chronic non-rheumatic carditis with lesions of the cardiac conduction system, with rhythm disturbances – ventricular polymorphic extrasystoles, ventricular tachycardia with a probable outcome in dilated cardiomyopathy (DCM). In March 2016, after recurrent arrhythmic syncope, it was decided to implant a Protecta DR D 364 DRG implantable cardioverter-defibrillator. He was discharged with recommendations to continue sotalol, metoprolol, captopril, verospiron, diuver, carbamazepine, and prednisolone.

From June 2016, the patient's condition deteriorated, and there was increasing heart and respiratory failure. According to data from 24-hour ECG monitoring: 15,819 polymorphic ventricular extrasystoles, decreased left ventricular ejection fraction (LVEF) according to echocardiography (EchoCG) up to 10% (Simpson). In January 2017, he was consulted by a council of physicians at Almazov National Medical Research Centre due to severe CHF on the background of dilatation of heart chambers: left ventricular end-diastolic diameter (LVEDD) 61 mm, decrease in myocardial contractility up to 10% (Simpson), formation of mitral and tricuspid regurgitation, life-threatening rhythm disturbances and low effectiveness of drug therapy, the only possible treatment for this patient is heart transplantation.

The child was admitted to Fortis Malar Hospital, Chennai, India from November 10, 2017 to March 20, 2018. During hospitalization, several cardiac arrest episodes were recorded, after which ECMO machine was connected. On January 17, 2018, orthotopic donor HT surgery was performed for health indications. The postoperative period was complicated by episodes of severe hypotension, as well as left ventricular dysfunction. Against the background of increasing left ventricular failure, a decision was made to reimplant the ECMO system. In view of inotropic therapy, the patient's hemodynamic state stabilized, LV contractility improved. On January 20, 2018, the ECMO system was explanted. On February 15, 2018, the cardiac monitor showed ventricular fibrillation with transition to asystole, cardiopulmonary resuscitation (CPR) was initiated. Acute rejection

was suspected and pulse therapy with glucocorticoids was initiated.

Coronary angiography was performed – a three-vessel lesion of the coronary arteries was detected, and percutaneous transluminal angioplasty of the right coronary artery and anterior interventricular coronary artery was performed. The diagnostic procedure was complicated by acute thrombosis in the right coronary arterial wall, CPR was initiated, and thrombolysis (eptifibatide) was performed. Due to unstable hemodynamics with a tendency to hypotension, the ECMO system was implanted again. Positive dynamics was observed for six days; against this background, the ECMO system was removed. Endomyocardial biopsy was performed – no signs of rejection. In March 2018, he was discharged due to his stabilized condition.

In May 2018, he suffered an ischemic stroke: mixed tetraparesis, seizure syndrome, asthenic syndrome in the early recovery period. Anticonvulsant therapy Kepra 33 mg/kg was prescribed; subsequently, positive dynamics were noted during the therapy; seizures did not recur, the therapy was discontinued.

According to EchoCG studies dated December 19, 2018, there was moderate dilatation of the left heart chambers (left atrium (LA) 30 mm, LVEDD 43 mm). LVEF 57%. Mitral valve (MV) leaflets of increased echogenicity. Slight acceleration of transmittal blood flow 1.6 m/s. Moderately dilated aortic root lumen, prolapse of aortic valve (AV) flaps, regurgitation grade 1. Estimated systolic pressure in the pulmonary artery (PA) 30 mm Hg. Moderate LV myocardial hypertrophy (interventricular septum (IVS) up to 8–9 mm, left ventricular posterior wall (LVPW) up to 9 mm).

The patient was re-hospitalized for evaluation at Fortis Malar Hospital, Chennai, India from February 17, 2019 to February 21, 2019. This hospitalization included percutaneous transluminal coronary angioplasty with left coronary artery stenting and anterior interventricular artery restenosis. The postoperative period was uneventful. According to EchoCG data over time, grade II mitral regurgitation, tricuspid insufficiency (TI) grade 2, pulmonary hypertension (LA pressure 51 mm Hg, inferior vena cava (IVC) 1.1 cm, collapsing more than 50%). LVEF 65%. He was discharged in stable condition with recommendations to continue immunosuppressive and antimycotic therapy, as well as statins.

As part of preoperative preparation, coronary angiography was performed on February 11, 2021 – a multivessel coronary lesion was determined (Fig. 1).

Chest CT scan shows that the right atrium and right ventricle are directly adjacent to the sternum in the lower

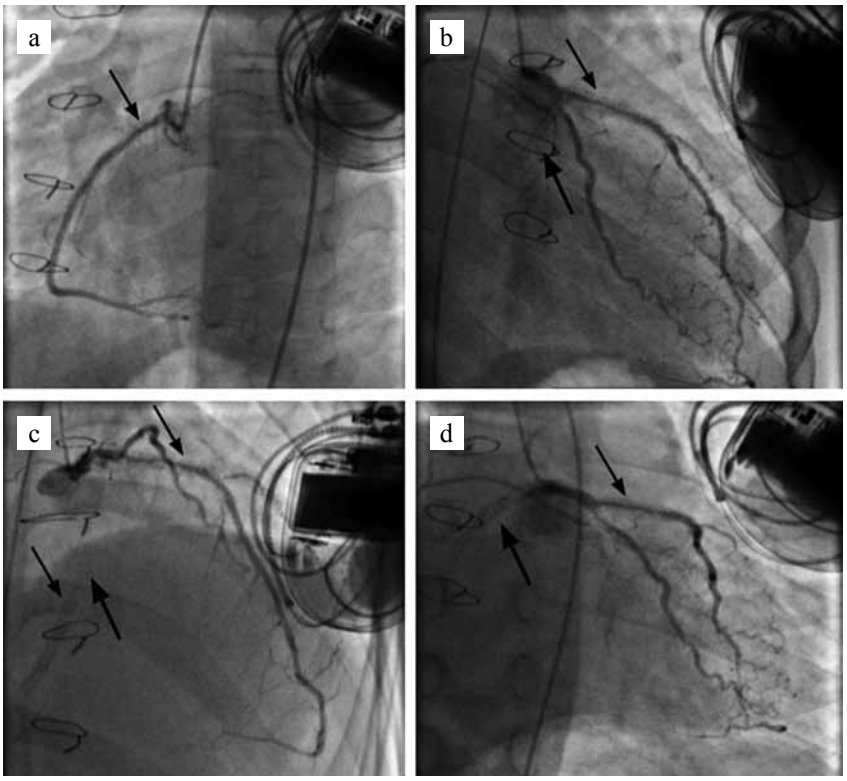

Fig. 1. Preoperative coronarography. The right coronary artery basin was previously stented, diffuse loss of stent lumen up to 40% maximum (a); left coronary artery basin, anterior interventricular artery (AIA): post-stenting condition in the proximal third from the orifice – up to 70% restenosis, the periphery is satisfactory. Circumflex artery (CA): represented by the main branch and the marginal artery (MA). The main branch is occluded in the proximal third, the periphery is hypoperfused, filled through intrasystem collaterals. MA – post-stenting condition – up to 70–80% restenosis, the periphery is satisfactory (b, c, d)

third, no focal and infiltrative changes were detected (Fig. 2).

On April 12, 2021, surgical treatment was performed in the scope of resternotomy, orthotopic heart transplantation by bicaval technique. After performing resternotomy and cardiolysis, the main stage of surgical intervention was performed according to the standard technique, it proceeded without peculiarities. Extracorporeal circulation lasted for 92 minutes, aortic clamping time was 63 minutes, and graft ischemia time was 155 minutes. The intraoperative stage proceeded without complications, and custodiol cardioplegia was used. After removing the aortic clamp, spontaneous recovery of cardiac activity was noted, no rhythm disturbances were registered. After control transesophageal echocardiography, decannulation was performed. At the end of extracorporeal circulation, we measured central hemodynamics: heart rate 125 beats/min, sinus rhythm, blood pressure 85–95/60–65 mmHg, cardiac index 2.53 l/min/m^2, stroke volume 24.5 ml, total peripheral resistance 1808 dyn·sec·cm^5, central venous pressure 4 mmHg, and pulmonary artery pressure 20/7 mmHg. On the background of inotropic therapy were dobutamine 5 mcg/kg/min and norepinephrine 0.6 mcg/kg/min.

The patient was then transferred to the aseptic ward of the Department of Anesthesiology and Reanimation in a stable condition, received dobutamine 5 mcg/kg/min, noradrenaline 0.6 mcg/kg/min as inotropic and vasopressor therapy. He was extubated 8 hours after the end of the operation, without any peculiarities. Subsequently, positive dynamics was noted in the form of decreasing doses of inotropic and vasopressor therapy; from day 2, the patient was activated within the bed, verticalization was also performed. Tacrolimus levels were monitored daily and immunosuppressive therapy was adjusted. On the 10th day after surgical treatment, the patient was transferred to the specialized department, where further rehabilitation and optimization of therapy was carried out. According to the standard protocol, endomyocardial biopsy was performed every 14 days. Histological examination revealed no signs of rejection (AMR0), EchoCG showed LVEF 70%, blood flow on the aortic valve was not accelerated, mitral regurgitation up to grade I, tricuspid regurgitation up to grade I, grade I pulmonary regurgitation, left ventricular global contractility was not reduced, no asynergy zones were reliably detected, right ventricular myocardium contractility was moderately reduced (TAPSE = 12 mm, S' = 8 cm/sec).

During hospitalization, immunosuppressive therapy was adjusted: gradual reduction of metipred to 10 mg per day, tacrolimus depending on serum levels (target values by the time of discharge), mycophenolate mofetil under control of white blood cell/neutrophil count. After administration of a course of granulocyte colony-stimulating factor (leukostim), the neutrophil level normalized. Mycophenolate mofetil was resumed. After a course of rehabilitation and adjustment of immunosuppressive therapy, the patient was discharged for outpatient follow-up.

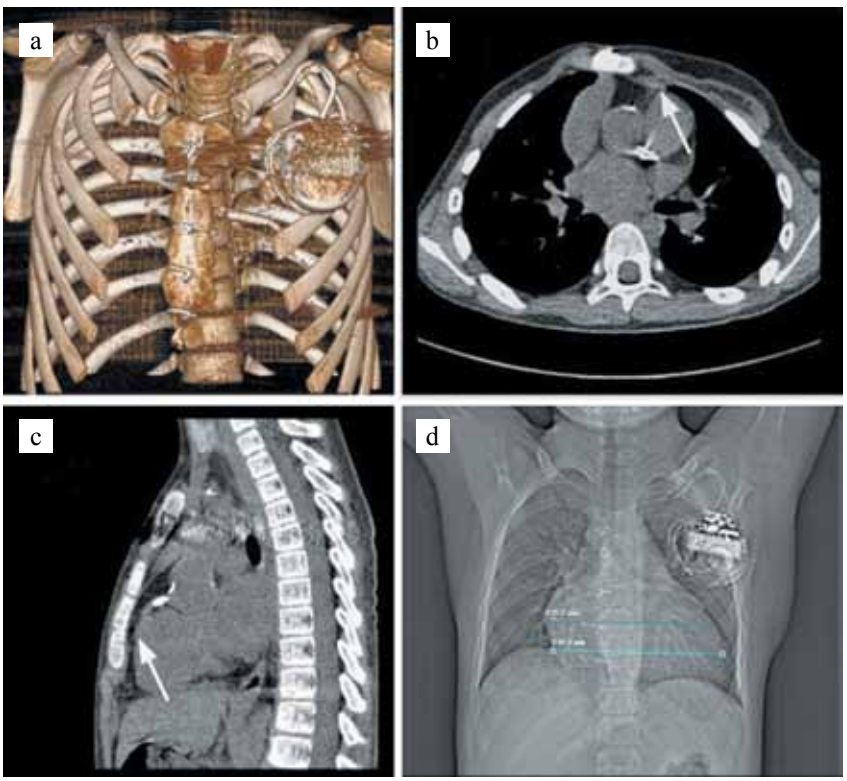

Fig. 2. Preoperative chest CT scan. CT volume rendering (a); transverse image (b); sagittal image (c); frontal image showing the transverse dimensions of the transplanted heart (d)

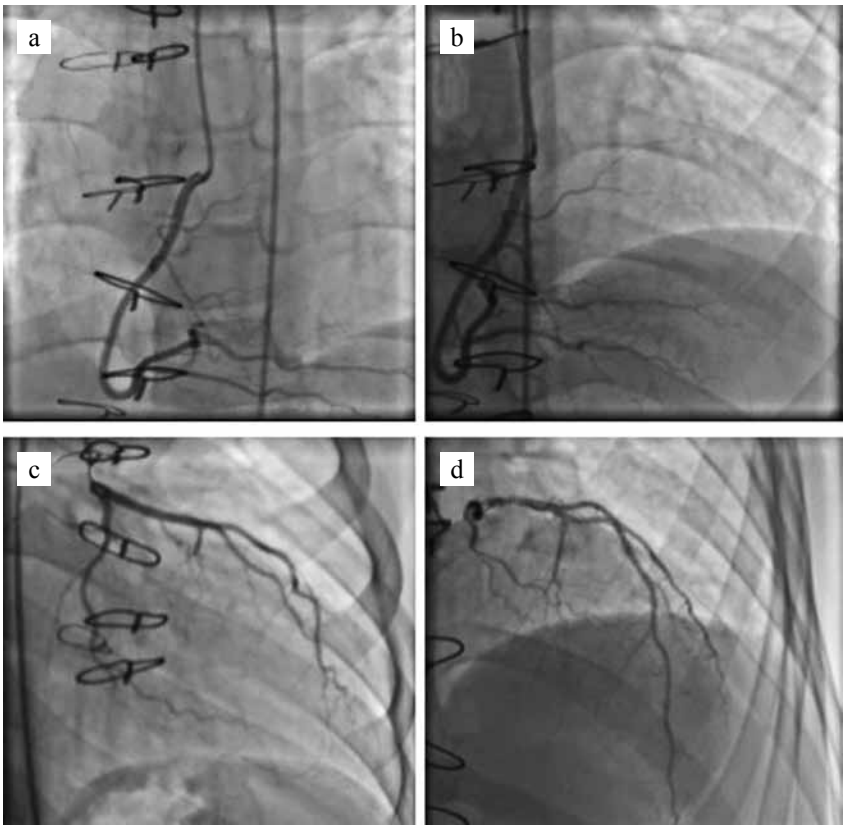

Fig. 3. Postoperative (12 months later) coronarography. Right and left coronary artery basin, without signs of atherosclerotic lesion of the coronary bed

Thereafter, planned hospitalizations and therapy adjustments, as well as examinations were carried out. On May 27, 2022, control coronarography was performed – coronary arteries without angiographic signs of atherosclerotic lesions. Blood flow through the coronary arteries was satisfactory (Fig. 3).

DISCUSSION

This case report describes a patient with CAV, one of the possible complications after heart transplantation. Patients with such a diagnosis are rarely helped by drug therapy; the most optimal treatment is surgical – heart retransplantation. According to the world literature, the number of repeated interventions does not exceed 5% in the age group from 18 to 39 years, but there is an increasing trend every year. However, the number of repeated HTs in the pediatric population in the Russian Federation, as well as in the world, still amounts to dozens of cases, which reflects the clinical significance of such reports.

As part of the preoperative preparation, the patient underwent chest CT scan, which, in our experience, allows us to assess cardiac syntopy and, in turn, is an important preparatory stage in planning repeat interventions. Preoperative preparation of the patient is also important, namely CHF compensation in a specialized department, if the severity of the patient's condition allows it.

The second stage of cardiac rehabilitation took place at the cardiology department. This clinical case demonstrates successful treatment of a CAV patient, in which case repeat HT was the optimal treatment.

CONCLUSION

The management of patients indicated for retransplantation, both preoperatively, as well as intraoperatively and postoperatively, requires multidisciplinary involvement. Repeat heart transplantation is the most optimal treatment modality for pediatric patients with CAV. However, further clinical evidence needs to be accumulated from which clear guidelines for this approach should be developed.

The authors declare no conflict of interest.

REFERENCES

1. *Chambers DC, Cherikh WS, Harhay MO, Hayes D Jr, Hsich E, Khush KK et al*. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-sixth adult lung and heart-lung transplantation Report-2019; Focus theme: Donor and recipient size match. *J Heart Lung Transplant*. 2019 Oct; 38 (10): 1042–1055. doi: 10.1016/j.healun.2019.08.001.
2. *Barghash MH, Pinney SP.* Heart Retransplantation: Candidacy, Outcomes, and Management. *Curr Transplant*

Rep. 2020; 7 (1): 12–17. doi: 10.1007/s40472-019-00257-y. Epub 2019 Dec 17. PMID: 32435573; PMCID: PMC7223608.

3. *Lund LH, Edwards LB, Kucheryavaya AY, Benden C, Christie JD, Dipchand AI et al.* International Society of Heart and Lung Transplantation. The registry of the International Society for Heart and Lung Transplantation: thirty-first official adult heart transplant report – 2014; focus theme: retransplantation. *J Heart Lung Transplant*. 2014 Oct; 33 (10): 996–1008. doi: 10.1016/j.healun.2014.08.003. Epub 2014 Aug 14. PMID: 25242124.
4. *Chou NK, Chi NH, Chen YS, Yu HY, Lee CM, Huang SC et al*. Heart retransplantation for heart allograft failure in Chinese heart transplant recipients: NTUH experience. *Transplant Proc*. 2006 Sep; 38 (7): 2147–2148. doi: 10.1016/j.transproceed.2006.06.109. PMID: 16980027.
5. *Gautier SV, Poptsov VN, Saitgareev RSh, Shumakov DV, Zakharevich VM, Khalilulin TA i dr.* Strategiya retransplantatsii serdtsa: rezul'taty i problemy. *Vestnik transplantologii i iskusstvennykh organov*. 2016; 18 (Prilozhenie): 32.
6. *Lund LH, Edwards LB, Kucheryavaya AY, Dipchand AI, Benden C, Christie JD et al.* International Society for Heart and Lung Transplantation. The Registry of the International Society for Heart and Lung Transplantation: Thirtieth Official Adult Heart Transplant Report – 2013; focus theme: age. *J Heart Lung Transplant*. 2013 Oct; 32 (10): 951–964. doi: 10.1016/j.healun.2013.08.006. PMID: 24054804.
7. *Rizvi SA, Luc JGY, Choi JH, Phan K, Moncho Escrivà E, Patel S et al*. Outcomes and survival following heart retransplantation for cardiac allograft failure: a systematic review and meta-analysis. *Ann Cardiothorac Surg*. 2018 Jan; 7 (1): 12–18. doi: 10.21037/acs.2018.01.09. PMID: 29492380; PMCID: PMC5827127.
8. *Srivastava R, Keck BM, Bennett LE, Hosenpud JD.* The results of cardiac retransplantation: an analysis of the Joint International Society for Heart and Lung Transplantation/United Network for Organ Sharing Thoracic Registry. *Transplantation*. 2000 Aug 27; 70 (4): 606–612. doi: 10.1097/00007890-200008270-00013. PMID: 10972218.
9. *Costanzo MR, Naftel DC, Pritzker MR, Heilman JK 3rd, Boehmer JP, Brozena SC et al*. Heart transplant coronary artery disease detected by coronary angiography: a multiinstitutional study of preoperative donor and recipient risk factors. Cardiac Transplant Research Database. *J Heart Lung Transplant*. 1998 Aug; 17 (8): 744–753. PMID: 9730422.
10. *Mehra MR, Crespo-Leiro MG, Dipchand A, Ensminger SM, Hiemann NE, Kobashigawa JA et al*. International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy – 2010. *J Heart Lung Transplant*. 2010 Jul; 29 (7): 717–727. doi: 10.1016/j.healun.2010.05.017. Erratum in: *J Heart Lung Transplant*. 2011 Mar; 30 (3): 360. PMID: 20620917.

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BIOLOGICAL PROPERTIES OF MACROPOROUS CRYOSTRUCTURATE BASED ON EXTRACELLULAR MATRIX COMPONENTS

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Objective: to study the biological properties of macroporous cryostructurate from multicomponent concentrated collagen-containing solution (MCCS) as a promising matrix for the formation of cell- and tissue-engineered constructs. **Materials and methods.** A macroporous spongy carrier was obtained by cryostructuring of collagen-containing extract, prepared by acetic acid hydrolysis of chicken connective tissue (BIOMIR Service, Russian Federation). N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (Sigma-Aldrich, USA) was used to make the cryostructurate water insoluble. The micromorphology of the sponge surface was studied using scanning electron microscopy. The cytotoxicity of the carrier was evaluated by reaction of the mouse NIH 3T3 fibroblast cell culture using automated microscope IncuCyte ZOOM (EssenBioscience, USA). Biocompatibility of the macroporous carrier was studied on cultures of human adipose tissue-derived mesenchymal stromal cells (AD-MSC), human hepatocellular carcinoma cell line *HepG2* and human umbilical vein endothelial cell line *EA.hy926*. The metabolic activity of cells was determined using PrestoBlue™ reagents (Invitrogen™, USA). Cell population development during long-term cultivation of the cell-engineered construct (CEC) was assessed by fluorescence-lifetime imaging microscopy over the entire surface of the sample using a Leica Dmi8 inverted microscope with Leica Thunder software (Leica Microsystems, Germany). **Results.** Optical microscopy and scanning electron microscopy (SEM) showed the presence of pores of different sizes in the resulting biopolymer material: large pores with 237 ± 32 μm diameter, medium-sized pores with 169 ± 23 μm diameter, and small-sized pores with 70 ± 20 μm diameter; large and medium-sized pores were predominant. The studied media did not exhibit cytotoxicity. Cell adhesion and proliferation on the surface of the material and their penetration into the underlying layers during long-term cultivation were observed. The highest metabolic activity of the cells was observed for human AD-MSC on day 14, which corresponds to the normal dynamics of development of a population of cells of this type. The functional activity of HepG2 cells – albumin and urea production – was shown in the liver CEC model. **Conclusion.** The good adhesion and active proliferation that were shown for the three cell types indicate that the resulting biopolymer carrier is biocompatible, and that the spread of the cells into the inner volume of the sponge and active population of the sponge under prolonged culturing indicates that this material can be used to create cell- and tissue-engineered constructs.

Keywords: cryogenic structuring, collagen, tissue engineering.

INTRODUCTION

One of the most important aspects of creating cell-engineered constructs (CEC) and tissue-engineered constructs (TEC) is the selection of a suitable scaffold which, on one hand, performs a structure-forming function, and, on the other hand, influences cell proliferation and differentiation processes. The internal architecture of TEC and CEC has been shown to be of fundamental importance for their structural and biological functions [1]. For instance, the use of a carrier with an ordered structure of microfibers that set the direction of growth of mesenchymal stromal cells (MSCs) in cartilage TECs led to increased glycosaminoglycan synthesis during cell differentiation compared to an unstructured scaffold [2]. Its structure is of even greater importance for the creation of TECs and CECs of the liver, where the normal functioning of cells of different types is possible only if there is a certain microarchitecture of the structure [3, 4].

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Various approaches such as electrospinning [5] and various types of bioprinting [6] are used to create structured cell carriers. Much focus is on the search for new bioprinting materials that mimic the extracellular matrix and methods of imparting a structure closer to the native tissue [7].

It should be noted that, despite the numerous advantages of these techniques, they are difficult to perform and require expensive equipment. In turn, the cryostructuring methodology allows to create effective scaffolds with pores of different sizes at lower costs, providing both mass transfer of nutrients and gases and expansion of cells from the surface into the inner volume of the scaffold material [8].

Cryostructuring techniques are used to obtain such macroporous polymeric materials as various cryogels and cryostructurates [9]. The former are formed when 3D polymer mesh nodes are formed in the volume of unfrozen microphase of a macroscopically frozen system (this process is called cryotropic gelation). If there is no gelation proper, then after removal of the frozen solvent, for example, by sublimation or cryoextraction, polymeric objects called cryostructurates are obtained. A fundamental feature of both cryogels and cryostructurates is their macroporosity formed by polycrystals of the frozen solvent. Most macropores are interconnected, and their morphology and sizes depend on the conditions of all successive stages of cryogenic structuring. It is this special macroporosity, combined in many cases with good mechanical properties of various cryogels and cryostructurates, especially those based on biocompatible polymers, that makes them promising materials for biomedical applications [10–15].

An important advantage of this technology is that the physical properties of cryogenically structured materials, especially the pore size, can be influenced quite easily by adjusting the preparation parameters, including polymer content, crosslinking mechanism, temperature, freezing time, and freeze/thaw rate and cycles [8, 16].

When choosing materials for the manufacture of CEC and TEC matrices, besides synthetic resorbable polymers such as polylactides [17], biopolymer hydrogels are most commonly used. Many hydrogels based on one of the extracellular matrix (ECM) components have been developed to stimulate the regenerative potential of damaged tissues by minimally invasive injection [18]. In this case, among commonly used biopolymers, preference is given to such natural polymers as collagen, gelatin, chitosan, hyaluronic acid, alginates, and polyesters of bacterial origin [19].

Given the multifunctional properties of ECM, the attention of researchers in recent years has been drawn to multicomponent biopolymer-based hydrogels – ECM biomimetics, which include all of its main components (proteins and polysaccharides) as well as growth factors and other signaling molecules necessary for cell adhesion, proliferation and differentiation [19, 20]. Such biopolymer-based hydrogels have been shown to provide greater cell proliferation compared to single ECM component substrates [21]. However, along with the pronounced advantages of hydrogels, there are several difficulties associated with the inability to perform the function of a scaffold for cells and the lack of sufficient pore space, which does not allow angiogenesis to proceed throughout the entire volume of CECs, complicates cell migration into the hydrogel volume, transport of nutrients to them, and removal of metabolites [10].

Consequently, the idea of creating a bioactive macroporous cryostructured matrix [22] that is based on a multicomponent concentrated collagen-containing solution (MCCS) obtained by acetic acid extraction from animal tissues seems promising [20].

This study is a continuation of our earlier works on obtaining and studying the physicochemical and biological properties of gelatine-based macroporous cryostructurate [23, 24]. The positive results obtained in the creation of CECs based on gelatin cryostructurate matrices allowed us to use them in this work as comparison samples.

MATERIALS AND METHODS

Preparation of MCCS-based cryostructured matrix

A commercially available product, "Collagen-containing extract" (TU 9389-008-54969743-2016, BIOMIR Service, Krasnoznamensk) was used as the initial raw material. MCCS level was 40 mg/mL, total protein content in the MCCS was 96%, pH = 5.8 ± 0.3. MCCS-based cryostructure samples were produced at the Nesmeyanov Institute of Organoelement Compounds in Moscow according to the modified method [22]. The MCCS was first heated for 1 hour at 42 °C, then diluted 1.5 times with deionized water and poured into 35 mm diameter plastic Petri dishes in a thin layer (2 mm). The dishes were placed in an ultracryostat K2 (Huber, Germany) and frozen at –20 °C for 3 hours. Then they were freeze-dried in a FreeZone[1] unit (Labconco, USA). Next, the resulting macroporous cryostructurate sponges were incubated in 0.1 M ethanol solution of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (Sigma-Aldrich, USA) at room temperature for 24 hours. Afterwards, they were washed with ethanol to remove excess carbodiimide and stored in 96% ethanol medium until used as a matrix for cell culture. For experiments, disks of 1 cm^2 area and 2 mm thickness were cut from the macroporous sponges.

Investigation of surface micromorphology of cryostructurates

The surface morphology of the samples was studied by phase-contrast microscopy using a Leica DMi8 Thunder super-resolution system with LAS X software.

The micromorphology of the collagen-containing matrix surface was studied using scanning electron microscopy (SEM) with lanthanide contrast of the sample, which allowed using low vacuum conditions, preserving the native structure of the material as much as possible [25]. Sample preparation included initial washing, exposure for 45 minutes in BioREE-A contrasting solution (Glaucon LLC, Russia) and final washing with distilled water. Next, excess moisture was removed from the surface of the specimen with an air brush and placed on the slide of an EVO LS10 microscope (Zeiss, Germany). Observations were performed in low vacuum mode (EP, 70 Pa), at an accelerating voltage of 20–25 kV. Images were captured using a backscattered electron detector (BSE mode).

Cell cultures

To evaluate cytotoxicity of the tested matrices, we used mouse NIH 3T3 fibroblast cell line (ATCC®CRL-1658™) from the American Type Culture Collection (ATCC). To study cell adhesion and proliferation, we used of human adipose tissue-derived mesenchymal stromal cell (AD-MSC) culture obtained at Shumakov National Medical Research Center of Transplantology and Artificial Organs according to the previously developed method [23]. Human umbilical vein endothelial cell line EA.hy926 (ATCC®CRL-2922™) from ATCC and human hepatocellular carcinoma cell line HepG2 from the cell culture collection owned by Shumakov National Medical Research Center of Transplantology and Artificial Organs were used to create CEC sponges.

Cell cultures were stored in liquid nitrogen at –196 °C. After thawing, the cells were seeded into standard 25 cm^2 culture vials (CELLSTAR® Greiner Bio-One, Germany) and cultured in appropriate complete growth medium (CGM). For fibroblast cell lines NIH 3T3 and endothelial cell lines EA.hy926, high glucose DMEM (PanEco, Russia) supplemented with 10% fetal calf serum (TS, Biosera, Germany) or fetal calf serum (HyClone, USA), respectively, antibiotic-antimycotic Anti-Anti (Gibco® by Life Technologies™, SC) and 2 mmol alanyl-glutamine (PanEco, Russia) were used. AD-MSC and HepG2 were cultured in DMEM/F12 medium (PanEco, Russia) supplemented with 10% fetal calf serum (HyClone, USA), 10 μg/mL of basic human fibroblast growth factor (FGF-2, Peprotech, AF-100-18B, USA), antibiotic-antimycotic Anti-Anti (Gibco® by Life Technologies™, SC), 1 mmol HEPES (Gibco® by Life Technologies™, SC) and 2 mmols alanyl-glutamine (PanEco, Russia). Vials with cells were cultured in a CO_2 incubator under standard conditions: 37 °C, humid atmosphere containing (5 ± 1) % CO_2.

For experiments, cells were washed off the culture plate using dissociation reagent TrypLE™ Express Enzyme (Gibco® by Life Technologies™, SC) and a suspension with the required cell concentration was prepared.

Cell count in the suspension was determined on an automated cell counter (TC20™ Automated Cell Counter, BIORAD, Singapore) with simultaneous viability test with trypan blue (BIORAD, # 145-0013, Singapore) according to the equipment manufacturer's methodology.

Matrix cytotoxicity assessment

The cytotoxicity of samples of MCCS-based media was determined by direct contact method according to GOST ISO 10993-5-2011 [26]. Mouse NIH 3T3 fibroblast cell lines were seeded into flat-bottomed 6-well culture plates (CELLSTAR® Greiner Bio-One, Germany) at a concentration of 5×10^5 cells per well and incubated for 24 hours at 37 °C under standard conditions until the formation of (80 ± 10)%. Next, the studied samples of cryostructurates were placed on the surface of the cell monolayer in the form of disks with a diameter of 6 mm and a thickness of 2 mm. The comparison samples were macroporous sponges in the form of gelatin-based cryostructurate disks of the same size as the samples of collagen-containing cryostructurates with proven absence of cytotoxic effect [23]. The disks were first thoroughly washed of ethanol residues with two portions of sterile distilled water and left for 24 hours in CGM at 37 °C. The CGM served as a negative control sample for cell culture, and 10 mg/mL single-element aqueous zinc standard (Sigma-Aldrich, USA) served as a positive control sample).

An additional test was performed for more detailed evaluation of growth dynamics and to identify a possible cytostatic effect. During the test, cell plates were incubated in the presence of samples using the IncuCyte ZOOM system (EssenBioscience, USA). The IncuCyte ZOOM system allowed the monolayer density to be assessed automatically every 2 hours throughout the experiment with simultaneous construction of growth curves. The experiment lasted for just over 50 hours.

Assessing the ability of the sponge carrier to support cell adhesion and proliferation

The ability of MCCS-based cryostructurate to support cell adhesion and proliferation was also compared with gelatin-based cryostructurate samples. To set up the experiment, cryostructurate samples were thoroughly washed from ethanol and placed in a CGM for 1 day until saturation. The required number of cells was grown in culture vials and a cell suspension with a working concentration of 1×10^5 cells/mL was prepared. Next, 1 ml of the cell suspension was applied to the sample surface drop by drop. The samples were placed in 50 ml centrifuge tubes and left in a CO_2 incubator for 1 hour for cell attachment, after which the CGM level in the tubes was brought to 5 ml and culturing was continued under standard conditions. On days 1, 3, 6, 9 and 14 of cultivation, three portions of the CGM were taken for metabolic activity test with PrestoBlue™ HS Cell

Viability Reagent (Invitrogen™ by Thermo Fisher Scientific, USA) according to the reagent manufacturer's protocol. Spectrophotometry was performed on a Spark 10M tablet reader (Tecan, Austria) with Spark Control™ Magellan V1.2.20 software at two wavelengths, 570 nm and 600 nm. Data from optical absorption measurements were used to calculate the coefficient of metabolic activity (K) according to the formula:

$$K = \frac{117,216 \cdot Abs_{570} - 80,586 \cdot Abs_{600}}{155,677 \cdot Abs_{600} - 14,652 \cdot Abs_{570}} \times 100\%,$$

where Abs_{570} is the optical absorption at 570 nm, and Abs_{600} is the optical absorption at 600 nm.

Cell count corresponding to coefficient K obtained was determined in an auxiliary experiment, where certain amounts of cells were seeded into the wells of a plate and their metabolic activity was measured after one day. A calibration graph was plotted based on the measurement results, which allowed to bring the value K to the number of AD-MSCs.

CECs based on collagen-containing cryostructurates and different cell types

To create CECs based on MCCS macroporous sponge, suspensions of appropriate cultures were prepared at a concentration of 1×10^5 cells/mL. Gelatin cryostructurate samples were also used as a control. Matrices from cryostructurates were immersed in the suspension and processed for 1 hour on a laboratory shaker in orbital stirring mode at 40 rpm for uniform distribution of cells on the surface and penetration into the inner volume of the sample. The resulting CECs were cultured under standard conditions for 7 and 10 days for HepG2, and 7 and 15 days for EA.hy926.

Cell surface adhesion, nature of cell distribution over the sample volume, viability, morphology and proliferative activity were assessed by in vivo microscopy with fluorescent dyes Live/Dead® Viability/Cytotoxicity Kit (Molecular Probes® by Life Technologies™, USA) using Leica DMi8 Thunder microscope with LAS X software for analyzing the 3-d structure of the sample.

Functional properties of HepG2 hepatocellular carcinoma cells when cultured on an MCCS-based macroporous matrix

HepG2 cells (5×10^5 kL), were plated on $10 \times 10 \times 2$ mm fragments of MCCS (n = 10) and gelatin (n = 10) samples. The resulting CECs were cultured in CGM under standard conditions for 10 days. On day 10, albumin content in the culture medium was determined by enzyme-linked immunosorbent assay using Human Albumin ELISA Kit (Invitrogen™ by Thermo Fisher Scientific, USA). Culture medium from cells that were cultured on plastic in the same quantity was used as a control.

Ammonia metabolism rate was determined after 90 minutes of incubation with 1 mmol ammonium chloride (Sigma-Aldrich, USA) diluted in culture medium on day 10 of the experiment. The amount of urea in the medium was estimated on a KonelabPrime 60i biochemical analyzer (ThermoFisher Scientific, Finland).

Reliability of differences was determined using Student's t-test (SPSS 26). Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Investigation of surface micromorphology of macroporous matrices

A microscopy of the samples revealed a porous structure of the material with numerous pores of different sizes (Fig. 1, a). Conventionally, the pores can be categorized into three groups by size: large (237 ± 32 μm), medium (169 ± 23 μm) and small (70 ± 20 μm). Thus, the MCCS-based cryostructured material is dominated by large-sized pores. In comparison, only medium-sized (169 ± 23 μm) and small-sized (70 ± 20 μm) pores were mainly present in the gelatin cryostructured material (Fig. 1, b). SEM images of the surface of both

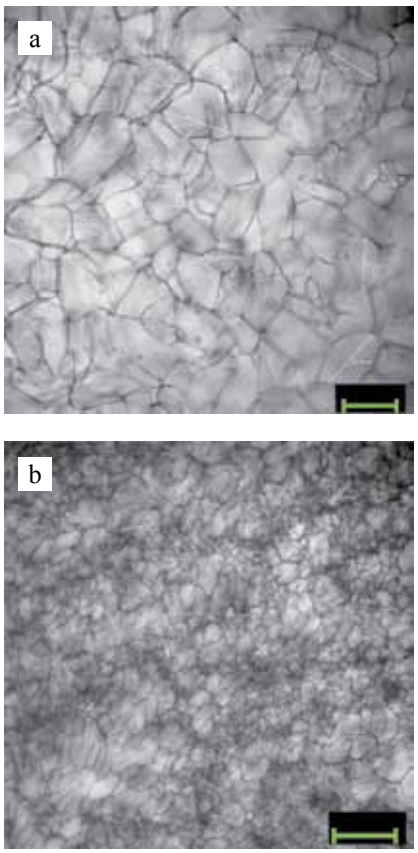

Fig. 1. Surface morphology of cryostructurates. Phase-contrast microscopy. Magnification 50×; a, MCCS-based cryostructurate; b, gelatin-based cryostructurate. Scale bar: 200 μm

sponges show that the pores form interconnected channels running through the entire volume of the material (Fig. 2).

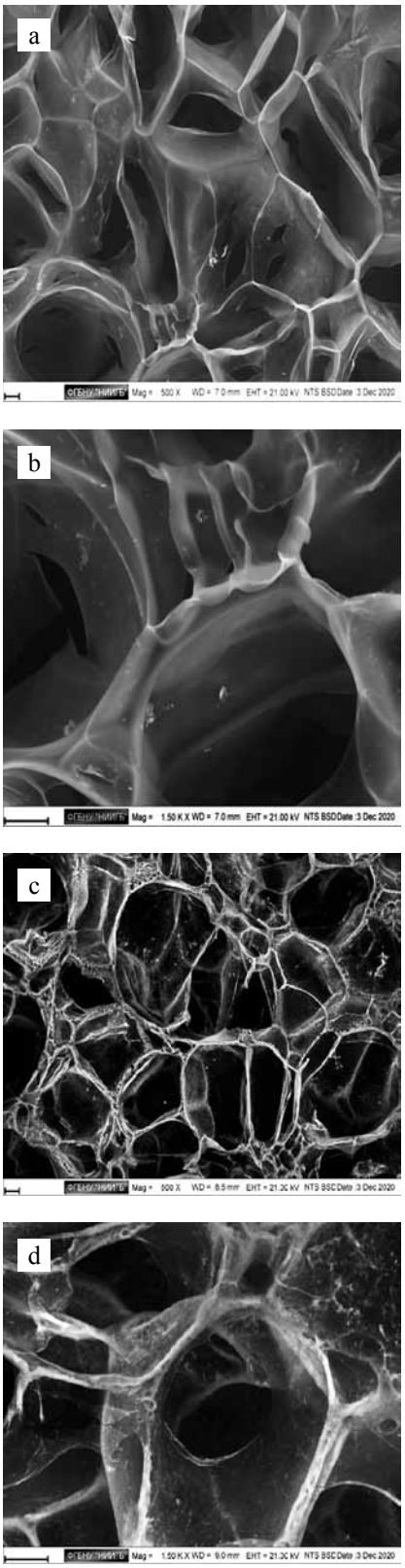

Fig. 2. Macroporous morphology of cryostructurates. SEM with lanthanide contrasting. a, b, MCCS-based cryostructurate; c, d, gelatin-based cryostructurate. a, b, magnification 500×; b, d, magnification 1500×. Scale bar: 20 μm

Cytotoxicity of the resulting cryostructurates

Assessment of the cytotoxicity of the MCCS-based cryostructurate revealed no negative effect when NIH 3T3 cells were cultured in the presence of fragments of macroporous matrices obtained from MCCS and gelatin. Cells actively proliferated over the entire area of the well of the plate, including the area of contact with the sample (Fig. 3). No rounded cells or cells with disturbed morphology were observed.

Determination of the degree of monolayer confluency in automatic mode showed that the most active cell growth begins after 20 hours of the experiment and, by the end of the experiment, this index reaches values of more than 70%, which corresponds to the normal development of NIH 3T3 cell population. There were no significant differences from the comparison sample (Fig. 3, b).

Biocompatibility. Cell adhesion and proliferation

The study of biocompatibility, i.e. the ability of cells to adhere to the surface and further proliferate, was carried out using an AD-MSC culture. In parallel, a gelatin-based cryostructurate was used as a control in the experiment. 1×10^5 cells were plated on a sponge fragment. Vital dye intravital microscopy showed that on day 3, cells adhered on the surface of both media. Distribution of AD-MSCs on the surface was quite uniform, the cells were spread out and had normal morphology. There were practically no dead cells. By day 12 of cultivation, a significant increase in cell mass was observed in the samples with the formation of large cell clusters. It was also noted that cells inhabited not only the surface of cryostructurates, but also penetrated deep into the sponge. Moreover, due to the presence of large, interconnected pores in the MCCS-based sponge, this effect was much more pronounced in this case (Fig. 4).

Data from the metabolic activity test with PrestoBlue™ reagent fully correspond to the microscopic picture and allow for quantitative assessment of their proliferative activity.

As shown in the graph (Fig. 5), after the lag phase that is necessary for cells to adapt, the logarithmic growth phase begins by day 4 of cultivation, and continues, with some slowdown by day 6–9, until the end of the experiment on day 14. In our opinion, the slowdown during this period is due to the filling of the available surface of the macroporous matrix by cells and the transition to colonization of the underlying layers of the cryostructurate. For both types of samples, we observed similar dynamics of cell mass growth, but AD-MSCs, when cultured on an MCCS matrix, proliferated more actively at late observation periods (days 9 and 14).

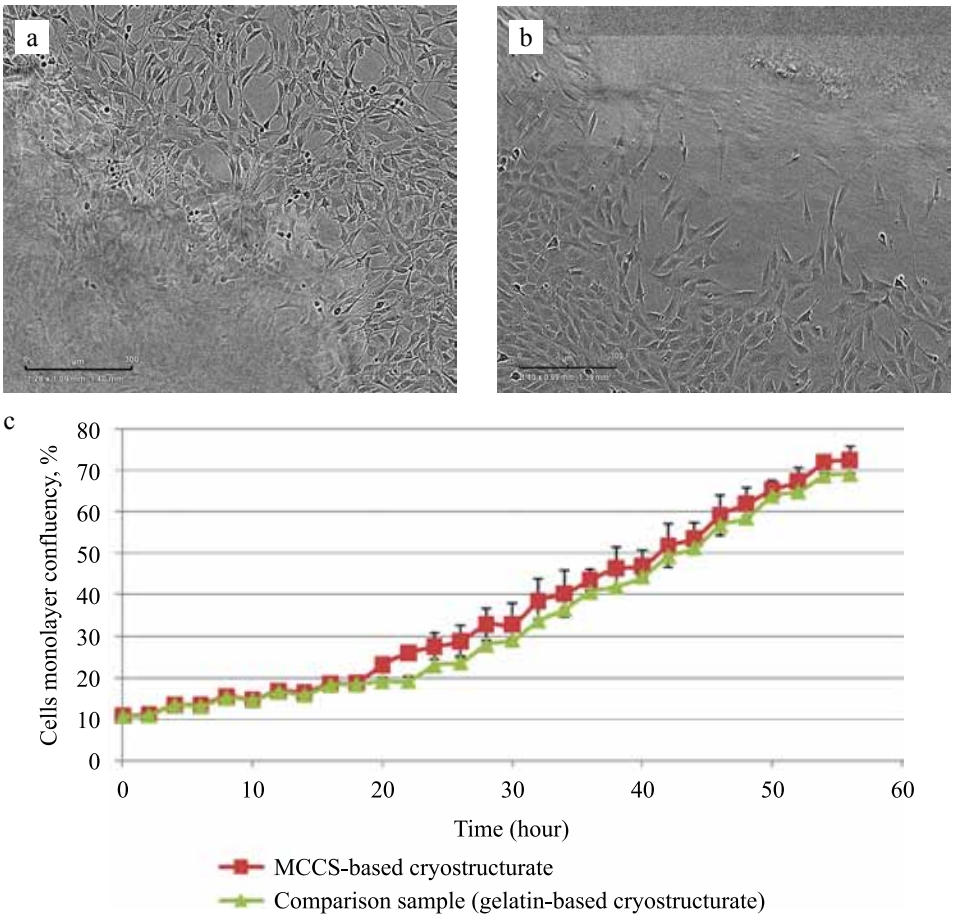

Fig. 3. Growth of mouse NIH 3T3 fibroblasts in the presence of macroporous matrix fragment. a, MCCS-based cryostructurate; b, gelatin-based cryostructurate (comparison sample). Phase-contrast microscopy. Magnification 100×. Scale bar: 300 μm; c, growth curve of NIH 3T3 cells on culture plastic in the presence of a fragment of MCCS-based macroporous carrier

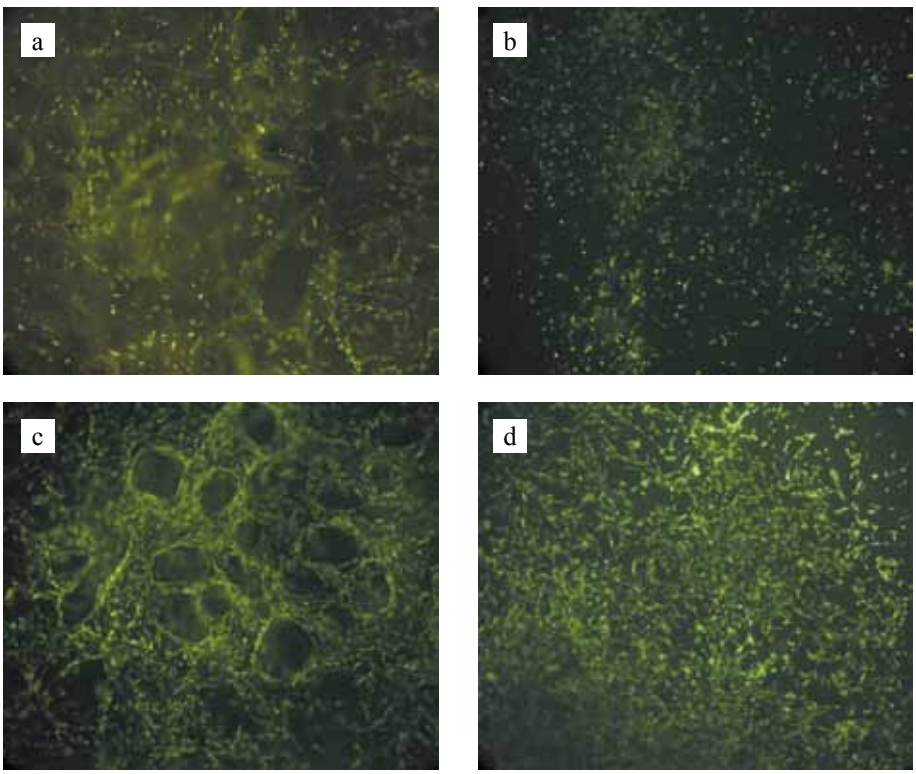

Fig. 4. Growth of human AD-MSC culture on MCCS-based matrix (a, c) and gelatin-based matrix (b, d); a and b, 3 days, c and d, 12 days of culture. Live/Dead™ stain. Magnification 100×. Scale bar: 100 μm

Creation of CECs from different cell types on an MCCS-based cryostructurate

When seeded with a large number of cells – 5×10^5 cells per sample – it was shown that for both HepG2 (Fig. 6, a–d) and EA.hy926 (Fig. 7, a–f) culture, almost all cells adhere to the sample surface and actively proliferate, forming significant clusters by the end of the experiment (on day 10 for HepG2, and day 15 for EA.hy926).

Adherent cells on gelatin-based cryostructurate also actively proliferated, but there were more dead cells in the case of HepG2 (Fig. 6, d). Note that significant differences were observed in the localization of cell clusters. In the case of the gelatin disk, we noted predominantly

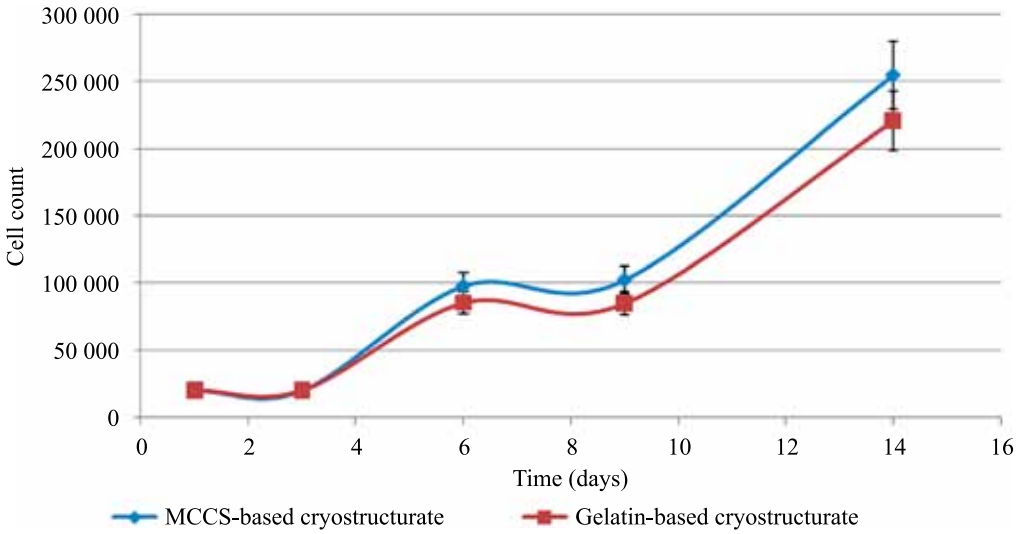

Fig. 5. Metabolic growth curve of human AD-MSCs on the tested matrices (Test with PrestoBlue™)

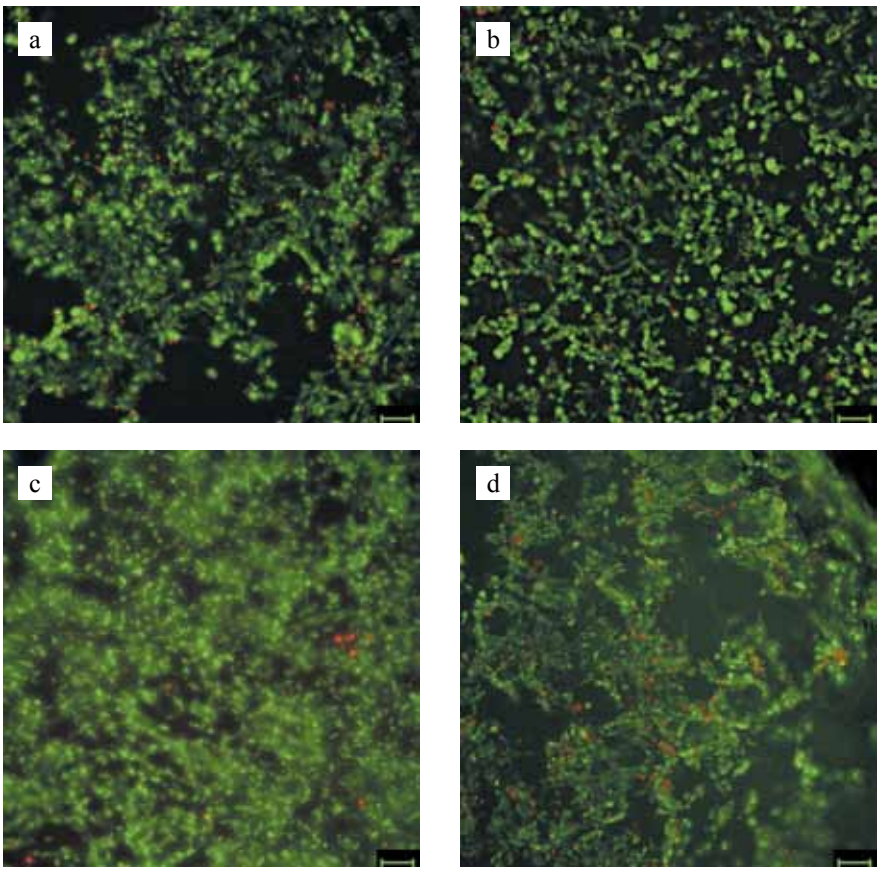

Fig. 6. HepG2 growth on a cryostructured matrix. a, 3 days of culture; b, 7 days, MCCS-based cryostructurate; c, 3 days of culture; d, 7 days, gelatin-based cryostructurate. Live/Dead™ stain, live cells are stained in green, dead cells are stained in red. Magnification 100×. Scale bar: 100 μm

cell spreading on the surface of the sample, whereas the MCCS disk showed a tendency to actively colonize the walls of large pores and channels with the colonization of the inner volume of the macroporous carrier. This was especially clear for endothelial cells at a late period of cultivation (Fig. 7, b).

The use of stacking technology", i.e. shifting the focal point of the microscope lens deep into the sample to a depth of more than 100 μm, followed by software processing of the image, showed that cells spread into the inner volume of the macroporous disk to a depth of up to 60 μm (Fig. 7, d).

For gelatin-based cryostructurates, this figure was smaller, not exceeding 50 μm (Fig. 7, e).

Assessment of the functional properties of HepG2 cells when cultured on an MCCS-based cryostructurate

The functional activity of HepG2 cells in CEC was analyzed by albumin synthesis and urea production

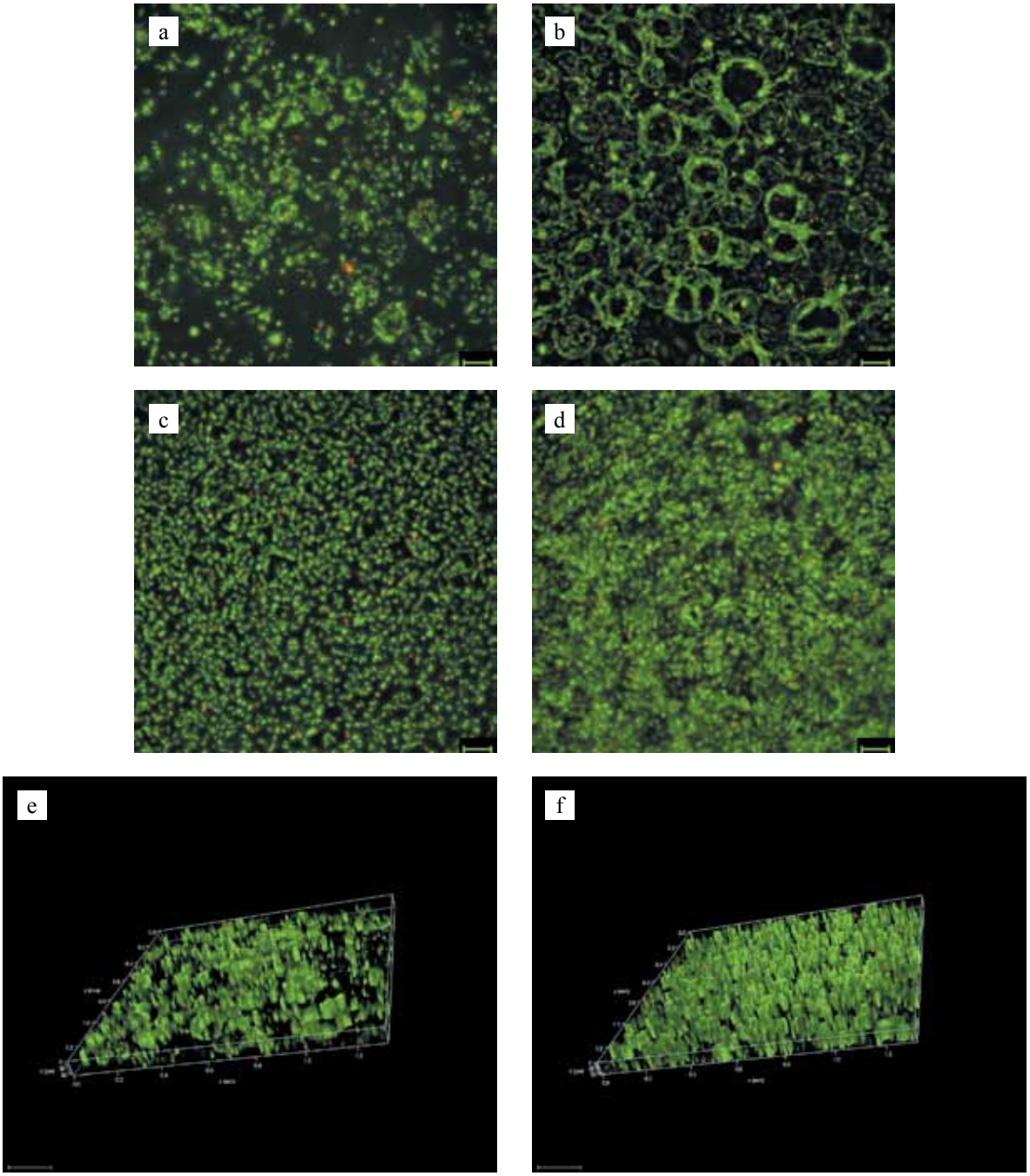

Fig. 7. EA.hy926 endothelial cell growth on cryostructured matrix. a, 7 days of culture; b, 15 days, MCCS-based cryostructurate; c, 7 days of culture; d, 15 days, gelatin-based cryostructurate; e, 15 days, cell distribution in the MCCS-based cryostructurate; f, 15 days, cell distribution in the gelatin-based cryostructurate. Live/Dead™ stain, live cells are stained in green, dead cells are stained in red. a–d, scale bar: 100 μm; e, f, scale bar: 200 μm

Table 1

Albumin content and urea level in culture medium on day 10 of culturing HepG2 cells on culture plastic (control)

	Albumin (mmol/mL)	Urea (mmol/mL)
Culture on plate (control)	960 ± 102	1.2 ± 0.2
Culture on MCCS-based cryostructurate	1413 ± 183	1.7 ± 0.3
p	<0.050*	0.051

* – the differences are statistically significant ($p < 0.05$).

Table 2

Albumin levels in culture medium on day 10 of CEC cultivation with different amounts of HepG2 cells

Cell count in CEC	Albumin (mmol/mL)
100,000	1323 ± 164
500,000	1413 ± 183
1,000,000	1963 ± 293

(Table 1). The culture medium of the same cell count on culture plastic was used as a control.

In a separate test, we compared the level of albumin production for CECs with different cell counts (Table 2).

Note that albumin and urea levels in the samples correlated with cell count in the CEC.

The obtained data indicate that HepG2 seeded on a MCCS-based cryostructurate can maintain its secretory function on day 10 of cultivation at a higher level, compared to cell cultivation on plastic.

CONCLUSION

The studies show that the macroporous cell carrier based on a cryostructured multicomponent concentrated collagen-containing solution has a large pore space, where large and medium-sized pores predominate, forming a network of branched channels in the matrix thickness. This macroporous structure significantly improves cell expansion deep into the MCCS-based cryostructurate compared to gelatin-based cryostructurates. The cell carrier studied in this work is non-cytotoxic, supports adhesion and proliferation of different cell types and formation of a cell-engineered construct in which cells can function normally.

The authors declare no conflict of interest.

REFERENCES

1. Transplantologija i iskusstvennye organy: uchebnik / Pod red. akad. RAN S.V. Gautier. M.: Laboratorija znanij, 2018; 319: il. (In Russ.).
2. *Yamamoto T, Randriantsilefisoa R, Sprecher CM, D'Este M*. Fabrication of collagen-hyaluronic acid cryogels by directional freezing mimicking cartilage arcade-like structure. *Biomolecules*. 2022 Dec 3; 12 (12): 1809. doi: 10.3390/biom12121809.
3. *Mirdamadi ES, Kalhori D, Zakeri N, Azarpira N, Solati-Hashjin M*. Liver tissue engineering as an emerging alternative for liver disease treatment. *Tissue Eng Part B Rev*. 2020 Apr; 26 (2): 145–163. doi: 10.1089/ten.teb.2019.0233.
4. *Zhang L, Guan Z, Ye JS, Yin YF, Stoltz JF, de Isla N*. Research progress in liver tissue engineering. *Biomed Mater Eng*. 2017; 28 (s1): S113–S119. doi: 10.3233/BME-171632.
5. *Wise JK, Yarin AL, Megaridis CM, Cho M.* Chondrogenic differentiation of human mesenchymal stem cells on oriented nanofibrous scaffolds: engineering the superficial zone of articular cartilage. *Tissue Eng Part A*. 2009 Apr; 15 (4): 913–921. doi: 10.1089/ten.tea.2008.0109.
6. *Daly AC, Kelly DJ.* Biofabrication of spatially organised tissues by directing the growth of cellular spheroids within 3D printed polymeric microchambers. *Biomaterials*. 2019 Mar; 197: 194–206. doi: 10.1016/j.biomaterials.2018.12.028.
7. *Schwab A, Hélary C, Richards RG, Alini M, Eglin D, D'Este M.* Tissue mimetic hyaluronan bioink containing collagen fibers with controlled orientation modulating cell migration and alignment. *Mater Today Bio*. 2020 Jun 1; 7: 100058. doi: 10.1016/j.mtbio.2020.100058.
8. *Lozinsky VI.* Polymeric cryogels as a new family of macroporous and supermacroporous materials for biotechnological purposes. *Russ Chem Bull*. 2008; 57 (5): 1015–1032. doi: 10.1007/s11172-008-0131-7.
9. *Lozinsky VI.* Cryostructuring of polymer systems. 50.† Cryogels and cryotropic gel-formation: terms and definitions. *Gels*. 2018; 4 (3): 77. doi: 10.3390/gels4030077.
10. *Henderson TMA, Ladewig K, Haylock DN, McLean KM, O'Connor AJ.* Cryogels for biomedical applications. *J Mater Chem B*. 2013; 1 (21): 2682–2695. doi: 10.1039/c3tb20280a.
11. *Memic A, Colombani T, Eggermont LJ, Rezaeeyazdi M, Steingold J, Rogers ZJ et al.* Latest advances in cryogel technology for biomedical applications. *Adv Ther*. 2019; 2 (4): 1800114. doi: 10.1002/adtp.201800114.
12. *Lozinsky VI.* Cryostructuring of polymeric systems. 55. Retrospective view on the more than 40-years studies performed in the A.N. Nesmeyanov Institute of Organoelement Compounds with respect of the cryostructuring processes in polymeric systems. *Gels.* 2020; 6 (3): 29. doi: 10.3390/gels6030029.
13. *Shiekh PA, Andrabi SV, Singh A, Majumder S, Kumar A.* Designing cryogels through cryostructuring of polymeric matrices for biomedical applications. *Eur Polym J.* 2021; 144; 110234. doi: 10.1016/j.eurpolymj.2020.110234.
14. *Ma Y, Wang X, Su T, Lu F, Chang Q, Gao J.* Recent advances in macroporous hydrogels for cell behavior and tissue engineering. *Gels*. 2022 Sep 21; 8 (10): 606. doi: 10.3390/gels8100606.
15. *Omidian H, Chowdhury SD, Babanejad N.* Cryogels: advancing biomaterials for transformative biomedical applications. *Pharmaceutics.* 2023 Jun 27; 15 (7): 1836. doi: 10.3390/pharmaceutics15071836.

16. *Lozinsky VI, Okay O*. Basic principles of cryotropic gelation. *Adv Polym Sci*. 2014; 263: 49–102. doi: 10.1007/978-3-319-05846-7_2.
17. *Sevastianov VI, Grigoriev AM, Basok YuB, Kirsanova LA, Vasilets VN, Malkova AP et al.* Biocompatible and matrix properties of polylactide scaffolds. *Russian Journal of Transplantology and Artificial Organs*. 2018; 20 (2): 82–90. (In Russ.). https://doi.org/10.15825/1995-1191-2018-2-82-90.
18. *Bhaskar B, Rao PS, Kasoju N, Nagarjuna V, Baadhe RR (Eds.)*. Biomaterials in Tissue Engineering and Regenerative Medicine. From Basic Concepts to State of the Art Approaches. Springer Nature Singapore Pte Ltd., 2021; 1039. https://doi.org/10.1007/978-981-16-0002-9.
19. *Sevastianov VI, Basok YB, Kirsanova LA, Grigoriev AM, Kirillova AD, Nemets EA et al.* A Comparison of the Capacity of Mesenchymal Stromal Cells for Cartilage Regeneration Depending on Collagen-Based Injectable Biomimetic Scaffold Type. *Life (Basel)*. 2021 Jul 27; 11 (8): 756. doi: 10.3390/life11080756.
20. *Sevast'yanov VI, Perova NV.* Biopolimernyy geterogennyy gidrogel' Sfero®GEL'' – in''ektsionnyy biodegradiruemyy implantat dlya zamestitel'noy i regenerativnoy meditsiny. *Prakticheskaya meditsina*. 2014; 8 (84): 120–126.
21. *DeQuach JA, Mezzano V, Miglani A, Lange S, Keller GM, Sheikh F, Christman KL.* Simple and high yielding method for preparing tissue specific extracellular matrix coatings for cell culture. *PLoS ONE.* 2010; 5 (9): e13039. doi: 10.1371/journal.pone.0013039.
22. *Lozinskiy VI, Kulakova VK, Kolosova OYu, Basok YuB, Grigor'ev AM, Perova NV, Sevast'yanov VI.* Biopolimernyy material dlya kletochno-inzhenernykh i/ili tkaneinzhenernykh konstruktsiy i sposob ego polucheniya. Pat. RF № 2774947 (2021); B.I. № 18 (2022).
23. *Grigoriev AM, Basok YuB, Kirillova AD, Surguchenko VA, Shmerko NP, Kulakova VK et al*. Cryogenically structured gelatin-based hydrogel as a resorbable macroporous matrix for biomedical technologies. *Russian Journal of Transplantology and Artificial Organs*. 2022; 24 (2): 83-93. https://doi.org/10.15825/1995-1191-2022-2-83-93.
24. *Lozinsky VI, Kulakova VK, Grigoriev AM, Podorozhko EA, Kirsanova LA, Kirillova AD et al*. Cryostructuring of polymeric systems: 63. Synthesis of two chemically tanned gelatin-based cryostructurates and evaluation of their potential as scaffolds for culturing of mammalian cells. *Gels.* 2022 Oct 28; 8 (11): 695. doi: 10.3390/gels8110695.
25. *Novikov I, Subbot A, Turenok A, Mayanskiy N, Chebotar I.* A rapid method of whole cell sample preparation for scanning electron microscopy using neodymium chloride. *Micron*. 2019; 124: 102687. doi: 10.1016/j.micron.2019.102687.
26. Mezhgosudarstvennyj standart GOST ISO 10993-5-2011 "Izdeliya medicinskie. Ocenka biologicheskogo dejstviya medicinskih izdelij. Chast' 5. Issledovanie na citotoksichnost': metody *in vitro*".

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EVALUATION OF THE EFFECT OF MESENCHYMAL STROMAL CELLS FROM DIFFERENT SOURCES ON HUMAN CHONDROCYTE PROLIFERATION

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Objective: to study the effect of a conditioned medium of mesenchymal stromal cells (MSCs) from different sources on human chondrocyte proliferation. **Materials and methods.** To confirm functional activity, chondrocytes were cultured in a cartilage cell-engineered construct (CEC), including 5×10^5 cells and 5 mg of tissue-specific matrix from decellularized cartilage. The conditioned medium was obtained after culturing MSCs derived from human adipose tissue (AT), MSCs derived from the pulp of primary teeth and MSCs isolated from umbilical cord-derived Wharton's jelly in a complete cell growth medium (CCGM). To evaluate the effect of MSC-derived secretome on chondrocyte proliferation, the conditioned medium, diluted 1 : 1 with CCGM, was added to wells containing chondrocytes. The effect of MSCs on human chondrocyte proliferation was studied by indirectly co-culturing cells in CCGM using Transwell inserts. 5×10^4 MSCs were applied to the bottom of the lower chamber, and 5×10^4 human chondrocytes and 5 mg of matrix were placed in the upper chamber. Chondrocyte proliferation was assessed at days 7 and 14 by DNA quantification. Interleukin-6 content was determined as a marker of secretory activity of MSCs in the conditioned medium. The morphology of the samples was studied using histological staining methods. **Results.** The ability of chondrocytes to produce cartilage-specific extracellular matrix was confirmed when forming cartilage CEC with tissue-specific matrix in a chondrogenic differentiation medium. When comparing the effect of the conditioned medium of MSCs obtained from different sources on the growth of human chondrocytes in vitro, increased proliferation was observed in all samples compared to controls. Indirect co-culture of MSCs with chondrocytes as part of CEC showed increased DNA amount in all samples at day 14, with the amount of DNA in the sample with MSC conditioned medium significantly higher than the control. **Conclusion.** Studies on the effect of MSC conditioned medium on chondrocyte proliferation in 2D culture indicate a possible regenerative potential of MSCs for cartilage tissue repair. Within the scope of this work, we did not identify significant differences in the effect of secretome derived from MSCs that were obtained from different sources on chondrocyte proliferation. However, additional in vivo studies are warranted in the future.

Keywords: cartilage tissue, mesenchymal stromal cells, conditioned medium, tissue engineering.

INTRODUCTION

Osteoarthritis (OA) is a disease resulting in "joint failure". It is based on destructive structural changes in the hyaline cartilage with subsequent degenerative processes of the underlying bone [1, 2]. OA incidence worldwide is rising every year, and this has seen an increasing level of disability globally [3].

Articular cartilage is composed of chondrocytes and extracellular matrix (ECM). The ECM is composed of collagens (mainly type II collagen), proteoglycans, and non-collagenous proteins [4]. In the early stages of knee OA, there are changes in the structure of collagen and proteoglycans, leading to articular cartilage erosion. In response to cartilage erosion, chondrocytes undergo a phase of hypertrophic activity, producing inflammatory mediators that promote further degradative changes in articular cartilage. The final stage is chondrocyte apoptosis, shifting the balance between synthesis and catabolism of collagen and proteoglycans toward catabolism. Expression of type II collagen, one of the prominent components of cartilage, decreases during the growth of chondrocytes; therefore, mature chondrocytes are unable to produce type II collagen *de novo* [3, 5–7]. The situation is aggravated by the lack of blood supply and low

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metabolic rate in cartilage, leading to its limited ability to self-repair [8, 9].

Treatment modalities for OA include symptomatic therapy consisting of the use of analgesics, as well as radical surgical intervention [10, 11]. However, the use of such methods does not always result in desired outcomes [12]. Therefore, in recent years, there has been a great interest in less invasive but more promising cellular technologies for cartilage structure restoration and OA treatment.

Over the past few decades, mesenchymal stem cells (MSCs) have been in the center of attention due to their high therapeutic potential. MSC cell therapy is used to treat various diseases, including OA, which affects 13% of the Russian population over 18 years of age [13–16]. The advantages of MSCs include their high chondrogenic potential, wide availability of sources for isolation (bone marrow, adipose tissue, pulp of primary teeth, Wharton's jelly, umbilical cord), as well as the fact that MSCs do not induce graft-versus-host disease [17–22].

Although MSCs are present in many tissues, their total count in the body is small, while cell therapy protocols typically require hundreds of millions of MSCs per treatment course, which requires additional time to culture them *in vitro*. Studies have shown that the implantation time of MSCs is usually too short to have an effective therapeutic effect [22]. In addition, several studies indicate that MSCs have a low survival rate (<1%) one week after their administration [23]. This suggests that the main effects of MSCs are based on paracrine mechanisms mediated by the production and secretion of a wide range of cytokines, chemokines, and growth factors by MSCs [24].

In this regard, the use of cell-free preparations based on MSC-derived secretome – conditioned media – is of great interest. *In vivo* models have demonstrated that a conditioned medium of MSCs obtained from various sources is as effective as transplantation of the corresponding MSCs [22]. It is hypothesized that the MSC-derived secretome will be able to stimulate the intrinsic regenerative potential of cartilage through secretion of various molecules such as interleukin (IL)-1β, IL-6, IL-10, vascular endothelial growth factor (VEGF), prostaglandin E2 (PGE2), transforming growth factor (TGF-β), and others [25].

Note that such a method is aimed not only at symptomatic treatment or at slowing down the development of OA, but most importantly at restoring the structure of cartilage tissue. Moreover, the advantage of using cell-free preparations is immunocompatibility, which excludes the choice of donors and recipients in therapy [26]. At the same time, MSCs have different characteristics depending on their origin, and the ideal source of MSCs for use in the treatment of knee OA has not yet been determined.

The aim of this work was to study the effect of a conditioned medium of MSCs obtained from different sources on human chondrocyte proliferation.

MATERIALS AND METHODS

Cell isolation

Cultures of MSCs isolated from umbilical cord-derived Wharton's jelly and MSCs derived from the pulp of primary teeth (PPT-derived MSCs) were obtained from the collection of cell cultures of the cell biology laboratory of the Institute of Biomedical Chemistry, Moscow. Human costal cartilage chondrocytes were obtained from the collection of cell cultures of the Priorov National Medical Research Center for Traumatology and Orthopedics, Moscow. The source of MSCs derived from human adipose tissue (hAT-derived MSCs) was subcutaneous adipose tissue from a healthy donor obtained with informed voluntary consent.

The phenotype of PPT-derived MSCs, hAT-derived MSCs, and Wharton's jelly MSCs was investigated for multipotency criteria by flow cytometry in previous studies [27].

Culture of chondrocytes on tissue-specific matrix

To confirm the ability to form a cell-engineered construct (CEC) of cartilage, human chondrocytes were cultured on a tissue-specific decellularized porcine cartilage scaffold, which is close in composition to natural ECM and was obtained according to our previously developed method [28].

The cartilage CECs consisted of 5×10^5 cells and 5 mg of matrix. The matrix was populated with cells by spinning in test tubes with the culture medium on a Multi Bio 3D programmable shaker (Biosan, Latvia). The first 5 days, CEC were cultured in a complete cell growth medium (CCGM) containing DMEM/F12 (PanEco, Russia) (1:1) supplemented with 10% fetal bovine serum (Cytiva, USA), 1% antibiotic/antimycotic solution (Thermo Fisher Scientific, USA) and 2 mM L-glutamine (PanEco, Russia). The CCGM was then replaced with chondrogenic differentiation medium containing DMEM/high Glucose, supplemented with GlutaMAX (Thermo Fisher Scientific, USA), 10% ITS+ (Corning, USA), 1% sodium pyruvate (Thermo Fisher Scientific, USA), 0.25% L-ascorbic acid 2-phosphate (Sigma-Aldrich, USA), 0.0001% dexamethasone (Merck, FRG), 0.002% TGF-β1 (Thermo Fisher Scientific, USA) and 1% antibiotic/antimycotic solution (Thermo Fisher Scientific, USA). The medium was changed every three days.

Cell viability in the CEC was assessed by fluorescent staining using the LIVE/DEAD dye (Thermo Fisher Scientific, USA) and Leica DMi8 Thunder microscope (Leica Microsystems, Germany).

CEC morphology was examined on day 21 of culture using histological staining.

Obtaining conditioned medium

hAT-derived MSCs, PPT-derived MSCs, umbilical cord-derived Wharton's jelly MSCs and human chondrocytes were cultured in 75 cm^2 vials. Third-passage cells were used for the experiment. The CCGM was replaced every three days. The conditioned medium was collected on day 10 of culture (when the monolayer confluency reached >70%) before the experiment and stored at +4 °C. The degree of cell monolayer confluency was determined visually using a Nikon Eclipse TS100 inverted light microscope (Nikon, Japan).

Study of chondrocyte proliferation in 2D culture

To evaluate the effect of the conditioned media of different types of MSCs, chondrocytes were cultured in 24-well plates. A conditioned medium diluted 1:1 with CCGM was introduced into wells containing chondrocytes (3000 cells per well). Intravital observation of cells and photography, as well as determination of the degree of confluency of the chondrocyte monolayer, were performed using the IncuCyte Zoom System (Essen BioScience, USA) for intravital observation of cells and analysis of dynamic processes in the culture medium. The conditioned medium of chondrocytes diluted 1:1 with CCGM served as a control.

Co-culture of chondrocytes and mesenchymal stromal cells

The effect of MSCs on human chondrocyte proliferation was studied by indirect cell co-culture in CCGM using a Transwell plate with polycarbonate membrane inserts for 24-well plates with a 3 μm pore size (Corning, USA). 5×10^4 MSCs were plated on the bottom of the lower chamber. In the upper chamber, 5×10^4 human chondrocytes and 5 mg of tissue-specific decellularized porcine cartilage scaffold were placed. Transwell plates containing chondrocytes in both chambers served as control. Chondrocyte proliferation on days 7 and 14 was assessed by DNA quantification using fluorescent dye Quant-iT PicoGreen (Thermo Fisher Scientific, USA).

DNA quantification

DNA was isolated using the DNeasy Blood&Tissue Kit (QIAGEN, Germany) according to the manufacturer's instructions. For DNA measurement, Quant-iT Picogreen kit (Thermo Fisher Scientific, USA) was used according to the manufacturer's instructions and a Spark 10M plate reader (Tecan Trading, Switzerland) at 520 nm wavelength.

Quantification of IL-6 level in the conditioned medium

As a marker of secretory activity of MSCs in conditioned medium, we determined the level of cytokine interleukin-6 (IL-6) by solid-phase enzyme-linked immunosorbent assay (Vector-Best, Russia). The method is based on a three-step analysis using mono- and polyclonal antibodies to IL-6. The procedure for the analysis is recommended by the manufacturer in the kit instructions. A plate reader was used to quantify IL-6 level at 450 nm wavelength.

Histological examination

The samples were fixed in a 10% formalin solution, washed in running water and dehydrated in alcohols of increasing concentration, kept in an ethanol + chloroform mixture, then in chloroform and embedded in paraffin. Sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin, Alcian blue, and Masson's trichrome. Analysis and photography of the preparations were carried out using an inverted Nikon Eclipse Ti microscope (Nikon, Japan).

RESULTS AND DISCUSSION

The immunophenotypic profile of marker expression in PPT-derived MSCs, hAT-derived MSCs and Wharton's jelly MSCs was investigated by us in previous work, and met the International Society for Cell & Gene Therapy (ISCT) criteria for multipotency of MSCs [29]. All primary cultures were characterized by high expression of CD29, CD44, CD49b, CD73 and CD90, while no expression of CD34, CD45 or HLA-DR was observed [27].

Staining of CEC with fluorescent dye LIVE/DEAD revealed a significant mass of viable chondrocytes on the surface of the matrix from decellularized cartilage (Fig. 1).

When chondrocytes were cultured in the differentiation medium as part of cartilage CEC on day 21 of cultivation, we observed the formation of large clusters of cartilage microparticles united by cells – conglomerates (Fig. 2). Cells were visualized on the surface of all cartilage microparticles, and the entire cell population was characterized by polymorphism. Thus, fibroblast-like cells could be detected in the periphery, whereas round-shaped cells were distributed in the central zone. In addition, cell growth was accompanied by significant ECM production.

The specimens were fixed in 10% formalin solution, washed in running water and dehydrated in alcohols of

ascending concentration, incubated in a mixture of ethanol and chloroform, then in chloroform and embedded in paraffin. Sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin, alcian blue, and Masson's trichrome. The preparations were analyzed and photographed using a Nikon Eclipse Ti inverted microscope (Nikon, Japan).

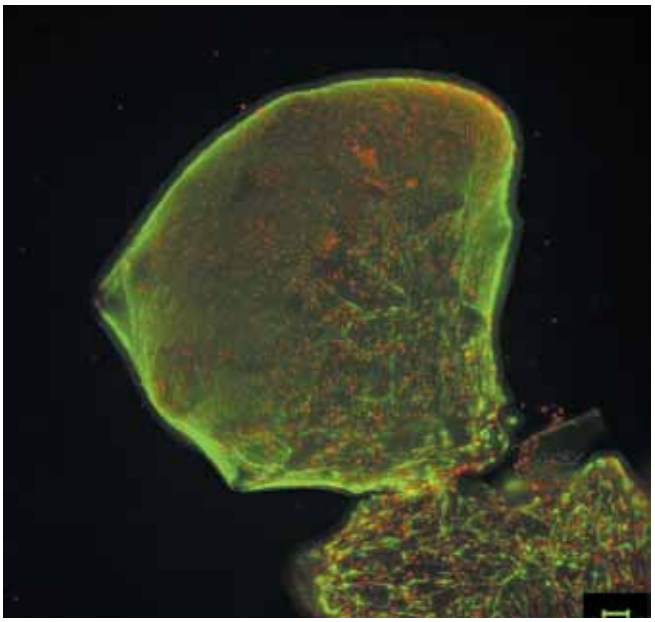

Fig. 1. Examination of human chondrocyte viability in cartilage CEC at day 21 of culturing. LIVE/DEAD staining. Scale bar: 100 μm

Due to their regenerative properties, MSC-derived secretome is considered as a promising treatment for articular cartilage diseases [30]. However, the composition and effect of a conditioned medium of MSCs will differ depending on the source of cells, and the methods and conditions of their cultivation. Therefore, we compared the effects of a conditioned medium, derived from MSCs that were obtained from different sources, on the growth of human chondrocyte culture *in vitro*.

Images obtained using a lifetime cell imaging system demonstrate an increase in the number of chondrocytes on the culture plate over time. Cell count in all samples did not differ significantly at day 24 of culturing. At both day 7 and 14 of observation, a decrease in proliferation could be observed in the control sample (CCGM diluted 1:1 with conditioned medium from chondrocytes) compared to the samples of the experimental groups.

Chondrocyte monolayer confluency on day 7, when cultured in the presence of a conditioned medium of PPT-

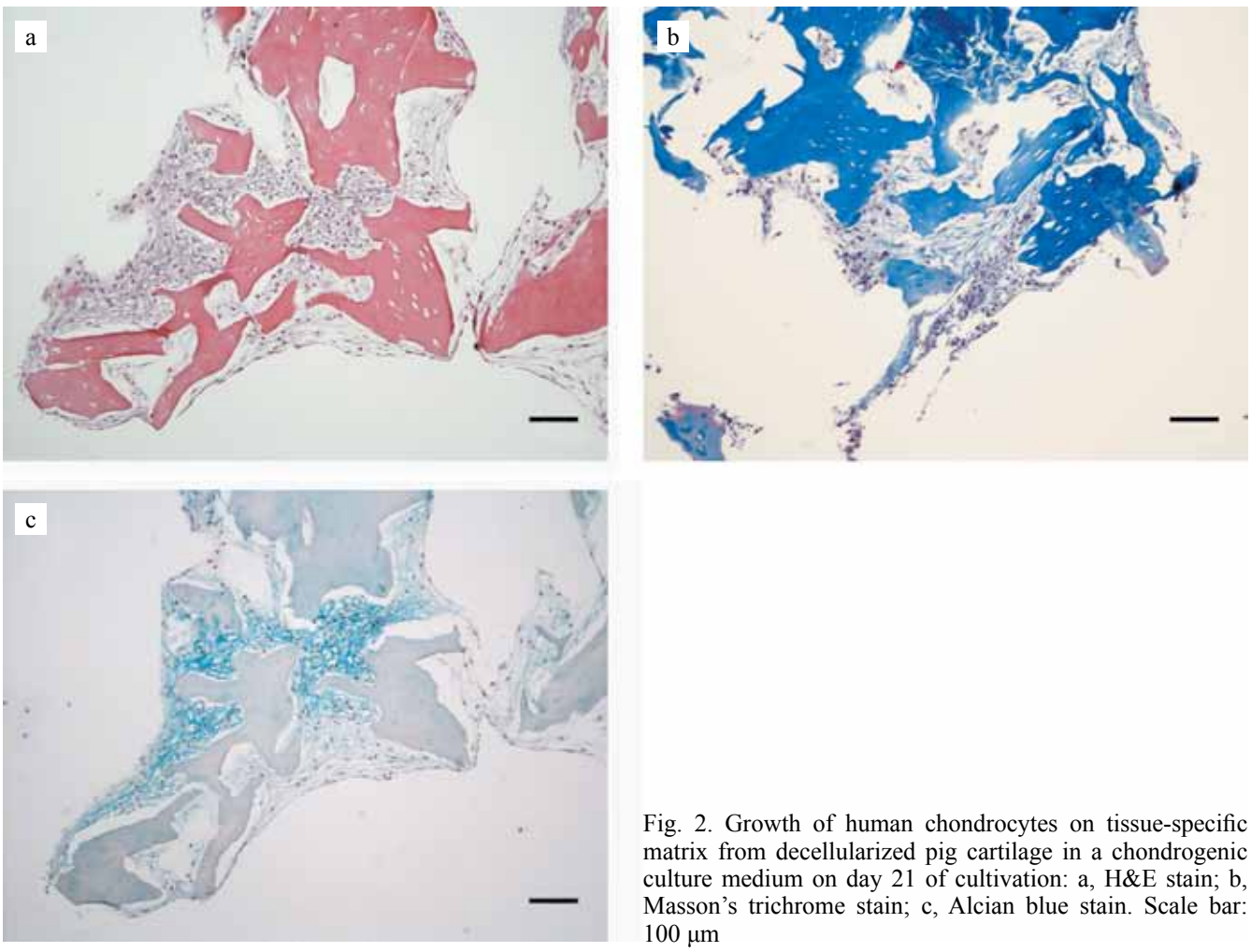

Fig. 2. Growth of human chondrocytes on tissue-specific matrix from decellularized pig cartilage in a chondrogenic culture medium on day 21 of cultivation: a, H&E stain; b, Masson's trichrome stain; c, Alcian blue stain. Scale bar: 100 μm

derived MSCs and hAT-derived MSCs was 1.2 times higher than that of the control sample, and 1.4 times when cultured in the presence of a conditioned medium of umbilical cord-derived Wharton's jelly MSCs. On day 14, monolayer confluency in the experimental samples was 1.2 times higher than that of the control (Fig. 4).

Table presents data on the influence of the paracrine effect of MSCs of different origins on the quantitative

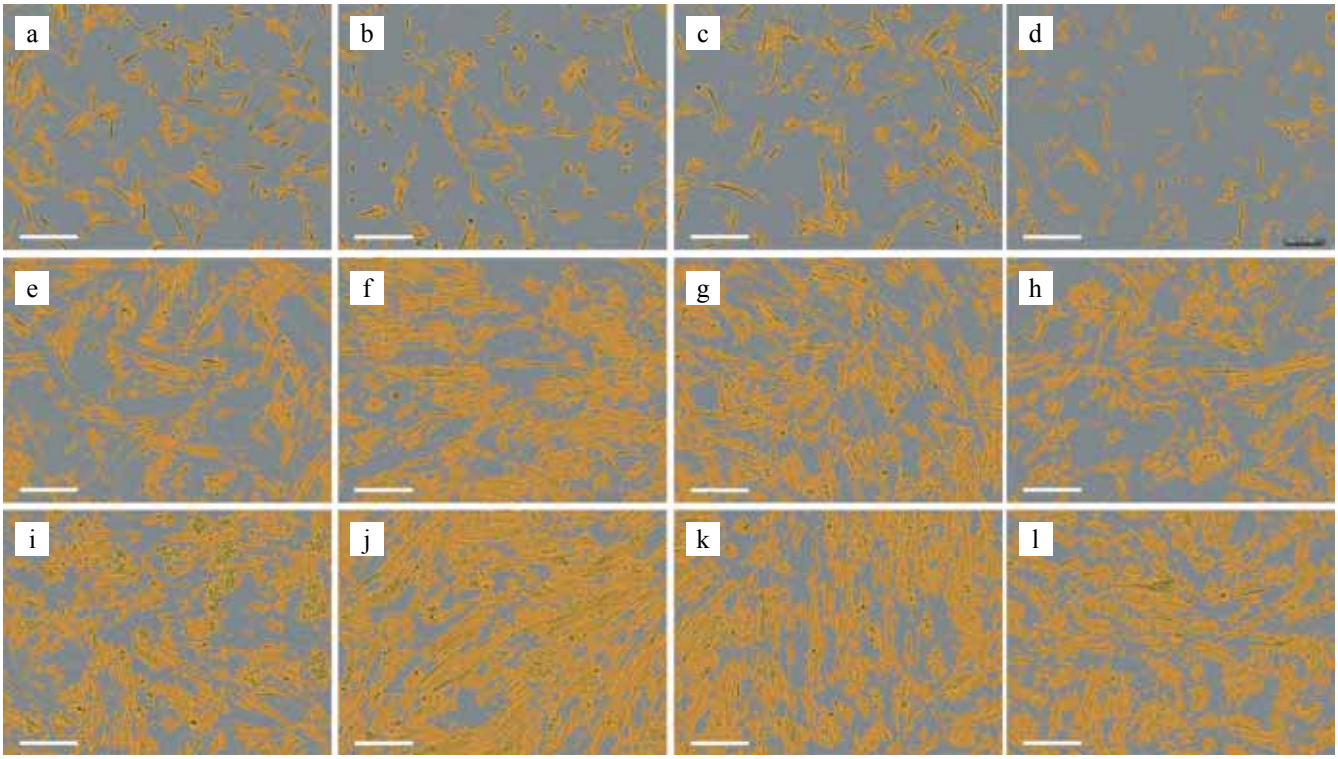

Fig. 3. Effect of conditioned medium on human chondrocyte proliferation in a 2D culture. (a, b, c, d), 24 hours; (e, f, g, h), day 7; (i, j, k, l), day 14; (a, e, i), conditioned medium of dental pulp MSCs; (b, f, j), conditioned medium of AT-MSCs; (c, g, k), conditioned medium of umbilical cord-derived Wharton's jelly MSCs; (d, h, l), conditioned medium of chondrocytes (control). Scale bar: 300 μm

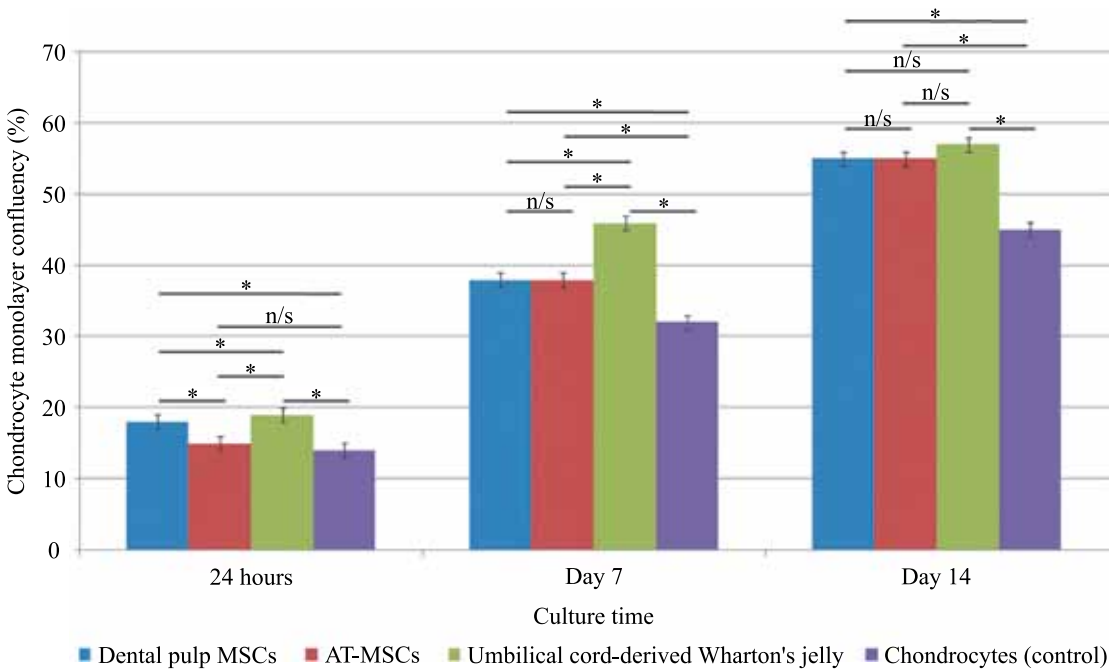

Fig. 4. Chondrocyte monolayer confluency in the presence of conditioned media from MSCs obtained from different sources (n/s, no statistically significant differences; *, there are differences at $p < 0.05$)

Table

Chondrocyte proliferation on decellularized porcine cartilage at days 7 and 14 of indirect co-culture in Transwell with mesenchymal stromal cells from different sources

Culture time	DNA (μg/CEC)			
	PPT-derived MSCs	hAT-derived MSCs	Umbilical cord-derived Wharton's jelly MSCs	Chondrocytes (control)
Day 7	0.57 ± 0.25	0.36 ± 0.14	0.80 ± 0.11	0.51 ± 0.25
Day 14	1.20 ± 0.09	1.34 ± 0.15	1.24 ± 0.27	0.90 ± 0.26

DNA content in CECs consisting of chondrocytes and tissue-specific matrix from decellularized porcine cartilage when they are indirectly co-cultured in Transwell cells. On day 7, the least amount of DNA was found in samples with conditioned medium of hAT-derived MSCs, while on day 14, all samples showed cell growth and, consequently, increased amount of DNA. DNA amount in the sample with conditioned medium of hAT-derived MSCs was significantly higher than that of the control ($p < 0.05$).

On day 14 of culture, the differences between the samples were insignificant, which may indicate a relatively similar effects of the conditioned medium of different MSCs on chondrocyte proliferation in this experiment.

Many research works have revealed the fundamental role of MSC-derived secretome as an active ingredient that can modulate cellular responses and signaling pathways, thereby promoting tissue repair [31]. One of the components of MSC-derived secretome is IL-6, a multifunctional cytokine that is an important factor in various physiological processes, including immune regulation, hematopoiesis and inflammation, and also modulates cell proliferation, differentiation and apoptosis [32].

To identify differences in cytokine secretion between PPT-derived MSCs, hAT-derived MSCs and Wharton's jelly MSCs, the IL-6 levels in the conditioned medium on days 1, 3 and 6 of culture were compared. So, on days 3 and 6 of culture, IL-6 levels in all samples were almost twofold higher (day 3: PPT-derived MSCs – 3.89 ± 0.31 ng/mL; hAT-derived MSCs – 26.99 ± 1.22 ng/mL, Wharton's jelly MSCs >70 ng/mL; day 6: 3.72 ± 0.44 ng/mL, 22.08 ± 3.71 ng/mL, and >70 ng/mL, respectively) than on day 1 of culture (1.85 ± 0.07 ng/mL, 16.94 ± 0.68 ng/mL, and >70 ng/mL, respectively). This confirms active cell proliferation and secretion of active factors over time.

Probably, the difference in the effect of conditioned medium of MSCs isolated from different sources on human chondrocyte proliferation, as well as the difference in IL-6 secretion, is mediated by the initial microenvironment (niche) of MSCs. It is worth noting that MSCs are found in many tissues of the body, but only MSCs from bone marrow and adipose tissue have been widely studied for the treatment of OA [12]. However, the number of MSCs in bone marrow is small, it also decreases with the age of the donor, and the cell collection procedure is quite traumatic. In this regard, research has begun to explore alternative sources of obtaining MSCs, including dental pulp and umbilical cord-derived Wharton's jelly. Umbilical cord-derived Wharton's jelly MSCs have been shown to have a positive effect on the regeneration of damaged hyaline cartilage in pigs [33]. Nowzari et al. showed the regenerative potential of human dental pulp MSCs and their secretome on a collagenase-induced OA model in rats [34].

CONCLUSION

Thus, studies on the effect of a conditioned medium of hAT-derived MSCs, PPT-derived MSCs and umbilical cord-derived Wharton's jelly MSCs on human chondrocyte proliferation in a 2D culture indicate that MSCs have a possible regenerative potential for cartilage tissue repair. Within the framework of this work, we did not identify any significant differences in the effect of the secretome of MSCs obtained from different sources on chondrocytes in indirect co-culture. However, further *in vivo* studies are warranted in the future.

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REFERENCES

1. *Karateev AE, Lila AM*. Osteoarthritis: current clinical concept and some promising therapeutic approaches. *Rheumatology Science and Practice*. 2018; 56 (1): 70–81. [In Russ, English abstract]. doi: 10.14412/1995-4484-2018-70-81.
2. *Hunter DJ, Bierma-Zeinstra S*. Osteoarthritis. *The Lancet*. 2019; 393 (10182): 1745–1759. doi: 10.1016/s0140-6736(19)30417-9. PMID: 31034380.
3. *Giorgino R, Albano D, Fusco S, Peretti GM, Mangiavini L, Messina C*. Knee osteoarthritis: epidemiology, pathogenesis, and mesenchymal stem cells: what else is new? An Update. *Int J Mol Sci*. 2023; 24 (7): 6405. doi: 10.3390/ijms24076405. PMID: 37047377.

4. *Hsueh MF, Önnerfjord P, Kraus VB*. Biomarkers and proteomic analysis of osteoarthritis. *Matrix Biol*. 2014; 39: 56–66. doi: 10.1016/j.matbio.2014.08.012. PMID: 25179675.
5. *Chow YY, Chin KY*. The role of inflammation in the pathogenesis of osteoarthritis. *Mediators Inflamm*. 2020; 2020: 8293921. doi: 10.1155/2020/8293921. PMID: 32189997.
6. *Aaron RK, Racine J, Dyke JP.* Contribution of circulatory disturbances in subchondral bone to the pathophysiology of osteoarthritis. *Curr Rheumatol Rep*. 2017; 19 (8): 49. doi: 10.1007/s11926-017-0660-x. PMID: 28718064.
7. *Sanchez C, Bay-Jensen AC, Pap T, Dvir-Ginzberg M, Quasnichka H, Barrett-Jolley R et al.* Chondrocyte secretome: a source of novel insights and exploratory biomarkers of osteoarthritis. *Osteoarthritis Cartilage*. 2017; 25 (8): 1199–1209. doi: 10.1016/j.joca.2017.02.797. PMID: 28232143.
8. *Sevastianov VI, Dukhina GA, Grigoriev AM, Perova NV, Kirsanova LA, Skaletskiy NN et al*. The functional effectiveness of a cell-engineered construct for the regeneration of articular cartilage. *Russian Journal of Transplantology and Artificial Organs*. 2015; 17 (1): 86–96. [In Russ, English abstract]. doi: 10.15825/1995-1191-2015-1-86-96.
9. *Shariatzadeh M, Song J, Wilson S*. The efficacy of different sources of mesenchymal stem cells for the treatment of knee osteoarthritis. *Cell Tissue Res*. 2019; 378 (3): 399–410. doi: 10.1007/s00441-019-03069-9. PMID: 31309317.
10. *Roos EM, Arden NK*. Strategies for the prevention of knee osteoarthritis. *Nature Reviews Rheumatology*. 2015; 12 (2): 92–101. doi: 10.1038/nrrheum.2015.135. PMID: 26439406.
11. *Urjas'ev OM, Zaigrova NK*. Osteoartrit: patogenez, diagnostika, lechenie. *Zemskij vrach.* 2016; 1–2 (29–30): 27–35.
12. *Loo SJQ, Wong NK*. Advantages and challenges of stem cell therapy for osteoarthritis (review). *Biomed Rep*. 2021; 15 (2): 67. doi: 10.3892/br.2021.1443. PMID: 34155451.
13. *Murphy JM, Fink DJ, Hunziker EB, Barry FP*. Stem cell therapy in a caprine model of osteoarthritis. *Arthritis Rheum*. 2003; 48 (12): 3464–3474. doi: 10.1002/art.11365. PMID: 14673997.
14. *Garay-Mendoza D, Villarreal-Martínez L, Garza-Bedolla A, Pérez-Garza DM, Acosta-Olivo C, Vilchez-Cavazos F et al*. The effect of intra-articular injection of autologous bone marrow stem cells on pain and knee function in patients with osteoarthritis. *Int J Rheum Dis*. 2018; 21 (1): 140–147. doi: 10.1111/1756-185X.13139. PMID: 28752679.
15. *Desancé M, Contentin R, Bertoni L, Gomez-Leduc T, Branly T, Jacquet S et al*. chondrogenic differentiation of defined equine mesenchymal stem cells derived from umbilical cord blood for use in cartilage repair therapy. *Int J Mol Sci*. 2018; 19 (2): 537. doi: 10.3390/ijms19020537. PMID: 29439436.
16. *Galushko EA, Bolshakova TYu, Vinogradova IB, Ivanova ON, Lesnyak OM, Menshikova LV et al*. Structure of rheumatic diseases among adult population of Russia according to data of an epidemiological study (preliminary results). *Rheumatology Science and Practice*. 2009; 47 (1): 11–17. [In Russ]. doi: 10.14412/1995-4484-2009-136.
17. *De Bari C, Roelofs AJ*. Stem cell-based therapeutic strategies for cartilage defects and osteoarthritis. *Curr Opin Pharmacol*. 2018; 40: 74–80. doi: 10.1016/j.coph.2018.03.009. PMID: 29625333.
18. *Basok YuB, Grigoryev AM, Kirsanova LA, Kirillova AD, Subbot AM, Tsvetkova AV et al*. Comparative study of chondrogenesis of human adipose-derived mesenchymal stem cells when cultured in collagen-containing media under *in vitro* conditions. *Russian Journal of Transplantology and Artificial Organs*. 2021; 23 (3): 90–100. doi: 10.15825/1995-1191-2021-3-90-100.
19. *Gronthos S, Mankani M, Brahim J, Robey PG, Shi S*. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc Natl Acad Sci USA*. 2000; 97 (25): 13625–13630. doi: 10.1073/pnas.240309797. PMID: 11087820.
20. *Zhou C, Yang B, Tian Y, Jiao H, Zheng W, Wang J et al*. Immunomodulatory effect of human umbilical cord Wharton's jelly-derived mesenchymal stem cells on lymphocytes. *Cell Immunol*. 2011; 272 (1): 33–38. doi: 10.1016/j.cellimm.2011.09.010. PMID: 22004796.
21. *Marmotti A, Mattia S, Castoldi F, Barbero A, Mangiavini L, Bonasia DE et al*. Allogeneic umbilical cord-derived mesenchymal stem cells as a potential source for cartilage and bone regeneration: an *in vitro* study. *Stem Cells Int*. 2017; 2017: 1732094. doi: 10.1155/2017/1732094. PMID: 29358953.
22. *Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R*. Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine. *Int J Mol Sci*. 2017; 18 (9): 1852. doi: 10.3390/ijms18091852. PMID: 28841158.
23. *Parekkadan B, Milwid JM*. Mesenchymal stem cells as therapeutics. *Annu Rev Biomed Eng*. 2010; 12: 87–117. doi: 10.1146/annurev-bioeng-070909-105309. PMID: 20415588.
24. *Maguire G*. Stem cell therapy without the cells. *Commun Integr Biol*. 2013; 6 (6): e26631. doi: 10.4161/cib.26631. PMID: 24567776.
25. *Mancuso P, Raman S, Glynn A, Barry F, Murphy JM*. Mesenchymal stem cell therapy for osteoarthritis: the critical role of the cell secretome. *Front Bioeng Biotechnol*. 2019; 7: 9. doi: 10.3389/fbioe.2019.00009. PMID: 30761298.
26. *Gunawardena TNA, Rahman MT, Abdullah BJJ, Kasim NHA*. Conditioned media derived from mesenchymal stem cell cultures: The next generation for regenerative medicine. *J Tissue Eng Regen Med*. 2019; 13 (4): 569–586. doi: 10.1002/term.2806. PMID: 30644175.
27. *Tsvetkova AV, Vakhrushev IV, Basok YuB, Grigor'ev AM, Kirsanova LA, Lupatov AYu et al.* Chondrogeneic po-

tential of MSC from different sources in spheroid culture. *Bull Exp Biol Med*. 2021; 170 (4): 528–536. doi: 10.1007/s10517-021-05101-x. PMID: 33725253.

28. *Sevastianov VI, Basok YuB, Grigoriev AM, Nemets EA, Kirillova AD, Kirsanova LA et al.* Decellularization of cartilage microparticles: Effects of temperature, supercritical carbon dioxide and ultrasound on biochemical, mechanical, and biological properties. *J Biomed Mater Res A*. 2023; 111 (4): 543–555. doi: 10.1002/jbm.a.37474. PMID: 36478378.
29. *Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006; 8 (4): 315–317. doi: 10.1080/14653240600855905. PMID: 16923606.
30. *Rosochowicz MA, Lach MS, Richter M, Suchorska WM, Trzeciak T*. Conditioned medium – is it an undervalued lab waste with the potential for osteoarthritis management? *Stem Cell Rev Rep*. 2023; 19 (5): 1185–1213. doi: 10.1007/s12015-023-10517-1. PMID: 36790694.
31. *Gangadaran P, Oh EJ, Rajendran RL, Oh JM, Kim HM, Kwak S et al*. Three-dimensional culture conditioned bone marrow MSC secretome accelerates wound healing in a burn injury mouse model. *Biochem Biophys Res Commun*. 2023; 673: 87–95. doi: 10.1016/j.bbrc.2023.05.088. PMID: 37364390.
32. *Fan Y, Ye J, Shen F, Zhu Y, Yeghiazarians Y, Zhu W et al*. Interleukin-6 stimulates circulating blood-derived endothelial progenitor cell angiogenesis *in vitro*. *J Cereb Blood Flow Metab*. 2008; 28 (1): 90–98. doi: 10.1038/sj.jcbfm.9600509. PMID: 17519976.
33. *Wu KC, Chang YH, Liu HW, Ding DC*. Transplanting human umbilical cord mesenchymal stem cells and hyaluronate hydrogel repairs cartilage of osteoarthritis in the minipig model. *Tzu Chi Med J*. 2019; 31 (1): 11–19. doi: 10.4103/tcmj.tcmj_87_18. PMID: 30692826.
34. *Nowzari F, Zare M, Tanideh N, Meimandi-Parizi A, Kavousi S, Saneian SM et al.* Comparing the healing properties of intra-articular injection of human dental pulp stem cells and cell-free-secretome on induced knee osteoarthritis in male rats. *Tissue Cell*. 2023; 82: 102055. doi: 10.1016/j.tice.2023.102055. PMID: 36948080.

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DIAGNOSTIC SIGNIFICANCE OF TGF-β1 IN KIDNEY RECIPIENTS WITH GRAFT DYSFUNCTION

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Development of minimally invasive diagnosis techniques for complications in recipients, based on analysis of the levels of molecular and genetic biomarkers, is an urgent task facing modern transplantology. Transforming growth factor beta 1 (TGF-β1), which has multiple effects in the body, among the potential indicators of complications. **Objective:** to assess the diagnostic significance of serum TGF-β1 in kidney recipients with graft dysfunction. **Materials and methods.** The study included 129 kidney recipients aged 17 to 68 years and 35 healthy subjects. Serum TGF-β1 levels in the recipients were determined by immunoenzyme technique. **Results.** Kidney recipients included 95 patients with laboratory and clinical signs of graft dysfunction, who underwent biopsy of the transplanted kidney, followed by morphological examination, and 34 recipients with normal graft function. Serum TGF-β1 levels in the kidney recipients were significantly higher than in their healthy counterparts ($p = 0.00001$); it did not correlate with most blood test parameters; with the glomerular filtration rate (GFR). Kidney recipients with graft dysfunction had significantly higher TGF-β1 levels than other recipients ($p = 0.018$). In recipients with graft dysfunction, morphological study revealed the following: acute tubular necrosis (ATN, n = 11), acute T-cell mediated rejection (ACR, n = 26), acute antibody-mediated rejection (AMR, n = 35), non-immune-mediated nephrosclerosis with signs of calcineurin inhibitor nephrotoxicity (CNI nephrotoxicity, n = 13), and recurrent glomerulonephritis (chronic graft rejection, n = 10). Recipients with immune-mediated graft injury (ACR, AMR and chronic rejection) had higher serum TGF-β1 levels than recipients with graft dysfunction resulting from other causes, $p < 0.0001$. Kidney recipients with serum TGF-β1 levels above the threshold value of 94.3 ng/mL had a higher risk of immune-mediated graft dysfunction than other kidney recipients (RR = 2.2 ± 0.22 [95% CI 1.46–3.46]) with 77.5% test sensitivity and 60.3% specificity. **Conclusion.** The calculated threshold serum TGF-β1 level in kidney recipients can be considered as an auxiliary indicator of graft dysfunction resulting from acute or chronic rejection.

Keywords: transforming growth factor beta, TGF-β1, kidney transplantation, graft dysfunction, diagnosis.

INTRODUCTION

Chronic kidney disease (CKD) has a high prevalence worldwide and is among the leading diseases with profound socioeconomic consequences [1]. Kidney transplantation (KT) is a radical and the most effective treatment for CKD [2].

Despite the high efficiency of KT, the risk of kidney graft injury and dysfunction persists throughout subsequent life. An objective method of verifying the pathology of a transplanted organ is biopsy, which is associated with all the limitations and risks of invasive interventions. The development of the concept of personalized methods of minimally invasive diagnosis of complications in the posttransplant period based on the analysis of the levels of molecular and genetic biomarkers and their combinations seems to be an urgent task [3]. Despite the obvious expediency of analyzing biomarkers in the urine of kidney recipients, such tests have not shown sufficient reliability for differentiating the processes of extracellular matrix accumulation associated with chronic rejection.

The list of potential biomarkers of kidney graft injury is constantly expanding and includes representatives of microRNA families, cell-free DNA, protein molecules, etc. [4]. There is a constant search for organ-specific biomarkers that signal not only the development of pathology of the transplanted kidney, but also the nature or degree of damage to the organ.

TGF-β1, which has multiple effects – it is involved in the regulation of immune response, has anti-inflammatory and immunosuppressive effects, and is involved in the synthesis of extracellular matrix proteins [5]. TGF-β1 is a cytokine that promotes collagen production by fib-

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roblasts with subsequent structural changes in the graft and the development of dysfunction [6].

Serum TGF-β1 level was found to be associated with liver fibrosis in children with congenital hepatobiliary abnormalities, and is also associated with the severity of liver, kidney, and heart graft fibrosis [7].

Assessment of serum TGF-β1 level in kidney recipients can be of practical implications for optimizing the diagnosis of complications in kidney recipients.

The aim of this work was to evaluate the diagnostic significance of serum TGF-β1 levels in kidney recipients with graft dysfunction.

MATERIALS AND METHODS

The study included 129 adult kidney recipients who underwent allotransplantation from a related kidney allotransplantation (RKAT) or cadaveric kidney allotransplantation (CKAT) in the period from 1999 to 2022 at Shumakov National Medical Research Center of Transplantology and Artificial Organs. The number of selected recipients included 95 with signs of graft dysfunction that required unscheduled punch biopsy and 34 without signs of graft dysfunction. The dysfunction criteria were elevated creatinine and urea levels and proteinuria. The comparison group consisted of 35 healthy individuals, selected randomly and not differing significantly in terms of age and gender from the recipients. In accordance with the patient management protocol at Shumakov National Medical Research Center of Transplantology and Artificial Organs and the clinical guidelines of the Russian Transplant Society, all recipients after KT underwent routine examinations, which included a clinical assessment of the condition, full blood count and blood chemistry tests with determination of tacrolimus levels, and graft biopsy.

Serum TGF-β1 levels were measured. Blood samples were collected in disposable tubes, centrifuged, serum was frozen and stored at –20 °C. The serum concentration of the biomarker was measured by enzyme immunoassay using specific reagent kits Human TGF-beta 1 ELISA Kit (RayBio®, USA) according to instructions. Blood samples were collected for analysis of TGF-β1 levels on the day of biopsy and other routine laboratory tests (full blood count, blood chemistry test, special blood test).

The pathology was verified via morphological studies of biopsy material. Graft glomerular filtration rate (GFR) was calculated using the CKD-EPI formula, which considers race, sex, age and serum creatinine level.

For comparative analysis of independent variables, nonparametric statistics methods – Mann–Whitney U test and Spearman correlation test – were used. Group differences were considered significant at $p < 0.05$. ROC analysis was used to determine the diagnostic significance of the biomarker and its threshold level. The main diagnostic characteristics of the test were evaluated: relative risk (RR), 95% CI limits, sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic efficiency. Statistical data processing was performed using the Statistica v.13.0 program package, StatSoftInc (USA).

RESULTS

The study included 129 kidney recipients aged 17 to 68 years, including 62 (48%) males and 67 (52%) females.

The major proportion of patients (78%) underwent CKAT and the remaining 22% underwent RKAT. The follow-up period for the recipients ranged from 2 to 4748 days (median, 345 days); 76% of the patients were examined in the long term (>1 month since transplantation). The main characteristics of the recipient group are presented in Table 1.

Serum TGF-β1 levels in the individuals included in the study varied widely, 92.38 [31.77; 129.70] ng/mL, did not differ significantly between men and women ($p = 0.37$), and did not correlate with age ($r = 0.09$; $p = 0.18$).

TGF-β1 levels in kidney recipients were significantly different and higher than in healthy individuals, $p = 0.00001$. A comparative analysis of TGF-β1 concentration in RKAT and CKAT groups showed no significant differences ($p = 0.32$).

There was no significant correlation between TGF-β1 level and length of time (days) since transplantation ($r = 0.137$; $p = 0.13$); there were no significant differences in TGF-β1 concentration in kidney recipients at early (<30 days) and late (>30 days) periods after transplantation ($p = 0.47$).

The relationship between TGF-β1 levels and the main indicators of full blood count, blood chemistry test, special blood test and urinalysis was studied (Table 2).

Correlation analysis showed that TGF-β1 level have no association with most blood test parameters, as well as with graft GFR, but there was a positive correlation with platelet count ($r = 0.206$; $p = 0.025$) and a negative correlation with aspartate aminotransferase (AST) activity ($r = –0.213$; $p = 0.024$). TGF-β1 levels were independent of recipients' serum tacrolimus level.

Evaluation of the association of serum TGF-β1 levels with urinalysis parameters showed a strong positive correlation with red blood cell count ($r = 0.354$; $p = 0.00001$), white blood cell count ($r = 0.245$; $p = 0.006$) and proteinuria ($r = –0.280$; $p = 0.001$).

Of all 129 patients included in the study, 95 patients were categorized as "with graft dysfunction" and 34 were designated as "normal function" recipients based on laboratory and clinical data. Graft function scores in both groups are shown in Table 3.

Kidney recipients with graft dysfunction had significantly higher levels of creatinine and urea, GFR and proteinuria ($p < 0.00001$) than those without. Comparati-

ve analysis of serum TGF-β1 levels in these groups also showed significant differences (p = 0.0004).

Based on the results of morphological study of biopsy specimens from recipients with graft dysfunction, the following pathology variants were identified: ATN (n = 11) in the early post-transplant period, ACR (n = 26), AMR (n = 35), non-immune response interstitial fibrosis with signs of calcineurin inhibitor nephrotoxicity (CNI nephrotoxicity, n = 13), recurrent glomerulonephritis (chronic graft rejection, n = 10). See Fig. 1.

A comparative analysis showed significantly higher TGF-β1 levels in recipients with ACR (p = 0.0003), AMR (p = 0.002) and chronic rejection (p = 0.001) compared to recipients without dysfunction (Fig. 2).

Table 1

Basic characteristics of kidney recipients and healthy subjects included in the study

Indicator		Kidney recipients	Healthy individuals
Number, n		129	35
Gender, n (%):	Male Female	62 (48%) 67 (52%)	18 (52%) 17 (48%)
Age, years:	Range Median [Interquartile range]	17 to 68 40 [33; 51]	21 to 64 38 [26; 50]
Type of transplantation, n (%):	Deceased donor (CKAT) Living-related donor (RKAT)	101 (78%) 28 (22%)	–
Graft function, n (%):	Normal function Signs of graft dysfunction	34 (26%) 95 (74%)	–
Follow-up period, days:	Range; Median [Interquartile range]	2 to 4748 325 [39; 1448]	–
Post-transplant period, n (%):	Early (≤1 month) Late (>1 month)	31 (24%) 98 (76%)	–
TGF-β1 level, ng/mL:	Median [Interquartile range]	104.0 [79.10; 138.80]	6.66 [3.87; 17.45]

Table 2

Correlation of TGF-β1 levels with full blood count, biochemical tests and urinalysis indicators in kidney recipients

Indicator	Spearman's rank correlation (r)	Significance level (p)
Full blood count		
Hemoglobin (g/L)	0.037	0.689
White blood cells (10^9/L)	0.075	0.496
Platelets (10^9/L)	0.206	0.025
Blood chemistry test		
Total protein (g/L)	–0.115	0.234
Urea (mmol/L)	0.111	0.219
Creatinine (μmol/L)	0.121	0.179
ALT (U/L)	–0.095	0.355
AST (U/L)	–0.246	0.015
Glucose (mmol/L)	0.102	0.308
Special blood test		
GFR (mL/min/1.73 m^2)	–0.026	0.76
Tacrolimus (ng/mL)	–0.044	0.630
Urinalysis		
Red blood cells (in the field of view)	0.354	0.00001
Leukocytes (in the field of view)	0.245	0.006
Proteinuria (g/L)	0.280	0.001

There were no significant differences in TGF-β1 levels in ATN or CNI nephrotoxicity compared to recipients with normal graft function (p = 0.82 and p = 0.36, respectively).

Kidney recipients with ACR, AMR and chronic rejection, in which immune processes play a leading role in their development, were grouped under "immune mechanisms" of graft injury. The ATN and CNI nephrotoxicity group constituted the group with graft dysfunction, labeled as "other processes". A comparative analysis of TGF-β1 levels and basic laboratory parameters of kidney function in recipients with normal graft function and dysfunction resulting from immune (ACR, AMR, chronic rejection) and other processes (ATN, CNI nephrotoxicity) was conducted.

In recipients with graft dysfunction resulting from immune mechanisms, TGF-β1 levels were not only significantly different from those in recipients with normal function (p < 0.000) but were also higher than in dysfunction caused by other processes (p = 0.0007; Fig. 3).

At the same time, the level of classical renal function parameters (creatinine, urea, proteinuria and GFR) did not differ significantly between recipients with immune and non-immune injury.

Based on the results obtained, the diagnostic significance of TGF-β1 level for identifying recipients with graft dysfunction resulting from immune mechanisms (ACR, AMR, chronic rejection) was assessed. The area under the ROC curve was 0.721 ± 0.04 [95% CI 0.64–0.80] and was significantly different from 0.5, $p < 0.001$ (Fig. 4).

The threshold serum TGF-β1 level for detection of kidney graft dysfunction arising from acute and chronic rejection mechanisms was 94.3 ng/mL. Kidney recipients with TGF-β1 levels exceeding this calculated threshold had a 2.2-fold higher risk of acute or chronic graft rejection resulting from immune mechanisms detected on morphological examination than other kidney recipients (RR = 2.2 ± 0.22 [95% CI 1.46–3.46] with 77.5% sensitivity, 60.3% specificity, and 70.0% overall diagnostic performance of the test). The positive and negative predictive values of serum TGF-β1 measurements for identifying patients at high risk of immunological complications after KT was 70.5% and 68.6%, respectively.

DISCUSSION

Active research in recent years in the field of biochemistry, immunology and genetics has not only expanded the understanding of the complex mechanisms of interaction between the recipient body and the donor organ, but also opened up additional opportunities for the development of innovative approaches to improving and predicting transplant outcomes. The mechanisms of tolerance and rejection of a transplanted organ include a whole set of complex immune processes [8].

One of the key milestones of transplantology was marked by the discovery, in the middle of the last century, of immunosuppressive drugs – calcineurin inhibitors – which became the basis of therapy that prevents graft rejection response [9]. At the same time, the need for lifelong use of immunosuppressive drugs comes with a number of negative effects, among which the nephrotoxic effect, the so-called CNI nephrosclerosis, especially critical for kidney recipients, is the main one. The risk of developing cellular and humoral rejection persists

Table 3

Comparative analysis of laboratory parameters in recipients with and without graft dysfunction

Indicator	Normal function	Graft dysfunction	Significance level (p)
Creatinine, μmol/L	85.30 [71.50; 95.00]	250.05 [160.76; 425.23]	<0.00001
Urea, mmol/l	7.69 [6.20; 8.80]	19. 88 [12.86; 28.10]	<0.00001
Proteinuria, g/L	0.03 [0.03; 0.04]	0.14 [0.04; 0.40]	<0.00001
GFR, mL/min	81.30 [68.50; 100.00]	20.80 [11.35; 36.50]	<0.00001
TGF-β1, ng/mL	86.41 [69.48; 109.70]	111.40 [87.06; 145.15]	0.0004

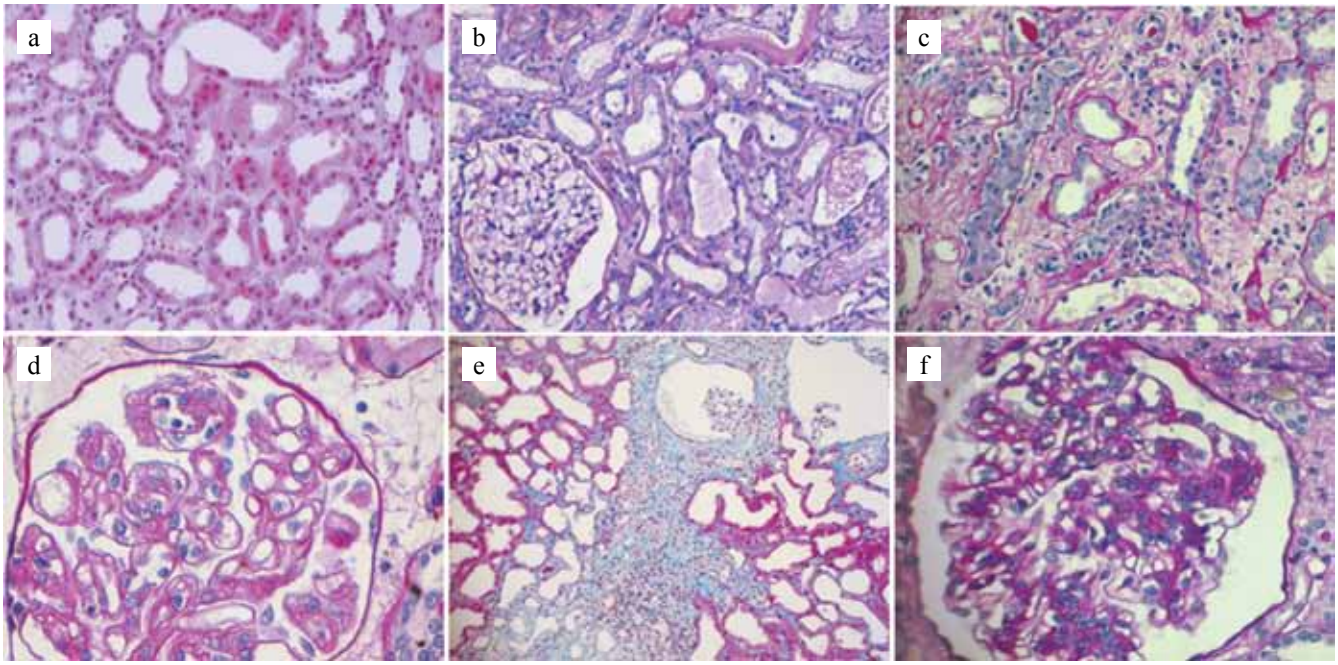

Fig. 1. Image of kidney biopsy specimens with H&E stain: (a) Normal, Masson's Trichrome stain ×40; (b) Acute tubular necrosis (ATN), PAS stain ×100; (c) Acute T-cell mediated rejection (ACR), PAS stain ×100; (d) Acute antibody-mediated rejection, transplant glomerulopathy (AMR), PAS stain ×200; (e) Interstitial fibrosis in CNI nephrotoxicity, Masson's Trichrome stain ×40; (f) Recurrent glomerulonephritis (IgA nephropathy)

throughout the life of the recipient. Repeated episodes of rejection lead to its chronic form with subsequent fibrosis and functional remodeling of the graft [10].

Kidney transplant injury is verified by morphological analysis of biopsy samples. However, the diagnostic value of this analysis is limited by the risk of taking an uninformative area of tissue, and the decision to perform an unscheduled biopsy is often made in the presence of a clear clinical picture of reduced renal function [11]. Studies on immune mechanisms of graft injury and improvement of diagnostic methods using minimally invasive laboratory technologies will allow not only to identify effective biomarkers of graft pathology at the early stage of complications, but also to consider them as a target for therapy.

Many studies have shown that TGF-β1 has a bright prospect as a marker for CKD [12]. Mediators of the TGF-β1 biological functions are Smad signaling pathways, including both Smad3, which is involved in the pathogenesis of kidney injury and fibrosis [13], and Smad2 and Smad7, which have a nephroprotective effect. These explain the ambiguity of data published by different authors on the role of TGF-β1 in kidney transplantation [14].

Considering our own and published data on the variability of the diagnostic and prognostic potential of TGF-β1 in solid organ recipients [7], this work was

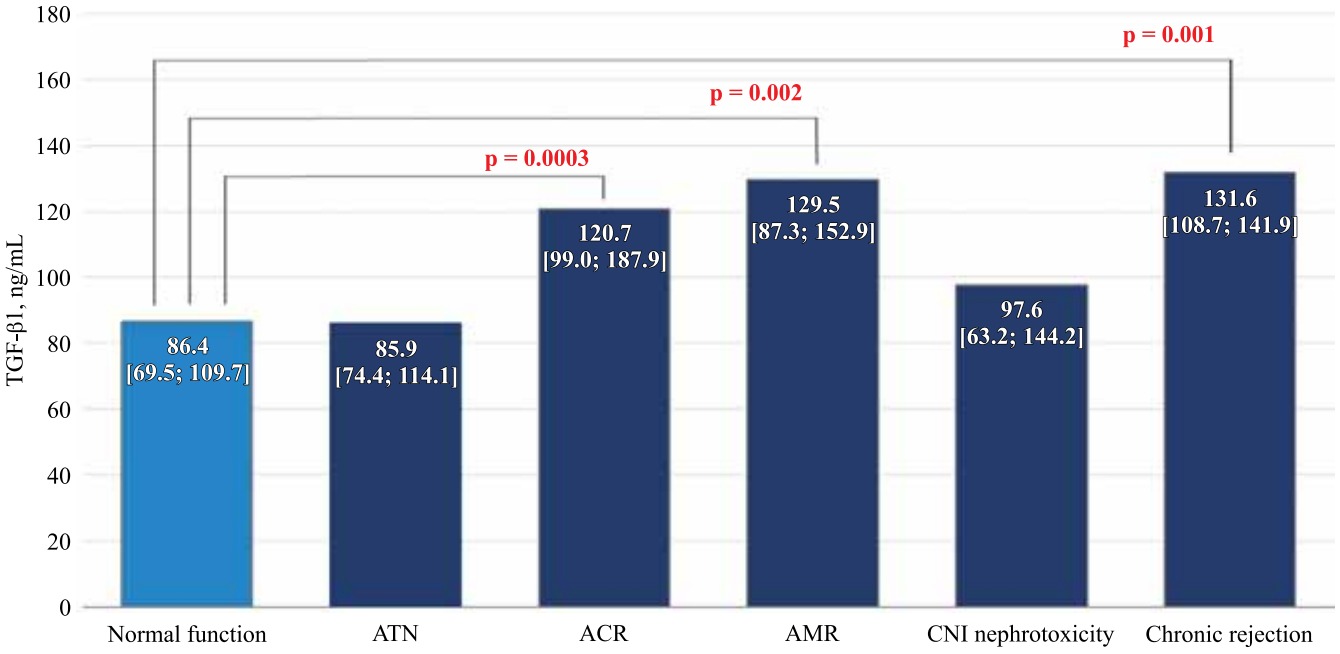

Fig. 2. Comparative analysis of serum TGF-β1 levels in kidney recipients with and without graft dysfunction of different nature

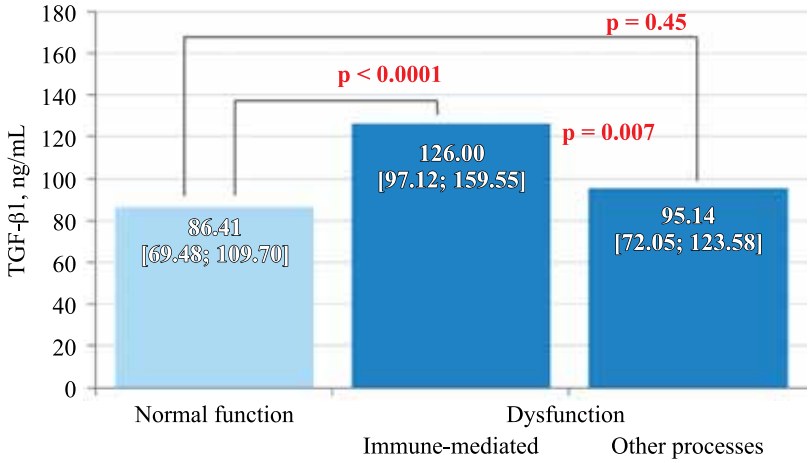

Fig. 3. Comparative analysis of TGF-β1 levels in kidney recipients with normal graft function, with immune-mediated graft dysfunction (acute cellular, humoral and chronic rejection), and with other processes (acute tubular necrosis, CNI nephrotoxicity)

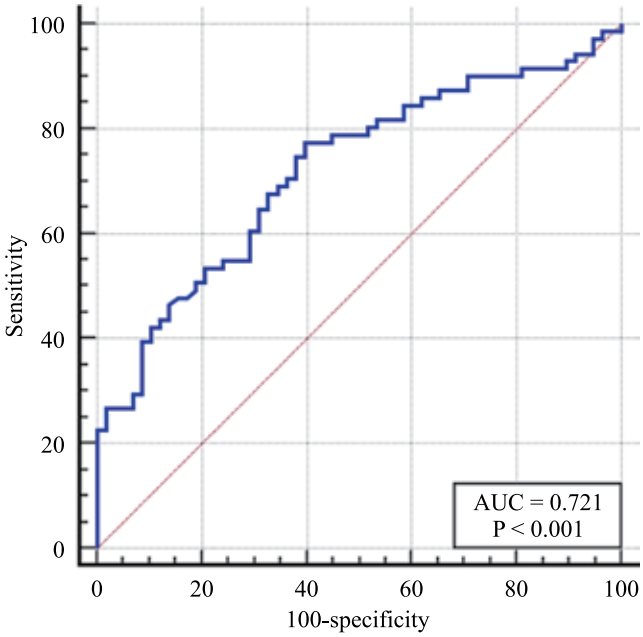

Fig. 4. ROC curve of serum TGF-β1 levels in kidney recipients with immune-mediated graft dysfunction

aimed at studying the diagnostic value of TGF-β1 in recipients with renal transplant dysfunction.

The study results showed that kidney recipients with serum TGF-β1 levels above 94.3 ng/mL had a 2.2-fold higher risk of acute or chronic rejection than other kidney recipients. In turn, differentiation of acute and chronic rejection requiring different approaches to therapy is possible by morphological examination.

Data on the association of high serum TGF-β1 levels with the presence of immune-mediated kidney graft injury are consistent with the results of foreign colleagues who showed higher TGF-β1 levels in kidney recipients with chronic rejection in comparison with recipients without rejection in the long term after transplantation [15]. However, it is worth noting that all patients had a history of several episodes of acute rejection, which allowed the authors to characterize TGF-β1 only as a marker for chronic rejection. In the present study, we have shown a significantly higher level of TGF-β1 in kidney recipients with acute rejection and in the early stages after transplantation, which allows us to count on the prospects of its use for identifying patients at risk of immune-mediated complications starting from the first days after KT.

Numerous animal experiments described by foreign authors demonstrate TGF-β1 participation in kidney injury mechanisms, as well as the association of increased TGF-β1 expression with decreased GFR, signs of tubular necrosis and fibrosis [16]. The positive correlation between TGF-β1 level and proteinuria ($r = 0.280$; $p = 0.001$), which we found, seems to be very significant taking into account the results of experiments by Kasuga et al., who managed to reduce the level of proteinuria in rats with glomerulonephritis by administering TGFβRII receptor antibodies [17]. This suggests that TGF-β1 can be used as a target for therapy. In another study by Du X.X. et al., serum TGF-β1 level was found to correlate with GFR and nephrograft survival period [18], which indicates a probable influence of a number of associated factors on TGF-β1 level that require additional study.

A study by Sugimoto et al. showed that in mice, morphogenic protein BMP, a member of the TGF-β superfamily, acts as an antagonist of TGF-β signal transduction, and oral administration of its agonist (THR-123) inhibits renal fibrosis [19]. Another activator of TGF-β signaling is thrombospondin-1, whose inhibition in mice, according to Sun et al., resulted in activation of angiogenesis and reduction of renal fibrosis in mice [20]. In a model of KT in rats, administration of the anti-inflammatory drug pirfenidone, which targets TGF-β, resulted in attenuation of inflammation and renal fibrosis [21].

In an experimental work by Border et al., an attempt was made to inhibit the process of fibrosis in glomerulonephritis by administering antibodies to TGF-β1 [22]. The experiments resulted in effective suppression of extracellular matrix protein accumulation, which was confirmed by histological studies [23]. Therapeutic use of TGF-β1 inhibitors is not yet possible due to the ambiguous role of the latter in tissue homeostasis and regeneration, which manifests both pro- and antifibrotic effects. It is suggested that the combination of antifibrotic therapy with protection of the tubular epithelium may be very promising [24].

The development of acute and chronic rejection based on immune mechanisms, contributes to accelerated formation of graft fibrosis [25]. Early detection of graft dysfunction is crucial for renoprotective treatment and can positively influence transplant outcomes.

Predicting allograft survival remains challenging, but a combination of clinical data and studies of potential biomarkers of the pathology can improve diagnostic accuracy. To date, several potential antifibrotic strategies have been identified, but no specific drug has yet been approved for the treatment of kidney transplant recipients because of the complexity of the cascade of pathologic processes in fibrosis and the intersection of many signaling pathways that mutually influence and compensate each other.

There is a need to develop ancillary minimally invasive diagnostic technologies, biomarkers to predict long-term outcome of transplantation or to differentiate fibrosis resulting from causes of different nature [26].

In the present study, a threshold serum TGF-β1 level in kidney recipients was calculated to identify patients at high risk of acute or chronic rejection who were recommended for unscheduled biopsy.

Obviously, all peculiarities of the mechanisms of TGF-β1 involvement in the development of transplanted kidney pathology are subject to further in-depth study.

The authors declare no conflict of interest.

REFERENCES

1. *Azegami T, Kounoue N, Sofue T, Yazawa M, Tsujita M, Masutani K et al.* Efficacy of pre-emptive kidney transplantation for adults with end-stage kidney disease: a systematic review and meta-analysis. *Ren Fail*. 2023: 45 (1): 2169618. doi: 10.1080/0886022X.2023.2169618.
2. *Stolyarevich ES, Zhilinskaya TR, Artyukhina LYu, Kim IG, Zaydenov Va, Tomilina NA.* Morphological structure of late renal graft dysfunction and its effect for long-term results. *Russian Journal of Transplantology and Artificial Organs*. 2018; 20 (1): 45–54. (In Russ.).
3. *Sharapchenko SO, Mamedova AA, Shevchenko OP.* Diagnostic and therapeutic potential of transforming growth factor beta 1 in solid organ transplantation: recent research findings. *Russian Journal of Transplantology and Artificial Organs*. 2023; 25 (2): 148–157. (In Russ.).
4. *Herath S, Erlich J, Au AYM, Endre ZH.* Advances in detection of kidney transplant injury. *Mol Diagn Ther*. 2019; 23: 333–351.
5. *Zhang H, Yang P, Zhou H, Meng Q, Huang X.* Involvement of Foxp3-expressing CD4+ CD25+ regulatory T cells in the development of tolerance induced by transforming growth factor-beta2-treated antigen-presenting cells. *Immunology.* 2008; 124: 304–314.
6. *Higgins CE, Tang J, Higgins SP, Gifford CC, Mian BM, Jones DM et al.* The Genomic Response to TGF-β1 Dictates Failed Repair and Progression of Fibrotic Disease in the Obstructed Kidney. *Front Cell Dev Biol.* 2021; 9: 678524. doi: 10.3389/fcell.2021.678524.
7. *Kurabekova RM, Shevchenko OP, Tsiroulnikova OM, Mozheyko NP, Tsiroulnikova IE, Monakhov AR, Gautier SV.* Level of transforming growth factor beta-1 relates to congenital liver disease severity in children of early age. *Russian Journal of Transplantology and Artificial Organs*. 2016; 18 (3): 16–21. (In Russ.).
8. *Buscher K, Rixen R, Schütz P, Hüchtmann B, Van Marck V, Heitplatz B et al.* Plasma protein signatures reflect systemic immunity and allograft function in kidney transplantation. *Transl Res.* 2023; 262: 35–43. doi: 10.1016/j.trsl.2023.07.007.
9. *Cohen DJ, Loertcher R, Rubin MF, Tilney NL, Carpenter CB, Strom TB.* Cyclosporine: A New Immunosuppressive Agent for Organ Transplantation. *Ann Intern Med.* 1984; 101: 667–682.
10. *Bentata Y.* Tacrolimus: 20 years of use in adult kidney transplantation. What we should know about its nephrotoxicity. *Artif Organs.* 2020; 44 (2): 140–152.
11. *Loupy A, Mengel M, Haas M.* Thirty years of the International Banff Classification for Allograft Pathology: the past, present, and future of kidney transplant diagnostics. *Kidney Int.* 2022; 101 (4): 678–691. doi: 10.1016/j.kint.2021.11.028.
12. *Lee SB, Kanasaki K, Kalluri R.* Circulating TGF-β1 as a reliable biomarker for chronic kidney disease progression in the African-American population. *Kidney Int*. 2009; 76: 10–12.
13. *Verrecchia F, Mauviel A.* Transforming growth factor-β signaling through the smad pathway: role in extracellular matrix gene expression and regulation. *J Invest Dermatol.* 2002; 118 (2): 211–215.
14. *Fujimoto M, Maezawa Y, Yokote K, Joh K, Kobayashi K, Kawamura H et al.* Mice lacking Smad3 are protected against streptozotocin-induced diabetic glomerulopathy. *Biochem Biophys Res Commun.* 2003; 305:1002–1007.
15. *Eikmans M, Sijpkens YW, Baelde HJ, de Heer E, Paul LC, Bruijn JA.* High transforming growth factor-β and extracellular matrix mRNA response in renal allografts during early acute rejection is associated with absence of chronic rejection. *Transplantation.* 2002; 73: 573–579.
16. *Meng XM, Nikolic-Paterson DJ, Lan HY.* Inflammatory processes in renal fibrosis. *Nat Rev Nephrol.* 2014; 10: 493–503.
17. *Kasuga H, Ito Y, Sakamoto S, Kawachi H, Shimizu F, Yuzawa Y, Matsuo S.* Effects of anti-TGF-β type II receptor antibody on experimental glomerulonephritis. *Kidney Int.* 2001; 60: 1745–1755.
18. *Du XX, Guo YL, Yang M, Yu Y, Chang S, Liu B et al.* Relationship of transforming growth factor-βl and arginase-1 levels with long-term survival after kidney transplantation. *Curr Med Sci.* 2018; 38: 455–460.
19. *Sugimoto H, LeBleu VS, Bosukonda D, Keck P, Taduri G, Bechtel W et al.* Activin-like kinase 3 is important for kidney regeneration and reversal of fibrosis. *Nat Med*. 2012; 18: 396–404.
20. *Sun D, Ma Y, Han H, Yin Z, Liu C, Feng J et al.* Thrombospondin-1 short hairpin RNA suppresses tubulointerstitial fibrosis in the kidney of ureteral obstruction by ameliorating peritubular capillary injury. *Kidney Blood Press Res.* 2012; 35: 35–47.
21. *Qiu ZZ, He JM, Zhang HX, Yu ZH, Zhang ZW, Zhou H.* Renoprotective effects of pirfenidone on chronic renal allograft dysfunction by reducing renal interstitial fibrosis in a rat model. *Life Sci.* 2019; 233: 116666.
22. *Border WA, Okuda S, Languino LR, Sporn MB, Ruoslahti E.* Suppression of experimental glomerulonephritis by antiserum against transforming growth factor beta 1. *Nature.* 1990; 346: 371–374.
23. *Voelker J, Berg PH, Sheetz M, Duffin K, Shen T, Moser B et al.* Anti-TGF-β1 antibody therapy in patients with diabetic nephropathy. *J Am Soc Nephrol*. 2017; 28: 953–962.
24. *Saritas T, Kramann R.* Kidney Allograft Fibrosis: Diagnostic and Therapeutic Strategies. *Transplantation*. 2021; 105 (10): e114–e130. doi: 10.1097/TP.0000000000003678.
25. *Loga L, Dican L, Matei HV, Mărunțelu I, Constantinescu I.* Relevant biomarkers of kidney allograft rejection. *J Med Life.* 2022; 15(11): 1330–1333. doi: 10.25122/jml-2022-0181.
26. *Williams WW, Taheri D, Tolkoff-Rubin N, Colvin RB.* Clinical role of the renal transplant biopsy. *Nat Rev Nephrol*. 2012; 8: 110–121.

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A RARE CASE OF ACUTE DESTRUCTIVE PANCREATITIS IN A PATIENT WITH CHRONIC KIDNEY DISEASE ON PERITONEAL DIALYSIS: DIAGNOSTIC AND TREATMENT CHALLENGES

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Patients with chronic kidney disease are susceptible to developing acute pancreatitis. We present a rare clinical case of acute pancreatitis with the formation of pancreatic necrosis in a patient on peritoneal dialysis (PD), debuted with PD–associated peritonitis. On hospitalization, there were no diagnostic criteria for acute pancreatitis; treatment for dialysis peritonitis was ineffective. Repeated ultrasound examination revealed signs of diffuse changes in the pancreas and multi-chamber formation of the small pelvis. Refractory peritonitis, inadequate ultrafiltration, and unclear nature of formation in the pelvic were the grounds for diagnostic laparoscopy and removal of the peritoneal catheter. Abdominal inspection revealed spots of stearin necrosis over the entire surface of the peritoneum and the greater omentum; in the pelvis there were adhesions between the uterus and the rectum. Development of pancreonecrosis was confirmed by abdominal CT scan. Treatment of acute pancreatitis was without effect, type 2 myocardial infarction developed, and with increasing symptoms of multiple organ failure, death occurred. Possible reasons for the development of destructive pancreatitis and the features of its course in the PD patient are discussed. Caution is necessary regarding this disease when dialysis peritonitis occurs.

Keywords: acute pancreatitis, peritoneal dialysis-associated peritonitis, continuous ambulatory peritoneal dialysis, chronic kidney disease.

INTRODUCTION

PD is a universally recognized method of renal replacement therapy (RRT) for stage 5 chronic kidney disease (CKD). In Russia, the PD program started in 1995 and over the past decades, thousands of CKD patients have received and are receiving treatment with this method [1]. PD patients are exposed to a variety of homeostatic disorders and complications peculiar to CKD and caused by the PD technique itself. Acute pancreatitis (AP) is one such rare but life-threatening complication.

The first description of two cases of AP in PD patients dates to 1985, and the authors suggested that the disease was a complication of PD [2]. AP incidence and severity in CKD patients, including those receiving replacement therapy (dialysis), remain unknown. Between 1985 and 2011, only 94 cases with 133 AP episodes in PD patients were reported [3]. In subsequent years, only single cases of AP in patients with CKD, including those who have undergone kidney transplantation, have been published [4, 5]. It is now generally recognized that patients with pre-dialysis and dialysis CKD are at higher risk of developing AP than the general population. There are also indications that AP is more common in CKD patients undergoing PD than hemodialysis (HD) patients, although not all researchers agree with this statement [6–8].

The duration of the PD program before the development of the first episode of AP varies, ranging from a few months to several years [3]. One of the first national studies of the incidence and severity of AP, based on the results of a questionnaire survey of dialysis centers in Germany, found a significantly higher incidence compared to the general population. Comparison of the two groups of dialysis patients showed that AP was more common in the PD group (266 per 100,000 per year, 67 per 100,000 per year in HD patients, 19.7 per 100,000 per year in the general population) and more severe, with half of them developing pancreonecrosis. Considering the methodology of the study, the authors do not exclude a higher incidence of AP among dialysis patients [8]. Analysis of the incidence and severity of AP in 67,078 patients with end-stage renal disease, who initiated dialysis between 1999 and 2007 in Taiwan, found that the cumulative incidence rates of AP were 0.6, 1.7, 2.6, 3.4, and 4% at 1, 3, 5, 7 and 9 years, respectively; patients on HD and PD had an AP incidence of 5.11 and 5.86 per 1000 person-years, respectively. Severe AP occurred in 44.9% of the HD patients and in 36% of the PD patients. According to the authors, CKD patients on PD were at a higher risk for AP than those on HD [9].

PD patients may have the same causes of AP as the general population [10, 11]. However, they have many

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additional factors that make the pancreas highly susceptible to inflammation [3, 12]. First, autopsies have revealed an increased prevalence of structural pancreatic abnormalities in deceased patients who had been on HD for a long time. Second, these patients exhibit a variety of metabolic disorders accompanying CKD (hyperglycemia, hypercalcemia, hypertriglyceridemia, etc.), whose involvement in the genesis of AP has been documented. Finally, the PD modality itself may predispose the patient to AP. Various explanations have been proposed for the negative impact of the PD procedure on the pancreas, but the question of whether this modality increases the risk of the disease remains open and controversial. Fig. 1 shows the main pathophysiologic mechanisms of AP development in CKD patients undergoing PD.

AP diagnosis in PD patients is difficult due to the presence of CKD and the peculiarities of the dialysis modality. In particular, the diagnostic accuracy of blood amylase enzyme activity is limited. This is because in CKD, increased blood amylase resulting from decreased urinary excretion is more common than in the general population, and the use of glucose polymer icodextrin in the PD program, on the contrary, decreases the activity of this enzyme. Often the development of AP in PD patients is masked by the clinical and laboratory picture of PD-associated peritonitis [3, 12].

The surgical community has witnessed a clear trend towards an increase in the frequency of the destructive form and the development of complications of AP in the general population over the past few years and the absence of a marked decrease in overall and postoperative mortality in this pathology, despite the use of modern detoxification techniques, development of new surgical intervention methods, and improvement in drug therapy [13]. Mortality from AP in the dialysis patient population is high, 8–58%; risk factors include severe disease, male gender, elderly age, and diabetes mellitus [3, 7–9, 12].

We present a rare clinical observation of the development of the first episode of AP with the formation of pancreatic necrosis in a CKD patient on PD, which debuted with PD-associated peritonitis and caused difficulties in early diagnosis and treatment.

CLINICAL CASE REPORT

1. Diagnosis and treatment of peritoneal dialysis-associated peritonitis

Patient V., female, 59 years old, was admitted to the intensive care unit for emergency indications with

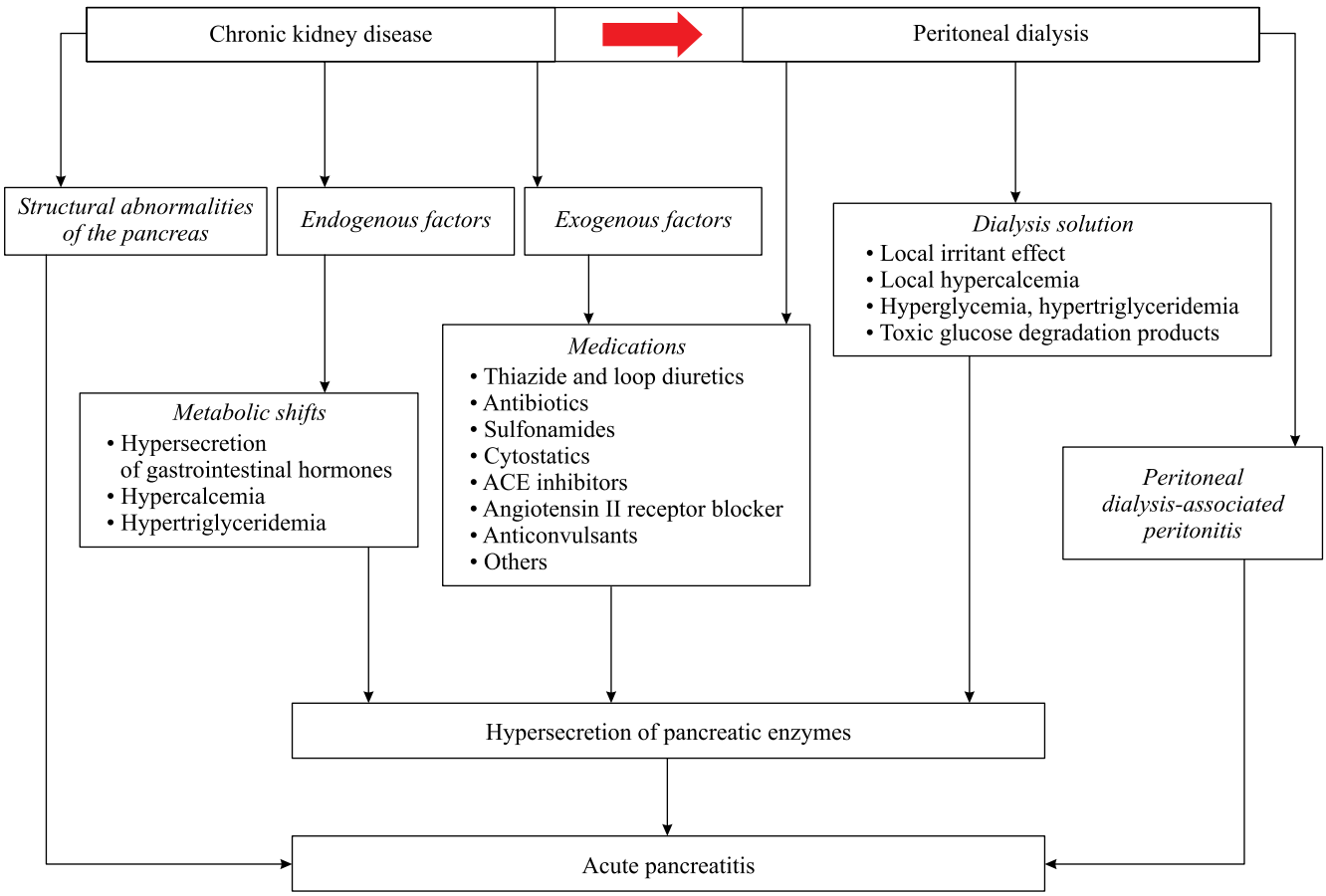

Fig. 1. Pathophysiologic mechanisms of acute pancreatitis in patients with chronic kidney disease on peritoneal dialysis treatment [3, 11, 12]

complaints of sharp abdominal pain without clear localization.

The patient has, for a long time, been suffering from CKD resulting in chronic glomerulonephritis (without histological verification). Renal replacement therapy (PD) has been ongoing for three years. A day before admission to the hospital, sharp abdominal pain without clear localization occurred and the dialysis solution turned cloudy.

Life history without peculiarities. Epidemiological anamnesis was absent. Harmful habits denied. Past diseases: childhood infections, acute respiratory diseases, and COVID-19 in 2021. No episodes of PD-associated peritonitis were recorded.

The patient was conscious, correctly oriented in place and time. Normosthenic physique, satisfactory nutrition. Skin and mucous membranes are of normal color, no peripheral edema. Respiratory rate 16/min, vesicular on auscultation. Heart rate 75/min, blood pressure 120/70 mmHg. The abdomen was not enlarged in size, was symmetrical, and participated in the act of breathing to a limited extent. On palpation, the abdomen was tense, more in the lower parts. There was pain, predominantly in the hypogastrium. Positive symptoms of peritoneal irritation. The liver was not enlarged. The external part of the dialysis catheter was located to the right of the umbilicus, the skin around it was not changed. Stool without pathologic impurities. Rectal examination: perianal area without changes; ampulla was empty; there was a trace of light brown feces on the glove. Kidneys were not palpable. Anuria.

The patient was undergoing PD in a continuous outpatient PD mode, 8 l/day (4 exchanges of 2 l each) solutions Physioneal (6 l) and Icodextrin (2 l). The PD program was adequate, he denied having episodes of PD-associated peritonitis and other complications.

The results of laboratory and instrumental examinations on the day of admission are presented in Table 1.

Table 1

Results of laboratory and instrumental examination of patient V. upon admission to the intensive care unit

Method	Result
Complete blood count	Red blood cells 3.7×10^{12}/l, hemoglobin 123 g/l, white blood cells 16.5×10^{9}/l, neutrophils 89.5%, platelets 288×10^{9}/l.
Biochemistry blood test	ALT 14 U/L, AST 12 U/L, alpha-amylase 87 U/L (norm 25–125), glucose 5.5 mmol/L, urea 14.8 mmol/L, creatinine 836 μmol/L, bilirubin (total) 5, 2 μmol/L, C-reactive protein 288 mg/L, procalcitonin ≥2 μg/L, fibrinogen 6.8 g/L, aPTT 31.6 s, prothrombin time 17.7 s.
Antibodies to Coronavirus (SARS-CoV-2)	IgM 3 (norm <2), IgG 446 (norm <10)
Dialysis fluid analysis	The color is grayish yellow. Cytosis 7128 ($\times 10^{6}$ per L), neutrophils 94%, lymphocytes 2%, monocytes 4%, erythrocytes 36×10^{6}/L
Abdominal ultrasound imaging	Intestinal pneumatization. The liver is not enlarged. The contours are clear and even. Echogenicity is increased. Sound conductivity is not reduced. The structure is homogeneous. Focal formations are not visualized. Intrahepatic bile ducts are not dilated. The portal vein and hepatic veins are not dilated and are passable. Gallbladder is not enlarged, 64 × 20 mm, the walls are not thickened 2 mm, homogeneous, anechogenic content in the cavity. No paravesical infiltration was revealed. The hepaticocholedochus is not dilated – 6 mm, located fragmentarily. The pancreas is visualized fragmentarily, head 25 mm, body 19 mm, tail 22 mm. Echogenicity is increased in the areas accessible for examination, the structure is homogeneous without focal changes. Wirsung duct is not dilated. Peripancreatic tissue is not infiltrated. Spleen is not enlarged, contours are smooth, structure is homogeneous without focal changes, echogenicity is average. Intestinal loops are not dilated, walls are not thickened, they are peristaltic. Free fluid is visualized in all sections. In the right subphrenic space 18 mm thick, in the subhepatic space 30 mm thick, in the subphrenic area on the left 15 mm thick, near the spleen 15 mm thick, along the lateral canals 40 mm thick, in the lesser pelvis 60 mm thick. Conclusion. Echo signs of free fluid in the abdominal cavity (peritoneal dialysis) and diffuse changes in the liver and pancreas
Chest X-ray	A chest X-ray in direct projection did not reveal focal or infiltrative shadows. The pulmonary pattern is not changed. The roots of the lungs are structural and not expanded. The heart shadow and mediastinum are not expanded. The contour of the diaphragm dome is clear and even. Sinuses are free.
Abdominal Plain radiography	There were no signs of violation of the integrity of the hollow abdominal organ. Single horizontal fluid levels in the intestinal lumen are determined.
Electrocardiography	Sinus rhythm. Heart rate 80 beats/min. PQ 0.12; QRS 0.08; QT 0.35. Deviation of the electrical heart axis to the left. Block of the left anterior branch of the bundle of His.
Dialysis solution sent for microbiological examination	

Based on the results of clinical and laboratory examination, the patient was diagnosed with PD-associated peritonitis. The following antibacterial and symptomatic therapy was prescribed:

- *Ampicillin / Sulbactam (1000 mg + 500 mg) intravenously, 3 times a day;*
- *Cefepin 500 mg (intraperitoneally) into dialysis solution, 4 exchanges per day;*
- *Sodium chloride 0.9% 1000 ml intravenously, 2 times per day;*
- *Tramadol 100 mg intramuscularly (as indicated).*

Three days later, the patient's condition improved slightly. The intensity of pain became less with localization in the umbilical region. Body temperature was normal. Pastosity appeared on the legs and eyelids. Lungs and heart without changes, BP 125/80 mm Hg. The abdomen participated in the act of breathing, soft on palpation. Local abdominal tension was determined in the peri-umbilical region, and there was limited pain on palpation. Symptoms of peritoneal irritation were negative. Stools tended to be constipated, brown in color. Turbidity of the dialysis solution became a little less, but there was no full transparency. The result of bacteriological examination of the dialysis solution was that there was no microflora growth. Leukocytosis, high level of C-reactive protein and procalcitonin remained (Table 2). Systemic antibacterial therapy was changed: tigecycline 100 mg intravenously twice a day was prescribed; intraperitoneal administration of cefepime 1.0 g at each exchange, infusion and symptomatic therapy (enoxaparin sodium 4000 anti-Xa IU subcutaneously, once a day, rabeprazole 20 mg intravenously once a day) were continued. The PD program was increased to 10 L/day (2 liters, 5 exchanges).

In the following days, the patient's condition remained severe with negative dynamics. Moderate pain in the umbilical region persisted. There were no symptoms of peritoneal irritation. A persistent syndrome of systemic inflammatory reaction was registered: blood leukocytosis with a shift of the leukocytic formula to the left, high concentrations of C-reactive protein and procalcitonin, although the level of the latter became lower. There was decreased total protein, increased hepatic transaminase activity by one and a half times and alpha-amylase activity by two times, and hypokalemia. The PD programs were inadequate for ultrafiltration (negative despite the correction performed). Dialysis solution remained turbid (on repeated negative cultures) with high leukocyte titers (Table 2).

The intraperitoneal antibiotic was changed to vancomycin 1.0 g twice a day and imipenem/cilastatin 500 mg three times a day.

Esophagogastroduodenoscopy and dynamic ultrasound imaging (DUI) of the abdominal cavity and lesser pelvis were performed. Conclusion of esophagogastroduodenoscopy: superficial gastritis; hemorrhagic duodenitis; axial cardiac hiatal hernia. Conclusion of ultrasound imaging of abdominal cavity and lesser pelvis: Echo signs of free fluid in the abdominal cavity (peritoneal dialysis), diffuse changes in the liver, pancreas and multi-chamber pelvic mass (in the projection of uterine appendages) with total dimensions of 90 × 45 mm.

Table 2

Dynamics of laboratory parameters of patient V. before diagnostic laparoscopy and removal of the peritoneal catheter

Parameter	Hospital stay	
	Day 3	Day 8
Complete blood count		
Red blood cells ($\times 10^{12}$/L)	3.65	3.44
Hemoglobin (g/L)	121	109
White blood cells ($\times 10^{9}$/L)	10.4	13.5
Band neutrophils (%)	7	2
Segmented neutrophils (%)	80	83
Platelets ($\times 10^{9}$/L)	246	257
Biochemistry blood test		
Total bilirubin (μmol/L)	4.2	5.4
– direct bilirubin (μmol/L)	2.4	2.6
– indirect bilirubin (μmol/L)	1.8	2.8
Glucose (mol/L)	4.8	5.4
ALT (U/L) (norm 5–34)	12	46
AST (U/L) (norm 5–31)	13	47
Alpha-amylase (U/L) (norm 25–125)	70	249
Total protein (g/L)	61	41
Alkaline phosphatase (U/L)	95	152
C-reactive protein (mg/L)	317	99
Procalcitonin (μg/L)	≥10	7
Creatinine (μmol/L)	764	730
Urea (mmol/L)	17.3	23.5
Triglycerides (mmol/L)	1.7	–
Parathyroid hormone (pg/mL)	287	–
Blood electrolytes		
Sodium (mmol/L)	130	132
Potassium (mmol/L)	4.0	3.6
Ionized calcium (mmol/L)	1.31	1.25
pH	7.39	7.24
Bicarbonate (mmol/L)	24	23
Dialysis solution analysis		
Color	Purulent	Yellow
Transparency	Turbid	Incomplete
Cytosis (cl. $\times 10^{6}$/L)	5076	318
Red blood cells (cl. $\times 10^{6}$/L)	4	227
Neutrophils (%)	96	74
Monocytes (%)	2	2
Lymphocytes (%)	2	24
Bacteriological examination of dialysis solution	No growth	No growth

2. Diagnosis and treatment of acute pancreatitis

There were no changes in the patient's condition against the background of complex conservative treatment. PD-associated peritonitis had a refractory course. The PD program was inadequate for ultrafiltration (negative). The nature of the pelvic mass was not clear according to DUI results. A decision was made to perform diagnostic laparoscopy [10] and remove the peritoneal catheter with transfer of the patient to HD.

Operation protocol. *Abdominal revision revealed no damage to the hollow organs; fibrin threads and stearin necrosis spots were found on the entire surface of the peritoneum and the greater omentum. The latter was vitreous edematous, with areas of bluish tint in the transverse colon area (Fig. 2). Intraoperative biopsy of the most altered area of the greater omentum was performed (histological examination showed fibrous-fatty tissue with necrosis).*

In the pelvic area, large serous cysts of both ovaries and adhesions between the uterus and a section of the rectum were detected (Fig. 3). There was a myomatous nodule on the uterus with a diameter of 3 cm. The abdominal cavity had residual turbid dialysis solution. The intestines were slightly distended, peristalsis was present. The abdominal cavity was washed with antiseptic solution, sanitized, fibrin was removed as much as possible. The peritoneal catheter was isolated and removed from a separate incision 3 cm above the projection of the internal cuff.

Laparoscopy results, as well as the negative dynamics of laboratory parameters (increasing leukocytosis, level of C-reactive protein, liver enzymes, alpha-amylase) before the operation, indicated acute destructive pancreatitis in the patient. In the postoperative period, abdominal CT scan was performed with bolus intravenous injection of 100 ml of Ultravist 370 at the rate of 4 ml per second. Conclusion. Postoperative condition. CT signs of peripancreatic cell thickening (parapancreatitis) and infiltrative changes in the retroperitoneal tissue in all sections with a tendency to the formation of delimited fluid accumulations:

- *along the posterior layer of the perirenal fascia on the right side with a layer up to 11 mm thick, approximate dimensions 38 × 68 mm;*
- *along the anterior layer of the perirenal fascia on the right side with a layer up to 20 mm thick, approximate dimensions 63 × 154 mm;*
- *along the lateral surface of the ascending colon with a layer up to 23 mm thick, approximate dimensions 64 × 131 mm;*
- *in the area of the greater omentum on the right side with approximate dimensions of 42 × 137 × 89 mm;*

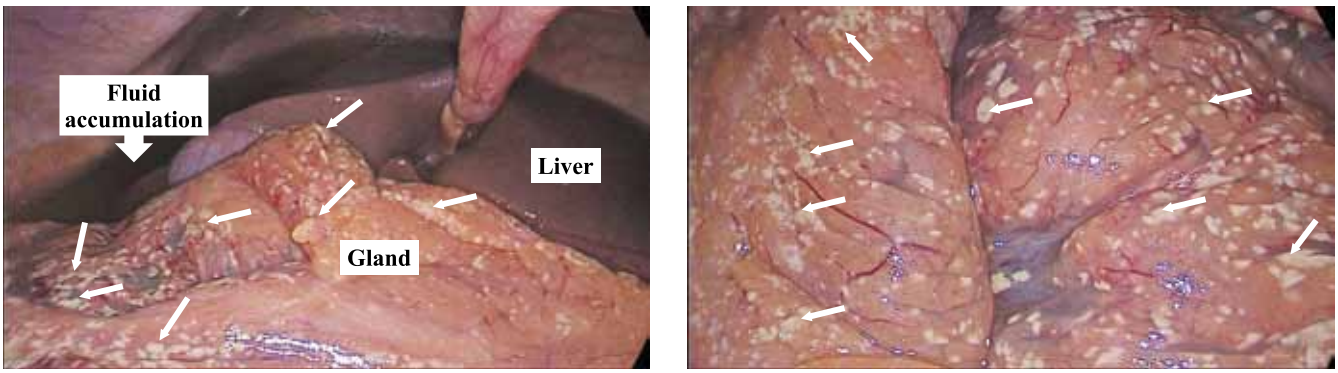

Fig. 2. Foci of stearin necrosis in the greater omentum during diagnostic laparoscopy in patient B.

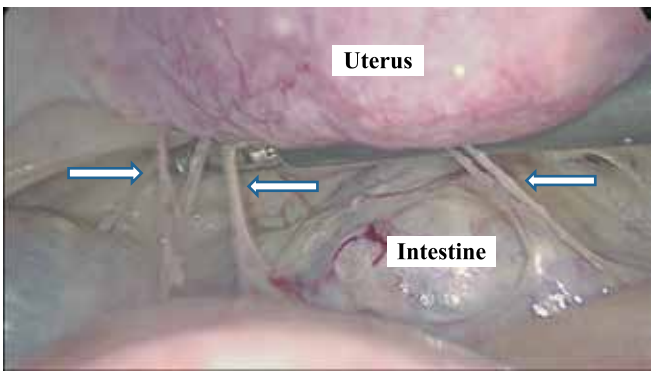

Fig. 3. Adhesions between the uterus and Intestine during diagnostic laparoscopy in patient B.

- *in the perinephric cellular tissue on the left side with a layer up to 30 mm thick, approximate size 61 × 64 mm;*
- *along the greater curvature of the stomach body with a layer up to 35 mm thick, approximate dimensions 53 × 53 mm.*

The patient was transferred to the intensive care unit for further treatment. On day 2 after surgery, she was bothered by pain in the postoperative wound area. The patient's general condition remained severe. Indicators of the system of integral assessment of the severity of the condition: APACHE II 16 points, SOFA 5 points. The patient was correctly oriented in space, time and person;

fully conscious, Glasgow Coma Scale: 15 points. Skin is pale, with normal moisture and reduced turgor. The lower limbs are pasty. Body temperature 36.5 °C. Breathing spontaneously; FIO_2: 21%; SPO_2: 94%. Respiratory rate: 18/min. On auscultation, breathing is rigid, no wheezes. Heart sounds are rhythmic, heart rate 90/min, blood pressure 110/60 mmHg. The abdomen was not swollen, participated in the act of breathing, soft on palpation, painful in the postoperative wound site; sluggish intestinal peristalsis was heard on auscultation; there were no symptoms of peritoneal irritation. In the area where the central vein catheter was installed, there were no signs of inflammation. The postoperative dressing was dry. There was 500 ml of serous-hemorrhagic discharge through the drainage. At laboratory examination, pronounced blood leukocytosis with a shift of leukocytic formula to the left, high levels of C-reactive protein and procalcitonin were all still present, increased blood bilirubin and severe hypoalbuminemia were noted; blood enzymes returned to normal levels (Table 3).

The patient continued multicomponent therapy, which included:

- *Daily hemofiltration sessions;*
- *Intravenous administration of antibiotics (Linezolid 1.2 g/day, Imipenem 1.0 g/day);*
- *Infusion therapy;*
- *Correction of hypoalbuminemia (albumin 25% 100 ml/day intravenously);*
- *Anti-ulcer and gastroprotective therapy (Rabeloc 20 mg/day intravenously);*
- *Parenteral nutrition (Dipeptiven 100 ml intravenously);*

Table 3

Dynamics of laboratory parameters of patient V. after diagnostic laparoscopy

Методика исследования	After diagnostic laparoscopy and peritoneal catheter removal		After sanitation re-laparoscopy
	Day 2	Day 5	
Complete blood count			
Red blood cells ($\times 10^{12}$/L)	3.4	2.4	3.3
Hemoglobin (g/L)	111	79	98
White blood cells ($\times 10^{9}$/L)	28.2	16.6	19.2
Neutrophils (%)	91	86	90
Platelets ($\times 10^{9}$/L)	71	150	70
Biochemistry blood test			
Total bilirubin (μmol/L)	31.7	41.4	24.7
– direct bilirubin (μmol/L)	14.1	9.1	6.5
– indirect bilirubin (μmol/L)	17.6	32.4	18.2
Glucose (mol/L)	4.4	2.7	2.1
ALT (U/L) (norm 5–34)	11	12	20
AST (U/L) (norm 5–31)	40	44	33
Alpha-amylase (U/L) (norm 25–125)	60	45	73
Albumin (g/L)	16.6	30	31
Alkaline phosphatase (U/L) (normal 30–112)	–	264	–
C-reactive protein (mg/L)	303	240	147
Procalcitonin (μg/L)	27	560	167
aPTT (s)	52.3	52.1	41.2
Prothrombin timem (s)	31.0	24.1	14.3
Fibrinogen (g/L)	4.3	3.1	1.9
Creatinine (μmol/L)	449	539	275
Urea (mmol/L)	13.8	19.6	22.8
Blood electrolytes			
Sodium (mmol/L)	128	128	131
Potassium (mmol/L)	4.0	4.5	4.7
Ionized calcium (mmol/L)	1.21	1.12	0.93
pH	7.23	7.21	7.34
Bicarbonate (mmol/L)	20	17	15
Microbiological examination			
Blood	No growth	No growth	
Sputum	*Streptococcus viridans* 10^{4}/mL Sensitivity: aztreonam, amikacin, imipenem	*Streptococcus viridans* 10^{6}/mL Sensitivity: aztreonam, amikacin	

– *Correction of blood electrolyte composition (intravenous injection of potassium chloride 4%);*
– *Correction of anemia (Erythropoietin 2500 IU/day, subcutaneously);*
– *Pain therapy (tramadol 200 mg/day, ketoprofen 200 mg/day, intravenously);*
– *Anticoagulant therapy (enixum 0.4 ml/day, subcutaneously);*
– *Treatment aimed at optimizing the gastrointestinal tract (metoclopramide 2.0 ml 3 times a day, intravenously).*

On day 5 after diagnostic laparoscopy (day 14 of hospitalization), the patient's condition was severe with negative dynamics. Increased abdominal pain was noted. Fully conscious, oriented in space, time and person, Glasgow coma scale: 15 points. Indicators of the system of integral assessment of the severity of the condition: APACHE II 20 points, on the SOFA 8 points. The skin is earthy. No peripheral edema. Body temperature 36.1 °C. Spontaneous breathing: FIO_2: 21%; SPO_2: 98%. Respiratory rate 18/min. Auscultatory respiration was vesicular, no wheezing. Heart sounds were clear, rhythmic. Heart rate 93/min. Blood pressure 104/68 mmHg. The abdomen was distended, soft on palpation, painful in the postoperative wound area. Peristalsis was sluggish, stool was single. Anuria. Postoperative wound dressings were dry and clean. The skin in the area where the main vein catheter was installed was without signs of inflammation. Laboratory examination showed that blood leukocytosis with a shift in the leukocyte formula was reserved and there were high levels of acute-phase proteins; a further increase in bilirubinemia and an increase in alkaline phosphatase activity were noted (Table 3). Increased abdominal pain, increasing intensity of bilirubinemia and systemic inflammatory response syndrome were indications for performing sanitation re-laparoscopy and abdominal revision [10].

Operation protocol. *At abdominal revision, plaques of stearin necrosis and fibrin threads were noted in all sections, 4000 mL of clear effusion was removed (no growth was detected at microbiological examination), sanitation and drainage of focal fluid accumulations in the parapancreatic region were performed, the abdominal cavity was sanitized with saline solution, silicone drains were installed in the left subphrenic and subhepatic spaces, in the lesser pelvis.*

In the postoperative period, the patient's condition remained very severe. A day after the second operation, blood pressure reduced, and vasotropic support with noradrenaline 0.2 mcg/kg/min was initiated. ECG showed signs of ischemia in the subendocardial layers of the myocardium of the anterolateral wall of the left ventricle. Echocardiography revealed impaired local contractility – hypokinesis of the apical anterior and septal segments of the left ventricle. Laboratory examination revealed an increase in cardiac-specific enzymes: total creatine phosphokinase 1896 U/L (norm 26–192), MB fraction of creatine phosphokinase 37 U/L (norm 0.0–24.0), lactate dehydrogenase 830 U/L (norm 81–234), troponin I 21999.9 pg/mL (norm 8.4–18.3). Given the results of laboratory and instrumental examination, the patient was diagnosed with type 2 myocardial infarction. With increasing symptoms of multiple organ failure, the patient died on day 18 of inpatient treatment.

At pathological autopsy, there were many gray-yellow curd-like foci of steatonecrosis on the peritoneum and in the omental tissue; the pancreas was yellow-gray in color, flabby consistency with obliterated lobular pattern and many dirty-yellow curd-like foci of steatonecrosis (histological examination shows extensive foci of steatonecrosis with focal leuko-lymphocytic infiltration); tissue around the gland is dirty gray in color, structureless, with many foci of steatonecrosis. The myocardium was red-brown in color with uneven blood filling with a pronounced clayey tinge (histological examination revealed diffuse and perivascular layers of connective tissue, uneven hypertrophy and contracture damage in cardiomyocytes, lipofuscin deposition in cardiomyocytes, vascular congestion, focal large-droplet fatty dystrophy).

DISCUSSION

The presented case report demonstrates the difficulties of clinical diagnosis of AP in a CKD patient on PD replacement therapy. According to the revised Atlanta-92 classification and national clinical guidelines for AP, the diagnosis of the disease requires two of the following three features [10, 14]:

1. Characteristic abdominal pain syndrome (intense persistent pain localized in the epigastrium, often radiating to the back);
2. Increased blood amylase and/or lipase activity exceeding the upper limit of the norm by three or more times;
3. Characteristic signs on CT/magnetic resonance imaging or transabdominal DUI.

At admission and during the first few days of hospitalization, none of the listed diagnostic criteria of AP was determined in our patient. The disease started with abdominal pain, but the pain did not have a clear localization and irradiation, there was no vomiting, at palpation the pain and slight muscle tension were localized mainly in the hypogastrium. Blood amylase activity was normal, although, as mentioned above, this situation is acceptable because the patient used a dialysis solution containing glucose polymer. Abdominal DUI revealed only diffuse changes in the pancreas and no characteristic symptoms. However, as is known, the diagnostic information value of DUI in AP is limited due to the special topography of the pancreas, as well as in the early stage of the disease. In our patient, the study was performed without removal of the dialysis solution from the abdominal cavity, which could also affect the results obtained. Thus, at the

time of admission and during the first week of hospitalization, clinical and laboratory symptoms – abdominal pain, cloudiness and inflammatory nature (high cytosis) of dialysis solution and signs of systemic inflammatory response (peripheral leukocytosis, serum elevation of C-reactive protein and procalcitonin) – corresponded to PD-associated peritonitis; there was no evidence for other etiology of peritonitis besides PD-associated peritonitis. A common complication of PD – PD-associated peritonitis – has similar manifestations to AP, so the diagnosis of the latter may be delayed. This was the case in our patient. Other authors also report about initial diagnosis of PD-associated peritonitis and delayed diagnosis of AP in PD patients [3, 10]. It is possible that contrast-enhanced CT, the gold standard for the diagnosis of AP, as well as determination of amylase and/or lipase in dialysis solution, could have helped in earlier detection of the disease, but there were no indications for these studies on admission [10].

The question remains open whether PD-associated peritonitis was the cause of AP or it was an early complication of AP – pancreatogenic (enzymatic) peritonitis. It seems that the second assumption is more realistic. This is confirmed by the persistent absence of microflora growth in the dialysis solution during repeated studies (although this could be a consequence of intensive antibacterial therapy) and the development of severe acute destructive peritonitis on the seventh-eighth day of hospitalization, confirmed by a twofold increase in blood amylase level, characteristic signs on CT and laparoscopy.

The cause of AP in our patient remained unknown. She had no classic causes of AP in the general population: alimentary factor, hepatobiliary diseases and pancreatic trauma. She also did not have metabolic disorders (hypercalcemia, secondary hyperparathyroidism, hypertriglyceridemia), which are common in CKD and predispose to the development of AP [3, 10, 12]. Probable risk factors were those associated with the PD method, including PD-associated peritonitis if it was primary. Another potential cause for the development of AP is drug therapy. Due to the duration and severity of the underlying disease, the patient was taking various medications, and she received intensive drug therapy for PD-associated peritonitis, while no high-risk drugs that were capable of initiating AP were prescribed [11]. The absence of a clearly identified single and specific etiologic factor for AP in our patient is a characteristic feature of the dialysis population. Some reports note that it is rare to identify risk factors for AP in dialysis patients; the proportion of such patients is small, from one third to one half of all cases [3, 12]. In the remaining patients, idiopathic AP is diagnosed; it occurs twice as often in PD patients as in the general population. The idiopathic nature of AP in dialysis patients implies the cumulative effect of individual pathogenic factors: risk factors found in the general population, risk factors associated with renal failure and its complications, and another risk factor – dialysis, especially PD, although its association with AP is still debated and not generally accepted [6–8].

In dialysis patients, conventional therapeutic tactics for AP with early administration of antibiotics are used to prevent acute necrotizing pancreatitis, and reduce mortality and infection rate [15]. Conservative treatment is often sufficient, but in this case, its full implementation proved ineffective. Surgical intervention is performed according to certain indications; in the observed patient, these were diagnostic difficulties (DUI signs of multichamber pelvic mass) and the need to interrupt the PD program in the first case and increased abdominal pain syndrome, increasing intensity of obstructive jaundice and systemic inflammatory response syndrome in the second case [10]. It should be noted that there are no clear guidelines regarding the modality of dialysis in PD patients with respect to AP – to continue this method or to transfer to HD. One of the indications for interruption of a PD program is insufficient (negative) ultrafiltration, which occurred in our patient. However, HD in AP is associated with a high incidence of hemorrhagic complications [6, 9].

Our patient had severe AP with the development of necrotizing pancreatitis and serious complications. The CT severity index developed by Balthazar was 8 points (6 zones of peripancreatic fluid accumulation, necrotizing pancreatitis), multi-organ dysfunction according to SOFA scale – 5–8 points [10]. At present, this situation is typical for both the general and the dialysis patient population; among the latter the mortality is several times higher and varies from 8% to 58% [7, 8, 12]. AP in PD patients is more severe than in HD patients, they require hospitalization more often and develop necrotizing pancreatitis more frequently [9]. In our patient, all known factors of poor prognosis of AP were present, which include: >50 years of age, presence of severe underlying disease, leukocytosis and increased activity of hepatic blood transaminases, APACHE II integral score >9 points. The most frequent cause of death in these patients is cardiovascular pathology, which was observed in this case.

CONCLUSION

Patients with CKD on PD are at high risk of developing AP, often due to the cumulative effect of various factors. The diagnosis of the disease may be delayed because of the similarity to PD-associated peritonitis and the lack of diagnostically significant elevation of blood amylase activity in some cases. A proactive approach to early diagnosis of AP in PD patients with abdominal pain syndrome and protracted PD-associated peritonitis, which includes investigation of serum lipase activity, amylase activity in dialysis solution and performance of abdominal CT scan is advisable. Treatment of severe

AP in PD patients presents certain difficulties. Early diagnosis of the disease and proper treatment promote a favorable outcome.

The authors declare no conflict of interest.

REFERENCES

1. *Andrusev AM, Peregudova NG, Shinkarev MB, Tomilina NA*. Kidney replacement therapy for end Stage Kidney disease in Russian Federation, 2016–2020. Russian National Kidney Replacement Therapy Registry Report of Russian Public Organization of Nephrologists "Russian Dialysis Society". *Nephrology and Dialysis*. 2022; 24 (4): 555–565. (In Russ, English abstract). doi: 10.28996/2618-9801-2022-4-555-565.
2. *Pitrone F, Pellegrino E, Mileto G, Consolo F*. May pancreatitis represent a CAPD complication? Report of two cases with a rapidly evolution to death. *Int J Artif Organs*. 1985; 8 (4): 235.
3. *Manga F, Lim C-S, Mangena L, Guest M*. Acute pancreatitis in peritoneal dialysis: a case report with literature review. *European Journal of Gastroenterology & Hepatology*. 2012; 24 (1): 95–101. doi: 10.1097/MEG.0b013e32834d4bcc.
4. *Hou SW, Lee YK, Hsu CY, Lee CC, Su YC*. Increased risk of acute pancreatitis in patients with chronic hemodialysis: a 4-year follow-up study. *PLoS One*. 2013; 8 (8): e71801.
5. *De Tersant M, Kwon T, Macher M-A, Maisin A, Deschênes G, Niel O*. Hypernatremia and acute pancreatitis in chronic kidney disease: back to the salt mines. Questions. *Pediatr Nephrol*. 2017. doi: 10.1007/s00467-017-3821-2.
6. *Lee YC, Hung SY, Wang HH, Wang HK, Lin CW, Chang MY et al*. Different risk of common gastrointestinal disease between groups undergoing hemodialysis or peritoneal dialysis or with non-end stage renal disease. A Nationwide population-based cohort study. *Medicine*. 2015; 94 (36): e1482. doi: 10.1097/MD.0000000000001482.
7. *Bruno MJ, van Westerloo DJ, van Dorp WT, Dekker W, Ferwerda J, Tytgat GN et al*. Acute pancreatitis in peritoneal dialysis and haemodialysis: risk, clinical course, outcome, and possible aetiology. *Gut*. 2000; 46 (3): 385–389. doi: 10.1136/gut.46.3.385.
8. *Lankisch PG, Weber-Dany B, Maisonneuve P, Lowenfels AB*. Frequency and severity of acute pancreatitis in chronic dialysis patients. *Nephrol Dial Transplant*. 2008; 23: 1401–1405. doi: 10.1093/ndt/gfm769.
9. *Chen HJ, Wang JJ, Tsay WI, Her SH, Lin CH, Chien CC*. Epidemiology and outcome of acute pancreatitis in end-stage renal disease dialysis patients: a 10-year national cohort study. *Nephrol Dial Transplant*. 2016; 1–6. doi: 10.1093/ndt/gfw400.
10. Ostryy pankreatit. Klinicheskie rekomendatsii [Acute Pancreatitis. Clinical Guidelines]. 2020. 66 p. http://obshhestvo-xirurgov.rf/stranica pravlenija/unkr/urgentnajaabdominalnajahirurgija/nacionalnye-klinicheskie-rekomendaci-po-ostromu-pankreatitu.html. (In Russian).
11. *Ostroumova OD, Akimova ES, Kochetkov AI*. Drug-induced acute pancreatitis: pathogenetic mechanisms. *Siberian Medical Review*. 2021; 3: 35–43. doi: 10.20333/25000136-2021-3-35-43.
12. *Vidal E, Alberici I, Verrina E*. Italian Registry of Pediatric Chronic Dialysis. Acute pancreatitis in children on chronic maintenance dialysis. *Pediatr Nephrol*. 2019; 34 (9): 1501–1512. doi: 10.1007/s00467-018-4043-y.
13. *Revishvili ASh, Sazhin VP, Olovyannyj VE, Zakharova MA*. Current trends in emergency abdominal surgery in the Russian Federation. *Pirogov Russian Journal of Surgery = Khirurgiya. Zurnal im. N.I. Pirogova*. 2020; 7: 6–11. (in Russ. English abstract). doi: 10.17116/hirurgia20200716.
14. *Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG et al*. Acute Pancreatitis Classification Working Group Classification of acute pancreatitis – 2012: revision of the Atlanta classification and definitions by international consensus. *Gut*. 2013; 62 (1): 102–111.
15. *Ukai T, Shikata S, Inoue M, Noguchi Y, Igarashi H, Isaji S et al*. Early prophylactic antibiotics administration for acute necrotizing pancreatitis: a meta-analysis of randomized controlled trials. *J Hepatobiliary Pancreat Sci*. 2015; 22: 316–321. doi: 10.1002/jhbp.221.

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NORMOTHERMIC EX VIVO HEART AND LUNG AUTOPERFUSION: ASSESSMENT OF FUNCTIONAL STATUS AND METABOLISM

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Objective: to carry out a comparative study of the efficacy of a 6-hour normothermic *ex vivo* heart and lung autoperfusion and cold cardioplegia using Bretschneider's solution (Custodiol®, Germany). **Materials and methods.** Landrace pigs weighing 50 ± 5 kg at the age of 4–5 months (n = 10) were used as a model for a series of acute experiments. In the experimental group (n = 5), the cardiopulmonary complex was conditioned by autoperfusion for 6 hours. In the control group, the heart pumping function was restored after 6-hour cold cardioplegia using Bretschneider's solution. The efficiency of graft preservation was assessed by measuring hemodynamic parameters, myocardial contractile function, and myocardial oxygen consumption. **Results.** After reperfusion and repeated isolation of the working cardiopulmonary complex, cardiac output was 0.63 [0.37; 0.8] L/min and 0.37 [0.23; 0.37] L/min in the experimental and control groups, respectively ($p < 0.05$). Indicators – global left ventricular stroke work index and preload recruitable stroke work – were significantly higher in the experimental group ($p < 0.05$). **Conclusion.** Normothermic autoperfusion is significantly more effective in preserving the morphofunctional status of a donor heart than static cold storage with Bretschneider solution for 6 hours.

Keywords: cold cardioplegia, cardiac transplantation, heart preservation, autoperfusion, normothermic perfusion, ischemia-reperfusion injury.

INTRODUCTION

Ischemia-reperfusion injury (IRI) is the main adverse outcome of the restoration of blood flow in a donor heart. IRI is also the main cause of early graft dysfunction. In most cases, donor heart harvesting begins with termination of blood supply and in situ washing with a cold preservative solution, followed by explantation and storage on ice [1]. Meanwhile, despite several negative consequences, this cold cardioplegia method remains the gold standard for donor organ preservation. Cooling of the organ to 4 °C slows down cellular metabolism, thereby reducing oxygen demand, but anaerobic metabolism and other cellular metabolic processes continue at lower temperatures [2–4].

Even though the IRI cascade is activated in all donor grafts, its reversibility as well as the limits of plasticity of compensatory mechanisms of endothelial autoregulation, are mainly determined by graft ischemic time. Prolonged ischemia is especially dangerous for cardiac grafts because of extreme sensitivity of the myocardium to hypoxia [5]. Using the cold cardioplegia preservation method, heart grafts can be safely preserved for 4–6 hours; further extension of the ischemic time leads to higher risk of early graft dysfunction [1, 6]. Machine perfusion technologies allow avoiding complications characteristic of static cold preservation methods. However, the search for the optimal scheme and mode of ex vivo coronary perfusion of the heart graft remains a subject of research. Despite the proven safety of ex vivo perfusion of donor heart at the stage of transportation, the problem of assessing graft contractility remains unresolved. The device used in clinical practice – OCS™ Heart Transmedics® system (Andover, MA, USA) – is based on the principle described by Oskar Langendorff in the late 1800s [7]. In this system, oxygenated perfusate is injected retrogradely into the aortic root, forcing the aortic valve to close, while venous blood, flowing from the coronary sinus, is drained actively, or due to the right ventricle's ejection into the reservoir [8, 9].

Coronary perfusion according to Langendorff is an effective way to meet the metabolic needs of the myocardium. However, since the left ventricle remains unloaded in this perfusion scheme, it is difficult to assess the pumping function of the heart and, therefore, to predict the functional outcome of transplantation. Described in 1926 by Ernest Starling and Maurice Visscher, experiments with hemodynamic isolation of the cardiopulmonary complex became widely known due to the pattern discovered by the authors describing the relationship

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between diastolic heart volume and the strength of heart contractions [10, 11]. At the same time, the use of the technique of isolating the autoperfused cardiopulmonary complex can provide not only a long and effective existence of the graft ex situ, but also a dynamic assessment of contractility using ultrasound diagnostic methods [12].

The inability to effectively assess the quality of a donor organ often leads to errors in predicting the consequences of its use, and the "better safe than sorry" idea leads to a significant number of organ rejections despite the fact that they may have been suitable for transplantation [13]. In addition to the evaluation of laboratory parameters of donor organ functional quality, normothermic autoperfusion will allow for more sophisticated diagnostic procedures such as ex vivo echocardiography or coronary angiography [14]. The technique of functional echocardiographic evaluation of the donor heart in experiment was described in detail in a recently published paper by Ruggeri et al. This study derived comparable results of ex vivo evaluation of the heart under the condition of volume loading with those of standard transesophageal or transthoracic cardiac echocardiography [15].

Thus, the possibility of replacing the cold asystole period with normothermic autoperfusion has the potential of eliminating the negative consequences of prolonged ischemia. This may allow long-distance delivery of donor organs, increase access to transplantation in remote regions, and ensure the selection of an optimal donor and recipient regardless of their geographic distance [16].

Objective: to carry out a comparative assessment of the functional and metabolic status of the cardiac graft after a 6-hour normothermic autoperfusion and cold cardioplegia using Bretschneider's solution (Custodiol®, Germany).

MATERIALS AND METHODS

Preparing animals for the experiment

Female Landrace pigs, weighing 50 ± 5 kg at the age of 4–5 months (n = 10) were used as a model for a series of experiments. Care, provision of the experiment, observation and withdrawal of animals from it were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 03/18/1986). In the experimental group (n = 5), cardiac conditioning was performed under 6-hour normothermic autoperfusion of the cardiopulmonary complex ex vivo, followed by cold cardioplegia using Bretschneider's solution at 4 °C for 1 hour, followed by reperfusion with a cardiopulmonary bypass machine. As a control group (n = 5), there were hearts preserved for 6 hours according to the clinically accepted protocol of cold cardioplegia using Bretschneider's solution.

On the day of the experiment, all animals were premedicated (zoletil-100) on an empty stomach. The dose was selected individually, according to weight and height parameters. After the onset of sleep, the surgical field and the area of catheterization of neck vessels were prepared. Then the animal was transported to the operating table and fixed in a supine position for subsequent tracheal intubation, installation of central arterial and venous catheters. The experiment was performed under endotracheal anesthesia with sevoflurane and muscle relaxation (rocuronium bromide). Mechanical ventilation was performed using FabiusPlus anesthesia-breathing apparatus (Draeger, Germany) with positive pressure on inhalation (20–30 cm water column) and exhalation (5–8 cm water column) with a respiratory volume of 8 ml/kg at a rate of 12–14 breaths per minute. Vital parameters were recorded using an IntelliVue MP70 monitor (Philips, Netherlands).

During the experiments, invasive blood pressure in the heart cavities and main vessels, cardiac arrhythmias (electrocardiography), and temperature of the organ complex were monitored. Blood analysis was performed using an automatic hematological analyzer XT-4000i (Sysmex, Germany) according to the manufacturer's guidelines. Central hemodynamic parameters were investigated by right heart catheterization with a Swan–Ganz catheter, as well as with the help of a portable multifunctional ultrasound system Philips CX50 (Philips Ultrasound, USA) with ECG synchronization using an S5-1 sector array probe. The ex vivo position of the probe was along the long axis of the left ventricle and in the apical four-chamber position. Left ventricular (LV) diastolic function was assessed by calculating the rate of change of LV pressure during isovolumic relaxation (–dP/dt). In the absence of mitral regurgitation, –dP/dt was calculated by an alternative non-invasive assessment method using the formula:

$$-dP/dt = (DBP - LVEDP) = IVRT$$

where DBP is diastolic blood pressure, LVEDP is left ventricular end-diastolic pressure.

LVEDP was estimated based on the ratio of mitral inflow peak velocity E/A: LVEDP = 10 mmHg if E/A 1.6 and 20 mmHg if E/A >1.6. IVRT is isovolumic relaxation time, which is calculated by subtracting T1 (time from the onset of QRS to the end of blood flow in the LV outflow tract) from T2 (time from the onset of QRS to the beginning of flow through the mitral valve) according to the methodology described in detail in the work by Parekh et al. [17]. Cardiac function was evaluated by calculating cardiac output (CO); global left ventricular stroke work index (LVSWI) by the formula SW = SV × (ESP – EDP), where SV is stroke volume, ESP is end-systolic pressure, and EDP is end-diastolic pressure; preload recruitable stroke work (PRSW) as a ratio of LVSWI and EDP.

To assess the efficiency of respiratory function of autologous lungs, the gas composition of blood samples taken from the left atrium was monitored using a Radiometer ABL800 FLEX analyzer (Denmark). Myocardial oxygen consumption was calculated by the formula:

$$\text{LV } O_2 \text{ cons} = \frac{([O_2]a) - ([O_2]cs) \times CAF}{\text{LV mass}},$$

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

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

Blood oxygen content was calculated by the formula:

$$O_2 =$$

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

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

Coronary vascular resistance (CVR) was calculated by the formula:

$$\text{CVR} = \frac{\left(\text{iARP(m)} - \text{iRAP(m)}\right)}{\text{CBF} \times 100 \text{ g}}$$

where iARP(m) is the mean invasive aortic root pressure, iRAP(m) is the mean invasive right atrial pressure, CBF is coronary blood flow, mL/min).

Surgical technique of the experiment

Explantation of the working cardiopulmonary complex (CPC) was performed through a midline sternotomy. Isolation of the CPC began with the removal of the pericardium and mobilization of the superior vena cava (SVC), then the brachiocephalic trunk (BCS), left subclavian artery (LSA), and inferior vena cava (IVC) were isolated. The trachea was carefully separated from the esophagus using an electrocoagulator, achieving hemostasis. After administration of heparin (3 mg/kg body weight), the LSA was ligated as distally as possible, and an introducer was placed through the arterial stump to measure the invasive blood pressure (iBP) in the aortic root and to guide diagnostic catheters. Then the BCS was ligated and crossed, and an 18 Fr arterial cannula was inserted into the arterial stump, which was connected to the arterial reservoir fixed at a height of 1 meter above the heart level. After clamping the descending thoracic aorta at the level of the isthmus, the arterial trunk was opened, and arterial blood was drawn into the reservoir. After stabilization of blood level and arterial pressure, 1–1.5 liters of Ringer's solution was injected into the femoral vein. After that, the vena cava was ligated and crossed, the trachea was crossed and reintubated with a cuffed tube. The functioning CPC was finally separated from the surrounding tissues, transferred to a container with warm saline solution (38 °C), the arterial trunk was clamped, and observation was continued for 6 hours. Throughout autoperfusion, a continuous infusion of 5% calcium chloride solution (3–5 ml/hour) and 10% glucose (5–10 ml/hour) was performed to maintain blood levels in the reference range.

After 6 hours of normothermic autoperfusion of CPC, cold cardioplegia was performed by injecting 2 liters of Bretschneider's solution (Custodiol®, Germany, HTK) into the aortic root. The CPC was then stored in Bretschneider's solution at 4 °C for 1 hour. After this time, the heart was perfused for 15–20 minutes using a heart-lung machine filled with the animal's own blood. If necessary, electrical defibrillation was performed. After warming and recovery of cardiac activity, the CPC was filled with blood, isolated and cardiac ultrasound was performed.

Tissue samples for histological examination were excised from the apical part of the left ventricle of the heart and the middle lobes of the left and right lungs, fixed in 10% neutral formalin, after fixation, dehydrated in alcohols of increasing strength and embedded in paraffin using a dispenser with heating and cooling plates. Histological sections, 4–5 μm thick, were prepared from paraffin blocks on a Microm HM 550 microtome. Before staining, the sections were deparaffinized for 10–15 minutes in two portions of pure xylene, followed by its removal in three portions of alcohol of decreasing strength (absolute 70°) to distilled water. Histological sections were stained according to standard methods: hematoxylin and eosin, Van Gieson with combined dyeing of elastic fibers with orsein, and PAS reaction was performed. Polarization microscopic examination of the myocardium was performed using an Axio Scope. A1 microscope (Zeiss, Germany) equipped with an analyzer and polarizer, AxioCam HRm and AxioCam HRc cameras (Zeiss, Germany) and ZEN blue software (Zeiss, Germany).

Statistical processing was performed using Statistica 10.0 software (StatSoft Inc., USA). The normality of distribution was checked using the Shapiro–Wilk test with subsequent assessment of equality of variance by Levene's test. The significance of differences between the comparison groups and the groups (p) for continuous data was calculated using the Mann–Whitney U nonparametric test in independent groups and Wilcoxon test in dependent groups. The level of significance between the comparison groups and the groups was considered reliable at $p < 0.05$, which corresponds to the criteria accepted in biomedical research.

RESULTS

In all experiments, 0.3–0.5 ml of 0.01% epinephrine solution was administered to compensate for adrenergic stimulation at the reperfusion stage. However, despite this, the hearts of the control group were not able to maintain aortic root blood pressure at the 60-mmHg level, and in most experiments, after 5–10 minutes of

independent functioning of the CPC, bradycardia and pronounced dilation of heart chambers were observed, requiring urgent drainage of chambers and reperfusion using a heart-lung machine.

Moreover, in the experimental group the reperfusion time required to wean the CPC from artificial circulation with the ability to independently maintain blood pressure in the aortic root at a level not lower than 60 mmHg was 87 [67; 102] minutes and 19 [17.5; 22.5] minutes ($p < 0.05$) in the control and experimental groups, respectively. At the same time, in all experiments of the control group, restoration of heart rhythm required multiple electrical defibrillation (up to 10 shocks) followed by electrical cardiac stimulation. The main hemodynamic parameters are presented in Table 1.

Cardiac pumping function was evaluated at baseline and within an hour after the end of reperfusion and repeated hemodynamic isolation of the CPC. The main parameters of cardiac ultrasound examination are summarized in Table 2.

Diastolic function was assessed at three points: 1) baseline, 2) immediately after reperfusion and restoration of autoperfusion, and 3) one hour after weaning from artificial circulation. When estimating the rate of change in LV pressure during isovolumic relaxation (–dP/dt), a significant decrease in –dP/dt was observed in the control group, which indicates a deterioration in the diastolic function of the heart, while in the experimental group this index changed insignificantly over time (Fig. 1).

At histological examination in myocardial samples taken after reperfusion in the control group, the phenomena of karyorrhexis and disappearance of nuclei were observed in cardiomyocytes. Despite the presence of free lumen of blood vessels, moderate perivascular edema was observed around them, spreading to the interstitium in which single leukocytes were found (Fig. 2).

In the experimental group, in contrast to the control group, the severity of intracellular and intercellular edema was significantly less, and the phenomena of leukodiapedesis in the interstitium were more pronounced. Preservation of transverse striation of cardiomyocytes with zones of increased anisotropy and subsegmental contractures was observed (Fig. 3).

DISCUSSION

Normothermic ex vivo perfusion is a promising method of donor heart conditioning. It can shorten cold ischemia period significantly, and allows for a wide screening of metabolic and functional parameters [9, 18, 19]. Moreover, the use of ex situ cardiac perfusion techniques has been proven to restore the function of the organ of borderline status and thereby increase the number and quality of grafts available for transplantation [20]. However, despite several studies, the choice of the optimal mode, as well as the sensitivity of diagnostic

Table 1

Basic hemodynamic parameters

Parameter \ Group	Control (n = 5)		Experimental (n = 5)	
	Before conservation	After conservation	Before conservation	After conservation
HR (bpm)	91.2 ± 14.8	100 (pacemaker)	93.3 ± 11.7	100 (pacemaker)
$iRAP_{(m)}$ (mmHg)	4.0 ± 1.4	11.4 ± 1.9*	7.4 ± 1.5	7.0 ± 2.3#
$iLAP_{(m)}$ (mmHg)	4.0 ± 1.9	10.3 ± 1.6*	3.4 ± 1.8	9.4 ± 1.6*
$iAP_{(m)}$ (mmHg)	70.6 ± 12.5	63.0 ± 15.8	67.2 ± 9.7	63 ± 7.5
$iPAP_{(m)}$ (mmHg)	15.5 ± 3.2	16.4 ± 3.7	15.3 ± 4.2	13.3 ± 3.3
CVR (mmHg)* min/mL/100 g	8.03 ± 1.5	13.9 ± 4.3*	7.1 ± 1.3	8.8 ± 1.1*#

Note. Data are presented as M ± SD; *, $p < 0.05$ compared with baseline values; #, $p < 0.05$ compared with control group; HR, heart rate; $iRAP_{(m)}$, mean invasive right atrial pressure; $iLAP_{(m)}$, mean invasive left atrial pressure; $iAP_{(m)}$, mean invasive aortic pressure; $iPAP_{(m)}$, mean invasive pulmonary artery pressure; CVR, coronary vascular resistance.

Table 2

Basic parameters of myocardial contractile function

Parameter \ Group	Control (n = 5)		Experimental (n = 5)	
	Before conservation	After conservation	Before conservation	After conservation
CO, l/min	0.83 [0.74; 1.86]	0.37* [0.23; 0.37]	0.84 [0.78; 0.94]	0.63# [0.37; 0.8]
LVSWI, ml·mmHg	26.09 [5.25; 48.25]	12.67* [1.9; 27.4]	33.89 [21.4; 47.9]	21.06# [15.28; 27.25]
PRSW, ml	0.63 [0.06; 1.2]	0.25* [0.03; 0.57]	0.65 [0.42; 0.88]	0.57# [0.34; 0.76]

Note. Data are presented as Me [Q1; Q3]. *, $p < 0.05$ compared with baseline values; #, $p < 0.05$ compared with the control group.

markers in predicting the functional outcome of transplantation is still a matter of debate.

Autoregulation of coronary blood flow is one of the most important properties, its preservation largely determines the compensatory plasticity of the graft in the resolution of IRI. In its turn, the preservation of this coronary bed property is largely determined by the coronary flow characteristics. The TransMedics Organ Care System (OCS), used today successfully, provides coronary flow in the range from 650 to 900 mL/min, with perfusion pressure from 60 to 80 mmHg [21, 22]. However, Hatami et al., in their studies, concluded that myocardial energy reserves can be preserved at aortic perfusion pressures as low as 40 mmHg [23]. According to Repse et al., vasomotor regulation of the coronary channel changes significantly with increasing duration of machine normothermic perfusion, which leads to excess coronary blood flow over time [24]. Controlled coronary perfusion under low perfusion pressure can potentially limit myocardial and endothelial injury [25]. In this regard, normothermic autoperfusion of the graft as a method of

Table 3

Myocardial oxygen consumption ($mL\text{-}O_2/min/100\ g$)

Group	Before conservation	After conservation
Control (n = 5)	12.44 [7.9; 18.5]	8.52 [4.25; 12.65]
Experimental (n = 5)	15.44 [8.7; 22.4]	117*# [106.5; 131]

Note. Data are presented as Me [Q1; Q3]; *, $p < 0.05$ compared to baseline values; #, $p < 0.05$ compared to the control group.

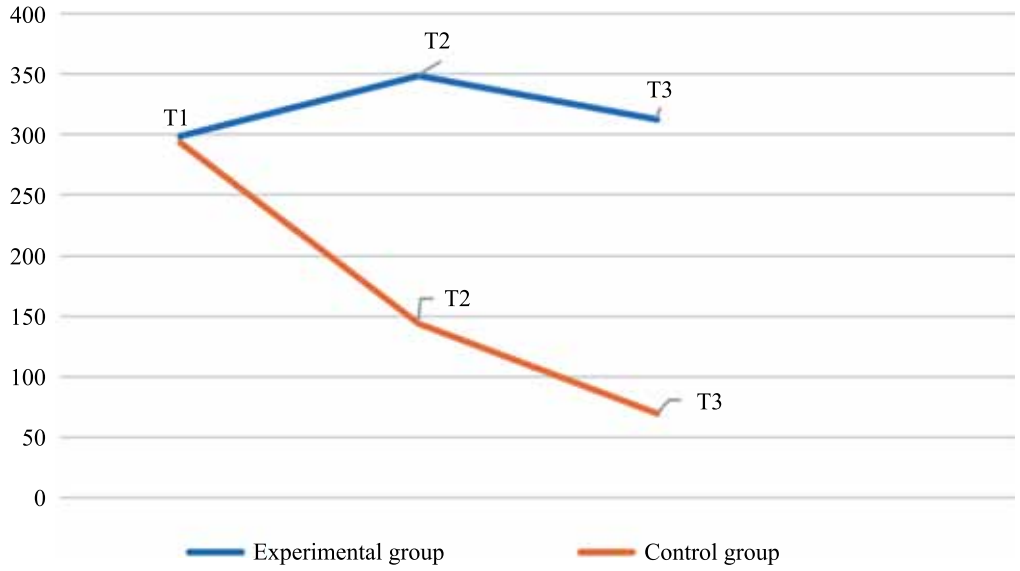

Fig. 1. Dynamics of –dP/dt changes during the experiment. T1, baseline; T2, immediately after reperfusion and restoration of autoperfusion; T3, one hour after weaning from cardiopulmonary bypass

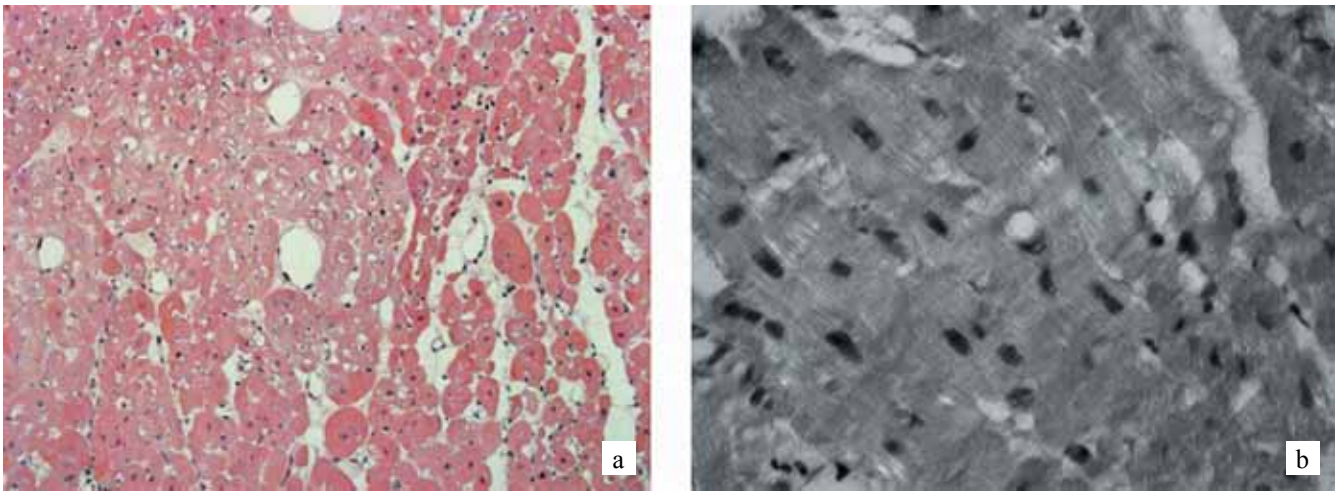

Fig. 2. Left ventricular myocardium after reperfusion, control group: a, H&E stain, magnification 400×; b, polarized light microscopy, magnification 630×

prolonged conditioning is of great interest, since in this case, hemodynamic parameters of the functioning of the complex are determined by the pumping function of the graft itself, taking into account its own metabolic needs.

The results of the conducted study prove that it is possible to provide effective coronary blood flow in an autoperfused cardiopulmonary complex. Thus, the previously established ability of the coronary arteries to vasodilate in response to increased myocardial oxygen consumption was confirmed in a series of experiments [26]. According to data obtained by Duncker et al., coronary resistance in the control group after reperfusion was statistically significantly higher than in the autoperfusion group. This fact indicates the preservation of regional vasomotor autoregulation in order to maintain adequate but not excessive oxygen supply to the myocardium.

The applied scheme of complete anatomical isolation of the autoperfused cardiopulmonary complex made it possible to create the necessary conditions for the effective functioning of the graft ex situ, as well as for the assessment of function and metabolism. However, along with the obvious advantages of autoperfusion technology over static cold preservation, the issue of preserving the structure and gas exchange function of autologous lungs remains open. Leukocyte sequestration observed during autoperfusion was described in earlier studies [27, 28]. However, this phenomenon was observed even when leukocyte-depleted perfusate was used. Taking into account the fact that the degree and rate of leukocyte sequestration in the lungs is inversely proportional to the rate of pulmonary blood flow, complete modeling of the initial cardiac output is the main condition for successful and long-term functioning of autologous lungs. Preservation of the initial hemodynamic parameters is important for predicting the functional outcome of transplantation and to evaluate the transplant from the standpoint of possibility to effectively provide cardiac output in the recipient's body.

Despite a number of successful trials of OCS, the validity of using lactate profiles as assessment markers has been questioned by multiple groups of researchers [29–31]. Also, it has been suggested that left ventricular contractility parameters can more accurately predict graft behavior after transplantation in contrast to metabolic markers, including lactate trend [32, 33].

According to Gellner et al., the working mode of perfusion with passive afterload, allows a more detailed prediction of post-transplant cardiac function [34]. In their works, White et al. and Xin et al. tried to load the left and right atria, facilitating ventricular ejection during active aortic root perfusion. In a series of experiments, the authors used perfusate injection both antegradely into the left atrium and retrogradely into the aorta [32, 35]. In this case, in systole, the left ventricle overcame aortic counterpressure, ejecting perfusate into the reservoir connected to the brachycephalic arteries, and in diastole, aortic counterpressure facilitated coronary perfusion. Also, a passive afterload perfusion regimen has been proposed as an alternative [28]. Instead of using retrograde flow to maintain coronary perfusion during diastole, an afterload module based on the Windkessel principle was used [36]. However, the only perfusion platform currently available for clinical use assumes "idle" operation of the left ventricle.

In our opinion, the use of a preserved thoracic aortic fragment as a Windkessel receiver is extremely insufficient and, in case of volume overload of the CPC, threatens serious endothelial damage. The intrinsic elastic properties of the aortic wall do not allow effective damping of cardiac output exceeding 1000–1500 mL/min preventing coronary hyperperfusion, while ensuring physiological blood pressure profile.

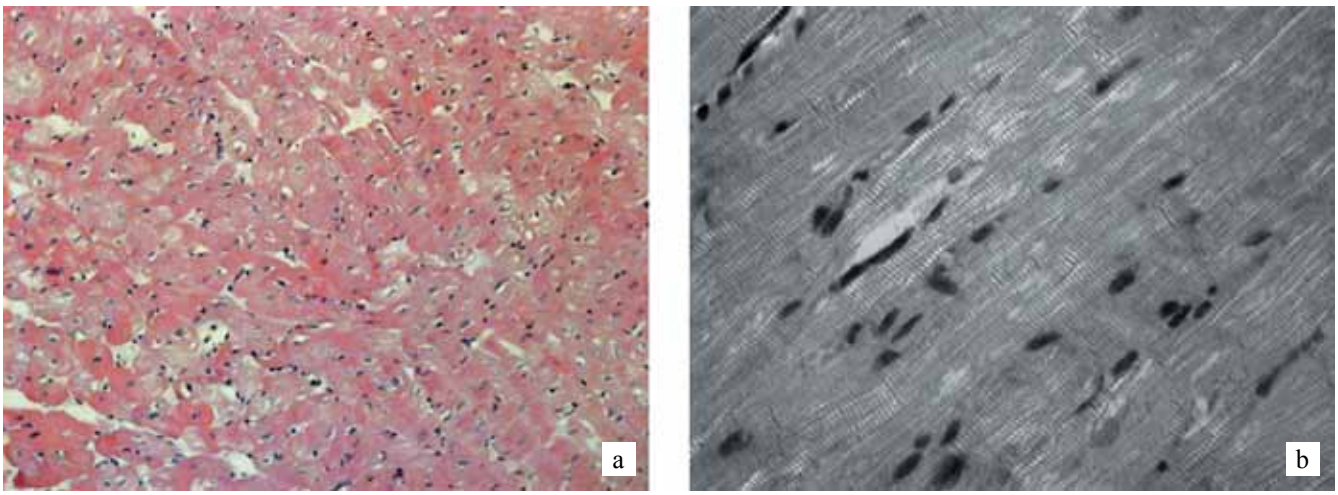

Fig. 3. Left ventricular myocardium after reperfusion, experimental group: a, H&E stain, magnification 400×; b, polarized light microscopy, magnification 630×

CONCLUSION

This study demonstrated significant advantages of normothermic autoperfusion of the CPC over static cold cardioplegia. However, pathomorphological changes caused by reduced blood flow in pulmonary circulation and in heart cavities, leading to leukocyte sequestration and pulmonary edema, require modification of the circulation circuit with inclusion of an effective cardiac output receiver and a pathway of blood return to the right heart.

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The authors declare no conflict of interest.

REFERENCES

1. *Guibert EE, Petrenko AY, Balaban CL, Somov AY, Rodriguez JV, Fuller BJ.* Organ Preservation: Current Concepts and New Strategies for the Next Decade. *Transfus Med Hemother*. 2011; 38 (2): 125–142. doi: 10.1159/000327033. Epub 2011 Mar 21. PMID: 21566713; PMCID: PMC3088735.
2. *Van Raemdonck D, Rega F, Rex S, Neyrinck A.* Machine perfusion of thoracic organs. *J Thorac Dis*. 2018 Apr; 10 (Suppl 8): S910–S923. doi: 10.21037/jtd.2018.02.85.
3. *Verhoven BM, Karim AS, Bath NM, Sarabia Fahl CJ, Wilson NA, Redfield RR 3rd et al.* Significant Improvement in Rat Kidney Cold Storage Using UW Organ Preservation Solution Supplemented With the Immediate-Acting PrC-210 Free Radical Scavenger. *Transplant Direct*. 2020 Jul 15; 6 (8): e578. doi: 10.1097/TXD.0000000000001032. PMID: 33134502; PMCID: PMC7581037.
4. *Natalini JG, Diamond JM.* Primary Graft Dysfunction. *Semin Respir Crit Care Med*. 2021 Jun; 42 (3): 368–379. doi: 10.1055/s-0041-1728794. Epub 2021 May 24. PMID: 34030200.
5. *Dhital KK, Chew HC, Macdonald PS.* Donation after circulatory death heart transplantation. *Curr Opin Organ Transplant*. 2017 Jun; 22 (3): 189–197. doi: 10.1097/MOT.0000000000000419. PMID: 28379853.
6. *Dani A, Vu Q, Thangappan K, Huang B, Wittekind S, Lorts A et al.* Effect of ischemic time on pediatric heart transplantation outcomes: is it the same for all allografts? *Pediatr Transplant*. 2022 Jun; 26 (4): e14259. doi: 10.1111/petr.14259. Epub 2022 Mar 8. PMID: 35258159; PMCID: PMC9159355.
7. *Skrzypiec-Spring M, Grotthus B, Szelag A, Schulz R.* Isolated heart perfusion according to Langendorff – still viable in the new millennium. *J Pharmacol Toxicol Methods*. 2007 Mar-Apr; 55 (2): 113–126. doi: 10.1016/j.vascn.2006.05.006. Epub 2006 May 26. PMID: 16844390.
8. *Schechter MA, Southerland KW, Feger BJ, Linder D Jr, Ali AA, Njoroge L et al.* An isolated working heart system for large animal models. *J Vis Exp*. 2014 Jun 11; (88): 51671. doi: 10.3791/51671. PMID: 24962492; PMCID: PMC4189428.
9. *White CW, Ali A, Hasanally D, Xiang B, Li Y, Mundt P et al.* A cardioprotective preservation strategy employing *ex vivo* heart perfusion facilitates successful transplant of donor hearts after cardiocirculatory death. *J Heart Lung Transplant*. 2013 Jul; 32 (7): 734–743. doi: 10.1016/j.healun.2013.04.016. PMID: 23796155.
10. *Starling EH, Visscher MB.* The regulation of the energy output of the heart. *J Physiol*. 1927 Jan 12; 62 (3): 243–261. doi: 10.1113/jphysiol.1927.sp002355. PMID: 16993846; PMCID: PMC1514842.
11. *Huisman PH, Schipperheyn JJ.* The isolated heart-lung preparation. The Hague: Martinus Nijhoff Medical Division, 1978: 36–36.
12. *Zhulkov MO, Zykov IS, Makaev AG, Protopopov AV, Murtazaliev MN, Kosimov FYu et al.* Surgical technique for explantation of a functioning cardiopulmonary complex in an experiment. *Russian Journal of Transplantology and Artificial Organs*. 2023; 25 (3): 122–128. [In Russ.]. https://doi.org/10.15825/1995-1191-2023-3-122-128.
13. *Zhulkov MO, Fomichev AV, Alsov SA, Cleaver EN, Chernyavsky AM.* Current state of the problem and results of *ex vivo* perfusion of donor hearts. *Russian Journal of Transplantology and Artificial Organs*. 2019; 21 (4): 143–146. [In Russ, English abstract]. https://doi.org/10.15825/1995-1191-2019-4-143-146.
14. *Koerner MM, Ghodsizad A, Schulz U, El Banayosy A, Koerfer R, Tenderich G.* Normothermic *ex vivo* allograft blood perfusion in clinical heart transplantation. *Heart Surg Forum*. 2014 Jun; 17 (3): E141–E145. doi: 10.1532/HSF98.2014332. PMID: 25002389.
15. *Ruggeri GM, Qua Hiansen J, Pivetta E, Mashari A, Ballocca F, Pinto Ribeiro RV et al.* Description of a Novel Set-up for Functional Echocardiographic Assessment of Left Ventricular Performance During *Ex Vivo* Heart Perfusion. *Anesth Analg*. 2018 Sep; 127 (3): e36–e39. doi: 10.1213/ANE.0000000000002866. PMID: 29505446.
16. *Zhulkov MO, Zykov IS, Sirota DA, Agaeva HA, Sabetov AK, Poveschenko OV et al.* Long-Term Conditioning of a Donor Heart by Autoperfusion. *Journal of experimental and clinical surgery*. 2022; 15 (3): 214–220. doi: 10.18499/2070-478X-2022-15-3-214-220 [In Russ].
17. *Parekh R, Kolias TJ.* A novel noninvasive method to assess left ventricular –dP/dt using diastolic blood pressure and isovolumic relaxation time. *Echocardiography*. 2013 Mar; 30 (3): 267–270. doi: 10.1111/echo.12042. Epub 2012 Nov 8. PMID: 23134266.
18. *Ardehali A, Esmailian F, Deng M, Soltesz E, Hsich E, Naka Y et al.* PROCEED II trial investigators. Ex-vivo perfusion of donor hearts for human heart transplantation (PROCEED II): a prospective, open-label, multicentre, randomised non-inferiority trial. *Lancet*. 2015 Jun 27; 385 (9987): 2577–2584. doi: 10.1016/S0140-6736(15)60261-6. Epub 2015 Apr 14. PMID: 25888086.
19. *Zhulkov MO, Zaynobudinov ShSh, Sirota DA, Protopopov AV, Zykov IS, Makaev AG et al.* Evaluation of the coronary status of an autoperfused donor heart in the experiment. *Clinical and Experimental Surgery.*

Petrovsky Journal. 2023; 11 (3): 00–00. https://doi.org/10.33029/2308-1198-2023-11-3-00-00. [In Russ.].
20. *Alomari M, Garg P, Yazji JH, Wadiwala IJ, Alamouti-Fard E, Hussain MWA et al.* Is the Organ Care System (OCS) Still the First Choice With Emerging New Strategies for Donation After Circulatory Death (DCD) in Heart Transplant? *Cureus*. 2022 Jun 24; 14 (6): e26281. doi: 10.7759/cureus.26281. PMID: 35754437; PMCID: PMC9229932.
21. *Dhital KK, Iyer A, Connellan M, Chew HC, Gao L, Doyle A et al.* Adult heart transplantation with distant procurement and ex-vivo preservation of donor hearts after circulatory death: a case series. *Lancet*. 2015 Jun 27; 385 (9987): 2585–2591. doi: 10.1016/S0140-6736(15)60038-1. Epub 2015 Apr 14. PMID: 25888085.
22. *Macdonald PS, Chew HC, Connellan M, Dhital K*. Extracorporeal heart perfusion before heart transplantation: the heart in a box. *Curr Opin Organ Transplant*. 2016 Jun; 21 (3): 336–342. doi: 10.1097/MOT.0000000000000309. PMID: 26967996.
23. *Hatami S, White CW, Shan S, Haromy A, Qi X, Ondrus M et al.* Myocardial Functional Decline During Prolonged *Ex Situ* Heart Perfusion. *Ann Thorac Surg*. 2019 Aug; 108 (2): 499–507. doi: 10.1016/j.athoracsur.2019.01.076. Epub 2019 Mar 12. PMID: 30872100.
24. *Repse S, Pepe S, Anderson J, McLean C, Rosenfeldt FL*. Cardiac reanimation for donor heart transplantation after cardiocirculatory death. *J Heart Lung Transplant*. 2010 Jul; 29 (7): 747–755. doi: 10.1016/j.healun.2010.02.009. Epub 2010 Apr 22. PMID: 20417128.
25. *Qi X, Hatami S, Bozso S, Buchko M, Forgie KA, Olafson C et al.* The evaluation of constant coronary artery flow versus constant coronary perfusion pressure during normothermic *ex situ* heart perfusion. *J Heart Lung Transplant*. 2022 Dec; 41 (12): 1738–1750. doi: 10.1016/j.healun.2022.08.009. Epub 2022 Aug 20. PMID: 36137869.
26. *Duncker DJ, Bache RJ*. Regulation of coronary blood flow during exercise. *Physiol Rev*. 2008 Jul; 88 (3): 1009–1086. doi: 10.1152/physrev.00045.2006. PMID: 18626066.
27. *Genco CM, Bernstein EA, Connolly RJ, Peterson MB, Zhang X, Somerville K et al.* Leukocyte redistribution and eicosanoid changes during the autoperfused working heart-lung preparation. *J Invest Surg*. 1991; 4 (4): 477–485. doi: 10.3109/08941939109141178. PMID: 1777442.
28. *Martin BA, Wright JL, Thommasen H, Hogg JC.* Effect of pulmonary blood flow on the exchange between the circulating and marginating pool of polymorphonuclear leukocytes in dog lungs. *J Clin Invest*. 1982 Jun; 69 (6): 1277–1285. doi: 10.1172/jci110567. PMID: 7085874; PMCID: PMC370200.
29. *Freed DH, White CW.* Donor heart preservation: straight up, or on the rocks? *Lancet*. 2015 Jun 27; 385 (9987): 2552–2554. doi: 10.1016/S0140-6736(15)60614-6. Epub 2015 Apr 14. PMID: 25888084.
30. *Hatami S, White CW, Ondrus M, Qi X, Buchko M, Himmat S et al.* Normothermic *Ex Situ* Heart Perfusion in Working Mode: Assessment of Cardiac Function and Metabolism. *J Vis Exp*. 2019 Jan 12; (143). doi: 10.3791/58430. PMID: 30688296.
31. *Page A, Messer S, Axell R, Naruka V, Colah S, Fakelman S et al*. Does the assessment of DCD donor hearts on the organ care system using lactate need redefining? *The Journal of Heart and Lung Transplantation*. 2017; 36 (4): S16–S17.
32. *White CW, Ambrose E, Müller A, Li Y, Le H, Hiebert B et al.* Assessment of donor heart viability during *ex vivo* heart perfusion. *Can J Physiol Pharmacol*. 2015 Oct; 93 (10): 893–901. doi: 10.1139/cjpp-2014-0474. Epub 2015 May 7. PMID: 26317524.
33. *Ribeiro R, Alvarez J, Xin L, Gellner B, Paradiso E, Yu F et al*. Contractility versus metabolic cardiac assessment during *ex situ* hear perfusion: a pre-clinical transplant study. *Canadian Journal of Cardiology*. 2019; 35 (10): S171.
34. *Gellner B, Xin L, Pinto Ribeiro RV, Bissoondath V, Adamson MB, Yu F et al.* The implementation of physiological afterload during *ex situ* heart perfusion augments prediction of posttransplant function. *Am J Physiol Heart Circ Physiol*. 2020 Jan 1; 318 (1): H25–H33. doi: 10.1152/ajpheart.00427.2019. Epub 2019 Nov 27. PMID: 31774696.
35. *Xin L, Gellner B, Ribeiro RVP, Ruggeri GM, Banner D, Meineri M et al*. A New Multi-Mode Perfusion System for *Ex Vivo* Heart Perfusion Study. *J Med Syst*. 2017 Dec 23; 42 (2): 25. doi: 10.1007/s10916-017-0882-5. PMID: 29273867.
36. *Kung EO, Taylor CA*. Development of A Physical Windkessel Module to Re-Create In-Vivo Vascular Flow Impedance for In-Vitro Experiments. *Cardiovasc Eng Technol*. 2011 Mar; 2 (1): 2–14. doi: 10.1007/s13239-010-0030-6. PMID: 26316899; PMCID: PMC4548962.

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SAFETY ASSESSMENT OF THE FEMTOSECOND LASER IN CORNEAL LIMBAL GRAFT EXCISION

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Objective: to study *in vitro* survival and preservation of the proliferative activity of limbal stem cells (LSCs) in femtosecond laser-cut limbal tissue fragments. **Materials and methods.** Limbal fragments were formed from donor cadaver eyes (n = 8) in the upper and lower limbus containing the highest number of limbal stem cells, using a Z8 femtosecond laser (FSL) (Ziemer, Switzerland). The limbal fragments were fragmented into 4 mini-grafts using different energy levels (100, 110, 120%). Mini-grafts from symmetrical sections of the cadaver eyes, which were manually isolated using a microsurgical blade, served as controls. The mini-grafts were cultured for two weeks in culture media intended for limbal epithelial stem cells (LESCs) (Epilife (0.06 mM Ca^{++}) and for multipotent mesenchymal stem cells (MMSCs) (DMEM/F12), with the addition of specific growth factors to selectively stimulate LESCs or MMSCs, respectively. The phenotype of the obtained cultured cells in the "laser" and "knife" groups was determined by flow cytometry using a set of markers (CD166, CD105, CD90, CD29, CD34) for the membrane proteins of LESCs and MMSCs. The ability of cultured cells to adhesion and proliferation in the "laser" and "knife" groups was determined by seeding the third passage of the resulting cultures on Bowman's membrane of acellular corneas. **Results.** Primary cell culture was obtained from mini-grafts of all donors in both groups. Cell morphology was consistent with the phenotype of corneal epithelial cells (cobblestone pattern). When cultured in the EpiLife medium (0.06 mM Ca^{++}), we determined the presence of LSCs proliferation from 38.6% of mini-grafts; in the DMEM/F12 medium (1 : 1) the presence was determined from 31.8%. Two weeks later, cell yield from mini-grafts in the "laser" and "knife" groups was 77.2% and 63.6%, respectively. Cell growth by the end of week 2 of culturing of mini-grafts obtained by FSL at 120, 110 and 100% energies was 87.5, 71.4 and 71.4%, respectively. It was found that the resulting cell cultures in the "laser" and "knife" groups and in the "120%", "110%" and "100%" subgroups were not different phenotypically. Cytofluorimetric analysis showed that cell cultures in the groups had a mixed pattern of marker expression of both LESCs (CD29+) and MMSCs (CD90+, CD105+). Seeding of the third passage of cell culture in the test groups in all cases demonstrated adhesion and formation of a cell monolayer on the Bowman's membrane of model corneas. **Conclusion.** The use of FSL for cutting out limbal grafts seems to be effective and safe in comparison with the traditional mechanical (knife) technique. Cell cultures obtained from FSL-cut mini-grafts were able to grow and migrate for at least 21 days.

Keywords: limbal stem cells, glueless simple limbal epithelial transplantation, limbal stem cell deficiency, femtosecond laser.

INTRODUCTION

Corneal transparency depends on several factors, among which the epithelial layer is one of the most important. The layer functions as an effective barrier separating the cornea both from the external environment and from the spread of conjunctival epithelium on it. The corneal epithelium is continuously renewed by LESC. The vector of cell movement is directed from Bowman's membrane to the corneal surface and from its periphery to the center [1]. LESC cells reside in the limbus, which is a complex micro-anatomical structure [2]. The proliferation, migration, and differentiation of LESCs are dependent upon their specialized microenvironment known as the limbal niche. In addition to limbal epithelial progenitor cells, the limbal niche contains MMSCs, melanocytes, immune cells, vascular and nerve cells, extracellular matrix and signaling molecules (growth factors and cytokines) [3–8].

Various pathologies affecting any component of the limbal niche can lead to limbal stem cell (LSC) dysfunction and, consequently, to limbal stem cell deficiency (LSCD) [7, 9, 10]. The causes of this condition may be primary, caused by genetic defects (congenital aniridia, Peters anomaly), systemic autoimmune diseases (Stevens–Johnson syndrome, ocular cicatricial pemphigoid) and acquired – due to trauma or chronic inflammatory processes (chemical and thermal burns, chronic long-term keratitis and keratoconjunctivitis, neurotrophic and

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bullous keratopathies, toxic-allergic reactions, ocular surface tumors, etc.) [11].

LSCD is classified into complete and incomplete depending on the amount of damage to the limbal zone. Depending on the involvement of each eye in this pathological process, bilateral or unilateral LSCD is distinguished [12].

The most promising and safe treatment option for unilateral LSCD (complete and incomplete), which has been widely used in the world, is simple limbal epithelial transplantation (SLET), described in 2012 by V. Sangwan et al. [12, 13]. For this technology, it is necessary to isolate a 2×2 mm section of the upper corneal limbus from a healthy eye and dividing it with a microsurgical blade into several (8–12) fragments, gluing them with fibrin glue to the amniotic membrane overlaid on top of the previously prepared corneal stroma of the damaged eye [13]. Since a relatively small volume of limbal tissue of the donor eye is taken, the risk of iatrogenic LSCD in the healthy eye is minimized. The efficiency of this operation is 80% or more in adult patients and 71.2% in children [14].

The glueless simple limbal epithelial transplantation technique (G-SLET) was proposed as an alternative to the SLET technique and does not involve the use of fibrin glue [15]. After removal of the fibrovascular pannus from the surface of the damaged eye, the resulting limbal flaps are fixed by placing them in tunnels formed on the periphery of the corneal stroma. Thus, a peculiar "depot" of limbal stem cells located in the peripheral part of the cornea is formed [15].

It should be noted that in both SLET and G-SLET techniques, all manipulations on cutting and fragmentation of the limbal graft are performed manually, mechanically using microsurgical instruments (a delaminator, a disposable non-dosed metal blade or a dosed diamond knife, microtweezers) [12, 13]. It is difficult to achieve uniformity in the limbal flap throughout its length; the quality of the obtained grafts depends on the surgeon's experience and is difficult to standardize. In the absence of methods to control the incision depth, excessively shallow or uneven dissection may significantly limit the production of sufficient LSC volume for successful reconstruction of the corneal epithelium. The limbal graft fragmentation stage and subsequent graft manipulation, in particular compression with forceps, can lead to damage and even death of a part of the LSC. Transferring an insufficient amount of LSC can significantly reduce the effectiveness of the operation.

The introduction into practice of FSL technologies for tissue dissection, ensuring the formation of uniform and dosed cuts, as well as the units having in their interface high-precision visualization systems based on optical coherence tomography (OCT), is an extremely relevant and promising direction in ophthalmic surgery. From our point of view, the use of FSL has a real potential to obtain a full-fledged limbal graft with full capture of the LSC niche with its microenvironment and minimal damage compared to the mechanical method. In the available literature, we did not find information about the use of FSL for SLET technology, as well as articles studying the effect of laser energy on LSC survival after corneal limbal laser extraction, which was the basis for this study.

The **objective of the study** was to investigate in an in vitro experiment the survival and preservation of the proliferative activity of LSCs in femtosecond laser-cut limbal tissue fragments.

MATERIAL AND METHODS

Obtaining limbal grafts containing LSCs

Experimental studies on tissues isolated from cadaveric human donor eyes were performed in accordance with the legislative and regulatory documents of the Russian Federation. Limbal eyeball transplants (n = 8) from deceased male donors (n = 4) aged 55.3 years (32–71 years), provided by the Eye Tissue Bank (ETB) from Fyodorov Eye Microsurgery after infectious screening and decontamination with 10% povidone-iodine solution (EGIS, Hungary) according to the algorithm for preparation of donor corneal cadaveric material in the ETB [16]. Cleaning from the corneal epithelium and removal of residual tissues at the limbus were not performed. Donor corneas that were unsuitable for transplantation in the clinic due to low endothelial density or stromal defects were used for the experiment. The time from the moment of ascertaining biological death to the extraction of tissue fragments was 18.8 ± 0.5 hours.

Limbal grafts were obtained in the operating room in compliance with all asepsis and antisepsis rules. The eyeballs were fixed in a sterile mechanical holder. The upper portion of the corneal limbus was determined by the remnants of the upper rectus muscle, and markings were made. Limbal flaps were excised using FSL in the meridian from 12 to 2 and from 4 to 6 of the conditional dial (experiment) (Fig. 1) at energy levels equal to 100%, 110%, and 120% (high-frequency low-energy

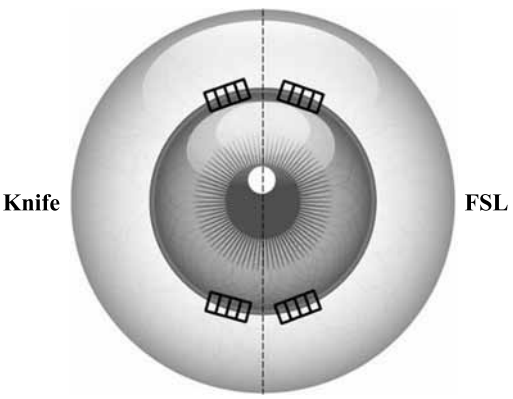

Fig. 1. Schematic of limbal mini-graft excision from donor cornea

FSL (nJ), horizontal cutting depth 200 μm), after which limbal flaps were excised in the meridian from 10 am to 12 noon and from 6 to 8 p.m with a manually dosed diamond knife and a dissecting knife (control) (Fig. 1). A 1.0 mm intact limbal section (lintel) was left between two limbal graft samples, different by the method via which they were obtained.

The technique for forming limbal fragments was as follows: a limbal graft 2.0 mm long, 1.5 mm wide was fragmented into 4 equal parts (mini grafts) (Fig. 1). In the upper and lower limbus of each eye on the left side, the limbal graft was cut out mechanically using microsurgical instruments (control). For this purpose, based on the marking in the upper and lower limbus, we made cuts with a diamond knife at a 200 μm depth, which, according to our estimations, is optimal for fully capturing the limbal niche with its microenvironment. After contouring, using a dissecting knife, the limbal graft was separated from the underlying tissues. Next, it was carefully transferred to a polymer substrate and divided into 4 equal parts (mini-grafts) using a steel microsurgical disposable blade. In the right part of the limbus (both above and below), formation of a limbal graft and its single-step division into 4 parts was carried out using FSL. For this purpose, after applanation of the laser handle to the cadaveric eye, the cutting trajectories were positioned, and the estimation of the horizontal cutting depth was controlled by the built-in OCT system. The laser operation time for the formation of one limbal graft with its fragmentation was 40 seconds. To evaluate the effect of different FSL energies on LSC growth, limbal grafts were cut out on different eyes at different energy levels (100%, 110%, and 120%). The selected levels were determined based on our previous studies of different energy values on the fragments formed. A total of 128 mini-grafts were obtained during the experiment (Fig. 2).

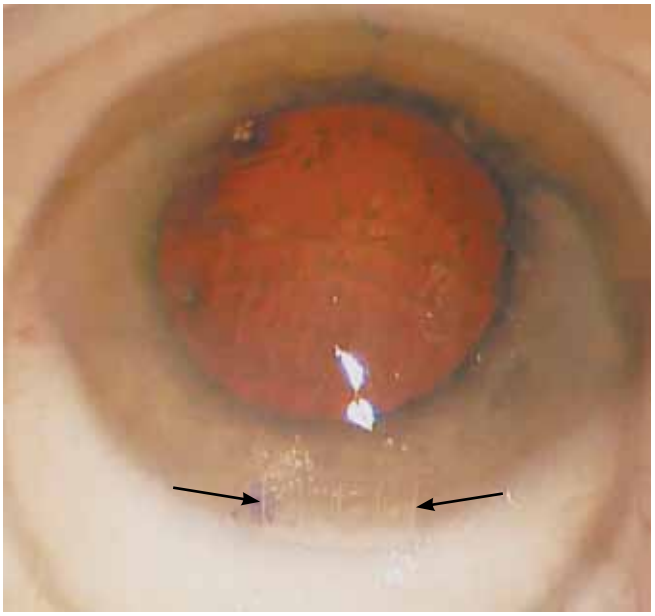

Fig. 2. Photo of the donor eye after cutting out limbal mini-grafts with a femtosecond laser. Four mini-grafts are visible, the lateral borders of the laser incisions are indicated by arrows

The resulting mini-grafts were placed into pre-prepared sterile microcentrifuge tubes with 500 μl of corneal storage solution (RU FSR № 2010106650, Eye Microsurgery, Russia). Then the labeled tubes were placed in a container and transported to a laboratory at Center for Fundamental and Applied Biomedical Problems (CFABP) under the head office Fyodorov Eye Microsurgery.

Cultivation of corneal limbal mini-grafts

Experiments on mini-grafts cultivation were performed at the CFABP laboratory under sterile in vitro conditions. Cultivation was carried out under standard conditions: +37 °C, 100% humidity and 5% CO_2 concentration (incubator NU-5510 NuAire, USA). For this purpose, each obtained mini-graft was placed in a separate well of a 48-well plate (#30048, SPL Lifesciences, Korea) with epithelial part upwards, 40 μl of culture medium was added and transferred to a CO_2 incubator; after 2 hours another 100 μl of medium was added. After 24 hours, the standard volume of medium was used (500 μl per well). The medium was changed every 2–3 days.

Cultivation was carried out in two media until the 3rd passage. To stimulate LESCs growth, EpiLife medium with 0.06 mM Ca^{++} (MEPICFPRF500, Gibco, USA) was used, with the addition of an antimycotic antibiotic (A5955, Sigma Aldrich, USA): 100 U/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B, 5% fetal bovine serum (SH30109. 03, HyClone Laboratories, USA), 5 μg/mL soluble human genetically engineered short-acting insulin (Humulin Regular, Eli Lilly and Company, USA), 5 μg/mL hydrocortisone (Pharmak, Ukraine) and 10 ng/mL human recombinant epidermal growth factor (hEGF) (FR-08000, PanEco, Russia) [17, 18]. The other part of the samples was cultured in DMEM/F12-based limbal MMSCs medium with 1.05 mM Ca^{++} (D6421, Sigma Aldrich, USA), supplemented with similar components [17, 18].

Once the cells reached 80–90% confluency, the culture was passaged using accutase enzyme (StemPro™ Accutase™ Cell Dissociation Reagent, A1110501, Gibco, USA). To do this, culture medium was removed from each well, 300 μL of enzyme was added and removed for debris clearance. Then 300 μL of accutase was re-added and placed in a CO_2 incubator at 37 °C for 10 minutes. The cell suspension was collected into a 15 mL centrifuge tube, precipitated for 5 minutes at 200 g at room temperature, and resuspended in 1 mL of culture medium. 10 μL of the suspension was used to count cells in a LUNA-II™ counter (Logos Biosystems, Korea).

Daily intravital observation of the mini-grafts and cell cultures was performed using an inverted phase-contrast microscope Olympus IX81 (Olympus, Japan). Images

were prepared in the internal program environment of the microscope (CellSence).

Statistical analysis

Categorical data were used to statistically analyze the results obtained. The categorical data included three numerical values (0, no adhesion and growth; 1, mini graft fixed; 2, mini graft fixed and there is cell yield). Statistical analysis included three tests:

The first test was to determine the effect of the media used (DMEM/F12 and EpiLife) on mini graft adhesion and cell yield using the "2×2 table" method with calculation of Fisher's exact test with two-sided hypothesis testing.

The second test was to determine the confidence limits, which allows to calculate the distribution of the attribute data over the sample and to determine the true value of the given parameters in the whole sample with a 90% probability. The modified Wald method was used to determine the confidence limits.

The third test was to calculate the probability of a positive event (we consider a positive event to be the attachment of a piece, as well as the attachment and active exit of cells from it). The binomial test was used, namely the sign test, which suggests that the event and its absence are equally likely to have a 50% probability.

Immunophenotyping of cell culture from corneal limbal mini-grafts

To determine the immunophenotype, the third passage culture suspension of cells, cultured on a DMEM/F12 medium, was divided into tubes of five equal parts (260,000 cells per tube), and washed from the complete cell culture medium in 2 mL of buffer (CellWASH, BD, USA), twice for 5 minutes each. The resulting cell sediment was stained using a set of markers to membrane proteins CD105, CD90, CD166, CD29, CD34 (Biolegend, USA); the markers were conjugated with fluorochromes according to the manufacturer's protocol. For this purpose, each tube was incubated at 25 °C in the dark for 15 minutes with antibodies (at the rate of 10 μL of antibody solution per 1 million cells). After incubation, the precipitate was resuspended in 1.0 mL of buffer and precipitated at 200 g for 5 min. The precipitate was then resuspended in 500 μL of buffer and analyzed on a CytoFLEX® flow cytofluorimeter (Beckman Coulter, USA). Immunoexpression curves were generated using the internal software of the device.

Study of the adaptation and adhesive properties of cell culture from corneal limbal mini-grafts

Donor human corneas that were unsuitable for transplantation were used for the experiment in the form of four corneoscleral discs.

Cornea preparation (cold cell elimination) was performed after completing all the donor corneal material preparation stages according to the previously described method [19]. Corneas were prepared in such a way that the entire epithelium was completely removed up to the Bowman's membrane, and the limbus was cleared of the remaining conjunctival tissue. Then, the resulting corneal discs were individually placed in new sterile transparent vials containing corneal storage solution (RU FSR № 2010106650, Eye Microsurgery, Russia) and they were left at +4 °C for 45–62 days. The condition of the corneal discs was monitored by the color of the solution (red-orange and transparent if not contaminated). Before cell seeding, the corneoscleral discs were washed twice in phosphate buffered saline (PBS) for 2 hours at room temperature, after which the central corneal section was cut out using a 6.0-mm-diameter trephine blade (Barron, Katena Products, Inc, USA) and placed in the well of a 96-well plate (32496, SPL Lifesciences, South Korea) with the Bowman's membrane to the outside and Descemet's membrane to the surface of the bottom of the well.

To assess the ability of cultured LSCs to adhere to the Bowman's membrane of the donor cornea, LSCs of the third passage were seeded onto prepared corneal discs located in the wells of the plate. Cell suspensions derived from mechanically excised mini-grafts (control) and using FSL (experiment) at different energy levels were used. LSC suspensions were seeded on the anterior surface of corneal discs at the rate of 140,000 cells per disc (1238.49 cells per 1 mm^2). Cultivation was performed under standard conditions for 14 days using EpiLife medium (0.06 mM Ca^{++}) and LESC supplements as described above. The culture medium was changed every 2–3 days. Intravital observation was performed using an Olympus IX81 inverted phase-contrast microscope. Images were acquired in the internal software environment of the microscope (CellSence).

Histological examination of corneas

After day 14 of culturing, the corneal discs were prepared for subsequent histological analysis. They were removed from the wells and washed three times in PBS for 10 minutes. Each was then fixed in 10% neutral formalin solution (141328, AppliChem, Germany) for 24 hours and cut in half to make transverse sections. Next, the corneal disc halves were washed with running water and dehydrated in alcohols of ascending concentration. Then they were embedded in paraffin, and a series of 10 μm-thick histological sections were stained with hematoxylin and eosin according to the standard technique. The preparations were studied and photographed using an Olympus IX81 inverted phase-contrast microscope in a transmitted light mode at 40× and 100× magnifications.

The resulting photographs were analyzed in Fiji software (ImageJ 2.0.0-rc69/1.52) [20]. Statistical pro-

cessing was performed on a personal computer using statistical programs.

RESULTS

Obtaining primary LSC culture *in vitro*

On day 3 of cultivation, it was noted that primary attachment of mini-grafts was not achieved in all wells. It is known that for full LSC growth, dense adhesion to the surface of the culture well is necessary, and the lack of fixation of mini-grafts leads to formation of debris, death of differentiated cells, and, in some cases, lack of formation of a monolayer of the primary cell culture. Therefore, to prevent this phenomenon, a cohesive viscoelastic ProVisc (Alcon, USA) containing 1.0% sodium hyaluronate and having a neutral pH was used once, according to the guidelines in a number of previously published works [21, 22]. For this purpose, the culture medium was completely removed from the well and 2 drops of viscoelastic were applied to the mini-graft. Then 500 μL of complete cell culture medium was added drop by drop and transferred to the incubator. For the first and subsequent examinations, slide plates were moved extremely carefully to the incubator, taking care to hold the incubator door when closing and opening to prevent shaking the mini grafts.

The first control examination after the beginning of cultivation was performed on day 7, further control examinations were performed every 3 days.

When examined on day 7 of cultivation, 31.25% of mini-grafts (n = 40) of the total number of wells were not fixed to the surface, they floated freely in the culture medium. At the end of culture on day 14, no growth was observed from the unfixed mini-grafts; large amounts of debris and dead undifferentiated cells were freely floating in the culture medium.

Primary cell culture was obtained in all samples that were adhered to the culture surface (68.75% of mini-grafts, n = 88). Morphologically, the primary culture cells followed the typical cobblestone pattern in all wells in both groups. Specifically, the cells formed a monolayer from the mini-graft, had a large nucleus and were tightly adhered to each other. At the same time, there was some variation in cell size and shape, more in line with the morphology of MMSCs, more pronounced for samples cultured in DMEM/F12 medium. Throughout the entire period of cultivation, the epithelial cell-specific morphology and the relative uniformity of their sizes were preserved in the primary culture.

In mini-grafts fixed to the surface of the wells and cultured in DMEM/F12, the first areas of cell growth were observed on day 5 from the start of cultivation. From the edge of the mini-graft, small cell clusters were formed, morphologically similar to LESCs, having a round shape and a large nucleus. In some samples, elongated cells with small nuclei (MMSCs) were observed by day 7. As they grew, they spread along the surface of the socket further from the mini-graft, forming cavities. The closer the formed cavities were to the mini-graft, the more they were filled with LESCs. It was noted that in samples where MMSCs were found, the number of LESCs was much higher (Fig. 3).

When cultivated on an EpiLife-based complete cell culture medium, the first signs of growth were also noted on day 5. On day 7 of observation, both MMSCs and LESCs were observed in the primary culture (Fig. 4).

Overall, cell growth in EpiLife medium from the initial passage to completion of culture on day 14 was slower compared to mini grafts cultured in DMEM/F12 medium. However, at the final observation period on day 14, the number of wells with adherent mini-grafts and cell growth was 38.6% in the EpiLife medium, while it was 31.8% in the DMEM/F12 medium (Table 1). Cell culture on the EpiLife medium had characteristic differences: mostly small polygonal cells with a large nucleus were present, there were small areas with larger cells and a relatively smaller nucleus, which is typical for maturing cells.

LSC proliferation depending on the mini-graft isolation method

After 7 days from the start of cultivation, there was a higher number of proliferating cells from the mini-grafts obtained by the traditional method using microsurgical instruments compared to the growth of cells recorded in the wells with mini-grafts obtained by FSL excision, 45.4% and 31.8%, respectively. However, at follow-up examination on day 11, there was an advance in the growth rate in the FSL-obtained samples. By the last observation period on day 14, the number of wells with recorded cell growth from the FSL mini-grafts was noticeably higher, 77.2% in contrast to the control group, 63.6% (Table 2).

Table 1

Percentage number of wells with fixed growth of LSCs depending on culture medium

Observation period	DMEM	EPL
Day 7	20.4%	18.1%
Day 11	22.7%	20.4%
Day 14	31.8%	38.6%

Table 2

Percentage number of wells with fixed growth of LSCs depending on the method of obtaining mini-grafts

Observation period	FSL	Mechanical method
Day 7	31.8%	45.4%
Day 11	59.0%	54.7%
Day 14	77.2%	63.6%

LSC proliferation depending on FSL energy level

When analyzing cell proliferation, from mini-grafts obtained by dissection at different energy levels, differences in growth rate and number of wells with cell growth were found. The best proliferation rates at the observation period of 7 days were demonstrated by the samples of mini-grafts obtained using a 110% FSL energy level (42.8%), and the lowest growth rates were recorded at an FSL energy level of 120% (25%). The growth pattern changed dramatically on day 11 of observation and the best growth rates were already demonstrated in the samples obtained with a 120% level of expended FSL energy (75%), the lowest growth rates in the samples with 100% level of FSL energy (42.8%). By the last observation period on day 14, in wells with mini-grafts obtained under 100%, 110% and 120% FSL energy levels, cell growth was 71.4%, 71.4%, and 87.5%, respectively (Table 3).

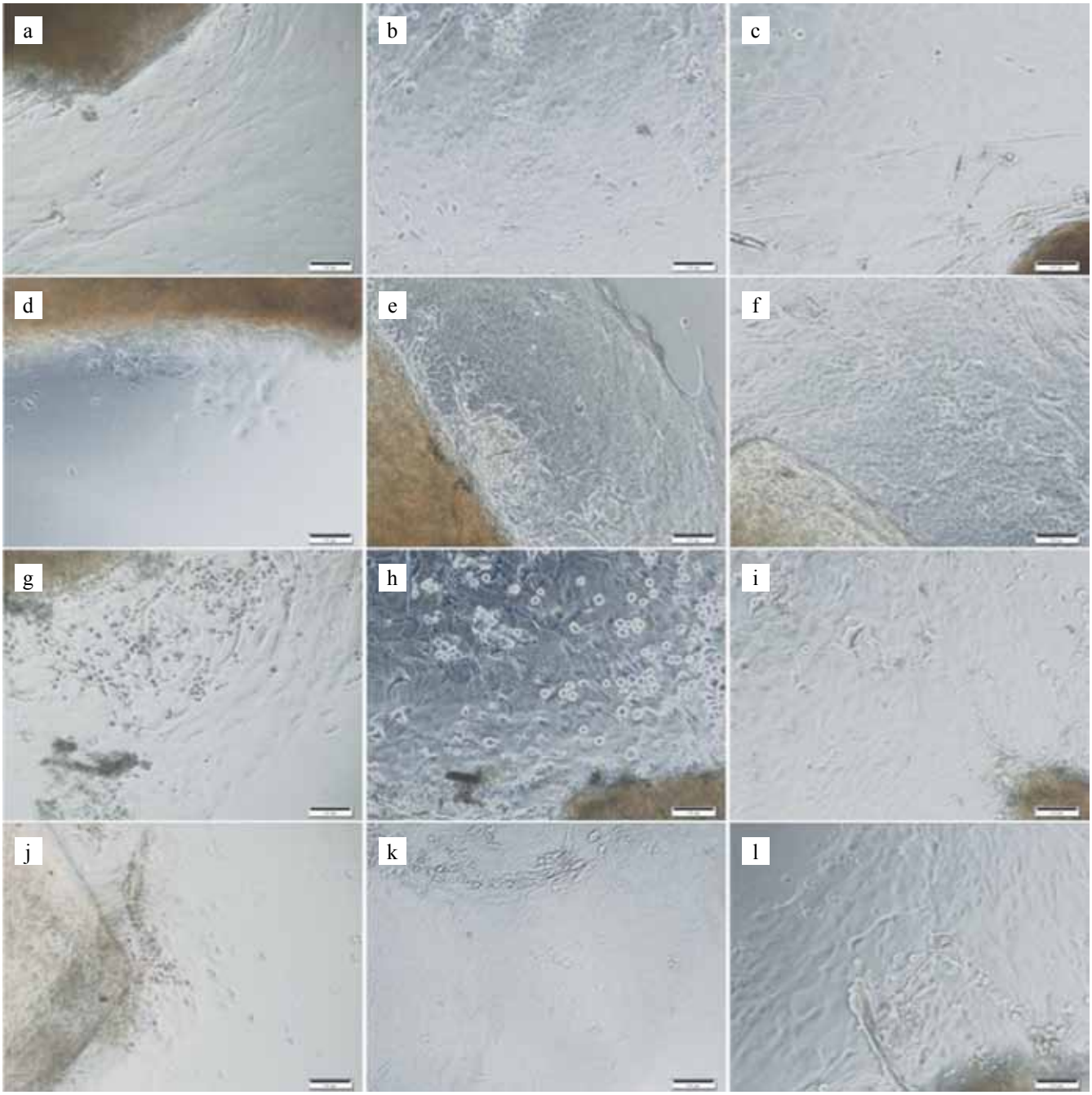

Fig. 3. Cell culture obtained from all types of mini-grafts on a DMEM/F12 medium. Different observation times (horizontally from left to right – days 7, 11 and 14 of culturing, respectively). (a, b, c), cell culture from mini graft obtained using 100% FSL energy level; (d, e, f), cell culture from a mini graft obtained using 110% FSL energy level; (j, h, i), cell culture from a mini-graft obtained using 120% FSL energy level; (j, k, l), cell culture from a mini graft obtained using microsurgical instruments. Light phase-contrast microscopy. Magnification 100×

Table 3

Percentage number of wells with fixed growth of LSCs depending on the FSL energy level expended to excise mini-grafts

Observation period	100% energy	110% energy	120% energy
Day 7	28.5%	42.8%	25.0%
Day 11	42.8%	57.1%	75.0%
Day 14	71.4%	71.4%	87.5%

Statistical analysis

When determining the influence of the used media (DMEM/F12 and EpiLife) on mini-graft adhesion and cell yield by the "2×2 table" method with the calculation of Fisher's exact test with the two-sided hypothesis testing, it was shown that the given compositions of the media do not statistically affect cell adhesion and cell yield in the knife and laser groups significantly ($p > 0.05$). However, it was shown that there is a statistically significant association of the signs between the "laser"

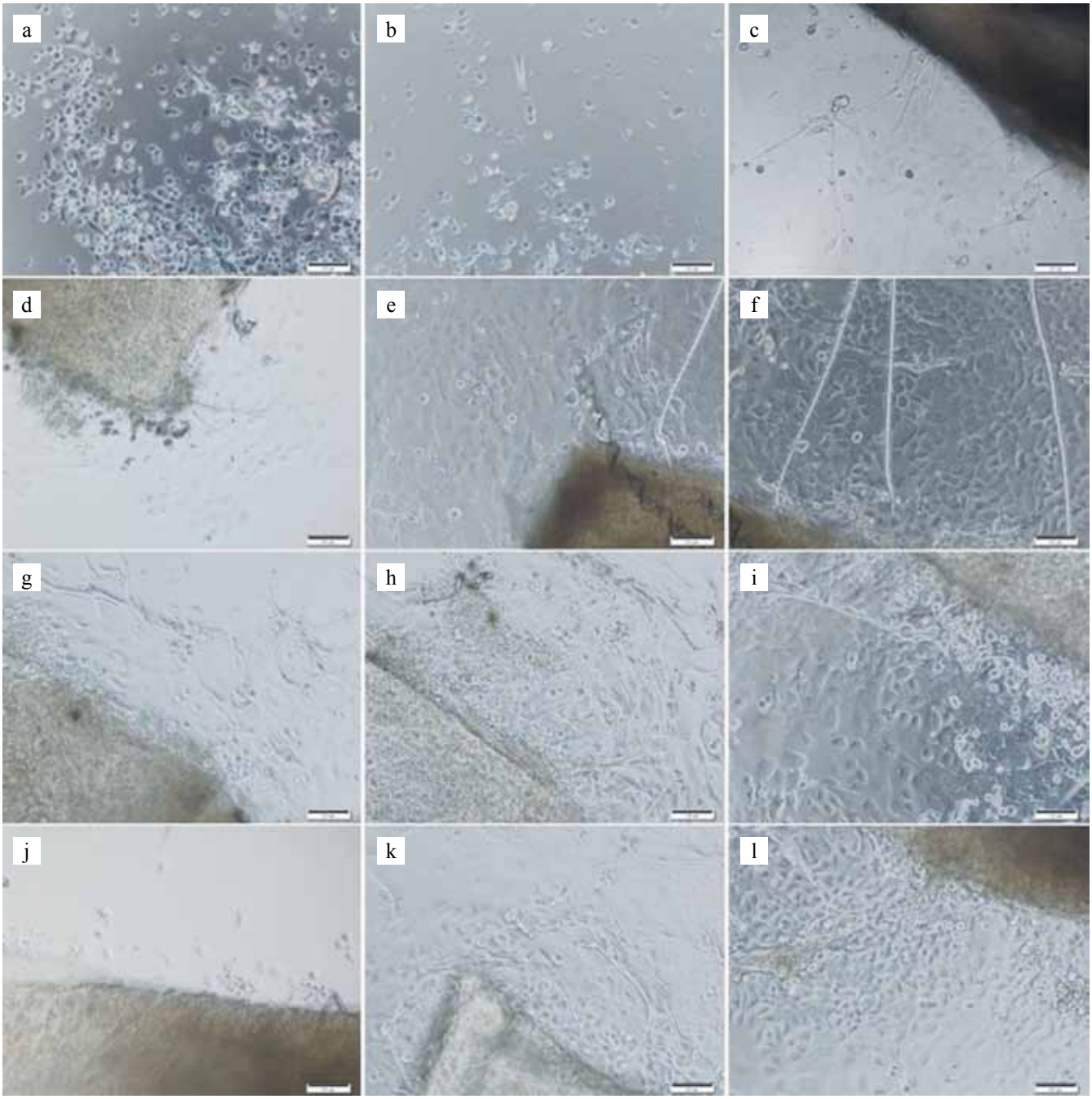

Fig. 4. Cell culture obtained from all types of mini-grafts on an EpiLife medium. Different observation times (horizontally from left to right – days 7, 11 and 14 of culturing, respectively). (a, b, c), cell culture from mini graft obtained using 100% FSL energy level; (d, e, f), cell culture from a mini graft obtained using 110% FSL energy level; (j, h, i), cell culture from a mini-graft obtained using 120% FSL energy level; (j, k, l), cell culture from a mini graft obtained using microsurgical instruments. Light phase-contrast microscopy. Magnification 100×

and "knife" methods of obtaining mini-grafts, where the best results came from the "laser" group ($p < 0.05$).

When determining the confidence limits using the modified Wald method, it was shown that on day 7, the confidence bounds in the "laser 100%" group were as follows [35%; 78%], "laser 110%" [57%; 98%], "laser 120%" [42%; 84%], "knife" [54%; 80%] (Fig. 5); and on day 14, the limits in the "laser 100%" group was [50%; 65%], "laser 110%" [80%; 92%], "laser 120%" [59%; 73%], "knife" [64%; 72%] (Fig. 6).

When calculating the confidence limits, the expected positive results on day 7 with 90% probability were obtained in the "laser 110%" and "knife" groups. On day 14, positive results were obtained in all groups. However, on day 14, maximum significant results were obtained in the "laser 110%" group (Diagram 2).

When calculating the probability of a positive event (we consider a positive event to be the attachment of a piece, as well as the attachment and active exit of cells from it) using the binomial test, it was revealed that in the "laser" group, with a one-sided sign test, the probability of getting a positive or successful result is 81.25% ($p = 0.003$). Accordingly, in the "knife" group, the probability of having a positive or successful result is 56.25% ($p = 0.029$).

Analysis of flow cytometry data

For immunophenotyping of the resulting cell culture along with analysis of the morphological picture, flow cytometry was performed. The cell cultures obtained on day 21 of cultivation of mechanically cut mini-grafts with FSL at 120% energy level were analyzed. We studied the expression levels of the following markers characterizing both MMSCs and LESCs: CD105 (endoglin) is a TGF-βIII receptor available in endothelial cells, syncytiotrophoblasts, macrophages and connective tissue fibroblasts; in MMSCs, endoglin plays mainly a signaling role in the processes of chondrogenic differentiation and is involved in the interaction of MMSCs and hematopoietic cells in the bone marrow; CD90 (Thy-1, T cell differentiation antigen) is widely used for MMSCs phenotyping, expressed by proliferating cells; CD166 and CD29 are markers of cells starting their differentiation pathway and not yet belonging to a specific cell type; CD34 is a negative marker for MMSCs. As a result, according to flow cytometry data, a heterogeneous cell culture, containing an insignificant number of MMSCs and a prevalent number of LESCs, was obtained. The morphological picture of the two samples allowed us to conclude that phenotypically identical cell cultures were obtained in both cases (Table 4).

Cultivation of LSC of the 3rd passage on cell-free donor corneas

Examination of cell culture on the corneal surface using a phase-contrast microscope was extremely difficult because the corneal stroma tissue lost transparency under constant presence in the culture medium. After two weeks of observation, a histological examination of sections of the corneas under study was conducted. As a result, a monolayer of cells fixed to Bowman's membrane was formed on all donor cornea samples. The cells were mostly small in size with a large polygonal nucleus, and spindle-shaped cells with a small nucleus were found among them. Groups of cells forming conglomerates were observed in the peripheral areas of the cornea during the formation of depressions (Fig. 7).

DISCUSSION

Currently, we are actively searching for an effective way to reconstruct the corneal epithelial layer in patients

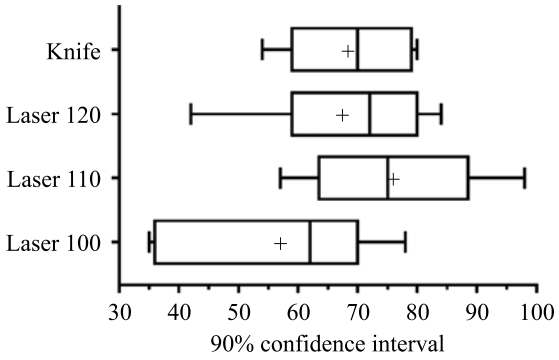

Fig. 5. Confidence limits on day 7 of observation in the test groups

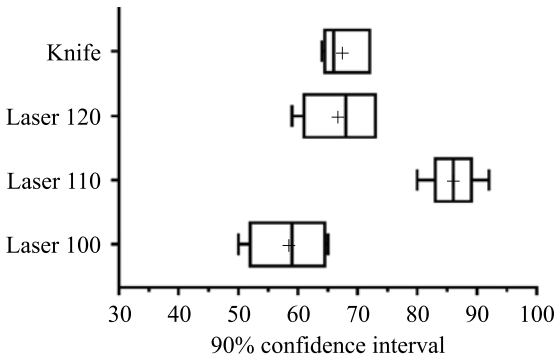

Fig. 6. Confidence limits on day 14 of observation in the test groups

Table 4

Immunophenotypic analysis of surface marker expression in a culture of passage 3 LSCs

Markers analyzed	Expression level	
	Femtosecond laser 120%	Knife
CD 105	0.49%	0.42%
CD 90	26.84%	28.26%
CD 166	99.89%	99.95%
CD 29	99.95%	99.96%
CD 34	0.11%	0.15%

with unilateral LSCD. Any surgical technique used for this disease should factor in the anatomical and functional features of the limbal zone.

The main component of the limbus is the limbal palisades of Vogt. These recesses have a unique gene expression and extracellular protein profile (extracellular matrix) that are specific and critical to LSC function. In the basal epithelial layer of the limbal niches, LESCs divide into identical cells in the horizontal plane or asymmetrically, thereby producing identical LESCs, and in the horizontal and vertical planes into transient amplifying cells (TACs). TACs then divide into postmitotic cells, which migrate centripetally. The postmitotic cells then differentiate into terminally differentiated cells (TDCs) and slough off the corneal surface. In addition to limbal epithelial progenitor cells, the limbal niche contains MMSCs, melanocytes, immune cells, vascular and neural cells, extracellular matrix and signaling molecules (growth factors and cytokines) [3–8].

MMSCs play a special role in LESC regulation. MMSC markers CD90 and CD105 are located beneath the basal membrane of the limbal crypt and interact closely with LESCs [23–25]. MMSCs contact LESCs through several molecular substrates and signaling pathways that include aquaporin-1 and vimentin [26], chondroitin sulfate [24], SDF-1/CXCR4 [27], BMP/Wnt [28], and IL-6/STAT3 [29]. Additional mechanisms of interaction are through intercellular contacts, secretion of growth factors, and cytokine expression [30].

Evidence on the structure of the limbal niche and its microenvironment, which is necessary for its full functioning, indicate that it is essentially important to preserve all components of the limbal niche during surgeries that are aimed at restoring the corneal epithelial layer.

The current availability of FSLs in ophthalmic practice, capable of operating at the lowest energy levels, minimizing trauma and death of LESCs, makes the approach to the use of FSLs in corneal epithelium reconstruction more attractive.

It should be noted that the first results of femtosecond lasers application in keratolimbal allograft (KLAL) were described by Korean scientists in 2010. They performed

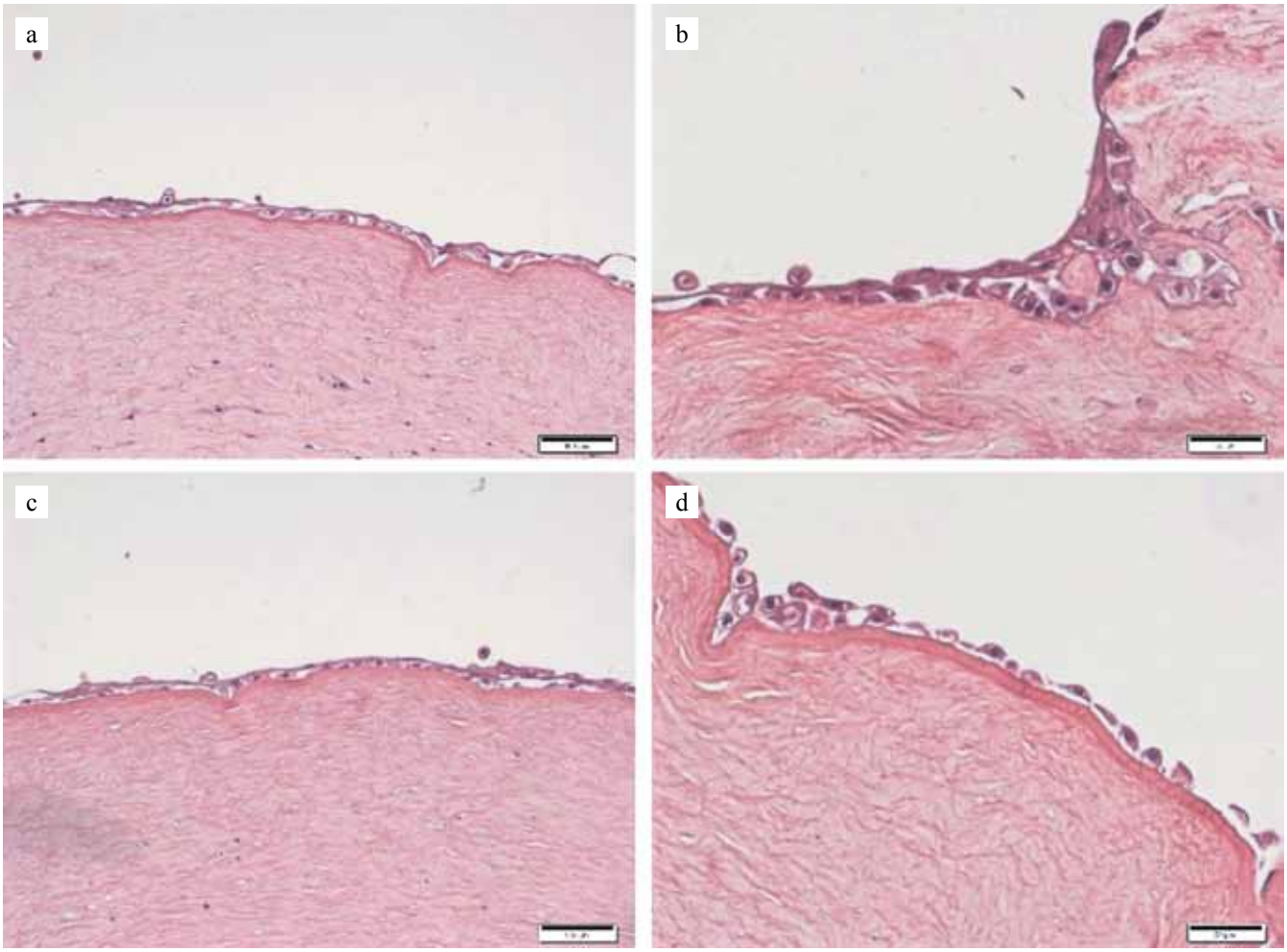

Fig. 7. Histological picture of donor inverted corneas with cell monolayer obtained from different cell cultures of the third passage. H&E stain: (a) culture obtained from a mechanically excised mini-graft, (c) culture obtained from a mini-graft excised by FSL at 100% energy, magnification 100×; (b) culture obtained from a mini-graft excised by FSL at 110% energy, (d) culture obtained from a mini-graft excised by FSL at 120% energy, magnification 50×

formation of a ring-type keratolimbal graft for further transplantation to the recipient. In this technology, a femtosecond laser (IntraLase, USA) was used to form a keratolimbal graft by cutting it out on the donor's eye so that only the distal border of the keratolimbal graft on the sclera side remained intact. It was cut out manually using a diamond knife because the applanation ring used in the kit of this laser had a maximum diameter of 9.5 mm [31]. The authors noted that the use of FSL in KLAL technology allows to cut the graft predictably thin and significantly reduces the risks and time spent on graft formation.

Currently, the following laser systems are used in the Russian Federation: VisuMax (Carl Zeiss Meditec, Germany), IntraLase (Abbot medical optics, USA), LensX and WaveLight (Alcon, USA), Femto Visum (Optosystems, Russia) and Femto LDV Z8 (Ziemer, Switzerland). The latter two laser versions differ in that they operate at the lowest possible energy level, which is in the nanoJoule (nJ) range, which allows for tissue dissection with minimal wound healing reaction and apoptotic cells along the incision plane [32]. This effect is achieved by operating the laser at the lowest possible energy levels but with a higher cutting frequency [32]. This effect is achieved by operating the laser at the lowest possible energy levels, but with a higher cutting frequency. A distinctive feature of the Femto LDV Z8 laser (Ziemer, Switzerland) is the presence of a mobile handle that can be positioned on the eye at any necessary angle; this laser is also equipped with a built-in OCT system, which allows to make the laser operation predictable, controlled and safe.

In this work, we evaluated the safety and efficacy of the Femto LDV Z8 low-energy high-frequency FSL (Ziemer, Switzerland) in relation to LESCs. Its use in the clinic within the G-SLET technology will significantly reduce the operation time, minimize the risks of obtaining uneven and incomplete limbal graft.

In the EpiLife medium, there was a preferential yield of polygonal epithelial-like cells, while in the DMEM/F12 medium, a yield of MMSC-like cells was also observed. It is known that MMSCs are essential for full maturation of LESCs.

During the cultivation process, we were able to find out that the yield of cells from FSL-cut mini-grafts is higher (77.2%) than in the group with manual isolation of mini-grafts (63.6%). This indicates that the use of FSL allows for precise cutting of limbal mini-grafts at a programmed depth, capturing the entire limbal niche with all its surroundings.

When comparing the cell cultures obtained by cutting out (using FSL) mini-grafts at different energy levels, the highest cell yield was observed in the 120% laser energy level group. However, at the time of the first control examination, indicators in this group were lower than the others. This can probably be attributed to the greater damaging effect of the energy on the peripheral borders of the mini-graft and the inhibition of the release of new cells overcoming the dead cell wall at the edges of the mini-graft. The higher number of cells (87.5%) in the obtained culture at the last observation period may indicate complete laser cutting out of all the necessary elements of the limbal niche functioning in a complex, without additional manipulations at the moment of evacuation of the mini-graft from the limbal bed, which was observed in the "laser 100%" and "laser 110%" groups. It should also be taken into account that statistical analysis showed that the probability of the mini-graft successfully adapting was higher in the "laser 110%" group.

CONCLUSION

This work presents a protocol for culturing limbal mini-grafts obtained using FSL, demonstrating active LSC growth. Cell cultures obtained from FSL-cut mini-grafts can grow for a long time, at least for 21 days. This indicates that FSL application in G-SLET technology is safe.

The use of FSL allows for precision cutting out of limbal mini-grafts at a controlled depth with its simultaneous fragmentation. This seems to us safer and more effective than the traditional mechanical "knife" cutting technique. This method has real prospects for further introduction into clinical practice.

The authors declare no conflict of interest.

REFERENCES

1. *Thoft RA, Friend J.* The X, Y, Z hypothesis of corneal epithelial maintenance. *Invest Opthalmol Vis Sci*. 1983; 24: 1442–1443.
2. *Del Monte DW, Kim T.* Anatomy and physiology of the cornea. *J Cataract Refract Surg.* 2011; 37: 588–598.
3. *Parfitt GJ, Kavianpour B, Wu KL, Xie Y, Brown DJ, Jester JV.* Immunofluorescence tomography of mouse ocular surface epithelial stem cells and their niche microenvironment. *Invest Ophthalmol Vis Sci*. 2015; 56: 7338–7344.
4. *Grieve K, Ghoubay D, Georgeon C, Thouvenin O, Bouheraoua N, Paques M et al.* Three-dimensional structure of the mammalian limbal stem cell niche. *Exp Eye Res*. 2015; 140: 75–84.
5. *Ramirez BE, Victoria DA, Murillo GM, Herreras JM, Calonge M. In vivo* confocal microscopy assessment of the corneoscleral limbal stem cell niche before and after biopsy for cultivated limbal epithelial transplantation to restore corneal epithelium. *Histol Histopathol*. 2015; 30: 183–192.
6. *Massie I, Dziasko M, Kureshi A, Levis HJ, Morgan L, Neale M et al.* Advanced imaging and tissue engineering of the human limbal epithelial stem cell niche. *Methods Mol Biol*. 2015; 1235: 179–202.
7. *Nubile M, Curcio C, Dua HS, Calienno R, Lanzini M, Iezzi M et al.* Pathological changes of the anatomical

structure and markers of the limbal stem cell niche due to inflammation. *Mol Vis*. 2013; 19: 516–525.
8. *Notara M, Shortt AJ, O'Callaghan AR, Daniels JT.* The impact of age on the physical and cellular properties of the human limbal stem cell niche. *Age*. 2013; 35: 289–300.
9. *Notara M, Refaian N, Braun G, Steven P, Bock F, Cursiefen C.* Short-term uvbirradiation leads to putative limbal stem cell damage and niche cell-mediated upregulation of macrophage recruiting cytokines. *Stem Cell Res*. 2015; 15: 643–654.
10. *Kim BY, Riaz KM, Bakhtiari P, Chan CC, Welder JD, Holland EJ et al.* Medically reversible limbal stem cell disease: clinical features and management strategies. *Ophthalmology*. 2014; 121: 2053–2058.
11. *Deng SX, Borderie V, Chan CC, Dana R, Figueiredo FC, Gomes JAP et al.* Global Consensus on Definition, Classification, Diagnosis, and Staging of Limbal Stem Cell Deficiency. *Cornea*. 2019; 38 (3): 364–375.
12. *Malyugin BE, Gerasimov MYu, Borzenok SA, Golovin AV*. Simple limbal epithelial transplantation (a literature review). *Fyodorov Journal of Ophthalmic Surgery*. 2019; (1): 77–86. [In Russ, English abstract]. https://doi.org/10.25276/0235-4160-2019-1-77-86.
13. *Sangwan VS, Basu S, MacNeil S, Balasubramanian D.* Simple limbal epithelial transplantation (SLET): a novel surgical technique for the treatment of unilateral limbal stem cell deficiency. *Br J Ophthalmol*. 2012; 96: 931–934.
14. *Basu S, Sureka SP, Shanbhag SS, Kethiri AR, Singh V, Sangwan VS*. Simple limbal epithelial transplantation: long-term clinical outcomes in 125 cases of unilateral chronic ocular surface burns. *Ophthalmology*. 2016; 123: 1000–1010.
15. *Malyugin BE, Gerasimov MY, Borzenok SA*. Glueless simple limbal epithelial transplantation. The report of the first two cases. *Cornea*. 2020; 39 (12): 1588–1591. doi: 10.1097/ICO.0000000000002467.
16. *Borzenok SA, Gerasimov MYu, Komakh YuA, Khubetsova MKh, Tonaeva HD, Malikova LM, Plaksa PI.* An algorithm for i nfectious screening of corneal donors in eye tissue bank of the S. Fyodorov Eye Microsurgery Federal State Institution. *Fyodorov Journal of Ophthalmic Surgery*. 2022; 2: 54–59. [In Russ, English abstract]. https://doi.org/10.25276/0235-4160-2022-2-54-59.
17. *Malyugin BE, Borzenok SA, Gerasimov MYu*. Clinical outcomes of autologous cultured oral mucosal epithelium transplantation for treatment of limbal stem cell deficiency. *Fyodorov Journal of Ophthalmic Surgery*. 2020; 4: 77–85. [In Russ, English abstract]. https://doi.org/10.25276/0235-4160-2020-4-77-85.
18. *Borzenok SA, Malyugin BE, Gerasimov MYu, Ostrovsky DS.* Cultivated autologous oral mucosal epithelial transplantation. *Russian Journal of Transplantology and Artificial Organs*. 2021; 23 (1): 171–177. [In Russ, English abstract] https://doi.org/10.15825/1995-1191-2021-1-171-177.
19. *Gerasimov MY, Ostrovskiy DS, Shatskikh AV, Borzenok SA, Malyugin BE.* Labial mucosal epithelium grafting in an ex vivo human donor cornea model. *Exp Eye Res*. 2022; 216: 108931. doi: 10.1016/j.exer.2022.108931.
20. *Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T et al.* Fiji: an open-source platform for biological-image analysis. *Nat Methods*. 2012; 9 (7): 676–682. doi: 10.1038/nmeth.2019.
21. *Andjelic S, Lumi X, Vereb Z, Josifovska N, Facsko A, Hawlina M et al.* A simple method for establishing adherent ex vivo explant cultures from human eye pathologies for use in subsequent calcium imaging and inflammatory studies. *J Immunol Res*. 2014; 2014: 232659. doi: 10.1155/2014/232659.
22. *Szabó DJ, Noer A, Nagymihály R, Josifovska N, Andjelic S, Veréb Z et al.* Long-Term Cultures of Human Cornea Limbal Explants Form 3D Structures Ex Vivo – Implications for Tissue Engineering and Clinical Applications. *PLoS One*. 2015; 10 (11): e0143053. doi: 10.1371/journal.pone.0143053.
23. *Yamanda K, Young RD, Lewis PN, Shinomiya K, Meek KM, Kinoshita S et al.* Mesenchymal-epithelial cell interactions and proteoglycan matrix composition in presumptive stem cell niche of the rabbit corneal limbus. *Mol Vis*. 2015; 21: 1328–1339.
24. *Dziasko MA, Armer HE, Levis HJ, Shortt AJ, Tuft S, Daniels JT.* Localisation of epithelial cells capable of holoclone formation *in vitro* and direct interaction with stromal cells in the native human limbal crypt. *PLoS One*. 2014; 9: e94283.
25. *Mathews S, Chidambaram JD, Lanjewar S, Mascarenhas J, Pranja NV, Muthukkaruppan V et al. In vivo* confocal microscope analysis of normal human anterior limbal stroma. *Cornea*. 2015; 34: 464–470.
26. *Higa K, Kato N, Yoshida S, Ogawa Y, Shimazaki J, Tsubota K et al.* Aquaporin 1-positive stromal niche-like cells directly interact with N-caderin-positive clustersin the basal limbal epithelium. *Stem Cell Res*. 2013; 10: 147–155.
27. *Xie HT, Chen SY, Li GG, Tseng SC.* Limbal epithelial stem/progenitor cells attract stromal niche cells by SDF-1/CXCR4 signaling to prevent differentiation. *Stem Cell*. 2011; 29: 1874–1885.
28. *Han B, Chen SY, Zhu YT, Tseng SC.* Integration of BMP/Wnt signaling to control clonal growth of limbal epithelial progenitor cells by niche cells. *Stem Cell Res*. 2014; 12: 562–573.
29. *Notara M, Shrott AJ, Galatowicz G, Calder V, Daniels JT.* IL6 and the human limbal stem cell niche: a mediator of epithelial-stromal interaction. *Stem Cell Res*. 2010; 5: 188–200.
30. *Polisetti N, Agarwal P, Khan I, Kondaiah P, Sangwan VS, Vemuganti GK.* Gene expression profile of epithelial cells and mesenchymal cells derived from limbal explant culture. *Mol Vis*. 2010; 16: 1227–1240.
31. *Choi S, Kim J, Lee D.* A New Surgical Technique: A Femtosecond Laser-Assisted Keratolimbal Allograft Procedure. *Cornea*. 2010; 29: 924–929.
32. *Riau AK, Liu YC, Lwin NC, Ang HP, Tan NY, Yam GH et al.* Comparative study of nJ- and μJ-energy level femtosecond lasers: evaluation of flap adhesion strength, stromal bed quality, and tissue responses. *Invest Ophthalmol Vis Sci*. 2014; 55: 3186–3194. doi: 10.1167/iovs.14-14434.

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SOCIAL BASES FOR THE DIALOGUE ON DECEASED ORGAN DONATION

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This article assesses the changes in the coverage of the problem of organ donation in Russia. The boundaries of the dialogue on posthumous organ donation are outlined, taking into account the current organ donation model in Russia. The paper defines the concept of social capital, the sociology of organ donation and their significance for the development of deceased organ donation (DOD). Steps to promote the DOD concept in Russia are proposed.

Keywords: deceased/posthumous organ donation, social capital, sociology of organ donation.

INTRODUCTION

Transplantation is the gold standard treatment for end-stage diseases of internal organs and is performed routinely [1–3]. At the same time, the concept of organ transplantation itself remains an attractive object of ethical reflection due to its paradoxical nature. It is the only type of medical care, where saving the life of one patient is, in most cases, inextricably linked to ending another patient's life. Organ shortage remains the main challenge of modern transplantation. The current situation is clearly illustrated by the latest reports from domestic and foreign publications. According to the 15th report of the Registry of the Russian Transplant Society, 2,555 transplants were performed in Russia in 2022 [4], and there were 8,378 people waitlisted for donor organs (2019 data from the Report by Academician Sergey Gautier, the chief freelance transplant specialist at the Ministry of Health of Russia [5]. According to the Health Resources and Services Administration of the United States of America (USA), 42,000 transplants were performed in 2022 in the USA and there were 104,000 people on the transplant waiting list [6]. Data from a recent publication by A.J. Matas, on the pages of JAMA Surgery, which reports that the transplant waiting list in the US for the past 20 years increased by 83%, while the number of transplants for the same period only doubled [7]. The latest statistical report for 2022 by Eurotransplant International Foundation, which unites 8 EU countries, shows that 6,454 transplants were performed in the year and that 13,277 patients were on the transplant waiting list [8].

Attempts to overcome the persistent organ shortage are shaping the modern image of transplantation, which in less than 70 years of its existence, has transformed from an experiment into the most complex type of organized medical care [9]. This has been made possible by the general progress in medical science, emergence and continuous improvement of immunosuppressive therapy protocols, introduction of new criteria for death based on neurological signs, expansion of criteria for the suitability of donor organs for transplantation, establishment and development of the concept of perfusion rehabilitation of donor organs, introduction of the institute of transplant coordination, and complex legal and administrative changes that have evolved into a national model of donation [4, 10–15]. The success of donor programs today depends, among other things, on the perception of the concept of deceased organ donation by the public.

This study of literary sources is aimed at defining the boundaries of the dialogue on deceased organ donation between medical professionals and non-professionals, assessing the influence of social capital on the development of donor programs, and initially marking the boundaries of such a phenomenon as sociology of posthumous organ donation.

ON THE BOUNDARIES OF DIALOGUE ABOUT DECEASED ORGAN DONATION AND TRANSPLANTATION BETWEEN MEDICAL PROFESSIONALS AND NON-PROFESSIONALS

The desire to become an organ donor is based on basic principles of bioethics, such as altruism, acting in the interest of the common good, justice, personal autonomy, integrity of the body, and non-harm [16]. Altruism, meanwhile, stands out as the main principle of bioethics when it comes to organ donation. In his seminal work "The Gift Relationship: From Human Blood to Social Policy", British sociologist Richard Titmuss concludes that voluntary, or altruistic donation reflects a sincere

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desire to help, and therefore such donors are free from the fear of being deceived and, through their behavior, contribute to the strengthening of social justice and are a kind of buffer for actions aimed at exploiting human resources [17]. Altruism is also defined as prosocial behavior and motivation to do good unselfishly, sometimes at the risk of life, and sometimes with the willingness to sacrifice oneself for the sake of others [18]. Therefore, an altruist does not need to be persuaded to become an organ donor, but should be educated so that he or she is left in no doubt about the altruistic nature of the act of organ donation.

An appropriate legal basis is needed in order to have a dialogue about cadaveric donation. In Russia, it is represented by Law № 4180-I "On Transplantation of Human Organs and(or) Tissues" of December 22, 1992 and Article #47 of Federal Law № 323-FZ "On the Fundamentals of Health Protection of Citizens in the Russian Federation" dated November 21, 2011, which contains important clarifications about the possibility of lifetime will and the right of a spouse or close relatives to refuse organ donation if the deceased has no lifetime will [19]. The amendments introduced in 2016 to the Federal Law № 323-FZ "On the Fundamentals of Health Protection of Citizens in the Russian Federation" dated November 21, 2011, outlined the boundaries within which it is possible to conduct a dialogue about cadaveric donation and transplantation between medical specialists and non-professionals. The need for such a dialogue will be confirmed below.

ABOUT THE PLACE FOR DIALOGUE ON POSTMORTEM DONATION AND TRANSPLANTATION

The ambiguous public perception of organ donation and transplantation is eloquently demonstrated by the results of recent sociological studies. In particular, in the results of a study by the STADA Group Health Report, which is being conducted for the fifth time by the consulting and marketing agency Kantar Health in 9 countries (Germany, Belgium, France, Great Britain, Italy, Poland, Russia, Serbia and Spain) with the participation of 18,000 people, about 2,000 in each country, it was shown that the existing model of donation in Russia, in the form of presumption of consent, is criticized by 64% of respondents, 65% at the time of the survey have not yet decided whether to be donors or not, and only 20% of respondents consider organ donation as their moral duty. This last indicator resonates favorably with the results of another well-known and for a long time the only available survey conducted by the Levada Center in 2013, where less than 10% of respondents indicated that they were willing to become a donor for a stranger [20, 21].

In countries where there was a presumption of refusal at the time of the study, such as Germany and the United Kingdom, only 36% and 38% of citizens, respectively, were registered as organ donors on specialized online resources. On the German Organspende, a special donor card can be ordered in plastic, or a paper version can be printed from the comfort of one's home [22]. The British register as organ donors or opt out by filling out special forms on the official portal of the National Health Service of Great Britain [23]. At the same time, 63% of respondents in Germany and 75% of respondents in the UK reported that they would be willing to become deceased donors automatically [20]. The UK's move to a presumption of consent model in May 2020 is likely to highlight the failure of the previous model, as reported on the official government resource: "*with widespread public support for deceased organ donation at 75–80% of the population, only 38% have opted in. This means families are often left with a difficult decision when a loved one dies*" [24], which has a negative impact on donation rates [24], which has a negative impact on donation rates.

In the United States, where the "informed consent" model is in place, 170 million people are registered as organ and tissue donors, approximately 51.2% of the population as of August 22, 2022 [25]. A recent article by Matas reports that the number of registered organ donors has increased to nearly 60% of the population in exactly one year but points out that there are many explantations from unregistered donors [7]. A search for up-to-date statistics on the number of explantations from unregistered donors proved to be of little use; all search queries lead to resources that offer people to register as organ donors. A 2014 publication from the National Bureau of Economic Research cites data from 2010, 2011, and 2012, when explantation rates among unregistered donors was 54%, 57%, and 55%, respectively, for brain-dead donors, and 42%, 38%, and 39%, respectively, for donors with sudden irreversible circulatory arrest. It is noteworthy, however, that the authors of the publication obtained these data, as indicated, by personal conversation [26]. A news article on the Newsday resource, in turn, reports that in New York City alone, almost 80% of donated organs were obtained from unregistered donors in 2022 [27]. In annual reports by the official government resource United Network for Organ Sharing [28], the national Organ Procurement and Transplantation Network [29] and the nonprofit organization Donate Life America [25] do not include data on unregistered donors. The true reasons for the lack of statistical information on the receipt of donor organs from unregistered donors are unknown; probably, this is due to the current model of donation in the United States, aimed at maximizing the number of registered donors, for which large-scale promotional campaigns to popularize posthumous organ donation are launched, which is financed both at the state

level and with the participation of over 100 different charitable organizations [25]. This intensive educational policy over the past 12 years has ensured record rates of postmortem donation in the USA, where 42,800 transplants were performed in 2022 [29]. It will be shown below why this approach can hardly be adapted to the domestic model of deceased donation.

According to 2021 data from the authoritative analytical platform Statista, the United States leads the world in terms of the number of donors per million population (41.6), with Spain in second place, with a slight lag (40.8) [30].

The "Spanish Model" of donation and transplantation originated in 1989, underwent major changes in 2007 and 2018, and has been considered the gold standard worldwide for the last 15 years [31]. The main elements of the Spanish Donation Model include: 1. A well-developed legal framework and technical support, 2. A three-tiered system of deceased donation coordination: national, regional, hospital, 3. A special profile of transplant coordination: partially employed ICU physicians and nurses as transplant coordinators, not involved in transplantation, appointed and reporting to the clinic management; their main task is to ensure postmortem donation with active involvement in its promotion, training of colleagues, interaction with the media and research activities, 4. Transplant coordinators are employed in clinics participating in donor programs, 5. The Spanish National Transplant Organization acts as a support service, 6. Continuous quality audit of the posthumous organ donation procedure, both external and internal, 7. Special attention to educational programs, 8. Close attention to the media and a special policy of interaction with them, 9. Financial reimbursement for clinics for participating in the donation process [32].

In May 2023, the journal Transplant International published a review article "Ten Lessons from the Spanish Model of Donation and Transplantation", whose authors summarize: "*Changes in the national donation and transplantation system of any country can be achieved by adapting elements of the Spanish Model, thus avoiding more complex measures.*" [31].

The work of the team of authors led by V. Papalos provides a careful analysis of the donation system in Spain, detailing the "components for success", where, in addition to changes in the legal framework, creation of an institute for transplant coordination, expansion of donation criteria, and development of clinical protocols and guidelines, working with public opinion and donor families to create "a culture of trust and confidence in donation and transplantation programs" is mentioned; also emphasized on is the importance of a thorough and ongoing training of healthcare professionals in the following areas: 1. Fundamentals of transplant coordination, 2. ICU training, 3. Training for emergency medical technicians, 4. Educational courses for neurologists in vascular centers, 5. Courses for non-medical specialties, 6. Separate educational programs on communication with relatives of patients, discussion of the procedure of consent to cadaveric organ donation in critical situations; correct conversation with media representatives, 7. Educational courses for media representatives [31, 32].

When comparing the best deceased donation models, their nominal status becomes apparent. Regardless of the current model of donation, the attention of the country's residents is actively being attracted to this problem, only the approaches differ. In Spain, a measured educational work is being carried out; a competent specialist can be consulted on the problem of postmortem donation at any time, consistent interaction with the media is carried out, and due to constant and accessible training, a high level of professionalism of medical specialists and representatives of non-medical specialties involved in deceased donation is maintained. This approach can be characterized as "soft" and/or "transparent", one of its main advantages being the invisible strengthening of social ties in society and closer interaction of seemingly unrelated social groups when it comes to posthumous organ donation, interaction in silence and for the common good.

In the United States, the practice is different; the model of mandatory informed consent for organ donation determines the need to actively "seek" such consent. In an article by A.B. Sterry, in the Cambridge Quarterly Journal of Health Ethics, it is reported that if a person waiting for a driver's license is asked directly if they would like to be a deceased donor, they are more likely to say no, not because they are against the concept, but because of the feeling of being forced to do something they have not thought through; the report cites the example of Chile, where the number of postmortem donors decreased by one-third after the introduction of the mandatory informed consent model [33]. This is probably why more than a hundred charities in the United States are engaged in postmortem organ donation education, as well as government and non-government foundations with multimillion-dollar advertising campaigns. Organ donation promotion programs have been criticized as being biased and often lacking a scientifically proven basis, secondly, providing false or incomplete information regarding risks for potential donors, and thirdly, having an obvious propaganda overtone [34]. Let us turn to Russian practice.

In recent years, unprecedented changes have occurred in the Russian practice of promoting deceased organ donation. According to the Report by Academician Sergey Gautier, the chief freelance transplant specialist at the Ministry of Health of Russia, no less than 80 speeches in the authoritative media were made in 2020 alone, and the formation of positive public opinion about organ donation and transplantation was singled out as a separate area of work [5]. In the Internet era, online resource "Or-

gan Donor = Life Donor", available at https://donorstvo.org/, the official platform of the Ministry of Health of the Russian Federation, which is constantly moderated, contains weekly statistics on operations performed, latest news on organ donation and transplantation, articles on current topics, interviews with leading national experts and, at the time of writing, contains about 70 news publications [35]. In recent years, the problems of perception of postmortem organ donation and attitude towards it have come to the attention of sociologists, now at the level of individual studies in small samples [36], but this already marks the vector of interaction between transplant specialists, sociologists and the society as a whole.

SOCIAL CAPITAL AS THE BASIS FOR DECEASED ORGAN DONATION

Social capital, in the first approximation, is defined as an indicator of the quality of social ties in society, for example, when there is a need to come to the aid of strangers.

The term was introduced by the French sociologist Pierre Bourdieu in 1980 and, in accordance with the author's definition, was considered as "the sum of the resources, actual or virtual, that accrue to an individual or a group by virtue of possessing a durable network of more or less institutionalized relationships of mutual acquaintance and recognition" [37]. The concept was then supplemented by the American sociologist James Coleman, who considered social capital as "a public good formed by all members of society and positively influencing it, and this influence determines the need for cooperation within society, as it is in the personal interest of each of its members [38]. Finally, political scientist Robert Putnam proposed his version: "social capital is the inherent properties of society, such as trust (primarily), social norms and networks that contribute to greater social cohesion due to cooperation for the common good" [39].

In a recent study of the impact of social capital on posthumous organ donation, Hans Schmiets observes that a posthumous organ donor does not become one for someone; it is a gesture of pure altruism toward society as such, based on trust in the health care system in general and the current donation model in particular [40].

Sociology of organ donation

There are few studies devoted to the sociology of postmortem organ donation. In fact, the term is coined by Laura L. Machin, a professor at Lancaster University Medical School, a sociologist and health care ethics researcher. Drawing on the works of Richard Titmuss, she discusses the sociology of postmortem organ donation as a system of interaction between professionals and representatives of civil society, based on altruism, a sense of solidarity, social cohesion, and anonymity [41, 42].

CONCLUSION

The organ donation system established in Russia is steadily developing, as evidenced by the steady increase in the number of operations performed year after year [4]. Thanks to breakthrough changes in the information field of Russian transplantation, we can state that the conditions for dialogue between specialists and members of the society on postmortem donation have not only been created, but the dialogue is already ongoing at a high level and in the right direction. The authors assume that the social capital of Russian citizens is sufficiently developed such that they can be interacted with in a partnership manner. In order to give specific outlines to the concept of sociology of posthumous donation as a means of its promotion in our country, the following steps are necessary: 1) Conducting more sociological research on focus groups (medical students, doctors of different specialties, specialists involved in donor programs both at large centers and in the regions); 2) Developing unified information and educational materials on the basis of the results obtained; 3) Transmission of knowledge on deceased donation as a form of social interaction aimed at developing social capital, and thus society, to regional donation and transplantation centers.

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REFERENCES

1. Kidney.org [Internet]. National Kidney Foundation [cited 2023 Nov 02]. Available from: https://www.kidney.org/news/nkf-releases-commentary-transplant-practices.
2. *Yamakawa M, Kyo S, Yamakawa S, Ono M, Kinugawa K, Nishimura T*. Destination therapy: the new gold standard treatment for heart failure patients with left ventricular assist devices. *Gen Thorac Cardiovasc Surg*. 2013; 61 (3): 111–117. doi: 10.1007/s11748-012-0181-5. PMID: 23264080.
3. *Finotti M, Auricchio P, Vitale A, Gringeri E, Cillo U.* Liver transplantation for rare liver diseases and rare indications for liver transplant. *Transpl Gastroenterol Hepatol*. 2021; 6: 27. doi: 10.21037/tgh-19-282. PMID: 33824931.
4. *Gautier SV, Khomyakov SM.* Organ donation and transplantation in the Russian Federation in 2022. 15th Report from the Registry of the Russian Transplant Society. *Russian Journal of Transplantology and Artificial Organs*. 2023; 25 (3): 8–30. [In Russ, English abstract]. doi: 10.15825/1995-1191-2023-3-8-30.
5. minzdrav.gov.ru [Internet]. Otchet glavnogo vneshtatnogo specialista transplantologa Minzdrava Rossii Gautier S.V. ob itogah raboty v 2020 godu [cited 2023 Nov 02]. Available from: https://static-0.minzdrav.gov.ru/system/attachments/attaches/000/057/001/original/%D0%9E%D1%82%D1%87%D0%B5%D1%82_%D

0%B7%D0%B0_2020_%D0%B3%D0%BE%D0%B4_%D0%93%D0%BE%D1%82%D1%8C%D0%B5.pdf?1625044703.
6. Organdonor.gov [Internet]. Organ Donation Statistics [cited 2023 Nov 02]. Available from: https://www.organdonor.gov/learn/organ-donation-statistics.
7. *Matas AJ, Montgomery RA, Schold JD*. The Organ Shortage Continues to Be a Crisis for Patients With End-stage Kidney Disease. *JAMA Surg*. 2023; 158 (8): 787–788. doi: 10.1001/jamasurg.2023.0526. PMID: 37223921.
8. Eurotransplant.org [Internet]. Annual Report [cited 2023 Nov 02]. Available from: https://www.eurotransplant.org/2023/01/23/preliminary-eurotransplant-annual-figures-2022-online/.
9. *Nordham KD, Ninokawa S.* The history of organ transplantation. *Proc (Bayl Univ Med Cent)*. 2021; 35 (1): 124–128. doi: 10.1080/08998280.2021.1985889. PMID: 34970061.
10. *Thongprayoon C, Hansrivijit P, Leeaphorn N, Acharya P, Torres-Ortiz A, Kaewput W et al.* Recent Advances and Clinical Outcomes of Kidney Transplantation. *J Clin Med*. 2020; 9 (1193): 1–18. doi: 10.3390/jcm9041193. PMID: 32331309.
11. *Weinhard J, Noble J, Jouve T, Malvezzi P, Rostaing L.* Tocilizumab and Desensitization in Kidney Transplant Candidates: Personal Experience and Literature Review. *J Clin Med.* 2021; 10 (4359): 1–9. doi: 10.3390/jcm10194359. PMID: 34640377.
12. *Reznik ON, Skvortsov AE, Moysyuk YaG.* Preservation and perfusion rehabilitation of donor organs: achievements of the last decade. *Almanac of Clinical Medicine*. 2020; 48 (3): 193–206. doi: 10.18786/2072-0505-2020-48-038.
13. *Jawitz OK, Milano C*. Commentary: An innovative strategy for expanding the donor pool. *J Thorac Cardiovasc Surg*. 2022; 164 (2): 571–572. doi: 10.1016/j.jtcvs.2021.08.007. PMID: 34417037.
14. *Etheredge HR*. Assessing Global Organ Donation Policies: Opt-In vs Opt-Out. *Risk Manag Healthc Policy*. 2021; 14: 1985–1998. doi: 10.2147/RMHP.S270234. PMID: 34012308.
15. *Polushin YuS, Bagnenko SF, Loginov IV, Reznik ON*. Role of transplantation coordination in the development of organ donorship. *Anesthesiology and reanimatology*. 2007; 3: 51–53.
16. *Cotrau P, Hodosan V, Vladu A, Daina C, Daina LG, Pantis C.* Ethical, Socio-Cultural and Religious Issues in Organ Donation. *Maedica (Bucur)*. 2019; 14 (1): 12–14. doi: 10.26574/maedica.2019.14.1.12. PMID: 31123506.
17. *Titmuss R.* The Gift Relationship: From Human Blood to Social Policy. London: George Allen and Unwin, 1970.
18. *Monday OI.* Is altruism always sufficient for organ donation? vroom's expectancy theory, for expanding the organ donor pool. *Saudi J Kidney Dis Transplant*. 2020; 31: 503–507. doi: 10.4103/1319-2442.284026. PMID: 32394924.
19. Konsul'tantPljus [Internet]. Federal'nyj zakon ot 21.11.2011 № 323-FZ (red. ot 24.07.2023) "Ob osnovah ohrany zdorov'ja grazhdan v Rossijskoj Federacii" (s izm. i dop., vstup. v silu s 01.09.2023) [cited 2023 Nov 02]. Available from: https://www.consultant.ru/document/cons_doc_LAW_121895/34050d0e38bdacc8a1fc52c8ef256a3ff2723b04/.
20. Stada.ru [Internet]. Bol'shinstvo rossijan kritikujut dejstvujushchuju v strane sistemu donorstva organov i tkanej [cited 2023 Nov 02]. Available from: https://www.stada.ru/press/news/bolshinstvo-rossiyan-kritikuyut-deystvuyushchuyu-v-strane-sistemu-donorstva-organov-i-tkaney.html#health_report.
21. Levada.ru [Internet]. Donorstvo chelovecheskih organov. Davat' ili ne davat'? [cited 2023 Nov 02]. Available from: https://www.levada.ru/2013/08/02/donorstvo-chelovecheskih-organov-davat-ili-ne-davat/.
22. Organspende-info.de [Internet]. Organspende [cited 2023 Nov 02]. Available from: https://www.organspende-info.de/.
23. NHS. Blood and Transplant [Internet]. Register your decision [cited 2023 Nov 02]. Available from: https://www.organdonation.nhs.uk/register-your-decision/.
24. GOV.UK [Internet]. Opt-out organ donation: Max and Keira's Bill passed into law [cited 2023 Nov 02]. Available from: https://www.gov.uk/government/news/opt-out-organ-donation-max-and-keira-s-bill-passed-into-law.
25. Donatelife.net [Internet]. Donate Life America 2022 Annual update [cited 2023 Nov 02]. Available from: https://donatelife.net/wp-content/uploads/2022_AnnualUpdate_Online.pdf.
26. *Kessler JB, Roth AE.* Don't Take 'No' For An Answer: An Experiment With Actual Organ Donor Registrations. *NBER Working Paper No. 20378*. 2014.
27. Newsday.com [Internet]. Long Island organ donations increase above pre-pandemic levels [cited 2023 Nov 2]. Available from: https://www.newsday.com/news/health/organ-donation-long-island-tcezz1qm.
28. Unos.org [Internet]. Data and trends [cited 2023 Nov 2]. Available from: https://unos.org/data/#Current.
29. Optn.transplant.hrsa.com [Internet]. National data [cited 2023 Nov 2]. Available from: https://optn.transplant.hrsa.gov/data/view-data-reports/national-data/.
30. Statista.com [Internet]. Rate of deceased organ donors in select countries in 2021 [cited 2023 Nov 2]. Available from: https://www.statista.com/statistics/406893/rate-of-organ-donation-by-deceased-donors-in-select-countries/.
31. *Papalois V, Streit S, Johnston-Webber C, Mah J, Prionas A, Wharton G et al*. Ten Lessons From the Spanish Model of Organ Donation and Transplantation. *Transpl Int*. 2023; 36: 11009. doi: 10.3389/ti.2023.11009. PMID: 37305337.
32. Nationalacademies.org [Internet]. Beatriz Dominguez-Gil. Organ Donation and Transplantation: The Spanish Model [cited 2023 Nov 02]. Available from: https://www.nationalacademies.org/documents/embed/link/LF2255DA3DD1C41C0A42D3BEF0989ACAECE-3053A6A9B/file/D4C248800043DE6CB28C1FD262BFEC3EE0541E2F76E0?noSaveAs=1.
33. *Sterri AB, Regmi S, Harris J.* Ethical Solutions to the Problem of Organ Shortage. *Camb Q Healthc Ethics*. 2022; 31 (3): 297–309. doi: 10.1017/S0963180121000955. PMID: 35899548.

34. *Rady MY, McGregor JL, Verheijde JL*. Mass media campaigns and organ donation: managing conflicting messages and interests. Medicine, *Health Care and Philosophy.* 2012; 15 (2): 229–241. doi: 10.1007/s11019-011-9359-3. PMID: 22020780.
35. Donorstvo.org [Internet]. Donor organov – donor zhiznej [cited 2023 Nov 2]. Available from: https://donorstvo.org/.
36. *Romanov SV.* The attitude of population to national programs of transplantation of organs and organ donorship. *Sociology of medicine*. 2019; 18 (1): 21–23. doi: 10.18821/1728-2810-2019-18-1-21-23.
37. *Bourdieu P, Wacquant LJD*. An Invitation to Reflexive Sociology. Chicago and London: University of Chicago Press, 1992.
38. *Coleman JS*. Social capital in the creation of human capital. *The American Journal of Sociology*. 1988; 94: 95–120. doi: 10.1086/228943.
39. *Putnam RD, Leonardi R, Nanetti RY*. Making democracy work: Civic tradition in modern Italy. New Jersey: Princeton University Press, 1991.
40. *Schmeets H, Peters F.* The Impact of Social Capital on Organ Donation: Evidence from the Netherlands. *Soc Indic Res.* 2021; 157: 863–897. doi: 10.1007/s11205-021-02666-y.
41. *Boylan AR, Locock L, Machin LL*. From waste product to blood, brains and narratives: developing a pluralist sociology of contributions to health research. *Sociol Health Illn.* 2019; 41 (3): 585–600. doi: 10.1111/1467-9566.12715. PMID: 29493796.
42. *Machin LL, Williams RA, Frith L*. Proposing a sociology of donation: The donation of body parts and products for art, education, research, or treatment. *Sociology Compass*. 2020; 14 (10): 1–16. doi: 10.1111/soc4.12826.

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