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Глубокоуважаемые коллеги!

Предваряя первый в 2022 году номер журнала, традиционно хотелось бы определить приоритеты нашей деятельности на ближайшее время. Требование сегодняшнего дня – развитие трансплантологии в регионах РФ и формирование в нашей стране единого профессионального поля, объединяющего не только крупные федеральные, но и региональные трансплантологические центры.

ФГБУ «НМИЦ ТИО им. ак. В.И. Шумакова» Минздрава России, выполняя функции национального медицинского исследовательского центра по профилю «хирургия (трансплантация органов и (или) тканей человека)», координирует и непосредственно участвует в развитии трансплантационной помощи в субъектах РФ. Регулярно осуществляются выезды в регионы для изучения состояния организации трансплантационной помощи, участия в выполнении первых трансплантаций органов.

Созданию единого профессионального поля способствует и налаженная система телемедицинских консультаций, которые проводятся с медицинскими организациями субъектов РФ в режиме 24/7 для повышения доступности квалифицированной медицинской помощи пациентам независимо от их места жительства.

Неотъемлемым условием является подготовка и повышение квалификации профессиональных кадров. Образовательный центр ФГБУ «НМИЦ ТИО им. ак. В.И. Шумакова» Минздрава России готовит медицинские кадры для всех субъектов РФ; с помощью дистанционных на-

OUR 2022 PRIORITIES: CONSOLIDATING THE TRANSPLANT COMMUNITY. ET MULTA ALIA...

Dear colleagues,

As we expect the release of the first issue of the Journal for the year 2022, traditionally we would like to set out our priorities for the nearest future. Our current commitment is to develop the transplantology industry across the length and breadth of the Russian Federation and to form a unified professional field that unites not only large national but also regional transplant centers in our country.

As a national medical research center in the area of surgery (transplantation of human organs and/or tissues), the Shumakov National Medical Research Center of Transplantology and Artificial Organs coordinates and directly participates in the development of transplant care across the Russian Federation. The Shumakov Center pays regular visits to regions in the country to study the state of transplant care there and participate in organ transplant surgeries being carried out for the first time.

A well-oiled system for 24/7 telemedicine consulting, held in conjunction with medical organizations in the federal subjects of the Russian Federation in order to increase access to quality healthcare for patients, regardless of their place of residence, also contributes to the creation of a unified professional field.

Professional training and upskilling are an essential condition. The educational training center at Shumakov Center trains medical personnel from all federal subjects in Russia; modern clinical protocols for prevention, diagnosis, treatment, and rehabilitation of specialized patients are transmit-

учно-практических мероприятий в субъекты РФ транслируются современные клинические протоколы профилактики, диагностики, лечения и реабилитации профильных пациентов.

Совершенствование системы донорства органов для трансплантации остается приоритетным направлением и одновременно ресурсом для дальнейшего развития трансплантационной помощи нашим согражданам в регионах. Опыт и успехи московской системы координации органного донорства демонстрируют потенциал развития, который пока еще предстоит реализовать в других субъектах РФ.

И безусловно, наша общая задача – развитие научных исследований, качество и уровень которых соответствуют современному мировому уровню. Наши читатели, конечно, обратили внимание, что тематика научных публикаций из года в год расширяется, в том числе за счет медико-биологических исследований, работ по регенеративной медицине. Такая тенденция будет сохраняться, отражая поступательное развитие трансплантологии как интегральной области медицины и биомедицинской науки.

Как сегодня, так и в будущем мы видим свое назначение в создании условий и обеспечении консолидации отечественного трансплантологического сообщества ради повышения доступности и улучшения клинических результатов трансплантации. Необходимы более активная трансляция технологий, обучение специалистов в области системы донорства для трансплантации, развитие научных исследований и многое другое (et multa alia, как говорили древние) – ведь в нашем деле нет мелочей. Этим вопросам мы по-прежнему будем уделять внимание и на страницах журнала, и на ежегодных всероссийских мероприятиях – съездах и конгрессах Российского трансплантологического общества.

От лица всей команды редколлегии и редакционного совета журнала желаю нашим постоянным и потенциальным авторам, а также читателям, всем членам профессионального сообщества успешной творческой деятельности в наступившем 2022 году.

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

ted to Russia's federal subjects by means of remote academic and research events.

Improving the system of organ donation for transplantation remains a priority and at the same time a resource for further development of transplantation care for our fellow citizens living in the regions of the country. The experience and successes of the Moscow system for organ donation coordination demonstrate the potential for development, which is yet to be realized in other federal subjects of the country.

And of course, our common objective is to develop scientific research, whose quality and level are consistent with today's global standards. Our readers must have noticed that the topics in our research publications are expanding from year to year, which is partly due to medical and biological research and works on regenerative medicine. This trend will continue, thereby reflecting the progressive development of transplantology as an integral field of medicine and biomedical science.

Both today and in the future, we see our purpose as creating conditions and consolidating the national transplant community in order to increase accessibility to and improve clinical outcomes of transplantation. Active technology transfer, training of specialists in the field of donation system for transplantation, development of scientific research and much more (et multa alia, as the ancients said) are all needed because there are no trifles in our business. These issues we will continue to pay attention to both on the pages of our Journal and at annual all-Russian events like the conventions and congresses of the Russian Transplant Society.

On behalf of the entire editorial team and editorial board, I would like to thank and wish our permanent and potential authors, as well as readers and all members of the professional community, a successful creative research and work in the year 2022.

Sincerely,
S.V. Gautier (Editor-in-chief)
Academician of the Russian Academy of Sciences

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COMBINED TREATMENT OF UNRESECTABLE HILAR CHOLANGIOCARCINOMA WITH SUBSEQUENT LIVER TRANSPLANTATION

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Objective: to demonstrate the experience of unresectable hilar cholangiocarcinoma treatment using neoadjuvant therapy followed by liver transplantation (LT). **Materials and methods**. From 2017 to 2021, six patients were included in the treatment protocol for unresectable Klatskin tumor followed by liver transplantation at Granov Russian Scientific Center for Radiology and Surgical Technology. The neoadjuvant therapy included endobiliary photodynamic therapy (PDT), as well as regional and systemic chemotherapy. Each method was used at least three times for 4 to 5 months with radiological evaluation and measurement of CA 19-9 levels. Patients were placed on the waiting list when the tumor marker reduced, or when there were no radiological signs of disease progression and there was no acute cholangitis. The recipients underwent laparoscopic abdominal revision for carcinomatosis and assessment of lymph nodes in the hepatoduodenal ligament with urgent morphological examination. Where there was no extrahepatic spread, LT was performed according to the classical technique with paracaval, para-aortic and hepatoduodenal lymphodissection, biliodigestive anastomosis by an isolated Roux loop of small intestine. The operation was performed in three patients, all of them were men aged 40 to 55 years (mean 48). The mean time from the start of treatment to transplantation was 9.3 months (range 6 to 14). Mean CA 19-9 level at the time of intervention was 81.3 IU/mL (8 to 212). **Results**. In three patients, CA 19-9 levels more than doubled on average over four months despite treatment. According to data from computed tomography RECIST assessment, two of the patients showed disease progression. In one patient, carcinomatosis was detected by diagnostic laparoscopy. In three patients, CA 19-9 levels decreased more than fourfold. Two of these patients were radiologically confirmed to have the disease stabilized, and one had a partial response. One patient died from sepsis three years after transplantation as a result of secondary biliary cirrhosis and biliary abscesses without signs of progression. Two patients are still alive after 6 and 21 months without signs of tumor progression. **Conclusion**. LT for unresectable Klatskin tumor is effective in controlling the bioactivity of the tumor through the use of neoadjuvant therapy.

Keywords: Klatskin tumor, hilar cholangiocarcinoma, liver transplantation, photodynamic therapy, regional chemotherapy.

INTRODUCTION

Hilar cholangiocarcinoma (HCC), or Klatskin tumor, arising from the epithelial cells of the bile duct, is a rare and extremely aggressive disease. It usually manifests in late stages, thus leading to late diagnosis and low survival rate. The best results are achieved by radical surgical intervention in the volume of liver resection with lymphodissection. However, some studies have shown that resectability in HCC is about 30–50%, 5-year survival rate under R0 resection is no more than 30–40% [1], and 5-year recurrence rate reaches 70% [2].

In addition, local recurrence occurs in 50% of cases after radical surgical intervention, and distant tumor metastasis occurs in 30–40% of patients [3]. The high frequency of "positive" surgical margins is down to the lack of detailed understanding of the spread of the process due to extended, predominantly proximal periductal tumor growth with a macroscopically unchanged bile duct wall.

Thus, it should be recognized that resection is currently considered the preferred treatment when technically possible. However, this technique is feasible only for a narrow group of patients, and oncological results, although the best available, are often not enough to achieve >30% 5-year survival rate. Most patients at the moment of referral already have unresectable forms of Bismuth-Corlette type IV, IIIa, IIIb with contralateral damage to vascular structures (branch of hepatic artery or portal vein) and correspond to TNM T4N0M0 stage IIIC. Such tumor spread does not allow for radical surgical intervention (liver resection in various volumes). Meanwhile, the role of palliative therapies and their combinations – systemic chemotherapy (CT), regional CT, photodynamic therapy (PDT), brachial/external beam

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radiation therapy – increases in these cases. Adequate biliary tree drainage and the control of cholangitis with regular bacteriological investigation of bile is of primary importance in the care of these patients due to the high risk of developing septic conditions. In some cases, with proper approach amidst palliative treatment, it is possible to stabilize the disease by reducing the bioactivity of the tumor.

In this situation, liver transplantation (LT) can be considered as an ideal treatment for patients with unresectable HCC due to complete removal of tumor tissue and the whole organ with potential macroscopically invisible micrometastases and substrate for recurrence. However, available studies have shown that the best LT outcomes in Klatskin tumor can be achieved only with proper patient selection and in combination with neoadjuvant therapy [4]. For example, the Mayo Clinic treatment protocol demonstrates an 82% 5-year survival [5].

MATERIALS AND METHODS

From 2017 to 2021, six patients were included in the treatment protocol for unresectable HCC followed by LT (Fig. 1) at Granov Russian Scientific Center for Radiology and Surgical Technology.

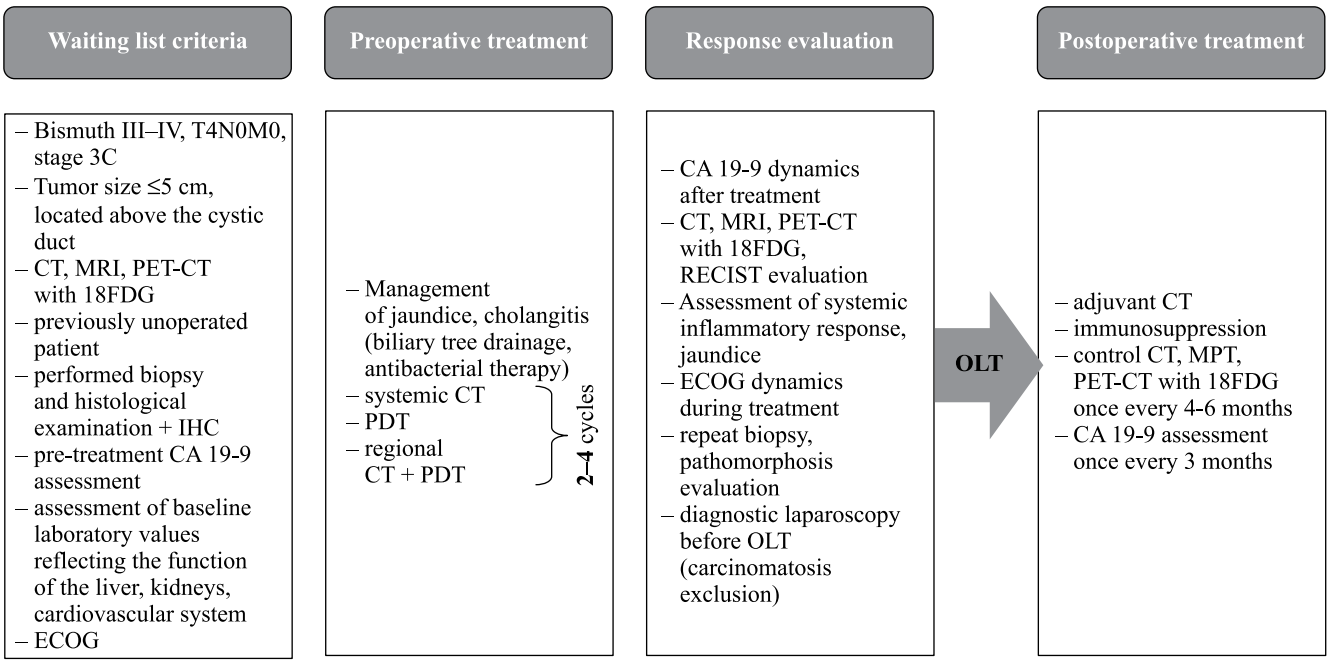

Fig. 1. Brief description of a multidisciplinary protocol for the treatment of unresectable Klatskin tumor with subsequent liver transplantation, developed at Granov Russian Scientific Center for Radiology and Surgical Technology

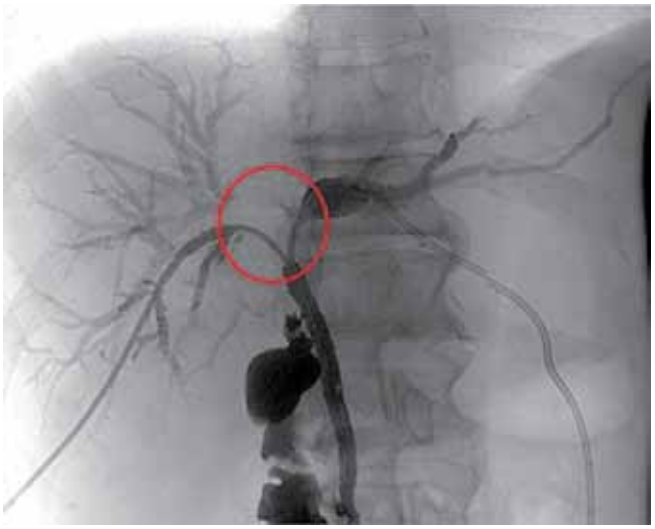

Fig. 2. X-ray picture of a 55-year-old patient with bilaterally installed external-internal cholangiodrainage. The red circle marks the area of confluence of the lobar bile ducts with the tumor stricture

The criterion for unresectability was segmental bile ducts lesion – Bismuth-Corlette type IV or IIIa, IIIb with contralateral lesion of vascular structures (branch of hepatic artery or portal vein). The stage of the disease was established via computed tomography, magnetic resonance imaging (MRI) and direct cholangiography (Fig. 2).

Patients with a tumor size <5 cm and located above the cystic duct were considered. Distant metastases were excluded by radiological methods of investigation. In all cases, histological confirmation by intravesical biopsy, assessment of CA 19-9 levels (in the absence of active cholangitis) before treatment, regular bacteriological examination of bile and appropriate antibacterial therapy were mandatory. Used as neoadjuvant therapy was a combination of endobiliary photodynamic therapy (PDT), regional CT (Fig. 3) and systemic CT.

Each technique was used at least three times for four to five months with radiological evaluation and determination of CA 19-9 levels in order to control tumor growth and bioactivity. Patients were placed on the LT wait list for only when the tumor marker was reduced, there were no radiological signs of disease progression, and there was no acute cholangitis. Prior to orthotopic liver transplantation (OLT), the potential recipient underwent laparoscopic abdominal revision for carcinomatosis and assessment of lymph nodes of the hepatoduodenal ligament with excision of suspicious tissue for morphological examination. Where extrahepatic spread was histologically confirmed, LT was not performed, otherwise, it was performed according to the classical technique with paracaval, para-aortic and hepatoduodenal lymphodissection, biliodigestive anastomosis by an isolated Roux loop of small intestine. All suspicious (enlarged/dense) lymph nodes in the area of hepatoduodenal ligament, ribs, aorta and inferior vena cava were removed. This, according to the classification of the Japanese Research Society for Gastric Cancer (JRSGC), corresponds to anatomic groups 5, 7, 8a, 8p, 9, 12a, 12b, 12p. LT was performed in three patients, all of whom were men. Their age ranged from 40 to 55 years (mean 48). The mean time from the start of treatment to transplantation was 9.3 months (6 to 14). The mean CA 19-9 level at the time of OLT was 81.3 IU/mL (8 to 212). A standard triple immunosuppression (tacrolimus, mycophenolic acid, prednisolone) was used in the postoperative period.

RESULTS

Despite inclusion in the treatment protocol and neoadjuvant therapy, three patients showed a more than twofold increase in CA 19-9 levels over an average of four months. RECIST CT scan showed that one of them had disease progression. Diagnostic laparoscopy demonstrated that one patient had carcinomatosis.

Against the background of a combination of methods (photodynamic therapy, regional chemotherapy, systemic chemotherapy) as neoadjuvant treatment, CA 19-9 was normalized in two patients, while a fourfold reduction of the tumor marker level was achieved in one patient. On follow-up CT scan, two of these patients responded to treatment as the disease stabilized, one patient had a partial response. Diagnostic laparoscopy and biopsy of hepatoduodenal lymph nodes in all patients with decreased CA 19-9 levels did not reveal tumor elements in the examined material, which allowed for OLT (Table). One of the examined specimens of the removed liver showed no macroscopic signs of tumor (Fig. 4).

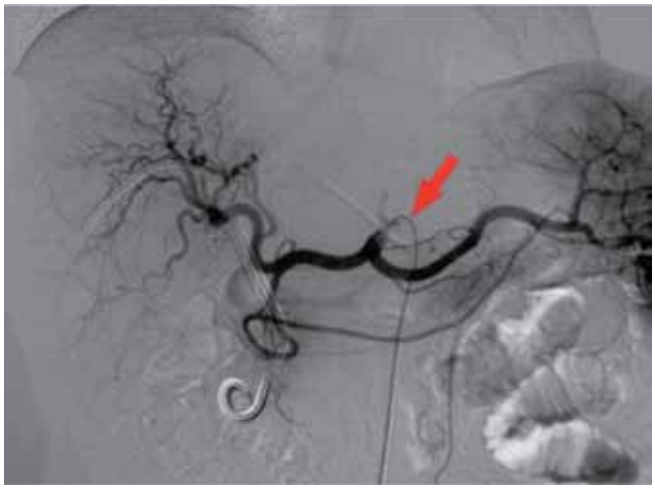

Fig. 3. Angiogram of a 40-year-old patient. Arterial introducer (indicated by red arrow) is inserted into the common hepatic artery for regional chemotherapy

Small single foci of cholangiocarcinoma could be detected only by additional slicing of the microslide (pathomorphosis stage IV) (Fig. 5).

One patient died from sepsis 3 years after OLT as a result of secondary biliary cirrhosis and biliary abscesses without signs of progression. It should be noted that the patient did not comply, had an episode of chronic rejection in the first six months after surgery due to violation of the medication regimen. Also, ischemic bile

Table

Neoadjuvant treatment with tumor marker dynamics and RECIST response for all patients included in the protocol. Treatment results

Patient	Age (years)	Number of PDT	Number of RCT	Number of SCT	CA 19-9 level before treatment	CA 19-9 level after treatment / at the time of OLT	RECIST response	Time to progression/OLT	Survival after OLT	Survival from initiation of treatment
1	49	7	11	8	986	8	CR	**OLT at 14 months**	36 months	50 months
2	40	4	4	5	754	24	SD	**OLT at 8 months**	21 months	29 months
3	37	4	4	4	337	754	SD	Carcinomatosis in diagnostic laparoscopy	–	11 months
4	56	2	2	3	3416	7256	PD	Progression at 4 months	–	7 months
5	55	4	3	5	864	212	SD	**OLT at 6 months**	6 months	12 months
6	46	5	6	6	789	1456	PD	Progression at 5 months	–	8 months

duct injury resulting from arterial insufficiency of blood supply to the graft cannot be ruled out. Two patients are still alive for 6 and 21 months without signs of cancer progression.

DISCUSSION

The use of LT as a treatment option for HCC patients has been attempted since the 1980s. Despite the reasonable potential advantage of complete removal of the affected organ with the achievement of a "negative" resection margin, the outcomes have left much to be desired. At the dawn of attempts to solve this problem, clinics performing LT in HCC reported a 3-year survival rate of about 30% [6]. Such results have led the global medical community to the conclusion that providing radical surgery by unilaterally hepatectomizing the recipient cannot significantly improve long-term outcomes. Moreover, immunosuppression is known to increase the risk of tumor progression and can lead to rapid patient death. However, a careful analysis of the accumulated materials revealed that cohorts of patients with "negative" resection margins and no metastases in regional lymph nodes had much better survival rates. Besides, a small group of patients at the Mayo Clinic who received only chemoradiotherapy without subsequent surgical treatment had a 22% 5-year survival rate [7]. Unsatisfactory outcomes of standard HCC treatment methods and the success of individual studies were the trigger for the active use of combined methods. With data indicating the effectiveness of chemoradiotherapy for HCC and the knowledge that disease progression is usually associated with local recurrence rather than distant metastases [8], a Nebraska transplant team first developed a strategy – high dose-rate neoadjuvant brachytherapy in combination with 5-fluorouracil (5-FU) chemotherapy and subsequent LT [9]. Of course, there were biliary, infectious and vascular complications associated with the use of high dose-rate brachytherapy and peculiarities of the course of the disease. However, early results were promising with regard to the development of local recurrences. Subsequently, the Mayo Clinic adopted this concept, developing a similar protocol for neoadjuvant therapy followed by LT in 1993. The protocol combined the benefits of radiation therapy, chemotherapy, and LT with appropriate selection of patients with localized, unresectable HCC. Preliminary results for 11 patients reported in 2000 were encouraging, and an update in 2004 reported an 82% 5-year survival rate in 28 patients [5].

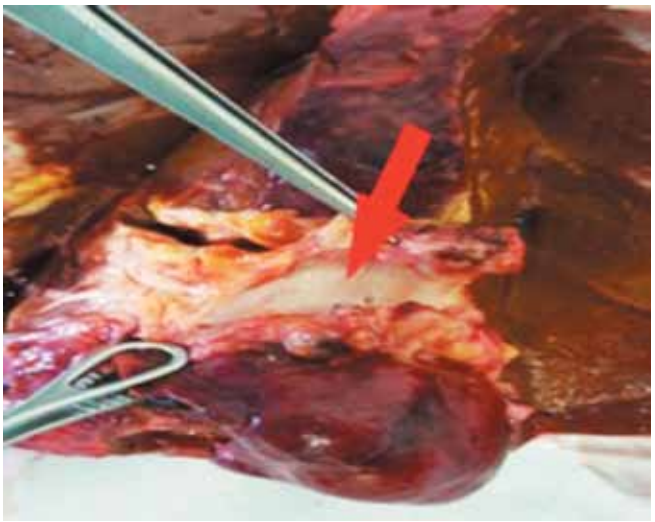

Fig. 4. Photo of a macroscopic specimen (removed recipient's liver). No macroscopic signs of tumor (indicated by red arrow) in the bile duct lumen

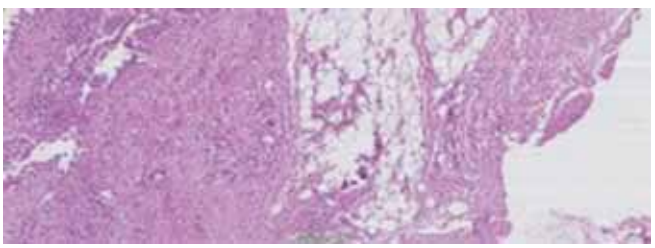

Fig. 5. Photo of a macroscopic specimen. Single foci of cholangiocarcinoma detected by additional slicing. Grade 4 pathomorphosis

Unfortunately, the domestic practice of LT in HCC appears to be extremely scarce and unsystematic, judging by the absence of a significant volume of publications. Treatment of technically unresectable HCC is palliative, and its results and prognosis differ little from those of disseminated process and, as a rule, are caused by rapidly progressing biliary obstruction and cholangitis. The primary task in treatment of such patients is biliary decompression in order to relieve mechanical jaundice and purulent cholangitis [10]. For this patient cohort, biliary decompression involves percutaneous transhepatic cholangiostomy due to the impossibility of performing retrograde drainage in more than a half of cases with proximal extrahepatic bile duct strictures [11].

The standard anti-tumor treatment for unresectable HCC, as well as for any form of inoperable locally disseminated or metastatic cholangiocellular carcinoma based on Russian and foreign clinical guidelines, is systemic polychemotherapy according to GemCis (gemcitabine/cisplatin) or GemCap (gemcitabine/capecitabine) scheme, as well as high-precision stereotactic conformal chemoradiotherapy with fluoropyrimidines [12, 13] or other options of chemotherapy and radiotherapy, depending on the patient's somatic status, individual intolerance and developing complications.

At the same time, according to the combined statistics of the effectiveness of these treatment methods for all inoperable malignant tumors of biliary structures, the median overall survival is 8–10 months [14]. Some of the best results achieved by chemoradiotherapy demonstrate a 30% 4-year survival rate [15].

Endobiliary PDT is a relatively new progressive treatment for unresectable HCC. The efficacy of PDT in combination with biliary decompression has been confirmed by numerous studies, some of which have shown a fivefold difference in life expectancy [16–19].

Being engaged in hepatobiliary and endovascular surgery and oncology in general, as well as HCC and LT in particular, we have been trying to use the entire arsenal of available options for this nosology for a long time. Like most of our colleagues, for biliary decompression we perform percutaneous transhepatic cholangiodrainage with mandatory evaluation of the bile microbial landscape and antibacterial therapy. The presence of percutaneous transhepatic drainage in the biliary tree in HCC patients implies that delivery of the emitter to the affected area is relatively easy and that multiple repetition of PDT procedure is possible, which has been confirmed by our own experience.

The ideological similarity between the world-renowned Mayo protocol and the treatment protocol developed by us is the stopping of tumor growth, reduction of biological activity of the tumor until radical treatment. Our neoadjuvant treatment includes PDT and no radiation therapy (RT). The efficacy of LT in unresectable Klatskin tumor is beyond doubt. However, as the authors themselves admit, implementation of external beam and intraductal brachytherapy is often accompanied by severe cholangitis, biliary abscesses, sepsis and vascular complications [5, 9], which, in our opinion, is manifested as pronounced proliferation of connective tissue and formation of rough cicatricial structures in the hepatoduodenal ligament. This cannot but affect the intraoperative precision of dissection of anatomical structures and formation of anastomoses, which significantly complicates vascular reconstruction during LT. The need to maintain a balance between the benefits and possible complications makes it necessary to keep the issue of RT use open. However, with regard to chemotherapy, we believe that in addition to the use of systemic chemotherapy, implementation of transarterial chemoinfusion (TAI) allows to create a high concentration of chemotherapy drug in a limited anatomical area, thereby increasing the cytostatic effect. In addition, direct angiographic examination allows to clearly assess the degree of involvement of vascular structures in the tumor process. In our opinion, alternating systemic chemotherapy and TAI with endobiliary PDT sessions is the best option for neoadjuvant therapy.

An additional advantage of the neoadjuvant protocol is the "test of time", as a cohort of patients with aggressive tumor biology experience disease progression despite ongoing treatment [20]. In such cases, LT is not indicated.

CONCLUSION

Indications for LT and its success in unresectable Klatskin tumor are determined by the effectiveness of palliative treatment for at least 3–4 months by reducing the tumor biological activity (assessment of tumor marker, size, metastatic lesion, extrahepatic spread) and control of acute cholangitis.

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REFERENCES

1. *Soares KC, Kamel I, Cosgrove DP, Herman JM, Pawlik TM.* Hilar cholangiocarcinoma: diagnosis, treatment options, and management. *Hepatobiliary Surg Nutr*. 2014 Feb; 3 (1): 18–34. doi: 10.3978/j.issn.2304-3881.2014.02.05. PMID: 24696835; PMCID: PMC3955000.
2. *Molina V, Sampson J, Ferrer J, Sanchez-Cabus S, Calatayud D, Pavel MC et al.* Klatskin Tumor: Diagnosis, Preoperative Evaluation and Surgical Considerations. *Cirugía Española (English Edition)*. 2015; 93 (9): 552–560. ISSN 2173-5077. https://doi.org/10.1016/j.cireng.2015.07.002.
3. *Groot Koerkamp B, Wiggers JK, Allen PJ, Besselink MG, Blumgart LH, Busch OR et al.* Recurrence Rate and Pattern of Perihilar Cholangiocarcinoma after Curative Intent Resection. *Journal of the American College of Surgeons*. 2015; 221 (6): 1041–1049. ISSN 1072-7515. https://doi.org/10.1016/j.jamcollsurg.2015.09.005.
4. *Rummo OO, Shcherba AE, Avdei EL, Fedoruk AM, Dzyadzko AM, Efimov DJu*. Evaluation of Different Methods Efficiency of Surgical Treatment in Patients with Liver Hilus Tumors of Surgical Treatment in Patients with Liver Hilus Tumors. *Annals of surgical hepatology*. 2013; 18 (2): 43–49 [In Russ, English abstract].
5. *Heimbach JK, Haddock MG, Alberts SR, Nyberg SL, Ishitani MB, Rosen CB et al.* Transplantation for hilar cholangiocarcinoma. *Liver Transpl*. 2004 Oct; 10 (10 Suppl 2): S65–68. doi: 10.1002/lt.20266. PMID: 15382214.
6. *Robles R, Figueras J, Turrión VS, Margarit C, Moya A, Varo E et al.* Spanish experience in liver transplantation for hilar and peripheral cholangiocarcinoma. *Ann Surg*. 2004 Feb; 239 (2): 265–271. doi: 10.1097/01.sla.0000108702.45715.81. PMID: 14745336; PMCID: PMC1356221.
7. *Foo ML, Gunderson LL, Bender CE, Buskirk SJ.* External radiation therapy and transcatheter iridium in the treatment of extrahepatic bile duct carcinoma. *Int J Radiat Oncol Biol Phys*. 1997 Nov 1; 39 (4): 929–935. doi: 10.1016/s0360-3016(97)00299-x. PMID: 9369143.
8. *Jarnagin WR, Ruo L, Little SA, Klimstra D, D'Angelica M, DeMatteo RP et al.* Patterns of initial disease recurrence after resection of gallbladder carcinoma and hilar cholangiocarcinoma: implications for adjuvant therapeutic strategies. *Cancer*. 2003 Oct 15; 98 (8): 1689–1700. doi: 10.1002/cncr.11699. PMID: 14534886.
9. *Rosen CB, Heimbach JK, Gores GJ.* Liver transplantation for cholangiocarcinoma. *Transpl Int*. 2010 Jul; 23 (7): 692–697. doi: 10.1111/j.1432-2277.2010.01108.x. Epub 2010 May 20. PMID: 20497401.

10. *Granov DA, Shapoval SV, Gapparov AC, Moiseenko AV*. Combination of regional therapy methods in the treatment of inoperable Klatskin tumor. *High-tech medicine*. 2020; 4: 8–16.
11. *Granov DA, Polikarpov AA, Tarazov PG, Timergalin IV, Polysalov VN*. Klatskin tumor complicated by obstructive jaundice and cholangitis in real practice: unresectable tumor or incurable patient? *Grekov's Bulletin of Surgery*. 2020; 179 (4): 9–16. [In Russ, English abstract]. https://doi.org/10.24884/0042-4625-2020-179-4-9-16.
12. *Benson AB, D'Angelica MI, Abbott DE, Anaya DA, Anders R, Are C et al*. Hepatobiliary Cancers, Version 2.2021, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw*. 2021; 19 (5): 541–565. doi: 10.6004/jnccn.2021.0022.
13. *Breder VV, Bazin IS, Kosyrev VYu, Ledin EV*. Practical recommendations for biliary cancer medication. *Malignant tumors*. 2021; 10 (3s2-1): 470–486. [In Russ]. doi: 10.18027/2224-5057-2020-10-3s2-26.
14. *Breder VV*. Cancer of the biliary system. *Practical Oncology*. 2012; 13 (4): 269–275. [in Russ].
15. *Polistina FA, Guglielmi R, Baiocchi C, Francescon P, Scalchi P, Febbraro A et al.* Chemoradiation treatment with gemcitabine plus stereotactic body radiotherapy for unresectable, non-metastatic, locally advanced hilar cholangiocarcinoma. Results of a five year experience. *Radiotherapy and Oncology*. 2011; 99 (Issue 2): 120–123. ISSN 0167-8140. https://doi.org/10.1016/j.radonc.2011.05.016.
16. *Ortner ME, Caca K, Berr F, Liebetruth J, Mansmann U, Huster D et al.* Successful photodynamic therapy for nonresectable cholangiocarcinoma: a randomized prospective study. *Gastroenterology*. 2003 Nov; 125 (5): 1355–1363. doi: 10.1016/j.gastro.2003.07.015. PMID: 14598251.
17. *Zoepf T, Jakobs R, Arnold JC, Apel D, Riemann JF.* Palliation of nonresectable bile duct cancer: improved survival after photodynamic therapy. *Am J Gastroenterol*. 2005 Nov; 100 (11): 2426–2430. doi: 10.1111/j.1572-0241.2005.00318.x. PMID: 16279895.
18. *Lee TY, Cheon YK, Shim CS, Cho YD.* Photodynamic therapy prolongs metal stent patency in patients with unresectable hilar cholangiocarcinoma. *World J Gastroenterol*. 2012 Oct 21; 18 (39): 5589–5594. doi: 10.3748/wjg.v18.i39.5589. PMID: 23112552; PMCID: PMC3482646.
19. *Wagner A, Kiesslich T, Neureiter D, Friesenbichler P, Puespoek A, Denzer UW et al.* Photodynamic therapy for hilar bile duct cancer: clinical evidence for improved tumoricidal tissue penetration by temoporfin. *Photochem Photobiol Sci*. 2013 Jun; 12 (6): 1065–1073. doi: 10.1039/c3pp25425a. Epub 2013 Apr 4. PMID: 23558738.
20. *Ito T, Butler JR, Noguchi D, Ha M, Aziz A, Agopian VG et al.* A 3-Decade, Single-Center Experience of Liver Transplantation for Cholangiocarcinoma: Impact of Era, Tumor Size, Location, and Neoadjuvant Therapy. *Liver Transpl*. 2021 Sep 5. doi: 10.1002/lt.26285. Epub ahead of print. PMID: 34482610.

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LIVER TRANSPLANTATION FOR PRIMARY BILIARY CHOLANGITIS (REVIEW)

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Primary biliary cholangitis (PBC) is an autoimmune liver disease resulting from the destruction and inflammation of intrahepatic bile ducts. This end-stage disease was once the most common cause of liver transplantation. The use of ursodeoxycholic and obeticholic acids as a first-line and second-line treatment, respectively, slows down the disease. However, treatment is not effective in about 40% of PBC patients, and the disease may progress to cirrhosis and end-stage liver disease. These patients undergo liver transplantation to save their lives. After surgery, recurrent PBC can develop in a milder form and rarely requires liver retransplantation.

Key words: primary biliary cholangitis, PBC, Liver transplantation, recurrence PBC, risk factors.

After the first clinical liver transplantation (LTx) in 1963, primary biliary cholangitis (PBC) was for a long time the leading indication for LTx. LTx in PBC patients accounted for 30–50% of all liver transplants [1–3]; in the first decade of the 21st century, there was a decline to 10% [1]. Despite the increasing prevalence of PBC, this disease is no longer the leading indication for LTx. In the last decade, there has been a trend toward an even greater decrease in the number of liver transplants for PBC [4], in Europe they represent about 9% of all liver transplants [5], and in Asia only 3.5% [6]. According to the European Liver Transplantation Registry, the proportion of liver transplantations for primary biliary cholangitis decreased from 20% in 1986 to 4% in 2015 ($P < 0.001$) [7].

In the United States between 1995 and 2006, while the total number of liver transplants increased, the absolute number of liver transplantations in the United States increased an average of 249 transplants per year between 1995 and 2006 ($P < 0.001$); the absolute number of transplants performed for PBC decreased an average of 5.4 cases per year ($P = 0.004$). A similar pattern was observed with respect to the absolute number of individuals added to the transplant waitlist showed a similar pattern: (1) an increase in total listings for transplants of all diagnoses ($P = 0.001$); 2) a decrease in the number of PBC patients ($P < 0.001$); (3) no significant change ($P = 0.083$) in the number of patients with primary sclerosing cholangitis [8].

Early detection of the disease by serological tests and treatment with ursodeoxycholic acid (UDCA) at the initial stage of the disease not only increased life expectancy, but also dramatically reduced the need for orthotopic LTx [8, 11], especially in patients with a favorable biochemical response to treatment [9, 10]. In the Netherlands, over the past three decades, there has also been a decrease in both the absolute and relative number of liver transplants in PBC [4]. The authors attribute this trend to the common use of UDCA as a standard treatment for PBC, but do not exclude the possibility of other mechanisms, given the many complex factors determining the final number of patients referred for transplantation and who eventually underwent it [4].

UDCA lowers serum hepatic enzyme levels and significantly reduces the likelihood of death after four years or the need for LTx [12]. Nevertheless, approximately 40% of patients have no biochemical response to UDCA treatment [13–15]. Inadequate response to UDCA treatment is directly associated with an increased risk of death or the need for LTx [16, 17]. Therefore, LTx remains the only option to prevent premature death in patients with advanced PBC [18]. The waitlist mortality rate in PBC patients is 12%, which is significantly higher than the mortality rate of liver failure of other etiologies [19].

There is an increase in the age of patients with PBC at the time of transplantation [20, 21]. In one study [22], the age of some patients exceeded 60 years. In addition, it has been found that an increasing number of men with PBC are undergoing LTx over time [7, 22]. According to the European Liver Transplantation Registry, between 1986 and 2015, the age at LTx increased from 54 (IQR 47–59) to 56 years (IQR 48–62), and the proportion of males increased from 11% to 15% [7].

Indications for liver transplantation in PBC patients. Patients with PBC require LTx if the disease proceeds to an end stage [23]. Liver transplantation is the only effective treatment for end-stage PBC with quite satisfactory results [18].

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Indications for transplantation are poor quality of life or possible death within less than one year. According to G.C. Mac Quillan and J. Neuberger (2003) [24], with many prognostic models available, the simplest guideline for timing of LTx is the level of serologic bilirubin. Deterioration of the condition of patients with PBC can be rapid in the end stages of the disease, requiring timely consideration of LTx [17].

Biochemical indicators of the restoration of transplanted liver function. After LTx, liver function is restored by the end of the fourth week [18]. Alanine aminotransferase and aspartate aminotransferase activities peaked once before this recovery [18]. In a retrospective study, transaminase activity on day 1 after LTx was 10–12 times the upper limit of normal and reached normal levels by day 9. Early peaks of transaminase activity represent a reflection of donor liver damage during its removal followed by reperfusion injury during transplantation [25]. Gamma-glutamyl transpeptidase levels peaked on day 7, and alkaline phosphatase levels peaked on week 2. Gamma-glutamyl transpeptidase levels peaked on day 9 after surgery, and then decreased. An early increase in gamma-glutamyl transpeptidase after LTx may be due to reperfusion injury, as well as surgical stress in the recipient [26]. Total bilirubin and direct bilirubin levels decreased after surgery to normal values at week 4. Bilirubin is an excellent prognostic biomarker for monitoring liver function recovery. Dynamic bilirubin monitoring is useful for early detection of biliary complications [18].

Post-liver transplant complications. According to recent data, the frequencies of infection and biliary complications are 62% and 21%, respectively [18]. Bacterial infections are the most common [27, 28]. Biliary complications occur in 10–30% of patients and can even cause mortality [29–31]. Hepatic artery thrombosis is a fatal vascular complication with high mortality (20–60%), with 3–5% incidence and usually requires repeated LTx [30, 32]. In a study by L. Chen et al. (2020) [18], 3 (4.3%) patients had hepatic artery thrombosis and 2 (2.9%) had portal vein thrombosis; fatal complications were abdominal bleeding, infection, and liver failure. Patients suffering from PBC had a high risk of chronic rejection [24].

Survival of PBC patients after liver transplantation. A retrospective analysis of the United Network for Organ Sharing database showed that the estimated patient survival at 1, 3, and 5 years for living donor liver transplants was 95.5%, 93.6%, and 92.5% and for deceased donor liver transplants was 90.9%, 86.5%, and 84.9%, respectively [33]. The overall survival rate of patients with PBC after LTx was reported to be 93–94%, 90–91% and 82–86% at 1, 3, 5 years, respectively [34]. A study by L. Chen et al. (2020) [18] reported that the overall patient survival was 98.6% at 1 year and 95.1% at 3 years. Over 70% of patients live more than 10 years [35, 36]. Long-term follow-up (up to 15 years) of patients after LTx for PBC showed excellent graft function and patient survival [37].

Recurrent PBC. PBC, as well as other autoimmune diseases (primary sclerosing cholangitis and autoimmune hepatitis), can recur after liver transplantation [38, 39]. Transplantation centers have recurrent PBC after both liver transplantation from a cadaveric liver [37, 40] and liver lobe from a living donor [41–43]. Recurrence did not affect patient and graft long-term survival [44]. Recurrent PBC has a mild clinical course, progresses slowly, and is rarely the cause of liver retransplantation [45, 46]. LTx outcomes in patients with PBC are excellent, with 5-year patient and graft survival rates exceeding 90% and 75%. However, in recent years, the problem of PBC recurrence after liver transplantation has been increasingly recognized as a cause of graft dysfunction and death, and the reason for repeated transplantation [47].

Frequency and timing of recurrent PBC. Recurrent PBC after liver transplantation occurs in a significant number of recipients [48]. Its frequency can reach from 32% [37] to 50% [24]. R.F. Liermann Garcia et al. (2001) [49] diagnosed recurrent PBC in 67 (17%) of 400 patients. Histological signs of the return of the disease were observed, on average, three years after liver transplantation. One patient required retransplantation eight years after primary liver transplantation.

During a median follow-up period of 114 months, R. Kurdow et al. (2003) [50] observed 18 patients with PBC who underwent liver transplantation. Six of the patients were reported to have developed a recurrence of primary biliary cirrhosis as indicated by liver biopsies, one patient also developed graft failure. Antimitochondrial antibodies were present in all patients within a period of 1 year after transplantation. Serological parameters were elevated in 16 of 18 patients. In another study [51], recurrent PBC developed in the transplanted liver in seven (15%) of 46 patients who underwent orthotopic LTx for PBC. All of these patients were alive 3 and 5 years after the diagnosis of disease recurrence.

J. Neuberger et al. (2004) [38] cited data on 485 patients with PBC in whom histological signs of disease recurrence were found in 114 (24.8%) patients after liver transplantation in annual protocol biopsy grafts, on average, 79 months after surgery.

J.E. Guy et al. (2005) [52] observed 48 patients with PBC after liver transplantation. One year after surgery, 27 (56.3%) patients had increased serum alkaline phosphatase levels. In graft biopsy specimens, there were reliable signs of recurrent disease in four (8%) patients, suspected recurrence in 11 (23%) patients and non-specific damage possibly related to PBC recurrence in two (4%) patients.

According to D.A. Jacob et al. (2006) [37], between April 1989 and April 2003, 1,553 liver transplants were performed in 1,415 patients at the Virchow Clinic in Berlin. Protocol liver biopsies were taken after 1, 3, 5, 7,

10 and 13 years after surgery. One hundred (7%) patients suffered from histologically proven PBC. Primary immunosuppression consisted of cyclosporine (n = 54) or tacrolimus (n = 46). Immediately after LTx, all patients received ursodeoxycholic acid. Corticosteroids were withdrawn three to six months after LTx. The median age of the 85 women and 15 men was 55 years (range 25–66 years). The median follow-up after liver transplantation was 118 months (range 16–187 months) and after recurrence 30 months (range 4–79 months). Actuarial patient survival after 5, 10 and 15 years was 87, 84 and 82% respectively. Ten patients (10%) died after a median survival time of 32 months. Two of these patients developed organ dysfunction owing to recurrence of PBC. Histological recurrence was found in 14 patients (14%) after a median time of 61 months (range 36–122 months). Patients with tacrolimus immunosuppression developed PBC recurrence more often ($p < 0.05$) and also earlier ($p < 0.05$). Fifty-seven patients developed an acute rejection and two patients a chronic rejection episode. Liver function did not alter within the first five years after histologically proven PBC recurrence.

A meta-analysis by M. Gautam et al. (2006) [53] found that recurrent PBC developed in 204 (16%) of 1,241 patients, averaging 46.5 months (range 25–78); the mean age of patients at the time of liver transplantation was 52 years (range 46.2–56); most were women (90%).

T. Kogiso et al. (2017) [54] conducted a retrospective multicenter study of recurrent PBC in 388 female patients after living-donor LTx. Postoperative factors were evaluated in 312 patients who survived for more than 1 year after living-donor LTx. Recurrent PBC was defined as abnormal hepatic enzyme levels with typical histological findings in liver biopsies. Fifty-eight patients (14.9%) developed recurrent PBC with a median of 4.6 (0.8–14.5) years post-LTx.

In a study by L. Chen et al. (2020) [18] included 69 patients with PBC who underwent living-donor LTx. Five-year overall survival and recurrence rates were estimated as 95.1% and 21.8%, respectively. A recipient aspartate aminotransferase-to-platelet ratio index greater than 2 was negatively associated with survival ($P = 0.0018$).

The incidence of recurrent PBC was quite high in Japan. At median follow up of 10.0 years (range 1.4–18.7 years), 29 (48%) patients were diagnosed with PBC after living-donor LTx at 4.6 years (range 1.3–14.5 years) [55].

Recurrent PBC develops, on average, 3 to 5.5 years after LTx [37, 49, 56]. Only in the study by J.E. Guy et al. (2005) [52] that the average time of PBC recurrence was found to be 1.6 years. PBC recurrence rate ranges from 1% to 35% [14, 58]. There were 9.6%, 20.6%, and 40.4% PBC recurrences at 5, 10, and 15 years, respectively, after LTx [57]. PBC recurrence in the short- and medium-term rarely affects patient and graft survival, but it can have a negative impact on these indicators in the long-term [18].

Etiology and pathogenesis of recurrent PBC. The etiology of recurrent PBC is the same as that of native liver disease. The mechanism of bile duct damage by antimitochondrial antibodies is related to an immune attack on expressed antigen molecules (e.g., E2 – pyruvate dehydrogenase complex) of bile duct epitheliocytes.

Risk factors for recurrent PBC. The risk of recurrent PBC increases with time following a LTx, but does not correlate with the frequency of loss of liver function [59]. In a study by H. Chen et al (2020) [18], the incidence of PBC recurrence after transplantation increased as the time after surgery increased: 3.5% after one year, 8.1% after three years, and 21.8% after five years. Half of the patients show histological signs of PBC recurrence 10 years after surgery, but they rarely have clinical problems [24]. As the follow-up period has increased, it becomes evident that the return of this disease in the transplanted liver is not a rare complication [60].

Immunosuppressive therapy can affect the timing and rate of PBC recurrence. However, there is no consensus on this issue; there are contradictory views in the literature. Some authors have found differences in the frequency of this complication depending on the immunosuppressive drugs used [59], while others, and most of them, deny such a dependence [36, 38, 51, 61]. Calcineurin inhibitors were previously thought to be associated with the risk of relapsed PBC [38, 62]. Later it was found that they have no significant effect on the development of relapsing PBC [36].

Relatively high incidence of recurrent PBC was associated with early steroid withdrawal after liver transplantation and withdrawal of calcineurin inhibitors. According to T.C. Schreuder et al. (2009) [59], the risk factor for recurrent PBC is the use of tacrolimus rather than cyclosporine. Multivariate analysis has confirmed this position [37].

A.J. Montano-Loza et al. (2019) [63] suggest that cyclosporine treatment prevents recurrent PBC. On the contrary, T. Kogiso et al. (2017) [54] believe that initial treatment with cyclosporine is significantly ($P < 0.05$) associated with recurrent PBC. A Japanese multicenter study also found that cyclosporine was a risk factor for recurrent PBC. However, switching from tacrolimus to cyclosporine one year after LTx significantly reduced its risk [57].

Many authors attach great importance to the recipient age [49, 54]. Recipients younger than 48 years of age are considered to be at greater risk of recurrent PBC [57]. In a recent study, multivariate regression analysis showed that the age of recipients younger than 48 years was an independent risk factor for PBC recurrence ($P = 0.03$) [18]. A multicenter study including 785 patients with PBC who underwent LTx showed that tacrolimus use and

liver dysfunction early after surgery were associated with increased risk of PBC recurrence at a younger age [63].

The risk of development of relapsed PBC is also associated with many other factors: shorter operation time [54], persistence of serum antimitochondrial antibodies, higher serum immunoglobulin M level [54], mismatch with donor gender [54, 57, 64], different HLA types, HLA mismatch [42, 61, 65–67] and presence of HLA B60 [54].

The development of recurrent PBC is influenced not only by recipient factors, but also by donor factors. However, they are less numerous and less significant. These include the age of the donor and warm ischemia time [49].

Recurrent PBC diagnosis. The main indicator in recurrent PBC diagnosis is the presence of histological signs in puncture biopsies of the transplanted liver; biopsies are performed not earlier than the first three months after surgery [59]. In contrast to biopsies performed only after the appearance of liver dysfunction, regular protocol biopsies can detect recurrent PBC earlier [40]. Therefore, biopsies performed according to the protocol play a particularly large role in early diagnosis of PBC [59]. They allowed to detect the return of the disease in 14 (14%) patients, only two of them developed graft dysfunction [37]. Antimitochondrial antibodies are not a reliable marker of recurrence, as they can persist in patients' serum after surgery [24]. Thus, liver puncture biopsy is the gold diagnostic standard for recurrent PBC [18].

Pathomorphology of recurrent PBC. Granulomatous destructive cholangitis or florid duct lesion is the most specific histological sign of PBC and its recurrence [68]. Unfortunately, it is often absent from biopsy specimens and has been found in only 4.8% of patients in transplanted liver biopsy specimens, on average, 2.75 years after surgery. [46]. Nevertheless, many authors regard granulomatous cholangitis, although rare, as the most characteristic feature of recurrent PBC [45, 56, 62].

Epithelioid granulomas in the portal tract, a less specific and also rare histological sign, only suggest a return of PBC. They were found in 3.8% of patients, on average, 2.75 years after surgery.

Other signs of recurrent PBC include bile duct injury and loss, chronic portal inflammation, reactive changes in the bile ducts, and fibrosis. It should be kept in mind that in the transplanted liver, there is often not one but a combination of two or more diseases simultaneously, which makes it difficult or impossible to make an accurate histologic diagnosis of recurrent PBC. Therefore, there may be hypodiagnosis because of the rigorous approach to evaluating histologic criteria and the short follow-up time for patients [46].

P.B. Sylvestre et al. (2003) [56] compared the morphology of transplanted liver biopsy specimens from 100 patients with PBC and 35 patients whose primary native liver disease was different. In the protocol biopsy specimens of the transplanted livers of the 100 patients with PBC, 14 of them showed florid duct lesion, and three had destructive lymphocytic cholangitis within dense portal infiltrate. In the control group of 35 patients, no such lesions were found.

Differential diagnosis. At differential diagnosis it is necessary to exclude viral hepatitis, biliary obstruction and acute or chronic rejection [68], graft-versus-host disease, drug influences [69], also hepatitis or injury to the large bile duct [68]. Differentiating PBC return from other late complications, more often from rejection, is difficult. Recurrent PBC is particularly difficult to differentiate from chronic rejection [38]. Therefore, the Banff Working Group on Liver Allograft Pathology proposes a set of consensus criteria for the most common and problematic causes of late liver allograft dysfunction occurring more than one year after surgery [70].

CONCLUSION

Primary biliary cholangitis was for a long time one of the leading indications for liver transplantation and accounted for up to 50% of such operations. Currently, there has been a significant decrease in the number of LTx in PBC. This is associated with early diagnosis and timely initiation of treatment with ursodeoxycholic acid and other drugs. However, the disease proceeds into an end stage in about 40% of patients, which still requires LTx. Patient and graft survival rates exceed 90% and 75%. After surgery, the frequencies of infection and biliary complications are 62% and 21%, respectively. In recent years, the problem of PBC recurrence following a LTx has been increasingly recognized as a cause of graft dysfunction, death and the reason for repeated transplantation. The risk of recurrent PBC increases with time after LTx. The presence of histological signs in puncture biopsies of the transplanted liver is the main method of diagnosis. It is necessary to consider that in the transplanted liver, there is often not one but a combination of two or more diseases simultaneously, which makes it difficult or impossible to make an accurate histological diagnosis of a recurrent PBC. To timely diagnose recurrent PBC, the Banff Working Group on Liver Allograft Pathology proposes a set of consensus criteria for the most common and problematic causes of liver allograft dysfunction occurring more than one year after surgery.

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REFERENCES

1. *Milkiewicz P.* Liver transplantation in primary biliary cirrhosis. *Clin Liver Dis*. 2008; 12 (2): 461–472. doi: 10.1016/j.cld.2008.02.015.
2. *Boonstra K, Beuers U, Ponsioen CY*. Epidemiology of primary sclerosing cholangitis and primary biliary cir-

rhosis: a systematic review. *J Hepatol*. 2012 May; 56 (5): 1181–1188. doi: 10.1016/j.jhep.2011.10.025.
3. *Griffiths L, Dyson JK, Jones DE.* The new epidemiology of primary biliary cirrhosis. *Semin Liver Dis*. 2014 Aug; 34 (3): 318–328. doi: 10.1055/s-0034-1383730.
4. *Kuiper EM, Hansen BE, de Vries RA, den Ouden-Muller JW, van Ditzhuijsen TJ et al.* Im-proved prognosis of patients with primary biliary cirrhosis that have a biochemical response to ursodeoxycholic acid. *Gastroenterology*. 2009; 136: 1281–1287.
5. *Schoning W, Schmeding M, Ulmer F, Andert A, Neumann U.* Liver transplantation for patients with cholestatic liver diseases. *Viszeralmedizin*. 2015; 31: 194–198. doi: 10.1159/000431017.
6. *Sun CK, Chen CL, Concejero AM, Wang CC, Wang SH, Liu YW et al.* Liver transplantation for primary biliary cirrhosis in a hepatitis endemic region: a single-center Asian experience. *Clinical Transplantation*. 2011; 25: 47–53. doi: 10.1111/j.1399-0012.2010.01288.x.
7. *Harms MH, Janssen QP, Adam R, Duvoux C, Mirza D, Hidalgo E et al.* European Liver and Intestine Transplant Association (ELITA). Trends in liver transplantation for primary biliary cholangitis in Europe over the past three decades. *Aliment Pharmacol Ther*. 2019 Feb; 49 (3): 285–295. doi: 10.1111/apt.15060.
8. *Lee J, Belanger A, Doucette JT, Stanca C, Friedman S, Bach N*. Transplantation trends in primary biliary cirrhosis. *Clin Gastroenterol Hepatol*. 2007 Nov; 5 (11): 1313–1315. doi: 10.1016/j.cgh.2007.07.015.
9. *Pares A, Caballeria L, Rodes J.* Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic Acid. *Gastroenterology*. 2006; 130 (3): 715–720. doi: 10.1053/j.gastro.2005.12.029.
10. *Corpechot C, Abenavoli L, Rabahi N, Chretien Y, Andreani T, Johanet C et al.* Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. *Hepatology*. 2008; 48 (3): 871–877. doi: 10.1002/hep.22428.
11. *Lammers WJ, van Buuren HR, Hirschfield GM, Janssen HL, Invernizzi P, Mason AL et al.* Global PBC Study Group. Levels of alkaline phosphatase and bilirubin are surrogate end points of outcomes of patients with primary biliary cirrhosis: an international follow-up study. *Gastroenterology*. 2014 Dec; 147 (6): 1338–1349.e5; quiz e15. doi: 10.1053/j.gastro.2014.08.029.
12. *Poupon R, Ping C, Chretien Y, Corpechot C, Chazouilleres O, Simon T et al*. Genetic factors of susceptibility and of severity in primary biliary cirrhosis. *J Hepatol*. 2008; 49: 1038–1045.
13. *Hohenester S, Oude-Elferink RP, Beuers U.* Primary biliary cirrhosis. *Semin Immunopathol*. 2009; 31: 283–307.
14. *Akamatsu N, Sugawara Y.* Primary biliary cirrhosis and liver transplantation. *Intractable & Rare Diseases Research*. 2012; 1: 66–80. doi: 10.5582/irdr.2012.v1.2.66.
15. *Aguilar MT, Carey EJ*. Current Status of Liver Transplantation for Primary Biliary Cholangitis. *Clin Liver Dis*. 2018 Aug; 22 (3): 613–624. doi: 10.1016/j.cld.2018.03.011.
16. *Ter Borg PC, Schalm SW, Hansen BE, van Buuren HR*. Dutch PBC Study Group. Prognosis of ursodeoxycholic Acid-treated patients with primary biliary cirrhosis. Results of a 10-yr cohort study involving 297 patients. *Am J Gastroenterol*. 2006 Sep; 101 (9): 2044–2050. doi: 10.1111/j.1572-0241.2006.00699.x.
17. *Hirschfield GM, Dyson JK, Alexander GJM, Chapman MH, Collier J, Hübscher S et al.* The British Society of Gastroenterology/UK-PBC primary biliary cholangitis treatment and management guidelines. *Gut*. 2018 Sep; 67 (9): 1568–1594. doi: 10.1136/gutjnl-2017-315259.
18. *Chen L, Shi X, Lv G, Sun X, Sun C et al.* The long-term outcomes of deceased-donor liver transplantation for primary biliary cirrhosis: a two-center study in China. *PeerJ*. 2020; 8: e9563. Published 2020 Aug 19. doi: 10.7717/peerj.9563.
19. *Singal AK, Fang X, Kaif M, Hasanin M, Mcguire BM, Kuo YF, Wiesner RH.* Primary biliary cirrhosis has high wait-list mortality among patients listed for liver transplantation. *Transpl Int*. 2017 May; 30 (5): 454–462. doi: 10.1111/tri.12877.
20. *Murillo Perez CF, Goet JC, Lammers WJ, Gulamhusein A, van Buuren HR, Ponsioen CY et al.* GLOBAL PBC Study Group. Milder disease stage in patients with primary biliary cholangitis over a 44-year period: A changing natural history. *Hepatology*. 2018 May; 67 (5): 1920–1930. doi: 10.1002/hep.29717.
21. *Webb GJ, Rana A, Hodson J, Akhtar MZ, Ferguson JW, Neuberger JM et al.* Twenty-Year Comparative Analysis of Patients With Autoimmune Liver Diseases on Transplant Waitlists. *Clin Gastroenterol Hepatol*. 2018 Feb; 16 (2): 278–287.e7. doi: 10.1016/j.cgh.2017.09.062.
22. *Lleo A, Jepsen P, Morenghi E, Carbone M, Moroni L, Battezzati PM et al.* Evolving Trends in Female to Male Incidence and Male Mortality of Primary Biliary Cholangitis. *Sci Rep*. 2016 May 19; 6: 25906. doi: 10.1038/srep25906.
23. *Silveira MG, Talwalkar JA, Lindor KD, Wiesner RH.* Recurrent primary biliary cirrhosis after liver transplantation. *Am J Transplant*. 2010; 10: 720–726.
24. *Mac Quillan GC, Neuberger J*. Liver transplantation for primary biliary cirrhosis. *Clin Liver Dis*. 2003; 7: 941–956.
25. *Naik P, Sritharan V, Bandi P, Madhavarapu M.* A single centre prospective study of liver function tests in post liver transplant patients. *Indian Journal of Clinical Biochemistry*. 2013; 28: 38–45. doi: 10.1007/s12291-012-0245-4.
26. *Zhang W, Wang M, Xie HY, Zhou L, Meng XQ, Shi J, Zheng S.* Role of reactive oxygen species in mediating hepatic ischemia-reperfusion injury and its therapeutic applications in liver transplantation. *Transplantation Proceedings*. 2007; 39: 1332–1337. doi: 10.1016/j.transproceed.2006.11.021.
27. *Li C, Wen TF, Mi K, Wang C, Yan LN, Li B.* Analysis of infections in the first 3-month after living donor liver transplantation. *World Journal of Gastroenterology*. 2012; 18: 1975–1980. doi: 10.3748/wjg.v18.i16.1975.
28. *Vera A, Contreras F, Guevara F.* Incidence and risk factors for infections after liver transplant: single-center ex-

perience at the University Hospital Fundacion Santa Fe de Bogota, Colombia. *Transplant Infectious Disease*. 2011; 130: 608–615. doi: 10.1111/j.1399-3062.2011.00640.x.
29. *Wojcicki M, Milkiewicz P, Silva M.* Biliary tract complications after liver transplantation: a review. *Digestive Surgery*. 2008; 25: 245–257. doi: 10.1159/000144653.
30. *Khalaf H.* Vascular complications after deceased and living donor liver transplantation: a single-center experience. *Transplantation Proceedings*. 2010; 42: 865–870. doi: 10.1016/j.transproceed.2010.02.037.
31. *Mejia GA, Olarte-Parra C, Pedraza A, Rivera JB, Benavides CA*. Biliary complications after liver transplantation: incidence. Risk factors and impact on patient and graft survival. *Transplantation Proceedings*. 2016; 48: 665–668. doi: 10.1016/j.transproceed.2016.02.033.
32. *Ma L, Lu Q, Luo Y.* Vascular complications after adult living donor liver transplantation: evaluation with ultrasonography. *World Journal of Gastroenterology*. 2016; 22: 1617–1626. doi: 10.3748/wjg.v22.i4.1617.
33. *Kashyap R, Safadjou S, Chen R, Mantry P, Sharma R, Patil V et al.* Living donor and deceased donor liver transplantation for autoimmune and cholestatic liver diseases – an analysis of the UNOS database. *Journal of Gastrointestinal Surgery*. 2010; 14: 1362–1369. doi: 10.1007/s11605-010-1256-1.
34. *Liberal R, Zen Y, Mieli-Vergani G, Vergani D.* Liver transplantation and autoimmune liver diseases. *Liver Transplantation*. 2013; 19: 1065–1077. doi: 10.1002/lt.23704.
35. *Maheshwari A, Yoo HY, Thuluvath PJ*. Long-term outcome of liver transplantation in patients with PSC: a comparative analysis with PBC. *Am J Gastroenterol*. 2004; 99: 538–542.
36. *Jacob DA, Neumann UP, Bahra M et al*. Liver transplantation for primary biliary cirrhosis: influence of primary immunosuppression on survival. *Transplant Proc*. 2005; 37: 1691–1692.
37. *Jacob DA, Neumann UP, Bahra M et al*. Long-term follow-up after recurrence of primary biliary cirrhosis after liver transplantation in 100 patients. *Clin Transpl*. 2006; 20: 211–220.
38. *Neuberger J, Gunson B, Hubscher S, Nightingale P*. Immunosuppression affects the rate of recurrent primary biliary cirrhosis after liver transplantation. *Liver Transpl*. 2004; 10: 488–491.
39. *Ziarkiewlcz-Wrobiewska B, Wroblewskl T, Wasiutynski A*. Morphological features and differential diagnosis of hepatitis C recurrence after liver transplantation – literature review and results of single transplantation center. *Ann Transplant*. 2008; 13 (2): 12–20.
40. *Charatcharoenwitthaya P, Pimentel S, Talwalkar JA et al*. Long-term survival and impact of ursodeoxycholic acid treatment for recurrent primary biliary cirrhosis after liver transplantation. *Liver Transpl*. 2007; 13: 1236–1245.
41. *Haga H, Miyagawa-Hayashino A, Taira K et al*. Histological recurrence of autoimmune liver diseases after living-donor liver transplantation. *Hepatol Res*. 2007; 37: 463–469.
42. *Hashimoto E, Taniai M, Yatsuji S et al*. Long-term clinical outcome of living-donor liver transplantation for primary biliary cirrhosis. *Hepatol Res*. 2007; 37: 455–461.
43. *Yamagiwa S, Ichida T*. Recurrence of primary biliary cirrhosis and primary sclerosing cholangitis after liver transplantation in Japan. *Hepatol Res*. 2007; 37: 449–454.
44. *Duclos-Vallee JC, Sebagh M*. Recurrence of autoimmune disease, primary sclerosing cholangitis, primary biliary cirrhosis, and autoimmune hepatitis after liver transplantation. *Liver Transpl*. 2009; 15 (Suppl 2): S25–34. doi: 10.1002/lt.21916.
45. *Neuberger J*. Liver transplantation for primary biliary cirrhosis: indications and risk of recurrence. *J Hepatol.* 2003; 39: 142–148.
46. *Hytiroglou P, Gutierrez JA, Freni M et al*. Recurrence of primary biliary cirrhosis and development of autoimmune hepatitis after liver transplant: A blind histologic study. *Hepatol Res*. 2009; 39 (6): 577–584.
47. *Mendes F, Couto CA, Levy C*. Recurrent and *de novo* autoimmune liver diseases. *Clin Liver Dis*. 2011; 15 (4): 859–878. doi: 10.1016/j.cld.2011.08.008.
48. *Carbone M, Neuberger J*. Liver transplantation in PBC and PSC: Indications and disease recurrence. *Clin Res Hepatol Gastroenterol*. 2011; 35: 446–454. doi: 10.1016/j.clinre.2011.02.007.
49. *Liermann Garcia RF, Evangelista Garcia C, McMaster P, Neuberger J*. Transplantation for primary biliary cirrhosis: Retrospective analysis of 400 patients in a single center. *Hepatology*. 2001; 33: 22–27.
50. *Kurdow R, Marks HG, Kraemer-Hansen H et al*. Recurrence of primary biliary cirrhosis after orthotopic liver transplantation. *Hepatogastroenterology*. 2003; 50: 322–325.
51. *Levitsky J, Hart J, Cohen SM, Te HS*. The effect of immunosuppressive regimens on the recurrence of primary biliary cirrhosis after liver transplantation. *Liver Transpl*. 2003; 9: 733–736.
52. *Guy JE, Qian P, Lowell JA, Peters MG*. Recurrent primary biliary cirrhosis: peritransplant factors and ursodeoxycholic acid treatment post-liver transplant. *Liver Transpl*. 2005; 11: 1252–1257.
53. *Gautam M, Cheruvattath R, Balan V*. Recurrence of autoimmune liver disease after liver transplantation: a systematic review. *Liver Transpl*. 2006; 12 (12): 1813–1824.
54. *Kogiso T, Egawa H, Teramukai S, Taniai M, Hashimoto E et al*. Risk factors for recurrence of primary biliary cholangitis after liver transplantation in female patients: A Japanese multicenter retrospective study. *Hepatol Commun*. 2017 May 16; 1 (5): 394–405. doi: 10.1002/hep4.1037.
55. *Yamashiki N, Haga H, Ueda Y, Ito T, Yagi S et al*. Use of Nakanuma staging and cytokeratin 7 staining for diagnosing recurrent primary biliary cholangitis after living-donor liver transplantation. *Hepatol Res*. 2020 Apr; 50 (4): 478–487. doi: 10.1111/hepr.13476.
56. *Sylvestre PB, Batts KP, Burgart LJ et al*. Recurrence of primary biliary cirrhosis after liver transplantation: his-

tologic estimate of incidence and natural history. *Liver Transpl*. 2003; 9: 1086–1093.
57. *Egawa H, Sakisaka S, Teramukai S, Sakabayashi S, Yamamoto M, Umeshita K et al.* Long-term outcomes of living-donor liver transplantation for primary biliary cirrhosis: a Japanese multi-center study. *Am J Transplant*. 2016; 16: 1248–1257.
58. *Bosch A, Dumortier J, Maucort-Boulch D, Scoazec JY, Wendum D, Conti F et al.* Preventive administration of UDCA after liver transplantation for primary biliary cirrhosis is associated with a lower risk of disease recurrence. *Journal of Hepatology*. 2015; 63: 1449–1458. doi: 10.1016/j.jhep.2015.07.038.
59. *Schreuder TC, Hubscher SG, Neuberger J*. Autoimmune liver diseases and recurrence after orthotopic liver transplantation: what have we learned so far? *Transpl Int*. 2009; 22: 144–152.
60. *Kotlyar DS, Campbell MS, Reddy KR*. Recurrence of diseases following orthotopic liver transplantation. *Am J Gastroenterol*. 2006; 101: 1370–1378.
61. *Sanchez EQ, Levy MF, Goldstein RM et al*. The changing clinical presentation of recurrent primary biliary cirrhosis after liver transplantation. *Transplantation*. 2003; 76: 1583–1588.
62. *Khettry U, Anand N, Faul PN, Lewis WD, Pomfret EA, Pomposelli J et al*. Liver transplantation for primary biliary cirrhosis: a long-term pathologic study. *Liver Transpl*. 2003; 9: 87–96.
63. *Montano-Loza AJ, Hansen BE, Corpechot C, Roccarina D, Thorburn D, Trivedi P et al*. Global PBC Study Group. Factors Associated With Recurrence of Primary Biliary Cholangitis After Liver Transplantation and Effects on Graft and Patient Survival. *Gastroenterology*. 2019 Jan; 156 (1): 96–107.e1. doi: 10.1053/j.gastro.2018.10.001.
64. *Grąt M, Lewandowski Z, Patkowski W, Wronka KM, Grąt K, Krasnodębski M et al.* Relevance of male-to-female sex mismatch in liver transplantation for primary biliary cirrhosis. *Ann Transplant*. 2015; 20: 116–123.
65. *Morioka D, Egawa H, Kasahara M, Jo T, Sakamoto S, Ogura Y et al.* Impact of human leukocyte antigen mismatching on outcomes of living donor liver transplantation for primary biliary cirrhosis. *Liver Transpl*. 2007; 13: 80–90.
66. *Balan V, Ruppert K, Demetris AJ, Ledneva T, Duquesnoy RJ, Detre KM et al.* Long-term outcome of human leukocyte antigen mismatching in liver transplantation: results of the National Institute of Diabetes and Digestive and Kidney Diseases Liver Transplantation Database. *Hepatology*. 2008; 48: 878–888.
67. *Manousou P, Arvaniti V, Tsochatzis E, Isgro G, Jones K, Shirling G et al*. Primary biliary cirrhosis after liver transplantation: influence of immunosuppression and human leukocyte antigen locus disparity. *Liver Transpl*. 2010; 16: 64–73.
68. *Hubscher SG, Portmann BC*. Transplantation pathology. In: Burt A.D., Portmann B.C., Ferrell L.D. editors. MacSween's Pathology of the Liver. 5th edn. London: Churchill Livingstone. 2007: 815–879.
69. *Faust ThW*. Recurrent Primary Biliary Cirrhosis, Primary Sclerosing Cholangitis, and Autoimmune Hepatitis After Transplantation. *Liver Transplantation*. 2001; 7 (11), Suppl 1: 99–108.
70. *Demetris AJ, Adeyi O, Bellamy CO, Clouston A, Charlotte F et al.* Liver biopsy interpretation for causes of late liver allograft dysfunction. Banff Working Group. *Hepatology*. 2006; 44 (2): 489–501. doi: 10.1002/hep.21280.

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HISTORY AND BACKGROUND OF KIDNEY TRANSPLANTATION IN UZBEKISTAN

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This paper presents a brief outline of the history of transplantation service in the Republic of Uzbekistan, which originated at the country's Center for Kidney Transplantation. The role played by outstanding scientists in Uzbekistan, their works and efforts towards the creation of a separate area of clinical and scientific medicine in Uzbekistan, are highlighted. Achievements by the research school of U.A. Aripov, Academician of the Academy of Sciences of the Republic of Uzbekistan, who performed the first successful kidney transplantation in 1972, are shown. The ups and downs of national transplantation nephrology, as well as the birth of a national school of kidney transplantation, domiciled at the Vakhidov Republican Specialized Scientific and Practical Medical Center of Surgery, headed by academician F.G. Nazirov, and giving a stimulus to the "second breath" of the national school of transplantology, is reflected. Separate attention is devoted to the actual problems of national transplantology, moral, ethical and regulatory issues that inevitably accompany this scientific and clinical direction are reflected. Kidney transplant outcomes in Uzbekistan are given, the prospects for further scientific and clinical directions are indicated.

Keywords: kidney transplantation, kidney transplant history, living related donors, immunosuppressive therapy.

Kidney transplantation (KTx) is known to be the only radical way to help patients suffering from chronic progressive kidney disease. According to WHO, the number of patients today suffering from end-stage kidney disease in the world is above 4 million; meanwhile, the number of surgical interventions to replace an organ that has lost its functions is steadily growing. With improvement in surgical technique, as well as a more detailed understanding of the mechanisms of immunosuppression, KTx allows to achieve 5- and 10-year survival rates of 90% and 74%, respectively (according to UNOS, USA) [1].

At the same time, the incidence of chronic progressive kidney disease, leading to chronic kidney disease (CKD) in its later stages, does not tend to decrease. In addition, due to the constantly expanding nosological range of diseases requiring KTx, donor organ shortage is still an acute problem. In connection with the above, the use of renal transplants from living related donors has acquired special relevance. Immediate restoration of transplanted kidney function and rare rejection crises in native transplantation, in addition to better immediate results, certainly make it possible to predict a higher long-term graft and patient survival rate than in cadaveric organ transplantation. This is associated not only with a higher degree of donor-recipient immunological compatibility, but also largely determined by reduced cold ischemia time and, accordingly, lower severity of reperfusion injuries [2].

The history of kidney transplantation in Uzbekistan is closely connected with the name, Uktam Aripovich Aripov, Academician of the Academy of Sciences of the Republic of Uzbekistan. In 1964, Aripov, having become the First Deputy Minister of Health of Uzbekistan, made great efforts to develop specialized medical services and train highly qualified scientific and pedagogical personnel to meet the country's needs. Being a polyvalent surgeon who performed the most complicated surgeries, he created all conditions for a comprehensive study of the pressing issues in abdominal surgery and transplantology. From 1971 to 1984, Aripov, being the rector of Tashkent State Medical University, organized a problematic research laboratory on overcoming tissue incompatibility in transplanted organs and tissues, he was also appointed to head the first kidney transplant center in Central Asia with a hemodialysis laboratory. At this center, he formed a team of young and talented like-minded scientists, uniting doctors of various specialties. Here, the problems of transplantation immunity, development of home-made immunosuppressants, clinical transplantology and treatment of CKD patients were developed. So, the first kidney transplant was performed in Uzbekistan on September 14, 1972, for end-stage CKD [3].

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Fig. 1. Academician U.A. Aripov

In 1974, Uktam Aripov was elected an Academician of the Academy of Sciences of the Republic of Uzbekistan and in 1978 he was elected an honorary doctor at the research-led medical Semmelweis University in Budapest. In 1983, Academician Aripov and a number of his coworkers were awarded the Beruni State Prize of Uzbekistan in Science and Technology for the development and introduction into clinical practice of new improved methods of treating CKD patients and for creation of domestic drugs [3].

So, in 1972, on the basis of the Problem Research Laboratory on overcoming tissue incompatibility in organ and tissue transplantation of the Tashkent Order of the Red Banner of Labor State Medical Institute, the Republican Center for Kidney Transplantation was organized. This center was a therapeutic, consultative, scientific, and educational center for the treatment of CKD patients [4].

The 40-bed kidney transplant center was located at the Clinical Hospital of the Uzbek SSR Ministry of Health. The work of the Center was headed by Academician of the Academy of Sciences of the Uzbek SSR, Uktam Aripov and professor N.P. Pak. Two senior and two junior researchers, and 11 residents worked at the Center. In addition, work at the Center was done in close cooperation with the Republican Nephrological Center [4].

The senior researcher and head of the clinical group on kidney transplantation of the Tashkent Kidney Transplantation Center was Pak Nikolay Petrovich, a laureate of the Beruni State Prize of Uzbekistan in Science and Technology, a medical doctor, a professor, an Academician of the International Academy of Sciences of Nature and Society, an Academician of the European Academy of Natural Sciences. Professor Pak was the chief specialist on hemodialysis and kidney transplantation at the Ministry of Health of the Republic of Uzbekistan for 19 years.

In addition, Uktam Aripov's initiative and scientific interest saw him forming his own scientific school and nurturing talented students. Such scientists as Academician M.S. Abdullakhojaeva, Professor R.N. Akalaev, Immunologist Professor F.Y. Garib, Dr. A.V. Barabash, Dr. K.G. Urazmetov, M.D. Urazmetova, Dr. B.F. Islamov and others worked under Uktam Aripov's leadership. In clinical and experimental studies, they showed that the drug gamma globulin, derived from a mixture of placental blood serum in contrast to similar drugs from venous (donor) blood, at 0.5–1.0 mg/mL, can inhibit the proliferative response of human lymphocytes to transplant antigens in mixed lymphocyte culture.

According to the results of the research activities of the above-mentioned scientists, there were formed and subsequently presented materials concerning kidney transplant complications in the clinic, results of studying the toxicity of "middle molecules" in CKD, experimental studies of the mechanisms of action of a number of drugs considered as potential immunomodulators.

A separate contribution to the development of the direction made by top doctor Evgenia Kholodova, who worked for more than 20 years as head of the department of hemodialysis and rehabilitation of transplant recipients in Republican Clinical Hospital No. 1 in Tashkent (formerly Kidney Transplant Center). For her excellent work as head of the Center, she was awarded the state Order of Mehnat Shukhrati award.

Some of Aripov's followers and students continued the program initiated by him. For example, Professor Dmitriy Arustamov, Honored Worker of Health of the Republic of Uzbekistan, laureate of the Abu Rayhan Beruyun Award, recipient of the Dustlik Order, was the director of the Republican Specialized Center for Urology. As head of the Department of Urology at the Tashkent State Medical Institute, he was the director of the Republican Specialized Center of Urology. Under his leadership, a number of scientific works, monographs, and methodological guidelines were written, author's

Fig. 2. Professor N.P. Pack

Fig. 3. E.G. Kholodova, Head of Department of Hemodialysis and Rehabilitation of Transplant Recipients.

certificates for inventions on organ and tissue transplantation were obtained.

The structure of the Nephrology Center as part of the organizational medical department included: a unit for kidney disease patients without renal failure, a unit for CKD patients, a unit for acute renal failure, a chronic dialysis unit, and a kidney transplant center.

The kidney transplant center itself included clinical groups: preoperative patient preparation, hemodialysis group, kidney harvesting and preservation group, postoperative patient management group, tissue typing and immunologic monitoring group.

The preoperative preparation group examined potential recipients, prepared them for kidney transplantation by conservative measures, and counseled patients in the units of the nephrology center and in other medical institutions.

The hemodialysis group was involved in hemodialysis programming and direct administration. The laboratory had a central dialysis fluid preparation station and 14 hemodialysis sites. During existence of the Center, the staff of the hemodialysis laboratory introduced such progressive and economic forms of treatment of patients as creation of various modifications of arterial-venous fistulas, transfer of some patients to outpatient hemodialysis, wide use of special methods of hemodialysis – diafiltration and hemosorption.

The kidney harvesting and preservation group had close ties with the intensive care, trauma and neurosurgical departments of city hospitals; it performed kidney harvesting and preservation for subsequent transplantation.

The postoperative management group was directly involved in nursing patients after surgical interventions, in issues of immunosuppressive therapy and in prevention of complications.

The patients spent their first post-kidney transplant month in wards of increased box sterility with a doctor, a nurse and a medical assistant, who were all on duty 24 hours a day.

The tissue typing and immunological control group conducted immunological study of potential recipients, dealt with selection of donor-recipient pairs, and carried out postoperative monitoring of the effectiveness of immunosuppressive therapy.

Hemodialysis units were opened in Samarkand, Fergana, Andijan, Bukhara, Almaty, Ashkhabad, Dushanbe, and Chimkent with the direct assistance and participation of the Center staff. The Center had a close connection with the Kidney Transplant Center of Almaty, with which donor kidneys and information about recipients was exchanged [4].

The Center provided consultative assistance in all regions of the country, as well as in the regions of neighboring Central Asian countries. Every year, the Center's staff attended to 200 calls by air ambulance.

Scientific research was conducted by the Center staff under a comprehensive plan to the assignment of the State Committee on Science and Technology under the USSR Council of Ministers, Uzbek SSR Ministry of Health and the All-Union Scientific Council for Transplantation and the Creation of Artificial Organs.

A search for and introduction of new immunosuppressive drugs into clinical practice was one of the problems of transplantology that required urgent attention. The accumulated experience allowed the native school of transplantology not only to study the effect of pharmaceutical drugs and their influence on the mechanisms of immunosuppression but also to synthesize their own drugs. So, in the 80s, the Institute of Bioorganic Chemistry of Uzbek SSR Academy of Sciences already had

Fig. 4. Prof. D.L. Arustamov

10 names of their own immunosuppressants in its arsenal. In turn, preparations batriden and megosyn were selected for clinical study, the first of which after clinical testing was approved for medical use as an immunosuppressant in kidney transplants and for clinical study in autoimmune diseases.

To solve the problems of prevention and treatment of immunosuppressive therapy complications in patients after KTx, the Kidney Transplant Center together with the Institute of Bioorganic Chemistry of Uzbek SSR Academy of Sciences and the Laboratory of Physical and Chemical Research Methods of the Central Research Laboratory, studied the metabolic pathways of immunosuppressants, target organs for aggressive metabolites of these drugs, search for possible protectors from adverse effects of immunosuppressors.

The accumulated experience also allowed to raise the scientific potential to an entirely new level; so by 1982, research workers at the Center defended two doctoral and nine phd dissertations, published two monographs and five methodical guidelines, published over 200 research papers; together with the tissue incompatibility problem laboratory, eight collections of scientific works were published.

The Tashkent Kidney Transplant Center hosted a meeting of experts of CMEA (Council for Mutual Economic Assistance) on KTx problems in 1977, and an All-Union Conference "Immunosuppression in allotransplantation" was held in 1979.

It should be noted separately the number of procedures performed; in 1972 the Center performed two KTx and 300 hemodialysis sessions, but by 1981 the number of kidney transplants had reached 227 (20–25 operations a year), and hemodialysis sessions had reached 25,500. The average 6-month survival rate was 65% [4].

The Center was frequently visited by foreign delegations and its staff had ties with the Semmelweis Medical University in Budapest (an agreement was signed on scientific cooperation with the renal transplant centers of the GDR, Poland, and Czechoslovakia). The Center's staff had frequent visits from foreign delegations to the Budapest Semmelweis Medical University (an agreement on scientific cooperation was signed), kidney transplantation centers in East Germany, Poland, and Czechoslovakia.

However, adoption of the Criminal Code of the Republic of Uzbekistan in its new version of 1994 had a significant impact on the entire kidney transplantation sector. The new law allowed the removal of organs from a corpse only with the permission of the relatives of the deceased or a consent given by the deceased while alive. This completely stopped kidney transplantation in our country.

During the existence of the Kidney Transplant Center, a total of 358 kidney transplants were performed. In 311 cases, cadaveric kidney transplants were performed, while 47 patients underwent kidney transplantation from a living related donor.

It was not until 2002 that the Ministry of Health issued an order authorizing kidney transplantation from a living related donor, which gave rise to a new program of living donation. The first such operation was performed at the Kidney Transplant Center (present-day Republican Clinical Hospital No. 1). However, 4 years later, this order was withdrawn, and such surgical interventions were stopped again. This situation, caused by lack of normative and legal support for the program, forced patients to seek help abroad.

Thus, as in other countries of the world, formation of organ and tissue transplantation service in Uzbekistan went through many difficult challenges in its development. The complicated logistical and political situation in post-Soviet countries at the end of the 1980s and beginning of the 1990s resulted in a complete loss of the previously created fundamental base and accumulated experience. Attempts to rehabilitate the transplantation service were faced with the lack of clearly formulated legislative acts, complicated moral and ethical background that excluded organ transplantation from brain-death donors.

Related transplantation remained the only available option for families with many children in Uzbekistan. Repeated attempts by academician Vasit Vakhidov, the director of the country's leading surgery center, to realize this direction were unsuccessful; more and more potential patients who needed this type of surgical treatment continued to go to foreign clinics. Only a decade later, his talented pupil, surgeon and organizer of health care, academician Feruz Nazirov managed to move this problem forward. At the cost of his enormous efforts, transplantology in Uzbekistan got a second wind. At the Vakhidov Republican Scientific and Practical Medical Center for Surgery (Vakhidov Center), the organ transplantation program was not only revived, but also took on completely new features.

Specialized departments were established, in particular, Department of Vascular Surgery and Kidney Trans-

Fig. 5. Academician V.V. Vakhidov

Fig. 6. Academician F.G. Nazyrov

plantation, headed until now by Professor Fazliddin Bahritdinov, and Department of Hemodialysis, headed by Ulugbek Yuldashev, presently head of Transplantology and Laboratory of the Republican Specialized Scientific and Practical Medical Center for Nephrology and Kidney Transplantation. Also there was a narrow profile transplantation commission, a material and technical base was strengthened, retraining of personnel was carried out in leading foreign clinics, indications for surgical intervention were expanded, a solid foundation for scientific work in an area which is very important for the country was created. All the above mentioned has seen some positive impacts not only by way of an increase in the number of interventions, but also in direct improvement in the quality of such interventions. Physicians started performing transplant surgeries in patients with diabetes mellitus and other comorbidities that previously did not allow one to count on a favorable outcome of the intervention.

Thus, since 2010, the Vakhidov Republican Specialized Scientific and Practical Medical Center of Surgery (Vakhidov Center) renewed kidney transplant surgeries, and in February 2018, after the publication of the draft law "on related kidney and liver lobe transplantation", academician F.G. Nazirov performed the first living related liver transplantation in the country. In addition, by the end of 2021, the number of kidney transplant operations performed at Vakhidov Center had reached 540.

At the same time, not only new areas of clinical transplantology were created, but also the already developed techniques were modified and improved. So, in 2015 the center performed the first kidney transplantation using laparoscopic donor nephrectomy.

The successes achieved at Vakhidov Center not only showed the safety, but also clearly demonstrated the need for further development of transplantology service in the country, being the impetus for further improvement of the legislative and regulatory framework. Thus, already in December 2019, amendments and additions were made to the draft law "on the order of related kidney and (or) liver lobe transplantation" significantly expanding the donor pool.

Currently, in Uzbekistan, surgical removal of organs for transplantation is possible only from living donors who are relatives of the recipient, and with their voluntary consent.

All patients with a transplanted kidney living in the Republic of Uzbekistan are registered at the department

Fig. 7. Staff at the Department of Vascular Surgery and Kidney Transplantation, headed by Prof. F.S. Bakhritdinov

of hemodialysis and rehabilitation of transplant recipients in the Republican Clinical Hospital No. 1 in Tashkent.

In addition, based on the results of scientific research at Vakhidov Center, two dissertations (PhD) have been defended and guidelines ("Optimization of tactical and technical aspects in kidney transplantation from a living related donor") developed. The proposed guidelines improved the efficiency of patient preparation for kidney transplantation and optimized the technical aspects of surgical intervention. A program for assessing the surgical risk of a kidney recipient was developed. Application of the proposed program with determination of 33 aggravating factors of the disease allows to estimate the surgical risk in the preoperative period and optimize the process of preparation for transplantation. The obtained scientific results have been implemented in the practical activity of public health service. For example, joint operations on kidney transplantation have been started in the regions (at the surgery department of Navoi Regional Multidisciplinary Medical Center and others). Implementation of the scientific research results allowed to reduce the incidence of complications in the early postoperative period from 29.5% to 11.2% and in the late period from 47.5% to 22.7% and overall mortality from 8.2% to 4.1%.

At the Vakhidov Center, teams of specialists of various fields also played a great role in the successful implementation of kidney transplants. They includes the department of General Resuscitation and Intensive Care under the leadership of Dr. Ibadov R.A., Department of Anesthesiology under the leadership of Dr. Nazirova L.A., Department of Hemodialysis under the leadership of Abdullaeva M.A., Department of Experimental Surgery under the leadership of Prof. Sadykov R.A., Department of Radioisotope Diagnostic Laboratory under the direction of Arifkhodjaev G.G., Biochemistry Department with a microbiology group under the leadership of Dr. Khaybullina Z.R., Functional Diagnostics Department under the leadership of Sharapov N.U., Department of Computed and Magnetic Resonance Imaging under the leadership of Dzhuraeva N.M., and Consultative and Diagnostic Department under the leadership of Ganihodjaev S.S.

In the development of transplantation of other organs, in particular, the liver, at the surgery center under the leadership of the director, academician F.G. Nazyrov, the chief researcher of the Department of Surgery of Portal Hypertension and Pancreatoduodenal Zone Dr. A.V. Devyatov, head of the Department of Liver and Bile Duct Surgery Dr. M.M. Akbarov and other employees of the departments contributed their share. In 2018 and 2019, 10 liver transplant surgeries were successfully performed.

Meanwhile, the work done has opened up additional opportunities in terms of development in this direction; so, in the future, the possibility of performing heart, cardiothoracic, pancreatic and lung transplantation surgeries is being considered.

In addition, organ and tissue transplantation is now being performed at several treatment and prevention centers in the country. For instance, a kidney transplantation program has been implemented at the Vakhidov Center since 2018 thanks to efforts by Khadzhibaev A.M.; achievements by the collective center correspond to those of leading international clinics.

At the same time, despite the successes achieved in the field of transplantology, there are still a number of unresolved issues in the country. Among these, issues with acute shortage of highly skilled specialists in the field of transplantology play a special role. It is known that transplantation service is not built only on transplant surgeons; for successful realization of the program, it is necessary to have morphologists, immunologists, specialists in the field of interventional interventions and many others. Thus, in spite of the work done, the development of this direction of clinical medicine undoubtedly requires even greater efforts aimed at creating and strengthening the solid material and technical base of specialized departments, improving its own scientific and clinical school. That said, one of the priority tasks can be considered the creation of a national transplantation center, allowing not only to unite specialists of different profiles specializing in clinical transplantology, but also allowing to significantly consolidate the efforts aimed at development of the national transplantology service.

Thus, related kidney transplantation, which opened the era of clinical transplantation of vital organs in the last century, has now acquired a new lease on life. Prospects for kidney transplantation in Uzbekistan in the 21st century are related to overcoming ethical problems of organ transplantation, improving tactical and technical aspects of KTx, opening new transplantation centers and centers for rehabilitation of organ recipients with all modern capabilities for examining and treating severe patients.

The authors declare no conflict of interest.

REFERENCES

1. *Parajuli S, Aziz F, Clark DF*. Kidney Transplant Management, Introduction to Kidney Transplantation. *Springer Nature Switzerland AG.* 2019; 1–3.
2. *Mojsjuk JaG, Sharshatkin AV, Arutjunjan SM.* Transplantacija pochki ot zhivogo rodstvennogo donora. *Nefrologija i dializ.* 2001; 3: 328–334.
3. Akademik Uktam Aripovich Aripov (k 90-letiju so dnja rozhdenija). *Annaly hirurgicheskoj gepatologii*. 2017; 1: 125–127.
4. *Pak NP, Abdurahmanov ShA.* Respublikanskij centr transplantacii pochek. T.: Prospekt, 1982. 15.

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APPLICATION OF INDOCYANINE GREEN FLUORESCENCE FOR URETER IMAGING: REVIEW

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Introduction. Indocyanine green has been used in medicine for more than 70 years in cardio-thoracic surgery, hepatobiliary and colorectal surgery, urology, gynecology, transplantology, etc. This review based on literature dates describing the use of ICG for intraoperative imaging and evaluation of the ureteral perfusion. **Method.** The research searched in the electronic database PubMed, Scopus, Elsevier, Springer, and Web of Science between January 2000 and December 2020, using the following search terms: ICG imaging, ureteral vessels. Additional literature data identified from separately published sources. **Results.** There are 21 articles were obtained in the specified database: 9 – with intravenous ICG, 12 – with intraureteral administration. The use of intravenous ICG followed by next clinical situation: ureteral imaging is described for ureteral strictures, for isolation and preservation of the ureteral branch of the uterine artery during radical hysterectomy, for robotic radical cystectomy with ureteroenterostomy, for laparoscopic removal of ureteral endometriosis, for evaluation of ureteral perfusion during kidney transplantation, and for identification and prevention of ureteral damage during pelvic surgery in patients with colorectal tumors and gynecological pathologies. **Conclusions.** Both intravenous and intraureteral ICG imaging are safe, easy to perform, and easily reproducible. It allows objectively identifying the degree of perfusion of the ureteral wall, clearly determining the boundaries of the stricture. It is effectively helps in the prevention of ureteral wall damage in extraurinal surgical interventions.

Keywords: ICG, ICG fluorescence, ureter detection.

INTRODUCTION

Indocyanine green (ICG) has been used in clinical medicine for more than 70 years, when it was first used to assess cardiac and hepatic function [1–3]. ICG is a tricarbocyanine molecule with a half-life of 150–180 seconds, completely excreted into the bile by the liver [4]. The literature also describes the metabolism of ICG in the renal parenchyma, which occurs with the participation of the protein bilitranslocase [5]. Intravenous administration of ICG is non-toxic and effective at low doses [6]. It was also shown that ICG has an effective intraoperative contrast identifier in real time with the possibility of penetration into tissues up to 5 mm [7].

The first report on the use of ICG for perfusion evaluation of a kidney transplant was reported in 2004 [8]. In the future, this method was used to assess the blood flow of the renal parenchyma during partial nephrectomy, donor nephrectomy, and vascular anastomoses of the renal graft [9–12]. Although there are many reports on the use of ICG in urology, there are few studies on the visualization and assessment of the ureteral condition.

This review focuses on the current literature that describes the use of ICG to intraoperative visualize and evaluate the condition of the ureter.

To do this, a search was conducted for studies in the electronic database PubMed, Scopus, Elsevier, Springer and Web of Science in the period from January 2000 to December 2020, using the following search terms: "ICG imaging, ureteral". Additional literature data were identified from separately published sources. As a result, 21 articles were received in the specified database. Of these, 9 articles describe the use of ICG imaging of the ureter with intravenous administration, 12 – with intraureteral administration.

VISUALIZATION OF THE URETER DURING INTRAVENOUS ADMINISTRATION OF ICG

Sekijima M. et al. reported in 2004 on the use of intravenous ICG (IV-ICG) to visualize the urinary system in a kidney transplant recipient. This method made it possible to record and reproduce images of organ vessels in real time during the operation [8].

IV-ICG imaging has proven to be particularly useful in robotic surgery in cases of ureteral scar strictures. At

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the same time, it allows you to completely resect the scar-altered ureteral tissue and perform ureteral reimplantation with preserved perfusion. Marc A. Bjurlin et al. 42 robotic operations for the reconstruction of the upper urinary tract were performed using this technique. At the same time, perioperative complications occurred in 14.3% of cases. The authors of this study hypothesized that the use of NIRF imaging can prevent postoperative ureteral stricture, especially during repeated operations, and also helps to ensure adequate blood flow in the area of the urinary anastomosis [13]. Although the authors indicate a lack of clinical data for long-term follow-up.

In two cases report by Ying Long et al. successful isolation and preservation of the ureteral branch of the uterine artery during radical hysterectomy with IV-ICG angiography for cervical cancer was demonstrated [14]. This procedure prevents damage to the ureteral branch with subsequent ischemia of the distal ureter leading to its necrosis and stricture. During 4 months of follow-up of these patients, there were no complications associated with the ureter.

After a robotic radical cystectomy with urinary diversion (RCUD), a severe complication is the development of ureteral anastomosis stricture. Jim K. Shen et al. conducted a comparative study of patients who underwent ureteroenterostomy in two groups of n = 93 in each: with IV-ICG imaging of the ureter, and without IV-ICG [15]. In the group of patients with IV-ICG imaging with a median follow-up of 12.0 months, there was no complication from ureteroenterostomy. While in the group without IV-ICG imaging (median follow-up 24.3 months), the number of complications was 7.5% (p = 0.01). Thus, the use of the IV-ICG method of visualization of ureteral perfusion allowed to significantly reduce the frequency of ureteroenterostomy strictures.

Another study analyzed 345 cases of ureteroenterostomy during robot-assisted radical cystectomy, of which 89 cases used IV-ICG imaging to assess vascularization of the distal ureter [16]. According to the results of this study, there was a significant decrease in the frequency of ureteral anastomosis strictures from 10.6% to 0% after IV-ICG evaluation of vascularization of the distal ureter. This highlights the clinical significance of this strategy for minimizing the complications of robot-assisted radical cystectomy.

The use of IV-ICG imaging of ureteral blood flow in open radical cystectomy with urinary diversion Chirag P. Doshi et al. studied in a group of 31 patients with 62 ureteroenteroanastomoses [17]. In the group with IV-ICG, ureteroenteroanastomosis stricture was observed in 3.2% of cases, in the group without IV-ICG – 16.7%. This study also confirms a decrease in the frequency of formation of ureteroenteroanastomosis structures when using the IV-ICG imaging technique to assess vascularization of the distal ureter.

Diego Raimondo et al. in preliminary study was conducted in 23 women who underwent laparoscopic removal of ureteral endometriosis using ICG for intraoperative assessment of ureteral perfusion [18]. Intraoperative assessment of the degree of ureteral blood flow made it possible to optimally choose the tactics of further surgical approach after removal of the endometrioid noudle: Double J ureteral stenting – 3 patients, ureteral stenting – 2 patients. In the remaining 28 cases (90.3%), the blood flow was assessed as satisfactory (regular fluorescence) and did not require any intervention. NIR-ICG has proven to be a safe and feasible tool for assessing residual ureteral vascularization after conservative surgery for ureteral endometriosis.

Currently, there are two preliminary studies on the use of ICG to assess ureteral perfusion in kidney transplantation. In the study (preliminary experience) Vignolini G. et al. 6 recipients with robotic kidney transplantation from a living donor were included [19]. The second study, conducted by H. Boullenois et al., included 11 recipients, 10 of which were conducted from a cadaveric donor (Fig. 1) [20]. In both studies, the use of ICG visualization of ureteral perfusion did not take time, and allowed objective and reliable visualization of ureteral vascularization. However, the correlation between ICG fluorescence and postoperative complications could not be studied due to the small number of patients, which requires further larger studies studies.

VISUALIZATION OF THE URETER DURING INTRAURETHRAL ADMINISTRATION OF ICG

Recently, intraurethral administration of ICG (IU-ICG) has been used for diagnostic purposes. The first mention in the available literature of ICG imaging with intraurethral administration describes a series of 7 patients with ureteral stricture who underwent robot-assisted reconstructive surgery [21]. Subsequently, intraoperative IU-ICG imaging was used to identify various ureteral pathologies in a larger cohort (8 and 25) of patients (Fig. 2) [22–25]. IU-ICG imaging made it possible to quickly and accurately identify the ureter, localize the level of structure with less tissue damage and protect its blood supply, and the technique itself is safe and easy to perform. This technique has been successfully used in operations on pathological kidneys [25, 26, 27].

The IU-ICG technique has been successfully used to identify and prevent ureteral damage during pelvic surgery in patients with colorectal tumors and gynecological pathologies [28–32]. Intraureteral ICG imaging was

effective for intraoperative identification of the ureter in complex gynecological and colorectal operations.

CONCLUSION

Thus, based on the described literature review, it can be concluded that ICG imaging (both intravenous and intraureteral) is safe, simple to perform and easily reproducible. It allows you to objectively identify both the degree of perfusion of the ureteral wall, and clearly determine the boundaries of the stricture. In case of extraurinary surgical interventions, such visualization effectively helps in the prevention of damage to the ureteral wall. Based on this, the use of ICG imaging in kidney transplant recipients will be the subject of our further study.

The authors declare no conflict of interest.

Fig. 1. Projection on a transplant diagram of an intraoperative image of indocyanin green fluorescence of a ureter in semi-quantitative analysis: a – complete and intense fluorescence of the ureter except the last centimeters in favor of good vascularization; b – partial and weak fluorescence of the ureter in favor of weak and partial vascularization; c – zero fluorescence of the ureterand the lower pole of the transplant in favor of no vascularization of the ureter and the lower pole of the transplant. Illustration: H. Boullenois et al. 2020

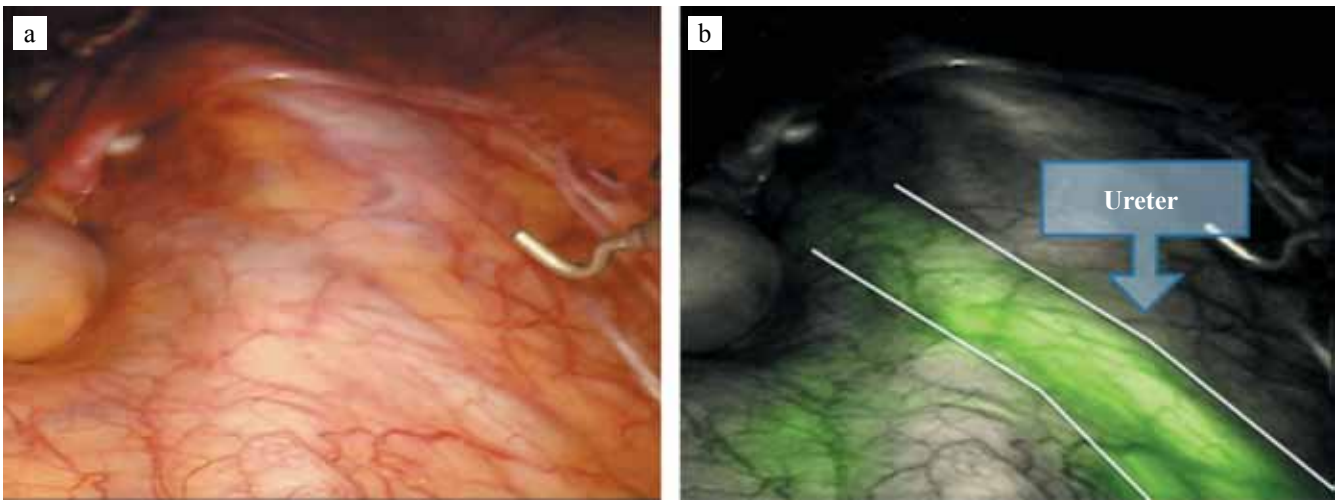

Fig. 2. Ureteral identification (a) in the absence of near-infrared fluorescence (NIRF) and (b) under NIRF. Reproduced with permission from Ziho Lee et al. 2015

REFERENCES

1. Symposium on diagnostic applications of indicator dilution techniques. *Proceedings of the Staff Meeting of Mayo Clinic*. 1957; 32: 463–508.
2. *Cherrick GR, Stein SW, Leevy CM, Davidson CS*. Indocyanine green: observations on its physical properties, plasma decay, and hepatic extraction. *J Clin Invest*. 1960; 39 (4): 592–600.
3. *Reinhart MB, Huntington CR, Blair LJ, Heniford BT, Augenstein VA*. Indocyanine Green: Historical Context, Current Applications, and Future Considerations. *Surgical Innovation*. 2016; 23 (2): 166–175. doi: 10.1177/1553350615604053.
4. *Kaplan-Marans E, Fulla J, Tomer N, Bilal K, Palese M*. Indocyanine Green (ICG) in Urologic Surgery. *Urology*. 2019 Oct; 132: 10–17. doi: 10.1016/j.urology.2019.05.008. Epub 2019 May 23. PMID: 31129192.
5. *Golijanin DJ, Marshall J, Cardin A, Singer EA, Wood RW, Reeder JE et al*. Bilitranslocase (BTL) is immunolocalised in proximal and distal renal tubules and absent in renal cortical tumors accurately corresponding to intraoperative near infrared fluorescence (NIRF) expression of renal cortical tumors using intravenous indocyanine green (ICG). *The Journal of Urology*. 2008; 179 (4): 137. doi: 10.1016/s0022-5347(08)60394-8.
6. *Majlesara A, Golriz M, Hafezi M, Saffari A, Stenau E, Maier-Hein L et al*. Indocyanine green fluorescence imaging in hepatobiliary surgery. *Photodiagnosis and Photodynamic Therapy*. 2017; 17: 208–215. doi: 10.1016/j.pdpdt.2016.12.005.
7. *Alander JT, Kaartinen I, Laakso A, Pätilä T, Spillmann T, Tuchin VV et al*. A review of indocyanine green fluorescent imaging in surgery. *Int J Biomed Imaging*. 2012; 2012: 940585. doi: 10.1155/2012/940585. Epub 2012 Apr 22. PMID: 22577366; PMCID: PMC3346977.
8. *Sekijima M, Tojimbara T, Sato S, Nakamura M, Kawase T, Kai K et al*. An intraoperative fluorescent imaging system in organ transplantation. *Transplant Proc*. 2004 Sep; 36 (7): 2188–2190. doi: 10.1016/j.transproceed.2004.09.001. PMID: 15518796.
9. *Tobis S, Knopf J, Silvers C, Yao J, Rashid H, Wu G, Golijanin D.* Near infrared fluorescence imaging with robotic assisted laparoscopic partial nephrectomy: initial clinical experience for renal cortical tumors. *J Urol*. 2011 Jul; 186 (1): 47–52. doi: 10.1016/j.juro.2011.02.2701. Epub 2011 May 14. PMID: 21571337.
10. *Boni L, David G, Mangano A, Dionigi G, Rausei S, Spampatti S et al.* Clinical applications of indocyanine green (ICG) enhanced fluorescence in laparoscopic surgery. *Surg Endosc*. 2015 Jul; 29 (7): 2046–2055. doi: 10.1007/s00464-014-3895-x. Epub 2014 Oct 11. PMID: 25303914; PMCID: PMC4471386.
11. *Aslim EJ, Lee FJ, Gan VHL*. The Utility of Intraoperative Near Infrared Fluorescence (NIR) Imaging with Indocyanine Green (ICG) for the Assessment of Kidney Allograft Perfusion. *J Transplant*. 2018 Aug 19; 2018: 6703056. doi: 10.1155/2018/6703056. PMID: 30210867; PMCID: PMC6120275.
12. *Arichi N, Mitsui Y, Ogawa K, Nagami T, Nakamura S, Hiraoka T et al.* Intraoperative fluorescence vascular imaging using indocyanine green for assessment of transplanted kidney perfusion. *Transplant Proc*. 2014; 46 (2): 342–345. doi: 10.1016/j.transproceed.2013.11.129. PMID: 24655959.
13. *Bjurlin MA, Gan M, McClintock TR, Volpe A, Borofsky MS, Mottrie A, Stifelman MD.* Near-infrared fluorescence imaging: emerging applications in robotic upper urinary tract surgery. *Eur Urol*. 2014 Apr; 65 (4): 793–801. doi: 10.1016/j.eururo.2013.09.023. Epub 2013 Sep 27. PMID: 24099660.
14. *Long Y, Yao Y, Yao DS*. Indocyanine green angiography for preserving the ureteral branch of the uterine artery during radical hysterectomy: Two case report. *Medicine (Baltimore)*. 2018 Oct; 97 (40): e12692. doi: 10.1097/MD.0000000000012692. PMID: 30290662; PMCID: PMC6200471.
15. *Shen JK, Jamnagerwalla J, Yuh BE, Bassett MR, Chenam A, Warner JN et al.* Real-time indocyanine green angiography with the SPY fluorescence imaging platform decreases benign ureteroenteric strictures in urinary diversions performed during radical cystectomy. *Ther Adv Urol*. 2019 Apr 4; 11: 1756287219839631. doi: 10.1177/1756287219839631. PMID: 31057669; PMCID: PMC6452578.
16. *Ahmadi N, Ashrafi AN, Hartman N, Shakir A, Cacciamani GE, Freitas D et al.* Use of indocyanine green to minimise uretero-enteric strictures after robotic radical cystectomy. *BJU Int*. 2019 Aug; 124 (2): 302–307. doi: 10.1111/bju.14733. Epub 2019 Apr 11. PMID: 30815976.
17. *Doshi CP, Wozniak A, Quek ML.* Near-infrared Fluorescence Imaging of Ureters With Intravenous Indocyanine Green During Radical Cystectomy to Prevent Ureteroenteric Anastomotic Strictures. *Urology*. 2020 Oct; 144: 220–224. doi: 10.1016/j.urology.2020.06.026. Epub 2020 Jun 30. PMID: 32619603.
18. *Raimondo D, Borghese G, Mabrouk M, Arena A, Ambrosio M, Del Forno S et al.* Use of Indocyanine Green for Intraoperative Perfusion Assessment in Women with Ureteral Endometriosis: A Preliminary Study. *J Minim Invasive Gynecol*. 2021 Jan; 28 (1): 42–49. doi: 10.1016/j.jmig.2020.04.004. Epub 2020 Apr 10. PMID: 32283326.
19. *Vignolini G, Sessa F, Greco I et al.* Intraoperative assessment of ureteral and graft reperfusion during robotic kidney transplantation with indocyanine green fluorescence videography. *Minerva Urologica e Nefrologica = The Italian Journal of Urology and Nephrology*. 2019 Feb; 71 (1): 79–84. doi: 10.23736/s0393-2249.18.03278-2.
20. *Boullenois H, Verrier C, Ingels A, Parier B, Serey-Eiffel S, Kozal S et al.* Visualiser la vascularisation urétérale des transplants rénaux par fluorescence au vert d'indocyanine: étude exploratoire [Indocyanine green fluorescence to visualize the ureteric vascularization of kidney transplants: An exploratory study]. *Prog Urol*. 2020 Mar; 30 (3): 155–161. French. doi: 10.1016/j.purol.2020.01.005. Epub 2020 Feb 28. PMID: 32122748.

21. *Lee Z, Simhan J, Parker DC, Reilly C, Llukani E, Lee DI et al.* Novel use of indocyanine green for intraoperative, real-time localization of ureteral stenosis during robot-assisted ureteroureterostomy. *Urology*. 2013 Sep; 82 (3): 729–733. doi: 10.1016/j.urology.2013.05.032. PMID: 23987169.
22. *Lee Z, Moore B, Giusto L, Eun DD.* Use of indocyanine green during robot-assisted ureteral reconstructions. *Eur Urol*. 2015 Feb; 67 (2): 291–298. doi: 10.1016/j.eururo.2014.08.057. Epub 2014 Sep 12. PMID: 25220372.
23. *Lee Z, Sterling ME, Keehn AY, Lee M, Metro MJ, Eun DD.* The use of indocyanine green during robotic ureteroenteric reimplantation for the management of benign anastomotic strictures. *World J Urol*. 2019 Jun; 37 (6): 1211–1216. doi: 10.1007/s00345-018-2493-2. Epub 2018 Sep 18. PMID: 30229414.
24. *Huang BW, Wang J, Zhang P, Li Z, Bi SC, Wang Q et al.* Application of indocyanine green in complex upper urinary tract repair surgery. *Beijing Da Xue Xue Bao Yi Xue Ban*. 2020 Aug 18; 52 (4): 651–656. Chinese. doi: 10.19723/j.issn.1671-167X.2020.04.010. PMID: 32773795; PMCID: PMC7433627.
25. *Morozov AO, Alyaev YG, Rapoport LM, Tsarichenko DG, Bezrukov EA, Butnaru DV, Sirota ES.* Near-Infrared Fluorescence with Indocyanine Green for Diagnostics in Urology: Initial Experience. *Urologia Journal*. 2017; 84 (3): 197–202. doi: 10.5301/uj.5000235.
26. *Lee M, Lee Z, Eun D.* Intraureteral and intravenous indocyanine green to facilitate robotic partial nephroureterectomy in a patient with complete ureteral triplication. *Korean J Urol*. 2015 Jun; 56 (6): 473–476. doi: 10.4111/kju.2015.56.6.473. Epub 2015 May 27. PMID: 26078846; PMCID: PMC4462639.
27. *Kanno T, Takahashi T, Somiya S, Ito K, Higashi Y, Yamada H.* Indocyanine Green Fluorescence-Guided Laparoscopic Lower-Pole Heminephrectomy for Duplex Kidney in Adult. *J Endourol Case Rep*. 2020 Dec 29; 6 (4): 384–387. doi: 10.1089/cren.2020.0123. PMID: 33457680; PMCID: PMC7803210.
28. *Siddighi S, Yune JJ, Hardesty J.* Indocyanine green for intraoperative localization of ureter. *Am J Obstet Gynecol*. 2014 Oct; 211 (4): 436.e1-2. doi: 10.1016/j.ajog.2014.05.017. Epub 2014 May 14. PMID: 24835212.
29. *Santi C, Casali L, Franzini C, Rollo A, Violi V.* Applications of indocyanine green-enhanced fluorescence in laparoscopic colorectal resections. *Updates Surg*. 2019 Mar; 71 (1): 83–88. doi: 10.1007/s13304-018-00609-w. Epub 2018 Dec 3. PMID: 30511261.
30. *Mandovra P, Kalikar V, Patankar RV.* Real-Time Visualization of Ureters Using Indocyanine Green During Laparoscopic Surgeries: Can We Make Surgery Safer? *Surg Innov*. 2019 Aug; 26 (4): 464–468. doi: 10.1177/1553350619827152. Epub 2019 Feb 8. PMID: 30734638.
31. *White LA, Joseph JP, Yang DY, Kelley SR, Mathis KL, Behm K, Viers BR.* Intraureteral indocyanine green augments ureteral identification and avoidance during complex robotic-assisted colorectal surgery. *Colorectal Dis*. 2021 Mar; 23 (3): 718–723. doi: 10.1111/codi.15407. Epub 2020 Nov 5. PMID: 33064915.
32. *Kamada T, Nakaseko Y, Yoshida M, Kai W, Takahashi J, Nakashima K et al.* Indocyanine green fluorescence-guided laparoscopic colorectal cancer surgery with prophylactic retrograde transileal conduit ureteral catheter placement after previous total cystectomy: a case report. *Surg Case Rep*. 2021 Mar 12; 7 (1): 67. doi: 10.1186/s40792-021-01153-3. PMID: 33710480; PMCID: PMC7954966.

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KIDNEY AUTOTRANSPLANTATION: A METHOD FOR TREATING URETERAL LESIONS IN UROLOGICAL AND ONCOLOGICAL PRACTICE

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The first successful kidney autotransplantation was performed in 1902. The technique has undergone several changes since then. The indications and surgical technique are presented in this literature review. Kidney autotransplantation is the treatment of choice for preserving renal function. Three clinical observations on the use of kidney autotransplantation in urological and oncological practice are described: a patient after iatrogenic ureteral injury and two patients with primary retroperitoneal tumor. Literature analysis and clinical observations from urological and oncological practice show that kidney autotransplantation could be safely used for strictly selected indications.

Keywords: kidney autotransplantation, ureteral injury, primary retroperitoneal tumors.

The history of kidney autotransplantation dates back to 1902. At the Vienna Medical Society Meeting, Hungarian surgeon Emerich (Imre) Ullmann, reported the first case of renal autotransplantation performed in a dog. In the same year, he performed the first autotransplantation of a kidney to a dog, using the recipient's carotid artery and jugular vein for vascular implantation. The operation technique was as follows: carotid artery and vein were ligated cranially, magnesium medical tubes were inserted into the proximal part of the vessels, to which a kidney, removed without flushing the vascular bed, wrapped in a napkin soaked in warm saline solution, was attached. The transplanted organ produced urine for 5 days. A few months later, Ullmann presented the first xenotransplantation of a kidney from a dog to the neck of a goat. In 1912, Nobel laureate A. Carrel, who developed the vascular anastomosis technique, repeated Ullmann's experiments. At that time, scientists and clinicians were not aware of the problems of ischemia-reperfusion injury. For more than 50 years, autotransplantation was not a hot topic, but during this time, researchers tested various kidney allotransplantation techniques using femoral and forearm vessels, as well as orthotopic position, alloimmunity mechanisms were discovered and the first successful kidney transplantation from a living donor into heterotopic (iliac fossa) position was performed [1, 2].

In 1956, Brazilian C. Freire performed this operation for the first time on a man with renal artery aneurysm, although an early thrombosis forced him to perform nephrectomy [3].

Only in 1961, R. Schackmann and W. Dampster successfully performed the same operation for the first time to preserve renal function in a patient suffering from renal artery stenosis and secondary arterial hypertension [4]. After the surgery, the patient's blood pressure normalized and did not require prescription of hypotensive therapy. This disease was previously considered incurable or treated by nephrectomy. In 1964, K. Ota performed renal autotransplantation in a 39-year-old patient for renal artery repair due to congenital vascular renal hypertension and complete obliteration of the right renal artery (Fig. 1, b) [5]. Autotransplantation of the right kidney into the left iliac fossa with microsurgical correction of the vessels was performed; the right renal artery was dilated using a venous graft patch.

American James Hardy performed autotransplantation of the right kidney to the right iliac region in 1963 due to proximal ureteral stricture resulting from traumatic injury (Fig. 1, a). Notably, J. Hardy used moderate whole-body hypothermia (32–36 °C) rather than graft to minimize ischemic injury [1, 2].

Rapid development of clinical transplantology in the 1970s gave impetus to the development of the topic of kidney autotransplantation. In 1970, J. Whitsell described a series of experiments on heterotopic autotransplantation in dogs without ureteral transection and a clinical case of successful treatment of a patient with extended (2.5 cm) arterial stenosis of the only right kidney. The renal vessels were reimplanted into the common iliac vessels, and the ureter was arched on the mesentery of the small intestine (Fig. 1, c) [6]. The first kidney autotransplantation for a malignant tumor was performed by famous pioneer of transplantology, R. Calne in 1971. A patient with bilateral renal tumor lesion with 1/3 of the right kidney parenchyma intact (according to selective angiogram) underwent left-sided nephrectomy with

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subtotal ex vivo extracorporeal nephrectomy resection and its implantation into the iliac area [3, 7]. In 1972 C. Linke and A. May were the first to describe the use of kidney autotransplantation to treat urological pathology (Fig. 1, e), more specifically, retroperitoneal fibrosis causing atrophy and extended ureteral stenosis [8]. Preoperative retrograde pyeloureterogram demonstrated bilateral hydroureteronephrosis with external compression and medial deviation of both ureters. Subsequently, a multistage correction of ureteral compression was performed, culminating in a right-sided renal autotransplantation.

Thus, the range of indications for kidney autotransplantation has been formed, including various vascular lesions of the renal pedicle, ureter and renal parenchyma of an infectious-inflammatory, metabolic, fibrotic, dysplastic and neoplastic nature (Table).

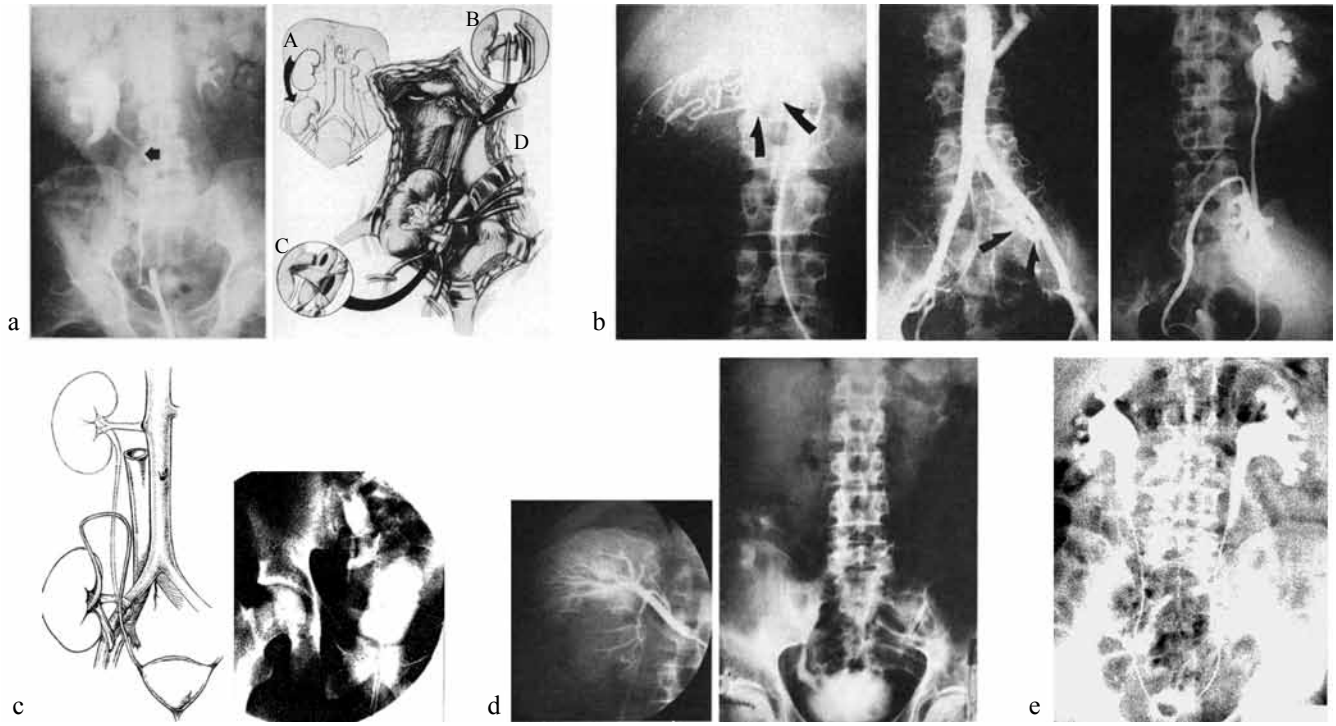

Fig. 1. a, left to right: right ureteral stricture diagnosed by right-sided retrograde pyeloureterogram (through nephrostomy tube) and retrograde urethrogram. Operation scheme; b, left to right: arrows indicate complete obliteration of the right renal artery. Aortogram – arrows point to the right renal artery, after microsurgical correction. Retrograde pyelogram; c, left to right: positions of the repositioned kidney and course of the ureter – diagram, cystoureteropyelogram; d, bilateral renal tumor lesion. Left to right: selective angiogram. After left-sided nephrectomy and subtotal extracorporeal nephrectomy and 1/3 kidney autotransplantation; e, Preoperative retrograde pyeloureterogram

Table

Indications for kidney autotransplantation

Vascular	Renal artery aneurysm
	Atherosclerosis of the renal artery and aorta (wall stenosis or dissection)
	Fibromuscular dysplasia
	Renal vein aneurysms
	Nutcracker syndrome (aortomesenteric compression of the left renal vein)
	Large saccular aneurysm of the renal artery
	Midaortic syndrome
Urological (main)	Extended ureteral strictures
	Ureteral avulsion
	Idiopathic retroperitoneal fibrosis
Oncological	Complex intraorganic lesion
	Bilateral tumor lesion
	Prior to radiotherapy
	Retroperitoneal sarcomas

VASCULAR INDICATIONS

Until recently, vascular pathology was the most common indication for kidney autotransplantation (Table). Currently, modern endovascular technologies have replaced autotransplantation in the treatment of arterial hypertension due to renal artery disease, having clear advantages – low invasiveness and possibility of repeated intervention without significant trauma to the patient. The same technologies are almost routinely used in the treatment of venous renal hypertension. But in some cases, for example, in large sized sac-shaped renal artery aneurysm, autografting is still the method of choice [2, 9].

Midaortic syndrome (a rare abdominal aortic coarctation syndrome) deserves special attention. This is a rare vascular pathology of various etiological nature, leading to narrowing of the descending aorta at the level of the L6 thoracic to L1 lumbar vertebrae. It is caused by congenital or acquired arteritis (Takayasu disease), neurofibromatosis, and fibromuscular dysplasia. Midaortic syndrome often leads to renovascular hypertension and decreased renal function. When endovascular intervention is ineffective, abdominal aortic bypass with bilateral orthotopic renal autografting becomes the method of choice [10].

UROLOGICAL INDICATIONS

Urological pathology is currently the main indication for kidney autotransplantation (Table). It is performed when ureteral prosthesis is necessary and plastic surgeries such as ureteroneocystostomy, ureteroureterostomy, pyelocystostomy, ipsilateral ureteroureterostomy, lower nephropexy, Boari surgery or psoas hitch are not possible due to tissue deficiency [11]. An alternative solution might be to replace the affected ureter with a section of the small intestine. However, the use of the small intestine leads to higher chances of complications of varying severity. Persistent urinary tract infection, unregulated metabolic acidosis, excessive mucus production, and adhesions can occur in combination in almost a third of patients, carrying additional risks of loss of kidney function and sepsis. In addition, the use of the small intestine may be limited by adhesions in the abdomen and retroperitoneum due to previous interventions [12].

The widespread use of endoscopic methods of lithoextraction and endourological treatment of uroteric tumors in recent decades has increased the number of extended lesions and proximal ureteric ruptures [13]. This leads to the need to perform temporary urinary diversion (nephrostomy) to preserve kidney function and provide multistage treatment. In such cases, kidney autotransplantation can be considered as a method allowing to solve the problem of urinary tract restoration in the shortest possible time and avoid complications associated with additional urinary derivation from the damaged kidney and subsequent delayed reconstructive intervention [11, 14].

Idiopathic retroperitoneal fibrosis (autoimmune periaortitis) is a rare disease with an annual incidence of 0.1–0.3 cases per 100,000 people, involving the abdominal aorta, iliac vessels and adjacent retroperitoneal space with frequent involvement of the middle and lower thirds of both ureters, leading to obstruction and terminal renal failure. The disease is caused by a chronic fibro-inflammatory process in which Ig4-secreting plasma cells are involved, and often requires differential diagnosis from retroperitoneal malignancies [15, 16]. If conservative treatment, including immunosuppressive therapy, progression of urinary tract obstruction is ineffective, bilateral kidney autotransplantation in heterotopic position allows to save a functioning renal parenchyma, preventing progression of chronic kidney disease [8].

ONCOLOGIC INDICATIONS

In the last decade, improvements in surgical techniques and complex, including chemotherapeutic, treatment of cancer patients, and increase in prognosis of patients' recurrence-free survival, has led to the development of the concept of "organ-sparing" surgical treatment of malignant tumors. A new sub-specialty, onconeurology, has appeared. The tasks of this subspecialty are: solving problems related to acute kidney injury and chronic renal failure in cancer patients, assessing nephrotoxic risks of antitumor therapy, both conventional chemotherapy and the latest molecular targeted therapy, treatment of renal manifestation of paraneoplastic process, treatment of patients who underwent nephrectomy for renal cancer, aspects of renal replacement therapy amidst active treatment of oncological process, possibility of performing kidney transplantation in patients who have undergone oncological treatment, treatment of oncological pathology in patients after kidney transplantation [17, 18]. The cornerstone of onco-nephrology is the concept of "nephron-sparing" treatment (Table). The importance of this approach is due to the fact that acute kidney injury or chronic kidney failure leads to a significantly increased risk of mortality in cancer patients from non-oncological causes, primarily from cardiovascular pathology [19, 20].

Organ-sparing treatment improves life expectancy in patients whose tumor has not spread beyond the kidney [21]. This is particularly important in patients with tumor lesions in one kidney, where all efforts should be focused on preserving the organ in order to avoid the need for chronic renal replacement therapy. Under such conditions, kidney autotransplantation with ex-vivo resection or tumor enucleation appears to be a feasible technique; this operation has significantly lost its popularity in the last decade [22]. This is due to the fact that minimally invasive nephron-sparing surgery in malignant kidney tumors, such as laparoscopic or robotic partial nephrecto-

my with superselective ischemic parenchyma, as well as ablative techniques, provide equivalent cancer-specific survival compared with radical nephrectomy [23].

Autografting with ex vivo tumor resection can be used in cases with complex intraorgan lesions involving the renal collar and/or the pelvicalyceal system, where resection carries risks of major blood loss or ischemia of the remaining, unaffected part of the renal parenchyma [24]. This operation can also be applied to multiple bilateral renal tumor lesions when organ-sparing treatment is absolutely obvious, but the standard approach to resection with bilateral local thermal ischemia carries a high risk of acute renal injury in the early postoperative period and chronic renal failure in the long term [7, 25].

On the other hand, autotransplantation for a kidney tumor can lead to a rather large range of complications, including bleeding (3.3–5% of cases), urinary tract infections (7.4%), renal vein thrombosis (4.1%), and loss of graft function (12.3%). Although it is necessary to take into account that patients with initially more anatomically complex spread of tumor process fall into the autotransplantation group [26].

Bolling described a casuistic case of kidney autotransplantation in a patient suffering from Ewing tumor arising from the 9th–11th ribs on the left side. In order to avoid radiation damage, the kidney was moved to the left iliac region before radiotherapy was started [27].

In 2010, V. Bonsal reported on the first removal of retroperitoneal liposarcoma in a block with the ureter, followed by kidney autografting into the iliac area to restore urine passage [28].

Surgical intervention is the main method of treatment for locally disseminated retroperitoneal sarcomas. Neither radiotherapy nor chemotherapeutic combination therapy significantly improves tumor prognosis and control. The need for multivisceral surgery in the removal of retroperitoneal sarcomas is due to the principles of radicalism in the removal of malignant tumors. However, the modern and reasonable desire to perform organ-sparing operations has led to the need to find a more rational, but also technically complex, surgical approach [29]. Statistically, up to 40% of surgical interventions performed for retroperitoneal sarcomas are combined with unilateral and sometimes bilateral nephrectomy. S. Mussi presents that there are 78.5% and 45.8% cases of kidney and ureter involvement in the tumoral process, respectively, but infiltrative damage occurs at a much smaller frequency – 10.7% and 12.5%, and in other cases, the involvement has a compressive nature, which is especially characteristic of liposarcoma. The noninfiltrative nature of the growth of fatty sarcoma makes it more likely to perform surgery, while maintaining the mass of functioning nephrons [30].

The use of transplantation and extracorporeal surgical techniques in complicated retroperitoneal anatomicotopographic conditions expands the possibilities of organ-sparing treatment without reducing the radical nature of the intervention. Prolonged involvement of the ureter in giant retroperitoneal sarcomas may require its removal en bloc with surrounding tumor tissues. It is almost impossible to compensate for the ureteral length deficit in such cases using standard urological approaches. Performing autotransplantation in the heterotypic position as a second step after tumor removal allows preserving the kidney function and urinary tract integrity [28, 31].

When retroperitoneal sarcoma spreads to the upper regions of retroperitoneal space, at the level of cavarenal segment of the inferior vena cava, the involvement of renal vascular pedicle and the difficulty of intraoperative differentiation of tumor tissue from parainephral tissue can lead to the need for tumor nephrectomy [32, 33]. In such cases, ex vivo dissection of the kidney from the surrounding tumor tissues followed by its autotransplantation is possible to preserve kidney function [28].

In Russia and in post-Soviet states, the use of kidney autotransplantation in oncological diseases was actively studied by A.E. Zotikov [34], I.B. Schepotin [35] and R.I. Rasulov [36].

TECHNICAL PECULIARITIES OF AUTOTRANSPLANTATION

The kidney autotransplantation technique does not differ fundamentally from that of allogeneic kidney transplantation, but there are a number of gray areas that require special attention.

The main condition for preserving the functioning renal parenchyma in autotransplantation is to minimize its ischemic injury. Controlled hypothermia is used as the first line of defense against hypoxic damage in organ transplantation. As a rule, organs are cooled to a temperature of 0 to +4 °C. Cooling reduces cellular metabolism and oxygen demand. However, at this temperature, a certain level of metabolism is preserved in human cells, which eventually leads to apoptosis and necrosis [37]. Therefore, the use of local hypothermia is indicated even at the tumor conglomerate explantation stage, when, due to traction at its extraction and mobilization stage, it is possible to kink the renal pedicle with blockage of organ blood flow. The use of pharmaco-cold protection during the ex vivo phase is also considered absolutely necessary. Kidney autotransplantation does not imply long periods of cold ischemia. Flushing of the vascular bed with 500.0 ml saline cooled to +4 °C with addition of 10,000 IU of heparin is considered sufficient for preservation of the renal autograft within 2–4 hours. On the other hand, the use of special preserving solutions (HTK, UW, IGL, etc.), which are now widely available, allows prolonging the cold ischemia time up to 24 hours without significant damage [38].

The most important factor for successful kidney autotransplantation is to obtain a renal artery and vein of

sufficient length and diameter c. Often prolonged compression of these vessels by tumor tissue leads to wall thinning and reduced diameter, which can lead to vascular complications after kidney reimplantation, both in the early and late postoperative period [39]. Marking the renal vessels during removal of the tumor-kidney block allows cutting off the vessels most proximally to obtain sufficient length and quickly find them in the conglomerate for rapid cannulation and perfusion with a preservative solution, minimizing warm ischemia time [39, 36].

At the stage of extracorporeal kidney dissection, it is necessary to use a precision surgical technique with the use of surgical binocular loupes (recommended magnification 2.5). This makes it possible to maximally protect important anatomical structures of the renal collar from damage during dissection, and to assess possible invasion into the collar and capsule of the removed kidney [36].

The choice of heterotopic position for kidney transplantation is not accidental. This position has certain surgical advantages that minimize complications compared with orthotopic autotransplantation. As a rule, the vessels of a renal autograft are somewhat shorter and thinner than those of an allograft. To prevent kinking and twisting of arterial and venous anastomoses, it is necessary to maintain some mobility in the anastomosis area. Wide mobilization of the external iliac vein, in some cases with intersection of the internal iliac vein, mobilization of the external iliac artery throughout, or use of the internal iliac artery, if its atherosclerotic lesion is excluded, can help avoid blood flow disturbances in the kidney and choose the optimal graft position in the iliac fossa [40, 41].

It should be noted that in patients with large-volume malignancies, balance in the blood coagulation system is shifted towards hypercoagulation. The use of anticoagulant therapy from day 1 after autotransplantation can reduce the likelihood of thrombosis in the vascular anastomoses area and in the renal microcirculatory bed [42, 43].

The second advantage of the heterotopic position is associated with the possibility of restoring urine passage in the transplanted kidney. The vast majority of urological complications following a kidney transplant surgery are associated with impaired blood supply to the ureter and pelvis. Since the autograft ureter is fed only from the renal vessels, there is always a risk of ischemization of its distal parts. Shortening the ureter usually solves this problem. Proximity to the bladder also allows any available repair options to be performed if the ureter length proves insufficient [44, 45].

CLINICAL CASE 1

Male patient Z., 29 years old, was admitted in October 2013 at the Lopatkin Research Institute of Urology and Interventional Radiology in Moscow with complaints of a left nephrostomy tube. In his medical history, 12 months before admission to our clinic, the patient had undergone an attempt of contact ureterolithotripsy on the left for a stone in the upper third of the left ureter, which resulted in iatrogenic detachment of the left ureter. The patient was placed with a percutaneous puncture nephrostomy tube on the left side.

When examined at the Research Institute of Urology, the secretory function of the left kidney was found to have reduced by 23% according to the radioisotope study, and the kidney function on the right side was satisfactory. According to ultrasound and multi-slice computed tomography (MSCT), the right kidney measured 12.5 × 6 cm, the parenchyma was 1.5 cm thick, there was no enlargement of the pelvicalyceal system. The type of blood supply was trunk. The left kidney was 11.8 × 5.8 cm, parenchyma was 1.5 cm thick, there was no enlargement of the pelvicalyceal system, a nephrostomy tube was visualized in the lumen of the pelvis. The type of blood supply was arterial. Left antegrade pyelography (Fig. 2, a) showed that the contrast medium was filling the pelvicalyceal system of the left kidney. No contrast agent was delivered to the left ureter from the pelvis.

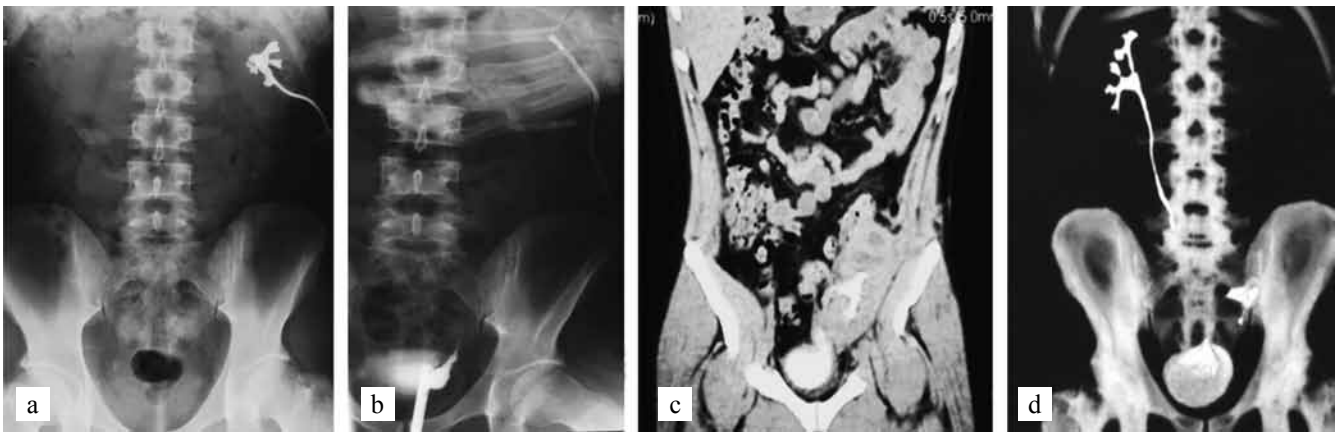

Fig. 2. a, antegrade pyelography (left); b, retrograde ureterography (left); c, d, renal MSCT with intravenous bolus contrast enhancement

During retrograde ureterography on the left, ureteral catheter was inserted 3 cm above the orifice of the left ureter, where an insurmountable obstacle was encountered. No contrast agent is delivered above 3 cm of the left ureter from its orifice (Fig 2, b).

Given the patient's young age, the intact function of the left kidney, and technical capabilities of the clinic, he underwent open autotransplantation of the left kidney. The left kidney with an artery and a vein was removed. Access to the left iliac fossa was performed. The artery and vein of the left kidney were anastomosed with the iliac artery and vein. The left ureter was modeled from the bladder according to the Boari technique and anastomosed on the inner stent No. 6 with the left renal pelvis. The operation lasted for 145 minutes; blood loss was 250 mL. The postoperative period was smooth. The patient was discharged on day 14 after surgery. Internal stent and nephrostomy tube were removed 8 weeks after the operation. Control computed tomography revealed that the left kidney was located in the left iliac region, passage of the contrast agent from the left kidney was not impaired (Fig. 2, c, d).

The patient has been under our observation for eight years. Control ultrasound examination in September 2020 and Doppler ultrasonography of the kidneys showed that the right kidney was intact, the left kidney was located in the left iliac region, without impaired blood supply. The left kidney was 11.4 × 5.8 cm in size, the parenchyma was 1.5 cm thick, the pelvicalyceal system was not enlarged.

The patient leads an active lifestyle. He works (office worker), does sports (runs half marathons).

CLINICAL CASE 2

Female patient D, 51 years old, diagnosed with stage IIIB primary retroperitoneal tumor with T4N0M0 (according to histological examination of biopsy material – retroperitoneal multinodular liposarcoma (G1–G2) (Fig. 3, a) was admitted to our clinic.

The primary retroperitoneal tumor was removed (Fig. 3, b) with autotransplantation of the left kidney, corpuscular resection of the pancreas and splenectomy, resection of the left diaphragmatic dome, left-sided hemicolectomy, extirpation of the uterus with appendages, and formation of suspended jejunostomy.

Stages of kidney autotransplantation: 1, formation of vascular anastomosis between the renal artery and the left internal iliac artery (Fig. 3, c, d); 2, final view of autotransplanted kidney in heterotopic position after the formed intervascular anastomosis and interureteric anastomosis (Fig. 3, e).

The surgery lasted for 435 minutes; intraoperative blood loss was 2800 mL. The postoperative period was according to the extent of the surgical intervention performed.

According to morphological examination, retroperitoneal multinodular liposarcoma (G1–G2), predominantly well-differentiated lipoma-like (G1) with overgrowth to the diaphragm area, spleen capsule, pancreas, adrenal gland, fouling of these organs and myxoid liposarcoma

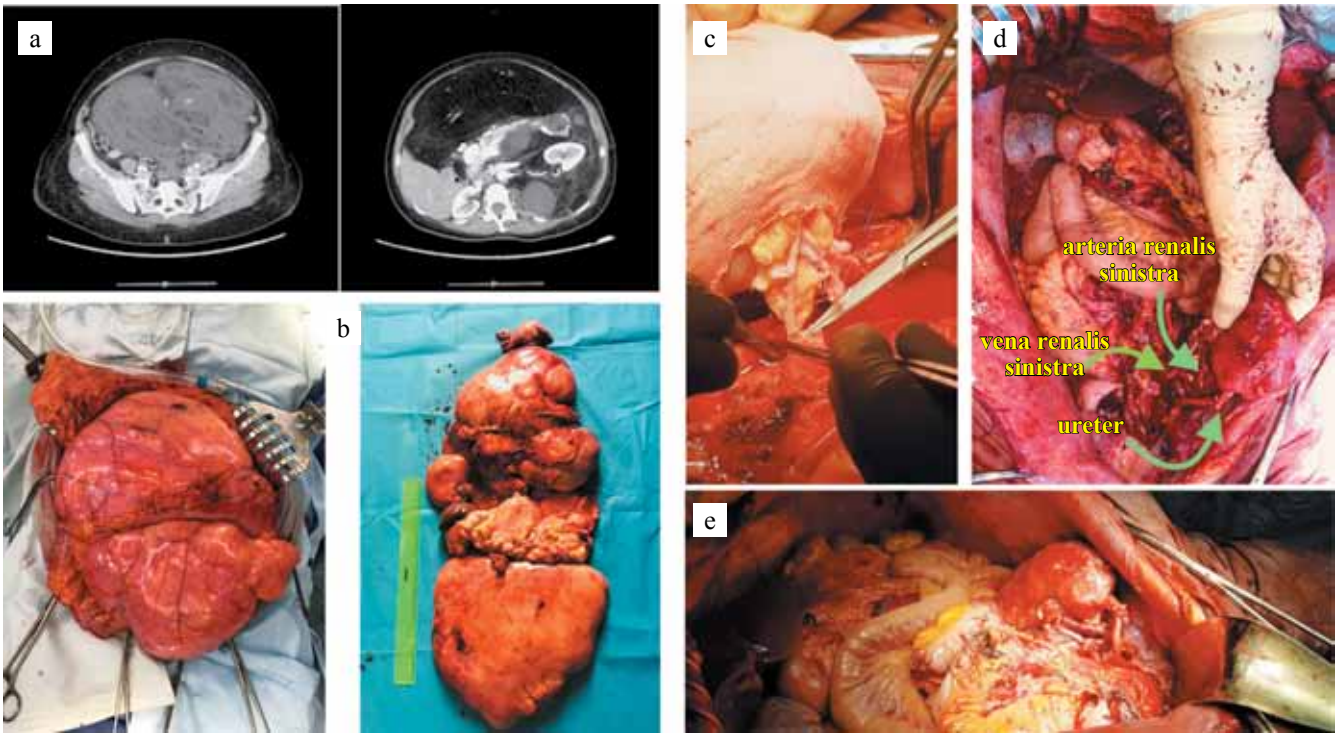

Fig. 3. a, MSCT of abdominal organs and retroperitoneal space with intravenous bolus contrast enhancement; b, intraoperative view of primary retroperitoneal tumor resection; c, d, e, kidney autotransplantation stages

node (G2) with ingrowth into the wall of one of the colon fragments were established.

Morphological examination of the removed specimen showed oncological radicality of the operation. The patient was discharged on day 18 after the surgery. She has been under our observation for 9 months with remission and intact function of the autotransplanted kidney.

CLINICAL CASE 3

Female patient V., 48 years old, diagnosed with stage IB primary retroperitoneal tumor pT4N0M0 (according to histological examination of biopsy material – low grade undifferentiated liposarcoma (G1)) (Fig. 4, a) was admitted to the clinic.

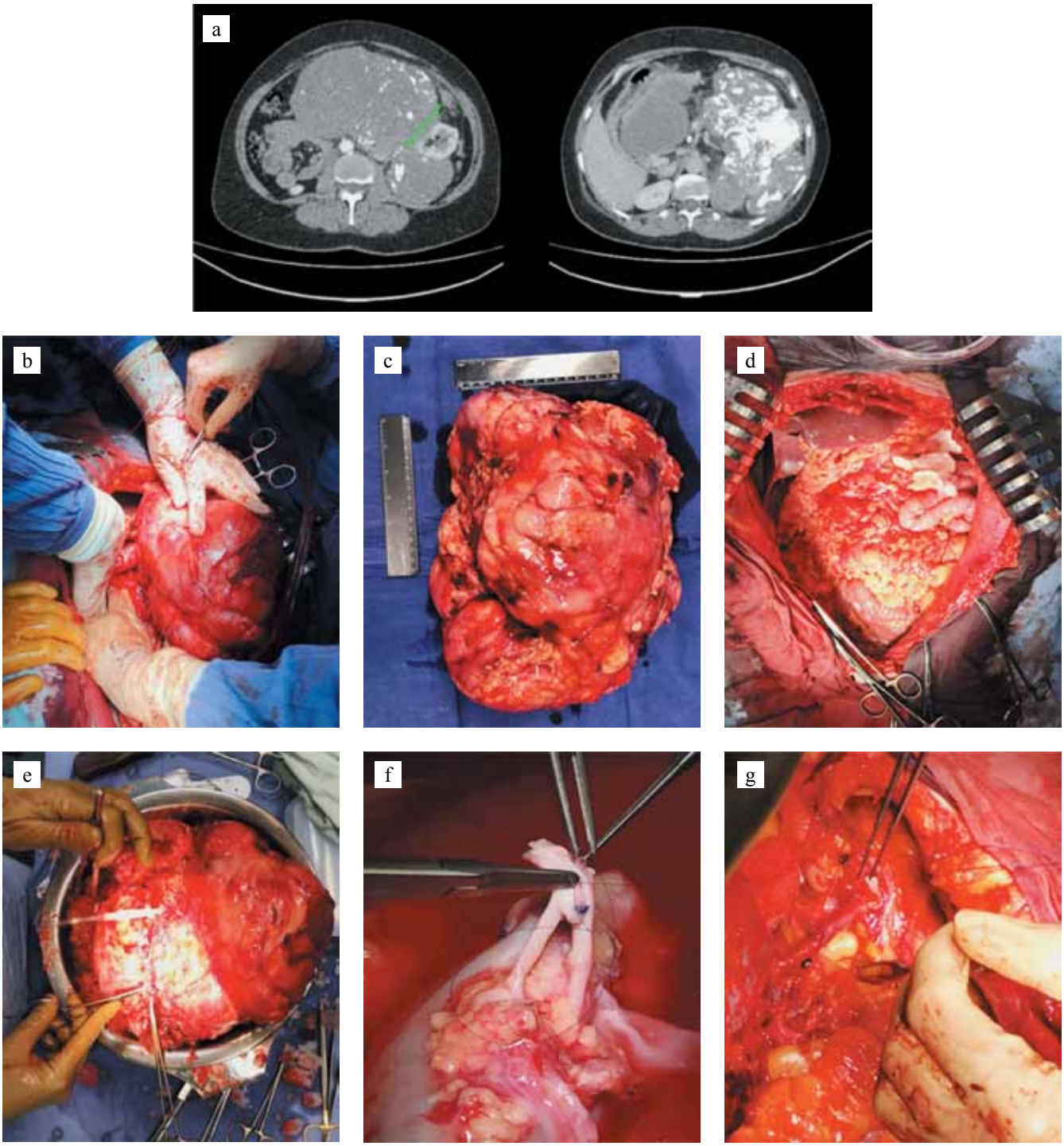

Fig. 4. a, MSCT of abdominal organs and retroperitoneal space with intravenous bolus contrast enhancement. b, intraoperative view of the tumor during resection; c, removed macro specimen; d, intraoperative view of the abdominal cavity after tumor resection; e, Ex vivo kidney isolation; f, kidney preparation for transplantation; g, completion of kidney autotransplantation stage

The primary retroperitoneal tumor was removed by left adrenalectomy, followed by extracorporeal resection of the upper pole of the left kidney and its autotransplantation to the iliac area; cholecystectomy, suspended jejunostomy were also performed (Fig. 4, b–g).

The surgery lasted for 370 minutes; intraoperative blood loss was 1200 mL. There were no complications during the postoperative period; it was according to the extent of the surgical intervention performed.

According to morphological study, undifferentiated low-grade liposarcoma (G1) of a spindle-cell structure, with small- and moderate-cell foci of necrosis, with 4 mitoses per 10 high power fields of view ×40 was established.

A morphological examination of the removed specimen showed oncological radicality of the operation. The patient was discharged on day 14 after surgery. Postoperative rehabilitation is ongoing (1.5 months after surgery).

DISCUSSION

Kidney autotransplantation is the method of choice for treatment aimed at preserving renal function. Its indications have varied since its introduction into clinical practice and up to the present time. New techniques, for example, endovascular surgery, has reduced the range of vascular indications for kidney autotransplantation.

Prolonged ureteric lesion remains one of the considered indications for kidney autotransplantation when there is a need for nephron-sparing treatment or social adaptation (sparing patients from lifelong use of nephrostomy tube or ureteric stent).

Recently, in the treatment of primary retroperitoneal tumors (PRT), there has been a tendency towards abandoning monobloc and cytoreductive surgery in favor of a balanced approach. A balanced approach in the treatment of primary retroperitoneal tumor includes: nephron-sparing interventions; removal of well-differentiated PRTs by separate "compartments" in order to maximize organ preservation; kidney autotransplantation. The importance of nephron-sparing interventions in PRT is due to minimization of the probability of acute kidney injury and chronic kidney disease, which increase the risk of mortality in cancer patients from non-cancer causes of stroke, and coronary heart disease [19, 20]. Preserved kidney function gives freedom in prescribing effective adjuvant therapy regimens. However, indications on the PRT side are extremely limited: well-differentiated (G1) liposarcomas; location of the kidney in the thickness of tumor nodules, involvement of renal vessels with preserved kidney function; extended involvement of the ureter; single kidney.

A multidisciplinary approach involving transplant specialists is necessary when extensive kidney involvement in the tumor process with kidney function intact is suspected. With proper planning of surgical intervention, it is possible to achieve good immediate and long-term treatment outcomes.

CONCLUSION

The literature and the clinical cases from urological and oncologic practice presented by us show that kidney autotransplantation can be safely used according to strictly chosen indications.

The authors declare no conflict of interest.

REFERENCES

1. *Langer RM, Kahan BD.* 100 years ago: Ullmann's pioneering operation – autotransplantation of the kidney. *Transplant Proc.* 2002 Mar; 34 (2): 429–433. doi: 10.1016/s0041-1345(02)02642-8. PMID: 12009580.
2. *Alameddine M, Moghadamyeghaneh Z, Yusufali A, Collazo AM, Jue JS, Zheng I et al.* Kidney Autotransplantation: Between the Past and the Future. *Curr Urol Rep*. 2018 Feb 5; 19 (3): 7. doi: 10.1007/s11934-018-0749-4. PMID: 29399714.
3. *Gil-Vernet JM.* Renal Allotransplantation. *Eur Urol*. 1982; 8: 61–73.
4. *Schackman R, Dempster WY*. Surgical kidney. A case demonstrated at the Post-Graduate Medical School of London. *Br med J.* 1924 i.v., (1963).
5. *Ota K, Mori S, Awane Y, Ueno A*. Ex situ repair of renal artery for renovascular hypertension. *Arch Surg*. 1967; 94 (3): 370–373. PMID: 5335236.
6. *Whitsell JC, Goldsmith EI, Nakamura H.* Renal autotransplantation without ureteral division: an experimental study and case report. *J Urol*. 1970; 103 (5): 577–582. doi: 10.1016/s0022-5347(17)62006-8.
7. *Calne RY*. Tumour in a single kidney: nephrectomy, excision, and autotransplantation. *Lancet Lond Engl*. 1971 Oct; 2 (7727): 761–762. doi: 10.1016/s0140-6736(71)92124-6.
8. *Linke CA, May AG.* Autotransplantation in retroperitoneal fibrosis. *J Urol.* 1972; 107 (2): 196–198. doi: 10.1016/s0022-5347(17)60981-9.
9. *Berloco PB, Levi Sandri GB, Guglielmo N, Lai Q, Melandro F, Poli L et al.* Bilateral ex vivo repair and kidney autotransplantation for complex renal artery aneurysms: a case report and literature review. *Int J Urol.* 2014 Feb; 21 (2): 219–221. doi: 10.1111/iju.12224. Epub 2013 Jul 10. PMID: 23841913.
10. *Zhang H, Li F, Ren H, Zheng Y*. Aortic bypass and orthotopic right renal autotransplantation for midaortic syndrome: a case report. *BMC Surg.* 2014; 14 (1): 86. doi: 10.1186/1471-2482-14-86.
11. *Zhang HX, Zhao L, Ma LL, Hou XF, Liu L, Deng SH.* Retroperitoneal laparoscopic nephrectomy with autotransplantation for severe iatrogenic ureteral injury. *Beijing Da Xue Xue Bao.* 2016 Feb; 48 (1): 622–626. PMID: 27538140.
12. *Chung BI, Hamawy KJ, Zinman LN, Libertino JA.* The Use of Bowel for Ureteral Replacement for Complex Ureteral Reconstruction: Long-Term Results, *J*

Urol. 2006; 175 (1): 179–183. doi: 10.1016/S0022-5347(05)00061-3.
13. *Geavlete P, Georgescu D, Niţă G, Mirciulescu V, Cauni V*. Complications of 2735 retrograde semirigid ureteroscopy procedures: a single-center experience. *J Endourol*. 2006 Mar; 20 (3): 179–185. doi: 10.1089/end.2006.20.179.
14. *Alizadeh M, Valizadeh R, Rahimi MM.* Immediate successful renal autotransplantation after proximal ureteral avulsion fallowing ureteroscopy: a case report. *J Surg Case Rep.* 2017; 2017 (2): rjx028. doi: 10.1093/jscr/rjx028.
15. *Stone JH, Khosroshahi A, Deshpande V, Chan JKC, Heathcote JG, Aalberse R et al.* Recommendations for the nomenclature of IgG4-related disease and its individual organ system manifestations. *Arthritis Rheum.* 2012; 64 (10): 3061–3067. doi: 10.1002/art.34593.
16. *Vaglio A, Maritati F.* Idiopathic retroperitoneal fibrosis. *J Am Soc Nephrol*. 2016; 27 (7): 1880–1889. doi: 10.1681/ASN.2015101110.
17. *Cosmai L, Porta C, Gallieni M, Perazella MA.* Onconephrology: a decalogue. *Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc – Eur Ren Assoc*. 2016; 31 (4): 515–519. doi: 10.1093/ndt/gfv320.
18. *Mansouri I, Alencar de Pinho N, Snanoudj R, Jacquelinet C, Lassalle M, Béchade C et al.* Trends and Outcomes with Kidney Failure from Antineoplastic Treatments and Urinary Tract Cancer in France. *Clin J Am Soc Nephrol CJASN*. 2020; 15 (4): 484–492 doi: 10.2215/CJN.10230819.
19. S*eylanova N, Crichton S, Zhang J, Fisher R, Ostermann M.* Acute kidney injury in critically ill cancer patients is associated with mortality: A retrospective analysis. *PloS One*. 2020; 15 (5): e0232370. doi: 10.1371/journal.pone.0232370.
20. *Bibkov BT, Tomilina NA*. Sostav bol'nyh i pokazateli kachestva lechenijana zamestitel'noj pochechnoj terapii terminal'noj hronicheskoj pochechnoj nedostatochnosti v Rossijskoj Federacii v 1998–2013. *Nefrologija i dializ.* 2016; 18 (2): 98–164.
21. *MacLennan S, Imamura M, Lapitan MC, Omar MI, Lam TBL, Hilvano-Cabungcal AM et al.* Systematic review of perioperative and quality-of-life outcomes following surgical management of localised renal cancer. *Eur Urol.* 2012; 62 (6): 1097–117. doi: 10.1016/j.eururo.2012.07.028.
22. *Moghadamyeghaneh Z, Hanna MH, Fazlalizadeh R, Obi Y, Foster CE, Stamos MJ, Ichii HA* Nationwide Analysis of Kidney Autotransplantation. *Am Surg.* 2017 Feb 1; 83 (2): 162–169. PMID: 28228203.
23. *Berger A, Crouzet S, Canes D, Haber G-P, Gill IS.* Minimally invasive nephron-sparing surgery. *Curr Opin Urol.* 2008 Sep; 18 (5): 462–466. doi: 10.1097/MOU.0b013e32830a4f10.
24. *Gwon JG, Kim YH, Han DJ.* Analysis of Solitary Kidney Autotransplantation Cases. *Transplant Proc.* 2017; 49 (9): 2055–2059. doi: 10.1016/j.transproceed.2017.09.030.
25. *Eisenberg ML, Lee KL, Zumrutbas AE, Meng MV, Freise CE, Stoller ML*. Long-Term Outcomes and Late Complications of Laparoscopic Nephrectomy With Renal Autotransplantation. *J Urol.* 2008; 179 (1): 240–243. doi: 10.1016/j.juro.2007.08.135.
26. *Kutikov A, Uzzo RG.* The R.E.N.A.L. Nephrometry Score: A Comprehensive Standardized System for Quantitating Renal Tumor Size, Location and Depth. *J Urol.* 2009; 182 (3): 844–853. doi: 10.1016/j.juro.2009.05.035.
27. *Bölling T, Janke K, Wolters HH, Glashörster M, Ernst I, Willich N et al.* Kidney-autotransplantation before radiotherapy: A case report. *Anticancer Res*. 2009; 29 (8): 3397–3400. PMID: 19661363.
28. *Bansal VK, Misra MC, Sharma A, Chabbra A, Murmu LR.* Giant Retroperitoneal Liposarcoma – Renal Salvage by Autotransplantation. *Indian J Surg*. 2013; 75 (2): 159–161. doi: 10.1007/s12262-012-0474-z.
29. *Stilidi IS, Nikulin MP, Davydov MM, Gubina GI.* "Kidney-preserving" operations in retroperitoneal tumors surgery. *Annaly hirurgii.* 2014; (3): 47–52.
30. *Mussi C, Colombo P, Bertuzzi A, Coladonato M, Bagnoli P, Secondino S et al.* Retroperitoneal sarcoma: Is it time to change the surgical policy? *Ann Surg Oncol.* 2011; 18 (8): 2136–2142. doi: 10.1245/s10434-011-1742-z.
31. *Paloyo SR, Ramirez AD, David-Paloyo FP, Dofitas RB.* Wide Excision of a Retroperitoneal Liposarcoma with En Bloc Ureterectomy and Renal Salvage by Autotransplantation. *Case Rep Transplant*. 2019; 2019 (Figure 2): 1–3. doi: 10.1155/2019/9725169.
32. *Fedorov VD, Cvirkun VV.* Hirurgicheskoe lechenie bol'nyh s neorgannymi zabrjushinnymi opuholjami. *Aktual'nye voprosy hirurgii (Sb. nauchnyh trudov k 50-letiju Instituta hirurgii im. A.V. Vishnevskogo RAMN).* 1995: 207–214.
33. *Kaprin AD, Ryabov AB, Khomyakov VM, Cheremisov VV, Vé K, Chissov VI et al.* Resection of the inferior vena cava in locally advanced non-organ retroperitoneal tumors. *Onkologiya. Zhurnal imeni P.A. Gertsena.* 2017; 6: 28–38. doi: 10.17116/ONKOLOG20176128-38 [In Russ, English abstract].
34. *Teplov AA, Gritskevich AA, Pyankin SS, Zotikov AE, Adirkhaev ZA, Kozhanova AV et al*. Extracorporeal resection of the kidney in the setting of the pharmacological and cold temperature ischemia with orthotopic replantation of the vessels without ureter transaction in patients with renal cell carcinoma. *Experimental and clinical urology*. 2015; 2: 52–63.
35. *Shchepotin IB, Lukashenko AV, Kolesnik EA, Vasylyev OV, Rozumiy DA, Priymak VV, Gukov UA*. Modern technologies in the surgery of retroperitoneal sarcomas. *Clinical Oncology.* 2011; 2 (2): 21–25.
36. *Rasulov RI, Muratov AA, Dvornichenko VV, Morikov DD, Teterina TP.* Renal replantation at extended and combined resection of retroperitoneal liposarcoma (case report). *Acta Biomed Sci.* 2017; 2 (1): 130–135.
37. *Taylor MJ, Baicu SC.* Current state of hypothermic machine perfusion preservation of organs: The clinical perspective. *Cryobiology*. 2010; 60 (3): S20–S35. doi: 10.1016/j.cryobiol.2009.10.006.
38. *Badet L, Abdennebi HB, Petruzzo P, McGregor B, Espa M, Hadj-Aissa A et al.* Evaluation of IGL-1, a new organ preservation solution: preclinical results in renal transplantation. *Progres En Urol J Assoc Francaise*

Urol Soc Francaise Urol. 2005; 15 (3): 481–448. PMID: 16097154.

39. *Novick AC, Stewart BH, Straffon RA.* Extracorporeal renal surgery and autotransplantation: Indications, techniques and results. *J Urol.* 1980; 123 (6): 806–811. doi: 10.1016/s0022-5347(17)56141-8.
40. *Kable T, Alcaraz A, Budde K, Humke U, Karam G, Lucan M et al.* Transplantatsiya pochki: Klinicheskie rekomendatsii Evropeyskoy assotsiatsii urologov. Per. s angl. M.Yu. Fedyanin. M.: ABV-Press, 2011.
41. *Breda A, Budde K, Figueiredo A, Lledó García E, Olsburgh J, Regele H et al.* EAU guidelines on renal transplantation. *Edn presented at the EAU Annual Congress London.* 2017, 2017.
42. *de Francisco ALM, Macía M, Alonso F, García P, Gutierrez E, Quintana LF et al.* Onco-Nephrology: Cancer, chemotherapy and kidney. *Nefrol Publicacion Of Soc Espanola Nefrol.* 2019; 39 (5): 473–481. doi: 10.1016/j.nefro.2018.10.016.
43. *Rubio-Jurado B, Balderas-Peña L-M-A, García-Luna EE, Zavala-Cerna MG, Riebeling-Navarro C, Reyes PA et al.* Obesity, Thrombotic Risk, and Inflammation in Cancer. *Adv Clin Chem*. 2018; 85: 71–89. doi: 10.1016/bs.acc.2018.02.006.
44. *Alberts VP, Idu MM, Legemate DA, Laguna Pes MP, Minnee RC*. Ureterovesical anastomotic techniques for kidney transplantation: A systematic review and meta-analysis. *Transpl Int.* 2014; 27 (6): 593–605. doi: 10.1111/tri.12301.
45. *Png JCD, Chapple CR*. Principles of ureteric reconstruction. *Curr Opin Urol*. 2000; 10 (3): 207–212. doi: 10.1097/00042307-200005000-00004.

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DEVELOPMENT OF APPROACHES TO ENZYME-FREE ISOLATION OF PANCREATIC ISLETS

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The success of pancreatic islet allotransplantation in the treatment of patients with a difficult-to-manage type 1 diabetes depends mainly on the quantity and quality of islets isolated from the pancreas of deceased donors using enzyme preparations, primarily collagenase. Numerous studies on improvement and standardization of islet isolation techniques have reached their limits in the last decade. This has made it impossible to further boost the number and quality of clinical transplants. Taking into account the negative impact of collagenase technique on the morphofunctional properties of isolated islets, this work has studied the possibility of enzyme-free isolation of islet tissue purified of exocrine ballast. Experiments using the pancreas of newborn and young rabbits showed that developing methodological approaches to obtaining islet-like cultures without the use of exogenous enzymes is feasible.

Keywords: pancreas, exocrine tissue, islets, isolation, collagenase, rabbits, floating islet-like cultures.

INTRODUCTION

The early 21st century saw a breakthrough in effective allotransplantation of pancreatic islets (PIs) in patients with type 1 diabetes mellitus by the emergence of the Edmonton Protocol [1]. Since then, there have been certain improvements in the outcomes and safety of islet transplantation [2]. At the same time, some transplantation centers have consistently been more successful than others [3], and this difference is largely, if not to a decisive extent, due to the quality of isolated islets, which depends on the methodological level and experience of the researchers involved in this problem.

The proper use of collagenase preparations, which are the most important reagents used for islet isolation, significantly affects the quantity and quality of islets (more precisely, islet equivalents) and ultimately determines the outcomes of their transplantation in diabetic patients [3]. For a number of years, Liberase H1 (Roche) was most widely used for islets isolation from collagenase preparations, which, in fact, was considered to be the enzyme of choice [4]. In 2007, however, concerns about its use arose after attention was drawn to the fact that it used raw material derived from bovine brains, which, in theory, could potentially transmit prion-related diseases [5]. Since then, efforts have been made to substitute this preparation by modifying its components and studying the digestive activity of new enzymes [6]. As a result, enzyme mixtures including Serva collagenase NB1, a mammalian tissue-free version of Liberase (Roche), and a new Vitacyte mixture were used [7–9]. Despite the fact that Liberase HI and Collagenase NB1 were the most widely studied enzymes for human islet isolation, quantitative and qualitative results obtained in a number of centers differed significantly and were often contradictory. At the same time, determination of islet β-cell function (basal and stimulated insulin secretion) was the criterion for qualitative assessment of islet samples isolated at multiple U.S. centers from donors with varying characteristics [10].

The lack of standardization of enzymatic treatment of human pancreatic tissue can, to a certain extent, explain the high variability of islet isolation results. In order to determine a reasonable choice of the most acceptable option for pancreatic tissue treatment, a comparative meta-analysis of the results of using mixtures of different enzyme preparations in islet isolation from deceased donors was performed [11]. The effect of different enzymes on the equivalent number of islets obtained from 1 gram of pancreas was evaluated, determining the degree of their purification, viability, and glucose-stimulated insulin secretion. The meta-analysis showed that it seems that research on standardization of isolation of sufficient numbers of islets from donor pancreas has reached its limits, and after a surge in achievements in the early 2000s there was a stagnation period in this field, which made it almost impossible to significantly increase effective islet transplantation in the clinic. Numerous experiments with the use of the pancreas of laboratory animals, primarily rodents, continuing to the present time, have not allowed to significantly improve islet isolation method [12–14] and extrapolate the obtained data to the protocol for islet isolation from the pancreas of deceased donors. Experiments on islet co-culture with

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mesenchymal stromal cells give some hope for increasing the survival time and functional capabilities of islets [15, 16].

It is important to note that, in addition to exogenous enzyme preparations, activation of its own proteolytic enzymes produced by acinar cells can have a significant impact on the number and quality of islets isolated from donor pancreas. As is known, the exocrine secretory function of the pancreas is to secrete pancreatic juice into the duodenum, which promotes the breakdown of protein from food into amino acids. Proteolytic enzymes represented by trypsin, chymotrypsin and carboxypeptidase, are secreted into the duodenal lumen in an inactive state, and they are activated under the influence of intestinal juice enterokinase. However, intraorgan activation of intrinsic enzymes in donor pancreas is quite real and is mainly associated with impaired oxygen supply during its extraction, cold storage and during islet isolation procedure [17]. Increased lactate production as a result of anaerobic glucose breakdown under hypoxia/anoxia causes intracellular acidosis, which is one of the main inducers of premature intracellular trypsinogen autoactivation and subsequent triggering of the enzyme cascade in acinar cells [18]. Since 90% of proteins synthesized by acinar cells are digestive enzymes, the inevitable ischemia periods provide "ideal" conditions for triggering autolytic processes in the pancreas [19]. The negative effect of endogenous pancreatic proteases on functional ability of islets was noted, in particular, in ischemia/reperfusion-induced pancreatitis developing during preservation of donor pancreas [20]. Islet survival during ischemia can also be hindered by the fact that islets are directly surrounded by acinar cells, which are characterized by higher density of zymogen granules compared to teleinsular cells. This histological feature makes islets particularly vulnerable to proteolytic damage [21].

Lack of progress in the development of islet isolation methods has made it necessary to search for new, more effective approaches to obtaining islet tissue in quantities sufficient for successful transplantation treatment of patients with diabetes mellitus. In this work, we have studied the possibility of obtaining islet cell cultures purified of exocrine ballast without using standard enzyme preparations, which, as mentioned above, significantly reduce survival rate and functional capabilities of isolated islets.

MATERIALS AND METHODS

Laboratory Soviet chinchilla rabbits of different age and body weight were used as pancreas donors. There were 60 newborns (1–2 days old) weighing 60–70 g and 12 one-month old weighing 600–700 g, i.e. much higher than that of newborn rabbits. The animals were obtained from a laboratory animal nursery belonging to KrolInfo LLC with presentation of a veterinary certificate.

Given the above-described deleterious effect of proteolytic enzymes released during prolonged manipulations with donor pancreas on islets, we used techniques that reduce this effect, minimizing, in particular, organ ischemia time. We assumed that moderate proteolysis by endogenous enzymes, inevitable even at the shortest treatment of pancreatic tissue, would lead to death of acinar cells only, but would not have a damaging effect on islets. Therefore, in order to prevent excessive autolysis of pancreatic tissue, immediately after euthanasia of the animals and extraction of the pancreas, the latter was placed in a cold (4 °C) Hanks' balanced salt solution (PanEco), then quickly, using ophthalmic forceps, the capsule, visible blood vessels and the excretory ducts were removed and the organ was cut into about 2 mm fragments, which were washed twice with cold Hanks' solution, then carefully crushed with sharp ophthalmic scissors for 7–10 minutes. The duration of microdissection depended on the visible features of the treated gland and was determined by the researcher in each particular case. The resulting thick tissue suspension was washed with cold Hanks' solution at least three times. As a result of these manipulations, the treated pancreatic tissue at room temperature (20–24 °C) was presumably exposed only to sparing proteolytic effect of endogenous pancreatic enzymes, which, after abundant washing of the resulting tissue suspension, were removed together with fragments of autolysed exocrine tissue. The tissue suspension obtained after treatment of 1 pancreas of a one-month-old rabbit, or 5 pancreases of newborn rabbits consisted mainly of microfragments smaller than 1 mm^3, which were transferred to a 25 cm^3 culture tube (Corning), where 10–12 mL of RPMI-1640 HEPES medium without glutamine (PanEco) was immediately added and 1 mL of fetal calf serum (HyClone) was added. The tubes were placed in an incubator and cultured at 37 °C. The growth medium was replaced with fresh medium every 2–3 days. Changes occurring during incubation were monitored through a Nikon Eclipse TS 100 inverted microscope by daily monitoring; significant changes were recorded using a digital camera.

Histological examination of native pancreas of the newborn and one-month-old rabbits, as well as samples of the resulting cultures, at different periods of incubation of pancreatic microfragments, was carried out. The studied material was fixed in formalin. After routine dehydration, the samples were embedded in paraffin. The 4 μm thick media were stained with hematoxylin and eosin, and subjected to immunohistochemical staining according to the horseradish peroxidase standard technique to detect the main types of islet cells using appropriate monoclonal antibodies: antinsulin and antiglucagon (Sigma).

RESULTS AND DISCUSSION

Histological examination of the pancreas of 1–2-day-old rabbits and the pancreas of one-month-old rabbits revealed a significant difference in the ratio of endocrine (islet) and exocrine (acinar) tissues in these animals of different ages. The proportion of exocrine tissue in young animals was significantly higher than in the newborns. At the same time, oval-shaped islets were clearly separated from the surrounding exocrine tissue by interlayers of connective tissue (Fig. 1, a). At the same time, in newborn animals, due to the natural absence of active digestion, the exocrine pancreas is poorly developed; islets in the neonatal pancreas are markedly smaller, irregular, jagged and have no pronounced connective-tissue interlayers at the border with exocrine cells (Fig. 1, b).

Such histological features made the treatment of pancreatic tissue of newborn rabbits with collagenase preparations inappropriate because of the practical absence of a point of action of such enzymes (collagen fibers of connective-tissue interlayers). Therefore, it was decided to study the changes occurring during cultivation of the pancreas of newborn rabbits subjected only to mechanical crushing without enzyme treatment.

Observations using an inverted microscope revealed a significant decrease in the mass of exocrine tissue already on day 2–3 of incubation of neonatal pancreatic microfragments and their compaction and "growing" against the background of final death and elimination of acinar cells by the end of day 5–7 (Fig. 2).

The detritus formed during acinar cell destruction was safely removed during the next replacement of the culture medium. As a result, a culture was formed consisting almost entirely of free-floating dense globular or ovoid structures (Fig. 3).

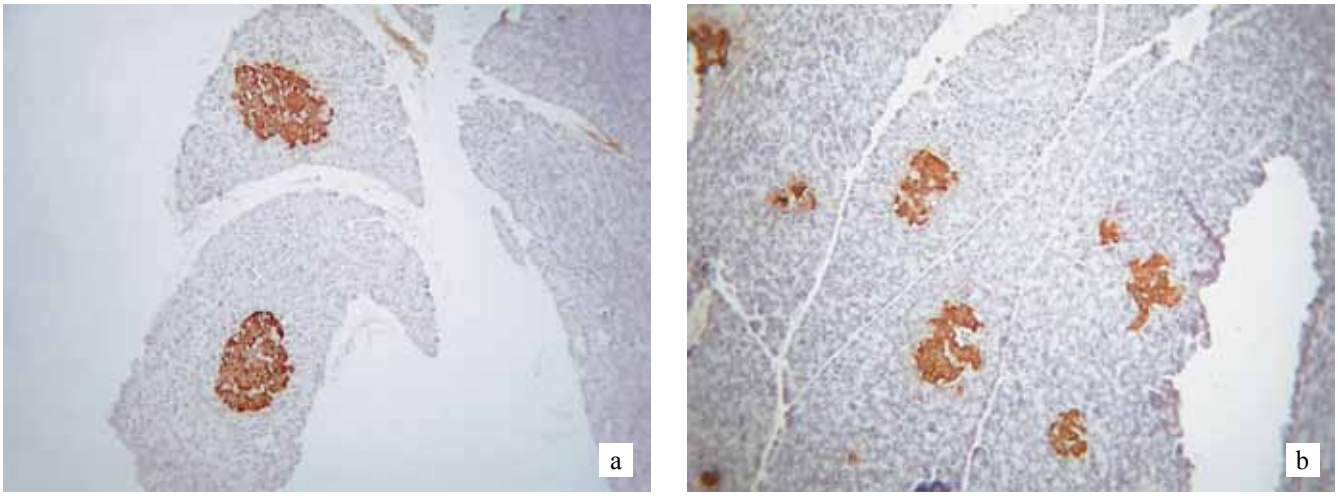

Fig. 1. a, pancreas of a one-month-old rabbit; b, pancreas of a one-day-old rabbit. Immunohistochemical staining of beta-cells of islets with insulin antibodies. 200×

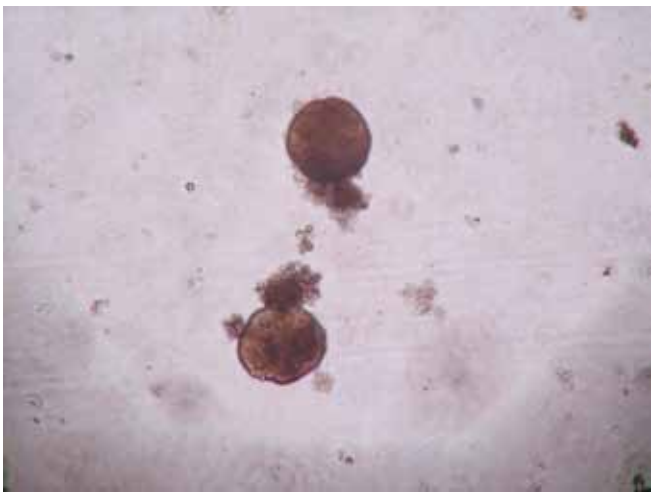

Fig. 2. Completion of spontaneous purification of newborn rabbit pancreatic microfragments from exocrine tissue. Inverted microscope. 100×

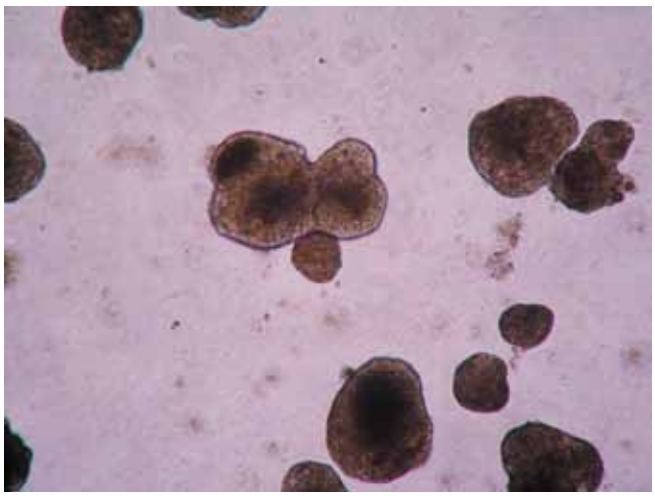

Fig. 3. Formation of floating cultures after 7-day incubation of pancreatic microfragments of newborn rabbits. Inverted microscope. 100×

Histological analysis of the cultures showed that they consisted of epithelium and were surrounded at the periphery by a layer of epithelium-like or fibroblast-like cells (Fig. 4).

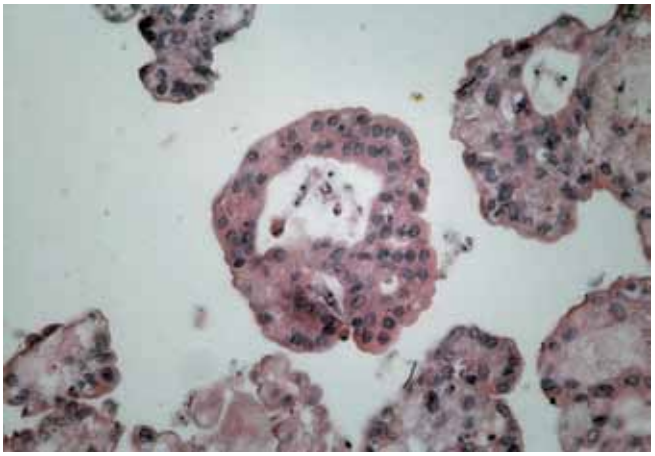

Fig. 4. Floating cultures obtained from the pancreas of newborn rabbits. H&E stain. 200×

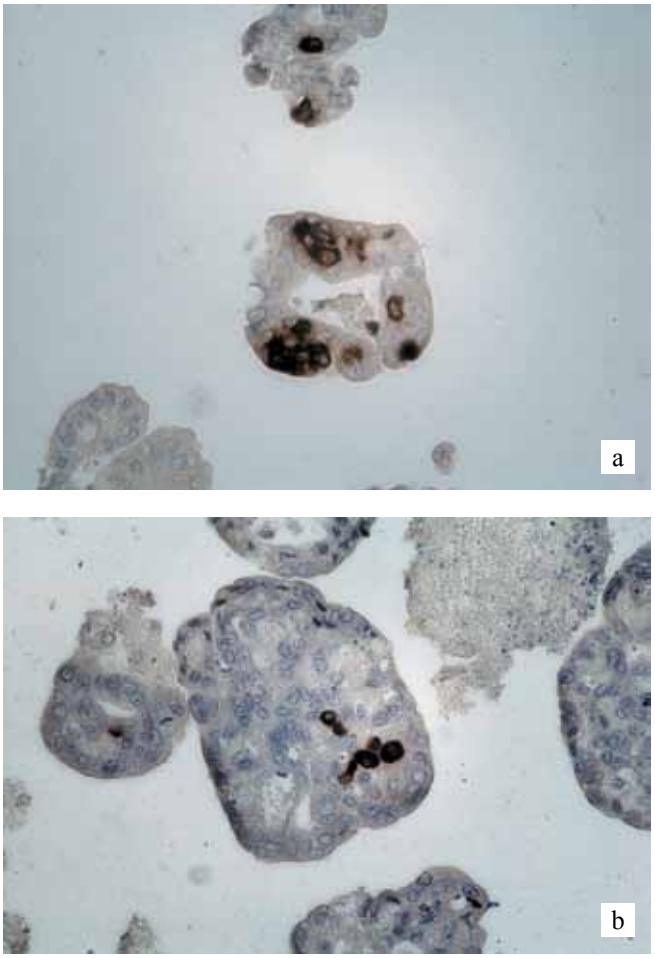

Fig. 5. Immunohistochemical staining of floating cultures obtained from the pancreas of newborn rabbits using insulin (a) and glucagon (b) antibodies. 200×

Immunohistochemical staining made it possible to identify the epithelium contained in the cultures as insulin-positive cells (to a greater extent) and glucagon-positive cells (Fig. 5).

The characteristic spherical and/or ovoid shape of the resulting free-floating cultures and detection of islet β- and α-cells in them gave grounds to call them floating islet-like cultures (FICs).

In contrast to obtaining cultures from the pancreas of newborn rabbits, the use of similar conditions when incubating pancreatic microfragments of one-month-old rabbits did not lead to pronounced elimination of exocrine tissue. Apparently, the reason for this failure was the presence of a significantly higher proportion of exocrine pancreatic tissue in young rabbits compared with the neonatal pancreas. Degradation by pancreatic acini was slow, and a significant amount of them persisted even after 8–10 days of incubation (Fig. 6).

At the same time, the prolonged effect of proteolytic enzymes released from acinar cells on the islet tissue apparently had a negative effect on its morphofunctional state, which prevented FICs formation. Therefore, we decided to increase the incubation temperature, which, presumably, could accelerate the death of exocrine tissue and provide more favorable competitive conditions for endocrine tissue survival.

In spite of the fact that the classical incubation condition is normothermia (temperature not higher than 37 °C), taking into account the natural resistance of pancreatic islets to unfavorable conditions and absence of such in exocrine tissue, we decided for the first time to incubate pancreatic microfragments at 38 °C, especially since normally the temperature inside the human and mammalian body can reach 38 °C.

As observations using an inverted microscope showed, such a regime (formally hyperthermic) is able to

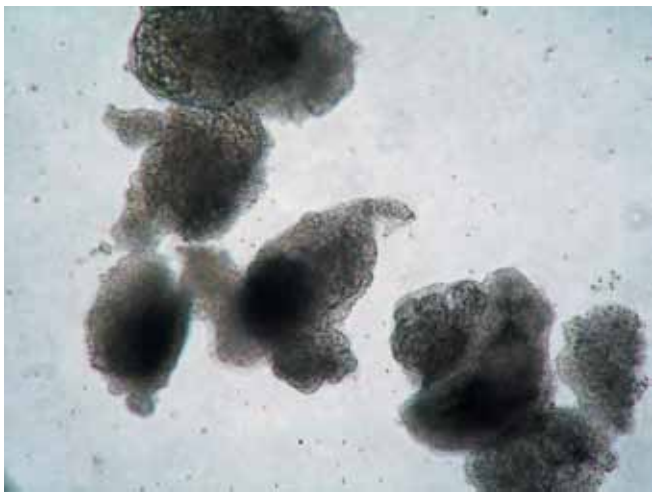

Fig. 6. Preserved exocrine tissue in the pancreatic microfragments of one-month-old rabbit after 10-day incubation. Inverted microscope. 100×

accelerate death and elimination of exocrine pancreatic tissue, which significantly reduces possible proteolytic effect on islets, contributing to their rapid cleansing and survival. Due to creation of such temperature conditions, a distinct degradation of exocrine tissue was observed already by day 3–4 of incubation (Fig. 7), and by day 8–10 ballast-cleared cultures were formed (Fig. 8).

Histological examination of the obtained floating cultures showed that they consisted mainly of viable epithelial cells (Fig. 9).

Using immunohistochemical staining, insulin granules were detected in their central part, indicating secretory activity of islet β-cells (Fig. 10). Thus, it was confirmed that islet-like cultures were obtained from the pancreas of one-month-old rabbits.

CONCLUSION

In our opinion, the very natural isolation, closeness of islets from the surrounding exocrine tissue, this "enzyme-boiling cauldron", allows us to hope that the native enzyme system of the pancreas is less dangerous for islets than the aggressive enzyme mixture that is unnaturally introduced into pancreatic tissue with the only purpose of "knocking out" islets, isolating them from the exocrine tissue. This consideration is confirmed by the fact that in acute pancreatitis, the endocrine (islet) tissue is very rarely affected, and only in cases of repeated

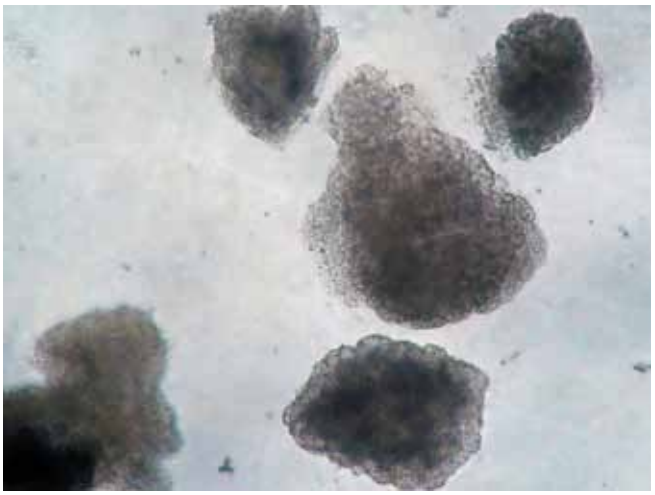

Fig. 7. Beginning of spontaneous purification against exocrine tissue at day 4 of incubation of pancreatic microfragments of one-month-old rabbits under hyperthermic conditions. Inverted microscope. 100×

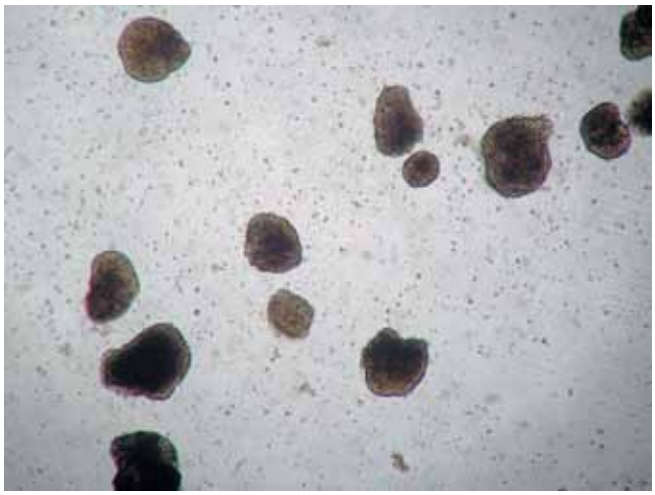

Fig. 8. Formation of floating cultures at day 10 of incubation of pancreatic microfragments of one-month-old rabbits under hyperthermic conditions. Inverted microscope. 40×

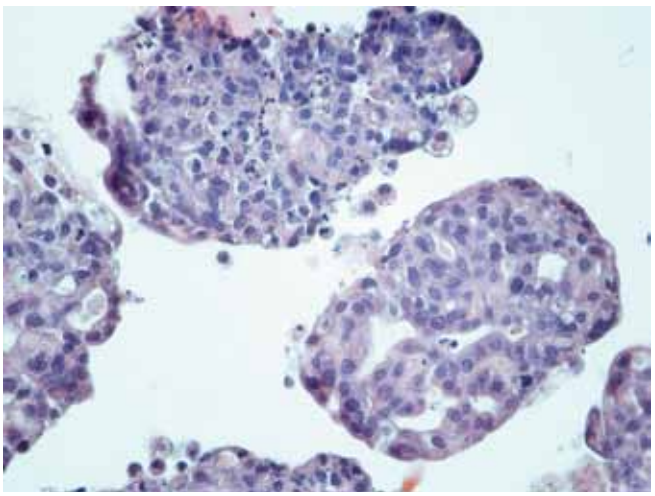

Fig. 9. Floating cultures obtained from the pancreas of one-month-old rabbits, day 8 of incubation under hyperthermic conditions. H&E stain. 400×

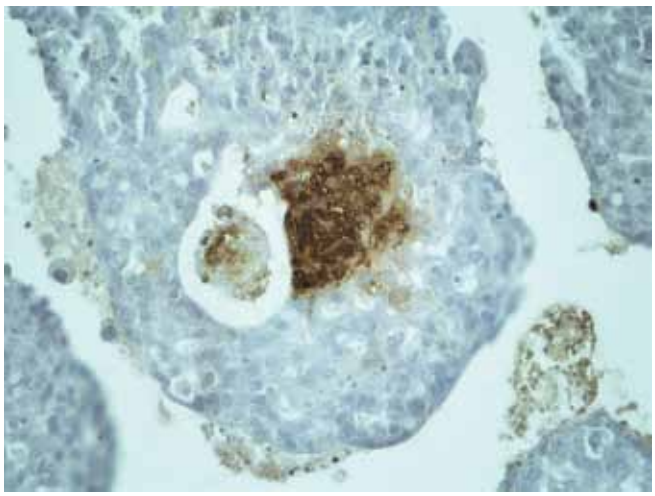

Fig. 10. Floating cultures obtained from the pancreas of one-month-old rabbits, day 8 of incubation under hyperthermic conditions. Immunohistochemical staining with insulin antibodies. 400×

episodes of recurrent pancreatitis or, more often, as a result of extensive pancreonecrosis, significant number of islets die, which leads to a pronounced deficit of insulin-producing β-cells, development of insulin deficiency and, naturally, to the clinical manifestation of insulin-dependent diabetes mellitus.

Results obtained in this study suggest that the use of exogenous enzymes for the treatment of donor pancreas tissue in the process of obtaining cultures consisting mainly of endocrine (islet) cells can be abandoned. Numerous observations using an inverted microscope showed that under standard conditions of incubation of the microfragments of mechanically crushed pancreas, its exocrine tissue dies spontaneously. This destructive process seems to be caused by autolysis of acinar cells under the influence of digestive enzymes contained in them during normothermic cultivation. The resulting detritus is removed in a timely manner during the next replacement of the culture medium. At the same time, naturally protected islets are not significantly damaged, which is due to the presence of islet-surrounding basement membranes consisting of various extracellular matrix proteins [22, 23]. The basement membrane serves as a peculiar interface between islets, endothelial cells and acinar cells through integrins and other cell receptors [24, 25], forming a protective barrier that ensures morphological integrity of islets during non-intensive self-digestion of the pancreas. As a result, the endocrine (islet) tissue seems to spontaneously cleanse itself from the, unnecessary ballast but highly immunogenic, exocrine tissue, while preserving the extracellular matrix necessary for survival and functioning of islet cells, represented, in particular, by the periosteal membrane mentioned above.

The most rapid process of getting rid of exocrine tissue occurs at the standard incubation temperature (37 °C) of microfragments of newborn rabbits. However, this mode turned out to be unsuitable for the pancreas of young (one-month-old) animals, which is due to the presence of a much higher percentage of exocrine tissue. It was decided to apply non-standard hyperthermic (38 °C) incubation for the first time in order to intensify autolytic processes. As expected, under the new temperature conditions, there was a significantly greater loss of exocrine tissue contained in the crushed pancreatic microfragments already at day 5–7, as well as their gradual compaction and scalding. These processes led to the formation of FICs similar to those we obtained from the pancreas of newborn rabbits.

Therefore, the rational methodological approaches developed in this study make it possible to obtain islet-like cultures purified of exocrine tissue without the use of the expensive and ambiguously effective enzyme preparations. In our opinion, further experiments on modification of the enzyme-free method of obtaining islet cell cultures from adult rabbits will provide data that can be extrapolated to develop a more rational and productive method of pancreatic islet isolation from deceased human donors.

The authors declare no conflict of interest.

REFERENCES

1. *Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL et al.* Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med.* 2000; 343: 230–238.
2. *Barton FB, Rickels MR, Alejandro R, Hering BJ, Wease S, Naziruddin B et al.* Improvement in outcomes of clinical islet transplantation: 1999–2010. *Diabetes Care.* 2012; 35: 1436–1445.
3. *Misawa R, Ricordi C, Miki A, Barker S, Molano RD, Khan A et al.* Evaluation of viable beta-cell mass is useful for selecting collagenase for human islet isolation: comparison of collagenase NB1 and liberase HI. *Cell Transplant.* 2012; 21: 39–47.
4. *Linetsky E, Bottino R, Lehmann R, Alejandro R, Inverardi L, Ricordi C*. Improved human islet isolation using a new enzyme blend, liberase. *Diabetes.* 1997; 46: 1120–1123.
5. *Alejandro R, Barton FB, Hering BJ, Wease S.* 2008 Update from the Collaborative Islet Transplant Registry. *Transplantation.* 2008; 86: 1783–1788.
6. *Sabek OM, Cowan P, Fraga DW, Gaber AO.* The effect of isolation methods and the use of different enzymes on islet yield and in vivo function. *Cell Transplant*. 2008; 17: 785–792.
7. *Bertuzzi F, Cainarca S, Marzorati S, Bachi A, Antonioli B, Nano R et al.* Collagenase isoforms for pancreas digestion. *Cell Transplant.* 2009; 18: 203–206.
8. *Szot GL, Lee MR, Tavakol MM, Lang J, Dekovic F, Kerlan RK et al.* Successful clinical islet isolation using a GMP-manufactured collagenase and neutral protease. *Transplantation.* 2009; 88: 753–756.
9. *Wang Y, Paushter D, Wang S, Barbaro B, Harvat T, Danielson K et al.* Highly purified versus filtered crude collagenase: comparable human islet isolation outcomes. *Cell Transplant.* 2011; 20: 1817–1825. PMID: 21396158.
10. *Kayton S, Poffenberger G, Henske J, Dai Ch, Thompson C, Aramandla R et al.* Human islet preparations distributed for research exhibit a variety of insulin-secretory profiles. *Am J Physiol Endocrinol Metab.* 2015 Apr 1; 308 (7): E592–E602. doi: 10.1152/ajpendo.00437.2014.
11. *Rheinheimer J, Klarmann Ziegelmann P, Carlessi R, Ross Reck L, Bauer AC, Leitão B.* Different digestion enzymes used for human pancreatic islet isolation: A mixed treatment comparison (MTC) meta-analysis. *Islets.* 2014; 6 (4): e977118. Published online 2014 Nov 7.
12. *Khatri R, Hussmann B, Rawat D, Gürol AO, Linn T.* Intraportal Transplantation of Pancreatic Islets in Mouse Model. *J Vis Exp.* 2018 May 5; (135): 57559. doi: 10.3791/57559.
13. *Saliba Y, Farès N.* Isolation, Purification, and Culture of Mouse Pancreatic Islets of Langerhans. *Methods Mol*

Biol. 2019; 1940: 255–265. doi: 10.1007/978-1-4939-9086-3_18. PMID: 30788831.
14. *Corbin KL, West HL, Brodsky S, Whitticar NB, Koch WJ, Nunemaker CS.* A Practical Guide to Rodent Islet Isolation and Assessment Revisited. *Biol Proced Online.* 2021 Mar 1; 23 (1): 7. doi: 10.1186/s12575-021-00143-x. PMID: 33641671.
15. *Dietrich I, Girdlestone J, Giele H.* Differential cytokine expression in direct and indirect co-culture of islets and mesenchymal stromal cells. *Cytokine.* 2021 Dec 17; 150: 155779. doi: 10.1016/j.cyto.2021.155779.
16. *Hubber EL, Rackham CL, Jones PM.* Protecting islet functional viability using mesenchymal stromal cells. *Stem Cells Transl Med.* 2021 May; 10 (5): 674–680. doi: 10.1002/sctm.20-0466.
17. *Brandhorst D, Brandhorst H, Johnson PRV.* Enzyme Development for Human Islet Isolation: Five Decades of Progress or Stagnation? *Rev Diabet Stud.* 2017 Spring; 14 (1): 22–38.
18. *Gorelick FS, Otani T.* Mechanisms of intracellular zymogen activation. *Baillieres Best Pract Res Clin Gastroenterol.* 1999; 13 (2): 227–240.
19. *Piton G, Barbot O, Manzon C, Moronval F, Patry C, Navellou JC et al.* Acute ischemic pancreatitis following cardiac arrest: a case report. *JOP.* 2010; 11 (5): 456–459.
20. *Dembinski A, Warzecha Z, Ceranowicz P, Tomaszewska R, Dembinski M, Pabianczyk M et al.* Ischemic preconditioning reduces the severity of ischemia/reperfusion-induced pancreatitis. *Eur J Pharmacol.* 2003; 473 (2–3): 207–216.
21. *Trimble ER.* In: Lanza RP, Chick WL. Pancreatic islet transplantation. 1994. Pancreatic islet-acinar relationships; 19–25.
22. *van Deijnen JH, Hulstaert CE, Wolters GH, van Schilfgaarde R.* Significance of the peri-insular extracellular matrix for islet isolation from the pancreas of rat, dog, pig, and man. *Cell Tissue Res.* 1992; 267 (1): 139–146.
23. *van Suylichem PT, van Deijnen JE, Wolters GH, van Schilfgaarde R*. Amount and distribution of collagen in pancreatic tissue of different species in the perspective of islet isolation procedures. *Cell Transplant.* 1995; 4 (6): 609–614.
24. *Otonkoski T, Banerjee M, Korsgren O, Thornell LE, Virtanen I.* Unique basement membrane structure of human pancreatic islets: implications for beta-cell growth and differentiation. *Diabetes Obes Metab.* 2008; 10 (Suppl): 119–127.
25. *Jiang FX, Naselli G, Harrison LC.* Distinct distribution of laminin and its integrin receptors in the pancreas. *J Histochem Cytochem*. 2002; 50 (12): 1625–1632.

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INDUCTION OF OSTEOGENESIS IN RABBIT MANDIBULAR BONE TISSUE USING AN ALBUMIN-BASED CRYOGENICALLY STRUCTURED POROUS 3D CARRIER LOADED WITH A BIOREGULATOR

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Objective: to study the induction of osteogenesis caused by introducing into the defect area broadly porous cryogenically structured 3D carriers, based on serum albumin and loaded with a bioregulator isolated from bovine serum on an experimental model of mandible defect in rabbits *in vivo*. **Materials and methods.** Cryogenically structured sponges in the form of cylindrical specimens, 5 mm in diameter and 5 mm in height, prepared from bovine serum albumin, were used as the bioregulator carrier. The experimental laboratory animals were male Chinchilla rabbits, weighing 2–2.5 kg. Bone tissue was skeletonized under anesthesia (intramuscular anesthetic Zoletil 100) with a 3-cm incision in the angle of the mandible and a 5-mm-diameter cutter was used to create a 2–3-mm deep defect to install an appropriate-size albumin sponge. A total of 24 animals participated in the experiment. X-ray control of the defect area was performed *in vivo* on day 14 using PanExam+ (Kavo) device (20 m X-ray). Histological examination of tissues was carried out at day 30 after the defect using a light microscope. **Results.** Experiments performed indicate an active restoration of bone tissue in the extensive defect area when using an albumin-based 3D carrier with the inclusion of a bioregulator as compared to the control experiments. There were osteointegrative and osteoinductive processes, almost complete decomposition (biodegradation) of albumin sponge with formation of islands of dense bone tissue with small foci of coarse fibrous tissue in the defect. This demonstrated good dynamics of recovery processes at this stage of healing. **Conclusion.** Under the action of a serum bioregulator contained in an albumin-based sponge, the repair process leads to restoration of normal bone tissue without formation of bone callus and altered bone tissue different from the native one.

Key words: osteoinduction, 3D technology, cryogels, bioregulators.

INTRODUCTION

In maxillofacial surgery, the problem of restoring bone defects with the use of special implant materials or compositions remains a hot topic. Moreover, it is important what materials and with what filling are used for replacement and restoration of bone tissue in various pathological processes. In particular, methods with replacement of bone defects using auto- and allografts as well as decalcified and decellularized artificial bones are used. However, our own bone material is not always available, especially for large defects, and the use of allografts and artificial materials can lead to complications, including rejection. Currently, there are still no implant materials and constructs that can fully meet all the requirements. In this regard, the development of new materials for implants, as well as means and methods of increasing their effectiveness should be considered very urgent [1]. Meanwhile, bone-made implants, subjected to the necessary pretreatment [2], as well as implants formed from biopolymer precursors suitable for this purpose are of interest [3, 4].

Membranous bones, such as cranial bones, provide better engraftment in the maxillofacial defect area [5]. Porous tissues such as cancellous bone can be used for faster sprouting of blood vessels in them and acceleration of ossification processes [6]. Grafts from decalcified bone matrix are also of interest since it has been shown that demineralized bone promotes osteoinduction processes [7, 8].

Previously, we investigated the effect of serum bioregulator in cryogenically structured sponge carriers (so-called cryogels and cryostructures [9]) on the repair of femoral defects in Wistar rats at different periods (day 7, 14, 30, 90). Moreover, it has been shown that during wound healing, there is an effective recovery of dense bone tissue, starting from early periods, leading to osteogenesis stimulation with complete recovery by late periods and with the formation of dense bone tissue in the experimental defect area [10, 11]. The best results in

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these experiments were achieved using albumin cryogels [12] as carriers of serum bioregulator [11]. The use of cryogels based on other proteins for the treatment of bone defects has also been reported [13]. For example, in the case of a cryogel formed from collagen filled with hydroxyapatite nanoparticles, safety, biodegradability and osseointegration induced by this material have been demonstrated over a long period (15 weeks) in vivo [14].

Given the positive results of the above experiments on the positive effect of serum bioregulator included in albumin cryogel on the regenerative process in the case of rat femur defect healing model, we planned to study the restoration of rabbit jaw bone, since there are significant differences between the tubular bones and the jaw bone. The jaw bone has a lamellar structure, the maxilla has a lot of spongy bone tissue and a thinner cortical plate of dense tissue, the mandible has a denser cortical plate [15]. Therefore, the jaw bones, as well as the skull bones, are less amenable to repair after damage. It was expected that the use of a bioregulator form adsorbed on a protein carrier as a transport conductor would accelerate osteogenesis stimulation, as a factor aimed at restoring the artificially created mandibular tissue defect.

MATERIALS AND METHODS

We used cryogenically structured sponge media in the form of cylindrical samples, 5 mm in diameter and 5 mm in height, prepared from bovine serum albumin according to a previously published technique [12]. Then, the resulting porous carriers were incubated in an aqueous solution of bioregulator, frozen, and lyophilically dried. The control samples were albumin carriers containing no bioregulator, also lyophilically dried.

The bioregulator was isolated from bovine blood serum (a commercial preparation used as a nutrient additive to culture media, BioLot) using a technique that included protein desalting with ammonium sulfate, dialysis, concentration, and then isoelectrophocusing in a sucrose density gradient at pH 3–10 [16]. The high degree of bioregulator purification was demonstrated by electrophoresis in polyacrylamide gel electrophoresis and reverse-phase HPLC. In this work, we used a commercial preparation Viorgon 1, manufactured by IPB.

The experimental laboratory animals were male Chinchilla rabbits, weighing 2–2.5 kg. Bone tissue was skeletonized under anesthesia (intramuscular anesthetic Zoletil 100) with a 3-cm incision in the angle of the mandible and a 5-mm-diameter cutter was used to create a 2–3-mm deep defect to install an appropriate-size albumin sponge. The study materials – 3D carriers containing and not containing the blood serum bioregulator – were placed into the formed defects. After filling the defects, soft tissues and skin were sutured. The animals were kept under standard vivarium conditions. The wound was sutured in layers after treatment with 3% hydrogen peroxide solution. The suturing was done with interrupted stitches with full coverage of the implant. Hemostasis was performed in the course of the operation. The skin wound was sutured with interrupted 4-0 polyglycolic acid sutures. Each rabbit was fitted with one test specimen of albumin sponge, either containing no serum bioregulator or containing serum bioregulator. In the negative control, nothing was inserted into the defect area and the wound was sutured. The following groups of 6 rabbits each were formed:

1. Native control (rabbits without defects).
2. Negative control (no albumin sponges were inserted in the defect area).
3. Control group (albumin sponges without serum bioregulator were inserted in the defect area).
4. Experimental group 1 (albumin sponges containing serum bioregulator at 10^{-10} mg/mL final concentration were inserted in the defect area).

A total of 24 animals participated in the experiment.

The defect area was subjected to X-ray imaging on day 14 in vivo on the PanExam+ (Kavo) device, (20 μR).

We divided the state of bone tissue on the radiographs into 3 points.

Score 1 – complete absence of osteoid tissue elements; the bone defect is determined radiographically as a shadow and defect zone, more filled with fibrous connective tissue.

Score 2 – defect area is 30–40% filled with islets of osteoid tissue and partially with coarse fibrous connective tissue, which presumably should restructure into young bone tissue with trabecular structure.

Score 3 – in the defect area, bone tissue density was almost equal to that of the parent tissue; small islets of connective tissue were observed against the background of active growth of normally structured bone tissue; but in general, identifying the defect area was difficult.

The state of bone defects was studied on day 14 via X-rays since the main regeneration processes, which further determine the quality of the bone tissue formed in the defect area, take place at this early stage. On day 30 after surgery, the animals were removed from the experiment, bone material was extracted from the defect area, fixed in formalin, decalcified, and embedded in paraffin to prepare 10 μm-thick histological sections. The histological sections were stained with hematoxylin and eosin and studied using light microscopy.

RESULTS AND DISCUSSION

As stated earlier, the main objective of this study was to test the possibility of osteogenesis induction caused by the action of serum bioregulator in the artificial bone defect area of a rabbit mandible, when such bioregulator was injected there in adsorbed manner on a cryogenically structured sponge cryogel prepared from denatured serum albumin. Formation of the above macroporous carrier is based on the previously discovered effect of cross-linking of the polypeptide chains of serum albumin

by disulfide bridges due to intermolecular thiol-disulfide exchange reactions resulting from the introduction of a denaturant (particularly urea or guanidine hydrochloride) and a small amount of a reducing agent (e.g., cysteine) into the initial protein solution, followed by freezing, freeze-drying, and further thawing [12, 17]. This sequence of operations leads to formation of a spongy protein cryogel (Fig. 1), which is easily degraded by proteases in case of a specific application [18]. It is this albumin carrier that was used in this work to introduce the serum bioregulator, adsorbed by it, into the body of laboratory animals.

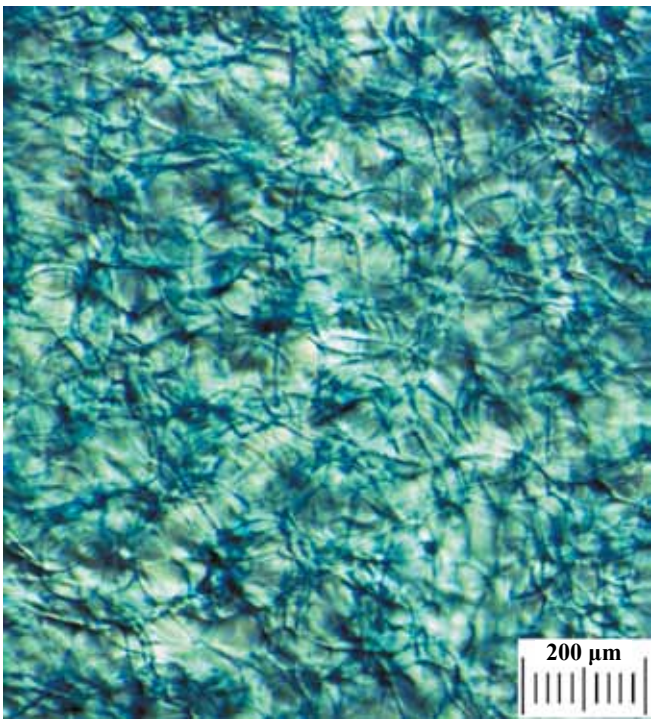

Fig. 1. Microstructure of serum-albumin-based cryogel contrasted with 0.125 mM aqueous methylene blue solution (optical stereomicroscope SMZ1000 (Nikon, Japan), equipped with digital system MMC-50C-M (MMCSoft, Russia) for image recording)

In the early stages after the defect (14 days), the state of the bone tissue was examined in vivo by X-ray.

The negative control on the mandible showed a defect zone without clear contours, there was a tendency to fill the formed defect. In the center, there are defined islands of dense substance, most likely zones of bone mass formation (score 1.5–2 on the scale described above). In the peripheral area of the defect, towards the center of the defect from the edge of the bone tissue, there were structurally more differentiated islands of bone substance. Radiographs show loose connective tissue located near the edge of the defect, which consists of intertwined bundles of collagen fibrils (Fig. 2).

In the control with a sponge, the defects were filled with connective tissue (coarse fibrous collagen tissue). In some areas closer to the edge of the maternal bone, islands of new bone substance deposition are detected (score 1 on the scale described above) (Fig. 3).

In the experimental group with the inclusion of serum bioregulator in the albumin sponge, exophytic growth of newly formed bone tissue with a trabecular structure interspersed with a fibrous matrix was determined in the defect area. Foci of osteogenesis in the form of osteoid deposition in the connective-tissue layer were observed (score 2.5–3 according to the above described scale) (Fig. 4).

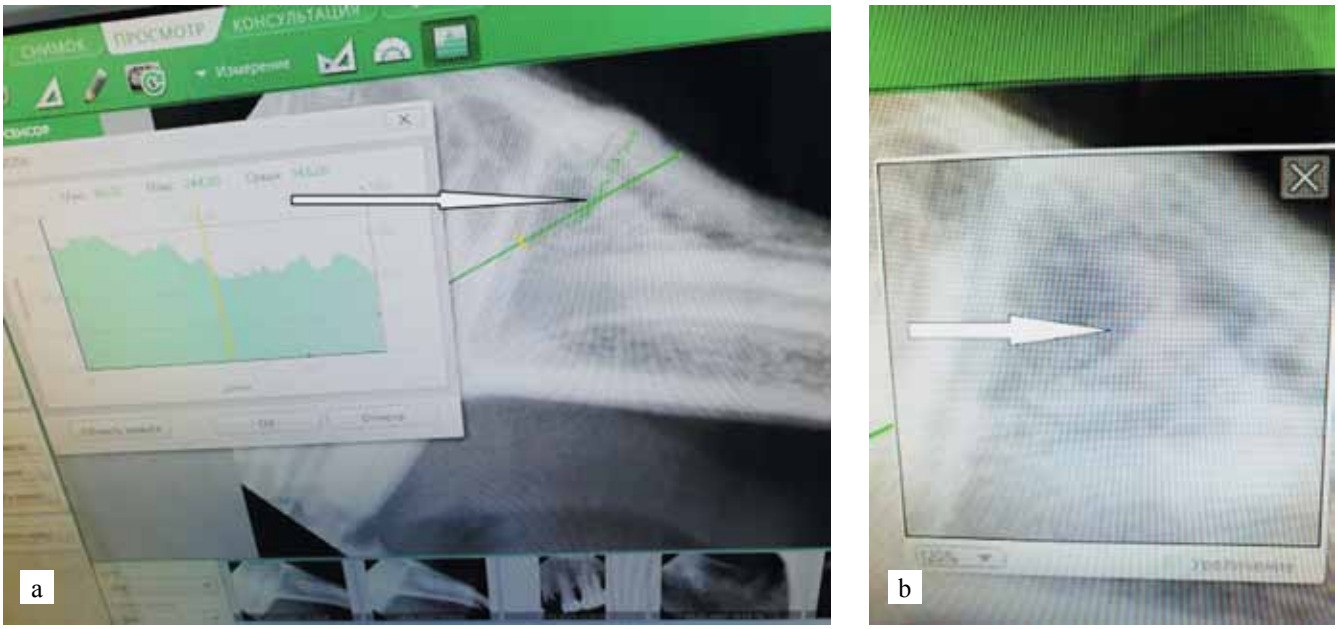

Fig. 2. X-ray of the rabbit mandible in the defect area (indicated by arrow) on day 14 in the control group: a, low magnification; b, high magnification

The histological description of the bone tissue state in the defect area on day 30 after its application in different groups gave the following picture.

Native control: Dense bone tissue, bone marrow (mostly yellow – fatty tissue, but red bone marrow is also presented) is visible inside cavities of bone beams. Elements of teeth with dentin, enamel and immature mesenchymal cells are well expressed. Dense bone tissue is well expressed, there are visible vessels. Osteons are well expressed, with small lacunae between the osteocytes. Nuclei of osteocytes are large, oval in shape. A mature lamellar osteoid bone is represented (Fig. 5, a).

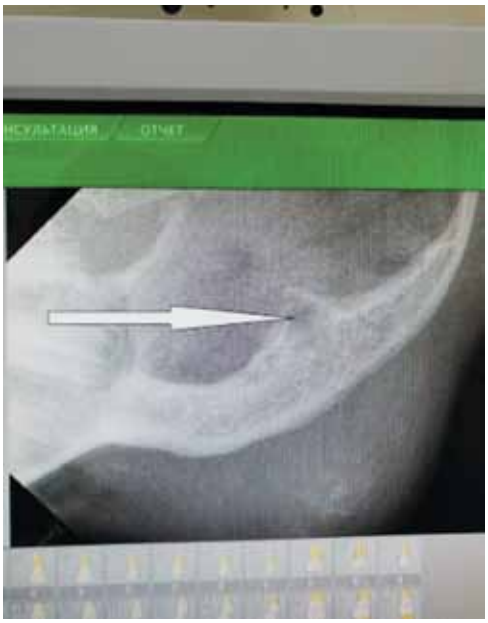

Fig. 3. X-ray of the rabbit mandible in the defect area (indicated by arrow) on day 14 in the albumin sponge group

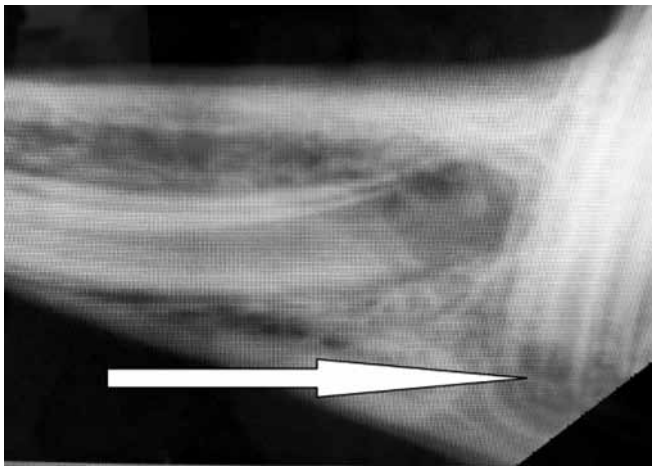

Fig. 4. X-ray of the rabbit mandible in the defect area (indicated by arrow) on day 14 in the group with albumin sponge, containing serum bioregulator

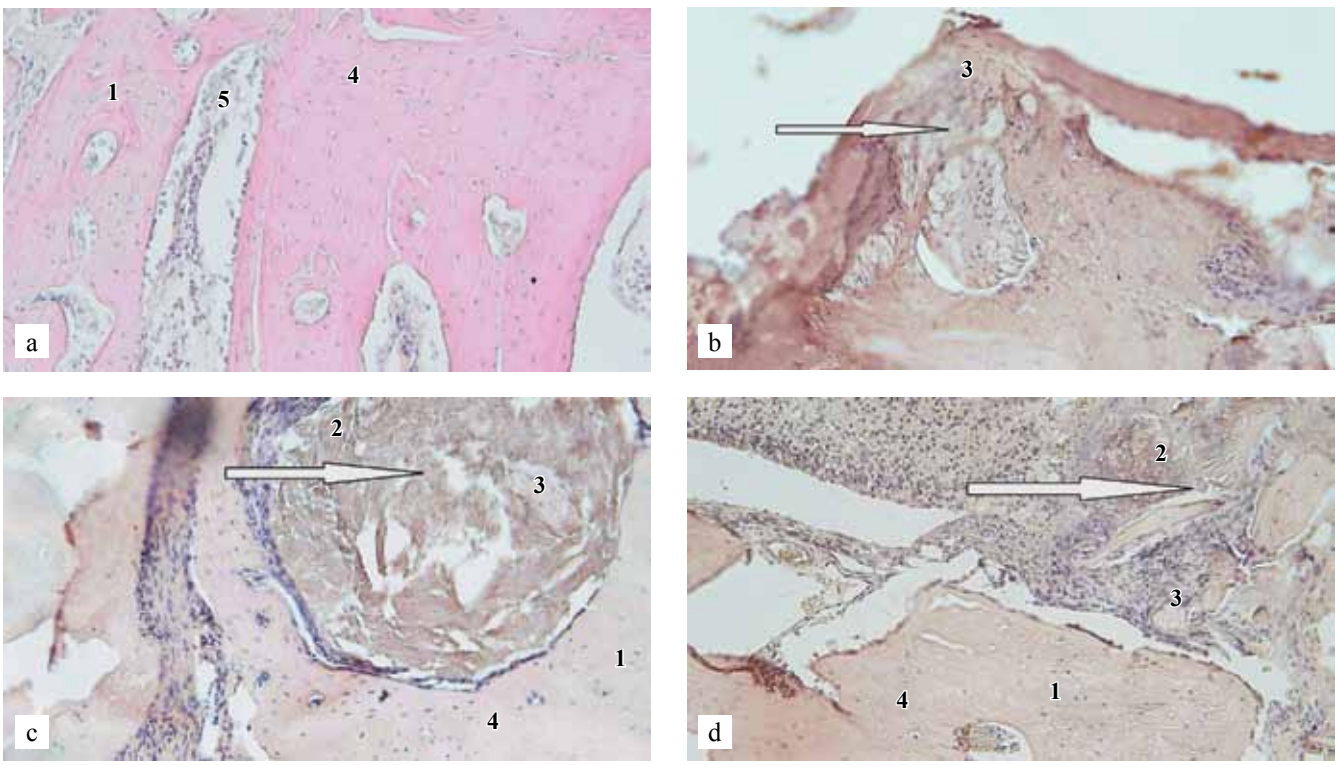

Fig. 5. Bone tissue of rabbit mandible: a, native; b, 30 days after application of defect (negative control, without introduction of any materials into the defect area; c, 30 days after application of defect (control, with introduction of albumin sponge, containing no serum bioregulator, into the defect area); d, 30 days after application of the defect (experimental group, with introduction of albumin sponge, containing serum bioregulator, into the defect area). 1, osteons; 2, remains of albumin sponge; 3, immature osteoid tissue; 4, dense bone tissue; 5, bone marrow. 200× magnification. The arrow indicates the defect area

In the negative control group (no filling of defect area with materials), there is no complete healing of defects. The wound cavity is filled with tissue detritus, between which a new bone is forming, bone marrow is not expressed. Immature osteoid tissue is observed, lacunae are not expressed. Fibrous bone tissue, vessels are poorly represented (Fig. 5, b).

In the control group (filling of defect with albumin sponge containing no serum bioregulator), the wound was healing without bone marrow formation. One can see active formation of bone tissue, osseointegration of albumin sponge into bone, formation of cavities in it and partial settlement by cells. And also on the border, there is active cell granulation with formation of immature osteoid tissue (Fig. 5, c).

In the experimental group (filling of defect with albumin sponge containing the serum bioregulator), the remains of decomposing albumin sponge are visible in the damage area, the newly formed bone is dense, with small lacunae. Inside the bone cavity, bone marrow is visible. Formation of dense mature osteoid tissue with formation of osteons and haversian canals and bone marrow is proceeding. The alveolar bone tissue and the lamellar dense bone are being rebuilt (Fig. 5, d).

Any connective tissue is a precursor of bone tissue. It is necessary for the growth of integration processes and filling of defects with bone tissue. In the negative control group with an albumin sponge containing no bioregulator, we observed the beginning of the bone repair process with the formation of immature bone, in contrast to the group in which albumin sponge containing a serum bioregulator was introduced into the defect area. Thus, the albumin sponge is a carrier, which is necessary for the settlement of newly formed cells in it as an osteoconductor, and the osteoinducer, which accelerates the process of mature bone tissue restoration, is a serum bioregulator included in it.

CONCLUSION

In the negative control, the picture of recovery of dense bone tissue of the rabbit's mandible is not pronounced, the restored tissue is mainly coarse-fibrous. In the control group with albumin sponge, one can see incomplete decomposition of this sponge and only the beginning of osseointegrative processes. Results obtained indicate active restoration of bone tissue in the extensive defect area when using a 3D carrier based on bovine serum albumin (albumin sponge) with inclusion of a bioregulator isolated from blood serum. We can see the processes of osteointegrative and osteoinductive activity, almost complete albumin sponge decomposition in the defect area, with the formation of islands of dense bone tissue with small foci of rough fibrous tissue in the defect site, which indicates good dynamics of restorative processes at the given stage of the defect healing. This may suggest that under the influence of serum bioregulator in the albumin sponge, the repair process leads to restoration of normal bone tissue without formation of bone callus and altered bone tissue different from the native one.

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The authors declare no conflict of interest.

REFERENCES

1. *Gunko VI.* Znachenie kostno-rekonstruktivnyh operacij pri medicinskoj reabilitacii s vrozhdennymi deformaciyami chelyustey. *Aktualnye voprosy stomatologii*. M., 2000: 117.
2. *Min'kov SA, Shkitov YuS, Sakovich GN, Kazimirskiy VA.* Klinicheskij opyt otsrochennoj implantacii nizhnej chelyusti vo vremya eyo rezekcii. *Aktualnye problemy stomatologii*. M., 2000: 126.
3. *Baker EJ, Onissema-Karimu S, Rivera-Galletti A, Fransis M, Wilkowski J, Salas-de-la-Cruz D, Hu X.* Protein-polysaccharide composite materials: fabrication and applications. *Polymers*. 2020; 12 (2): 464. doi: 10.3390/polym12020464.
4. *Mbundi L, Gonzalez-Perez M, Gonzalez-Perez F, Juanes-Gusano D, Rodriguez-Cabello JC.* Trends in the development of tailored elastin-like recombinamer-based porous biomaterials for soft and hard tissue applications. *Frontiers in Materials.* 2021; 7 (1): 601795. doi: 10.3389/fmats.2020.601795.
5. *Zins JE, Whitaker LA.* Membranous versus endochrondral bone: Implications for craniofacial surgery. *Plast reconstr Surg*. 1983; 72: 778.
6. *Gadzhiev AP.* Auto- i allotransplantaciya kompaktnoj i gubchatoj kostnoj tkani pri zameshhenii defekta nizhnej chelyusti [Dissertation]. M., 1986. 214.
7. *Polezhaev LV.* Regeneraciya putyom indukcii. *Zhurnal obschey biologii*. 1966; 27 (2): 223–233.
8. *Sampath TK, Reddi AH.* Dissociative extraction and reconstitution matrix components involved in local bone differentiation. *Cell Biology*. 1981; 78 (12): 7599–7603.
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. *Krasnov MS, Shayhaliev AI, Korshakov EV, Efimenko MV, Soloshenkov PP, Davidova TR et al.* Induction of osteogenesis of rat bone tissue using cryogenically structured porous 3D materials containing a bioregulator. *Bulletin of Experimental Biology and Medicine.* 2019; 168 (7): 113–117. doi: 10.1007/s10517-019-04657-z.
11. *Krasnov MS, Shaikhaliev AI, Korshakov EV, Gasbanov GA, Korgoloev RS, Sinitskaya ES et al.* Changes in rat bone tissue at the site of the defect *in vivo* under the effect of a cryogenically structured albumin sponge containing a bioregulator. *Bulletin of Experimental Biology and Medicine.* 2021; 170 (12): 805–808. doi: 10.1007/s10517-021-05160-0.
12. *Rodionov IA, Grinberg NV, Burova TV, Grinberg VYa, Lozinsky VI.* Cryostructuring of polymeric systems. 40.

Proteinaceous wide-pore cryogels generated by the action of denaturant/reductant mixtures on bovine serum albumin in moderately-frozen aqueous media. *Soft Matter*. 2015; 11 (24): 4921–4931. doi: 10.1039/c4sm02814g.

13. *He Y, Wang C, Xiao Y, Lin W.* An overview on collagen and gelatin-based cryogels: fabrication, classification, properties and biomedical applications. *Polymers.* 2021; 13 (14): 2299. doi: 10.3390/polym13142299.
14. *Vilela MJC, Colaço BJA, Ventura J, Monteiro FJM, Salgado CL.* Translational research for orthopedic bone graft development. *Materials*. 2021; 14: 4130. doi: 10.3390/ma14154130.
15. *Pavlova IA, Vinogradova AV, Sergeeva ND, Spasich TA*. Anatomiya, fiziologiya chelyustno-licevoj oblasti v vozrastnom aspekte: metodicheskoe posobie. Irkutsk: N CRVH SO RAMN, 2014. 59.
16. *Yamskova VP, Krasnov MS, Yamskov IA*. Mehanizm deystviya membranotropnyh gomeostaticheskih tkane-specificheskih bioregulyatorov. Saarbrucken: Lambert Academic Publishing, 2012. 136.
17. *Lozinsky VI, Konstantinova NR, Solov'eva NI.* Method for the preparation of porous protein gel. Russ. Pat. No. 2,058,083 (1994).
18. *Lozinsky VI, Shchekoltsova AO, Sinitskaya ES, Vernaya OI, Nuzhdina AV, Bakeeva IV et al.* Influence of succinylation of a wide-pore albumin cryogels on their properties, structure, biodegradability, and release dynamics of dioxidine loaded in such spongy carriers. *Int J Biol Macromol*. 2020; 160 (1): 583–592. doi: 10.1016/j.ijbiomac.2020.05.251.

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DETERMINING THE OPTIMAL PANCREATIC DECELLULARIZATION PROTOCOL, TAKING INTO ACCOUNT TISSUE MORPHOLOGICAL FEATURES

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Introduction. Developing a tissue-engineered pancreatic construct (TEPC) involves a search for matrices/scaffolds capable of mimicking the structure and composition of the natural extracellular matrix (ECM), which is an important component of the tissue microenvironment. A cell-free, tissue-specific matrix obtained from pancreas decellularization seems to be the most suitable for creation of a TEPC. The choice of pancreatic tissue decellularization protocol should take into account the morphological characteristics of the original pancreas. Preservation of the architectonics and composition of the native tissue in the decellularized pancreas matrix (DPM), and the presence of native ECM components allow for creation of conditions for prolonged vital activity of functionally active islet (insulin-producing) cells when creating TEPC. **Objective:** to determine the optimal parameters for decellularization of deceased donor pancreas with fibrosis, lipomatosis, and without pronounced signs of fibrosis and lipomatosis. **Materials and methods.** We used the caudal part of the pancreas obtained after multiorgan procurement from deceased donors, which was unsuitable for transplantation. Tissue-specific matrix was obtained by a combination of physical and chemical methods of pancreatic decellularization. A freeze-thaw cycle protocol and two protocols using osmotic shock were used. Samples of initial pancreatic tissue and decellularized fragments were subjected to histological analysis. **Results.** It was shown that a physico-chemical method with freeze-thaw cycles is suitable for effective pancreatic decellularization in severe lipomatosis; a physico-chemical method using osmotic shock, but different protocol variants, is suitable for pancreas with diffuse fibrosis and for pancreas without pronounced signs of fibrosis and lipomatosis. **Conclusion.** For complete human pancreatic decellularization, the protocol should be correlated with histological features of the original tissue.

Keywords: pancreas, lipomatosis, fibrosis, decellularization, tissue-specific scaffold.

INTRODUCTION

Developing tissue-engineered constructs of tissues and organs, including the pancreas, includes the search for matrices (scaffolds, frameworks) capable of mimicking the structure and composition of the natural extracellular matrix (ECM). ECM secreted by cells is a network of macromolecules, including polysaccharide glycosaminoglycans (GAG) and proteins (collagens, laminins, fibronectin), and is an important component of the tissue microenvironment [1]. ECM performs many functions in the tissue, such as provision of structural integrity, mechanical properties and tissue organization, cell-matrix and signaling interactions such as cell attachment sites, regulation of cell adhesion, proliferation, migration, differentiation and death [2]. A tissue-specific matrix, in which the features of the native tissue architectonics and composition are preserved, seems to be the most suitable for TEPC creation [3–5].

One of the promising methods for obtaining tissue-specific matrices is decellularization of tissues and organs. An efficient decellularization process ensures removal of cellular material, including DNA, and cell surface antigens from the native tissue, while keeping as much as possible the structural, biochemical and biomechanical properties of the native ECM using different tissue processing techniques [1].

Most protocols describe the combined and sequential application of various physical, chemical and enzymatic methods to achieve effective decellularization. Decellularization of a large tissue fragment is quite a long process due to the need for penetration of all reagents into the target cells [4, 6]. In this case, physical exposure can disrupt the matrix structure, while chemical and enzymatic methods can cause reactions that will damage ECM components and even change ECM chemical composition [1]. Decellularization protocols must also take into account the characteristics of the original

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tissue, such as density and thickness, and the presence of lipids. Identical tissue may have different characteristics of structure and composition depending on the characteristics of the donor. For these reasons, optimization of the decellularization protocol is of paramount importance on a case-by-case basis.

Among the physical methods of decellularization, freeze-thaw cycles, osmotic shock method, mechanical agitation, perfusion, ultrasound and others are widespread. When tissue is frozen, intracellular ice crystals are formed, resulting in destruction of cell membranes and cell lysis. However, ECM protein structures can also be destroyed, so it is necessary to monitor the rate of temperature change in order to control the size of ice crystals formed [7]. Hypotonic and hypertonic solutions (osmotic shock method) [1] promote cell lysis but do not remove cell fragments from the matrix. Moreover, removal of DNA residues is of paramount importance in all decellularization protocols because of the tendency of nuclear material to attach to ECM proteins. Removal of cellular detritus is facilitated by a mechanical stirring process carried out with a magnetic stirrer, an orbital shaker, or a roller system [5, 8].

Physical methods alone are not sufficient for complete tissue decellularization. However, they are effective when combined with chemical and enzymatic processes. Surfactants are used as the chemicals (detergents) for dissolution of cell membranes and detritus dissociation in the decellularization process. Triton X100 detergent, targeting lipid-lipid and lipid-protein interactions, is often used to treat tissues with high protein content in ECM, while it is used with caution to treat tissues with high GAG content [9]. Sodium dodecyl sulfate (SDS) is used to effectively remove nuclear and cytoplasmic fragments. SDS dissolves both cellular and nuclear membranes but tends to denature proteins and can alter the natural structure of matrix [1]. For this reason, short-term SDS treatment is the most common – in order to minimize possible damage to proteins and to the overall matrix structure.

When creating TEPCs, the presence of native ECM components in the decellularized pancreatic matrix (DPM) allows creating conditions for prolonged life activity of functionally active islet (insulin-producing) cells [3–6, 10]. Maximum complete removal of cellular material from DPM minimizes immune response during further TEPC implantation [6]. Pancreatic islets cultured in the presence of DPM have been shown to increase insulin secretion compared to isolated islets in monoculture [11].

The aim of our study was to determine the optimal parameters for decellularization of deceased donor pancreas with fibrosis, lipomatosis and without pronounced signs of fibrosis and lipomatosis.

MATERIALS AND METHODS

Baseline

A significant number of deceased donor pancreas cannot be used for transplantation because organ transplantation requires strict pancreas selection criteria [12]. As a result of strict requirements for the quality of donor pancreas [13], many organs are rejected on the basis of medical history, fibrosis, lipomatosis, anthropometric characteristics and other parameters, even if the pancreas is healthy and functioning. Such organs can be reprocessed (including undergoing a decellularization process) for creation of biomaterials and can be used for tissue engineering rather than disposed of.

For the study, we used the caudal portion of the pancreas obtained as a result of multiorgan harvesting from deceased donors (n = 10, age of donors 34–63 years) and unsuitable for transplantation. Pancreatic tissue was stored at –80 °C until decellularization.

Decellularization of pancreatic fragments using freeze-thaw cycles

We have previously proposed a protocol for decellularization of donor pancreatic fragments with lipomatosis, which allows to obtain a tissue-specific matrix/scaffold free of cells and cell fragments, with low DNA content and preserved morphofunctional properties of the pancreatic ECM [14]. Pancreatic tissue was subjected to three freeze-thaw cycles to –80 °C and thawing to +37 °C followed by mechanical grinding (2 × 1 × 1 mm) and processing at room temperature under constant stirring in a CellRoll rotary system (INTEGRA Biosciences AG, Switzerland), with the buffer solution (pH = 7.4) containing 0.1% SDS solutions changed three times and increasing Triton X100 concentration (1, 2, and 3%, respectively) (Sigma, USA). Decellularized pancreatic fragments were then thoroughly washed of surface-active agent residues for 72 hours in phosphate-buffered saline with addition of an antibiotic/antimycotic.

In our work, we used this protocol for decellularization of pancreatic tissue that had no signs of fibrosis and lipomatosis.

Decellularization of pancreatic fragments using osmotic shock (two protocol variants)

Focusing on known methods of decellularization of different parenchymatous organs [1, 3–6, 15], we used physicochemical method with osmotic shock (exposure to ionic strength) in two versions for pancreas with fibrosis and for pancreas without fibrosis and lipomatosis symptoms.

Mechanically crushed pancreatic tissue (to a size of no more than 2 × 1 × 1 mm) was treated with detergents

at room temperature under continuous stirring in a Cell-Roll rotary system (INTEGRA Biosciences AG, Switzerland). In the first variant, 0.1% SDS solution and low and high ionic strength phosphate-buffered saline were used (variant I), and in variant II, 0.1% SDS solution and high and low ionic strength phosphate-buffered saline were used (variant II). Both variants were followed by thorough washing off of residual detergents from decellularized pancreatic tissue fragments – matrix (DPM) in three changes of the phosphate-buffered saline containing antibiotic and antimycotic.

Histological examination

Samples of the original pancreatic tissue and decellularized fragments were subjected to histological analysis. The material was fixed in 10% buffered formalin, dehydrated in ascending alcohols, incubated in a mixture of chloroform and ethanol, chloroform, and embedded in paraffin. Sections, 4–5 μm thick, were obtained using an RM2245 microtome (Leica, Germany) and further subjected to hematoxylin and eosin staining and Masson's trichrome stain; cell nuclei were visualized by DAPI staining (Sigma, USA). Obtained histological preparations were analyzed using a Nikon Eclipse 50i microscope (Nikon, Japan) equipped with a digital camera.

RESEARCH RESULTS

Histological analysis of the original pancreas

The morphological study of the original material revealed three types of pancreatic tissue samples: a pancreas with pronounced signs of lipomatosis (Fig. 1, a–c), a pancreas with diffuse fibrosis (Fig. 1, d–f) and a pancreas without pronounced morphological signs of pathology (Fig. 1, g–i). In spite of the identified differences, all samples showed preserved islets, which, as a rule, had a round (less often, elongated) shape and compact, sometimes lobular, structure. The compact structure was characteristic of smaller islets, while lobularity was determined in some larger islets. Specific DAPI staining confirmed the presence of cell nuclei both in the islet and in the surrounding acinar tissue.

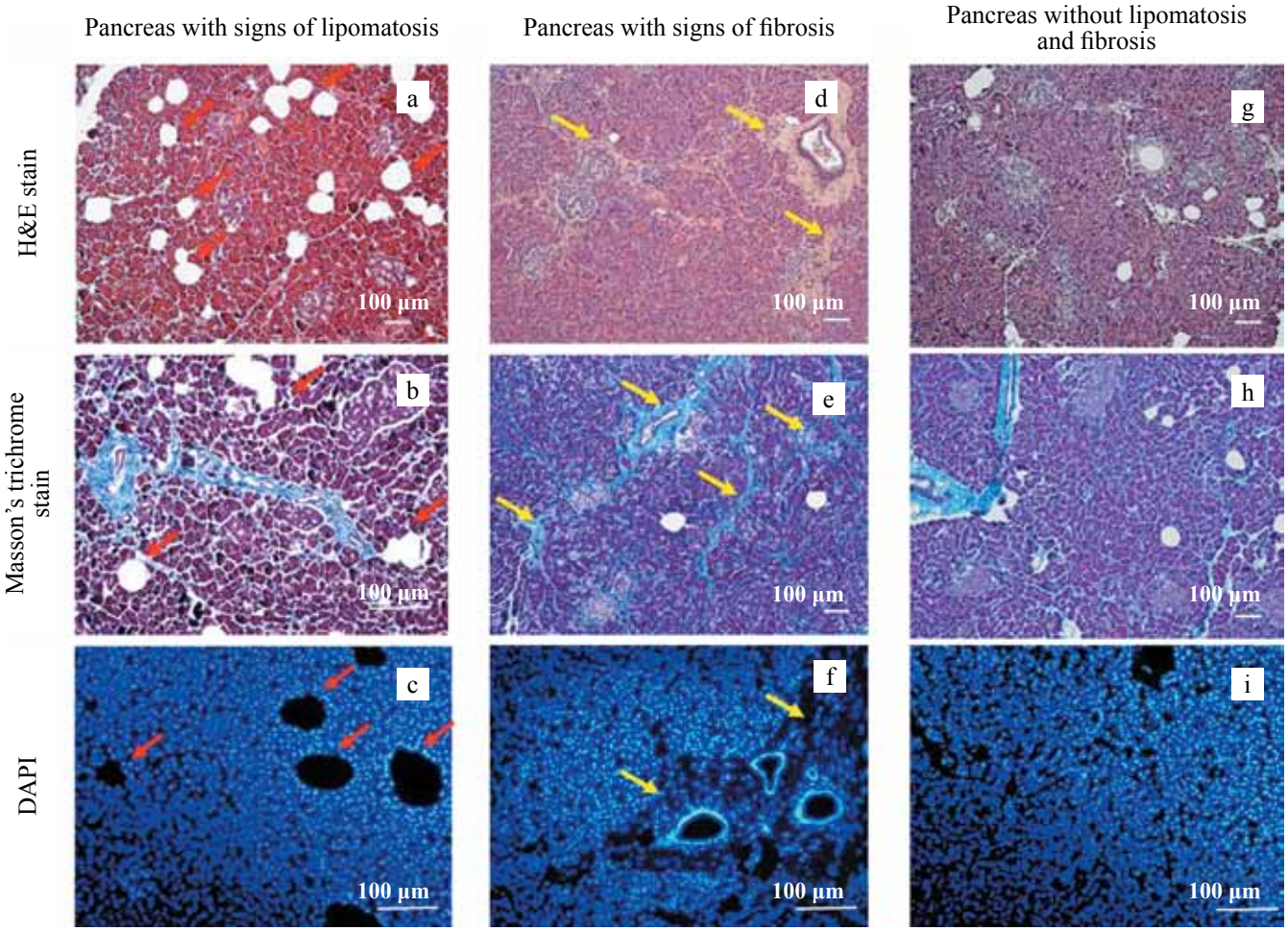

Fig. 1. Histological picture of deceased donor pancreas: a, d, g, H&E staining; b, e, h, Masson's trichrome staining; c, f, i, DAPI staining. Red arrows indicate lipomatosis features, yellow arrows indicate fibrous cords

Histological analysis of pancreas decellularized using freeze-thaw cycles

In samples of pancreatic fragments with lipomatosis after three consecutive freeze-thaw cycles and treatment with detergents, we observed a complete absence of preserved cells, complete absence of individual cell nuclei, and complete absence of small fragments of cellular detritus in the obtained connective tissue scaffold (Fig. 2, b). The morphological picture showed that a purified thin-fibrous matrix was obtained (Fig. 2, a).

After full protocol decellularization of a pancreas with diffuse fibrosis, the histological picture, in general, was different from that obtained when treating a pancreas with lipomatosis. Along with separate, well-purified openwork thin-fiber fragments, the samples showed dense areas (Fig. 2, c) in which a significant number of cells and nuclei were preserved, detected by DAPI staining (Fig. 2, d). This led to the conclusion that the tissue was incompletely decellularized, while a similar treatment of pancreas with lipomatosis allowed to obtain a completely purified matrix.

After decellularization of pancreatic tissue without pronounced signs of fibrosis and lipomatosis, we observed, in the obtained samples, preserved thin collagen fibers in the stroma and absence of preserved cells and cell nuclei (Fig. 2, e). However, numerous small grains of cellular detritus were detected in the thickness of the sample (Fig. 2, f), indicating that the decellularization procedure was ineffective.

Thus, the studies showed that the proposed protocol is suitable only for pancreas with lipomatosis, which seems to be related to the morphological features of the tissue.

Histological analysis of pancreas decellularized using osmotic shock (I and II versions of the protocol)

To determine optimal conditions of effective decellularization of pancreatic tissue with diffuse fibrosis and without marked signs of fibrosis and lipomatosis, we tested a physico-chemical pancreas decellularization method using osmotic shock – variant I of the protocol.

Decellularization of fibrotic pancreatic tissue by osmotic shock method (variant I of the protocol) resulted in samples in which the main part looked dense, non-porous due to the collapsed framework. At the same time, the preparations stained by Masson's method showed rough, densely packed collagenous strands (Fig. 3, a). The presence of cellular detritus (mainly in the peripheral zone), including nuclear material, was confirmed by DAPI staining (Fig. 3, b). Based on these results, we can conclude that the proposed decellularization protocol does not allow to obtain a purified, porous connective tissue scaffold and cannot be further recommended for decellularization of a pancreas with diffuse fibrosis.

Samples of decellularized matrix from the pancreas without evident signs of fibrosis and lipomatosis, obtained by the osmotic shock method (variant I of the protocol) demonstrated complete absence of preserved cells and cell nuclei (confirmed by DAPI staining) (Fig. 3, d)

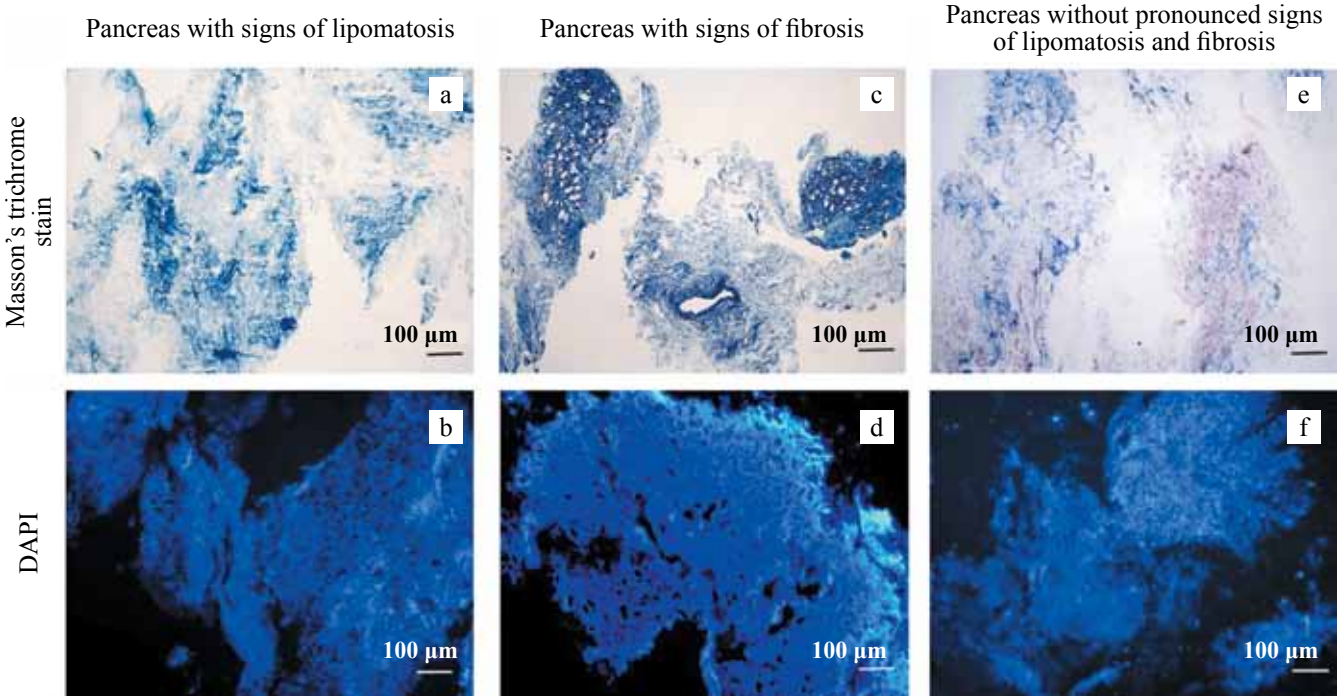

Fig. 2. Histological picture of pancreas, decellularized using freeze-thaw cycles: a, c, e, Masson's trichrome staining; b, d, f, DAPI staining

and a well-defined fine-cell thin-fiber structure tissue scaffold with blue collagen fibers (Fig. 3, c). Thus, the proposed protocol was effective in obtaining a matrix as a result of decellularization of pancreas without pronounced signs of fibrosis and lipomatosis.

Decellularization of a pancreas with diffuse fibrosis using the osmotic shock method according to variant II of the protocol was successful. In contrast to the samples obtained using variant I, the obtained matrix was characterized by a porous, fine-cell, thin-fiber structure with preserved collagen fibers (Fig. 4, a). At the same time, preserved cells, cell nuclei, and fragments of cellular detritus were not visualized in the samples when stained with DAPI (Fig. 4, b). The use of this protocol for decellularization of a pancreas with diffuse fibrosis allows to obtain tissue-specific matrix/framework free of cells and cellular fragments.

Thus, histological analysis of deceased donor pancreas revealed morphological features of the samples associated with the presence of signs of lipomatosis and diffuse fibrosis, which requires the use of different processing regimens for effective decellularization. The study of physical and mechanical properties of pancreatic tissue, such as density, stiffness, elasticity, is also an important aspect of decellularization protocol optimization [5]. It can be assumed that physico-mechanical properties will correlate to a certain extent with histological features of pancreas. Confirmation of this hypothesis needs further investigation.

We have not found any information in the published domestic and foreign scientific literature on the influence of human pancreas morphological features on the choice of decellularization protocol.

CONCLUSION

On the basis of the obtained data, we can state that in order to perform full decellularization of human pancreatic fragments, the treatment protocol should correlate with the histological features of the original tissue. It has been shown that physico-chemical method with freeze-thaw cycles is suitable for effective decellularization; for a pancreas with severe lipomatosis and for a pancreas

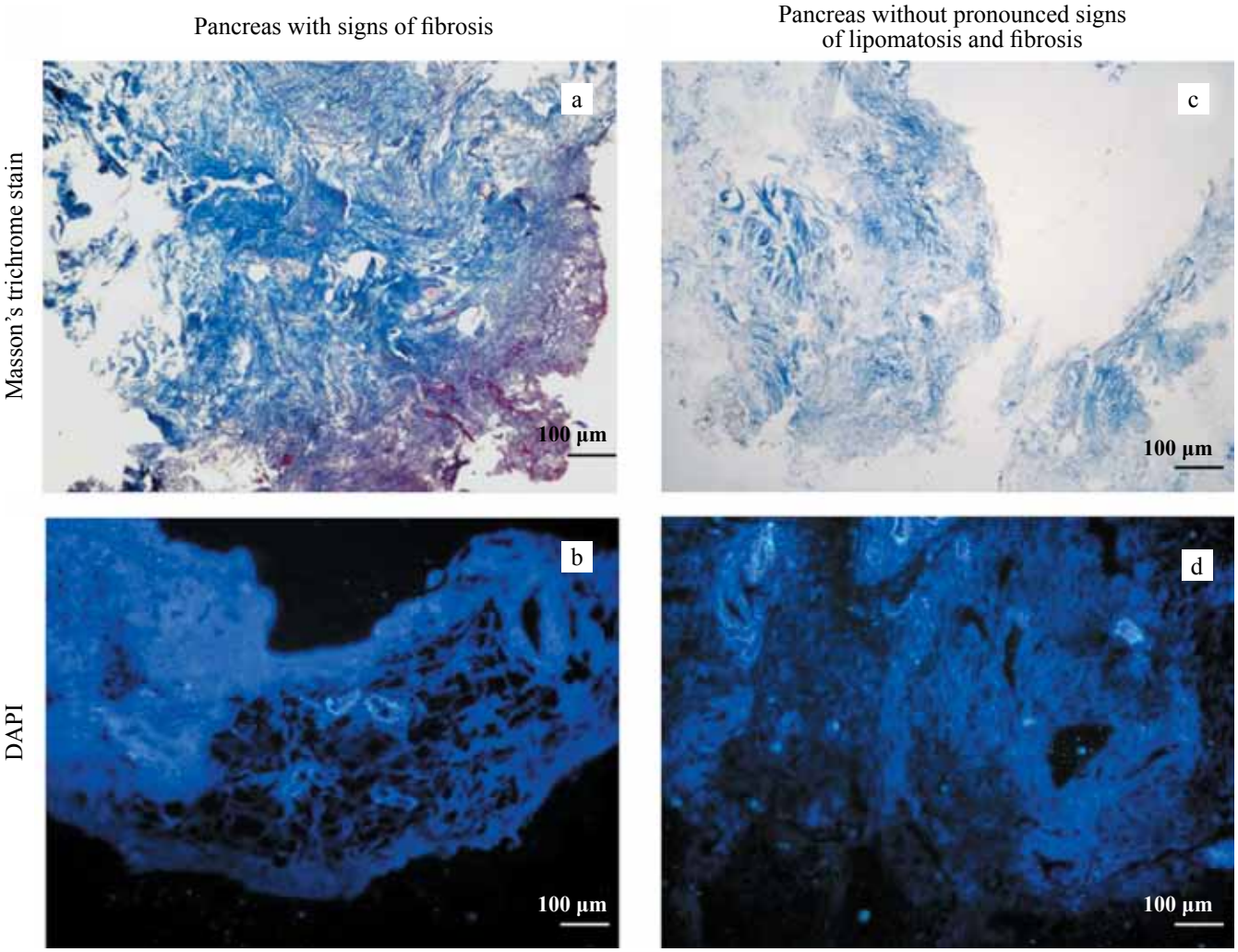

Fig. 3. Histological picture of the pancreas, decellularized using osmotic shock (protocol option I): a, c, Masson's trichrome staining; b, d, DAPI staining

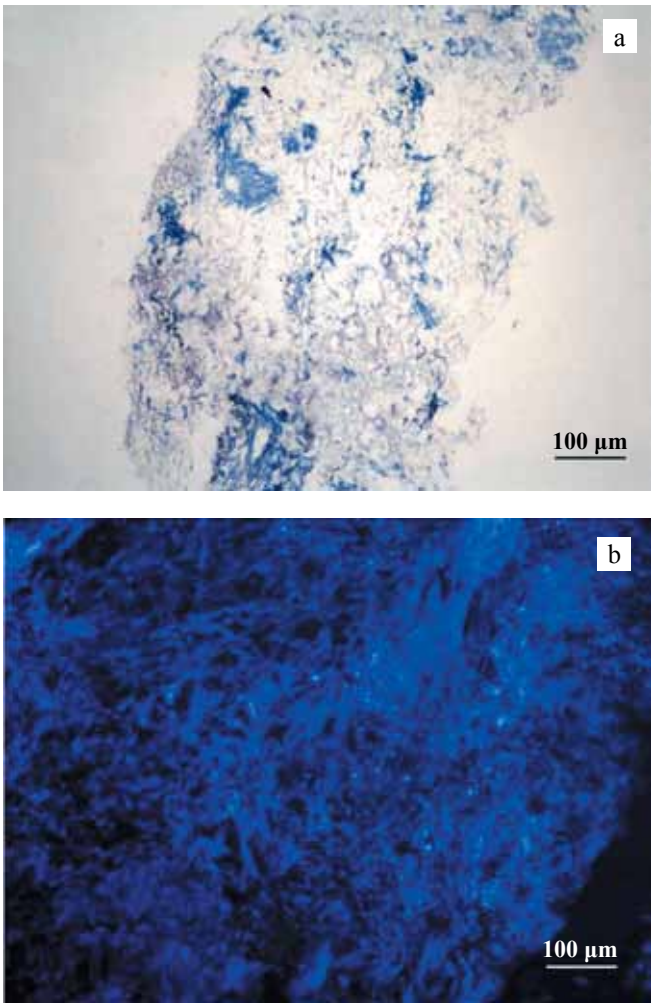

Fig. 4. Histological picture of the pancreas with diffuse fibrosis, decellularized using osmotic shock (protocol option II): a, Masson's trichrome staining; b, DAPI staining

without pronounced signs of fibrosis and lipomatosis, physico-chemical method using osmotic shock, but different variants of the protocol, is suitable.

The authors declare no conflict of interest.

REFERENCES

1. *Mendibil U, Ruiz-Hernandez R, Retegi-Carrion S, Garcia-Urquia N, Olalde-Graells B, Abarrategi A.* Tissue-Specific Decellularization Methods: Rationale and Strategies to Achieve Regenerative Compounds. *Int J Mol Sci*. 2020; 21, 5447. doi: 10.3390/ijms21155447.
2. *Stendahl JC, Kaufman DB, Stupp SI.* Extracellular Matrix in Pancreatic Islets: Relevance to Scaffold Design and Transplantation. *Cell Transplant*. 2009; 18 (1): 1–12. doi: 10.3727/096368909788237195.
3. *Damodaran G, Vermette P.* Decellularized pancreas as a native extracellular matrix scaffold for pancreatic islet seeding and culture. *J Tissue Eng Regen Med*. 2018; 12 (5): 1230–1237. doi: 10.1002/term.2655.
4. *Goh SK, Bertera S, Olsen P., Olsen P, Candiello JE, Halfter W et al.* Perfusion-decellularized pancreas as a natural 3D scaffold for pancreatic tissue and whole organ engineering. *Biomaterials*. 2013; 34 (28): 6760–6772. doi: 10.1016/j.biomaterials.2013.05.066.
5. *Sackett SD, Tremmel DM, Ma F, Feeney AK, Maguire RM, Brown ME et al.* Extracellular matrix scaffold and hydrogel derived from decellularized and delipidized human pancreas. *Scientific Reports*. 2018; 8: 10452. doi: 10.1038/s41598-018-28857-1.
6. *Crapo PM, Gilbert TW, Badylak SF.* An overview of tissue and whole organ decellularization processes. *Biomaterials*. 2012; 32: 3233–3243. doi: 10.1016/j.biomaterials.2011.01.057.
7. *Rabbani M, Zakian N, Alimoradi N.* Contribution of Physical Methods in Decellularization of Animal Tissues. *Journal of Medical Signals & Sensors*. 2021; 11 (1): 1. doi: 10.4103/jmss.JMSS_2_20.
8. *Starnecker F, König F, Hagl C, Thierfelder N.* Tissue-engineering acellular scaffolds-The significant influence of physical and procedural decellularization factors. *J Biomed Mater Res B Appl Biomater*. 2018; 106 (1): 153–162. doi: 10.1002/jbm.b.33816.
9. *Klak M, Łojszczyk I, Berman A, Tymicki G, Adamiok-Ostrowska A, Sierakowski M et al.* Impact of Porcine Pancreas Decellularization Conditions on the Quality of Obtained dECM. *Int J Mol Sci*. 2021; 22, 7005. doi: 10.3390/ijms22137005.
10. *Salg GA, Giese NA, Schenk M, Hüttner FJ, Felix K, Probst P et al.* The emerging field of pancreatic tissue engineering: A systematic review and evidence map of scaffold materials and scaffolding techniques for insulin-secreting cells. *Journal of Tissue Engineering*. 2019; 10: 1–25. doi: 10.1177/2041731419884708.
11. *Baranova NV, Kirsanova LA, Ponomareva AS, Nemets EA, Basok YuB, Bubentsova GN i dr.* Sravnitel'nyy analiz sekretornoy sposobnosti ostrovkov Langergansa, kul'tivirovannykh s biopolimernym mikrogeterogennym kollagensoderzhashchim gidrogelem i tkanespetsificheskim matriksom. *Vestnik transplantologii i iskusstvennykh organov*. 2019; 4: 45–53. doi: 10.15825/1995-1191-2019-4-45-53.
12. *Venturini M, Angeli E, Maffi P, Fiorina P, Bertuzzi F, Salvioni M et al.* Technique, complications, and therapeutic efficacy of percutaneous transplantation of human pancreatic islet cells in type 1 diabetes: the role of US. *Radiology*. 2005; 234: 617–624. doi: 10.1148/radiol.2342031356.
13. *Matsumoto S, Gala-Lopez B, Pepper AR.* Islet cell transplantation for type 1 diabetes. *J Diabetes*. 2010; 2 (1): 16–22. doi: 10.2147/DMSO.S50789.
14. *Ponomareva AS, Kirsanova LA, Baranova NV, Surguchenko VA, Bubentsova GN, Basok YB et al.* Decellularization of donor pancreatic fragment to obtain a tissue-specific matrix scaffold. *Russian Journal of Transplantology and Artificial Organs*. 2020; 22 (1): 123–133. doi: 10.15825/1995-1191-2020-1-123-133.
15. *Porzionato A, Stocco E, Barbon S, Grandi F, Macchi V, De Caro R.* Tissue-Engineered Grafts from Human Decellularized Extracellular Matrices: A Systematic Review and Future Perspectives. *Int J Mol Sci*. 2018; 19, 4117. doi: 10.3390/ijms19124117.

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PROGRAMMED CELL DEATH AND LIVER DISEASES

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Cell death represents the most critical pathologic entity in liver disease, which dictates pathologic consequences such as inflammation, fibrosis, and cell transformation. We analyzed the conclusions of studies on the involvement of different types of programmed cell death (PCD) in the pathogenesis of liver diseases. Three main forms of PCD (autophagy, apoptosis, necrosis) and five additional, still insufficiently studied PCD – necroptosis, ferroptosis, pyroptosis, partanatosis and entosis – observed in the liver in various acute and chronic diseases are considered. The involvement of several PCD at once in the development of any one pathology and one type of PCD in different pathologies was established. This indicates the existence of cross-regulation of metabolism in the liver cells with different levels of damage in the formation of the main dominant type of PCD. Available results indicate the possibility of attenuation (correction) of functional and morphological manifestations of PCD in the organ by controlled blocking of effector-mediated PCD pathways, as well as targeted induction of autophagy, anti-apoptotic and anti-necrotic mechanisms in liver cells.

Keywords: programmed cell death, autophagy, apoptosis, necrosis, liver diseases.

The study of molecular mechanisms of diseases in order to improve their treatment has become the main focus of modern medical science. Development of ideas about active participation in adaptation, morphogenesis and cellular homeostasis of the evolutionarily developed mechanism of regulation of cell activity – programmed cell death (PCD) – has contributed to the expanded study of the role of PCD in pathological processes in the body [1–4]. This review assesses the role of different forms of PCD in the pathogenesis of acute and chronic liver diseases, as well as substantiates possible ways to correct them.

1. DIFFERENT FORMS OF PCD ACTIVATED IN LIVER DISEASES

Cell death represents the most critical pathologic entity in liver disease, which dictates pathologic consequences such as inflammation, fibrosis, and cell transformation [5]. Most of the described cell death mechanisms, except for direct physical or chemical destruction, are mediated by evolutionary mechanisms and therefore belong to a type of programmed cell death. Depending on the nature of the damaging effect and the mechanisms of death initiation, there are 3 main, most studied PCD types (autophagy, apoptosis, necrosis) and 5 types of additional, still insufficiently studied PCD (necroptosis, pyroptosis, ferroptosis, partanatosis, entosis) (see Table 1).

These different types of PCD can manifest and coexist simultaneously in hepatocytes, cholangiocytes or nonparenchymal liver cells. The degree of expression and involvement of each form of PCD depends on the etiology and stage of the pathological process, and on the degree of cross-effects of other types of PCD on them. It is also important to note that among all the listed forms of PCD, autophagy and apoptosis occupy a special place because in the early stages of their development, the cell death process can be stopped or even prevented. This means that autophagy and apoptosis can serve as mechanisms for therapeutic regulatory (regenerative) effects.

1.1. Autophagy and autophagic cell death

Autophagy is the process of lifelong intracellular degradation and utilization of altered cytoplasmic contents through formation of autophagosomes. Autophagy plays a key role in the processes of cell adaptation and survival [7], as it provides short-term maintenance of cellular and energy homeostasis [8–10] due to the release of nutrient and energy-intensive substances into the cytoplasm and subsequent utilization. That is why some authors [11] consider autophagy as a way of "predominantly programmed survival" of cells due to the fact that when activated, autophagy provides protective rather than cytotoxic effects [12]. As a result of stress, altered proteins of the cytoplasm, damaged mitochondria, endoplasmic reticulum and peroxisomes translocate to organelle membranes, where they form a protein complex involved in formation of an autophagosome with a double membrane. Autophagosome formation (D = 0.3–1.0 μm) occurs with the participation of Vps 34, Beclin-1, Atg4 – Atg12/Atg16L1 proteins, etc. Subsequent formation of an autophagolysosome occurs through fusion of the autophagosome with lysosomes

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[13]. Degradation (hydrolysis) of altered proteins and release of nutrient and energy-intensive substances that can support the life-support of these cells into the cytoplasm takes place in the autophagolysosome [10, 11, 13]. With excessive degradation of altered proteins in cells, autophagic death can be prevented only by inhibiting autophagy [14].

Autophagy plays an important role in protecting the liver from toxic factors, particularly in alcoholic liver disease [15–17] and toxic effects of pharmaceuticals [18, 19]. The function of autophagy under these influences is to attenuate oxidative stress, to inhibit the excessive accumulation of altered proteins and damaged organelles in cells [20, 21]. Inhibition of autophagy by removal of

Table 1

Characteristics of various types of PCD [4–6]

Forms of PCD in liver	Mechanism of action	Effectors (markers)	Cell morphology	Liver diseases
1. Autophagy	Sequential formation in cytoplasm of phagophore, autophagosome, autophagosome lysosome	LC3-P, ULK1, Atg12, Atg4, GABA-RAP	Vacuolization of cell cytoplasm, formation of autophagosomes and autophagolysosomes	Toxic effects, viral hepatitis, alcoholic liver disease
2. Apoptosis (degradation of dying cells by phagocytosis without inflammation)	Caspase-dependent (receptor-mediated) and mitochondrial-dependent pathways	PS of outer membrane, FAS/ TNFR1, CASPs-3,7,8,9,10 BAX/BAK APAF1	Cell compaction, chromatin condensation, nuclear fragmentation, formation of apoptotic bodies	Cholestatic, autoimmune, diseases, viral hepatitis, alcoholic disease, non-alcoholic steatohepatitis, hepatocarcinoma
3. Necrosis mediated by increased mitochondrial permeability transition – mPT-driven necrosis – (cell destruction and inflammation)	ROS and RNS generation, cytosolic Ca accumulation in mitochondria (Mx).	CypD, BAX/BAK	Wrinkling and disorganization of cytoplasm and Mx structure, nuclear thickening and fragmentation, cytoplasmic membrane rupture, cell lysis	Ischemia-reperfusion injury, nonalcoholic fatty disease, alcoholic disease
4. Necroptosis (necrotoxic cell destruction)	Activation of FAS/ TNFR1, TLRs-3/4, ZBP1; CASP-8 inhibition, necrosome formation	RIPK1, RIPK3 MLKL	Cell lysis due to increased plasma membrane permeability	Drug toxicity, non-alcoholic steatohepatitis, alcoholic disease, autoimmune diseases
5. Ferroptosis (develops when the cell lacks glutathione)	Fe-catalyzed formation of ROS, lipid peroxidation (PL)	GPX4	Necrotic morphology, mitochondria destructured, loss of cristae, outer membrane ruptures	Drug toxicity, autoimmune hepatitis, alcoholic disease, non-alcoholic steatohepatitis
6. Pyroptosis (combines signs of apoptosis, necrosis and inflammation)	Removal of grand-tricellular pathogens (LPS and/ or bacteria) by inflammasome formation	NLRP1,-3; CASP-1, CASPs-4,-5,-11; GSDMD	Cell lysis due to formation of pores in the outer membrane;	Drug toxicity, cholestasis, autoimmune and viral hepatitis, non-alcoholic steatohepatitis, alcoholic disease
7. Partanatosis	Alkylating DNA damage, exposure to ROS, RNS, hypoxia	PARP-1	DNA fragmentation, nuclear condensation	Toxic drugs, hepatocarcinoma
8. Entosis	Disappearance of integrin signaling from matrix; cancer cell competition	Myosin/ Rho A/ ROCK	Invasion, engulfment of some cells by others (cell cannibalism)	Hepatocarcinoma, fibrosis progression

Note. APAF1, apoptotic protease activating factor 1; ROS, reactive oxygen species; RNS, reactive nitrogen species; LP, lipid peroxidation; BAK, Bcl-2 homologous antagonist killer; BAX, Bcl-2-associated X protein; CASPs, cysteine-dependent aspartate-directed proteases; CypD, Cyclophilin D; Fas TNF, ligand TNF cell death receptor encoded by the FAS gene; TNFR1, tumor necrosis factor receptor 1; GABA-RAP, Gamma-aminobutyric acid receptor-associated protein, encoded by the GABARAP gene, which takes part in autophagosome formation; GPX4, Glutathione peroxidase 4; GSH, Glutathione; GSDMD, Gasdermin D; LC3, soluble microtubular-associated protein 1A/1B – light chain-3, which is conjugated in the autophagosome with phosphatidylethanolamine to form LC3-II, an autophagy marker; MLKL, mixed lineage kinase domain like pseudokinase; mPT-driven necrosis, necrosis caused by increased mitochondrial (Mt) membrane permeability; NLRP1,-3, NOD-like receptor (NLR) family, pyrin domain-containing proteins; PS, phosphatidylserine (marker of apoptosis); PARP-1, Poly (ADP-ribose) polymerase 1; RIPK1,-3, receptor-interacting serine/threonine-protein kinase 1 and 3; Rho A/ROCK, Rho-associated protein kinase (ROCK); TLRs $^{3}/_{4}$, toll-like receptors $^{3}/_{4}$; TNFR1, tumor necrosis factor receptor 1; ULK1, Unc-51 like autophagy activating kinase; ZBP1, Z-DNA-binding protein.

proteins involved in the formation of autophagosomes (Atg5 knockout gene) leads to liver cell death, inflammation and fibrosis in a mice alcohol stress model [22]. At the same time, activation of autophagy by removal of lipid peroxidation products, lipid droplets and aggregates of altered protein – adenosine monophosphate-activated protein kinase (AMPK or AMP-activated protein kinase) – promotes protection of mitochondria against apoptosis when modeling alcoholic disease [16, 23].

It was found that ethanol induces autophagy through oxidative stress mechanisms, which are associated with AMPK activation and suppression of the mammalian target of rapamycin complex 1 (mTORC-1) pathway [17, 21]. Indeed, autophagy activation by rapamycin, an inhibitor of mTORC-1, reduced ethanol-induced liver damage, whereas inhibition of autophagy by chloroquine exacerbated the damage [17]. A single alcohol intake activates autophagy; with chronic intake and in high doses, alcohol can suppress autophagy [17, 21] and exacerbate liver damage through toxic effects.

Liver cell death during toxic exposure to acetaminophen (paracetamol) – (APAP) – does not occur as a result of their autophagic death. Studies in mice have shown that activation of autophagy actually protects liver cells from APAP-induced death, whereas inhibition of autophagy aggravates APAP toxicity [24–27]. Mitochondrial dysfunction, mitochondrial protein breakdown products, accumulation of reactive oxygen species (ROS) and adenosine triphosphate (ATP) depletion all contribute to cell necrosis, but also serve as a catalyst for autophagy initiation upon APAP exposure [18, 24]. Autophagy initiation limits ROS generation by damaged mitochondria, and the autophagosomes that form create a barrier to the expansion of necrosis. Consequently, the resulting changes contribute to activation of mitochondrial biogenesis and liver regeneration [18, 19].

1.2. Apoptosis

Apoptosis, like autophagy, is an active player in morphogenesis and regulation of cell count in the body; it supports cell homeostasis and stimulates physiological cell regeneration [28]. Meanwhile, apoptosis is also triggered under various pathological conditions, which leads to death of cells that are undesirable for the body [29]. Cell death and its removal in apoptosis is carried out by phagocytosis without inflammation. Produced apoptotic cells are utilized by neighboring parenchymal and nonparenchymal cells, fibroblasts, macrophages and dendritic cells [30, 31]. Mediators released by apoptotic cells selectively inhibit neutrophil migration [31, 32]. At the same time, there is increased chemotaxis of macrophages, which, using their numerous receptors, detect the appearance of expression of phosphatidylserine (PS) and other oxidized lipids on the surface of apoptotic cells, which are markers of early apoptosis, and quickly remove them.

Apoptosis includes 3 phases: signaling (induction phase), effector (realization phase) and degradation (destruction phase). The signaling phase can be accomplished via two pathways: extrinsic (involving cell death receptors – caspase-dependent pathway) and intrinsic (involving mitochondria) [5, 28]. Both pathways eventually lead to activation of initiating caspases (CASPs-8,-9,-10,-12) and subsequent activation of effector caspases (CASPs 3,6,7,14), which results in proteolysis, nuclear fragmentation and apoptotic cell death by phagocytosis [28].

In the extrinsic caspase-dependent pathway, the apoptosis signal is triggered by extracellular microenvironment factors: hypoxia, ischemia/reperfusion, exposure to physical and chemical agents, disturbances in cell cycle signaling, etc. These factors stimulate transmembrane cell receptors of two types: type 1 – Tumor necrosis factor (TNF)-family death receptors (TNF receptors or DRs), such as, FAS (CD 95, APO-1), TNFR-1 (p55, CD 120A) and others; type 2 – pattern recognition receptors (PRR) [12], which include toll-like receptors (TLRs). TLRs are part of a multiprotein complex containing Receptor-interacting protein kinase 1 (RIPK1), cellular inhibitor of apoptosis proteins 1 and 2 (cIAP-1 and cIAP-2) and several other proteins [5], which can participate in cell death prevention.

The apoptosis triggering process occurs through interaction of a specific TNF-family death receptor with its adaptor, which further interacts with effectors, procaspases, inactive precursors that initiate caspases [28]. As a result of the interaction of ligand, receptor, adaptor and procaspases, apoptosomes are formed in which cell death processes are initiated due to autolytic activation of caspases [33]. First, the initiating caspases, CASPs-2,-8,-9,-10,-12, are activated in apoptosomes, which further participate in activation of effector caspases, CASPs-3,-7, etc. [5, 34], which leads to proteolysis, nuclear fragmentation, and apoptotic cell death.

The intrinsic or mitochondrial apoptosis signaling pathway is initiated by stressor or cytotoxic DNA damage that activates nuclear protein p53. Induction of p53 increases the activity of the Bcl-2 family regulatory proteins: Bcl-2, BID (BH3 – interacting-domain death agonist BH3) and tBID (BID – after the post-transcriptional modification). These activated proteins, moving into mitochondria, interact with the mitochondrial pool of pro-apoptotic proteins of the Bcl-2 family: with the Bcl-2 associated X apoptosis regulator BAX (BAX) and/or BAK, Bcl-2 homologous antagonist killer (BAK) [12]. Such interaction leads to conformational changes in mitochondrial proteins, pore formation in the outer mitochondrial membrane and release of mitochondrial apoptotic components – cytochrome C, procaspases-2,-3,-9 and apoptotic protease activating factor 1 (APAF1), which

are involved in apoptosome formation [35]. Further, as in the extrinsic apoptosis pathway, procaspase-9 is initiated in the apoptosome, which interacts with the effector procaspase-3, activates it to caspase-3, and triggers the caspase cascade of the effector phase of apoptosis.

Apoptosis, being a regulated process, can be cancelled in the inducer phase (reversible apoptosis phase). The ability to cancel apoptosis is regulated by multidomain protein RIPK1, which is part of the multiprotein type 2 transmembrane receptor complex (see above). RIPK1 has a direct effect on the outcome of type 1 TNF-death receptor activation and causes the affected cell to survive or die, depending on its posttranslational modifications [36, 37]. It is known that E3-ubiquitin (a protein involved in regulation of the functioning and degradation of intracellular proteins), when interacting with cIAP, catalyzes RIPK1 polyubiquitination and promotes activation of nuclear factor kappa B (NF-kB). This interaction leads to gene transformation, ensuring survival and prevention of cell death (cancellation of the irreversible apoptosis phase) [38, 39].

Cellular structures are destroyed (cytoskeleton destruction, cleavage of adhesive proteins, hydrolysis of the nuclear membrane) in the effector phase of apoptosis. In the degradation phase of apoptosis, deep morphological and biochemical changes occur, leading to formation of apoptotic cells, 0.2 μm in diameter, which leave the apoptosis zone, appear in blood and are subsequently phagocytosed [40]. In the degradation phase, apoptosis acquires secondary necrosis features, in which the release of damage-associated molecular patterns (DAMPs), including nucleosomes, from cells, occurs. Nucleosomes contain fragments of genomic DNA, nuclear protein HMGB-1, heat shock proteins HSP and other autoantigens that induce an antigen-specific immune response [32, 41].

Both intrinsic and extrinsic pathways of apoptosis are usually involved in cholestatic and autoimmune liver damage, alcoholic and non-alcoholic steatohepatitis, mild hepatotoxic liver injury and viral hepatitis [42–44].

It has been shown in animal models and in vitro experiments that ethanol causes metabolic, toxic and inflammatory damage to liver cells. Cell damage leads to dysfunction of mitochondria and other organelles, ROS formation, BAX (proapoptotic Bcl-2 protein) translocation into mitochondria, cytochrome C release, caspase activation [45–47] and apoptotic cell death [42]. It is known that acute and chronic alcohol exposure increases intestinal permeability to bacterial products such as lipopolysaccharides (LPS) and this leads to inflammation, Kupffer cells stimulation, increased TNF production by them [48] and TNF expression of apoptosis receptors FAS and TNFR1 [49].

In vitro and in vivo studies using pan-caspase inhibitor showed a significant attenuation of alcohol-induced hepatocyte apoptosis without transition to necroptosis (i.e. without induction of RIPK1 and RIPK3 markers) [50, 51]. Despite the lack of influence of pan-caspase inhibitor on inflammatory markers, less pronounced liver fibrosis was observed when the damage was modeled with combined use of alcohol and CCl_4 [52].

In non-alcoholic steatohepatitis, the pathological process in the liver proceeds against the background of detection of caspase-3,-7 and an increased number of TUNEL*-positive cells in liver biopsy specimens, which proves the inflammatory nature of apoptosis. Such results were obtained in a study of CASP3 and CASP-8 knockout mice that were fed a diet deficient in methionine and choline. These mice were protected against apoptosis, had decreased activity of proinflammatory cytokines, and reduced expression of morphological signs of inflammation and liver fibrosis [53–55]. Meanwhile, mice fed a high-fat diet demonstrated increased ROS levels and signs of apoptosis – increased caspase-3 and caspase-8 activity – as well as increased content of TUNEL*-positive cells in liver biopsy specimens [56]. The importance of apoptosis in non-alcoholic steatohepatitis is also supported by other studies. Thus, it was shown [57] that along with caspase-3 and -7 in nonalcoholic steatohepatitis, caspase-6 is activated, which begins to play an important role in the progression of this disease due to the disturbance of regulatory interaction of metabolic sensor AMPK and apoptotic process participants. In a healthy liver, AMPK is known to phosphorylate proapoptotic caspase-6 and inhibit its activation.

Meanwhile, when non-alcoholic steatohepatitis was simulated in mice on a choline-deficient diet in combination with a high-fat diet, AMPK activity was suppressed; at the same time, simultaneous use of a caspase-6 inhibitor attenuated the morphological signs of apoptosis and liver fibrosis and decreased the level of transaminases[57].

It was also shown that hepatocyte swelling in non-alcoholic steatohepatitis [58] causes stress in cells and their organelles, which induces apoptosis, release of cellular degradation products from damaged molecular patterns (DAMPs, damage-associated molecular patterns) and activation of TLRs. In turn, TLRs activation turns the inflammation and cell death signals into a permanent damaging factor. This is facilitated by interaction of hepatocytes with liver macrophages (Kupffer cells) and leukocytes (natural killers), which, by supplying

* TUNEL (terminal deoxyribonucleotide transferase – mediated dUTP nick end labeling) is a method of recording free 3'-OH DNA ends in a condensed chromatin nucleus using light, laser, confocal or transmission electron microscopy. In apoptosis, DNA fragmentation leads to a significant increase in the number of 3'-OH DNA ends (TUNEL-positive cells) in both intrinsic and extrinsic pathways of apoptosis.

TNF to the body, maintain inflammation in nonalcoholic steatohepatitis [59].

Consequently, apoptosis in nonalcoholic steatohepatitis and nonalcoholic fatty liver disease is the predominant mode of cell death. It involves both extrinsic (via cell surface receptors) and intrinsic pathways of apoptosis activation (via lipotoxicity and organelle stress).

In cholestatic liver diseases accompanied by impaired bile excretion and cholangiopathy, apoptotic death of cholangiocytes is also noted [60]. In liver biopsy specimens from patients with primary biliary cholangitis, cholangiocyte apoptosis, in which cytoplasm thickening and nuclear condensation are observed in cells, is detected [61]. It is believed that apoptosis in these patients is mediated by TNF receptor FAS (CD95), since FAS expression in the cytoplasm of bile duct cells is accompanied by increased FasL expression in the surrounding lymphocytes and other immune cells [61, 62]. FAS-mediated apoptosis of hepatocytes in cholestasis is accompanied by activation of hepatic stellate cells (HSCs) and development of fibrosis, which indicates a connection between apoptosis and formation of fibrosis in the liver [63]. FAS (apoptosis antigen 1) is not the only cell death receptor involved in cholangiocyte apoptosis. Takeda et al. [64] showed that expression of DR5 (death receptor-5) gene was also increased in the liver of patients with primary biliary cholangitis and primary sclerosing cholangitis, and DR5 knockout mice were resistant to cholestatic cell death after bile duct ligation [64]. Cubero et al. [65] reported increased expression of activated proteolytic enzymes caspase-3 and -8 as well as RIPK3 protein in liver biopsy specimens of patients with primary biliary cholangitis, indicating activation of apoptosis and association of apoptosis with other forms of PCD. Using a model of bile duct ligation in CASP-3 knockout mice, the researchers reported a decrease in transaminases AST and ALT, a decrease in caspase-3 activity and RIPK1 and RIPK3 protein levels [65], i.e. a concomitant inhibition of necroptosis mechanisms. Other works [66, 67] have shown the association between decreased apoptosis and decreased caspase-3 and -7 -positive cells, decreased inflammation, hepatic stellate cell (HSC) activity, fibrosis, portal hypertension and improved survival with pan-caspase inhibitors. These data support the importance of apoptosis in cholestatic liver disease.

Liver cell apoptosis is also thought to play an important role in the pathogenesis of hepatitis B and C virus (HCV and HBV) [68, 69]. By histological markers (cell cytoplasm thickening, DNA fragmentation and detection of TUNEL-positive cells), apoptosis has long been detected in liver biopsy specimens of patients with viral hepatitis [70]. Increased expression of FAS receptors in hepatocytes detected by increased FASL expression in lymphocytes in HBV and HCV suggests that apoptosis is the main cause of liver cell death during active hepatitis [70, 71].

1.3. Necrosis caused by increased mitochondrial membrane permeability (mPT-driven necrosis)

Necrosis is the final state of a severe pathological process in cells, which is accompanied by cell swelling, membrane rupture, release of DAMPs and subsequent development of inflammatory response. mPT-driven necrosis described for liver is characterized by pore formation and increased permeability of inner and outer mitochondrial membranes, decreased membrane potential, cessation of ATP synthesis, osmotic destruction of both membranes and cell death [12, 72]. The exact mechanisms of mPT-driven necrosis are not yet known. It has been suggested that a decrease in mitochondrial membrane potential leads to pore enlargement due to disruption of the interaction between the ATP synthase of the pores and mitochondrial protein cyclophilin D (CypD) involved in their formation [73]. The involvement of mPT-driven necrosis in a number of liver diseases, in which oxidative stress and mitochondrial Ca^{2+} overload play an important pathogenetic role, has been proved [12]. For example, it has been shown that in APAP toxicity, APAP is converted into toxic metabolite NAPQI (N-acetyl-p-benzoquinone imine) by direct oxidation involving cytochromes; then NAPQI is effectively detoxified by glutathione (GSH) to form APAP-GSH conjugates [74]. However, when GSH and cysteine reserves in cells are depleted, the toxic metabolite NAPQI binds to thiol (-SH) protein groups, and the resulting NAPQI degradation products cause stress damage to the endoplasmic reticulum and mitochondria [75]. Subsequent mitochondrial damage occurs as a result of ROS formation, nitric oxide (RNS) accumulation in mitochondria [76] and developing Ca^{2+} overload. Continued ROS generation increases mitochondrial stress, which leads to activation of mitogen-activated protein kinases (MAPK) that leads cells to mPT-driven necrosis [77]. mPT-driven necrosis develops as a consequence of mitochondrial membrane rupture, translocation of apoptosis-inducing factor (AIF) into the nucleus and release of endonuclease, followed by DNA fragmentation [78]. The importance of mPT-driven necrosis in the development of APAP toxicity, nonalcoholic steatohepatitis and nonalcoholic fatty liver disease has been confirmed in a number of works [79–81].

1.4. Necroptosis

Necroptosis, as a necrotoxic type of PCD, is involved in most chronic liver diseases, including viral hepatitis, autoimmune hepatitis, nonalcoholic steatohepatitis and alcoholic liver disease [82, 83]. Necroptosis has been shown to be initiated by TNF-family receptors (FAS

and TNFR1), pattern recognition receptors (PRRs)-, (TLRs) or intracellular sensor Z-DNA binding protein 1 (ZBP1). Necroptosis is activated when caspase-8 is inhibited, when receptors for interacting protein kinase-1 and -3 – (RIPK1 and RIPK3) are activated, and when a mixed lineage kinase domain-like protein (MLKL) is activated. Necroptosis is manifested by the formation of necrosomes, a rapid increase in cell membrane permeability and release of DAMPs from cells into the extracellular space [84–86]. The role of RIPK1 and RIPK3 proteins, MLKL and other participants in necroptotic liver cell damage has been actively studied in recent years [87–90]. In a model of autoimmune hepatitis using concanavalin-A (ConA), it was observed that administration of NEC-1, a RIPK1 inhibitor, protects the liver from damage [91–93]. Mice with the MLKL knockout were also protected against ConA damage [90]. However, additional studies on mice with ConA – liver damage and MLKL knockout – failed to reveal differences in the liver condition in the control and experimental groups. The studies could not also confirm the involvement of necroptosis [90, 94]. In a study of patients with HBV and patients with chronic viral hepatitis, increased serum RIPK3 and MLKL levels in the liver were found when compared to controls (healthy controls) [95, 96]. However, the authors could not relate these results to necroptosis, since it is known that RIPK1 and RIPK3 acquire functions that are independent of necroptosis in inflammation [5]. Besides, the results obtained may be a consequence of cross-influence of other PCD types activated under these conditions.

1.5. Pyroptosis

Cell pyroptosis has features of apoptosis and necrosis and is designed to remove intracellular pathogens. Pyroptosis is characterized by formation of an inflammasome containing a complex of caspases activated in the cell and producers of proinflammatory cytokines – IL-1β, IL-18. It is classified as an inflammatory necrosis coding type, which closely interacts with innate immunity [97]. There are two distinct pathways through which pyroptosis can occur – canonical and non-canonical. The canonical pathway is triggered if inflammasome sensors belonging to the NOD-like receptor (NLR) family, pyrin domain-containing proteins 1 and 3 (NLRP1 and NLRP3) are stimulated by PAMPs (pathogen-associated molecular patterns) pathogens and DAMPs. These sensors use caspase-1 to activate intracellular protein Gasdermin-D (GSDMD), which forms pores in the cytoplasmic membrane and promotes cell death [12]. When pyroptosis is activated via the non-canonical pathway, cytosolic lipopolysaccharides (LPS) and PAMPs are directly stimulated by caspases -4, -5 and -11. These caspases in turn activate GSDMD, which, by binding membrane phospholipids, initiates pore formation and leads to cell death [98–100].

Involvement of pyroptosis in liver diseases such as alcoholic disease [101–103], nonalcoholic steatohepatitis [104–107], APAP-induced toxic liver injury [108–110], autoimmune hepatitis [111–113], cholestatic liver diseases [114–117], and viral hepatitis [118, 119], has been proven mainly by examining the activation of key mediators of pyroptosis, NLRP3 inflammasome activity, and GSDMD protein.

1.6. Ferroptosis

Ferroptosis is a type of PCD, which depends on intracellular iron content that catalyzes ROS formation and subsequent oxidative cell damage. Ferroptosis is activated upon depletion of cellular GSH, which promotes activation of iron-dependent lipid peroxidation (LPO) of cell membranes [120, 121]. Ferroptosis develops independently of apoptosis, necrosis, autophagy, and pyroptosis, and has subcellular characteristics of necrosis that are caused by the release of DAMPs [12]. The small size of compressed-density mitochondria, absence of cristae in them, and ruptures of the outer cell membrane, are morphological signs of ferroptosis [122, 123]. GSH-dependent enzyme glutathione peroxidase 4 (GPX4) is the main endogenous inhibitor of ferroptosis due to its ability to limit PL processes [124]. Inhibition of GPX4 activity leads to accumulation of ROS and LPO, and therefore reduced GPX-4 activity is considered a marker of ferroptosis. Reducing the accumulation of ROS and PL products can be achieved by the use of iron chelates (deferroxamine) and LPO inhibitors – (ferrostatin) [125]. The role of ferroptosis in the pathogenesis of liver disease has been investigated in alcohol disease [126–128], non-alcoholic steatohepatitis [128, 129], APAP toxicity [128, 130–132], and autoimmune hepatitis [133, 134]. It has been suggested that ferroptosis plays a role in various liver diseases and, therefore, it can coexist in cells along with other types of PCD (apoptosis, mPT-driven necrosis, necroptosis, etc.) [6].

1.7. Partanatosis

Partanatosis is a type of PCD caused by excessive cell response to DNA damage mediated predominantly by poly (ADP-ribose) polymerase 1 (PARP-1). Partanatosis occurs after severe and prolonged alkylating DNA damage, oxidative stress, hypoglycemia or inflammation [12]. Reactive nitrogen species (RNS), such as NO, are a trigger for PARP-1 activation, which causes depletion of nicotinamide adenine dinucleotide (NAD) and ATP in cells; RNS also contribute to the accumulation of poly (ADP-ribose) polymerase and poly (ADP-ribosylation) proteins, causing loss of mitochondrial membrane potential. In addition, poly (ADP-ribose) polymerases bind AIF and promote AIF nuclear translocation, causing

DNA fragmentation and nuclear condensation. It has recently been shown that a factor that inhibits macrophage migration in various liver diseases can bind AIF and catalyze DNA breakdown [135]. These data suggest that there is a cross-linkage between some necrotic types of PCD (mPT-driven necrosis, necroptosis) and parthenatosis. This is confirmed by the ability of activated RIPK1 and RIPK3 – markers of necroptosis – to stimulate the enzymatic activity of PARP-1, as well as contribute to ATP depletion and AIF release [136]. The role of partanatosis in liver diseases has not yet been studied; however, it is known that PARP-1 is involved in liver cell death [6].

1.8. Entosis

Entosis is a type of PCD related to cell cannibalism, which occurs in healthy and malignant tissues, involving the engulfment of viable cells by non-phagocytic cells of the same (homotypic) or a different (heterotypic) type [12]. Entosis of epithelial cells usually occurs when the cells lose integrin signaling as a result of detachment from the extracellular matrix. Enthosis is accompanied by cell invasion which depends on the activity of E-cadherin, catenin alpha 1, RhoA and Rho-associated kinase (ROCK). Entosis occurs under conditions of cancer cell competition and downregulation of myosin, a component of cytoplasmic membranes in engulfing cells, which allows penetration into these target cells [137]. Cells displaying high AMRK activity due to a lack of nutrients, succumb to entosis, designed to restore their nutrition [138]. In chronic liver diseases such as chronic hepatitis B and autoimmune hepatitis, entrainment of activated T-lymphocytes by hepatocytes occurs, indicating the involvement of entolysis in liver damage and the development of immune tolerance [139]. Recently, HSCs have been shown to be involved in the entosis of antifibrotic natural killer cells in HBV cirrhotic patients as a potentially novel mechanism of fibrosis enhancement [140].

2. CROSS-REGULATION OF DIFFERENT PCD PATHWAYS

Analysis of the involvement of various forms of PCD in liver diseases shows that separate forms of PCD can participate simultaneously with others in any one pathology and, besides, certain forms of PCD, having common markers with other forms of PCD, participate in formation of various nosological types of diseases. Since different forms of PCD, having different mechanisms, nevertheless cross-regulate each other, it gives the grounds to assume that at least some forms of PCD (ferroptosis, necroptosis, pyroptosis, partanatosis) are intermediate stages of formation of basic forms of PCD, such as apoptosis and mPT-driven necrosis. The best-known mechanisms of cross-regulation of the interaction between different PCD forms in liver diseases are presented in Table 2.

It has also been shown that CypD, an indispensable participant in mPT-driven necrosis, reverses the inhibitory effect of cIAP on RIPK1 to promote necrosome formation to facilitate the necrotic cell death pathway [141]. Activated RIPK3 and MLKL activate NLRP3 inflammasomes [142], and this mechanism promotes a direct link between cell necrosis and inflammation in addition to the existing mechanism of inflammasome activation by DAMP. Caspase-8, an effector of the extrinsic pathway of apoptosis similar to caspase-1, is also capable of activating NLRP3 inflammasome to trigger pro-IL-1β to stimulate inflammatory processes [143].

Apparently, cross-regulation of PCD in the liver is designed to effectively ensure cell death of different phenotypes with different levels of damage. In addition, cross-regulation seems to be designed to promote the formation of the main form of PCD that will dominate in cells at the final stages of the pathological process –

Table 2

Cross-regulation of metabolic pathways in different forms of PCD [6]*

PCD pathway	Effector	Mechanism of Action	Regulated PCD pathway
Apoptosis	CASP8	Inactivates RIPK3	Inactivates Necroptosis
		Inactivates CypD	Inhibits mPT-driven necrosis
		Activates NLRC4 inflammasome	Induces pyroptosis
	CASP3	Activates GSDM-E – regulated pyroptosis	Regulates pyroptosis
	CASP3/ CASP-7	Inactivates GSDMD	Inactivates pyroptosis
mPT-driven necrosis	CypD	Reverses the inhibitory effect of cIAP on RIPK1	Regulates necroptosis
Necroptosis	RIPK1	Inhibits CASP8	Inhibits CASP8-dependent apoptosis
		Stimulates anti-apoptotic activation of NF-kB	Regulates apoptosis
	RIPK1/ RIPK3	Activates PARP-1	Stimulates partanatosis

* for Table 2 legend, see Table 1 legend and the list below. cIAP, cellular inhibitor of apoptosis proteins; GSDME, Gasdermin E; NLRC-4, NOD-like receptor family CARD domain-containing protein 4; NF-kB, Nuclear factor kappa-light-chain-enhancer of activated B cells.

inflammation, fibrosis, cell transformation or even cell recovery with induction of autophagy and regulation of early reversible apoptosis mechanisms. The possibility of reorienting PCD processes towards restorative and regenerative processes in damaged cells is supported by numerous studies on the positive role of optimally selected schemes of postconditioning of ischemically damaged organs used to attenuate ischemic (necrotic) and reperfusion (apoptotic) injury. Postconditioning limits cell necrosis, and the severity of the anti-necrotic effect depends on ischemia time and postconditioning protocol used [144]. Postconditioning also inhibits development of cell apoptosis and enhances autophagy processes in cells. Such restorative processes can be stimulated in the liver not only when optimal postconditioning schemes of an ischemically damaged organ are used, but also when adequate medication and cellular therapy for disorders caused by PCD are applied.

3. WAYS TO REGULATE PCD PROCESSES IN LIVER DISEASES

The analysis of mechanisms of involvement of various forms of PCD in liver diseases allows to outline several ways for their inhibition.

Ability to cross-regulate PCD pathways, preserved in dying liver cells at various diseases, indicates the feasibility of controlled correction of emerging disorders, first of all, primarily with the help of agents whose action is directed on inhibition of effector mechanisms of PCD. Among such remedies, new drugs are known and are undergoing clinical trials: emricane (IDN-6556), a pan-caspase inhibitor for patients with nonalcoholic steatohepatitis with fibrosis F1–F3 or liver cirrhosis; Selonsertib (GS-4997), inhibitor of Apoptosis signal-regulating kinase 1 (ASK-1) for patients with nonalcoholic steatohepatitis and progressive liver cirrhosis; drugs: GSK-2982772 and GSK-2983559, RIPK1 inhibitors for treatment of various chronic inflammatory diseases [6], as well as ferrostatin [125] and Artesunate, ferroptosis regulator with antifibrogenic effect [145]; Montelukast, inhibitor of TNF-α/JNK signaling pathway [146]; DAPT, inhibitor of Notch signaling pathway [147], and others.

With sluggish restorative (regenerative) processes, as well as in the early reversible stages of apoptosis, when the liver cells still retain the ability to autoregulate and independently survive, adaptogens may be effective for the correction of liver failure and restoration of liver cells structure. These include autophagy inducers and regulators of cell apoptosis, promoting activation of metabolic pathways – AMPK and NF-kB and inhibition – mTORC-1-dependent metabolic pathways. It is also advisable to use metabolic inducers with antioxidant and anti-inflammatory activity [148–154] since oxidative stress and inflammation are constant companions of almost all forms of PCD.

Besides drug therapy for chronic (non-oncological) liver diseases, for inhibition of PCD processes and activation of repair processes in damaged liver cells, it is reasonable to develop methods of cellular regeneration therapy, which are based on the use of apoptotically altered donor liver cells and donor bone marrow [155]. These cells produce numerous paracrine factors that act as targeted carriers of the regenerative signal complex – regulators of regenerative processes. So, isolated cultured donor hepatocytes have already found application in the clinic. They are used as the basic element in auxiliary liver support systems, in the perfusion circuit of which the patient's blood (or plasma) continuously comes in contact with isolated donor hepatocytes cultivated in it and is enriched with paracrine factors released by apoptotic donor cells [156]. Isolated donor hepatocytes began to be used also as part of cell-engineered constructs implanted directly into the damaged patient's liver. The ratio of cellular elements (hepatocytes/mesenchymal stem cells) in the implanted cell-engineered constructs was optimized to increase the efficiency of their use [157, 158]. Induction of repair processes in the damaged liver is also performed by application of total RNA from bone marrow cells, which is a nonspecific factor of regulation of repair processes and acts as a carrier of a complex of multiple regenerative signals to the damaged liver cells [159].

CONCLUSION

Analysis of the role of programmed cell death in liver diseases allows us to conclude as follows:

1. Among the known forms of PCD, autophagy, apoptosis and mPT-driven necrosis should be considered the main forms; other forms of PCD (ferroptosis, necroptosis, pyroptosis, partanatosis, entosis) remain insufficiently studied and, apparently, should be considered as intermediate stages of the main forms of PCD (apoptosis and mPT-driven necrosis).
2. Cross-regulation of different forms of PCD in the liver is intended to increase the efficiency of formation of cell death of different phenotypes with different levels of damage, as well as to ensure development of one of the dominant consequences of PCD such as inflammation, fibrosis, cellular transformation or reparative regeneration.
3. Preservation of cross-regulation in different forms of PCD in the liver indicates the possibility of controlled correction of the resulting disorders with the help of drugs and cell technologies. Among the drugs, the promising ones are those that inhibit effector-mediated PCD pathways and have an adaptive effect on liver cells by induction of autophagy, early reversible apoptosis and enhancement of antioxidant and anti-inflammatory activity. Application of cell technologies is based on the use of apoptotic donor

liver and bone marrow cells that produce paracrine regulatory factors, which inhibit PCD and specifically induce reparative regeneration in liver cells.

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REFERENCES

1. *Yarilin AA*. Apoptosis: The nature of the phenomenon and its role in the norm and with pathology. *Actual problems of pathophysiology: selected lectures under the ed. B.B. Moroz*. M.: Medicine, 2001: 13–56.
2. *Tak H, Matsui Y, Sadoshima J.* The role of autophagy in mediating cell survival and death during ischemia and reperfusion in the heart. *Antioxidants and Redox Signaling.* 2007; 9 (9): 1373–1381.
3. *Gubsky YuI*. Death Cells: Free radicals, necrosis, apoptosis: monograph. Vinnitsa: Nova Book. 2015. 360.
4. *Potapnev MP.* Autophagia, apoptosis, cell necrosis and immune recognition of their and someone else's. *Immunology*. 2014; 2: 95–102.
5. *Shojaie L, Iorga A, Dara L.* Cell Death in Liver Diseases: A Review. *Int J Mol Sci*. 2020 Dec; 21 (24): 9682. doi: 10.3390/ijms21249682.
6. *Aizawa S, Brar G, Tsukamoto H.* Cell Death and Liver Disease. *Gut Liver*. 2020 Jan; 14 (1): 20–29. doi: 10.5009/gnl18486.
7. *Xie Z, Klionsky DJ.* Autophagosome Formation: Core Machinery and Adaptations. *Nat Cell Biol*. 2007: 1102–1109. doi: 10.1038/ncb1007-1102.
8. *Kuballa P, Nolte WM, Castoreno AB, Xavier RJ.* Autophagy and the immune system. *Ann Rev Immunol*. 2012; 30: 611–646.
9. *Romao S, Gannage M, Munz C.* Checking the garbage bin for problems in the house, or how autophagy assists in antigen presentation to the immune system. *Semin Cancer Biol*. 2013; 23 (5): 391–396.
10. *Rubinsztein DC, Marino G, Kroemer G.* Autophagy and aging. *Cell*. 2011; 146 (5): 682–695.
11. *Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT.* PAMPs and DAMPS: signal Os that spur autophagy and immunity. *Immunol Rev*. 2012; 249 (1): 158–175.
12. *Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P et al*. Molecular Mechanisms of Cell Death: Recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death Differ*. 2018; 25: 486–541. doi: 10.1038/s41418-017-0012-4.
13. *Liu G, Bi Y, Wang R, Wang X.* Self-eating and self-defense: autophagy controls innate immunity and adaptive immunity. *J Leukoc Biol*. 2013; 93 (4): 511–519.
14. *Galluzzi L, Vitale I, Abrams JM, Alnemri ES, Baehrekke EH, Blagosklonny MV et al.* Molecular Definitions of Cell Death Subroutines: Recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death Differ*. 2012: 107–120. doi: 10.1038/cdd.2011.96.
15. *Yin XM.* Autophagy in Liver Diseases: A Matter of What to Remove and Whether to Keep. *Liver Res*. 2018: 109–111. doi: 10.1016/j.livres.2018.09.001.
16. *Ding W, Li M, Chen X, Ni H, Lin C, Gao W et al.* Autophagy Reduces Acute Ethanol-Induced Hepatotoxicity and Steatosis in Mice. *Gastroenterology*. 2010; 139: 1740–1752. doi: 10.1053/j.gastro.2010.07.041.
17. *Lin CW, Zhang H, Li M, Xiong X, Chen X, Chen X et al.* Pharmacological Promotion of Autophagy Alleviates Steatosis and Injury in Alcoholic and Non-Alcoholic Fatty Liver Conditions in Mice. *J Hepatol*. 2013; 58: 993–999. doi: 10.1016/j.jhep.2013.01.011.
18. *Ni HM, McGill MR, Chao X, Du K, Williams JA, Xie Y et al.* Removal of Acetaminophen Protein Adducts by Autophagy Protects against Acetaminophen-Induced Liver Injury in Mice. *J Hepatol*. 2016; 65: 354–362. doi: 10.1016/j.jhep.2016.04.025.
19. *Ni HM, Williams JA, Jaeschke H, Ding WX.* Zonated Induction of Autophagy and Mitochondrial Spheroids Limits Acetaminophen-Induced Necrosis in the Liver. *Redox Biol*. 2013: 427–432. doi: 10.1016/j.redox.2013.08.005.
20. *Ding WX, Yin XM.* Sorting, Recognition and Activation of the Misfolded Protein Degradation Pathways through Macroautophagy and the Proteasome. *Autophagy*. 2008: 141–150. doi: 10.4161/auto.5190.
21. *Khambu B, Wang L, Zhang H, Yin X-M.* The Activation and Function of Autophagy in Alcoholic Liver Disease. *Curr Mol Pharmacol*. 2017; 10: 165–171. doi: 10.2174/1874467208666150817112654.
22. *Ni HM, Woolbright BL, Williams J, Copple B, Cui W, Luyendyk JP et al.* Nrf2 Promotes the Development of Fibrosis and Tumorigenesis in Mice with Defective Hepatic Autophagy. *J Hepatol*. 2014; 61: 617–625. doi: 10.1016/j.jhep.2014.04.043.
23. *Eid N, Ito Y, Maemura K, Otsuki Y.* Elevated Autophagic Sequestration of Mitochondria and Lipid Droplets in Steatotic Hepatocytes of Chronic Ethanol-Treated Rats: An Immunohistochemical and Electron Microscopic Study. *J Mol Histol*. 2013; 44: 311–326. doi: 10.1007/s10735-013-9483-x.
24. *Ni HM, Bockus A, Boggess N, Jaeschke H, Ding WX.* Activation of Autophagy Protects against Acetaminophen-Induced Hepatotoxicity. *Hepatology*. 2012; 55: 222–232. doi: 10.1002/hep.24690.
25. *Hu C, Zhao L, Shen M, Wu Z, Li L.* Autophagy Regulation Is an Effective Strategy to Improve the Prognosis of Chemically Induced Acute Liver Injury Based on Experimental Studies. *J Cell Mol Med*. 2020: 8315–8325. doi: 10.1111/jcmm.15565.
26. *Lin Z, Wu F, Lin S, Pan X, Jin L, Lu T et al.* Adiponectin Protects against Acetaminophen-Induced Mitochondrial Dysfunction and Acute Liver Injury by Promoting Autophagy in Mice. *J Hepatol*. 2014; 61: 825–831. doi: 10.1016/j.jhep.2014.05.033.
27. *Baulies A, Ribas V, Núñez S, Torres S, Alarcón-Vila C, Martínez L et al*. Lysosomal Cholesterol Accumulation Sensitizes to Acetaminophen Hepatotoxicity by Impairing Mitophagy. *Sci Rep*. 2015; 5. doi: 10.1038/srep18017.
28. *Dyatlova AS, Dudkov AV, Linkova NS, Khavinson VKh.* Molecular markers of Kaspaza-dependent and mitochondrial apoptosis: the role in the development of pathology and in the processes of cellular aging. *Suc-*

cess of modern biology. 2018; 138 (2): 126–137. doi: 10.7868/S0042132418020023.
29. *Ryzhov SV, Novikov VV.* Molecular mechanisms of apoptotic processes. *Russian biotherapeutic magazine*. 2002; 1 (3): 17–25.
30. *Janssen WJ, Henson PM.* Cellular regulation of the inflammatory response. *Toxicol Pathol*. 2012; 40 (2): 166–173.
31. *Zitvogel L, Kepp O, Kroemer G.* Decoding cell death signals in inflammation and immunity. *Cell*. 2010; 140 (6): 798–804.
32. *Peter C, Wesselborg S, Herrman M, Lauber K.* Dangerous attraction: phagocyte recruitment and danger signals of apoptotic and necrotic cells. *Apoptosis*. 2010; 15 (9): 1007–1028.
33. *Creagh EM.* Caspase crosstalk: integration of apoptotic and innate immune signaling pathways. *Tren Immunol*. 2014; 35 (12): 631–639.
34. *Lewin B.* Cells. M.: Binom. Laboratory of Knowledge, 2011. 951 s.
35. *Riedl SJ, Salvesen GS.* The Apoptosome: Signalling Platform of Cell Death. *Nat Rev Mol Cell Biol*. 2007; 8: 405–413. doi: 10.1038/nrm2153. 35.
36. *Gerlach B, Cordier SM, Schmukle AC, Emmerich CH, Rieser E, Haas TL et al.* Linear Ubiquitination Prevents Inflammation and Regulates Immune Signalling. *Nature*. 2011; 471: 591–596. doi: 10.1038/nature09816.36.
37. *O'Donnell MA, Legarda-Addison D, Skountzos P, Yeh WC, Ting AT.* Ubiquitination of RIP1 Regulates an NF-KB-Independent Cell-Death Switch in TNF Signaling. *Curr Biol*. 2007; 17: 418–424. doi: 10.1016/j.cub.2007.01.027. 37.
38. *Ashkenazi A, Salvesen G.* Regulated Cell Death: Signaling and Mechanisms. *Annu Rev Cell Dev Biol*. 2014; 30: 337–356. doi: 10.1146/annurev-cellbio-100913-013226. 38.
39. *Brenner D, Blaser H, Mak TW.* Regulation of Tumour Necrosis Factor Signalling: Live or Let Die. *Nat Rev Immunol*. 2015; 15: 362–374. doi: 10.1038/nri3834.
40. *Golubev AM, Moskaleva EYu, Severin SE et al.* Apoptosis at critical states. *Total resuscitation*. 2006; 2 (6): 184–190.
41. *Vandenabeele P, Galluzzi L, Vanden Berghe T, Kroemer G.* Molecular Mechanisms of Necroptosis: An Ordered Cellular Explosion. *Nat Rev Mol Cell Biol*. 2010; 11: 700–714. doi: 10.1038/nrm2970.
42. *Wang S, Pacher P, De Lisle RC, Huang H, Ding WX.* A mechanistic review of cell death in alcohol-induced liver injury. *Alcohol Clin Exp Res*. 2016; 40: 1215–1223. doi: 10.1111/acer.13078.
43. *Luedde T, Kaplowitz N, Schwabe RF.* Cell death and cell death responses in liver disease: mechanisms and clinical relevance. *Gastroenterology*. 2014; 147: 765–783. doi: 10.1053/j.gastro.2014.07.018.
44. *Schwabe RF, Luedde T.* Apoptosis and necroptosis in the liver: a matter of life and death. *Nat Rev Gastroenterol Hepatol*. 2018; 15: 738–752. doi: 10.1038/s41575-018-0065-y.
45. *Cahill A, Cunningham CC, Adachi M, Ishii H, Bailey SM, Fromenty B, Davies A.* Effects of Alcohol and Oxidative Stress on Liver Pathology: The Role of the Mitochondrion. *Alcohol Clin Exp Res*. 2002; 26: 907–915. doi: 10.1111/j.1530-0277.2002.tb02621.x.
46. *Adachi M, Higuchi H, Miura S, Azuma T, Inokuchi S, Saito H et al.* Bax Interacts with the Voltage-Dependent Anion Channel and Mediates Ethanol-Induced Apoptosis in Rat Hepatocytes. *Am J Physiol Gastrointest Liver Physiol*. 2004; 287. doi: 10.1152/ajpgi.00415.2003.
47. *Malhi H, Gores GJ.* Cellular and Molecular Mechanisms of Liver Injury. *Gastroenterology*. 2008; 134: 1641–1654. doi: 10.1053/j.gastro.2008.03.002.
48. *Hartmann P, Seebauer CT, Schnabl B.* Alcoholic Liver Disease: The Gut Microbiome and Liver Cross Talk. *Alcohol Clin Exp Res*. 2015: 763–775. doi: 10.1111/acer.12704.
49. *Natori S, Rust C, Stadheim LM, Srinivasan A, Burgart LJ, Gores GJ.* Hepatocyte Apoptosis Is a Pathologic Feature of Human Alcoholic Hepatitis. *J Hepatol*. 2001; 34: 248–253. doi: 10.1016/S0168-8278(00)00089-1.
50. *Hao F, Cubero FJ, Ramadori P, Liao L, Haas U, Lambertz D et al*. Inhibition of Caspase-8 Does Not Protect from Alcohol-Induced Liver Apoptosis but Alleviates Alcoholic Hepatic Steatosis in Mice. *Cell Death Dis*. 2017; 8: e3152. doi: 10.1038/cddis.2017.532.
51. *Wilson CH, Kumar S.* Caspases in Metabolic Disease and Their Therapeutic Potential. *Cell Death Differ*. 2018: 1010–1024. doi: 10.1038/s41418-018-0111-x.
52. *Roychowdhury S, Chiang DJ, Mandal P, McMullen MR, Liu X, Cohen JI et al.* Inhibition of Apoptosis Protects Mice from Ethanol-Mediated Acceleration of Early Markers of CCl_4-Induced Fibrosis but Not Steatosis or Inflammation. *Alcohol Clin Exp Res*. 2012; 36: 1139–1147. doi: 10.1111/j.1530-0277.2011.01720.x.
53. *Feldstein AE, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD, Gores GJ.* Hepatocyte Apoptosis and Fas Expression Are Prominent Features of Human Nonalcoholic Steatohepatitis. *Gastroenterology*. 2003; 125: 437–443. doi: 10.1016/S0016-5085(03)00907-7.
54. *Thapaliya S, Wree A, Povero D, Inzaugarat ME, Berk M, Dixon L et al.* Caspase 3 Inactivation Protects against Hepatic Cell Death and Ameliorates Fibrogenesis in a Diet-Induced NASH Model. *Dig Dis Sci*. 2014; 59: 1197–1206. doi: 10.1007/s10620-014-3167-6.
55. *Hatting M, Zhao G, Schumacher F, Sellge G, Al Masaoudi M, Gaßler N et al.* Hepatocyte Caspase-8 Is an Essential Modulator of Steatohepatitis in Rodents. *Hepatology*. 2013; 57: 2189–2201. doi: 10.1002/hep.26271.
56. *Barreyro FJ, Holod S, Finocchietto PV, Camino AM, Aquino JB, Avagnina A et al.* The Pan-Caspase Inhibitor Emricasan (IDN-6556) Decreases Liver Injury and Fibrosis in a Murine Model of Non-Alcoholic Steatohepatitis. *Liver Int*. 2015; 35: 953–966. doi: 10.1111/liv.12570.
57. *Zhao P, Sun X, Chaggan C, Liao Z, Wong K, He F et al.* An AMPK–Caspase-6 Axis Controls Liver Damage in Nonalcoholic Steatohepatitis. *Science*. 2020; 367: 652–660. doi: 10.1126/science.aay0542.
58. *Maiers JL, Malhi H.* Endoplasmic Reticulum Stress in Metabolic Liver Diseases and Hepatic Fibrosis. *Semin*

Liver Dis. 2019; 39: 235–248. doi: 10.1055/s-0039-1681032.
59. *Roh YS, Kim JW, Park S, Shon C, Kim S, Eo SK et al.* Toll-Like Receptor-7 Signaling Promotes Nonalcoholic Steatohepatitis by Inhibiting Regulatory T Cells in Mice. *Am J Pathol*. 2018; 188: 2574–2588. doi: 10.1016/j.ajpath.2018.07.011.
60. *Faubion WA, Guicciardi ME, Miyoshi H, Bronk SF, Roberts PJ, Svingen PA et al*. Toxic Bile Salts Induce Rodent Hepatocyte Apoptosis via Direct Activation of Fas. *J Clin Investig*. 1999; 103: 137–145. doi: 10.1172/JCI4765.
61. *Harada K, Ozaki S, Gershwin ME, Nakanuma Y.* Enhanced Apoptosis Relates to Bile Duct Loss in Primary Biliary Cirrhosis. *Hepatology*. 1997; 26: 1399–1405. doi: 10.1002/hep.510260604.
62. *Iwata M, Harada K, Hiramatsu K, Tsuneyama K, Kaneko S, Kobayashi K, Nakanuma Y.* Fas Ligand Expressing Mononuclear Cells around Intrahepatic Bile Ducts Co-Express CD68 in Primary Biliary Cirrhosis. *Liver*. 2000; 20: 129–135. doi: 10.1034/j.1600-0676.2000.020002129.x.
63. *Canbay A, Higuchi H, Bronk SF, Taniai M, Sebo TJ, Gores GJ.* Fas enhances fibrogenesis in the bile duct ligated mouse: a link between apoptosis and fibrosis. *Gastroenterology*. 2002; 123: 1323–1330. doi: 10.1053/gast.2002.35953.
64. *Takeda K, Kojima Y, Ikejima K, Harada K, Yamashina S, Okumura K et al*. Death Receptor 5 Mediated-Apoptosis Contributes to Cholestatic Liver Disease. *Proc Natl Acad Sci USA*. 2008; 105: 10895–10900. doi: 10.1073/pnas.0802702105.
65. *Cubero FJ, Peng J, Liao L, Su H, Zhao G, Eugenio Zoubek M et al.* Inactivation of Caspase 8 in Liver Parenchymal Cells Confers Protection against Murine Obstructive Cholestasis. *J Hepatol*. 2018; 69: 1326–1334. doi: 10.1016/j.jhep.2018.08.015.
66. *Canbay A, Feldstein A, Baskin-Bey E, Bronk SF, Gores GJ.* The Caspase Inhibitor IDN-6556 Attenuates Hepatic Injury and Fibrosis in the Bile Duct Ligated Mouse. *J Pharmacol Exp Ther*. 2004; 308: 1191–1196. doi: 10.1124/jpet.103.060129.
67. *Eguchi A, Koyama Y, Wree A, Johnson CD, Nakamura R, Povero D et al*. Emricasan, a Pan-Caspase Inhibitor, Improves Survival and Portal Hypertension in a Murine Model of Common Bile-Duct Ligation. *J Mol Med*. 2018; 96: 575–583. doi: 10.1007/s00109-018-1642-9.
68. *Lau JYN, Xie X, Lai MMC, Wu PC.* Apoptosis and Viral Hepatitis. *Semin Liver Dis*. 1998; 18: 169–176. doi: 10.1055/s-2007-1007152.
69. *Kountouras J, Zavos C, Chatzopoulos D.* Apoptosis in Hepatitis C. *J Viral Hepat*. 2003: 335–342. doi: 10.1046/j.1365-2893.2003.00452.x.
70. *Ehrmann J, Galuszková D, Ehrmann J, Krè I, Jezdinská V, Vojtì Ek B et al.* Apoptosis-Related Proteins, BCL-2, BAX, FAS, FAS-L and PCNA in Liver Biopsies of Patients with Chronic Hepatitis B Virus Infection. *Pathol Oncol Res*. 2000; 6: 130–135. doi: 10.1007/BF03032363.
71. *Luo KX, Zhu YF, Zhang LX, He HT, Wang XS, Zhang L. In situ* Investigation of Fas/FasL Expression in Chronic Hepatitis B Infection and Related Liver Diseases. *J Viral Hepat*. 1997; 4: 303–307. doi: 10.1046/j.1365-2893.1997.00053.x.
72. *Galluzzi L, Bravo-San Pedro JM, Vitale I, Aaronson SA, Abrams JM, Adam D et al*. Essential versus Accessory Aspects of Cell Death: Recommendations of the NCCD 2015. *Cell Death Differ*. 2015; 22: 58–73. doi: 10.1038/cdd.2014.137.
73. *Giorgio V, Von Stockum S, Antoniel M, Fabbro A, Fogolari F, Forte M et al.* Dimers of Mitochondrial ATP Synthase Form the Permeability Transition Pore. *Proc Natl Acad Sci USA*. 2013; 110: 5887–5892. doi: 10.1073/pnas.1217823110.
74. *Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB.* Acetaminophen Induced Hepatic Necrosis. IV. Protective Role of Glutathione. *J Pharmacol Exp Ther*. 1973; 187: 211–217.
75. *Pumford NR, Hinson JA, Wayne Benson R, Roberts DW.* Immunoblot Analysis of Protein Containing 3-(Cystein-S-Yl) Acetaminophen Adducts in Serum and Subcellular Liver Fractions from Acetaminophen-Treated Mice. *Toxicol Appl Pharmacol*. 1990; 104: 521–532. doi: 10.1016/0041-008X(90)90174-S.
76. *Ramachandran A, Jaeschke H.* Acetaminophen Hepatotoxicity. *Semin Liver Dis*. 2019; 39: 221–234. doi: 10.1055/s-0039-1679919.
77. *Moles A, Torres S, Baulies A, Garcia-Ruiz C, Fernandez-Checa JC.* Mitochondrial-Lysosomal Axis in Acetaminophen Hepatotoxicity. *Front Pharmacol*. 2018. doi: 10.3389/fphar.2018.00453.
78. *Bajt ML, Ramachandran A, Yan HM, Lebofsky M, Farhood A, Lemasters JJ, Jaeschke H*. Apoptosis-Inducing Factor Modulates Mitochondrial Oxidant Stress in Acetaminophen Hepatotoxicity. *Toxicol Sci*. 2011; 122: 598–605. doi: 10.1093/toxsci/kfr116.
79. *Chen D, Ni HM, Wang L, Ma X, Yu J, Ding WX, Zhang L.* P53 Up-Regulated Modulator of Apoptosis Induction Mediates Acetaminophen-Induced Necrosis and Liver Injury in Mice. *Hepatology*. 2019; 69: 2164–2179. doi: 10.1002/hep.30422.
80. *Wang X, Du H, Shao S et al.* Cyclophilin D deficiency attenuates mitochondrial perturbation and ameliorates hepatic steatosis. *Hepatology*. 2018; 68: 62–77. doi: 10.1002/hep.29788.
81. *Laker RC, Taddeo EP, Akhtar YN, Zhang M, Hoehn KL, Yan Z.* The mitochondrial permeability transition pore regulator cyclophilin D exhibits tissue-specific control of metabolic homeostasis. *PLoS One*. 2016; 11: e0167910. doi: 10.1371/journal.pone.0167910.
82. *Schwabe RF, Luedde T.* Apoptosis and necroptosis in the liver: a matter of life and death. *Nat Rev Gastroenterol Hepatol*. 2018; 15: 738–752. doi: 10.1038/s41575-018-0065-y.
83. *Roychowdhury S, McMullen MR, Pisano SG, Liu X, Nagy LE.* Absence of receptor interacting protein kinase 3 prevents ethanol-induced liver injury. *Hepatology*. 2013; 57: 1773–1783. doi: 10.1002/hep.26200.

84. *Murphy JM, Vince JE.* Post-Translational Control of RIPK3 and MLKL Mediated Necroptotic Cell Death. *F1000Research*. 2015. doi: 10.12688/f1000research.7046.1.
85. *Sun L, Wang H, Wang Z, He S, Chen S, Liao D et al.* Mixed Lineage Kinase Domain-like Protein Mediates Necrosis Signaling Downstream of RIP3 Kinase. *Cell*. 2012; 148: 213–227. doi: 10.1016/j.cell.2011.11.031.
86. *Newton K, Manning G.* Necroptosis and Inflammation. *Annu Rev Biochem*. 2016; 85: 743–763. doi: 10.1146/annurev-biochem-060815-014830.
87. *Dara L, Liu ZX, Kaplowitz N.* Questions and Controversies: The Role of Necroptosis in Liver Disease. *Cell Death Discov*. 2016. doi: 10.1038/cddiscovery.2016.89.
88. *Dara L.* The Receptor Interacting Protein Kinases in the Liver. *Semin Liver Dis*. 2018; 38: 73–86. doi: 10.1055/s-0038-1629924.
89. *Kaplowitz N, Win S, Than TA, Liu ZX, Dara L.* Targeting Signal Transduction Pathways which Regulate Necrosis in Acetaminophen Hepatotoxicity. *J Hepatol*. 2015: 5–7. doi: 10.1016/j.jhep.2015.02.050.
90. *Günther C, He GW, Kremer AE, Murphy JM, Petrie EJ, Amann K et al.* The Pseudokinase MLKL Mediates Programmed Hepatocellular Necrosis Independently of RIPK3 during Hepatitis. *J Clin Investig*. 2016; 126: 4346–4360. doi: 10.1172/JCI87545.
91. *Jouan-Lanhouet S, Arshad MI, Piquet-Pellorce C, Martin-Chouly C, Le Moigne-Muller G, Van Herreweghe F et al.* TRAIL Induces Necroptosis Involving RIPK1/RIPK3-Dependent PARP-1 Activation. *Cell Death Differ*. 2012; 19: 2003–2014. doi: 10.1038/cdd.2012.90.
92. *Arshad MI, Piquet-Pellorce C, Filliol A, L'Helgoualc'h A, Lucas-Clerc C, Jouan-Lanhouet S et al.* The Chemical Inhibitors of Cellular Death, PJ34 and Necrostatin-1, down-Regulate IL-33 Expression in Liver. *J Mol Med*. 2015; 93: 867–878. doi: 10.1007/s00109-015-1270-6.
93. *Zhou Y, Dai W, Lin C, Wang F, He L, Shen M et al.* Protective Effects of Necrostatin-1 against Concanavalin A-Induced Acute Hepatic Injury in Mice. *Mediat Inflamm*. 2013; doi: 10.1155/2013/706156.
94. *Hamon A, Piquet-Pellorce C, Dimanche-Boitrel MT, Samson M, Le Seyec J.* Intrahepatocytic Necroptosis Is Dispensable for Hepatocyte Death in Murine Immune-Mediated Hepatitis. *J Hepatol*. 2020: 699–701. doi: 10.1016/j.jhep.2020.05.016.
95. *Chen L, Cao Z, Yan L, Ding Y, Shen X, Liu K et al.* Circulating Receptor-Interacting Protein Kinase 3 Are Increased in HBV Patients with Acute-on-Chronic Liver Failure and Are Associated with Clinical Outcome. *Front Physiol*. 2020; 11. doi: 10.3389/fphys.2020.00526.
96. *Han L, Teng Y, Fan Y, Gao S, Li F, Wang K.* Receptor-Interacting Protein Kinase 3 (RIPK3) MRNA Levels Are Elevated in Blood Mononuclear Cells of Patients with Poor Prognosis of Acute-on-Chronic Hepatitis B Liver Failure. *Tohoku J Exp Med*. 2019; 247: 237–245. doi: 10.1620/tjem.247.237.
97. *Orning P, Weng D, Starheim K, Ratner D, Best Z, Lee B et al.* Pathogen Blockade of TAK1 Triggers Caspase-8–Dependent Cleavage of Gasdermin D and Cell Death. *Science*. 2018; 362: 1064–1069. doi: 10.1126/science.aau2818.
98. *Man SM, Kanneganti TD.* Regulation of Inflammasome Activation. *Immunol Rev*. 2015: 6–21. doi: 10.1111/imr.12296.
99. *Aachoui Y, Sagulenko V, Miao EA, Stacey KJ.* Inflammasome-Mediated Pyroptotic and Apoptotic Cell Death, and Defense against Infection. *Curr Opin Microbiol*. 2013: 319–326. doi: 10.1016/j.mib.2013.04.004.
100. *Kelley N, Jeltema D, Duan Y, He Y.* The NLRP3 Inflammasome: An Overview of Mechanisms of Activation and Regulation. *Int J Mol Sci*. 2019; 20: 3328. doi: 10.3390/ijms20133328.
101. *Wu J, Lin S, Wan B, Velani B, Zhu Y.* Pyroptosis in Liver Disease: New Insights into Disease Mechanisms. *Aging Dis*. 2019; 10: 1094–1108. doi: 10.14336/AD.2019.0116.
102. *Petrasek J, Iracheta-Vellve A, Saha B, Satishchandran A, Kodys K, Fitzgerald KA et al.* Metabolic Danger Signals, Uric Acid and ATP, Mediate Inflammatory Cross-Talk between Hepatocytes and Immune Cells in Alcoholic Liver Disease. *J Leukoc Biol*. 2015; 98: 249–256. doi: 10.1189/jlb.3AB1214-590R.
103. *Heo MJ, Kim TH, You JS, Blaya D, Sancho-Bru P, Kim SG.* Alcohol Dysregulates MiR-148a in Hepatocytes through FoxO1, Facilitating Pyroptosis via TXNIP Overexpression. *Gut*. 2019; 68: 708–720. doi: 10.1136/gutjnl-2017-315123.
104. *Beier JI, Banales JM.* Pyroptosis: An Inflammatory Link between NAFLD and NASH with Potential Therapeutic Implications. *J Hepatol*. 2018: 643–645. doi: 10.1016/j.jhep.2018.01.017.
105. *Xu B, Jiang M, Chu Y, Wang W, Chen D, Li X et al.* Gasdermin D Plays a Key Role as a Pyroptosis Executor of Non-Alcoholic Steatohepatitis in Humans and Mice. *J Hepatol*. 2018; 68: 773–782. doi: 10.1016/j.jhep.2017.11.040.
106. *Mehal WZ.* The Inflammasome in Liver Injury and Non-Alcoholic Fatty Liver Disease. *Dig Dis*. 2014; 32: 507–515. doi: 10.1159/000360495.
107. *Mridha AR, Wree A, Robertson AAB, Yeh MM, Johnson CD, Van Rooyen DM et al.* NLRP3 Inflammasome Blockade Reduces Liver Inflammation and Fibrosis in Experimental NASH in Mice. *J Hepatol*. 2017; 66: 1037–1046. doi: 10.1016/j.jhep.2017.01.022.
108. *Williams CD, Farhood A, Jaeschke H.* Role of Caspase-1 and Interleukin-1β in Acetaminophen-Induced Hepatic Inflammation and Liver Injury. *Toxicol Appl Pharmacol*. 2010; 247: 169–178. doi: 10.1016/j.taap.2010.07.004.
109. *Williams CD, Antoine DJ, Shaw PJ, Benson C, Farhood A, Williams DP et al.* Role of the Nalp3 Inflammasome in Acetaminophen-Induced Sterile Inflammation and Liver Injury. *Toxicol Appl Pharmacol*. 2011; 252: 289–297. doi: 10.1016/j.taap.2011.03.001.
110. *Zhang C, Feng J, Du J, Zhuo Z, Yang S, Zhang W et al.* Macrophage-Derived IL-1α Promotes Sterile Inflammation in a Mouse Model of Acetaminophen Hepatotoxicity. *Cell Mol Immunol*. 2018; 15: 973–982. doi: 10.1038/cmi.2017.22.

111. *Luan J, Zhang X, Wang S, Li Y, Fan J, Chen W et al.* NOD-like Receptor Protein 3 Inflammasome-Dependent IL-1β Accelerated ConA-Induced Hepatitis. *Front Immunol*. 2018; 9. doi: 10.3389/fimmu.2018.00758.
112. *Wang J, Ren H, Yuan X, Ma H, Shi X, Ding Y.* Interleukin-10 Secreted by Mesenchymal Stem Cells Attenuates Acute Liver Failure through Inhibiting Pyroptosis. *Hepatol Res*. 2018; 48: E194–E202. doi: 10.1111/hepr.12969.
113. *Lan P, Fan Y, Zhao Y, Lou X, Monsour HP, Zhang X et al.* TNF Superfamily Receptor OX40 Triggers Invariant NKT Cell Pyroptosis and Liver Injury. *J Clin Investig*. 2017; 127: 2222–2234. doi: 10.1172/JCI91075.
114. *Maroni L, Agostinelli L, Saccomanno S, Pinto C, Giordano DM, Rychlicki C et al*. Nlrp3 Activation Induces Il-18 Synthesis and Affects the Epithelial Barrier Function in Reactive Cholangiocytes. *Am J Pathol*. 2017; 187: 366–376. doi: 10.1016/j.ajpath.2016.10.010.
115. *Gong Z, Zhou J, Zhao S, Tian C, Wang P, Xu C et al.* Chenodeoxycholic Acid Activates NLRP3 Inflammasome and Contributes to Cholestatic Liver Fibrosis. *Oncotarget*. 2016; 7: 83951–83963. doi: 10.18632/oncotarget.13796.
116. *Liao L, Schneider KM, Galvez EJC, Frissen M, Marschall HU, Su H et al.* Intestinal Dysbiosis Augments Liver Disease Progression via NLRP3 in a Murine Model of Primary Sclerosing Cholangitis. *Gut*. 2019; 68: 1477–1492. doi: 10.1136/gutjnl-2018-316670.
117. *Xu WF, Zhang Q, Ding CJ, Sun HY, Che Y, Huang H et al.* Gasdermin E-Derived Caspase-3 Inhibitors Effectively Protect Mice from Acute Hepatic Failure. *Acta Pharmacol Sin*. 2020. doi: 10.1038/s41401-020-0434-2.
118. *Serti E, Werner JM, Chattergoon M, Cox AL, Lohmann V, Rehermann B.* Monocytes Activate Natural Killer Cells via Inflammasome-Induced Interleukin 18 in Response to Hepatitis C Virus Replication. *Gastroenterology*. 2014; 147. doi: 10.1053/j.gastro.2014.03.046.
119. *Yu X, Lan P, Hou X, Han Q, Lu N, Li T et al.* HBV Inhibits LPS-Induced NLRP3 Inflammasome Activation and IL-1β Production via Suppressing the NF-KB Pathway and ROS Production. *J Hepatol*. 2017; 66: 693–702. doi: 10.1016/j.jhep.2016.12.018.
120. *Yang WS, Stockwell BR.* Ferroptosis: Death by Lipid Peroxidation. *Trends Cell Biol*. 2016: 165–176. doi: 10.1016/j.tcb.2015.10.014.
121. *Mao L, Zhao T, Song Y, Lin L, Fan X, Cui B et al.* The Emerging Role of Ferroptosis in Non-Cancer Liver Diseases: Hype or Increasing Hope? *Cell Death Dis*. 2020. doi: 10.1038/s41419-020-2732-5.
122. *Doll S, Conrad M.* Iron and Ferroptosis: A Still Ill-Defined Liaison. *IUBMB Life*. 2017; 69: 423–434. doi: 10.1002/iub.1616.
123. *Capelletti MM, Manceau H, Puy H, Peoc'h K.* Ferroptosis in Liver Diseases: An Overview. *Int J Mol Sci*. 2020; 21: 4908. doi: 10.3390/ijms21144908.
124. *Yang WS, Sriramaratnam R, Welsch ME, Shimada K, Skouta R, Viswanathan VS et al*. Regulation of Ferroptotic Cancer Cell Death by GPX4. *Cell*. 2014; 156: 317–331. doi: 10.1016/j.cell.2013.12.010.
125. *Xie Y, Hou W, Song X et al.* Ferroptosis: process and function. Cell Death Differ. 2016; 23: 369–379. doi: 10.1038/cdd.2015.158.
126. *Zhou Z, Ye TJ, Bonavita G, Daniels M, Kainrad N, Jogasuria A, You M.* Adipose-Specific Lipin-1 Overexpression Renders Hepatic Ferroptosis and Exacerbates Alcoholic Steatohepatitis in Mice. *Hepatol Commun*. 2019; 3: 656–669. doi: 10.1002/hep4.1333.
127. *Zhou Z, Ye TJ, DeCaro E, Buehler B, Stahl Z, Bonavita G et al.* Intestinal SIRT1 Deficiency Protects Mice from Ethanol-Induced Liver Injury by Mitigating Ferroptosis. *Am J Pathol*. 2020; 190: 82–92. doi: 10.1016/j.ajpath.2019.09.012.
128. *Macías-Rodríguez RU, Inzaugarat ME, Ruiz-Margáin A, Nelson LJ, Trautwein C, Cubero FJ.* Reclassifying Hepatic Cell Death during Liver Damage: Ferroptosis-A Novel Form of Non-Apoptotic Cell Death? *Int J Mol Sci*. 2020 Feb 28; 21 (5): 1651. doi: 10.3390/ijms21051651.
129. *Qi J, Kim JW, Zhou Z, Lim CW, Kim B.* Ferroptosis Affects the Progression of Nonalcoholic Steatohepatitis via the Modulation of Lipid Peroxidation–Mediated Cell Death in Mice. *Am J Pathol*. 2020; 190: 68–81. doi: 10.1016/j.ajpath.2019.09.011.
130. *Wang M, Liu CY, Wang T, Yu HM, Ouyang SH, Wu YP et al.* (+)-Clausenamide Protects against Drug-Induced Liver Injury by Inhibiting Hepatocyte Ferroptosis. *Cell Death Dis*. 2020; 11. doi: 10.1038/s41419-020-02961-5.
131. *Yamada N, Karasawa T, Kimura H, Watanabe S, Komada T, Kamata R et al.* Ferroptosis Driven by Radical Oxidation of N-6 Polyunsaturated Fatty Acids Mediates Acetaminophen-Induced Acute Liver Failure. *Cell Death Dis*. 2020; 11. doi: 10.1038/s41419-020-2334-2.
132. *Yamada N, Karasawa T, Takahashi M.* Role of Ferroptosis in Acetaminophen-Induced Hepatotoxicity. *Arch Toxicol*. 2020: 1769–1770. doi: 10.1007/s00204-020-02714-5.
133. *Zeng T, Deng G, Zhong W, Gao Z, Ma S, Mo C et al.* Indoleamine 2,3-Dioxygenase 1 enhanceshepatocytes Ferroptosis in Acute Immune Hepatitis Associated with Excess Nitrative Stress. *Free Radic Biol Med*. 2020; 152: 668–679. doi: 10.1016/j.freeradbiomed.2020.01.009.
134. *Deng G, Li Y, Ma S, Gao Z, Zeng T, Chen L et al.* Caveolin-1 Dictates Ferroptosis in the Execution of Acute Immune-Mediated Hepatic Damage by Attenuating Nitrogen Stress. *Free Radic Biol Med*. 2020; 148: 151–161. doi: 10.1016/j.freeradbiomed.2019.12.026.
135. *Wang Y, An R, Umanah GK et al.* A nuclease that mediates cell death induced by DNA damage and poly (ADP-ribose) polymerase-1. *Science*. 2016; 354. doi: 10.1126/science.aad6872. aad6872.
136. *Park EJ, Min KJ, Lee TJ, Yoo YH, Kim YS, Kwon TK.* β-Lapachone induces programmed necrosis through the RIP1-PARP-AIF-dependent pathway in human hepatocellular carcinoma SK-Hep1 cells. *Cell Death Dis*. 2014; 5: e1230. doi: 10.1038/cddis.2014.202.
137. *Sun Q, Luo T, Ren Y et al.* Competition between human cells by entosis. *Cell Res*. 2014; 24: 1299–1310. doi: 10.1038/cr.2014.138.

138. *Hamann JC, Surcel A, Chen R et al.* Entosis is induced by glucose starvation. *Cell Rep*. 2017; 20: 201–210. doi: 10.1016/j.celrep.2017.06.037.
139. *Sierro F, Tay SS, Warren A et al.* Suicidal emperipolesis: a process leading to cell-in-cell structures, T cell clearance and immune homeostasis. *Curr Mol Med*. 2015; 15: 819–827. doi: 10.2174/1566524015666151026102143.
140. *Shi J, Zhao J, Zhang X et al.* Activated hepatic stellate cells impair NK cell anti-fibrosis capacity through a TGF-beta-dependent emperipolesis in HBV cirrhotic patients. *Sci Rep*. 2017; 7: 44544. doi: 10.1038/srep44544.
141. *Hitomi J, Christofferson DE, Ng A et al.* Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. *Cell*. 2008; 135: 1311–1323. doi: 10.1016/j.cell.2008.10.044.
142. *Conos SA, Chen KW, De Nardo D et al.* Active MLKL triggers the NLRP3 inflammasome in a cell-intrinsic manner. *Proc Natl Acad Sci USA*. 2017; 114: E961–E969. doi: 10.1073/pnas.1613305114.
143. *Chung H, Vilaysane A, Lau A et al.* NLRP3 regulates a non-canonical platform for caspase-8 activation during epithelial cell apoptosis. *Cell Death Differ*. 2016; 23: 1331–1346. doi: 10.1038/cdd.2016.14.
144. *Maslov LN, Naryzhnaya NV, Sementsov AS, Mohamoyedzian AV, Gorbunov AS.* The influence of the post-conditioning of the heart on necrosis, apoptosis, oncosis and autophage cardiomyocytes. *Pathophysiology and experimental therapy*. 2016; 60 (2): 94–100.
145. *Kong Z, Liu R, Cheng Y.* Artesunate alleviates liver fibrosis by regulating ferroptosis signaling pathway. Biomed Pharmacother. 2019 Jan; 109: 2043–2053. doi: 10.1016/j.biopha.2018.11.030.
146. *El-Kashef DH, Abdelrahman RS.* Montelukast ameliorates Concanavalin A-induced autoimmune hepatitis in mice via inhibiting TNF-α/JNK signaling pathway. *Toxicol Appl Pharmacol*. 2020 Apr 15; 393: 114931. doi: 10.1016/j.taap.2020.114931.
147. *Zhang M, Wu P, Li M, Guo Y, Tian T, Liao X, Tan S.* Inhibition of Notch1 signaling reduces hepatocyte injury in nonalcoholic fatty liver disease via autophagy. *Biochem Biophys Res Commun*. 2021 Apr 2; 547: 131–138. doi: 10.1016/j.bbrc.2021.02.039.
148. *Peng Z, Liao Y, Wang X, Chen L, Wang L, Qin C et al.* Heme oxygenase-1 regulates autophagy through carbon-oxygen to alleviate deoxynivalenol-induced hepatic damage. Arch Toxicol. 2020 Feb; 94 (2): 573–588. doi: 10.1007/s00204-019-02649-6.
149. *Yu X, Hao M, Liu Y, Ma X, Lin W, Xu Q et al.* Liraglutide ameliorates non-alcoholic steatohepatitis by inhibiting NLRP3 inflammasome and pyroptosis activation via mitophagy. *Eur J Pharmacol*. 2019 Dec 1; 864: 172715. doi: 10.1016/j.ejphar.2019.172715.
150. *Hongming Lv, Liu Y, Zhang B, Zheng Y, Ji H, Li S.* The improvement effect of gastrodin on LPS/GalN-induced fulminant hepatitis via inhibiting inflammation and apoptosis and restoring autophagy. *Int Immunopharmacol*. 2020 Aug; 85: 106627. doi: 10.1016/j.intimp.2020.106627.
151. *Zhao S, Liu Y, Pu Z.* Bone marrow mesenchymal stem cell-derived exosomes attenuate D-GaIN/LPS-induced hepatocyte apoptosis by activating autophagy *in vitro*. *Drug Des Devel Ther*. 2019 Aug 19; 13: 2887–2897. doi: 10.2147/DDDT.S220190.
152. *Pervaiz S, Bellot G L, Lemoine A, Brenner C.* Redox signaling in the pathogenesis of human disease and the regulatory role of autophagy. *Int Rev Cell Mol Biol*. 2020; 352: 189–214. doi: 10.1016/bs.ircmb.2020.03.002.
153. *Veskovic M, Mladenovic D, Milenkovic M, Tosic J, Borozan S, Gopcevic K et al*. Betaine modulates oxidative stress, inflammation, apoptosis, autophagy, and Akt/mTOR signaling in methionine-choline deficiency-induced fatty liver disease. *Eur J Pharmacol*. 2019 Apr 5; 848: 39–48. doi: 10.1016/j.ejphar.2019.01.043.
154. *Nasiri-Ansari N, Nikolopoulou C, Papoutsi K, Kyrou I, Mantzoros CS, Kyriakopoulos G et al.* Empagliflozin Attenuates Non-Alcoholic Fatty Liver Disease (NAFLD) in High Fat Diet Fed ApoE$^{(-/-)}$ Mice by Activating Autophagy and Reducing ER Stress and Apoptosis. *Int J Mol Sci*. 2021 Jan 15; 22 (2): 818. doi: 10.3390/ijms22020818.
155. *Beer L, Mildner M, Gyöngyösi M, Ankersmit HJ*. Peripheral blood mononuclear cell secretome for tissue repair. *Apoptosis*. 2016, 21: 1336–1353. doi: 10.1007/s10495-016-1292-8.
156. *He YT, Qi YN, Zhang BQ, Li JB, Bao J*. Bioartificial liver support systems for acute liver failure: A systematic review and meta-analysis of the clinical and preclinical literature. *World J Gastroenterol*. 2019 Jul 21; 25 (27): 3634–3648. doi: 10.3748/wjg.v25.i27.3634.
157. *Weng J, Han X, Zeng F, Zhang Y, Feng L, Cai L et al.* Fiber scaffold bioartificial liver therapy relieves acute liver failure and extrahepatic organ injury in pigs. *Theranostics*. 2021; 11 (16): 7620–7639. doi: 10.7150/thno.58515.
158. *Shagidulin MYu, Onishchenko NA, Basok YuB, Grigoriev AM, Kirillova AD, Nemets EA et al.* Functional efficiency of cell-engineered liver constructs based on tissue-specific matrix (experimental model of chronic liver failure). *Russian Journal of Transplantology and Artificial Organs*. 2020; 22 (4): 89–97. https://doi.org/10.15825/1995-1191-2020-4-89-97.
159. *Onishchenko NA, Fomenko EV, Nikolskaya AO, Gonikova ZZ, Shagidulin MYu, Balyasin MV et al.* Activation of regenerative processes in the liver when using cell-bone marrow total RNA. *Russian Journal of Transplantology and Artificial Organs*. 2020; 22 (3): 134–142. https://doi.org/10.15825/1995-1191-2020-3-134-142.

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EFFECT OF TRANSDERMAL IMMUNOMODULATION ON LIVER REGENERATION

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Introduction. The use of immunomodulators to regulate reparative processes in affected organs and tissues remains a pressing issue. Of greatest interest is liver regeneration after extended hepatic resection (EHR) in donors in right lobe living related donor liver transplantation. We propose a transdermal therapeutic system (TTS) with an immunomodulator to enhance the natural process of liver tissue regeneration. **Objective:** to study the effect of transdermal administration of immunomodulator sodium aminodihydrophthalazinedione on early recovery processes in the liver after EHR in in vivo experiments. **Materials and methods.** Sodium aminodihydrophthalazinedione was used as an active substance in TTS in the form of powder for preparation of intramuscular injection solution (Galavit®, SELVIM LLC). An experimental EHR model was performed on 22 male Wistar rats weighing 350–380 g. After HER, all animals were divided into two groups. Group 1 (n = 10) consisted of untreated animals. In group 2 (n = 12), TTS was applied immediately after liver resection. The experiment lasted for 48 hours; the TTS was changed once after 24 hours from the beginning of application. **Results.** In either group, there was no significant difference in the weight of liver remnant gain and in biochemical blood parameters at 48 hours after EHR. Assessment of the mitotic index (MI) of hepatocytes 48 hours after EHR revealed a significant increase in MI in both groups in comparison with the baseline (before liver resection) equal to 0.14 ± 0.07‰. The MI in group 1 and group 2 animals was 12.70 ± 4.9‰ and 17.43 ± 4.90‰, respectively ($p \leq 0.05$). **Conclusion.** Studies on the regenerative activity of sodium aminodihydrophthalazinedione TTS on an experimental EHR model in rats showed that this drug form had a pronounced stimulating effect on the mitotic activity of liver cells.

Keywords: transdermal therapeutic system, sodium aminodihydrophthalazinedione, immunomodulator, extended liver resection, mitotic index.

INTRODUCTION

The immune system, whose entire elements are actively involved in restoring the structure and function of damaged tissue cells, play an important role in regulation of regenerative processes in the body. This raises the question about the feasibility of using immunomodulators (IM) to influence reparative processes in affected organs and tissues [1, 2]. Of greatest interest is the problem of accelerated recovery of the liver after extended resection in cancer patients, as well as in donors in right lobe living related liver transplantation [3]. The extended hepatic resection (EHR) model is usually used in experimental studies. This type of surgery belongs to the critical injury class, because it removes 60% or more of the total mass of the organ, and often there are clinical manifestations of acute liver failure in the postoperative period [3].

There have been studies confirming the positive effect of a single injection of various immunomodulators on liver tissue repair [1, 4]. It should be noted that the question of duration of IM effect in a single injection remains open.

It is known that mitotic activity of hepatocytes is reduced in the first day after the operation but increases already by the second day [5]. Maximum mitotic and functional activity of hepatocytes is observed between day 2 and day 5 after the resection [5, 6]. It can be assumed that the use of prolonged drug form in the form of a transdermal therapeutic system will enhance the natural process of liver tissue regeneration by maintaining a constant concentration of IM in the blood for the required period.

The authors developed a transdermal therapeutic system containing a synthetic low molecular weight drug substance sodium aminodihydrophthalazinedione (Galavit®) [7]. In vivo experiments showed that the use of Galavit® transdermal therapeutic system (TTS) provides bioavailability of the immunomodulator, equal to the bioavailability of intramuscular injection of the drug substance at the same dose. This significantly reduces the maximum blood levels of the drug, but the retention time of sodium aminodihydrophthalazinedione in the body increases by more than 10 times, which may contribute to prolonged drug effect [8].

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In view of the above, the **objective** of the study was to investigate the effect of transdermal administration of immunomodulator aminodihydrophthalazinedione on the early stage of recovery in the liver in the experimental EHR model.

MATERIALS AND METHODS

Sodium aminodihydrophthalazinedione was used as an active substance in TTS in the form of a powder for preparing a solution for intramuscular injection (trade name Galavit®, Selvim LLC).

Excipients and materials approved for medical purposes were used in the manufacture of laboratory samples of Galavit® TTS.

The experimental animals were 22 male Wistar rats weighing 350–380 g, in which the EHR model was reproduced. Before EHR modeling, the operated rats were anesthetized by inhaled diethyl ether. Then, observing the rules of aseptics and antiseptics, the abdominal cavity was opened, the liver was exposed to the wound, and ligatures were sequentially applied to the bases of the medial, left lateral, and right upper lobes of the liver, after which ~70% of the total liver mass was removed. The operation was always performed in the morning hours (between 10 am and 12 noon), when the daily rhythm of mitotic activity of the liver cells was minimal.

All animals after EHR were divided into two groups. The first group (n = 10) consisted of animals with EHR without treatment.

In the second group (n = 12), immediately after liver resection, Galavit® TTS (10 cm^2) was applied in the back area of the rats to the skin areas with previously removed hair. Each TTS contained 40 mg of the drug substance. The experiment lasted for 48 hours with a single change of TTS 24 hours after the start of application.

To assess the dynamics of liver weight recovery in each operated animal, the removed part of the liver was weighed on Ohaus Explorer (Switzerland) electronic scale immediately after EHR, which was taken as 70% of the total liver weight. Then the initial mass of the residual liver was calculated for each animal based on these data. Then, after 48 hours, the remaining portion of the liver was excised, its weight was measured, and the values obtained were compared with the calculated initial mass of the residual liver for the particular animal.

In addition, the following biochemical blood parameters were determined: total protein, albumin, urea, creatinine and hepatic cytolysis enzymes: alanine aminotransferase (ALT), asparagine aminotransferase (AST) and alkaline phosphatase (ALP). For this purpose, the tail tip of the rat was incised under ether anesthesia, 28–32 μL blood was pipetted and applied to Reflotron™ test strips, which were immediately placed in a Reflotron™ biochemical analyzer (Roche, Switzerland). Blood from intact animals (n = 4) was used as a control.

Efficiency of stimulating effect of transdermal immunomodulation on liver regeneration processes after EHR was evaluated by mitotic (proliferative) activity of hepatocytes in the resected liver remnant. For this purpose, histological preparations of the resected liver were prepared, then the mitotic index (MI) – the number of mitotically dividing cells per 1000 analyzed cells – was calculated. For each specimen on the histological section of liver tissue stained with hematoxylin and eosin under 400× magnification (Leica LMLS microscope), we determined the number of mitotic figures and the average (total) number of cells. The formula used was:

$$MI = \frac{M}{N} \times 1000,$$

where *M* is the sum of dividing cells, *N* is the total number of cells analyzed. Mitotic index was expressed in ppm.

The significance of difference between the studied indicators in the compared groups was assessed using the parametric Student's t-test.

RESULTS AND DISCUSSIONS

It is known that after EHR, the process of organ recovery up to its initial mass occurs through proliferation and polyploidization of hepatocytes. It was noted that distinct signs of increased proliferative activity of the liver cells after EHR appear only 48 hours after this operation [9]. Considering the above, we evaluated the effect of percutaneous immunomodulator injection on stimulation of regeneration of the remaining part of the resected liver at 48 hours from the start of TTS.

The degree of recovery of rat liver weight in groups 1 and 2, 48 hours after EHR can be judged from the calculated results presented in Table 1.

As can be seen from Table 1, the increase in the liver remnant weight in the experimental animals, 48 hours

Table 1

Changes in rat liver weight 48 hours after extended resection

Animal group	Taken (~70%), g	Remnant (calculated, ~30%), g	Liver weight (calculated), g	Remnant (after 48 hours), g	Weight gain of liver remnant, g	Weight gain of liver, %
Group 1 (EHR; n = 10)	7.77 ± 0.754	3.33 ± 0.32	11.10 ± 1.06	8.08 ± 1.26	4.75 ± 1.17	43.06 ± 10.49
Group 2 (EHR + TTS; n = 12)	7.16 ± 1.05	3.07 ± 0.45	10.24 ± 1.50	7.00 ± 0.96	3.93 ± 0.79	39.03 ± 9.30

after resection, was 43 ± 10%; while in the group of animals with EHR and Galavit® TTS, it was 39 ± 9% for the same period. There was no significant difference in the weight of the liver remnant gain.

The effect of immunomodulator on restoration of liver homeostasis in rats at 48 hours after EHR was estimated by comparing the calculated blood biochemical indicators in the groups. Results of blood tests are presented in Table 2.

As can be seen from Table 2, biochemical blood parameters in both groups were significantly higher than in the intact animal group.

Analysis of results of the two experimental groups showed that such biochemical blood parameters as total protein (50.5 ± 2.9 g/L in group 2 and 52.3 ± 3.6 g/L in group 1) and albumin (24.1 ± 2.8 g/L and 24.9 ± 2.6 g/L respectively), in both groups, 48 hours after EHR, were practically the same.

Table 2

Biochemical blood parameters of rats after EHR

Biochemical blood parameters	ALT, U/I	AST, U/I	GGT, U/I	ALP, U/I	Total protein, g/l	Albumin, g/l	Urine, mmol/l	Creatinine, μmol/l
Intact group (n = 4)								
Mean	**52.6**	108.4		**184.2**	**60.8**	**30.0**	**7.5**	**24.7**
SD	6.7	21.0		87.0	1.5	12.0	0.4	3.3
Group 1 (EHR; n = 10)								
Mean	**407.3**	**657.5**	**6.7**	**559.7**	**52.3**	**24.9**	**8.6**	**49.1**
SD	203.5	225.6	4.9	239.5	3.6	2.6	7.4	28.9
Group 2 (EHR + TTS, n = 12)								
Mean	**470.3**	**830.9**	**10.8**	**568.1**	**50.5**	**24.1**	**12.1**	**62.8**
SD	225.7	334.0	7.1	193.9	2.9	2.8	14.4	54.8

Table 3

Mitotic index of hepatocytes 48 hours after EHR

Sample name / group	No. of histological sample	MI, ‰	MI mean, ‰	SD
Source tissue (n = 5)	2536	0.19	0.14	0.07
	2540	1.54		
	2542	0.14		
	2559	0.11		
	2621	0.05		
Group 1 (EHR; n = 10)	2535	10.21	**12.70**	4.9
	2539	10.80		
	2541	5.38		
	2544	8.37		
	2617	16.4		
	2619	16.8		
	2620	22.08		
	2622	13.26		
	2623	8.82		
	2624	15.13		
Group 2 (EHR + TTS, n = 12)	2537	19.40	**17.43**	4.9
	2545	10.84		
	2548	7.18		
	2626	25.09		
	2636	21.3		
	2637	12.69		
	2638	17.04		
	2639	17.6		
	2640	19.2		
	2614	17.5		
	2616	24.6		
	2617	13.7		

Urea levels were 12.1 ± 14.4 mmol/L in group 2 and 8.6 ± 7.4 mmol/L in group 1, while *creatinine* levels were 62.8 ± 54.8 μmol/L and 49.1 ± 28.9 μmol/L in group 2 and group 1, respectively. No significant difference was found.

The levels of liver cell cytolysis enzymes (ALT, AST, GGT, ALP) were slightly higher on average in group 2 (experimental) (EHR + TTS) – 470.3 ± 225.7 U/L; 830.9 ± 334.0 U/L; 10.8 ± 7.1 U/L; 568.1 ± 193.9 U/L, respectively. In group 1, ALT, AST, GGT, and ALP values were lower on average – 407.3 ± 203.5 U/L; 657.5 ± 225.6 U/L; 6.7 ± 4.9 U/L; 559.7 ± 239.5 U/L, respectively. However, the observed differences in cytolysis enzymes at 48 hours after EHR in the compared groups were not significant.

So, in a comparison of biochemical blood values at 48 hours after EHR, transdermal administration of immunomodulator Galavit® was not found to have any positive effect on restoration of hepatic homeostasis in rats.

The effect of using immunomodulator TTS to accelerate liver regeneration in EHR at 48 hours was found when comparing the results of histological study of hepatocyte proliferation by determining the MI of hepatocytes (see Table 3).

Research on the mitotic activity of liver hepatocytes at 48 hours after EHR, assessed according to the MI, revealed a significant increase in MI in both groups (0.14 ±

0.07‰) when compared to the baseline (before liver resection). As can be seen from Table 3, the MI in group 1 (EHR without treatment) at 48 hours of EHR averaged 12.70 ± 4.9‰, whereas in group 2 (with Galavit® TTS application immediately after EHR), the MI averaged significantly higher – 17.43 ± 4.90‰, ($p \leq 0.05$). Thus, percutaneous administration of immunomodulator Galavit® had a pronounced stimulating effect on the mitotic activity of liver cells.

The found pattern is supported by results from comparative morphological analysis of histological liver tissue preparations, in which higher mitotic activity of hepatocytes after 2 days in group 1 with EHR (Fig. 2) compared with the original tissue (Fig. 1) and more pronounced manifestations of mitotic activity of hepatocytes in group 2 with application of IM TTS (Fig. 3) are determined.

Figs. 1–3 show, as examples, photos of histological samples of rat liver tissue: initial; 48 hours after EHR, 48 hours after EHR and application of IM TTS.

Note that there was no death in all groups of animals throughout the duration of the experiment.

So, the study of the mitotic activity of hepatocytes after EHR in the groups of animals investigated showed that EHR induces proliferative activity in hepatocytes, and that transdermal administration of immunomodulator Galavit® after EHR has a stimulating effect on proliferation of hepatocytes in the resected liver. Increased proliferative activity of hepatocytes is accompanied by adaptive restructuring of the metabolism of these cells, which seems to have predetermined the absence of a positive effect of the immunomodulator TTS on biochemical indicators of hepatic homeostasis at 48 hours after EHR.

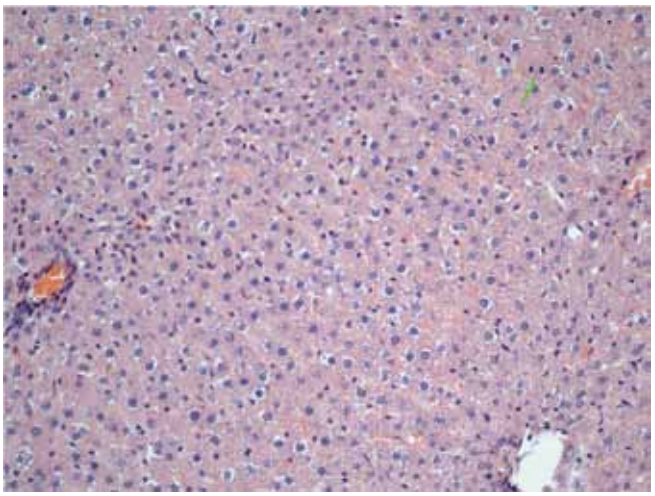

Fig. 1. Histological picture of the original rat liver tissue. Single figures of mitoses in the parenchyma (indicated by arrow). H&E staining. 200×

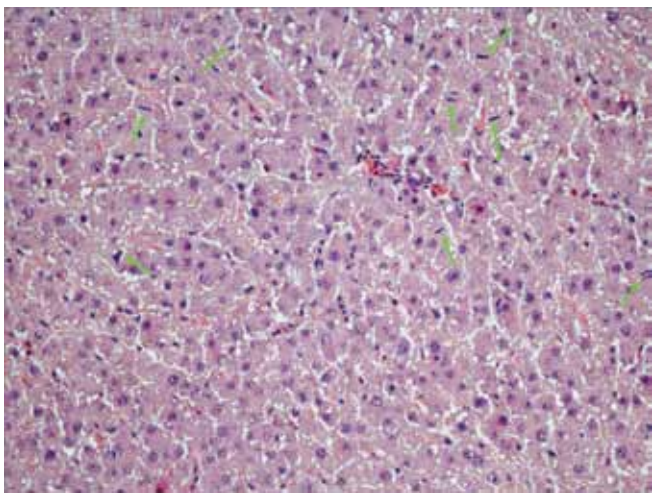

Fig. 2. Mitotic activity of hepatocytes 48 hours after EHR. Arrows indicate hepatocytes in the mitosis stage. H&E staining. 200×

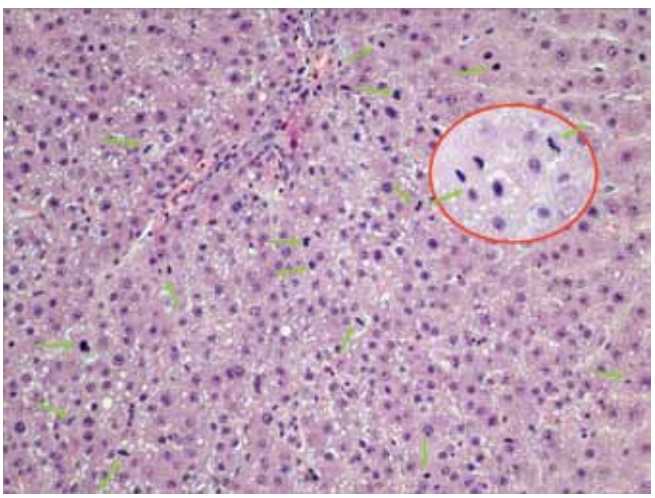

Fig. 3. Mitotic activity of hepatocytes 48 hours after EHR and application of the immunomodulator TTS. Multiple mitosis figures in the field of view (indicated by arrows). H&E staining. 200× (400× in the selected area)

CONCLUSION

The conducted studies on the regenerative activity of sodium aminodihydrophthalazinedione transdermal therapeutic system on an experimental EHR model of rats showed the prospects for percutaneous administration of this immunomodulator, as well as the need to further study its effect on liver repair processes at different periods of TTS application.

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The authors declare no conflict of interest.

REFERENCES

1. *Jushkov BG, Danilova IG, Hramcova JuS.* Vlijanie immunomoduljatorov na regeneraciju pecheni. *Jeksperimental'naja i klinicheskaja farmakologija*. 2006; 69 (1): 53–55. [In Russ]. doi: 10.30906/0869-2092-2006-69-1-53-55.
2. *Jushkov BG*. Kletki immunnoj sistemy i reguljacija regeneracii. *Bjulleten' sibirskoj mediciny*. 2017; 16 (4): 94–105. [In Russ]. doi: 10.20538/1682-0363-2017-4-94-105.
3. *Gonikova ZZ, Nikol'skaja AO, Kirsanova LA, Shagidulin MJu, Onishchenko NA, Sevast'janov VI*. Sravnitel'nyj analiz jeffektivnosti stimuljacii processov regeneracii pecheni kletkami kostnogo mozga i obshhej RNK jetih kletok. *Vestnik transplantologii i iskusstvennyh organov.* 2019; 21 (1): 113–121. [In Russ, English abstract]. doi: 10.15825/1995-1191-2019-1-113-121.
4. *Krasovskij VS, Sentjurova LG, Zurnadzhan SA*. Opyt primenenija "Lajfferona" pri travmah pecheni v jeksperimente. *Mezhdunarodnyj zhurnal prikladnyh i fundamental'nyh issledovanij*. 2015; 10 (2): 240–243. [In Russ, English abstract].
5. *Krotova OA, Granov DA, Rutkin IO.* Sindrom "nedostatochnogo razmera pecheni" posle rezekcii i transplantacii fragmenta pecheni. *Vestnik hirurgii im. I.I. Grekova.* 2012; 171 (3): 113–116. [In Russ].
6. *Andreev AA, Ostroushko AP, Laptijeva AJu, Gluhov AA.* Reparativnaja regeneracija pecheni posle segmentarnoj rezekcii (literaturnyj obzor). *Aspirantskij vestnik Povolzh'ja.* 2018; 18 (5–6): 183–190. [In Russ, English abstract]. doi: 10.17816/2072-2354.2018.18.3.183-190.
7. *Kuznecova EG, Kuryleva OM, Salomatina LA, Sevast'janov VI.* Jeksperimental'noe issledovanie diffuzii immunomoduljatora Galavit® v model'noj sisteme. *Razrabotka i registracija lekarstvennyh sredstv.* 2020; 9 (1): 92–97. [In Russ, English abstract]. doi: 10.33380/2305-2066-2020-9-1-92-97.
8. *Kuznecova EG, Kuryleva OM, Salomatina LA, Kursakov SV, Gonikova ZZ, Nikol'skaja AO et al.* Sravnitel'nyj analiz farmakokineticheskih parametrov transdermal'nogo i vnutrimyshechnogo vvedenij preparata Galavit®. *Vestnik transplantologii i iskusstvennyh organov.* 2021; 23 (2): 114–121. [In Russ, English abstract]. doi: 10.15825/1995-1191-2021-2-114-121.
9. *El'chaninov AV, Fathudinov TH, Makarov AV, Glinkina VV, Bol'shakova GB*. Regeneracija pecheni mlekopitajushhih. *Klinicheskaja i jeksperimental'naja morfologija.* 2012; 4: 57–61. [In Russ, English abstract].

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STRUCTURAL VALVE DEGENERATION: ARE THERE COMMON MECHANISMS WITH ATHEROSCLEROSIS AND CALCIFIC AORTIC STENOSIS?

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Current research shows that some of the pathogenetic processes behind structural destruction of bioprosthetic valves are largely similar to those involved in the development of atherosclerotic vascular lesions and native valve calcification. These processes include lipid and leukocyte infiltration, typical for both prosthetic and native tissues. They are accompanied by formation of foam cells, excessive production of matrix-degrading enzymes and increased oxidative stress. This fact suggests that some approaches to conservative treatment of atherosclerosis may be useful for prolonging the lifespan of bioprosthetic valves.

Keywords: bioprosthetic heart valves, structural valve degeneration, atherosclerosis, calcific aortic stenosis, conservative therapy, pathophysiology, risk factors.

INTRODUCTION

To date, the main way of correcting severe valvular heart disease is by replacing the affected valves [1–3]. According to various estimates, 250,000 to 400,000 such operations are performed annually in the world [4–7], about 10,000 in Russia. Moreover, there seems to be higher number of interventions on the heart valve apparatus across the globe. This is associated with increased accessibility of surgical care in developing countries, as well as the aging population of Western countries, accompanied by increasing prevalence of acquired valve defects [7]. By the year 2050, expectations are that annually 850,000 valves will be implanted [4]. Mechanical valves (MV) and bioprosthetic heart valves (BHV) are used as substitutes for native valves [9, 10]. Silence, optimal hemodynamic parameters and low thrombogenicity favorably distinguish BHVs from MVs [10]. At the same time, BHVs have a significant drawback – limited period of functioning, which is caused by occurrence of degenerative changes in the prosthetic biomaterial over time [9, 10]. In some sources, this phenomenon is called structural valve degeneration (SVD) [11, 12]. Due to SVD, 20% to 50% of conventional stented bioprostheses require replacement as early as 15 years after implantation [6]. Moreover, faster rates of SVD directly correlate with younger age [5, 6]. This peculiarity of BHVs predetermines the need for reprosthetic surgeries and is a significant limitation for the wide use of this type of medical devices, especially in young patients [1–3].

It is important to emphasize that the mechanisms responsible for SVD development are poorly understood and studied. As recently as 15–20 years ago, many researchers believed that only passive physical and chemical processes were behind the destruction and calcification of the biological component of BHVs [13–15], but these views are now regarded as simplistic [16]. Numerous original studies conducted over the past two decades show that the recipient's immune response may significantly contribute to the degeneration of the biological tissues of BHVs. Thus, researchers today increasingly consider SVD as an active cell-regulated process [9, 17], whose pathophysiology partially resembles that of atherosclerosis (ATS) and calcification of native aortic valves (AVs) [6, 18, 19].

Given the increase in the number of BHVs used in global surgical practice in recent decades [4, 20], there is an increasing need to find methods to reduce the rate of SVD, which is the main cause of BHV dysfunction. At the same time, the concept of SVD as an active cell-regulated process opens up new opportunities in the development of ways to modify the xenobiomaterial used for valve prosthetics, as well as medication support for operated patients in order to prevent early failure and increase the duration of BHVs functioning. Thus, our review concentrates on the analysis of current information on SVD pathophysiology and the similarity of the mechanisms behind it with those responsible for ATS and calcific aortic stenosis (CAS). Recent advances in the development of methods to reduce the immune response to BHV tissues are also reviewed.

SVD, ATS AND CAS: WHAT DO THEY HAVE INCOMMON?

It is known that ATS and CAS share many common risk factors, such as age, smoking, hypertension, metabolic syndrome, diabetes mellitus and hypercholesterol-

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emia [21, 22]. Some clinical studies have also indicated the association of the latter with SVD and deterioration of the hemodynamic parameters of BHVs due to degeneration of the prosthetic biomaterial [23–27]. Given that SVD, ATS and CAS share common risk factors, it can be assumed that these pathological conditions partially share similar mechanisms.

ATS and CAS are slowly progressive chronic inflammatory diseases characterized by lipid accumulation and activation of processes of maladaptive extracellular matrix remodeling in affected parts of vascular wall and AV leaflets respectively [28]. Intensive lipid and leukocytic infiltration accompanied by formation of foamy cells, which are lipid-laden macrophages, act as key histopathological events uniting the pathogenesis of ATS and CAS [28–33]. Through the release of proinflammatory cytokines, macrophages and foamy cells induce excessive activation of resident smooth muscle cells (SMCs) of vessels and valve interstitial cells (VICs) of valves, which becomes the main driving force behind the pathological changes observed in the development of the diseases under consideration [28–33].

In its turn, SVD is a process of gradual and irreversible destruction of the biological component of BHVs, apparently caused mainly by passive cell-independent mechanisms [14, 15]. At the microstructural level, SVD is mainly manifested by stratification, fragmentation and calcification of collagen and elastin fibrils of the extracellular matrix, and at the macrostructural level by perforations, tears and/or mineralization of the flaps, which eventually cause prosthesis dysfunction due to stenosis and/or transprosthetic regurgitation [11, 12]. However, like the affected areas of vessels and native valves, the tissues of implanted BHVs are subject to infiltration by immune cells, among which macrophages are the predominant type [34–41]. Also, some authors note the presence of lipid stains and foamy cells in explanted BHVs due to dysfunction [34, 41, 42], which is a key sign of atherogenic processes. It should be noted that cellular and lipid infiltrates in BHVs are usually co-localized with areas of damaged or calcified matrix.

Although the primary role of lipids and immune cells (particularly macrophages and foam cells) in the progression of ATS and CAS is generally understood [28–33], their contribution to BHV degeneration is largely unclear. Apparently, macrophages and other immune cells can contribute to additional destruction and calcification of the prosthetic biomaterial through several mechanisms. For instance, macrophages and foam cells are capable of producing numerous matrix-degrading enzymes, including almost the entire spectrum of matrix metalloproteinases (MMPs) and cathepsins B/K/L/S/V [43–46]. Increased expression of a number of MMPs and cathepsins has been noted in resected atherosclerotic plaques [47] and stenotic AVs [29], but BHVs have hardly been studied for the presence of proteolytic enzymes in their tissues. Nevertheless, BHV-infiltrating macrophages and foam cells have been shown to actively secrete MMP-9 [41] and plasminogen proenzyme [40]. It has been shown that non-calcified pericardial BHVs explanted due to leaflet ruptures show higher MMP-9 content compared to calcified prostheses and intact bovine (cattle) pericardium [48]. It is also known that activated macrophages and granulocytes create high concentrations of reactive oxygen species (ROS) in the surrounding space, sufficient to cause DNA damage and death of co-cultured monocytes [49]. As in the case of atherosclerotic plaques and calcified AV leaflets [28, 29], ROS provoke increased oxidative stress in degenerating BHV tissues and immune cells infiltrating them, presumably contributing to oxidative damage of the prosthetic biomaterial [50, 51] and its dystrophic calcification, partially caused by mineralization of apoptosed macrophages [40]. Finally, macrophages can produce calcium-binding proteins, in particular osteopontin and osteonectin [52, 53], as well as produce vesicles resembling matrix vesicles secreted by bone osteoblasts, which mediate bone biomineralization [54, 55]. It is important to note that a number of non-collagenous bone matrix proteins, including osteopontin, osteonectin and osteocalcin, were detected by immunohistochemistry in the tissues of explanted BHVs, and their expression levels correlated with the degree of cellular infiltration and calcification of the leaflets [56]. Again, this pattern largely resembles that of mineralization of native AVs [57, 58].

The study of BHV flaps by immunohistochemical staining showed that lipid deposits found in them consist mainly of oxidized low-density lipoproteins (oxLDL) [41, 42], which is also typical of ATS-affected vessels and calcified AV flaps [28–33]. The contribution of oxLDL to the development of SVD and to BHV dysfunction is still unknown. Potentially, lipid infiltration of BHV flaps can accelerate their degeneration by stimulating inflammatory activation of implant-infiltrating macrophages, formation of froth cells and their increased production of proteolytic enzymes. Experimental data support this hypothesis: the results of immunohistochemical staining of tissues of explanted BHVs show that macrophages penetrating them in the presence of oxLDL express high MMPs-9 levels, which is not observed in the samples without pronounced lipid infiltration [41]. These findings are consistent with the results of studies that have indicated an important role of oxLDL in stimulation of MMP secretion by immune cells [59–63]. It is also known that oxLDL enhance the production of various proinflammatory cytokines and chemokines by macrophages, such as interleukin-1β/-6/-8, tumor necrosis factor alpha, monocyte chemoattractant protein-1, macrophage inflammatory proteins, etc. [32, 62–64]. The release of cytokines and chemoattractant molecules may help recruit new immune cells in the inflammation site, although this process has not been studied in BHV tissues.

Notably, clinical studies reveal an association between BHV degeneration and lipid metabolism disor-

ders. For example, it was found that the risk of early SVD is higher in patients with increased low-density lipoproteins (LDL) and apolipoprotein B in relation to high-density lipoproteins and apolipoprotein A-I, respectively [65, 66]. In addition, elevated circulating levels of proprotein convertase subtilisin/kexin type 9 also correlate with the more rapid SVD and deterioration of the hemodynamic characteristics of BHVs [66, 67]. Finally, a macrophage-produced and/or LDL-borne enzyme, lipoprotein-associated phospholipase A2 (Lp-PLA2), seems to be associated with BHV degeneration [42]. It is known that Lp-PLA2 is involved in the development of both ATS and CAS by enhancing inflammation and calcification of native tissues through generation of such proinflammatory, proapoptotic and proosteogenic mediators as lysophosphatidylcholine and oxidized fatty acids [29, 68].

Probably, another mechanism can unite SVD and CAS pathogenesis. It has been established that the degree and rate of progression of native AV calcification correlate with the presence of intraleaflet haemorrhage (ILH) in the valve tissues [69], with the areas with ILH usually co-localized with calcium deposits [70, 71]. The relationship between ILH and AV mineralization is poorly studied [72]. It is assumed that iron accumulation in the matrix, which originates from the dead erythrocytes and induces differentiation of VICs into osteoblast-like cells through elevated oxidative stress, promotes calcification enhancement [72]. Recently, a group of researchers from China noted that there are also erythrocyte iron deposits co-localized with mineralized matrix areas in the flaps of explanted BHVs [73]. Probably, the iron accumulated in BHVs contributes to ROS generation through Fenton and Haber-Weiss redox reactions and subsequent oxidation-conditioned degeneration of the prosthetic biomaterial [50, 51]. In addition, fragments of erythrocytes diffusing into loosened tissues and then dying in them can serve as calcium phosphate nucleation nuclei.

Some commonalities of pathophysiological features characteristic of SVD with those of other inflammatory diseases of the cardiovascular system can also be seen in the results obtained by a group headed by Dr. Skowasch (Skowasch et al.) [74]. These studies showed increased expression of C-reactive protein (CRP) by BHV-infiltrating cells, and CRP levels in BHV-degenerating tissues correlated with those in the blood serum [74]. In addition to those described above, other mechanisms typical of ATS and CAS pathogenesis may also be involved in SVD. For example, activation of renin-angiotensin-aldosterone system [75–77] and autotoxin accumulation [78] are largely responsible for elevated oxidative stress and, as a consequence, inflammatory and fibroproliferative processes in the affected vessels and AVs. Presumably, these same factors may also play a role in the development of SVD, but their involvement in BHV degeneration has not yet been studied.

SVD, ATS AND CAS: FUNDAMENTAL DIFFERENCES

Despite the obvious similarity of a number of processes uniting the pathophysiology of SVD, ATS and CAS, they have notable differences. The most important of these is the absence of a fibroproliferative response to inflammatory infiltration on the part of BHV tissues, since they usually do not have living mesenchymal cells that could mediate it. A possible exception is only homovital allogeneic valve conduits, which have in their tissues significant populations of endothelial cells, SMCs and VICs of donor origin. Also living cells in allografts can be preserved after antibiotic treatment and cryopreservation, though their quantity in this case is usually small [79, 80]. Small clusters of cells with endothelial and fibroblast phenotype were also detected in xenogeneic BHVs [39, 81, 82], but there are no examples in current literature of when their number would be comparable with that in native tissues.

Hypothetically, fibrosis and ossification of BHV flaps controlled by myofibroblasts and osteoblast-like cells, respectively, can occur during SVD [6]. At least all necessary components for this are present in BHV tissues [83]. Nevertheless, it seems extremely unlikely that a small population of mesenchymal cells can contribute to fibrous and/or osteogenic remodeling of the prosthetic biotissue matrix in such a way that it would be visible against the background of passive degenerative-dystrophic processes. Modern research supports these views. For example, a group of scientists from Japan could not find cells with myofibroblast or SMC phenotypes in explanted BHVs, while fibrosis and mineralization of their flaps, apparently, were associated with deposition of fibrinogen from blood plasma and macrophage apoptosis [40]. Another research group attempted to study the expression of components of the cytokine system OPG/RANKL/RANK in explanted BHV tissues (the latter is known to be responsible for osteogenic differentiation of cells in native AVs), which showed that this system is not involved in SVD [84].

Another important difference SVD has from ATS and CAS lies in the triggers of the processes of lepid accumulation and leukocyte infiltration. For instance, endothelial dysfunction, accompanied by changes in the endothelial layer secretory profile and/or its partial loss, is the main cause of pathological changes in the affected vessels and AVs [28, 29]. Because of this, LDL start penetrating into the subendothelial space and deeper layers of the vascular wall or cusps. Oxidizing, they provoke intense aseptic inflammatory reactions with further recruitment of immune cells. With the exception of homovital allogeneic valve conduits, BHVs lack endothelial lining (although small reendothelized areas may occur on their surface [40]), thus, the considered mechanism cannot be involved in their case. Analysis of current literature sources shows that the main trigger of inflammatory

infiltration of xenogeneic BHVs is most likely residual xenoglycans, the end links of the polysaccharide chains of which are represented by sugars such as galactose-alpha-1,3-galactose and N-glycolylneuraminic acid [85]. Moreover, the main trigger of immune response to allogeneic valve substitutes seems to be residual molecules of human leukocyte antigen [86–88]. Lipid infiltration of LDL in this case is secondary to macrophage infiltration [41, 83].

Based on the above, we conclude that unlike in contrast to ATS and CAS, SVD is unlikely to be mediated by resident or alien mesenchymal cells. Thus, immune cells, primarily macrophages, are responsible for cell-mediated degradation of BHVs. The main trigger of inflammatory infiltration of both xenogeneic and allogeneic BHVs are foreign carbohydrate and protein molecules, which allows to consider the active processes behind SVD not as an ATS-like process, but rather as one of the variants of chronic implant rejection [9, 17], which has some features of atherosclerotic lesion. A comparative characteristic of SVD, ATS and CAS is shown in Table.

ATS THERAPY IN SVD INHIBITION

To date, there are no conservative therapies to slow down SVD. Nevertheless, the partial similarities between SVD and ATS suggest that anti-atherosclerotic drugs may be effective in inhibiting BHV degeneration. Some authors had previously believed that better clinical results could be achieved with lipid-lowering therapy in patients with appropriate indications [89]. Two small-scale retrospective studies demonstrated lower rates of increase in peak flow velocity, decrease in effective valve opening

Table

Comparative characteristics of some pathophysiological features between SVD, ATS and CAS

Sign	Structural valve degeneration	Atherosclerosis	Calcific aortic stenosis
Presence of inflammatory cellular infiltrates.	Present, but not in all cases.	Always present.	Always present.
Deposition of oxidized low-density lipoproteins and formation of foam cells.	Noted by several research groups, but apparently, rarely accompanies cellular infiltration in prosthetic biotissues.	A key sign of the disease.	Key sign of the disease.
Increased production of proteolytic enzymes, proteolysis activation.	A significant increase in MMP-9 expression was detected in some samples. However, interaction of proteolytic enzymes with stabilized matrix is poorly studied.	Increased expression of various MMPs, cathepsins and other matrix-degrading enzymes. Active matrix remodeling.	Increased expression of various MMPs, cathepsins and other matrix-degrading enzymes. Active matrix remodeling.
Release of inflammatory mediators, including various cytokines and chemokines.	Virtually unexplored. At least one study noted an increase in CRP expression in degenerating bioprostheses. There is indirect evidence pointing to the involvement of Lp-PLA2 in the destruction of prosthetic biotissues.	Increased production of a wide range of cytokines, chemoattractant and other proinflammatory agents.	Increased production of a wide range of cytokines, chemoattractant and other proinflammatory agents.
Increase in intracellular oxidative stress, intensification of extracellular oxidation.	Oxidation-dependent damage to the prosthetic biotissue has been noted in at least two studies.	One of the main mechanisms of pathogenesis.	One of the main mechanisms of pathogenesis.
Involvement of noncollagenous bone matrix proteins in biomineralization.	Increased expression of osteopontin, osteonectin, and osteocalcin was detected in calcified areas of the matrix.	Involved in those cases where calcification in atherosclerotic plaque is observed.	One of the main participants in aortic valve calcification processes.
Initiating causes of lipid and leukocyte infiltration.	Residual xenoglycans and other foreign molecules.	Endothelial layer dysfunction and damage.	Endothelial layer dysfunction and damage.
Active fibroproliferative response to inflammatory infiltration on the tissue side.	Probably impossible due to the complete absence or extremely small population of mesenchymal cells. Passive mechanisms (stratification of biotissue fibers, deposition of fibrinogen and other proteins from blood plasma) are responsible for leaflet fibrosis.	One of the main mechanisms of disease development; it is mediated by activated valvular interstitial cells.	One of the main mechanisms of disease development; it is mediated by activated valvular interstitial cells.
Heterotopic ossification	Apparently, it cannot be realized because no osteoblast-like cells are found in the prosthetic tissues.	Partial mineralization is due to the activity of smooth muscle cells with an osteogenic phenotype.	One of the main mechanisms of aortic valve calcification.

area and increase in regurgitation in patients with BHVs that were treated with statins compared to patients in the control group [90, 91]. In another study, statins reduced plasma CRP levels in patients with BHVs, indicating their anti-inflammatory effect [74]. However, the results of the latest and the largest observational study to date, which included data on 1193 patients, was unable to show whether lipid-lowering therapy could delay the SVD process for BHVs in the aortic position at 1, 5 and 10 years after implantation [92]. Therefore, the use of statins for prevention of early failure of BHVs became skeptical [92, 93].

To date, it is still not possible to draw a definitive conclusion about the effectiveness of statins in slowing down the SVD process due to the limited number of studies and the inconsistency of their results. There is a possibility that lipid-lowering therapy can be effective only for a subset of patients, for example, young people, whose immune system is more reactive, and the processes of degeneration of BHVs, presumably, are more associated with their cellular infiltration rather than fatigue breakdown of the prosthetic biomaterial. Thus, according to Dr. G. Nollert and his group (Nollert et al.) [27], cigarette smoking, high cholesterol and triglyceride levels were associated with accelerated BHV failure in patients aged 57 years or younger. No such association was observed in patients older than 57 years. However, it should be noted that the 2010 observational study included patients older than 63 years [92].

OTHER WAYS TO REDUCE INFLAMMATORY RESPONSE TO BIOPROSTHETIC VALVES

Since SVD somewhat resembles chronic immune rejection of living organ and tissue transplants, it is logical to assume that immunosuppressive therapy may be useful in delaying valve degeneration. This hypothesis is supported by experiments on laboratory animals. Specifically, experiments with inbred rats showed a direct correlation between the inflammation intensity and degree of calcification in glutaraldehyde-preserved guinea pig aortic valve, as well as reduced inflammatory response and degree of implant degeneration in patients who had been given steroid treatment [94]. A number of clinical observations also suggest that long-term use of corticosteroids reduces the rate of bioprosthetic valve calcification in young patients [95, 96]. However, immunosuppressive therapy can hardly be considered a viable option: due to significant side effects, this strategy is not applicable to most patients with bioprosthetic valves. In addition, the efficacy of immunosuppression in inhibiting bioprosthetic valve degeneration has not been validated by clinical trials.

An acceptable alternative to immunosuppressive therapy is decellularization or additional enzymatic treatment of prosthetic biomaterial aimed at eliminating xenoglycans, the most immunogenic components of animal biotissues [85]. Also, over time, it will probably become possible to obtain biomaterial from genetically modified animals whose tissues do not express the most immunoreactive carbohydrate xenoglycans [85]. Currently, porcine [97] and cattle [98] knockout for galactose-alpha-1,3-galactose and N-glycolylneuraminic acid have already been bred. The first experimental models of BHVs from the tissues of knockout pigs have also been made [99]. If future clinical trials prove the benefits of using BHVs created from the tissues of modified animals, they are likely to enter clinical practice [100].

CONCLUSION

According to current views, SVD is not simply a passive degenerative-dystrophic process and is partly realized through cell-dependent mechanisms. The triggers and nature of cellular infiltration of bioprosthetic valves allow us to attribute this reaction, occurring both on chemically stabilized xenogeneic biological tissues and on unfixed allogeneic biomaterial, to chronic immune rejection. It is noteworthy that some of the identified mechanisms resemble those involved in vascular ATS and native aortic valve calcification. They include lipid accumulation, foam cell formation, increased production of matrix-destroying enzymes, release of inflammation mediators and elevated oxidative stress. The clinical significance of these phenomena is still poorly understood.

Unfortunately, there are currently no drug therapies that can delay bioprosthetic valve deterioration. Suggestions that lipid-lowering therapy might be useful in this regard have not been confirmed, although there is a possibility that it might still play a role in patients younger than 57 years of age. Besides, there are opinions that special biomaterial processing aimed at eliminating immunogenicity and the manufacture of bioprosthetic valves from the tissues of genetically modified animals, will reduce the inflammatory response to the implants and increase their shelf-life in young patients. Given the global trend towards an increase in the number of heart valve replacement surgeries and an increase in the proportion of bioprosthetic valves used for this purpose, even a slight improvement in the latter, accompanied by an increase in their average lifespan by 3–5 years, will have a significant clinical impact.

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REFERENCES

1. *Baumgartner H, Falk V, Bax JJ, De Bonis M, Hamm C, Holm PJ et al*. 2017 ESC/EACTS Guidelines for the management of valvular heart disease. *Eur Heart J*. 2017; 38 (36): 2739–2791. doi: 10.1093/eurheartj/ehx391.
2. *Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP 3rd, Fleisher LA et al*. 2017 AHA/ACC focused update of the 2014 AHA/ACC guideline for the management of patients with valvular heart disease: a report of the American College of Cardiology American Heart Association task force on clinical practice guidelines. *Circulation*. 2017; 135 (25): e1159–e1195. doi: 10.1161/CIR.0000000000000503.
3. *Nishimura RA, Otto CM, Bonow RO, Carabello BA, Erwin JP 3rd, Guyton RA et al*. 2014 AHA/ACC guideline for the management of patients with valvular heart disease: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol*. 2014; 63 (22): 2438–2488. doi: 10.1016/j.jacc.2014.02.537.
4. *Bax JJ, Delgado V*. Bioprosthetic heart valves, thrombosis, anticoagulation, and imaging surveillance. *JACC Cardiovasc Interv*. 2017; 10 (4): 388–390. doi: 10.1016/j.jcin.2017.01.017.
5. *Fiedler AG, Tolis GJr*. Surgical treatment of valvular heart disease: overview of mechanical and tissue prostheses, advantages, disadvantages, and implications for clinical use. *Curr Treat Options Cardiovasc Med*. 2018; 20 (1): 7. doi: 10.1007/s11936-018-0601-7.
6. *Pibarot P, Dumesnil JG*. Prosthetic heart valves: selection of the optimal prosthesis and long-term management. *Circulation*. 2009; 119 (7): 1034–1048. doi: 10.1161/CIRCULATIONAHA.108.778886.
7. *Zilla P, Brink J, Human P, Bezuidenhout D*. Prosthetic heart valves: catering for the few. *Biomaterials*. 2008; 29 (4): 385–406. doi: 10.1016/j.biomaterials.2007.09.033.
8. *Bockeria LA, Milievskaya EB, Kuzdoeva ZF, Pryanishnikova VV*. Cardiovascular surgery – 2017. Diseases and congenital anomalies of the circulatory system. M.: NMITsSSKh im. Bakuleva MZ RF, 2018. 252.
9. *Manji RA, Lee W, Cooper DKC*. Xenograft bioprosthetic heart valves: past, present and future. *Int J Surg*. 2015; 23 (PtB): 280–284. doi: 10.1016/j.ijsu.2015.07.009.
10. *Tillquist MN, Maddox TM*. Cardiac crossroads: deciding between mechanical or bioprosthetic heart valve replacement. *Patient Prefer Adherence*. 2011; 5: 91–99. doi: 10.2147/PPA.S16420.
11. *Capodanno D, Petronio AS, Prendergast B, Eltchaninoff H, Vahanian A, Modine T et al*. Standardized definitions of structural deterioration and valve failure in assessing long-term durability of transcatheter and surgical aortic bioprosthetic valves: a consensus statement from the European Association of Percutaneous Cardiovascular Interventions (EAPCI) endorsed by the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J*. 2017; 38 (45): 3382–3390. doi: 10.1093/eurheartj/ehx303.
12. *Dvir D, Bourguignon T, Otto CM, Hahn RT, Rosenhek R, Webb JG et al*. Standardized definition of structural valve degeneration for surgical and transcatheter bioprosthetic aortic valves. *Circulation*. 2018; 137 (4): 388–399. doi: 10.1161/CIRCULATIONAHA.117.030729.
13. *Schoen FJ, Levy RJ*. Tissue heart valves: current challenges and future research perspectives. *J Biomed Mater Res*. 1999; 47 (4): 439–465. doi: 10.1002/(SICI)1097-4636(19991215)47:4<439::AID-JBM1>3.0.CO;2-O.
14. *Schoen FJ, Levy RJ*. Calcification of tissue heart valve substitutes: progress toward understanding and prevention. *Ann Thorac Surg*. 2005; 79 (3): 1072–1080. doi: 10.1016/j.athoracsur.2004.06.033.
15. *Simionescu DT*. Prevention of calcification in bioprosthetic heart valves: challenges and perspectives. *Expert Opin Biol Ther*. 2004; 4 (12): 1971–1985. doi: 10.1517/14712598.4.12.1971.
16. *Rodriguez-Gabella T, Voisine P, Puri R, Pibarot P, Rodés-Cabau J*. Aortic bioprosthetic valve durability: incidence, mechanisms, predictors, and management of surgical and transcatheter valve degeneration. *J Am Coll Cardiol*. 2017; 70 (8): 1013–1028. doi: 10.1016/j.jacc.2017.07.715.
17. *Manji RA, Ekser B, Menkis AH, Cooper DKC*. Bioprosthetic heart valves of the future. *Xenotransplantation*. 2014; 21 (1): 1–10. doi: 10.1111/xen.12080.
18. *Barbarash LS, Rogulina NV, Rutkovskaya NV, Ovcharenko EA*. Mechanisms underlying bioprosthetic heart valve dysfunctions. *Complex Issues of Cardiovascular Diseases*. 2018; 7 (2): 10–24. doi: 10.17802/2306-1278-2018-7-2-10-24.
19. *Cote N, Pibarot P, Clavel MA*. Incidence, risk factors, clinical impact, and management of bioprosthesis structural valve degeneration. *Curr Opin Cardiol*. 2017; 32 (2): 123–129. doi: 10.1097/HCO.0000000000000372.
20. *Head SJ, Çelik M, Kappetein AP*. Mechanical versus bioprosthetic aortic valve replacement. *Eur Heart J*. 2017; 38 (28): 2183–2191. doi: 10.1093/eurheartj/ehx141.
21. *Lindman BR, Clavel MA, Mathieu P, Iung B, Lancellotti P, Otto CM et al*. Calcific aortic stenosis. *Nat Rev Dis Primers*. 2016; 2: 16006. doi: 10.1038/nrdp.2016.6.
22. *Rajamannan NM*. Mechanisms of aortic valve calcification: the LDL-density-radius theory: a translation from cell signaling to physiology. *Am J Physiol Heart Circ Physiol*. 2010; 298 (1): H5–15. doi: 10.1152/ajpheart.00824.2009.
23. *Briand M, Pibarot P, Després JP, Voisine P, Dumesnil JG, Dagenais F et al*. Metabolic syndrome is associated with faster degeneration of bioprosthetic valves. *Circulation*. 2006; 114 (1 Suppl): I512–I517. doi: 10.1161/CIRCULATIONAHA.105.000422.
24. *Farivar RS, Cohn LH*. Hypercholesterolemia is a risk factor for bioprosthetic valve calcification and explantation. *J Thorac Cardiovasc Surg*. 2003; 126 (4): 969–975. doi: 10.1016/s0022-5223(03)00708-6.
25. *Lorusso R, Gelsomino S, Luca F, De Cicco G, Bille G, Carella R et al*. Type 2 diabetes mellitus is associated with faster degeneration of bioprosthetic valve: results from a propensity score-matched Italian multicenter study. *Circulation*. 2012; 125 (4): 604–614. doi: 10.1161/CIRCULATIONAHA.111.025064.
26. *Nitsche C, Kammerlander AA, Knechtelsdorfer K, Kraiger JA, Goliasch G, Dona C et al*. Determinants of bio-

prosthetic aortic valve degeneration. *JACC Cardiovasc Imaging*. 2020 Feb; 13 (2 Pt 1): 345–353. doi: 10.1016/j.jcmg.2019.01.027. [Epub 2019 Mar 13].

27. *Nollert G, Miksch J, Kreuzer E, Reichart B*. Risk factors for atherosclerosis and the degeneration of pericardial valves after aortic valve replacement. *J Thorac Cardiovasc Surg*. 2003; 126 (4): 965–968. doi: 10.1016/s0022-5223(02)73619-2.
28. *Gulyaev NI, Varavin NA, Korovin AE, Kuznetsov VV, Yakovlev VV, Gordienko AV*. Modern aspects of pathogenesis of calcification of the aortic valve. *Bulletin of Saint-Petersburg State University*. 2016; 3: 20–34. doi: 10.21638/11701/spbu11.2016.302.
29. *Kostyunin AE, Yuzhalin AE, Ovcharenko EA, Kutikhin AG*. Development of calcific aortic valve disease: do we know enough for new clinical trials? *J Mol Cell Cardiol*. 2019; 132: 189–209. doi: 10.1016/j.yjmcc.2019.05.016.
30. *Li H, Horke S, Förstermann U*. Vascular oxidative stress, nitric oxide and atherosclerosis. *Atherosclerosis*. 2014; 237 (1): 208–219. doi: 10.1016/j.atherosclerosis.2014.09.001.
31. *Parisi V, Leosco D, Ferro G, Bevilacqua A, Pagano G, de Lucia C et al*. The lipid theory in the pathogenesis of calcific aortic stenosis. *Nutr Metab Cardiovasc Dis*. 2015; 25 (6): 519–525. doi: 10.1016/j.numecd.2015.02.001.
32. *Schaftenaar F, Frodermann V, Kuiper J, Lutgens E*. Atherosclerosis: the interplay between lipids and immune cells. *Curr Opin Lipidol*. 2016; 27 (3): 209–215. doi: 10.1097/MOL.0000000000000302.
33. *Weber C, Noels H*. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med*. 2011; 17 (11): 1410–1422. doi: 10.1038/nm.2538.
34. *Bottio T, Thiene G, Pettenazzo E, Ius P, Bortolotti U, Rizzoli G et al*. Hancock II bioprosthesis: a glance at the microscope in mid-long-term explants. *J Thorac Cardiovasc Surg*. 2003; 126 (1): 99–105. doi: 10.1016/s0022-5223(03)00131-4.
35. *Butany J, Zhou T, Leong SW, Cunningham KS, Thangaroopan M, Jegatheeswaran A et al*. Inflammation and infection in nine surgically explanted Medtronic Freestyle stentless aortic valves. *Cardiovasc Pathol*. 2007; 16 (5): 258–267. doi: 10.1016/j.carpath.2007.01.009.
36. *Grabenwöger M, Fitzal F, Gross C, Hutschala D, Böck P, Brucke P et al*. Different modes of degeneration in autologous and heterologous heart valve prostheses. *J Heart Valve Dis*. 2000; 9 (1): 104–111. PMID: 10678382.
37. *Lepidi H, Casalta JP, Fournier PE, Habib G, Collart F, Raoult D*. Quantitative histological examination of bioprosthetic heart valves. *Clin Infect Dis*. 2006; 42 (5): 590–596. doi: 10.1086/500135.
38. *Manji RA, Hara H, Cooper DK*. Characterization of the cellular infiltrate in bioprosthetic heart valves explanted from patients with structural valve deterioration. *Xenotransplantation*. 2015; 22 (5): 406–407. doi: 10.1111/xen.12187.
39. *Nair V, Law KB, Li AY, Phillips KR, David TE, Butany J*. Characterizing the inflammatory reaction in explanted Medtronic Freestyle stentless porcine aortic bioprosthesis over a 6-year period. *Cardiovasc Pathol*. 2012; 21 (3): 158–168. doi: 10.1016/j.carpath.2011.05.003.
40. *Sakaue T, Nakaoka H, Shikata F, Aono J, Kurata M, Uetani T et al*. Biochemical and histological evidence of deteriorated bioprosthetic valve leaflets: the accumulation of fibrinogen and plasminogen. *Biol Open*. 2018; 7 (8): bio034009. doi: 10.1242/bio.034009.
41. *Shetty R, Pibarot P, Audet A, Janvier R, Dagenais F, Perron J et al*. Lipid-mediated inflammation and degeneration of bioprosthetic heart valves. *Eur J Clin Invest*. 2009; 39 (6): 471–480. doi: 10.1111/j.1365-2362.2009.02132.x.
42. *Mahmut A, Mahjoub H, Boulanger MC, Fournier D, Després JP, Pibarot P, Mathieu P*. Lp-PLA2 is associated with structural valve degeneration of bioprostheses. *Eur J Clin Invest*. 2014; 44 (2): 136–145. doi: 10.1111/eci.12199.
43. *Abd-Elrahman I, Meir K, Kosuge H, Ben-Nun Y, Weiss Sadan T, Rubinstein C et al*. Characterizing cathepsin activity and macrophage subtypes in excised human carotid plaques. *Stroke*. 2016; 47 (4): 1101–1108. doi: 10.1161/STROKEAHA.115.011573.
44. *Bühling F, Reisenauer A, Gerber A, Krüger S, Weber E, Brömme D et al*. Cathepsin K – a marker of macrophage differentiation? *J Pathol*. 2001; 195 (3): 375–382. doi: 10.1002/path.959.
45. *Kessenbrock K, Brown M, Werb Z*. Measuring matrix metalloproteinase activity in macrophages and polymorphonuclear leukocytes. *Curr Protoc Immunol*. 2011; Chapter 14: Unit 14.24. doi: 10.1002/0471142735.im1424s93.
46. *Yasuda Y, Li Z, Greenbaum D, Bogyo M, Weber E, Brömme D*. Cathepsin V, a novel and potent elastolytic activity expressed in activated macrophages. *J Biol Chem*. 2004; 279 (35): 36761–36770. doi: 10.1074/jbc.M403986200.
47. *Johnson JL*. Metalloproteinases in atherosclerosis. *Eur J Pharmacol*. 2017; 816: 93–106. doi: 10.1016/j.ejphar.2017.09.007.
48. *Simionescu A, Simionescu DT, Deac RF*. Matrix metalloproteinases in the pathology of natural and bioprosthetic cardiac valves. *Cardiovasc Pathol*. 1996; 5 (6): 323–332. PMID: 25851789.
49. *Ponath V, Kaina B*. Death of monocytes through oxidative burst of macrophages and neutrophils: killing in trans. *PLoS One*. 2017; 12 (1): e0170347. doi: 10.1371/journal.pone.0170347.
50. *Christian AJ, Lin H, Alferiev IS, Connolly JM, Ferrari G, Hazen SL et al*. The susceptibility of bioprosthetic heart valve leaflets to oxidation. *Biomaterials*. 2014; 35 (7): 2097–2102. doi: 10.1016/j.biomaterials.2013.11.045.
51. *Lee S, Levy RJ, Christian AJ, Hazen SL, Frick NE, Lai EK et al*. Calcification and oxidative modifications are associated with progressive bioprosthetic heart valve dysfunction. *J Am Heart Assoc*. 2017; 6 (5): e005648. doi: 10.1161/JAHA.117.005648.
52. *Rittling SR*. Osteopontin in macrophage function. *Expert Rev Mol Med*. 2011; 13: e15. doi: 10.1017/S1462399411001839.

53. *Rosset EM, Bradshaw AD*. SPARC/osteonectin in mineralized tissue. *Matrix Biol*. 2016; 52–54: 78–87. doi: 10.1016/j.matbio.2016.02.001.
54. *New SE, Aikawa E*. Role of extracellular vesicles in de novo mineralization: an additional novel mechanism of cardiovascular calcification. *Arterioscler Thromb Vasc Biol*. 2013; 33 (8): 1753–1758. doi: 10.1161/ATVBAHA.112.300128.
55. *New SE, Goettsch C, Aikawa M, Marchini JF, Shibasaki M, Yabusaki K et al*. Macrophage-derived matrix vesicles: an alternative novel mechanism for microcalcification in atherosclerotic plaques. *Circ Res*. 2013; 113 (1): 72–77. doi: 10.1161/CIRCRESAHA.113.301036.
56. *Srivatsa SS, Harrity PJ, Maercklein PB, Kleppe L, Veinot J, Edwards WD et al*. Increased cellular expression of matrix proteins that regulate mineralization is associated with calcification of native human and porcine xenograft bioprosthetic heart valves. *J Clin Invest*. 1997; 99 (5): 996–1009. doi: 10.1172/JCI119265.
57. *Mohler ER 3rd, Adam LP, McClelland P, Graham L, Hathaway DR*. Detection of osteopontin in calcified human aortic valves. *Arterioscler Thromb Vasc Biol*. 1997; 17 (3): 547–552. doi: 10.1161/01.atv.17.3.547.
58. *Pohjolainen V, Taskinen P, Soini Y, Rysä J, Ilves M, Juvonen T et al*. Noncollagenous bone matrix proteins as a part of calcific aortic valve disease regulation. *Hum Pathol*. 2008; 39 (11): 1695–1701. doi: 10.1016/j.humpath.2008.04.015.
59. *Ardans JA, Economou AP, Martinson JM Jr, Zhou M, Wahl LM*. Oxidized low-density and high-density lipoproteins regulate the production of matrix metalloproteinase-1 and -9 by activated monocytes. *J Leukoc Biol*. 2002; 71 (6): 1012–1018. PMID: 12050187.
60. *Huang Z, Meng S, Wang L, Wang Y, Chen T, Wang C*. Suppression of oxLDL-induced MMP-9 and EMMPRIN expression by berberine via inhibition of NF-κB activation in human THP-1 macrophages. *Anat Rec (Hoboken)*. 2012; 295 (1): 78–86. doi: 10.1002/ar.21489.
61. *Sanda GM, Deleanu M, Toma L, Stancu CS, Simionescu M, Sima AV*. Oxidized LDL-exposed human macrophages display increased MMP-9 expression and secretion mediated by endoplasmic reticulum stress. *J Cell Biochem*. 2017; 118 (4): 661–669. doi: 10.1002/jcb.25637.
62. *Yang K, Liu X, Liu Y, Wang X, Cao L, Zhang X et al*. DC-SIGN and Toll-like receptor 4 mediate oxidized low-density lipoprotein-induced inflammatory responses in macrophages. *Sci Rep*. 2017; 7 (1): 3296. doi: 10.1038/s41598-017-03740-7.
63. *Ye J, Wang C, Wang D, Yuan H*. LncRBA GSA5, up-regulated by ox-LDL, aggravates inflammatory response and MMP expression in THP-1 macrophages by acting like a sponge for miR-221. *Exp Cell Res*. 2018; 369 (2): 348–355. doi: 10.1016/j.yexcr.2018.05.039.
64. *Bae YS, Lee JH, Choi SH, Kim S, Almazan F, Witztum JL et al*. Macrophages generate reactive oxygen species in response to minimally oxidized low-density lipoprotein: toll-like receptor 4- and spleen tyrosine kinase-dependent activation of NADPH oxidase 2. *Circ Res*. 2009; 104 (2): 210–218. doi: 10.1161/CIRCRESAHA.108.181040.
65. *Nsaibia MJ, Mahmut A, Mahjoub H, Dahou A, Boucharеb R, Boulanger MC et al*. Association between plasma lipoprotein levels and bioprosthetic valve structural degeneration. *Heart*. 2016; 102 (23): 1915–1921. doi: 10.1136/heartjnl-2016-309541.
66. *Mahjoub H, Mathieu P, Sénéchal M, Larose E, Dumesnil J, Després JP et al*. ApoB/ApoA-I ratio is associated with increased risk of bioprosthetic valve degeneration. *J Am Coll Cardiol*. 2013; 61 (7): 752–761. doi: 10.1016/j.jacc.2012.11.033.
67. *Salaun E, Mahjoub H, Dahou A, Mathieu P, Larose É, Després JP et al*. Hemodynamic deterioration of surgically implanted bioprosthetic aortic valves. *J Am Coll Cardiol*. 2018; 72 (3): 241–251. doi: 10.1016/j.jacc.2018.04.064.
68. *Wilensky RL, Macphee CH*. Lipoprotein-associated phospholipase A(2) and atherosclerosis. *Curr Opin Lipidol*. 2009; 20 (5): 415–420. doi: 10.1097/MOL.0b013e3283307c16.
69. *Akahori H, Tsujino T, Naito Y, Matsumoto M, Lee-Kawabata M, Ohyanagi M et al*. Intraleaflet haemorrhage is associated with rapid progression of degenerative aortic valve stenosis. *Eur Heart J*. 2011; 32 (7): 888–896. doi: 10.1093/eurheartj/ehq479.
70. *Morvan M, Arangalage D, Franck G, Perez F, Cattan-Levy L, Codogno I et al*. Relationship of iron deposition to calcium deposition in human aortic valve leaflets. *J Am Coll Cardiol*. 2019; 73 (9): 1043–1054. doi: 10.1016/j.jacc.2018.12.042.
71. *Stam OCG, Daemen MJAP, van Rijswijk JW, de Mol BAJM, van der Wal AC*. Intraleaflet hemorrhages are a common finding in symptomatic aortic and mitral valves. *Cardiovasc Pathol*. 2017; 30: 12–18. doi: 10.1016/j.carpath.2017.06.002.
72. *Deutsch MA, Gummert JF*. Intraleaflet hemorrhage and iron-dependent pathomechanisms in calcific aortic valve disease: epiphenomenon or major actor? *J Am Coll Cardiol*. 2019; 73 (9): 1055–1058. doi: 10.1016/j.jacc.2018.12.041.
73. *Lee S, Ferrari G, Levy RJ*. Abstract 14677: oxidative damage in failed clinical bioprosthetic heart valve explants. *Circulation*. 2015; 132 (3): A14677.
74. *Skowasch D, Schrempf S, Preusse CJ, Likungu JA, Welz A, Lüderitz B et al*. Tissue resident C reactive protein in degenerative aortic valves: correlation with serum C reactive protein concentrations and modification by statins. *Heart*. 2006; 92 (4): 495–498. doi: 10.1136/hrt.2005.069815.
75. *Kattoor AJ, Pothineni NVK, Palagiri D, Mehta JL*. Oxidative stress in atherosclerosis. *Curr Atheroscler Rep*. 2017; 19 (11): 42. doi: 10.1007/s11883-017-0678-6.
76. *Kostyunin AE, Ovcharenko EA, Barbarash OL*. The renin-angiotensin-aldosterone system at a potential target for therapy in patients with calcific aortic stenosis: a literature review. *Kardiologiia*. 2019; 59 (11S): 4–17. doi: 10.18087/cardio.n328.
77. *Sata M, Fukuda D*. Crucial role of renin-angiotensin system in the pathogenesis of atherosclerosis. *The Journal of Medical Investigation*. 2010; 57 (1–2): 12–25. doi: 10.2152/jmi.57.12.

78. *Zhao Y, Hasse S, Zhao C, Bourgoin SG*. Targeting the autotaxin – lysophosphatidic acid receptor axis in cardiovascular diseases. *Biochem Pharmacol*. 2019; 164: 74–81. doi: 10.1016/j.bcp.2019.03.035.
79. *Armiger LC*. Viability studies of human valves prepared for use as allografts. *Ann Thorac Surg*. 1995; 60 (2 Suppl): S118–S121. doi: 10.1016/0003-4975(95)00217-9.
80. *Oei FB, Stegmann AP, van der Ham F, Zondervan PE, Vaessen LM, Baan CC et al*. The presence of immune stimulatory cells in fresh and cryopreserved donor aortic and pulmonary valve allografts. *J Heart Valve Dis*. 2002; 11 (3): 315–325. PMID: 12056721.
81. *Mukhamadiyarov RA, Rutkovskaya NV, Kokorin SG, Odarenko YuN, Mil'to IV, Barbarash LS*. Cell typing of biological heart valves prosthesis explanated due to the development of calcium-associated dysfunctions. *Bulletin of Siberian Medicine*. 2018; 17 (2): 94–102. doi: 10.20538/1682-0363-2018-4-94-102.
82. *Mukhamadiyarov RA, Rutkovskaya NV, Sidorova OD, Barbarash LS*. Cellular composition of calcified bioprosthetic heart valves. *Annals of the Russian Academy of Medical Sciences*. 2015; 70 (6): 662–668. doi: 10.15690/vramn560.
83. *Kostyunin AE, Ovcharenko EA, Klyshnikov KY*. Modern understanding of mechanisms of bioprosthetic valve structural degeneration: a literature review. *Russian Journal of Cardiology*. 2018; 11: 145–152. doi: 10.15829/1560-4071-2018-11-145-152.
84. *Steinmetz M, Skowasch D, Wernert N, Welsch U, Preusse CJ, Welz A et al*. Differential profile of the OPG/RANKL/RANK-system in degenerative aortic native and bioprosthetic valves. *J Heart Valve Dis*. 2008; 17 (2): 187–193. PMID: 18512489.
85. *Kostyunin AE, Rezvova MA*. The role of residual xenoanthigens in the degeneration of xenogenic bioprosthetic heart valves. *Immunologiya*. 2019; 40 (4): 56–63. doi: 10.24411/0206-4952-2019-14006.
86. *Bibevski S, Ruzmetov M, Fortuna RS, Turrentine MW, Brown JW, Ohye RG*. Performance of SynerGraft decellularized pulmonary allografts compared with standard cryopreserved allografts: results from multiinstitutional data. *Ann Thorac Surg*. 2017; 103 (3): 869–874. doi: 10.1016/j.athoracsur.2016.07.068.
87. *Hoekstra F, Knoop C, Vaessen L, Wassenaar C, Jutte N, Bos E et al*. Donor-specific cellular immune response against human cardiac valve allografts. *J Thorac Cardiovasc Surg*. 1996; 112 (2): 281–286. doi: 10.1016/S0022-5223(96)70250-7.
88. *Hogan P, Duplock L, Green M, Smith S, Gall KL, Frazer IH et al*. Human aortic valve allografts elicit a donor-specific immune response. *J Thorac Cardiovasc Surg*. 1996; 112 (5): 1260–1267. doi: 10.1016/S0022-5223(96)70139-3.
89. *Colli A, Gherli T, Mestres CA, Pomar JL*. Degeneration of native and tissue prosthetic valve in aortic position: do statins play an effective role in prevention? *Int J Cardiol*. 2007; 116 (2): 144–152. doi: 10.1016/j.ijcard.2006.03.047.
90. *Antonini-Canterin F, Popescu BA, Zuppiroli A, Nicolosi GL*. Are statins effective in preventing bioprosthetic aortic valve failure? A need for a prospective, randomized trial. *Ital Heart J*. 2004; 5 (2): 85–88. PMID: 15086137.
91. *Antonini-Canterin F, Zuppiroli A, Popescu BA, Granata G, Cervesato E, Piazza R et al*. Effect of statins on the progression of bioprosthetic aortic valve degeneration. *Am J Cardiol*. 2003; 92 (12): 1479–1482. doi: 10.1016/j.amjcard.2003.08.066.
92. *Kulik A, Masters RG, Bédard P, Hendry PJ, Lam BK, Rubens FD et al*. Postoperative lipid-lowering therapy and bioprosthesis structural valve deterioration: justification for a randomised trial? *Eur J Cardiothorac Surg*. 2010; 37 (1): 139–144. doi: 10.1016/j.ejcts.2009.06.051.
93. *Gilmanov D, Bevilacqua S, Mazzone A, Glauber M*. Do statins slow the process of calcification of aortic tissue valves? *Interact Cardiovasc Thorac Surg*. 2010; 11 (3): 297–301. doi: 10.1510/icvts.2009.230920.
94. *Manji RA, Zhu LF, Nijjar NK, Rayner DC, Korbutt GS, Churchill TA et al*. Glutaraldehyde-fixed bioprosthetic heart valve conduits calcify and fail from xenograft rejection. *Circulation*. 2006; 114 (4): 318–327. doi: 10.1161/CIRCULATIONAHA.105.549311.
95. *Eishi K, Ishibashi-Ueda H, Nakano K, Kosakai Y, Sasako Y, Kobayashi J et al*. Calcific degeneration of bioprosthetic aortic valves in patients receiving steroid therapy. *J Heart Valve Dis*. 1996; 5 (6): 668–672. PMID: 8953446.
96. *Shimazaki Y, Kuraoka S, Takeda F, Watanabe T, Inui K*. Mitral valve re-replacement for impaired bioprosthesis after 19 years in a patient undergoing steroid treatment. *J Heart Valve Dis*. 2003; 12 (1): 45–47. PMID: 12578334.
97. *Zhang R, Wang Y, Chen L, Wang R, Li C, Li X et al*. Reducing immunoreactivity of porcine bioprosthetic heart valves by genetically-deleting three major glycan antigens, GGTA1/β4GalNT2/CMAH. *Acta Biomater*. 2018; 72: 196–205. doi: 10.1016/j.actbio.2018.03.055.
98. *Perota A, Lagutina I, Duchi R, Zanfrini E, Lazzari G, Judor JP et al*. Generation of cattle knockout for galactose-α1,3-galactose and N-glycolylneuraminic acid antigens. *Xenotransplantation*. 2019; 26 (5): e12524. doi: 10.1111/xen.12524.
99. *Rahmani B, McGregor C, Byrne G, Burriesci G*. A durable porcine pericardial surgical bioprosthetic heart valve: a proof of concept. *J Cardiovasc Transl Res*. 2019; 12 (4): 331–337. doi: 10.1007/s12265-019-09868-3.
100. *Smood B, Hara H, Cleveland DC, Cooper DKC*. In search of the ideal valve: optimizing genetic modifications to prevent bioprosthetic degeneration. *Ann Thorac Surg*. 2019; 108 (2): 624–635. doi: 10.1016/j.athoracsur.2019.01.054.

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PROTEIN COMPOSITION AND FUNCTIONAL PARAMETERS OF RBC MEMBRANES IN LIVER AND KIDNEY TRANSPLANTATION

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Organ transplantation is an effective treatment for many end-stage diseases. However, reperfusion injury constitutes a major complication of transplantation, which is associated with microcirculatory disorders and aggregation of blood corpuscles. Red blood cells (RBC) play an essential role in maintaining hemodynamic and rheological properties of the blood. Moreover, the study of mechanisms of changes in RBC functional indices is an urgent task. The main indicator of RBC functioning is the stability of RBC membrane structure. The issue of RBC membrane modification in organ transplantation has not been studied so far. **Objective:** to study the protein composition of RBC membranes, their aggregation and electrokinetic parameters in liver and kidney recipients, as well as in related kidney and liver fragment donors before and after operation. **Research materials.** Blood of 12 kidney recipients and 5 related kidney donors, 8 liver recipients and 4 related liver fragment donors – 1–2 hours before surgery, 1 week, 1, 2, 7, 10, 12 months after surgery. The control group consisted of 8 healthy volunteers. **Research methods.** Protein separation was done by Laemmli electrophoresis. RBC electrophoretic mobility, which characterizes the electrokinetic properties of cells, was measured by microelectrophoresis. Aggregation was calculated microscopically by counting unaggregated RBCs. Obtained values were compared by Mann-Whitney U test. **Results.** Examination of the RBC membrane of kidney recipients revealed a significant decrease in the amount of Band 3 protein and glycophorin before and after transplantation. Band 3 protein levels reduced at 1 month, glycophorin reduced at 7 months after surgery, with a maximum decrease in these protein fractions by more than 50% by 7 days compared with control values. There was also a decrease in spectrin content for 2 months after surgery with a maximum decrease of 30% by 1 month. In liver recipients, analysis of RBC membrane proteins revealed a decrease in the amount of glycophorin before surgery and further decrease at 2 months of post-transplant period. The maximum decrease in this index was 72% by 7 days after surgery. In addition, there was a fall in spectrin and Band 3 protein levels at 1 month by more than 60% relative to the control values. In donors, there were changes in the protein fraction of RBC membranes in the long-term post-operative period: spectrin and Band 3 protein levels reduced by 2 times at month 2 in kidney donors, while glycophorin levels reduced by 2.3 times at month 1 after operation in liver donors. Similarly, both groups of donors had increased actin levels at month 1 after surgery. The revealed changes in protein levels in the protein phase of RBC membranes were combined with functional indices of RBCs. In kidney recipients, decreased RBC electrophoretic mobility and increased aggregation were detected at 2 months. In liver recipients, the changes in these indicators were at 1 month. A decrease in RBC electrophoretic mobility was detected in donors of both groups. **Conclusion.** Changes in RBC membrane electronegativity are associated with changes in glycophorin and Band 3 protein levels, whereas in RBC aggregation process in liver/kidney recipients, the structural and functional disorders in the interrelationships of such membrane proteins as spectrin, Band 3 protein, and glycophorin, are significant factors. Alteration of actin determines inhibition of RBC aggregation growth in donors.

Keywords: kidney transplantation, liver transplantation, red blood cells.

INTRODUCTION

Organ transplantation has become a very effective method of treatment for a number of severe end-stage diseases [1]. However, transplantation is a technically complex surgical intervention that can be accompanied by massive blood loss, which causes complications in the early postoperative period [2]. Besides, with this type of surgical intervention, changes in homeostasis are associated with an imbalance in the coagulation system [3, 4]. The pathogenesis of coagulopathy is mediated through endothelial damage and activation of hypercoagulation cascade, which leads to microcirculatory disorders in the early post-transplant period [3].

Erythrocytes, in turn, have a significant impact on the rheological properties of blood and microcircula-

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tion [16]. Disturbance of the membrane structure leads to changes in their stiffness, decreases deformation, increases erythrocyte aggregation and initiates thrombosis [5, 6]. Increased cell aggregation and increased release of such procoagulants as erythrocytin and adenosine triphosphate stimulate blood clotting [7, 8]. Erythrocyte aggregation can cause tissue hypoxia, as the aggregates fill the lumen of capillaries and do not leave space for the parietal layer of plasma, causing blood stasis [9]. Thus, red blood cells (RBCs) play an essential role in maintaining the hemodynamic and rheological properties of the blood; a study of the mechanisms of changes in their functional indices is an important task. The stability of the membrane structure is taken as a major indicator of RBC functioning [10]. However, the issue of erythrocyte membrane modification in organ transplantation has not been studied so far. From this point of view, not only recipients, but also related donors, in whom the risk of hemocirculatory disorders increases manifold when a kidney or a liver fragment is removed, have not been studied postoperatively.

The **objective** in this work was to study protein composition of erythrocyte membranes, their aggregation and electrokinetic indices in liver and kidney recipients, as well as in related kidney and liver fragment donors before and after operation.

MATERIALS AND METHODS

The blood of kidney or liver transplant recipients and that of related donors in the postoperative period was studied. Kidney and liver explantation and transplantation were performed at Volga District Medical Center in Nizhny Novgorod, Russia, where such medical interventions have been performed since 2006 [11]. All patients gave voluntary informed consent via a form approved by Order No. 517n of the Russian Ministry of Health, dated August 11, 2017. The study was approved by the local ethics committee of Volga District Medical Center. Enrolled were 12 deceased-donor kidney transplant recipients, 5 living related kidney donors, 8 deceased-donor liver transplant recipients and 4 living related fragment donors, aged from 40 to 58 years. From deceased kidney donors, the mean preservation time was 510 ± 219.33 minutes, from related kidney donors, 22 ± 2.73 minutes, from deceased liver donors, 330 ± 32.07 minutes, and from related liver donors, 26.5 ± 1.73 minutes. The control group consisted of 8 healthy volunteers. All study participants were observed at the outpatient transplant center of Volga District Medical Center according to the approved standards [12]. Blood for analysis was taken from the ulnar vein of the patients 1–2 hours before surgery, 1 week, 1, 2, and 7 months after surgery, to study protein fractions of erythrocyte membranes, and additionally 10 and 12 months after surgery to study the degree of aggregation and RBC electrophoretic mobility, which characterizes their electrokinetic properties.

Protein separation was performed via Laemmli's sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [13] using mini gel electrophoresis system Mini-PROTEAN Tetra cell (Bio-Rad, U.S.A.). The gels were prepared using a 30% acrylamide/methylene bis-acrylamide solution. The buffer used to prepare the stacking gel contained 0.5 M Tris-OH, 0.4% DSN, pH 6.8. A buffer containing 1.5 M Tris-ON, 0.4% DSN, pH 8.8 was used to prepare the separating gel. Electrophoresis chambers were filled with a buffer containing 0.025 M Tris-OH, 0.192 M glycine, 0.1% DSN, pH 8.3. Polymerization was performed in the presence of tetramethylethylenediamine (TEMED) and 10% ammonium persulfate (APS) at room temperature. The applied samples were 20 µl in volume. Before application, samples were diluted with sample buffer (0.0625 M Tris-HCl, 10% glycerol, 0.001% bromophenol blue, 5% mercaptoethanol, 2.3% DSN, pH 6.8) and heated in a water bath (100°C) for 10 minutes. While the samples were passing through the stacking gel, electrophoresis was performed at a constant current strength of 20 mA. In the separating gel, the current strength was 40 mA. At the end of electrophoresis, the gel was stained for 30–60 minutes in a solution containing Coomassie Blue R250, 40% methanol, and 10% acetic acid. Unbound dye was removed by washing the gel in a solvent (40% methanol, 10% acetic acid). The resulting gel tracks were processed using the ImageJ program. Standard protein samples (Bio-Rad, U.S.A.) were used as markers.

By electrophoresis, about 15 major membrane proteins with a molecular weight ranging from 15 to 250 kD were detected in the erythrocyte membrane. Spectrin, glycophorin, and band 3 protein account for about 60% of the membrane proteins. Given that the main cytoskeleton proteins are spectrin (band 1 and 2), ankyrin (band 2.1), band 4.1 and 4.9 proteins, and actin (band 5), while in functional and quantitative relation among integral proteins, band 3 protein or anion channel and glycophorins prevail [14, 15]. In our work, we analyzed exactly these fractions of erythrocyte membrane proteins.

Electrokinetic and aggregation properties were determined by measuring RBC electrophoretic mobility and optical measurement of erythrocyte aggregation. RBC electrophoretic mobility was determined by microelectrophoresis using a cytospherometer in our modification [16]. We recorded the time of erythrocytes passing the distance of 100 µm in Tris-HCl buffer with pH 7.4 at 8 mA current strength. Erythrocyte aggregation was studied by optical microscopy by counting single RBCs and their aggregates in blue dextran T-2000 solution (GE Healthcare Company, 20 mg/mL) in a Tris HCl buffer [17].

Data obtained was statistically processed using Statistica 12, R. The distribution was checked for compliance

with the normal law using the Kolmogorov–Smirnov goodness of fit test. When analyzing differences in individual groups, statistical significance was calculated using a multiple t-test by the Sidak–Bonferroni method. Differences between recipient and donor groups were analyzed using nonparametric Mann–Whitney U test. We used the following confidence intervals to indicate statistical significance: $p < 0.05$, data differ (*); $p < 0.01$, data differ (#).

RESULTS

The study of erythrocyte membrane proteins showed a significant quantitative change in the protein fractions in RBC membrane from both kidney/liver recipients and related donors.

Analysis of erythrocyte membrane proteins in kidney recipients revealed a decrease in the main integral proteins of the erythrocyte membrane – band 3 protein and glycophorin – by 24% and 25% before surgery relative to control values (Fig. 1). In the postoperative period, there was a further 60% decrease in band 3 protein levels at day 7 after surgery relative to control values, followed by a gradual recovery to control values. By day 7, spectrin and glycophorin levels also decreased by 34% and 58%, respectively, relative to control values, after which the reduced spectrin level remained stable for two months postoperatively, and glycophorin – for the entire follow-up period.

Related kidney donors showed a 50% decrease in spectrin levels, a 65% decrease in band 3 protein by month 2 of the postoperative period, and a 78% increase in actin by month 1 relative to control (Fig. 2). At month 7 after surgery, protein composition of membranes was restored to control values.

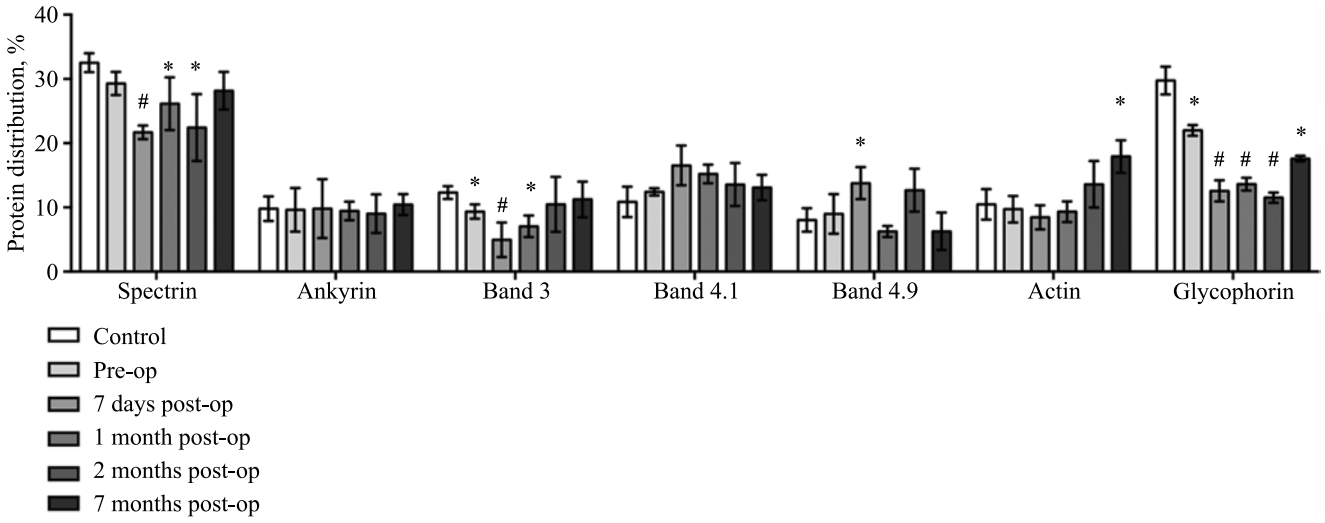

Fig. 1. Dynamics of protein composition of RBC membranes in kidney transplant recipients. Here and below in Fig.: Pre-op, before surgery; post-op, after surgery; * – statistically significant differences versus control ($p < 0.05$); # – statistically significant differences versus control ($p < 0.01$). The error bars represent standard deviations

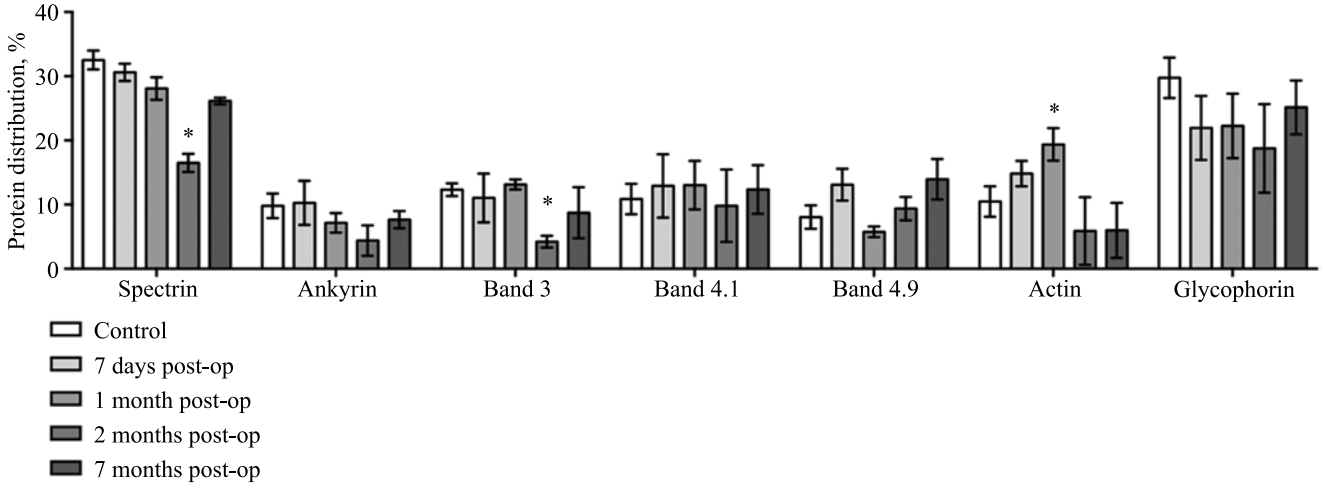

Fig. 2. Dynamics of protein composition of RBC membranes in related kidney donors

Comparison of erythrocyte protein composition between kidney recipient and donor groups revealed differences in the dynamics of spectrin, band 3 protein and glycophorin at all registration points until month 2 after surgery ($p < 0.05$), indicating more pronounced changes in recipients' RBC protein composition.

The study of erythrocyte membrane in liver transplant recipients revealed a significant decrease in glycophorin levels before and after surgery for 2 months with a maximum 72% decrease by day 7 after surgery relative to control values (Fig. 3). On day 7 after surgery, there was an 80% decrease in band 3 protein, and by month 1, there was a 66% decrease in band 3 protein and a 25% decrease in spectrin levels relative to the control. By the end of the study, the protein spectrum was restored to control.

In the related liver fragment donors, changes in the protein composition were detected only with respect to glycophorin content by month 1 of follow-up (65% decrease) and actin, whose levels increased by 49%. The levels of the other fractions were maintained at the control values (Fig. 4).

Comparison of concentrations of protein fractions of erythrocyte membranes in liver recipients and donors revealed significant differences in spectrin levels at month 1 after surgery, band 3 protein at day 7 and month 1 after surgery and glycophorin at day 7 and month 2 after surgery ($p < 0.05$).

Thus, in the postoperative period we observed changes in both peripheral and integral proteins of the erythrocyte membrane, combined with changes in functional indicators of erythrocytes: RBC electrophoretic mobility – an indicator reflecting cell surface charge and erythrocyte aggregation properties. It was shown that in kidney transplant recipients, RBC electrophoretic mobility significantly decreased in the period up to the second month after surgery (Fig. 5). Kidney donors had decreased RBC electrophoretic mobility between

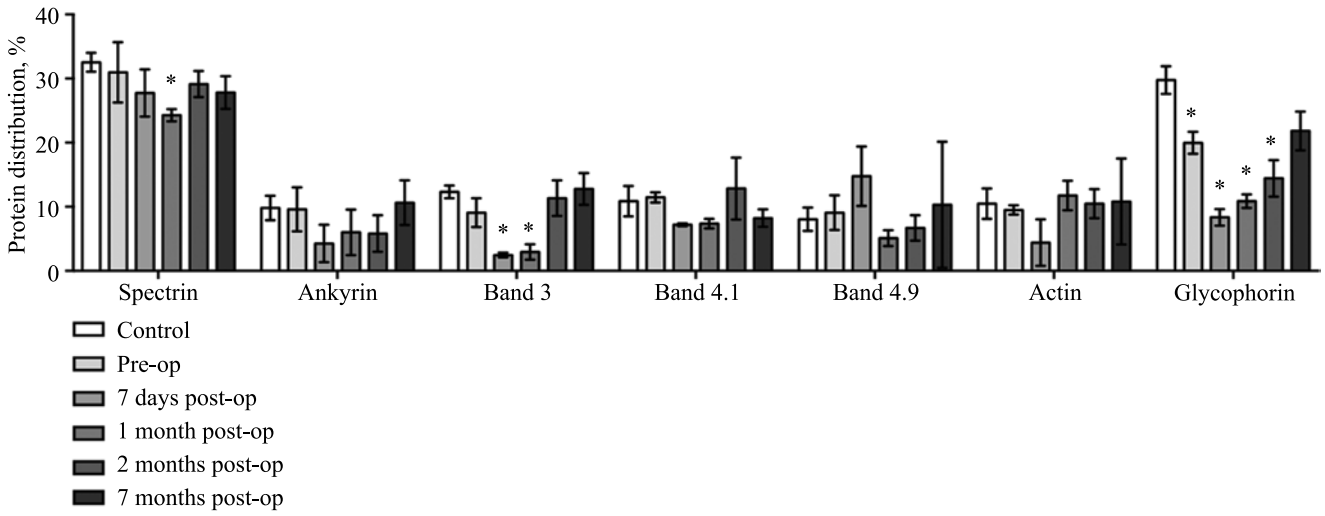

Fig. 3. Dynamics of protein composition of RBC membranes in liver transplant recipients

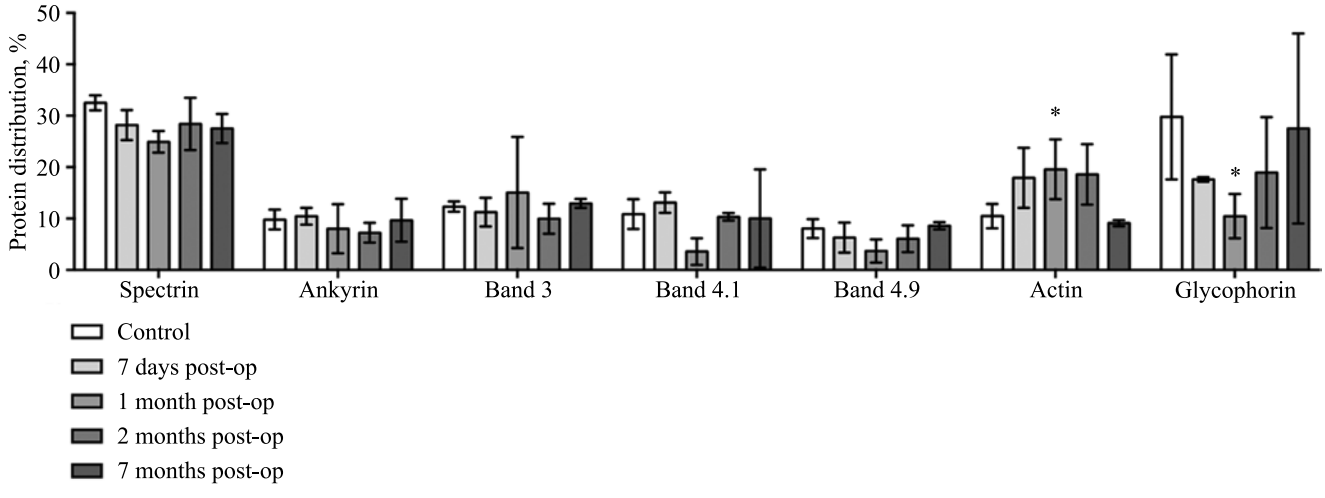

Fig. 4. Dynamics of protein composition of RBC membranes in related liver donors

months 1 and 2 after surgery (Fig. 6). RBC electrophoretic mobility was restored after month 2. The study of erythrocyte aggregation properties revealed an increase in erythrocyte aggregation in kidney transplant recipients, which is consistent with a decrease in RBC electrophoretic mobility in this patient cohort (Fig. 7). No significant change in aggregation was observed in related kidney donors (Fig. 8).

Liver recipients had decreased RBC electrophoretic mobility during the first month after surgery (Fig. 9). In related liver fragment donors, there was decreased RBC electrophoretic mobility at day 30 after surgery (Fig. 10). A significant increase in erythrocyte aggregation up to 1 month was shown in liver recipients (Fig. 11). No significant changes in the studied index were observed in related liver fragment donors (Fig. 12).

DISCUSSION

It is known that structural changes in erythrocyte membranes in pathological processes play a crucial role in the functional activity of cells. In this pilot study, we have shown that changes in the protein composition of the membrane had an influence on RBC electronegativity and RBC aggregation in recipients, and on RBC electronegativity in liver and kidney donors. It should be noted that reperfusion injury, which is associated with microcirculatory disorders and cell aggregation, is a major complication of transplantation [18].

Results show that liver and kidney recipients, as well as related donors had unidirectional dynamics in the form of decreased number of integral proteins. Moreover, in recipients, changes in integral protein, glycophorin, were registered before surgery and persisted in the postopera-

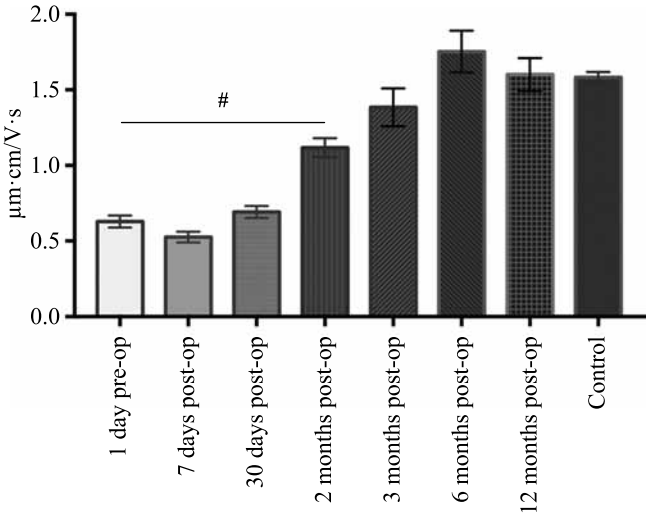

Fig. 5. Dynamics of changes in RBC electrophoretic mobility in kidney transplant recipients

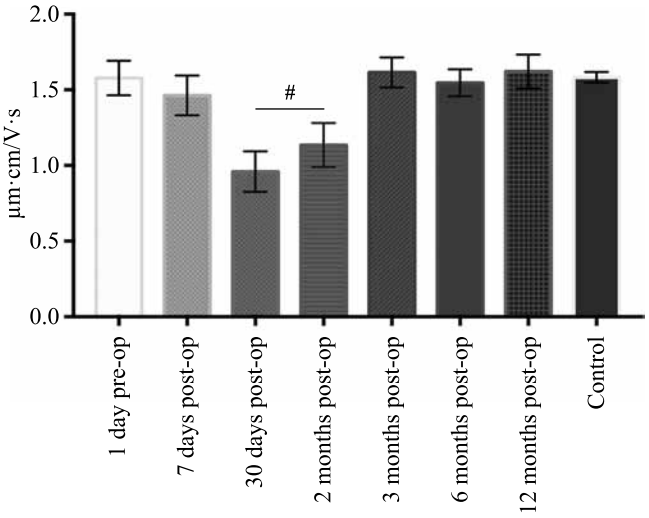

Fig. 6. Dynamics of change in RBC electrophoretic mobility in related kidney donors

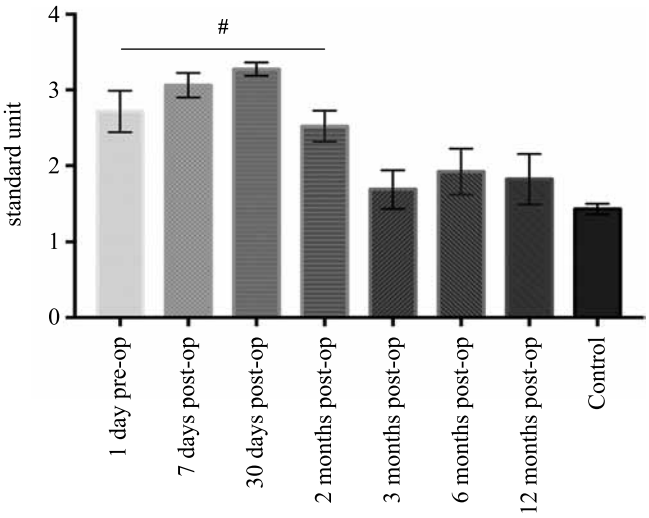

Fig. 7. Dynamics of RBC aggregation in kidney transplant recipients

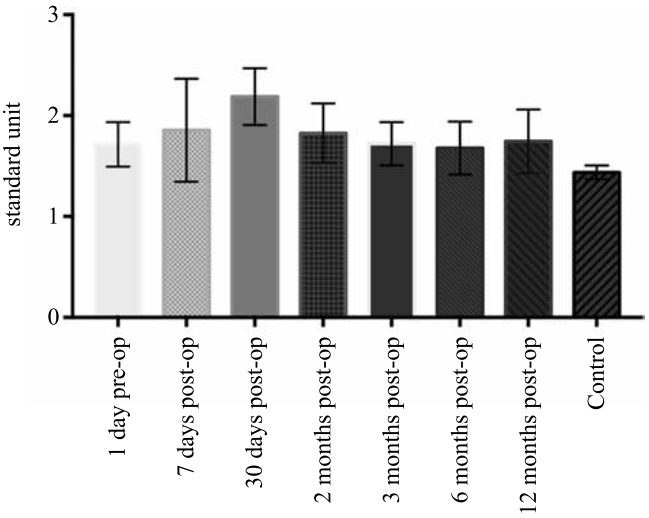

Fig. 8. Dynamics of RBC aggregation in related kidney donors

tive period. Given the high correlation of RBC electrophoretic mobility with deviation of glycophorin and band 3 protein, we can assume that the above dynamics of changes in the level of these membrane proteins is one of the leading factors determining the change in RBC membrane electronegativity. For instance, it is known that band 3 and glycophorin proteins belong to sialoglycoproteins and strongly promote the creation of a negative surface charge [19, 20]. Surface charge modification can promote erythrocyte aggregation, but is not the underlying factor, which proves the identified decrease in RBC electrophoretic mobility and the absence of aggregation in the donors of the studied groups.

The change in aggregation appears to be of a more complex nature and depends on multiple interactions between both integral and peripheral proteins. Cytoskeleton proteins, which determine membrane plasticity, may act as significant factors in the aggregation process: when spectrin levels fall, there is reduced ankyrin binding sites and membrane surface viscosity [21]. Probably, the band 3 protein contributes in a way to such characteristics as erythrocyte plasticity and aggregation. Thus, the cytoplasmic region of band 3 protein has binding sites for a number of glycolysis enzymes [22], decreased glycolysis activity reduces ATP concentration, Na^+/K^+-ATPase activity and erythrocyte plasticity [23]. Inhibition of Na^+/K^+-ATPase activity leads to increased intracellular Ca^{2+} levels [24]. Accumulation of Ca^{2+} ions activates calmodulin, which determines the growth of erythrocyte aggregation [22, 25].

Thus, the totality of results obtained indicates that in the process of erythrocyte aggregation in liver/kidney recipients, structural and functional disorders, determined by spectrin, band 3 protein and glycophorin,

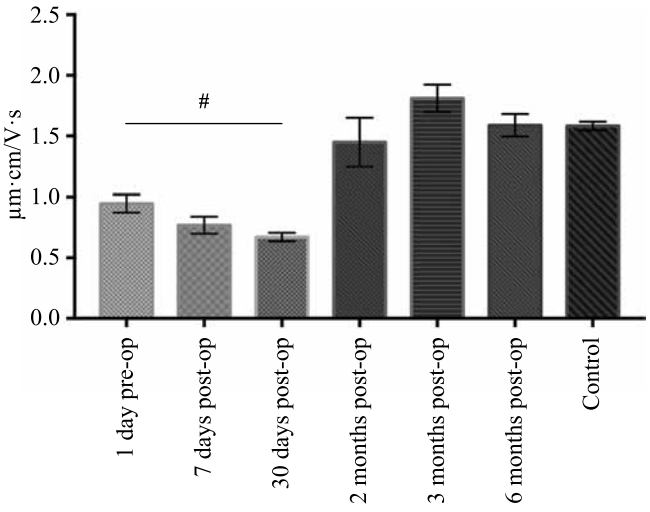

Fig. 9. Dynamics of changes in RBC electrophoretic mobility in liver transplant recipients

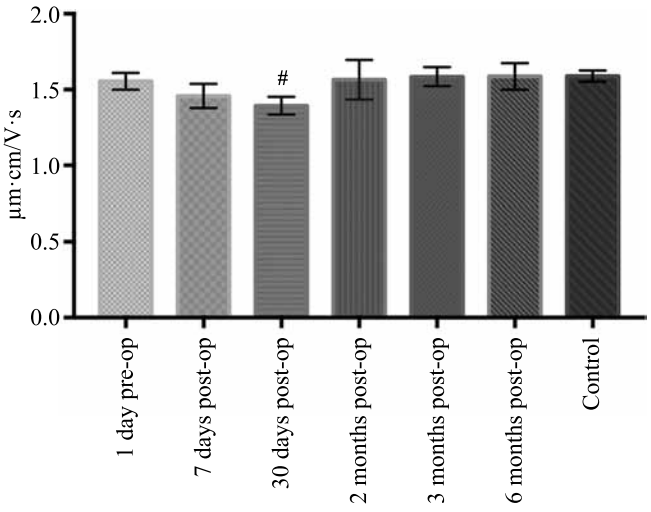

Fig. 10. Dynamics of changes in RBC electrophoretic mobility in related liver donors

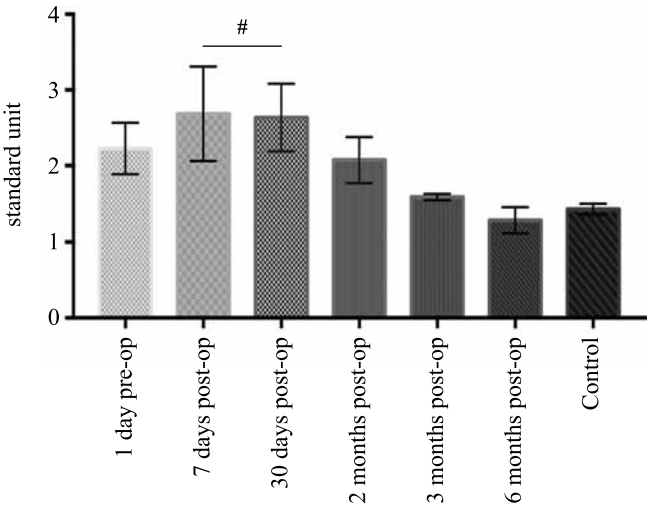

Fig. 11. Dynamics of RBC aggregation in liver transplant recipients

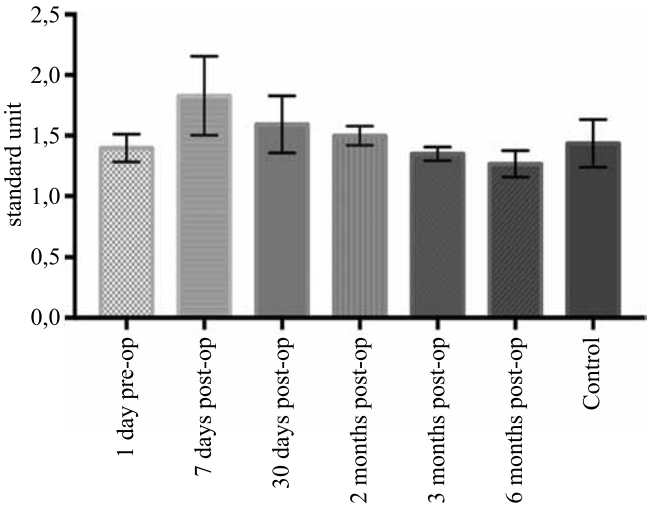

Fig. 12: Dynamics of RBC aggregation in related kidney donors

are significant factors. Analysis of the dynamics of the protein composition of donor erythrocytes show that increased actin levels restrain an increase in erythrocyte aggregation.

CONCLUSION

During organ transplantation, in particular liver and kidney, the protein structure of erythrocyte membranes undergoes damage, expressed both in the recipient and in the related donor. This can initiate a decrease in erythrocyte electronegativity and an increase in erythrocyte aggregation.

The authors declare no conflict of interest.

REFERENCES

1. *Gautier SV.* Transplantology of the 21st century: High technologies in medicine and innovations in biomedical science. *Russian Journal of Transplantology and Artificial Organs*. 2017; 19 (3): 10–32. [In Russ, English abstract]. doi: 10.15825/1995-1191-2017-3-10-32.
2. *Zhuravel SV, Kuznetsova NK, Chzhao AV, Timerbayev VKh*. Transfusion of blood components during orthotopic hepatic transplantation. *General reanimatology*. 2007; 3 (4): 28–30. [In Russ].
3. *Tarabarko NV, Epifanov SU, Pinchook AV.* The complex correction of blood coagulability in early terms after kidney transplantation. *Russian Journal of Transplantology and Artificial Organs*. 2016; (5): 24–25. [In Russ].
4. *Khubutiya MSh, Zhuravel SV, Gulyaev VA, Kabanova SA, Khvatov VB, Nikulina VP.* Usage of red blood cells from cadaveric donor during orthotopicliver transplantation. *Vestnik Rossiiskoi Voenno-medicinskoi academii.* 2014; 4 (48): 152–157. [In Russ, English abstract].
5. *Manchenko EA, Kozlova EK, Sergunova VA, Chernysh AM.* Homogeneous deformation of native erythrocytes during long-term storage. *General reanimatology.* 2019; 15 (5): 2–10. [In Russ, English abstract]. doi: 10.15360/1813-9779-2019-5-4-10.
6. *Muravyov AV, Mikhailov PV, Tikhomirova IA.* Microcirculation and hemorheology: points of interaction. *Regional blood circulation and microcirculation.* 2017; 16 (2): 90–100. [In Russ, English abstract].
7. *Boyarinov GA, Boyarinova LV, Deryugina AV, Solov'eva OD, Zaytsev RR, Voyennov OV et al.* Role of secondary brain damage factors in activation of vascularplatelet hemostasis in traumatic brain injury. *General reanimatology.* 2016; 12 (5): 42–51. [In Russ, English abstract]. doi: 10.15360/1813-9779-2016-5-42-51.
8. *Kuhn V, Diederich L, Keller TCS 4th, Kramer CM, Lückstädt W, Panknin C et al*. Red Blood Cell Function and Dysfunction: Redox Regulation, Nitric Oxide Metabolism, Anemia. *Antioxidants & redox signaling*. 2017; 26 (13): 718–742. doi: 10.1089/ars.2016.6954.
9. *Deryugina AV, Gracheva EA.* Dynamics of the morphofunctional indicators of erythrocytes under the action of sulfhydryl inhibitor of angiotensin-converting enzyme fentiaprilin the experimental model of arterial hypertension. *Russian journal of physiology.* 2019; 105 (9): 1163–1170. [In Russ, English abstract]. doi: 10.1134/S0869813919090048.
10. *Skoumalová A, Herget J, Wilhelm J*. Hypercapnia protects erythrocytes against free radical damage induced by hypoxia in exposed rats. *Cell Biochem Funct.* 2008; 26 (7): 801–807. doi: 10.1002/cbf.1509. PMID: 18683905.
11. *Romanov SV, Abaeva OP, Alexandrova OY, Smirnova GY.* Issues and perspectives of building a regional system of donor services (on the example of Nizhny Novgorod region). *Russian Journal of Transplantology and Artificial Organs*. 2019; 21 (1): 57–63. [In Russ, English abstract]. doi: 10.15825/1995-1191-2019-1-57-63.
12. *Romanov SV, Zhukov SN, Dzyubak SA*. Economic and social problems of providing medical care to kidney and liver recipients on an outpatient basis (on the example of a regional outpatient transplantation center) Chief Medical Officer. 2020; (1): 23–33. [In Russ, English abstract]. doi: 10.33920/med-03-2002-02.
13. *Laemmli UK.* Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 1970; 227 (259): 680–685.
14. *Borovskaya MK, Kuznetsova EE, Gorokhova VG, Koriakina LB, Kurilskaya TE, Pivovarov JuI.* Structural and functional characteristics of membrane's erythrocyte and its change at pathologies of various genesis. *Bulletin of ESCC SB RAMS.* 2010; 3 (73): 334–354. [In Russ, English abstract].
15. *Bonarska-Kujawa D, Cyboran-Mikołajczyk S, Kleszczyńska H.* Molecular mechanism of action of chlorogenic acid on erythrocyte and lipid membranes. *Molecular Membrane Biology.* 2015; 32 (2): 46–54.
16. *Deryugina AV, Ivashchenko MN, Ignatiev PS, Lodyanoy MS, Samodelkin AG*. Alterations in the phase portrait and electrophoretic mobility of erythrocytes in various diseases. *Modern Technologies in Medicine*. 2019; 11 (2): 63–68. [In Russ, English abstract]. doi: 10.17691/stm2019.11.2.09.
17. *Deryugina AV, Abaeva OP, Romanov SV, Vedunova MV, Ryabova EN, Vasenin SA, Titova NA.* Electrokinetic, oxidative and aggregation properties of red blood cells in the postoperative period following kidney transplantation. *Russian Journal of Transplantology and Artificial Organs.* 2020; 22 (2): 72–79. [In Russ, English abstract]. doi: 10.15825/1995-1191-2020-2-72-79.
18. *Iskenderov E, Kandoga A, Mende K.* Effects of amplifier liver regeneration on the currency *in vivo* reperfusion injury. *Avicenna bulletin.* 2012; (4): 154–158. [In Russ, English abstract].
19. *Simmonds MJ, Herbert JM, Oguz KB.* Blood rheology and agin. *Journal of Geriatric Cardiology.* 2013; 10 (3): 291–301. doi: 10.3969/j.issn.1671-5411.2013.03.010.
20. *Shumilova AV, Deryugina AV, Gordleeva SYu, Boyarinov GA*. Cytoflavin action on electro-kinetic and aggregation indices of erythrocytes in the post-traumatic period of cerebrocranial injury in experiment. *Éksperimentalnaya i klinicheskaya farmakologiya.* 2018; 81 (3): 20–23. [In Russ, English abstract]. doi: 10.30906/0869-2092-2018-81-3-20-23.

21. *Moroz VV, Chernysh AM, Kozlova EK, Borshegovskaya PY, Bliznjuk UA, Rysaeva RM, Gudkova OY.* Comparison of red blood cell membrane microstructure after different physicochemical influences: atomic force microscope research. *Journal of Critical Care*. 2010; 25 (3): e531–512. doi: 10.1016/j.jcrc.2010.02.007.
22. *Nunomura W, Takakuwa Y, Parra M, Conboy J, Mohandas N.* Regulation of Protein 4.1R, p55, and Glycophorin C Ternary Complex in Human Erythrocyte Membrane. *The Journal of biological chemistry*. 2000; 275 (32): 24540–24546. doi: 10.1074/jbc.M002492200.
23. *Yamaguchi T, Fukuzaki S.* ATP effects on response of human erythrocyte membrane to high pressure. *Biophysics and physicobiology.* 2019; 16: 158–166. doi: 10.2142/biophysico.16.0_158.
24. *Glitsch HG.* Electrophysiology of the sodium-potassium-ATPase in cardiac cells. *Physiol Rev.* 2001; 81: 1791–1826. doi: 10.1152/физрев.2001.81.4.1791.
25. *Muravyov AV, Tikhomirova IA, Maimistova AA, Bulaeva SV, Zamishlayev AV, Batalova EA.* Crosstalk between adenylyl cyclase signaling pathway and Ca^{2+} regulatory mechanism under red blood cell microrheological changes. *Clin Hemorheol Microcirc*. 2010; 45 (2–4): 337–345. doi: 10.3233/CH-2010-1317.

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Q-METHODOLOGY TO IDENTIFY PERCEPTIONS OF DECEASED ORGAN DONATION IN THE UK

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Background. Attitude towards organ donation is predominantly positive in the UK, however, donation rate remains low. To develop more effective interventions, this research aims to examine the behavioural barriers in organ donations using Q methodology to elicit patterns of overlap among different barriers and motivators. **Method.** A Q methodology study was conducted with 40 participants aged 19–64 were asked to rank 47 statements on issues that are associated with organ donation. By-person factor analysis using Centroid method and Varimax rotation was conducted to bring out patterns in the way statements were ranked to obtain groupings of participants who had arranged the statements in similar fashion. **Results.** Four viewpoints were extracted: The Realist, the Optimist Hesitant, the Pessimist Determinant and the Empathetic. Salient barriers to organ donation presented in each viewpoint suggest that perceived lack of knowledge, anxiety, mistrust in the healthcare system and lack of cue to action are the main barriers to organ donation. Consensus statements suggest that religion and family agreement are inconsequential if attitude to organ donation is well formed. **Conclusion.** There are different attitudes around deceased organ donation that were uncovered using Q methodology. These results suggest that people respond to behavioural change campaigns differently depending in their own perceptions on organ donation. We argue that a paradigm shift in behavioural interventions is underpinned by understanding the overlapping yet distinctive nature perceived perspectives.

Keywords: Organ donation, Q methodology, behavioural interventions.

INTRODUCTION

Despite the joined effort of hundreds of researchers to improve the rate of organ donation, there has only been a slight increase in donation rates in the UK averaging at 2% growth rate annually (NHS, 2019b) [1].

Organ donation decision is extremely complex. It invokes countless beliefs, symbols, sentiments, and emotions as well as numerous rituals and social practices. A meta-analysis Feeley and Moon (2009) [2] and Li et al. in (2015) [3] showed weak performance and low effect size for those interventions, likely caused by the extensive emotional reactions organ donation triggers (Miller, Currie, & O'carroll, 2018) [4] that can influence information processing (Handley & Lassiter, 2002) [5] and communication (Stefanelli & Seidl, 2017) [6]. When asked about barrier to organ donation, participants usually respond with familiar notions triggered intuitively (Greene & Haidt, 2002) [7]. Religion, fear of death and "I don't know much about it" are, unsurprisingly, the most common barriers reported in qualitative studies. Most interventions to increase donation rate are based on the main modifiable barrier reported in literature, which is knowledge and information. We propose, however, that people have heterogenous views about organ donation, an amalgam of different components jumbled together to shape the attitude.

Subjective perception to barriers to donation have not been fully explored in organ donation. Literature suggests that what is considered as a barrier might act as a motivator depending on individual subjective perception. This study uses Q methodology to identify how people in UK perceive barrier to organ donation and how such perception creates distinctive views. Views on organ donation further our understanding of barriers against organ donation and inform behavioural interventions to produce more targeted and effective approaches.

MATERIALS AND METHODS

To investigate attitudes towards deceased organ donation in people residing temporarily or permanently in UK for six months or longer, we conducted a Q methodology study. Q methodology combines the strengths of both qualitative and quantitative research practises and allows for a systematic investigation of human subjectivity (McKeown & Thomas, 2013) [8]. It is neither a survey nor an interview. The sample size for Q methodology studies is small and "does not need large numbers of subjects as does survey analysis" (Smith, 2001) [9]. It is especially suitable for research with "many, potentially complex and contested answers" (Watts & Stenner, 2005) [10]. In Q methodology research, attitudes represent "prototypical exemplars" (Valenta & Wigger,

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1997) [11] rather than disconnected, non-overlapping ideas with cut-off points such opposite to the attitudes presented in the current literature.

In a Q study, participants are presented with a set of statements around the area of study. Participants are then asked to rank those statements according to their agreement with each statement on a quasi-normal grid. Q methodology is completed through several stages (Fig. 1). The first step is to create a concourse. A concourse refers to the collection of all discussions around the topic (Stephenson, 1980) [12]. This includes statements made around the topic of organ donation collected from existing literature (interviews and surveys), social media contents, essays, publications, and any other source related to the issue.

Initially, 224 statements were collected to account for all possible views, statements and opinions around the topic (Stephenson, 1980) [12]. A comprehensive literature review using several databases was the major source of these statements, complemented by Google searches and informal conversations to enrich the collection of concourses beyond the published. Statements from social media, like Facebook comments, YouTube videos, blogs, and NHS (National Health Services in the UK) websites were collected. Concourse statements were structured into 8 themes: religion, body, death, healthcare, knowledge, awareness, recipient, and others.

The statements were then reduced to a manageable-sized list to form the Q set, 47 statements representing barriers and motivators falling under all themes. The participants sample in Q methodology, the P set; was strategically selected (Brown, 1980) [13]. P set does not represent the population, it represents the variety of views in a population, thus the sample size in Q methodology is smaller than that of a survey, and it is generalisable in representing the variety in population. Data collected from 40 participants recruited through snowball sampling strategy, aged between 19–64 years' old (45% female and 55% male). A conscious effort was put to ensure that participants hold different religions and cultural origins. Data were collected online on qmethodsoftware.com.

Participants were provided with instructions to arrange the statements from +5 (similar to what I think) to –5 (opposite to what I think) with the zero column representing statements that (do not concern me) (Brown, 1980) [13]. The grid distributions forces participants to rank statements from 2 statements per column on ends to 7 statements at the middle. The resulting outcome is the Q sort, a genuine 'operationalised' representations of personal point of views (McKeown & Thomas, 2013) [8].

RESULTS

Q sorts resulted in 7 factors initially. We used Pearson correlation for this study and opted for Varimax rotation and centroid method for analysis. This is followed by creating factors arrays, which represent a hypothetical Q sort that loads perfectly onto a factor. Kaiser-Guttman criteria, Humphrey's Rule and Scree Test were used to reduce the number of factors into distinguishable attitudes to facilitate interpretations. We found three factors that satisfy all three criteria used for factor extraction, those three factors account for 31 participants and explain 39% of total variance (Table 1).

Factors' Interpretation. Factor interpretation was carried out using the "*crib sheet*" method (Watts & Stenner, 2012) [14] to ensure systematic and holistic approach in the interpretation process. The crib sheet lists the two statements at each end (on location +5 and –5) then lists the statements ranked the lowest and the highest by that factor. The support factor interpretation and comparison between factors.

Factor Interpretation

The interpretation is conducted by applying abductive strategy in interpretation. By the end of interpretation, we created a qualitative account each viewpoint, a story to describe each viewpoint comprehensively.

Factor 1 – I want to know more; Factor 1 explains 11% of variance in the study. Eight people loaded significantly on this factor (Table 2). Only participant is registered as an organ donor (Fig. 2).

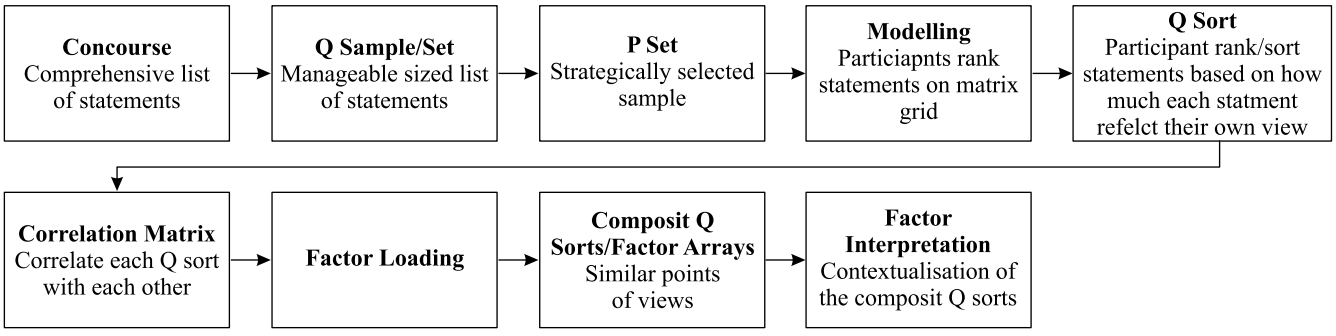

Fig. 1. Q Methodology Stages

Table 1

Statements and Factor Arrays

Statement	Factor 1	Factor 2	Factor 3
1 – I believe my religion does not allow it	–2	–1	0
2 – I think rich or famous people can receive organs before the people with the most need	–1	–2	2
3 – I do not think I have ever thought about it	–2	1	1
4 – I think the process of registration is complicated	0	–2	–1
5 – I think anyone can register and be a donor even if old or have a disease	0	1	2
6 – I think there is no special need for organs for Asian, African, and Middle Eastern groups	–3	–3	–2
7 – I think giving out organs to save someone's life is a noble act	4	4	5
8 – I think doctors might not do their best to save someone's life if they know they are on the Organ Donor Register	1	–3	–4
9 – I think I am too old to donate	–4	–2	–3
10 – I believe I will be haunted if I donate	–4	0	–2
11 – I think it is non-religious to take organs	–5	–1	0
12 – I do not know anyone who donated an organ	5	–2	4
13 – I believe there is a great need for organs especially in minority groups	2	1	3
14 – Brain death is confusing to me, but I think experts know better	1	2	2
15 – I feel I cannot decide to donate because I do not know all the facts	5	3	–1
16 – I believe transplantation results are successful and they are improving people's health	3	5	5
17 – If someone religious says it is not allowed, then I will not do it	–3	1	–5
18 – I feel talking about death and after life is important to appreciate our lives	0	3	3
19 – I think doctors will prematurely declare my death If I am a donor just so they can harvest my organs	0	–4	–2
20 – It feels scary to donate, but once I pass that emotional hurdle, I feel better about myself	1	3	2
21 – I believe the human body is not a machine	–1	–1	0
22 – I think brain dead people can regain consciousness	1	0	1
23 – I thought about registering as a donor but I never did	2	5	1
24 – I do not want doctors or the healthcare system to be in control of my organs	3	–5	0
25 – When someone asks me to register to donate, it feels like he is waiting for my death to get my organs	–2	–5	–1
26 – I trust the donation system to be fair	–1	4	3
27 – I do not mind organ donation but my family disagree	0	0	0
28 – I trust doctors and nurses to always provide the best care they can	2	4	4
29 – I think people exaggerate on the importance of the whole organ donation subject	0	–3	–1
30 – I think people who have medical conditions cannot donate	4	–1	1
31 – I feel I have no responsibility towards anyone else	–1	–4	–3
32 – I think transplant recipients do not live more than 10 years after a transplant operation	2	–1	0
33 – People on the waiting lists are ill and I believe they need my help	1	2	4
34 – I believe donated organs can be bought and sold	–1	–3	1
35 – I might feel easy to donate because my family encourages me to donate	–2	1	2
36 – I believe the present need for transplant organs is fully covered	–3	–2	–3
37 – I believe people would not need transplants if they took better care of their health	4	–4	–4
38 – I do not mind donating some organs, but not my heart or eye	–1	2	0
39 – I believe organs are a gift from god, we are not allowed to give them away	–5	0	–5
40 – No matter how hard it is to think about organ donations, it makes me feel good about myself	2	2	3
41 – I do not think I have the courage to donate	0	3	–2
42 – I think it is just easier to say no than to think about it	3	1	–4
43 – I think my religion encourages organ donation in order save other people's lives	–3	2	–1
44 – I do not mind donating when I am alive, not when I am dead	–2	0	–3
45 – I want to be cremated and if I donated organs, I cannot do that	–4	–1	–1
46 – Talking about death is creepy	1	0	–2
47 – I think I am not dead if my heart is still beating	3	0	1

–5	–4	–3	–2	–1	0	1	2	3	4	5
39	9	17	44	31	19	8	32	42	37	15
11	10	36	25	38	41	46	23	24	30	12
	45	6	1	21	29	22	13	47	7	
		43	3	34	4	20	40	16		
			35	2	27	14	28			
				26	5	33				
					18					

Fig. 2. Factor 1 Array

People on this factor value information and hold themselves responsible for seeking information to make better decisions for themselves. They show a positive view on the success of transplantation procedures. They also have good information on the registration process, but they show a misunderstanding on the donation criteria. They tend to stay rational and in control of their emotional attachments with their bodies keep the religion influence on their decisions to minimum, their religion is personal reflects a positive relationship with their religions, however, they do not tend to follow religious leaders.

The pattern of barriers in this factor shows that both religious and non-religious individual may share similar views. It also shows that the mechanic view of the body may not be related to non-religious views. Our analysis shows that knowledge is not an abstract term, and educational campaigns targeting this view may prioritise targeting certain themes (such as eligibility criteria and the reasons for organ failures) over other aspect.

Factor 2: I need inspiration & I will never do it; Factor 2 explains 12% of variance in the study. Eight people loaded significantly on this factor (Table 3). None of them is registered as an organ donor. Three out of eight are loaded negatively on this factor, thus; interpretation will be divided into two halves, one for the positively loaded participants and then for the negatively loaded ones.

Factor 2-A: I need inspiration, People on this factor show a high level of trust in healthcare professionals and they extend this trust to the harvesting and allocation systems as well (Fig. 3).

People who loaded positively on this factor show a high level of trust[1]. They trust the healthcare professionals[2] and extend this trust to the harvesting and allocation system as well[3]. This trust acts as the main motivator for people loading positively on this factor. People loading on this factor show spiritual connections with religion[4] and with their body[5].however, they do not perceive religion as a barrier to donation[6].

Table 2

Sorts Weights on F1

Q Sort	Weight	Gender	Age	Education	Socio-Economic Class	Ethnicity	Religion	Years in UK	Donor
I6205	10	M	27	Mid	Mid	Asian (Nepalese)	N/A	2	No
I4585	5.49	M	33	Mid	Mid	Middle East British	Atheist	3	No
I4584	5.30	F	26	Mid	Mid	Netherlands	Atheist	4	No
I5931	4.10	F	19	Low	Mid	White American	Christian	2	No
I4609	4	M	25	Mid	Mid	White Ukrainian	Atheist	16	Yes
I4652	4	F	36	Mid	Mid	Middle East	Muslim	2	No
I6018	3.89	M	34	Mid	Mid	African	Christian	3	No
I4572	–6.89	M	22	Mid	Mid	Indian	Sikh	2	No

[1] Statement 16 – I believe transplantation results are successful and they are improving people's health is on +5 rank, 26 – I trust the donation system to be fair and 28 – I trust doctors and nurses to always provide the best care they can on +4 highest among factors.

[2] Statements 24 – I don't want doctors or the healthcare system to be in control of my organs and 25 – When someone asks me to register to donate, it feels like he is waiting for my death to get my organs both on –5 and distinguishing statements for this factor, 19 – I think doctors will prematurely declare my death If I am a donor just so they can harvest my organs on –4 and distinguishing factor as well and 8 – I think doctors might not do their best to save someone's life if they know they are on the Organ Donor Register on –3 both are lowest among factors.

[3] Statement 34 – I believe donated organs can be bought and sold on –3 a distinguishing statement and 2 – I think rich or famous people can receive organs before the people with the most need on –2 rank and lowest among factors.

[4] Statement 43 – I think my religion encourages organ donation in order save other people's lives on +2 and 17 – If someone religious says it is not allowed, then I will not do it on +1, both are distinguishing statements.

[5] Statement 38 – I do not mind donating some organs, but not my heart or eye on +2 and distinguishing statement and 21 – I believe the human body is not a machine on –2 and the highest among factors.

[6] Statement 17 – If someone religious says it is not allowed, then I will not do it is on +1 and a distinguishing statement, statement 11 – I think it is non-religious to take organs and 1 – I believe my religion does not allow it on –1, and 39 – I believe organs are a gift from God, we are not allowed to give them away a distinguishing statement on 0.

–5	–4	–3	–2	–1	0	1	2	3	4	5
25	31	6	9	11	39	17	43	41	26	23
24	19	34	36	45	10	3	38	18	28	16
	37	29	2	1	44	35	14	20	7	
		8	4	21	27	5	33	15		
			12	32	46	13	40			
				30	22	42				
					47					

Fig. 3. Factor 2 Array

They exhibit a significant fear from the process of organ donation[7]. Several statements show how consistent this group of people in expressing their fear from donating organs and their hesitancy to register. That fear seems to be crippling, and it might be the main barrier against donation[8]. However, positive views from their family and friends may help alleviate such fear[9].

Knowledge on brain death is not the main drive for the attitude, for people loading on this factor (both positively and negatively), related statements lie in the middle region of the grid, indicating these statements are irrelevant to the decision to donate[10]. Most knowledge related statements were ranked in the middle area of the grid (–2 to +2) indicating that these statements are not extremely relevant to their views on organ donation[11].

People loaded positively on this factor demonstrate a trustworthy view of the healthcare system and healthcare providers. They show a spiritual view of the body despite a generally positive view on organ donation. They also show brain death knowledge is not relevant to them and religion may or may not hold negative to organ donation but that does not seem to be the main drive for their attitude. Fear and emotional distress play a major role for people loaded positively on this factor. Despite a great trust in the healthcare system, they appear to be hesitant to take a positive step towards organ donation.

This pattern of barriers shows that messages on religious view on organ donation or myth busting campaigns on brain death may not be relevant. It is the irrational fear that plays the major role regardless of any information they may hold on organ donation.

Factor 2-B: I will never do it**,** this group represents the people who loaded negatively in Factor 2 (Table 3). People in this group, contrary to the group loaded positively on this factor; show a great mistrust in the healthcare system represented by healthcare providers and allocation system. They largely show an extreme negative view

Table 3

Sorts Weights on F2

Q Sort	Weight	Gender	Age	Education	Socio-Economic Class	Ethnicity	Religion	Years in UK	Donor
4567	5.18	F	46	Low	Low	White American	Christian	12	No
4616	3.98	M	29	Mid	Mid	White European	Atheist	2	No
5897	5.97	F	22	Low	Low	White European	N/A	5	No
6263	5.73	F	52	Low	Mid	White Australian	Christian	25	No
6291	7.9	F	56	Mid	Mid	Chinese	Taoism	7	No
4586	–8.66	F	26	Mid	Mid	Chinese Malaysian	Christian	7	No
6216	–10.3	M	40	Mid	Mid	Latino	Christian	3	No
4648	–13.74	M	25	Mid	Mid	White European	Christian	10	No

[7] Statement 23 – I thought about registering as a donor but I never did is on +5 and a distinguishing statement for this factor, 41 – I don't think I have the courage to donate and 20 – It feels scary to donate, but once I pass that emotional hurdle, I feel better about myself, and 15 – I feel I cannot decide to donate because I don't know all the facts, all on +3 and are distinguishing statements as well, and 40 – No matter how hard it is to think about organ donations, it makes me feel good about myself on +2 as well as 42 – I think it is just easier to say no than to think about it on +1 and a distinguishing statement.

[8] Statement 20 – It feels scary to donate, but once I pass that emotional hurdle, I feel better about myself a distinguishing statement and scored the highest among factors and Statement 41 – I do not think I have the courage to donate scored the highest among factors and both statements are on +3.

[9] Statement 35 – I might feel easy to donate because my family encourages me to donate on +1.

[10] Statement 44 – I do not mind donating when I am alive, not when I am dead a distinguishing statement, 47 – I think I am not dead if my heart is still beating, the lowest among factors and 22 – I think brain dead people can regain consciousness, the lowest among factors, all on 0.

[11] They ranked 13 – I believe there is a great need for organs especially in minority groups on +1, 36 – I believe the present need for transplant organs is fully covered on –2 and 6 – I think there is no special need for organs for Asian, African, and Middle Eastern groups on –3. Regarding the registration process, transplantation results and eligibility criteria, they ranked statement 4 – I think the process of registration is complicated on –2, statement 32 – I think transplant recipients do not live more than 10 years after a transplant operation on –1 and statement 30 – I think people who have medical conditions cannot donate on –1 as a distinguishing statement and 9 – I think I am too old to donate on –2.

on organ donation. They may or may not have enough knowledge about registration process, brain death or allocation system, but they certainly have strong negative attitude towards it.

This group of people seems to be determined in their decision regarding organ donation. Their mind is set potentially from death anxiety, poor knowledge or by organ donation scandals in different countries. Behavioural interventions on this group of people seems futile. Further examinations of their views might uncover individual reasons for those views. Either way, behavioural changes on this group require individualised and long-term campaigns to alter the negative views which might exhaust the limited resources for such interventions.

–5	–4	–3	–2	–1	0	1	2	3	4	5
25	31	6	9	11	39	17	43	41	26	23
24	19	34	36	45	10	3	38	18	28	16
	37	29	2	1	44	35	14	20	7	
		8	4	21	27	5	33	15		
			12	32	46	13	40			
				30	22	42				
					47					

Fig. 4. Factor 3 Array

As a group, they correlate reasonably high with each other. Moreover, Factor 2 is closer to Factor 3 than to Factor 1.

Factor 3: It is a good deed, Factor 3 explains 16% of variance in the study. Fifteen people loaded significantly on this factor (Table 4). Four of them are registered as organ donors (Fig. 4).

People on this factor view organ donation as a noble act[12] and they are willing to fight their own fear to help those who are ill and in need of those organs[13]. Their fear does not stem from death anxiety or mistrust in the healthcare system. They show trust in healthcare providers[14], while recognising possible corruption in the allocation system[15]. They are motivated by their responsibility towards others without assigning any blame towards those who fall ill[16]. Popole loaded on this factor

Table 4

Sorts Weights on F3

Q Sort	Weight	Gender	Age	Education	Socio-Economic Class	Ethnicity	Religion	Years in UK	Donor
4526	6.22	F	27	Mid	Mid	Middle East	Muslim	3	No
4527	4.40	F	26	Mid	Mid	Middle East	Christian	1	No
4565	5.42	F	34	Low	Low	African	Atheist	4	No
4583	5.15	M	38	Mid	Mid	Indian	Hindu	13	No
4606	3.611	M	46	low	High	Indian British	Sikh	46	No
4607	3.76	F	56	Mid	Mid	White British	COE	56	No
4633	4.45	M	30	Mid	Low	Indian	Hindu	2	No
4658	5.43	F	37	Mid	Mid	White British	Christian	37	Yes
4725	6.144	M	35	Mid	Mid	White European	Agnostic	11	Yes
4726	6.56	M	25	Mid	Mid	White European	Atheist	7	No
5839	4.26	M	27	Mid	High	Middle East British	Muslim	12	No
5850	3.91	F	53	Low	Mid	White British	COE	53	Yes
6271	44.25	F	24	Mid	Low	White European	Atheist	5	No
6277	5.04	M	47	Mid	Mid	Indian British	Buddhist	47	Yes
4570	–4.14	M	47	Low	Mid	Middle East British	Atheist	20	No

[12] Statement 7 – I think giving out organs to save someone's life is a noble act on +5.

[13] Statement 33 – People on the waiting lists are ill and I believe they need my help on +4 and a distinguishing statement for this factor, 40 – No matter how hard it is to think about organ donations, it makes me feel good about myself on +3 and the highest among all factors, and 20 – It feels scary to donate, but once I pass that emotional hurdle, I feel better about myself on +2. Statement 46 – Talking about death is creepy on –2 a distinguishing statement and lowest among all factors and 42 – I think it is just easier to say no than to think about it on –4 as a distinguishing statement and the lowest among all factors as well.

[14] Statement 28 – I trust doctors and nurses to always provide the best care they can on +4, 8 – I think doctors might not do their best to save someone's life if they know they are on the Organ Donor Register on –4 and higher among all factors, 14 – Brain death is confusing to me, but I think experts know better on +2 and highest among all factors.

[15] Statement 2 – I think rich or famous people can receive organs before the people with the most need on +2, 34 – I believe donated organs can be bought and sold on +1 and 19 – I think doctors will prematurely declare my death If I am a donor just so they can harvest my organs on –2 all are distinguishing statements for this factor.

[16] Statement 31 – I feel I have no responsibility towards anyone else on –3 and the lowest among all factors and statement 37 – I believe people would not need transplants if they took better care of their health on –4.

shares a more mechanical view and they do not perceive religion to be a barrier to organ donation[17].

The awareness level in this group is high[18] with a considerable knowledge about organ donation registration criteria[19]. This awareness is mixed with a certain level of misinformation especially in information related to brain death[20]. Although they show some comfort with their knowledge level, and they do not perceive is as a barrier against becoming a donor[21].

As a group, the Q sorts loading on this factor do not correlate with each other, and they load reasonably high on their factor. This indicates that participants loading on Factor 3 have a homogenous view on organ donation. Moreover, Factor 3 is closer to Factor 1 than to Factor 2.

Consensus Statements, Consensus statements are statements with similar Z scores across factors. In this study, there were six statements that were consensus among all three factors (Table 5). Three out of the six statements are related to the general and special need of organs for minority groups. It signifies the relative awareness in the need for organ donation, possibly, brought about by the active campaigns related to the new change in law in organ donation from opt-in to opt-out system. These results suggest that future campaigns can afford to shift their focus on issues other than awareness.

Another consensus statement was statement 9 – I think I am too old to donate, it implies a that age as a criterion for donation is not a major concern for participants, even for older participants, however, other criteria such as medical conditions as an eligibility criterion to donate was important to highlight especially in Factor 1.

The last statement that surprisingly, all factors agreed upon is statement 27 – I do not mind organ donation but my family disagree on rank 0, and it was non-significant even at $P > 0.05$. This results contradicts existing literature that used the Theory of Reasoned Action where subjective norm (a function of normative beliefs) is affected by perceptions of specific salient others' preferences about behaviour (Ryan & Carr, 2010) [15]. Many campaigns to support organ donation focused on improving family approval of their loved one's decision to donate, our study suggests that is not a significant barrier against donation.

DISCUSSION

Behavioural Intervention Insights, identifying three factors (four viewpoints), each with distinguishing combination of barriers and motivators suggests that campaigns with "one size fits all" strategy are ineffective and inefficient. Building on our analysis, we uncovered

Table 5

Consensus Statements

Statement	F1		F2		F3	
	Rank	Z Score	Rank	Z Score	Rank	Z Score
Those That Do Not Distinguish Between ANY Pair of Factors						
All Listed Statements are Non-Significant at P > 0.01, and Those Flagged with an * are also Non-Significant at P > 0.05)						
6 – I think there is no special need for organs for Asian, African, and Middle Eastern groups*	–3	–0.947	–3	–0.995	–2	–0.746
9 – I think I am too old to donate	–4	–1.47	–2	–0.89	–3	–1.02
13 – I believe there is a great need for organs especially in minority groups	2	0.955	1	0.58	3	1.06
27 – I do not mind organ donation but my family disagree*	0	–0.095	0	0.087	0	–0.211
36 – I believe the present need for transplant organs is fully covered*	–3	–1.171	–2	–0.827	–3	–1.022
40 – No matter how hard it is to think about organ donations, it makes me feel good about myself*	2	0.911	2	0.773	3	1.064

[17] Statement 39 – I believe organs are a gift from god, we are not allowed to give them away and 17 – If someone religious says it is not allowed, then I will not do it which is distinguishing statement for this factor, both on –5, 11 – I think it is non-religious to take organs, 43 – I think my religion encourages organ donation in order save other people's lives on –1 and a distinguishing statement and 1 – I believe my religion does not allow it on 0.

[18] Statement 13 – I believe there is a great need for organs especially in minority groups on +3, 6 – I think there is no special need for organs for Asian, African, and Middle Eastern groups on –2 and 36 – I believe the present need for transplant organs is fully covered on –3.

[19] Statement 5 – I think anyone can register and be a donor even if old or have a disease on +2 a distinguishing statement for this group, 9 – I think I am too old to donate on –3; registration process, 4 – I think the process of registration is complicated on –1 and transplantation results, 16 – I believe transplantation results are successful and they are improving people's health on +5.

[20] Statement 30 – I think people who have medical conditions cannot donate and 47 – I think I am not dead if my heart is still beating both on +1 and are distinguishing statements, and 22 – I think brain dead people can regain consciousness on +1.

[21] Statement 15 – I feel I cannot decide to donate because I do not know all the facts on –1 as a distinguishing statement.

insights on potentially effective intervention design for each factor.

Factor 1, The hallmark for this factor is a thirst for knowledge and information with perceived lack of knowledge. For this factor, behavioural change campaigns should focus on providing detailed information about organ donation. However, information should not focus on need (S15/+5), but rather on information about eligibility criteria and brain death. Eligibility criteria might exclude people suffering from certain diseases but not necessarily age (S9/–4). For example, campaigns should focus on the fact that you can still register and even donate even if you have an illness (S30/+4). The eligibility criteria on NHS website which enlists very few diseases that excludes donation, they are Creutzfeldt-Jakob Disease (CJD), Ebola virus disease, Active cancer, and HIV. One can donate organs even if they have had cancer (but not active) or even if they cannot donate blood.

Another important part of information is brain death. Campaigns should focus on the fact that brain death is irreversible, and the patient cannot regain consciousness (S22/+1) (NHS, 2019a) [16] using preferably expert opinion (S14/+1). Campaigns should also focus on the diagnostic criteria of brain death and shows that strict measures for brain death diagnosis eliminates the risk of misdiagnosis.

The campaigns message for this group should stay away from religious messages, family agreement, and easy registration process (although this does not apply top UK anymore with the opt-out system). One effect about opt-out system however, that is important to this group, it is important to highlight that despite the opt-out system, a potential donor will not be forced to donate organs. No organ will be harvested without the permission of the family, thus, the decision to donate is still held by the hands of the person (S24/+3) as participant 4586 explains "If I die and then doctors ask my family for my organs, may be my mother would be so sad she will say no, I want to give her that chance, to say no". Messages here should encourage to communicate decision to the family if one wants to be a donor.

Q methodology analysis for this factor show that knowledge is categorical, and the level of knowledge is irrelevant to the perception of knowledge level. People may perceive their knowledge level to be low despite potentially scoring well in a survey for knowledge level. It shows how perception is at the core of behavioural barrier to organ donation. That is a similar case for religion. People may hold different religions with similar views and vice versa. To address knowledge perception, interventions that are founded on self-efficacy theory can be most relevant to this group.

Factor 2, the hallmark of this factor is the hesitation and anxiety. For this factor, behavioural change campaigns should focus on real-life stories that inspire others to become a donor. However, campaigns messages should avoid evoking images of "wasted organs" (S38/+2) but rather visuals playing a nice emotional tune that fills the heart with warmth. Examples of emotionally stimulating have been implemented globally and in the UK (NHS, 2019c; Nicholas, 2017) [17, 18].

The campaigns message for this group should focus on emotionally attractive messages to encourage people to overcome their fear and decide to become an organ donor, especially promoting organ donation as a selfless noble act that will help save or improve people's lives. Messages involve positive religious views and religious leaders advocating for organ donation might be impactful. Campaigns promoting sharing decision with family might be helpful as well, especially if the family holds positive views as that would help ease the tension when it comes to considering donating.

This group shows anxiety as the main barrier to donation. Consistently, they show a great emotional reaction throughout the array. For this group, Terror Management Theory may be the most effective theory to be used in behavioural interventions. A study used this theory to alter organ donation behaviour showed that misconceptions mediate the relationship between death thoughts and organ donation intention, this study supports that finding and shows people loaded on this factor perceive their information on organ donation to be insufficient. Interventions to address the hesitancy and death anxiety in this groups should promote organ donation as a selfless noble act that will help save or improve people's lives.

Factor 3, The hallmark of this factor is the need for a cue for action suggesting that interventions based on Immediacy Theory may be most effective for this group. For this factor, behavioural campaigns should focus on providing information about allocation system and the laws that prevents unethical management of organs, this also includes highlighting if there are financial rewards for the donors, the nationality and race of the donors if possible. Complete transparency in the organ donation data on both ends; donation and transplantation are essential for this factor (S2/+2 and S34/+1). Other medical information regarding brain death definition is important too (S14/+2, S47/+1 and S30/+1).

The campaigns message for this group should maintain the organ donation is a selfless act (S7/+5) offering the gift of life (NHS, 2020) and improving the life of people in need, picturing donors as hero and asserting organ donation as the ultimate charitable act especially at certain holidays like Christmas and Eid (NHS, 2019a) [16]. this suggests that Self-Affirmation Theory by emphasising their roles as givers and their values such as selflessness to be effective for this group. Campaigns however should avoid religiously motivated messages and avoid awareness about the need for the organs as well (S13/+3 and S36/–3).

CONCLUSION

There is a chronic and severe shortage of donated organs in UK (NHS, 2018) [19] and a valid argument to a continuously increased demand (Cheetham et al., 2016; Jox, Assadi, & Marckmann, 2015) [20, 21]. There is a complex net of social, religious, and psychological barriers against organ donation, in addition to a potential lack of knowledge and awareness and a history of mistrust in the medical profession. Designing more effective interventions is crucial to increase donated organs.

We examined viewpoints on organ donation using Q methodology. Our results show four distinctive viewpoints. We make no claim to generalise the results for general population, instead, Q methodology examine the variations of views in the population. Our research suggests that people with different viewpoints are influenced by different behavioural change strategies, and we predict that interventions designed with these factors in mind will produce better outcomes than "one-size-fits-all" strategy.

Our data suggests that busting myths and improving knowledge level about organ donation is more effective for people loading on Factor 1, people loading on all other factors may benefit from different strategies that are seldom applied in focused and strategic ways. Moreover, the consensus statement on family agreement on rank zero implies that participants do not consider family disagreement as a barrier which contradicts the fundamental theoretical idea of theory of planned behaviour that is commonly used in the organ donation field. Taken together, the results indicate that there are many folds on the viewpoints about organ donation that we need to unfold. Further research should be conducted to assess the prevalence of each factor and experiments to validate the conclusions on the effective behavioural intervention designs.

The authors declare no conflict of interest.

REFERENCES

1. NHS. (2019b). Organ Donation Activity. Retrieved from https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/16420/section-3-organ-donation-activity.pdf.
2. *Feeley TH, Moon S-I.* A meta-analytic review of communication campaigns to promote organ donation. *Communication Reports*. 2009; 22 (2): 63–73.
3. Li AT, Wong G, Irving M, Jan S, Tong A, Ralph AF, Howard K. Community-Based Interventions and Individuals' Willingness to be a Deceased Organ Donor: Systematic Review and Meta-Analysis. *Transplantation*. 2015; 99 (12): 2634–2643. doi: 10.1097/tp.0000000000000897.
4. Miller J, Currie S, O'carroll RE. 'What if I'm not dead?' – Myth-busting and organ donation. *British journal of health psychology*. 2019 Feb; 24 (1): 141–158.
5. *Handley IM, Lassiter GD*. Mood and information processing: When happy and sad look the same. *Motivation and Emotion*. 2002; 26 (3): 223–255.
6. *Stefanelli A, Seidl R.* Opinion Communication on Contested Topics: How Empirics and Arguments can Improve Social Simulation. *Journal of Artificial Societies and Social Simulation*. 2017; 20 (4): 1–3.
7. *Greene J, Haidt J.* How (and where) does moral judgment work? *Trends in cognitive sciences*. 2002; 6 (12): 517–523.
8. *McKeown B, Thomas DB*. Q methodology (Vol. 66): Sage publications. 2013.
9. *Smith N.* Operant subjectivity: Objectivity of subjectivity. NW Smith, Current systems in psychology: History, theory, research, and applications Belmont, CA: Wadsworth/Thomson Learning. 2001.
10. *Watts S, Stenner P.* Doing Q methodology: theory, method and interpretation. *Qualitative research in psychology*. 2005; 2 (1): 67–91.
11. *Valenta AL, Wigger U.* Q-methodology: definition and application in health care informatics. *Journal of the American Medical Informatics Association: JAMIA*. 1997; 4 (6): 501–510. doi: 10.1136/jamia.1997.0040501.
12. *Stephenson W*. Consciring: A general theory for subjective communicability. *Annals of the International Communication Association*. 1980; 4 (1): 7–36.
13. *Brown SR*. Political subjectivity: Applications of Q methodology in political science: Yale University Press. 1980.
14. *Watts S, Stenner P*. Doing Q methodological research: Theory, method & interpretation: Sage. 2012.
15. *Ryan S, Carr A*. Applying the biopsychosocial model to the management of rheumatic disease. In Rheumatology (pp. 63–75): Elsevier. 2010.
16. NHS. (2019a). A lifesaving gift is on the Christmas list of thousands of people this year. Retrieved from https://www.organdonation.nhs.uk/get-involved/news/a-lifesaving-gift-is-on-the-christmas-list-of-thousands-of-people-this-year/.
17. NHS. (2019c). Real life stories. Retrieved from https://www.organdonation.nhs.uk/helping-you-to-decide/real-life-stories/.
18. *Nicholas R.* 6 Campaigns Using Emotion to Boost Organ Donations. 2017. Retrieved from https://www.mmm-online.com/home/channel/campaigns/6-campaigns-using-emotion-to-boost-organ-donations/.
19. NHS. (2018). Organ Donation and Transplantation – Activity figures for the UK as at 6 April 2018. Retrieved from NHS Blodd and Transplant Website: https://nhsbtdbe.blob.core.windows.net/umbraco-assets/1343/annual_stats.pdf.
20. *Cheetham OV, Thomas MJC, Hadfield J, O'Higgins F, Mitchell C, Rooney KD*. Rates of organ donation in a UK tertiary cardiac arrest centre following out-of-hospital cardiac arrest. *Resuscitation*. 2016; 101: 41–43. doi: https://doi.org/10.1016/j.resuscitation.2016.01.003.
21. *Jox RJ, Assadi G, Marckmann G.* Organ Transplantation in Times of Donor Shortage: Challenges and Solutions (Vol. 59): Springer. 2015.

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ANALYSIS OF IMPLICATIONS OF ORGAN DONATION ON LIVING DONORS IN SOUTHEASTERN IRAN: A QUALITATIVE STUDY

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Objectives: despite the annual increase in living donors and the positive and negative implications following organ donation, this issue had become a significant challenge for donors. The present study aimed to analyze the experiences and views of living donors to organ donation implications. **Material and Methods.** The present study was performed using qualitative content analysis. Twenty participants were selected using the purposive sampling method; data were collected by semi-structured interviews and analyzed based on Lundman and Graneheim contractual content analysis method after implementing MAX 12. **Results.** Data analysis elicitated 721 codes, 20 subcategories, six main categories, and two themes, including positive and negative implications of organ donation from the viewpoint of living donors. The main categories of positive effects resulting from organ donation included the "donor's peace of mind", "fundamental strength", and "recipient's achievements". On the other hand, the main categories of negative implications resulting from organ donation included "donor's physical suffering", "damaged interactions", and "abandonment". **Conclusion.** Increasing the number of living donors makes us consider it essential to understand the efficiency of its two-way implications on many aspects of donor and recipient. Thus, managing the negative impacts of living organ donation and strengthening its positive side emphasizes the need to increase the awareness of organ donation associations, develop health policies at higher levels, and, most importantly, improve the satisfaction of live organ donors.

Keywords: qualitative study, implications, living donors, organ donation.

INTRODUCTION

In the current century, significant advances in providing health care services have shifted the pattern of disease to non-contagious diseases. These advances have led to an increase in life expectancy, life span, followed by the spread of chronic diseases and organ failure [1]. Iran is one of the 23 countries with a high burden of these diseases and low and middle income [2]. Organ transplantation is the best treatment for patients with end-stage organ failure. However, the demand for transplanting organs does not match the supply of living and deceased donors [3]. The lack of organs for transplantation is one of the major problems worldwide [4]. This deficiency is much more severe among Black and Asian societies than in European ones [5]. The unavailability of the donated organ is a global concern, as in most cases, this donation takes place when a person is brain dead [6]. Obviously, deceased donors cannot meet the growing demand, especially for organs such as the kidneys, and their families may not even be consent to organ donation [7]. For this reason, voluntary organ donation attracted the attention of living people all over the world, including Iran. This donation can include organ donation during the wellbeing period and voluntary consent to donate organs after death by receiving an organ donation card [8].

Dong et al. (2011) stated that the transplantation of living organs has more benefits. For example, the liver is an organ that possesses the capacity to regenerate. In particular, after transplantation of a living organ, the donor and recipient's liver regrow and regenerate to complete organs. In terms of survival and transplant rejection, transplantation of living organs is better than that of dead organs [9].

Regardless of the benefits of living organ donation to the recipient, living donors may experience many positive and negative implications after donating an organ. For example, a recent literature review about these implications on living liver and kidney donors has shown that generally, they feel optimistic about the organ donation experience and are not regretful. They have a high level

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of health related quality of life (HRQOL) [10, 11]. Some other studies have suggested that a considerable number of living donors experience psychosocial problems after donation. For example, one study found an increase in detectable psychiatric disorders among one in four living donors, including cases without a history of pre-donation disorders. In this analysis, at most one-third of donors reported poor health conditions or significantly got worse than before the donation, and persistent fatigue and pain were relatively common complaints [10]. Others agree on considering the potential benefits of donating to living kidney donors. They agreed that organ donation candidates should be aware of the risks and benefits of donation despite the confirmation of guidelines; there is, unfortunately, no scientific evidence on the benefits of live donation [12, 13]. However, some studies showed that the experience after transplantation, the same as a new chronic condition, leads to an uncertainty about the future and affects life accommodation [14].

Despite the growing number of living donors, few qualitative studies measured the implications of organ donation. The present study examined the positive and negative implications of organ donation from participants' perspectives in southeastern Iran's cultural context, using a qualitative approach and in-depth analysis of the phenomenon from the standpoint of living donors regarding the high frequency of organ donations in this region. It directs the community's policies toward describing potential risks and enhancing its benefits for potential donors when making informed decisions and informed consent.

MATERIALS AND METHODS

Study design and setting

A qualitative approach of contractual content analysis was used to explain organ donation's impact on living donors, applying purposive sampling of living organ donors in the southeastern part of Iran. In the present study, samples were referred to the organ donation center, Kidney and Bone Marrow Donation Commission in Afzalipour hospital, and Kidney Donation Association. The interview place, chosen by participants, was cozy and comfortable so that people safe during the interview (hospital, private home, park, nursing school, etc.).

Participants

In the present study, a total of 20 participants, including 16 organ donors, one member from the family of the donor, one organ recipient, one surgeon from the Organ Donation Commission, and one psychologist, were studied. Nine of the donors participating in the study included kidney donors, five were non-related (for sale), and four were related (not for sale). Four of them had bone marrow donations, one non-related (for sale) and three related (not for sale), and the remaining three donors, who were related (not for sale), donated a portion of the liver.

Data collection

The collected data analysis determined the number of participants; sampling continued without any restrictions until all levels and codes were saturated and completed. Proper communication was established between categories. The first author conducted interviews. However, all the researchers reviewed the interviews like an outside supervisor. After each interview, the researchers studied the interviews, identified the interview's strengths and weaknesses, and reviewed the items considered in the following interview. According to the written reminders, the proposed questions required researchers to refer to two participants for the interview during the analysis of the interviews. Two interviews were conducted with participants 2 and 1. Researchers conducted a total of 22 interviews with 20 participants. The interview questions centered on the implications of organ donation in living donors. First, the interview started with open questions like "Would you mind sharing your experience of positive and negative implications of the organ donation you did?", then a follow-up question was asked to clarify the concept. The interview took 45–90 minutes. At the end of the interview, participants were given the interviewer's mobile phone number and asked to discuss any issues with the interviewer if they remembered any of the implications of organ donation and the possibility of further interviews. Finally, participants were appreciated with a small gift.

Analysis

Data collection and analysis were performed simultaneously. The MAXQDA.12 used to facilitate organization and comparison of the data. The transcription of each interview was reviewed several times. The qualitative data content analysis process was performed according to the method proposed by Graneheim and Lundman, including writing the entire interview, reading the entire text of interviews several times to achieve a general understanding of its content and immersion in the data, determining semantic units and summarizing them, extracting the primary codes, classifying the similar primary codes under the same subcategories, classifying similar codes under more comprehensive categories, extracting latent and manifest concepts from the data, and formulating the final themes [15]. To this end, after preparing the transcriptions, each text was reviewed several times. Later, the semantic units were identified based on the research questions and appropriate codes were written for each semantic unit. As shown in Table 1, the preliminary codes were categorized and labeled based

on their conceptual similarity (subcategories). The subcategories were compared and placed under the main categories, which were more abstract (categories). The main categories were categorized under a more abstract concept (theme). All extracted codes and categories were reviewed and approved by the second and fifth authors of this study. The initial extracted codes were reduced by continuous data analysis and comparison; finally, the categories and subcategories were abstracted. Lincoln and Guba criteria (credibility, dependability, confirmability, and transferability) were used to ensure The data trustworthiness [16]. To ensure the results credibility, participants were asked to confirm the extracted codes from the interview and resolve the contents on demand (member check). Data-source triangulation from interviews with family caregivers with variety in relationship with patient, ethnicity and religion established credibility. Regarding confirmability of the findings, all texts of the interviews, codes, and categories were reviewed and confirmed by the second, third, and fifth authors of this study (peer check) as well as a faculty member outside the research area (faculty check). To ensure the dependability of the results, all stages of the study were recorded. Participants were selected by maximum variation sampling in terms of ethnicity, level of education, religion, economic status, relation to the patient, and social class, which enhanced the transferability of the study.

Ethics approval and consent to participate

To observe ethical considerations, the researcher asked participants to complete the informed consent form, and before starting the interview, they were allowed to record audio and take notes. They were assured all demographic information would remain confidential. After the final report, the audio files would be removed, and, if desired, they could obtain the audio file of the interview from the researcher and be informed of the overall results. Participants were reassured that they were free to leave the study at any stage of the study. The Ethics Committee of Kerman University of Medical Sciences approved this study with the code of IR.KMU.REC.1398.222.

RESULTS

A total of 20 participants, including 16 organ donors, one donor family member, one organ recipient, a surgeon member of the donation commission, and a psychologist included in the present study. Participants were in the 26–58 age range. Table 1 displays other participants' features including gender, marital status, education level, age, etc. Based on the participants' statements about organ donation implications, we extracted 721 codes, 20 subcategories, six main categories, and two themes (Table 2). Table 3 also shows the narrations of the participants according to the subcategories.

Positive Implications of Organ Donation

The Peace of Mind

The first major category of positive implications was living donor's "peace of mind". Based on a thorough analysis of interviews with living donors, this category includes the subcategories of "Donor's Sense of calm and Satisfaction", "Satisfaction of donor's Spiritual Needs", "Improvement of donor's Economic status", and "Donor's Adaptation and acceptance and his family with a donation".

Donor's Sense of Tranquilization and Satisfaction: This subcategory was more evident in related donors. Participants felt disburdened after donating, thus leading to peace of mind and happiness. Some participants considered donation an honor and never regret it. They stated that their family members pay attention to them more than before, which leads to strengthening their relationship and finally their satisfaction.

Satisfaction of donor's Spiritual Needs: Donating an organ is a humanitarian and God-pleasing act and can save the lives of patients who need organs or transplanted tissues due to having various diseases. Hence, some participants showed great interest in filling the form of transplant cards. Participants described it God-pleasing act and anticipated a reward in the other world. Some of the participants who donated the organ for charitable purposes stated that they felt light, disburdened, and satisfied.

Improvement of donor's Economic Status: Due to the economic plight governing the society, selling the organs in towns has increased. We see Organ For Sale Ads distributed in social networks beside urban graffiti. Improving the donor's economic state is what some donors consider as a positive consequence of organ donation.

Donor's Adaptation and acceptance with a donation: When a person steps into the process of acquiring a new identity, he or she may anticipate and experience many challenges and concerns along the way. What awareness the donor face or what reaction their family member show facilitates the acceptance. In this regard, we have seen better acceptance and adaptation in related donor families and non-related donor families who did this great job to save their family's lives.

The present study showed that most related donors felt satisfied, peace of mind, acceptance, and adaptation, but non-related donors did not feel them. However, improving the economic state was seen more in non-related donors in the early days due to receiving money from the receivers; and in the long term, due to compensating for the hard-working and suffering of patient's family by the receivers in related donors. Spiritual needs of

both non-related and related donors of kidney and bone marrow were satisfied.

Donor's Fundamental Strength

The second major category of positive implications of organ donation, in living donors, is the fundamental strength of the donor. This main category includes three subcategories of donor's "Paying more attention to life", "Taking more care of the physical condition", and "Resistance against the difficulties".

Paying more attention to life: This is the first and most leading subcategories. Evidence has shown that those who experience life-threatening diseases appreciate the renewed life.

Taking more care of physical condition: Because these people once experienced illness and transplantation, they prefer to take more care and pay more attention to their physical condition so that they do not get sick again. Post-donation self-control and self-care are positive aspects.

Resistance against the difficulties: A number of donors believed, it is normal to have troubles, unhappiness, and failures in our lives. Problems and inconveniences arise in people's lives and are not specific to one person. When people face problems and losses, they have to solve them to the best of their abilities. Life has its ups and downs and is not always meant to be. Sometimes the hardships and misfortunes grow and evolve us. This increases capacity, patience, and forbearance. Both related and non-related donors of kidney, liver, and bone marrow experienced these subcategories. They were more intense in related donors.

Table 1

Participants demographic characteristics

Row	Gender	Age	Education	Economic state	Marital status	Donation	Period	Donation state	Relation with recipient
1	Female	30	Bachelor's Degree	average	single	kidney	1 year	not for sale (related)	sister
2	Male	35	Master's	poor	single	kidney	1 year	not for sale (related)	brother
3	Female	33	Diploma	poor	others	kidney	5 years	for sale (non-related)	non-related
4	Female	27	Bachelor's Degree	good	single	kidney	5 months	not for sale (related)	sister
5	Female	43	Master's	good	others	bone marrow	8 months	not for sale (humanitarian aids)	non-related
6	Male	45	Diploma	good	married	bone marrow	4 months	not for sale (related)	father
7	Male	28	Diploma	poor	single	bone marrow	2 years	for sale ads (non-related)	non-related
8	Male	36	Bachelor's Degree	poor	married	kidney	6 years	for sale by organ donation association (non-related)	non-related
9	Female	35	illiterate	poor	married	kidney	10 years	not for sale (related)	mother
10	Male	38	Bachelor's Degree	average	married	liver	2 years	not for sale (related)	father
11	Male	32	Diploma	good	others	liver	1 year	not for sale (related)	mother
12	Male	40	Diploma	average	single	kidney	5 years	for sale by organ donation association (non-related)	non-related
13	Male	56	Bachelor's Degree	average	married	kidney	15 years	for sale by organ donation association (non-related)	non-related
14	Female	29	Master's	good	single	liver	11 months	not for sale (related)	sister
15	Male	40	Diploma	average	others	kidney	7 years	for sale by organ donation association (non-related)	non-related
16	Female	46	Diploma	good	married	bone marrow	1 year	not for sale (related)	mother
17	Male	56	Super-specialized	good	married	–	–	–	Surgeon
18	Female	38	PhD	good	married	–	–	–	psychologist
19	Female	34	Diploma	poor	married	–	–	–	donor's family member
20	Male	26	Bachelor's Degree	average	single	–	–	–	recipient

Table 2

Theme, Categories and subcategories extracted from the data

Theme	Main category	Subcategory
Positive consequences of organ donation	Peace of mind	Donor's sense of tranquilization and satisfaction
		Satisfaction of donor's Spiritual Needs
		Improvement of donor's Economic status
		Donor's Adaptation and with a donation
	Fundamental strength	Taking more care of the physical condition
		Paying more attention to life
		Strengthening the donor against the difficulties
	Recipient's achievements	Recipient's Mental state Improvement
		Recipient's more attention to life
		Reducing the time limits of disease
		Recipient's More Accomplishments in life
Negative consequences of organ donation	Physical suffering	Change in the donor's body image
		Physical effects on the donor
	Damaged interaction	The emotional gap in married life
		Threats and the collapse of intimacy
		Chaos and differences in relationships
	Abandonment	Donor's psychological disorders
		Donor's Fear of the unknown future
		Regret for the decision to donate
		Economic, social, educational collapse on the donor

Table 3

Examples of quotes from study participants

Subcategories	Participant Narratives
Donor's Sense of Tranquilization and Satisfaction	*"We were all happy after the donation. I had a strange feeling after donating my kidney, as if I was feeling peace and calm. I was feeling on top of the world. I had never experienced it before. "It was a calm after a storm." (P1).* *"I feel intimate with my partner more than before, Elahe tells me that she feels a piece of my organ in her body, and she is into me. I am not regretful; It was the best decision I had ever made. I talk about it with honor." (P4).*
Satisfaction of donor's Spiritual Needs	*"By doing so, I proved to myself and my children that this world is transient and hereafter is important, and God gives me a hand. I felt I took one of thousand responsibilities God assigned me." (P5).* *"In our religion, just as taking the life of a human being is highly condemned, giving life to people is also highly valued. I strongly believe in this expression. By doing so, I feel released from concerns of daily life" (P6).*
Improvement of donor's Economic Status	*"I could clear my debts. At least I did not feel ashamed in front of my family. I did not have any mental concern" (P15).* *"I could run a business, it was not so profitable, but I am happy with it." (P7).*
Donor's Adaptation and acceptance with a donation	*"Before making this decision, I was anticipating these days, so I tried to deal with the issue of living with one kidney, so that this issue would not be challenging for me in the future, and thank God, My family and I accepted this issue, and I cope with it easily." (P4).* *"I sold my kidneys in a tough situation and my wife supported me greatly. Even after the donation, she paid more attention to me physically and mentally; I feel better and better. As she accepted this matter, I could deal with it." (P15).*
Paying more attention to life	*"Sometimes I find this event a flip, believe it or not, I don't waste my times after donating, and I plan for every moment of life" (P14).*
Taking more care of physical condition	*"I am careful of my about healthy nutrition and safety. For example, in the fall, I will get the influenza vaccine. Thank God I did not face any problem." (P8).* *"My sister and I are much more careful about our health than before. We strengthen ourselves. We try to stay far away from someone who has an infectious disease because now our body system is more vulnerable." (P4).*
Resistance against the difficulties	*"Now I do not make life hard as I did before and am not greedy for many things. I don't get tired with any difficulty. I am extremely patient now". (P11).* *"I stand on my own feet now. I think I will be able to cope with many problems alone, either economically or psychologically and ...". (P12).*

End of Table 3

Subcategories	Participant Narratives
Recipient's Mental state Improvement	*"I remember exactly a few days after the donation, that his mental state had improved. He was no longer sad. I never imagined that he would get better quickly and get out of his loneliness." (P2).* *"I saw my patient's wellness. They were no longer as frustrated and depressed as they were during the disease period", said the psychologist. (P18).*
Recipient's More Accomplishments in life	*"My sister has won a place in chess and tennis and even won a medal. She is very successful at work as well." (P1).* *"I promised that if I returned to normal life, I would work hard and be able to compensate for my family's attempts", said one of the participants, who was a recipient and related donor (P20)*
Recipient's more attention to life	*A related participant who is close to the recipient stated: "When he talks to relatives and acquaintances, he advises them not to waste their life and appreciate it. He keeps repeating these words at home." (P16).* *the experience of the organ recipient was as follows: "I no longer need to follow a strict diet, I am more active, more outgoing, now I feel like I live like other people." (P20).*
Changes in the body image	*"Now, when I see the scar on my body, I feel bad. In the early days, I felt malformed, and I was upset. Or if someone saw my scars, I would tell them that I fell and wounded." (P15).* *"I think because I am different financially and physically from the others, it has caused me to lose my self-confidence." (P8).* *"I think I am entirely different from others. This weakens my morale. I feel a change in people's behavior to me. Is it a true feeling, or I became crazy?" (P3).*
Physical Complications	*"Although I underwent transplantation long ago, sometimes I feel pain on my scar; I went to the doctor several times, had a general examination and sonography, and finally told me that you have no problem, another doctor told me that it could be caused by damage and you have to put up with it." (P10).* *"Evidence has shown that patients undergoing kidney transplantation may have long-term complications such as high blood pressure, diabetes, etc.", the participant surgeon said. "Therefore, they should be monitored for a long time and given the necessary training." (P17).* *"When I researched the complications of kidney donation, I got information about the risk of death, not about the physical damage." (P 9).*
The emotional gap in married life	*"After the operation, my wife distance herself from me. She did not express her feelings at all. She did not say anything. Later, I found out that she was under the care of a urologist and she is physically malformed." I turned the blind eye. I was damaged emotionally." (P19).*
Chaos and differences in relationships	*"Every time I dispute with my wife, she blames this as my weakness. It makes me even more nervous. We were on the verge of getting divorced once." (P13).* *"My wife did not have the slightest idea of my donation when she found out, e had conflicts and dispute. I am regretful. Although I am happy with this act, I would consult my wife if I returned." (P15).* *"I objected because I did not want my husband to be defective and a friend and acquaintance would tease me. Although I turn a blind eye, I blame him sometimes (P19).*
The threat of intimacy	*"I lost my favorite girl because I told a lie to her about it. At first, I thought she would get angry and come back, but she left me forever. So sometimes I say, 'I wish I had not done it' Now I think I cannot start a family." (P12).*
Fear of the future	*"I'm afraid of any possible problem in the future because I am a man and I have to work. If I get exhausted, my family do nothing", said one participant. (P12).* *"Before making a decision, I did not think about the future at all, I thought only about the present, I wanted to make those conditions better, but now after a few years, I am regretful. I know I was under a lot of pressure at the time, and I really could not make the right decision, I could not collect the correct information because I did not have much time." (P3).*
Economic, social, and educational collapse	*"The tests and visits were costly for me because I was not insured. Unfortunately, the recovery process took a long time because of the extreme stress I had. I am the head of my family." (P9).* *"I did not attend the classes for a while due to the situation at home, my sister's illness, and the decision I made for the donation. I was excluded from the class because of my frequent absence. I got fired from a part-time job due to troubles we go into." (P4).*
Mental disorders	*"After the operation, I was completely depressed. I am no longer as happy as before. I am not hopeful for life. I just got better. The first few months after I had severe depression, I was targetless. I used to go mountain climbing and travel, but now I don't even like to attend family events." (P13).* *the psychologist believed: "Donors are not physically monitored, except for some alarming cases. Psychological symptoms are like flames in the ashes, which, if not constantly monitored, can lead to more severe mental disorders." (P18).*

Recipient's achievements

The last category of positive implications of organ donation in living donors was the achievements of the recipient. Because we used theoretical sampling, our participants included organ recipients, psychologists, and donor families. In the present study, most participants were related donors, regularly interacted with the recipient, and saw the implications of organ donation clearly in him, so this main category was elicitated from their conversations. It includes four subcategories of "Recipient's Mental state Improvement", "Recipient's More Accomplishments in life", "Recipient's more attention to life", and "Time constraints reduction of diseases".

Recipient's Mental state Improvement: Recipients experience lots of pressure and stress due to enduring the disease's stressful conditions and involving family members in similar situations. Thus, it causes severe psychological problems. Related donors, friends, relatives, co-workers, psychologists, and even the recipient believed in improving the mental state after the operation was successful. The psychologist participating in the study also agreed with the patient's family members.

Recipient's More Accomplishments in life: Most organ recipients became successful in various life areas after the transplantation. The reason for their belief in working hard in life is to compensate for the wasted time.

Recipient's more attention to life: Another subcategory of recipient achievements is the recipient's more attention to life, which was also the case with organ donors.

Reducing the time limits of disease: During the illness, we hear about restrictions on diet, physical, recreational, and social activity in interviews with the patient and his relatives. These restrictions greatly affected the patient and those around him. Organ transplants significantly reduced the limitations of the disease.

Negative Implications of Organ Donation

Despite the positive implications of living organ donation, participants believed that the other side of the spectrum was dark. It is clear that the expansion of living organ donation, like any other phenomenon in the treatment system, along with undeniable achievements, has undesirable implications. The negative implications of organ donation include "donor's physical suffering", "damaged interactions" and "Donor abandonment".

Donor's physical suffering: The first category of perceived negative implications of living-organ donation is donor's physical suffering, which includes two subcategories of "Changes in the body image" and "Physical effects".

Changes in the body image: Many participants believed that organ donation caused them unforeseen physical experiences. This mental image results from sensory perceptions over the years, social interactions with other people, and responses. The body image changes with the gradual body change over the years. Any change in the body image of the body seriously disturbs the balance of the person. These changes can result from disease, accidents, or evolutionary changes in the body's structures and function. People are distressed by the slightest change in appearance or bodily functions. Significant changes can be devastating. Some people express their feelings easily and freely in such cases, but others even refuse to look or touch the area. Some people are so preoccupied with a change in their body's mental image that they become depressed or resort to self-destructive behaviors.

Physical Complications: Participants believed that organ donation caused unpredictable physical complications for them. In this regard, doctors in this study pointed to the unforeseen short-term and long-term complications of organ donation.

Damaged interactions

The second category associated with perceived negative implications is damaged interactions, which included three subcategories of the emotional gap in married life, intimacy threats, conflict, and turmoil in the relationship. This category was more common in non-related donors.

The emotional gap in married life: Participants believed that organ donation caused an emotional gap following marital dissatisfaction in their lives.

Chaos and differences in relationships: Some donors had donated organs without consulting them due to the possible opposition against donations by the family. When their family learned this issue, implications such as chaos, quarrels, and disputes would arise. Family members of some patients saw organ donation as a defect in organs and a factor in labeling others, which they believed caused family members to be embarrassed by friends and acquaintances.

The threat of intimacy: Loss of intimacy and closeness between family members, especially spouses, was an important implication of organ donation.

Abandonment: The third category was related to the negative implications of organ donation in living donors. This category was elicited from the participants' statements. They stated that there was no monitoring after the donation, including three subcategories of "occurrence of psychological problems in the donor", "fear of the future", "regret for donating", and "the economic, social and educational collapse".

Fear of the future: Most participants with different intensities expericed fear of the future. This fear was more prevalent among non-related donors. It was evident in related donors whose transplant was rejected but was

more accepted by the related donors, arguing that the transplant would be done to save the life of their beloved ones and they acted resorting to God. A small number of organ donors regretted the decision sometime after the organ donation. They believed that the decision was made in a critical situation and alone; perhaps a safer decision would have been made if there was enough time.

Economic, social, and educational collapse: Another subcategory was donor abandonment after the donation that was more evident in related donors. Participants believed that no insurance covered their costs, and they were completely ignored after the donation in many ways, which disrupted their lives after the donation. This was more evident in poor donors.

Mental disorders: Several participants believed that they experienced different symptoms after donating an organ. These symptoms were more severe in non-related donors, while the related donors, except in cases of forcible donation (lack of time to find a suitable donor, physician's recommendation due to multiple rejections of recipient's transplantation) did not have symptoms. Psychologists believe that donors get mental disorders after organ donation due to lack of monitoring and sudden abandonment.

DISCUSSION

The present study aimed to investigate living donors' experiences and views about the implications of organ donation. One of the positive implications of organ donation from the participant's perspective was "donor's peace of mind". In this regard, Rasmussen et al. Stated that donors experienced a sense of tranquility and reduced anxiety after donation. The donation has strengthened their relationship, caused a positive change, dynamism, and fortified the whole family [17]. These donors believe that the pleasure of seeing the recipient's everyday life compensates for the donor's adverse experiences, besides the appreciation by the recipient's family and constant respect from others makes the donors feel proud and privileged [18–20]. In contrast, some donors express dissatisfaction that their recipient does not appreciate them [21].

In the present study, many related donors described the donation as an honor and believed that they would not be regretful in the future. Donors consider the increased attention and care of other family members to improve relationships and their satisfaction. From the spiritual perspective, recent studies consistent with the present study have shown that this decision has philosophical or spiritual nature and strengthens spiritual beliefs [17, 19, 22]. From an economic point of view, although some studies report financial problems after organ donation [23–25], others suggest the improvement of the economic state. In this regard, a qualitative study showed the recipient's back to work and a decrease in donor's responsibility [17].

However, related donors had difficulties in making a living. They had financial problems due to the prolonged recovery period after donation, the cost of surgery and tests, and even job loss. After recovery, they became motivated to return to life; thus, their economic state improved (with more intensity in the related liver and kidney donors). On the other hand, the improvement of economic state was evident in the non-related donors in the first days of donation, after receiving financial assistance from the recipient's family. In this category, we saw donors' acceptance and adaptations. They accepted the possibility of danger in the future and did so by trusting in God. In another study, donors decided that they would live with the implications, no matter what happens to them, and that the donor's risk and implications would be acceptable to them with the prospect of improving the recipient's life. The donor was adapted to it mentally and physically [18].

The second category for positive outcomes was donor's "fundamental strengthening" and was more experienced in related donors. Previous studies, consistent with the present study, have shown that donation caused a change in donor's viewpoints to life [26], increased self-esteem [19], personal growth [27], feelings of success and pride [28, 29]. They felt no physical difference [26, 28, 30]. According to a qualitative study, donors believed that donation led donors to pay greater attention to their physical condition, independence, improved social life, and return to normal by related donors [17]. In contrast, in our study, most related donors noted that donation was a turning point in their lives. Most donors returned to life at an even higher level than the pre-donation period. The opposite was true for some non-related donors.

The last category was the positive implications of organ donation, the "achievements of the recipient", and was more intense in recipients who received organs from a related donor than those who received it from a non-related donor. However, some studies have negatively described the overall experience of donating. They showed that some recipients experienced stress, symptoms of depression, or anxiety, reported to occur despite the desired medical outcome [21, 29]. It can result from stress, adaptation and effects of steroids, so it affects their relationships. These recipients said that despite the successful transplantation, they did not feel stronger or better due to comorbidities such as diabetes and thought that they wasted the donor's effort and kidneys so that the donor and other family members blamed him [21]. Another study reported the significant effect of transplantation on health for both the recipient and his family [30]. Rasmussen's qualitative study addressed the ending up of limitations (dialysis, diet, and reduced impact on the family), returning recipient to full-time work, handling

more tasks and responsibilities, and then reducing the care and financial burden of the donor as well as the recipient's potential to participate in activities such as hanging out with friends, eating out, and gaining independence. The present study stated that the donor's and recipient's lives had excelled to a higher level than before the operation (routine) [17]. Organ recipients had great success in various areas of life after the transplantation. Most of them agreed on compensating for the wasted time resulting from their diseases and all efforts of their family and donor.

Negative Implications: The first category of negative implications of organ donation is "physical suffering", which was equally evident in all donors. Other studies have shown that common concerns of liver donors include bloating, shrinking of the Muscle tone [11], fatigue [11, 20], abdominal pain, back pain, or interfering pain [20]. In contrast, kidney donors showed an increased risk of gestational hypertension or preeclampsia after donation compared to non-donors [31–33]. Another study showed that body image-related concerns were low in kidney donors [34] but evident in liver donors [11].

The second emerging category of negative implications is "damaged interactions". The items mentioned in the present study were primarily experienced in non-related donors and men. This category was manifested in sexual and emotional disorders and even chaos in living donors' normal relationships. However, previous studies have shown that even some related donors experienced increased family relationships and tensions, even years after donation [18, 29, 35]. It was not evident in related donors due to family intimacy and informed pre-donation decisions. Some studies suggested that the relationship between donor and recipient has remained constant or even sometimes improved [19, 28, 30]. Halpern et al. confirmed that sexual dysfunction is common among living kidney donors [21, 36]. Other studies have shown that related donors who donate their organs to spouses describe an improvement in their marital relationship resulting from the donation. Participants were closer to their partners and reported that kidney donation strengthened their relationships and family [17, 21]. Di Martini et al. showed a positive change in relationships even after donating a living liver. In this study, marital, family, and recipient relationships were improved after donation, respectively [20]. This study's results are inconsistent with those of the present study due to the kinship relationship between the donor and recipient.

The last category of the negative implications was "Donor abandonment." A qualitative study showed that donors trusted physicians' master to monitor their health and medical risks and appreciated medical follow-up. Donors believed that they felt safe and valued when the hospital followed up on their condition regularly [18]. Regarding follow-up, another study reported that most donors expressed satisfaction with the care received after medical follow-up, but some donors expressed frustration with unfulfilled expectations from health professionals [28]. Although the present study showed that participants trusted in physicians before donation, they mentioned that patients were left alone after donation. It leads to a physical defect, psychological disorders, fear of the future, and feelings of regret for the donation that affected other aspects of life, such as economic, social, and educational collapse. Other studies have shown that donors feel positive about the experience of organ donation and show little regret for donating [10, 11].

Furthermore, donors unanimously agreed on making such a decision again [19, 30]. In the study of Meyer et al., none of the participants regretted their decision [18]. Perhaps this difference results from non-related donors' presence in the present study, which was mentioned as a study limitation in Meyer's study. Some studies showed that many living donors experienced psychosocial problems after donation [10, 37, 38]. The present study suggested that these problems were more common in women than in men, and so did Erim et al. in their studies [39].

In general, recent studies have emphasized the importance of pre-and post-donation evaluation. The transplantation team should pay attention to donors' emotional state and quality of life, especially those with chronic diseases or poor perception [40].

Limitations: The present study had several limitations based on which the implications should be interpreted. First, this study was conducted in southeastern Iran, so cultural beliefs, economic, and even educational problems in this region may cause difficulty in generalizing the results to the other areas. However, it was attempted to include participants with maximum diversity of socio-cultural, work experience, and different educational levels, which has made the results of the study applicable widely in similar units. Second, the analysis was performed during the pandemic; only those whose recovery had long been passed and reached a stable condition were included in the study due to their high-risk conditions. Therefore, it is recommended to perform future studies on living donors, especially in the early days after transplantation and due to corona's impact on their decision and other concerns.

CONCLUSION

Based on the present study results, understanding the implications of organ donation is like a double-edged sword that can be interpreted positively or negatively from the donor's perspective. In this study, the negative implications were primarily observed in non-related donors and those who decided under emergency conditions, while the positive implications were observed in related donors who were close to the recipient and knew his problems. However, there were fewer negative and

positive implications in related and non-related donors, respectively. Commenting on this issue requires further studies on both groups of donors.

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REFERENCES

1. *James SL, Abate D, Abate KH*. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*. 2018; 392 (10159): 1789–1858.
2. *Kilpi F, Webber L, Musaigner A et al.* Alarming predictions for obesity and non-communicable diseases in the Middle East. *Public health nutrition*. 2014; 17 (5): 1078–1086.
3. *Lai Q, Vitale A, Iesari S et al.* The Intention-to-Treat Effect of Bridging Treatments in the Setting of Milan Criteria – In Patients Waiting for Liver Transplantation. *Liver Transplantation*. 2019; 25 (7): 1023–1033.
4. *Beard TR, Kaserman DL, Osterkamp R.* The global organ shortage: Economic causes, human consequences, policy responses: Stanford University Press; 2013.
5. *Wise J*. Organ donation: opt-out system should be in place by 2020 in England. *BMJ: British Medical Journal (Online)*. 2018; 362.
6. *Popoola AA, Olanrewaju TO, Bolaji BO et al.* Expanding renal transplantation organ donor pool in Nigeria. *Saudi Journal of Kidney Diseases and Transplantation*. 2018; 29 (5): 1181.
7. *Smith SW, Nazione S, LaPlante C et al*. Living kidney donor decision making and communication. *Journal of health communication*. 2011; 16 (8): 870–888.
8. *Berkman B*. Organ Donor Card Effectiveness. *AMA Journal of Ethics*. 2002; 4 (8).
9. *Dong Y, Zha Q, Zhang H et al.* Consensus reaching in social network group decision making: Research paradigms and challenges. *Knowledge-Based Systems*. 2018; 162: 3–13.
10. *Dew MA, Zuckoff A, DiMartini AF et al.* Prevention of poor psychosocial outcomes in living organ donors: from description to theory-driven intervention development and initial feasibility testing. *Progress in Transplantation*. 2012; 22 (3): 280–292.
11. *Parikh ND, Ladner D, Abecassis M et al.* Quality of life for donors after living donor liver transplantation: a review of the literature. *Liver Transplantation*. 2010; 16 (12): 1352–1358.
12. *Van Pilsum Rasmussen S, Henderson ML, Kahn J et al.* Considering tangible benefit for interdependent donors: Extending a risk – benefit framework in donor selection. *American Journal of Transplantation*. 2017; 17 (10): 2567–2571.
13. *Allen M, Abt P, Reese P.* What are the harms of refusing to allow living kidney donation? An expanded view of risks and benefits. *American Journal of Transplantation*. 2014; 14 (3): 531–537.
14. *McCormick KM.* A concept analysis of uncertainty in illness. *Journal of Nursing Scholarship*. 2002; 34 (2): 127–131.
15. *Graneheim UH, Lundman B*. Qualitative content analysis in nursing research: concepts, procedures and measures to achieve trustworthiness. *Nurse education today*. 2004; 24 (2): 105–112.
16. *Anney VN*. Ensuring the quality of the findings of qualitative research: Looking at trustworthiness criteria. 2014.
17. *Rasmussen SEVP, Robin M, Saha A et al.* The Tangible Benefits of Living Donation: Results of a Qualitative Study of Living Kidney Donors. *Transplantation Direct*. 2020; 6 (12).
18. *Meyer KB, Bjørk IT, Wahl AK et al*. Long-term experiences of Norwegian live kidney donors: qualitative in-depth interviews. *BMJ open*. 2017; 7 (2): e014072.
19. *Brown JB, Karley ML, Boudville N et al.* The experience of living kidney donors. *Health & social work*. 2008; 33 (2): 93–100.
20. *Butt Z, DiMartini AF, Liu Q et al.* Fatigue, Pain, and Other Physical Symptoms of Living Liver Donors in the Adult-to-Adult Living Donor Liver Transplantation Cohort Study. *Liver Transplantation*. 2018; 24 (9): 1221–1232.
21. *Ralph AF, Butow P, Craig JC et al.* Living kidney donor and recipient perspectives on their relationship: longitudinal semi-structured interviews. *BMJ open*. 2019; 9 (4): e026629.
22. *Walsh A*. Living kidney donor experiences: implications for counselling. *EDTNA-ERCA Journal*. 2004; 30 (4): 196–200.
23. *Rodrigue JR, Schutzer ME, Paek M et al.* Altruistic kidney donation to a stranger: psychosocial and functional outcomes at two US transplant centers. *Transplantation*. 2011; 91 (7): 772–778.
24. *Mjøen G, Stavem K, Westlie L et al.* Quality of life in kidney donors. *American Journal of Transplantation*. 2011; 11 (6): 1315–1319.
25. *Li S, Thiessen C, Gannon J et al., editors.* Financial Burdens and Coping Mechanisms of Living Kidney Donors. American journal of transplantation; 2016: Wiley-Blackwell 111 River St, Hoboken 07030-5774, NJ USA.
26. *Williams AM, Colefax L, O'Driscoll CT et al.* An exploration of experiences of living renal donors following donation. *Nephrology Nursing Journal*. 2009; 36 (4).
27. *Andersen MH, Mathisen L, Øyen O et al.* Living donors' experiences 1 wk after donating a kidney. *Clinical transplantation*. 2005; 19 (1): 90–96.

28. *Andersen MH, Bruserud F, Mathisen L et al.* Follow-up interviews of 12 living kidney donors one yr after open donor nephrectomy. *Clinical transplantation*. 2007; 21 (6): 702–709.
29. *Heck G, Schweitzer J, Seidel-Wiesel M.* Psychological effects of living related kidney transplantation – risks and chances. *Clinical transplantation*. 2004; 18 (6): 716–721.
30. *Gill P, Lowes L.* Gift exchange and organ donation: donor and recipient experiences of live related kidney transplantation. *International journal of nursing studies*. 2008; 45 (11): 1607–1617.
31. *Garg AX, Nevis IF, McArthur E et al.* Gestational hypertension and preeclampsia in living kidney donors. *New England Journal of Medicine*. 2015; 372 (2): 124–133.
32. *Okoli J, Watt J.* Crisis decision-making: the overlap between intuitive and analytical strategies. *Management Decision*. 2018.
33. *Mjøen G, Hallan S, Hartmann A et al.* Long-term risks for kidney donors. *Kidney international*. 2014; 86 (1): 162–167.
34. *Rodrigue J, Schold J, Morrissey P et al.* Mood, body image, fear of kidney failure, life satisfaction, and decisional stability following living kidney donation: findings from the KDOC study. *American Journal of Transplantation*. 2018; 18 (6): 1397–1407.
35. *Lennerling A, Qureshi AR, Fehrman-Ekholm I.* Spouses who donate seem to be the winners – a questionnaire study of kidney donors long-term. *Open J Nephrol*. 2012; 2 (3): 44–48.
36. *Halpern S, Thomas A, Holscher C et al., editors.* Sexual Dysfunction Among Living Kidney Donors. American journal of transplantation; 2017: Wiley 111 River St, Hoboken 07030-5774, NJ USA.
37. *Zheng X-Y, Han S, Wang L-M et al., editors.* Quality of life and psychology after living-related kidney transplantation from donors and recipients in China. Transplantation proceedings; 2014: Elsevier.
38. *Chen G, Wang C, Ko DSC et al*. Comparison of outcomes of kidney transplantation from donation after brain death, donation after circulatory death, and donation after brain death followed by circulatory death donors. *Clinical Transplantation*. 2017; 31 (11): e13110.
39. *Erim Y, Kahraman Y, Vitinius F et al.* Resilience and quality of life in 161 living kidney donors before nephrectomy and in the aftermath of donation: a naturalistic single center study. *BMC nephrology*. 2015; 16 (1): 164.
40. *Hsieh C-Y, Chien C-H, Liu K-L, editors.* Positive and negative affects in living kidney donors. Transplantation proceedings; 2017: Elsevier.

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ATTITUDE OF THE YOUTH IN THE REPUBLIC OF TATARSTAN TOWARDS ORGAN DONATION

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Objective: to study the views of the youth in the Republic of Tatarstan on organ donation and transplantation, to analyze their awareness and ideas about donor transplants and potential their willingness to become organ donors. **Materials and methods.** An anonymous sociological survey of 880 respondents aged 18 to 35 from the Republic of Tatarstan was conducted in the period from January 1 to July 1, 2021. An 11-question questionnaire was developed using online service Google Forms. Participation in the survey was voluntary. **Results.** Female and male respondents accounted for 79.0% and 21.0%, respectively; 34.2% of the respondents have or are receiving medical education. Among the respondents, 71.5% have a clear understanding of the term "organ donation", 27.4% are not sure of their understanding, 1.1% do not have a clear understanding. 56.8% consider the issue of organ donation for transplantation in the Republic of Tatarstan as a pressing matter, 3.5% do not see it so, while 39.7% found it difficult to answer. After death, 35.9% would agree to become donors, 39.5% probably would agree, 9.3% probably would disagree, 5.6% strongly disagrees, 9.7% found it difficult to answer. The most common associations with organ donation were positive: 34.5% associate it with "life", 25.1% with "help", and 22.0% with "lifeline". **Conclusion.** Young people in the Republic of Tatarstan are ready for a healthy debate of the problem of organ donation and most of them see it as a noble course. Given the interest in the problem and lack of awareness by the target audience, it is advisable to include independent academic disciplines on transplantology and organ donation in the curriculums of medical universities in the country. It is necessary to attract modern interdisciplinary information and educational resources to promote organ donation among the Russian public.

Keywords: organ donation, transplantation, public opinion, sociology, public attitude.

INTRODUCTION

At the turn of the 21st century, amid an unprecedented growth in scientific and technological progress, organ transplantation, having accumulated the latest achievements in surgery, resuscitation, immunology, pharmacology and other medical and biological sciences, has become part of the arsenal of many medical institutions, opening a new era in modern medicine. This is due to the fact that transplantation, on one hand, is the only radical treatment for a number of end-stage diseases of vital organs (kidneys, liver, heart, lungs, pancreas), allowing most patients to return to full activity. On the other hand, transplantology in medicine, as a multidisciplinary science that raises practical healthcare to a new level, can be compared without exaggeration to astronautics in engineering. The level of development of transplantology reflects the level of development of medicine in a country or region and is an important indicator of a country's economic well-being. In the Russian Federation, despite the annual positive dynamics in higher number of organ transplant surgeries, the need for donor organs dwarfs available transplant care [1]. In 2019, the number of deceased donors in Russia was 5.14 per million population. This is the 48th deceased organ donation rate among all countries where organ transplant surgeries are officially allowed [2].

In the Republic of Tatarstan with a 3 million population, 900,000 people annually require 206 kidney transplantations, 76 liver transplantations and 40 heart transplantations. Today, there are two organ transplant centers in the region, one (Republican Clinical Hospital) of which performs kidney and liver transplantation, and the other (Interregional Clinical and Diagnostic Center) performs heart transplantation. In 2018, 32 organ transplants (8.2 per million population) were performed in Tatarstan. In 2019, there were 57 organ transplants (14.6 per million population). In 2020, 64 organ transplants (16.4 per million population) were performed. For comparison, in the Russian Federation with a population of 146.8 million people in 2019, a total of 2,427 organ transplants (16.5 per million population) were performed [1]. Donor activity per population was 5.7 (23), 6.2 (24), and 6.9 (27) in 2018, 2019 and 2020 respectively. In 2019 in Russia, it was 7.2 per million population (1062) [1].

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In 2018, 27 kidney transplants (6.9 per million) were performed. In 2019, we had 39 (10.0 per million). In 2020, there were 40 (10.3 per million). In the Russian Federation in 2019 – 1,473 (10.0 per million population) [1]. The number of cadaveric kidney transplants and living-related donor transplants in 2018 were 8 and 19, respectively; it was 30 and 9 in 2019, 34 and 6 in 2020, respectively. In 2018, 4 liver transplants were performed (1.0 per million). In 2019, 14 (3.6 per million). In 2020, there were 20 (5.1 per million). In the Russian Federation in 2019, we had 584 (4.0 per million population) [1]. In 2018, 1 heart transplant was performed (0.3 per million); 4 (1.0 per million) in 2019 and 4 (1.0 per million) in 2020. In the Russian Federation in 2019, there were 335 (2.3 per million population) [1]. So, results from the last three years demonstrate that organ transplants in the Republic of Tatarstan are increasing, despite the severe epidemiological situation in 2020 due to the new coronavirus infection (COVID-19). However, the need for organ transplantation still exceeds the available transplant care. The number of waitlisted candidates for organ transplantation continues to grow due to organ shortage.

Organ shortage in Russia is man-made. Low public awareness on the principles of how organ transplantation authorities function, lack of organized educational work and portrayal of transplant surgeons in a negative light by mass media have led to the fact that potential donor reserves are used extremely inefficiently [3, 4]. Despite the fact that "presumption of consent" is proclaimed in the country, 78% of Russians are not ready to become donors after death [5]. Analyzing the results obtained from different age groups, the authors note a more "benevolent" willingness to become a donor among the younger generation as opposed to older respondents. In this context, the young generation of citizens who grew up in an era of easy access to information and brought up on the principles of voluntary activity may become potential target audience for promotion of organ donation as a socio-humanitarian phenomenon.

Proceeding from the foregoing, the **objective** of the present report is to study the position of young citizens of the Republic of Tatarstan concerning organ donation and transplantation, analyze their awareness and perception of donor transplants and potential willingness to become organ donors.

MATERIALS AND METHODS

An anonymous sociological survey of 880 respondents aged 18 to 35 years old in the Republic of Tatarstan was conducted in the period from January 1 till July 1, 2021. An 11-question questionnaire was developed with the use of online service Google Forms. The survey was voluntary in nature and dealt with the issues of understanding the terminology, awareness and perception of donor transplants and potential willingness to become organ donors.

RESULTS

Among the respondents, 79.0% were females, and 21.0% were males. With respect to Religious affiliation, 33.5% where Muslims, 31.4% were Christians, 4.8% were supporters of Buddhism, Judaism and other religions, 25.1% were atheists, while 5.2% were agnostics, who do not deny the existence of God, but are not adherents of any religion. Theoretically, having a medical education should probably have a positive effect on a citizen's attitude towards transplantation. In this regard, we decided to find out the nature of the respondents' education. It turned out that 65.8% were not related to medicine, 8.5% had higher medical education, and 25.7% were studying at a medical university or college.

Among the respondents, 71.5% had a clear understanding of the term "organ donation", 27.4% were not sure of their understanding, 1.1% did not have a clear understanding. About 56.8% considered the issue of organ donation for transplantation in the Republic of Tatarstan as a pressing matter, 3.5% did not see it that way, while 39.7% found it difficult to answer.

From our point of view, it is especially interesting that the overwhelming majority of respondents support post-mortem organ donation. After death, 35.9% would agree to become donors, 39.5% probably would agree, 9.3% probably would disagree, 5.6% strongly disagrees, 9.7% found it difficult to answer (Fig. 1).

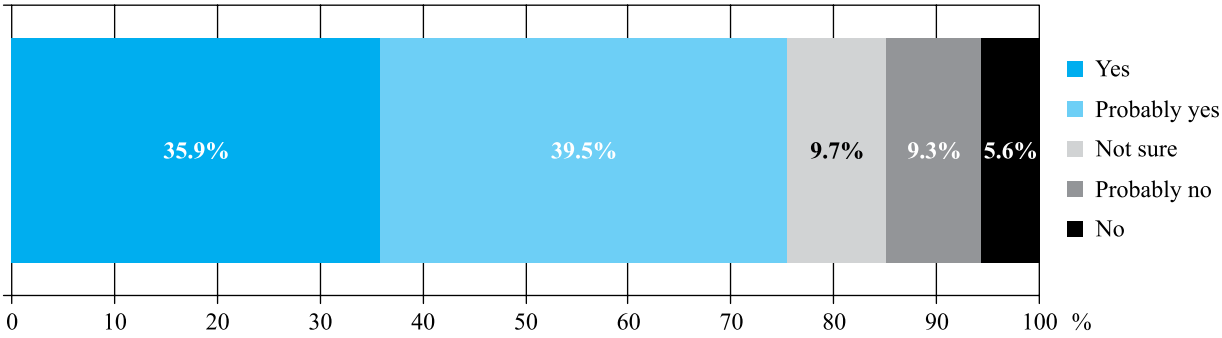

Fig. 1. Distribution of responses to the question: "Do you agree to become a posthumous organ donor (donate your organs to patients in need when you die)?" (in %, N = 880 people)

At the same time, 9.9% of the respondents argued why they were unwilling to donate their organs to another person: 6.9% were unwilling due to their religious beliefs, and 3.0% could not explain their unwillingness. Among other reasons, 0.7% cited their low awareness of the issue, and 0.7% said they were afraid of criminal death. About 0.45% of young Tatarstan respondents had not thought about death and its consequences, 0.34% were worried about their relatives.

At first sight, physicians, due to the specifics of their education, should be more confident about the problem. However, analysis shows that there is no significant qualitative difference between the answers of respondents with and without medical education.

The topic of organ donation, due to its close connection with death, is of great interest to the media, journalists, film and television show directors. On this basis, one of the questions of the questionnaire was formulated as follows: "*When you hear about organ donation, what kind of information do you most often receive?*" Neutral information was received by 49.7%, positive information by 30.3%, negative information by 15.5%, and no information on this topic by 4.5% of respondents. Among the notes, our attention was drawn to the comment: "*I don't receive any information on Russia, only from other countries*."

When compiling the questionnaire, we allowed respondents to suggest several options on how to raise public awareness of organ donation issues. So, 69.5% noted the need to publish cases of successful transplants in Russia; 68.4% suggested meeting real patients in need of organ transplantation, 51.1% proposed involving transplant surgeons in open discussion of the problem, 45.1% preferred engaging popular bloggers and opinion leaders, 45.1% proposed developing publishing educational video clips and materials, while 32.0% went with the view that religious leaders and religious associations should be involved in the discussion of the problem.

At the end of the questionnaire, we asked respondents to name the first 1 to 3 associations they could think of about organ donation. Analysis of the results showed that young people in most cases have positive associations with organ donation: 34.5% associate it with "life", 25.1% with "help", and 22.0% with "lifeline" (Fig. 2).

DISCUSSION

Despite the fact that the majority of respondents identify themselves as belonging to a particular religious denomination, religion has almost no influence on the position regarding organ donation among today's youth. At the same time, 32.0% of the respondents supported the idea of involving religious associations in the discussion of the problem. This indicates expediency of potential

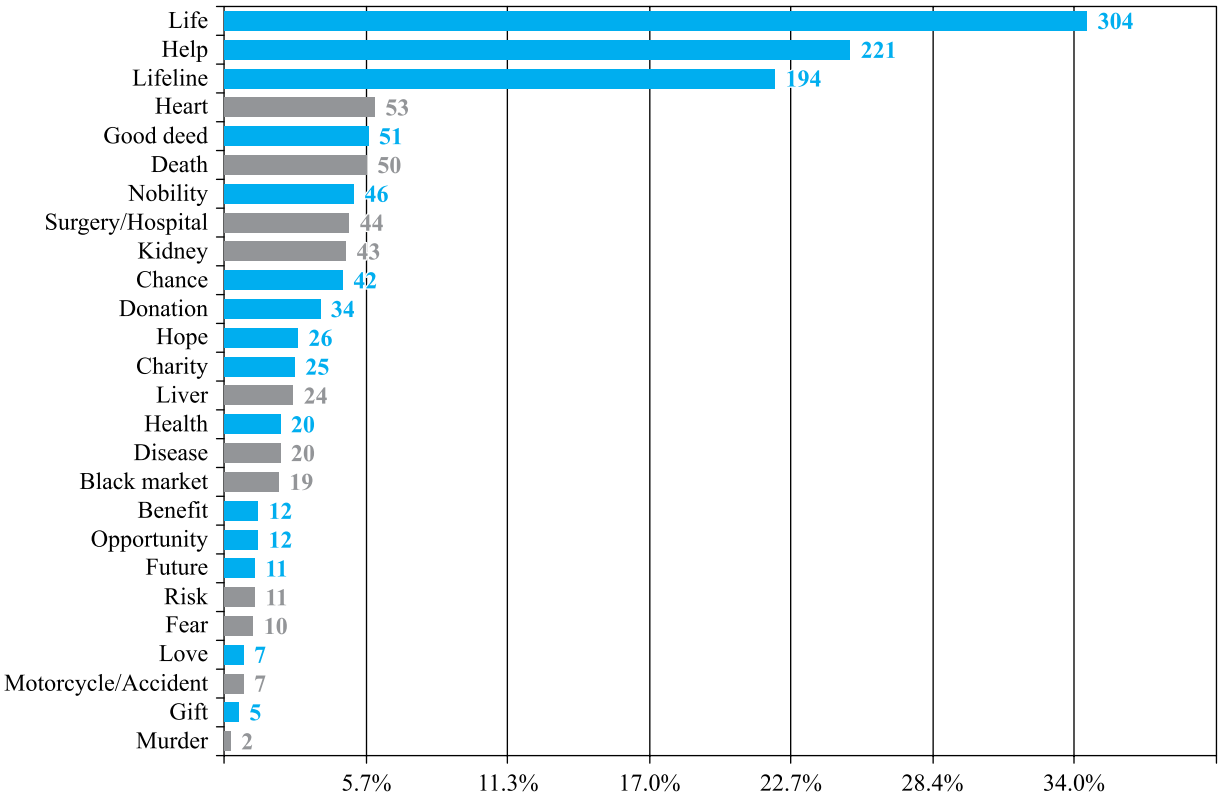

Fig. 2. Distribution of responses to the question: "Write 1 to 3 first associations that come to mind when you hear about organ donation" (up to 3 answer options, N = 880 people)

cooperation with religious organizations in the issue of popularizing organ donation.

In our opinion, the lack of increased involvement of people with medical education in the problem of donation is ambiguous. Despite the dominant positive attitude toward the problem, unfortunately, myths about the criminal nature of organ donation are widespread among "medics" as well as among "non-medics". In our opinion, this requires modernizing medical education with compulsory inclusion of the discipline "Basics of transplantology and organ donation" in the curriculums of medical universities [6]. On one hand, this will increase the level of professional skills of the graduates, and, on the other hand, it will give impetus to the development of transplantology not only in megacities, but also in Russian regions. In this regard, elective course "*Basics of Clinical Transplantology and Organ Donation*" was introduced into the educational program for the 2021 academic year at the Department of Emergency Medical Care and Simulation Medicine, Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, and a student research and practice group of transplantology, organ donation and experimental surgery was organized.

Analysis of questionnaires shows that the respondents have a general positive attitude towards organ donation. On one hand, this fact can be connected with the young age, maximalist and, in some way, frivolous approach to their life and health. However, the reasoned position, the request for specific information initiatives and the positive nature of the associative series, on the other hand, allow us to speak about the personal traits of a modern young person that fit into the generational theory [7–9]. In this regard, we consider it expedient to develop an accessible, interdisciplinary format of interaction with young people to involve them in the discussion of the problem and strategically stimulate their donor potential following the example of foreign countries [10].

On the basis of the above-mentioned, with the participation of the staff from the Department of Emergency Medical Care and Simulation Medicine, Institute of Fundamental Medicine and Biology, Kazan (Volga Region) Federal University, an organ donation development center "*Donate Life Russia*" was organized in 2020 at the Medical Law Clinic, Kazan. This is the first social project in the Republic of Tatarstan to promote organ donation. In 2020, the project received grant support from the Federal Agency for Youth Affairs (Rosmolodezh) [11]. The project raises awareness among the youth of the republic through modern and comprehensible content. Lectures and discussions are attended by specialists in the field of health care, transplantology, charity, volunteering, as well as lawyers and opinion leaders.

So, in general, the youth of the Republic of Tatarstan are ready for an open discussion of the problem of organ donation. The majority of the respondents support organ donation, associating it with noble causes. Nevertheless, there is a need to develop understandable and supportive resources to raise awareness among young people about organ donation.

CONCLUSION

1. Young people in the Republic of Tatarstan are ready for a meaningful discussion of the problem of organ donation; they mostly associate this topic with noble causes.
2. Taking into account the interest in the problem and the insufficient awareness among the target audience, independent academic disciplines on transplantology and organ donation should be included in the curricula of medical universities in Russia.
3. Modern interdisciplinary information and educational resources should be attracted to promote organ donation among the Russian public.

The authors declare no conflict of interest.
Each author contributed 25% to the study.

REFERENCES

1. *Gautier SV, Khomyakov SM*. Organ donation and transplantation in the Russian Federation in 2019. 12th report from the Registry of the Russian Transplant Society. *Russian Journal of Transplantology and Artificial Organs*. 2020; 22 (2): 8–34. [In Russ, English abstract]. doi: 10.15825/1995-1191-2020-2-8-34.
2. Newsletter. Final Numbers 2019. International Registry in Organ Donation and Transplantation, December 2020. (Data obrashhenija: 02.07.2021). URL: https://www.irodat.org/img/database/pdf/Newsletter%20Dec%202020%20.pdf.
3. Delo vrachej-transplantologov: trizhdy obvinennye / Pod red. T. Batenjova [Jelektronnyj resurs]. (Data obrashhenija: 02.07.2021). URL: https://iz.ru/news/312449.
4. *Bagnenko SF, Shherbuk JuA, Polushin JuS i dr*. Prichiny deficita donorskih organov i puti ego preodolenija. *Medicinskij akademicheskij zhurnal*. 2011; 11 (4): 13–24. [In Russ].
5. Donorstvo organov: problemy i perspektivy razvitija v Rossii. 21.10.2013. Levada-centr [sajt]. (Data obrashhenija: 04.07.2021). URL: https://www.levada.ru/sites/default/files/otchet_donorstvo_organov_v_rossii_levada-centr.pdf.
6. *Bagnenko SF, Reznik ON*. Key problems of transplantation development and the objectives of higher medical education. *Transplantologiya. The Russian Journal of Transplantation*. 2017; 9 (3): 192–210. [In Russ, English abstract]. doi: 10.23873/2074-0506-2017-9-3-192-210.
7. *Howe N, Strauss W.* Millennials Rising: The Next Great Generation. New York: Vintage Books, 2000.

8. *Astashova JuV*. Teorija pokolenij v marketinge. *Vestnik Juzhno-Ural'skogo gosudarstvennogo universiteta*. 2014; 8 (1): 108–114. [In Russ].
9. *Popov NP*. Russian and American generations of the 20th century: where have Millennials come from? *Monitoring of Public Opinion: Economic and Social Changes*. 2018; 4: 309–323. [In Russ, English abstract]. doi: 10.14515/monitoring.2018.4.15.
10. *Tishchenko PD*. The problem of the social-humanitarian assessment for the new legislation on organ donor-ship. *Clin. Experiment. Surg. Petrovsky J*. 2019; 7 (3): 8–14. [In Russ, English abstract]. doi: 10.24411/2308-1198-2019-13001.
11. Prikaz Federal'nogo agentstva po delam molodezhi (Rosmolodjozh') ot 22 sentjabrja 2020 g. № 298 "Ob utverzhdenii spiska pobeditelej Vserossijskogo konkursa molodezhnyh proektov sredi fizicheskih lic v ramkah molodezhnyh obrazovatel'nyh forumov v 2020 godu" [In Russ].

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EARLY EXPERIMENTS WITH HYPOTHERMIC OXYGENATED MACHINE PERFUSION OF KIDNEY GRAFTS FROM EXTENDED CRITERIA DONORS

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Objective: to evaluate the safety and efficacy of hypothermic oxygenated machine perfusion (HOPE) for kidney grafts obtained from expanded criteria donors (ECD). **Materials and methods.** From June 2018 to June 2021, 200 surgeries involving kidney transplants from deceased donors were performed at Botkin City Clinical Hospital. Of these, 123 were men (61.5%) and 77 were women (38.5%). The mean age was 47.62 ± 11.69 (20–73) years. In 102 cases, kidney grafts were procured from ECD. In 92 recipients (90.2%) of kidney transplants from an expanded criteria donor, static cold storage done according to the standard technique was used to preserve the organ; these patients constituted observation group 1. In 10 recipients (9.8%), hypothermic oxygenated perfusion was used in addition to static cold preservation; these patients formed observation group 2. **Results.** No 30-day mortality was recorded in both observation groups. The mean static cold storage time in group 1 patients was 612.33 ± 178.88 (133–1180) minutes. Overall incidence of delayed graft function was 26.5% (53/200). Incidence of delayed graft function was 19.3% (19/98) for organs from standard donors using static cold storage and 35.8% (33/92) for ECD organs. Twenty-five patients (12.5%) had postoperative complications. Postoperative complications with delayed graft function were diagnosed in 12 patients, which was 22.6% (12/53), with immediate function in 13 patients, which was 8.8% (13/147). Mean cold storage time in group 2 patients was 319.11 ± 110.24 (311–525) minutes. Mean HOPE time was 202.34 ± 21.48 (150–210) minutes. Delayed graft function was recorded in 1 group 2 patient (10%). No complications, including perfusion-related one, were recorded in this group. **Conclusion.** The unique technique used at Botkin City Clinical Hospital for HOPE in kidney transplant is safe. It provides a low risk of delayed graft function for ECD kidneys.

Keywords: kidney transplant, expanded criteria donors, hypothermic oxygenated machine perfusion.

INTRODUCTION

Long-term outcomes in kidney transplantation are constantly improving. At present, the 10-year survival of kidney transplants is over 80% [1]. The effectiveness of transplantation as a method of treatment of end-stage chronic organ failure has consistently increased the number of patients in waiting lists both worldwide and in Russia [2, 3]. The increasing shortage of donor organs prompts the search for new ways to tackle this problem. The most widespread practice throughout the world is the practice of expanding the criteria for organ donation [4]. It should be noted that long-term outcomes of solid organ transplantation from a standard donor and from an expanded criteria donor differ slightly. Specifically, the 1- and 5-year survival rates of a renal transplant from a standard donor are 92% and 70%, respectively, and from an expanded criteria donor, the figure is 80% and 44%, respectively [5]. This difference is explained by the poorer tolerability of cold ischemia of organs from expanded criteria donor. Oxygen consumption in tissues decreases significantly at 4–10 °C, but the corresponding metabolism is still observed. Additional oxygen can support mitochondrial adenosine triphosphate (ATP) synthesis and, in turn, restrain the damage process [6].

In preclinical trials, HOPE was found to reduce the incidence of cell damage and macrophage activation [7, Experiments suggest that this technology in clinical conditions should improve kidney transplant outcomes. Currently, there are several clinical studies evaluating the effectiveness of this technology in liver and kidney transplantation in the world.

MATERIALS AND METHODS

From June 2018 to June 2021, 200 surgeries involving kidney transplants from deceased donors were performed at Botkin City Clinical Hospital. Of these, 123 were men (61.5%) and 77 were women (38.5%). The mean age was 47.62 ± 11.69 (20–73) years. In 102 cases,

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kidney grafts were procured from ECD. In 92 recipients (90.2%) of kidney transplants from an expanded criteria donor, static cold storage done according to the standard technique was used to preserve the organ; these patients constituted observation group 1. In 10 recipients (9.8%), hypothermic oxygenated perfusion was used in addition to static cold preservation; these patients formed observation group 2.

Expanded criteria donors were defined according to the UNOS definition of 2003. [R.A. Metzger, F.L. Delmonico, S. Fenf et al. Expanded criteria donors for kidney transplantation. Am J of Transplantation. 2003; 3, suppl 4: 114–125] as a donor 50–59 years of age with at least two of the following conditions: cause of death from cerebrovascular accident, hypertension history, serum creatinine >133 μmol/L, or a donor ≥60 years of age had all, any two factors, any one factor or none of the factors presented above.

Protocol for HOPE in renal graft: renal graft perfusion was in the operating room with strict adherence to aseptic rules. The temperature of the preservative solution was measured using a non-contact Testo 805 thermometer, the target level was 4–8 °C.

The packaged kidney graft was removed from the shipping container. Punch biopsy of the kidney was performed. The graft was examined to assess its appearance and vascular anatomy. A soft silicone cannula previously attached to the arterial line was inserted into the mouth of the renal artery and fixed to the aortic site with 2–3 knotted sutures (Fig. 1).

If there were two or more renal arteries, each was cannulated using a Y-shaped adapter.

The cannula was connected to a circuit consisting of a roller pump of a stationary AIC apparatus, an oxygenator, and a pressure sensor built into the CPB pump (Fig. 2). The required volume circulation rate of the preservative depends on the hydrostatic pressure in the arterial line. The preservative delivery rate was gradually increased by the regulator until 40 mmHg target pressure was reached. The procedure was performed under constant monitoring by a surgeon. The tightness of the connection between the arterial cannula and the renal artery was constantly monitored, the renal graft temperature was determined every 15 minutes, the pH of the perfusate to assess oxygen saturation was determined every 30 minutes. Thawed refrigerants were replaced as needed. When the pressure in the system decreased, indirectly indicating a decreased resistance in the renal microcirculatory bed, the preservative delivery rate was adjusted to maintain 40 mmHg target pressure. Vascular resistance index was calculated as the ratio of pressure in the system to the volumetric blood flow rate.

Perfusion was completed when the recipient was admitted to the operating room. Before the kidney was incorporated into the recipient's bloodstream, another biopsy of the kidney from the primary punch biopsy site was performed. Intraoperative ultrasound examination with determination of the resistance index was performed before suturing the surgical wound. In the first week of the postoperative period, the following parameters were assessed daily: diuresis, urea, creatinine, K^+, concentration of blood calcineurin inhibitors, resistance index in ultrasound examination was estimated, based on which the decision on the need for renal replacement therapy was made. Postoperative complications were assessed.

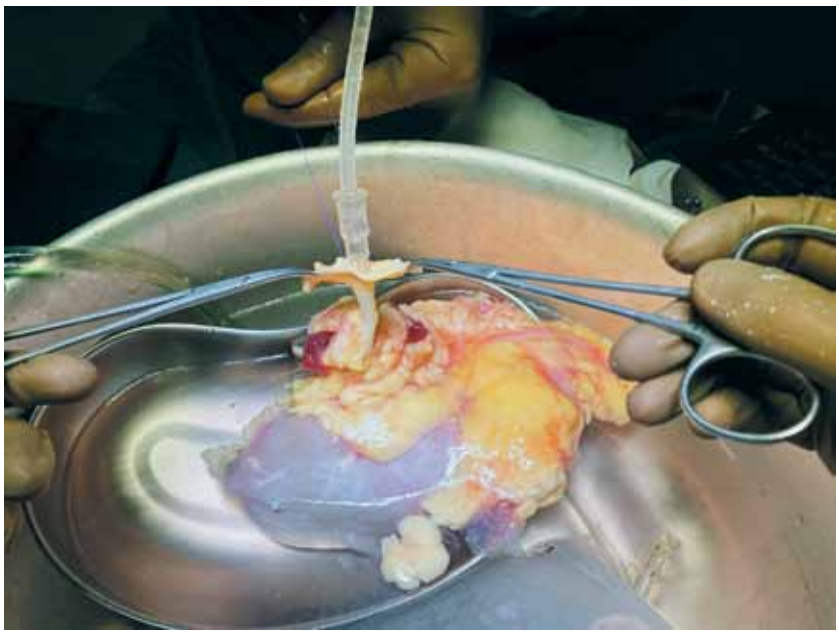

Fig. 1. Cannulation of kidney graft artery for oxygenated cold perfusion

RESULTS

No 30-day mortality was recorded in both observation groups. The mean static cold storage time in group 1 was 612.33 ± 178.88 (133–1180) minutes. The overall incidence of delayed renal graft function was 26.5% (53/200). When using an organ from a standard donor using static cold storage, the incidence of delayed graft function was 19.3% (19/98), and it was 35.8% (33/92) for ECD organs. Postoperative complications occurred in 25 patients (12.5%). Postoperative complications with delayed graft function were diagnosed in 12 patients, which was 22.6% (12/53), with immediate function in 13 patients, which was 8.8% (13/147).

The mean cold storage time in group 2 was 319.11 ± 110.24 (311–525) minutes. The mean hypothermic oxygenated perfusion time was 202.34 ± 21.48 (150–210) minutes. Mean renal graft temperature during perfusion ranged from 4.7 to 6.8 °C (Fig. 3).

The mean partial pressure of oxygen in the perfusate ranged from 323 to 574 mmHg (Fig. 4).

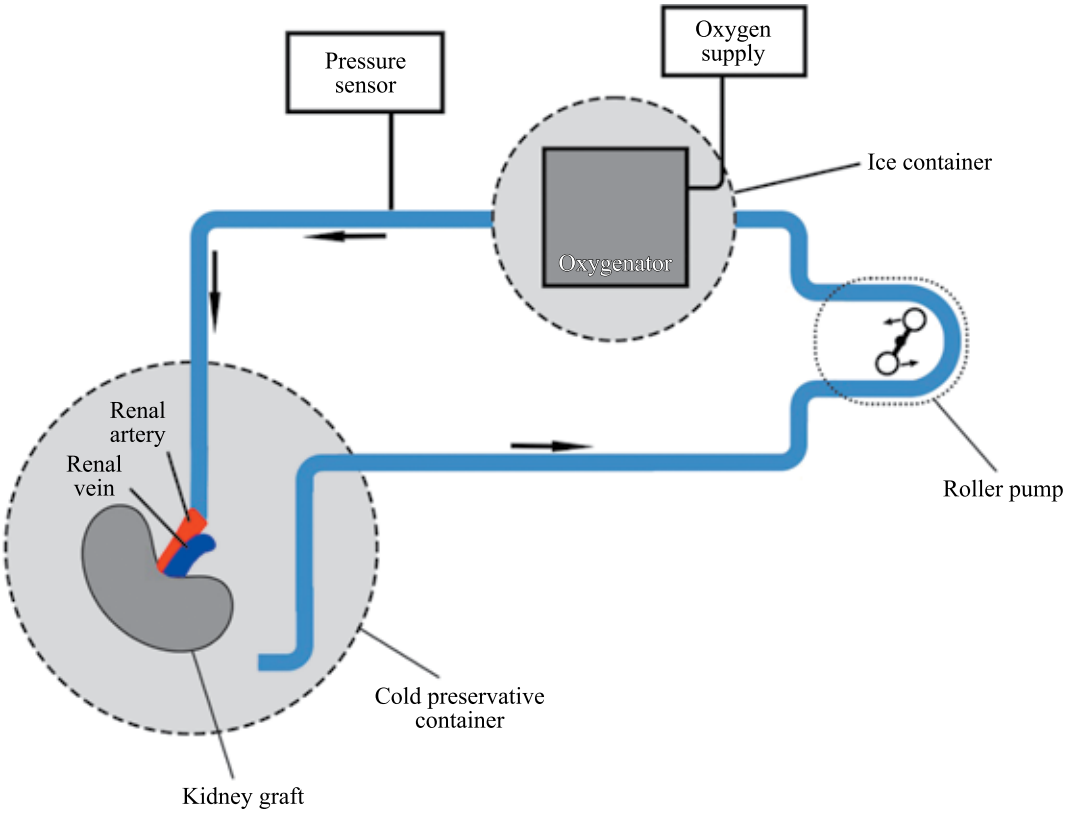

Fig. 2. Schematic layout of oxygenated cold perfusion of a kidney graft

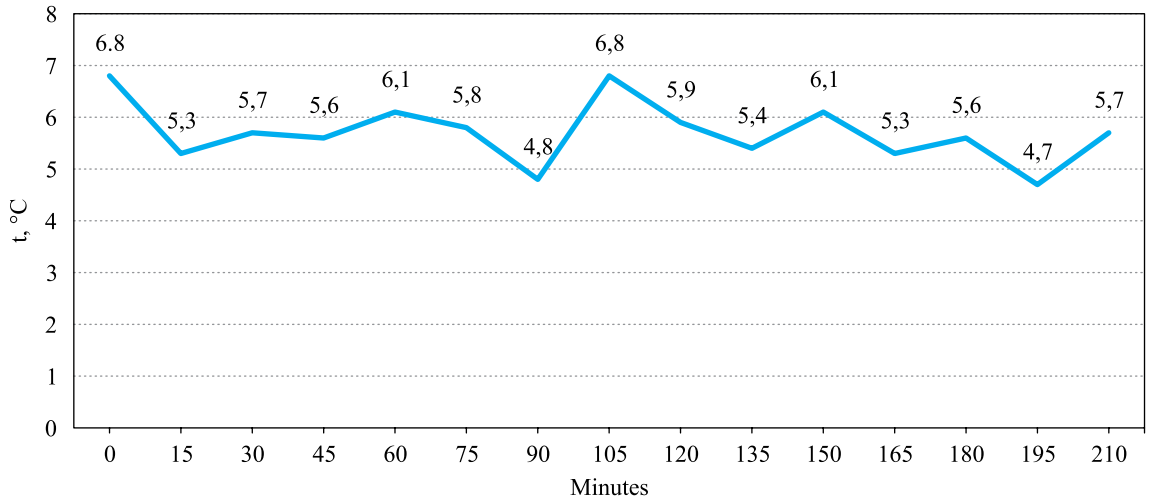

Fig. 3. Dynamics of average renal graft temperature during oxygenated cold perfusion

In one case, the vascular resistance index during perfusion increased by 0.02 (Table 1).

This group 2 patient (10%) had a delayed graft function. No complications, including perfusion-related complications, occurred in this group.

Electron microscopy of nephrobiopsy specimens from group 1 patients showed pronounced negative dynamics expressed in mitochondrial destruction (Fig. 5).

Electron microscopy of nephrobiopsy specimens from group 2 patients showed mitochondrial preservation after perfusion (Fig. 6).

DISCUSSION

Shortage of donor organs is a problem that limits the number of transplants performed. One solution is to expand the indications for donation, which leads to increased use of ECD renal transplants. According to our data, the use of ECD renal transplants significantly increases the likelihood of developing delayed function (35.8 vs 19.3%, p = 0.021). Development of delayed function, in turn, is associated with a significantly higher incidence of postoperative complications (22.6 vs 8.8%, p = 0.015), resulting in an increase in the average length of hospital stay and increased treatment costs. When evaluating our experience with the use of ECS organs, we obtained data on the effect of high donor body mass index (BMI) and cold ischemia time of the donor kidney on incidence of delayed function (Table 2).

In most cases, reducing the static cold storage time appears to be a difficult task. The patient has to come to the transplant center, undergo preoperative examination, in some cases dialysis is required. All this leads to

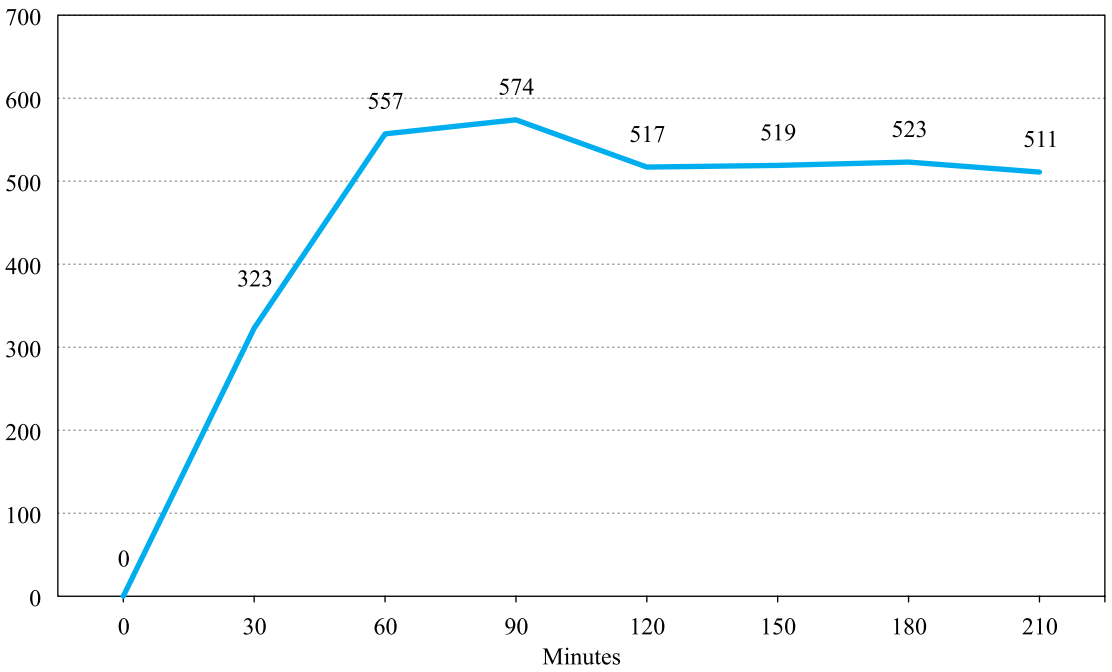

Fig. 4. Dynamics of mean partial pressure of oxygen in perfusate during oxygenated cold perfusion

Table 1

Indicators of oxygenated cold perfusion of the renal graft

S/N	Beginning-of-perfusion pressure	Beginning-of-perfusion volumetric blood flow rate	Beginning-of-perfusion vascular resistance	End-of-perfusion pressure	End-of-perfusion volumetric blood flow rate	End-of-perfusion vascular resistance
1	41	70	0.58	42	90	0.46
2	40	120	0.33	40	150	0.26
3	42	110	0.38	40	120	0.30
4	39	90	0.43	41	90	0.45
5	42	60	0.70	40	70	0.57
6	40	120	0.33	42	140	0.3
7	41	100	0.41	41	120	0.34
8	42	90	0.46	40	90	0.44
9	40	80	0.50	39	100	0.39
10	40	90	0.44	41	100	0.41

an average cold storage time of more than 10 hours at Botkin Hospital. Organs from standard donors tolerate the long cold ischemia period satisfactorily, ECD organs do so significantly worse. In our opinion, the solution to this problem is to replace static cold storage with HOPE. Delivery of oxygen dissolved in the preserving solution to the cells at a preserved low temperature constitutes the theoretical advantage of this technology. Oxygen supports aerobic metabolism in the cells, which slows down dramatically but does not stop completely in cold environments. Aerobic metabolism eliminates uncoupling of the mitochondrial respiratory chain with formation of reactive oxygen species, prevents the development of intracellular acidosis, and also maintains Na^+/K^+-ATPase activity at a normal level, which in turn reduces the risk of cell apoptosis.

Our proposed heart-lung machine for hypothermic oxygenated perfusion has all the qualities necessary for machine perfusion: it can control pressure and volumetric blood flow rate and oxygenate the preservation solution. An important problem we encountered in the development of the perfusion protocol is ensuring and maintaining the necessary temperature of the solution. The fact is that during perfusion the solution in the circuit is heated. So, on one hand, careful temperature control of the renal graft is required, and on the other, effective constant cooling of the solution is needed. We use a remote thermometer to dynamically measure the temperature of the kidney graft. For constant cooling of Custodiol, we use special sterile refrigerants, which are placed near the renal graft without touching it or mixing with the preservative, and we cover the oxygenator with ice as an element of the circuit with the largest area of contact with the solution. These measures allow to maintain the temperature of the graft within 4–8 °C throughout the perfusion period.

Addition of oxygen to the perfusion solution is the most important part of the protocol. Oxygen dissolves well in Custodiol; we managed to achieve an average partial pressure of oxygen in perfusate at 500 mmHg at a delivery rate of 4 liters per minute.

To assess the effectiveness of perfusion in preserving cell mitochondria, we performed electron microscopy of nephrobiopsy specimens before and after HOPE. In all cases after completion of hypothermic machine perfusion, preserved mitochondria with cristae were detected

Table 2

Impact of expanded criteria donor risk factors on delayed renal graft function

Risk factor	Immediate function (n = 59)	Delayed function (n = 33)	p
Donor's age:			
55 to 65 years old	42	23	0.65
>65 years old	17	10	
Donor's gender:			
Male	27	16	0.73
Female	32	17	
Donor's BMI:			
<25	26	12	0.04
>25	33	21	
Recipient's BMI:			
<25	29	15	0.63
>25	30	18	
Donor's hospitalization time:			
<72 hours	44	22	0.29
>72 hours	15	11	
Cold ischemia time:			
<10 hours	26	12	0.03
>10 hours	33	21	

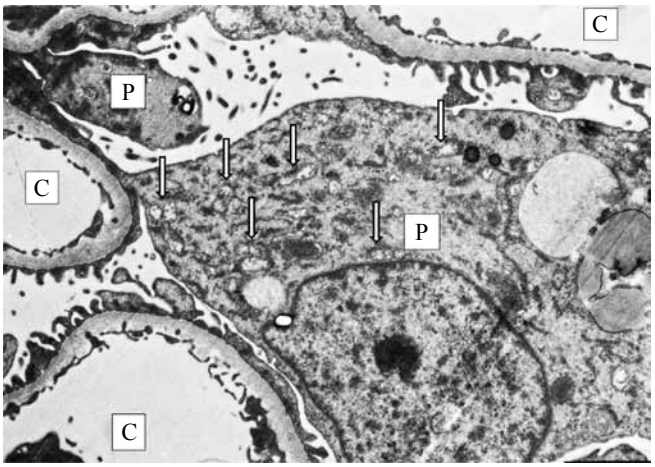

Fig. 5. Renal glomerulus fragment before HOPE. The podocyte (P) cytoplasm contains: small mitochondria with partially preserved cristae (arrows); short profiles of granular endoplasmic reticulum. C, capillaries. 9000× magnification

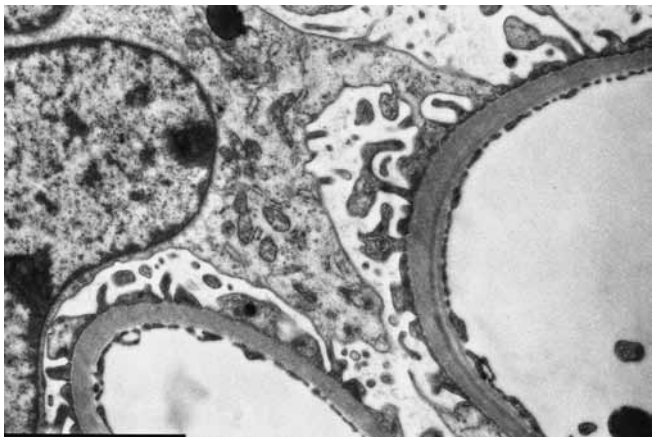

Fig. 6. Renal glomerulus fragment. The podocyte cytoplasm contains: small mitochondria with partially preserved cristae, short profiles of granular endoplasmic reticulum. 12000× magnification

in the mitochondria of the renal glomerulus, distal and proximal nephron fragments.

So, the developed technique makes it possible to maintain the energy balance of cells for a long time and prevent mitochondrial destruction, which inevitably occurs in the process of static cold storage.

The revealed morphological advantages of HOPE also correlate with clinical manifestations. Out of 10 patients who underwent kidney transplantation from a deceased ECD using the perfusion used, delayed graft function was observed in 1 patient (10%), which was significantly lower compared with Group 1 ($p = 0.035$). It was in this case that there was an increase in vascular resistance index from 0.43 to 0.45 during perfusion; in other cases, the index decreased on average by 0.07 ± 0.04 (0.02–0.13). This suggests that this parameter can be regarded as a predictor of delayed renal graft function.

The first experience of using our own HOPE protocol for an ECD renal graft showed its safety and efficiency. Further studies will clarify the optimal perfusion parameters, identify predictors of delayed organ function and primary nonfunction. Further reduction of the incidence of delayed function is possible through introduction of transport systems for hypothermic oxygenated perfusion.

CONCLUSION

The original technique used at Botkin Hospital for renal graft HOPE is safe. It is associated with a low risk of delayed renal graft function for ECD organs. Further studies will expand the indications for this technique.

The authors declare no conflict of interest.

REFERENCES

1. *Lee HS, Kang M, Kim B, Park Y*. Outcomes of kidney transplantation over a 16-year period in Korea: An analysis of the National Health Information Database. *Plos one*. 2021; 16 (2): e0247449.
2. *Gautier SV, Khomyakov SM.* Donorstvo i transplantatsiya organov v Rossiyskoy Federatsii v 2016 godu. IX soobshchenie registra Rossiyskogo transplantologicheskogo obshchestva. *Vestnik transplantologii i iskusstvennykh organov*. 2017; 19 (2): 6–26.
3. *Gautier SV, Khomyakov SM.* Donorstvo i transplantatsiya organov v Rossiyskoy Federatsii v 2019 godu. XII soobshchenie registra Rossiyskogo transplantologicheskogo obshchestva. *Vestnik transplantologii i iskusstvennykh organov*. 2020; 22 (2): 8–34.
4. *Wang Z, Durai P, Tiong HY*. Expanded criteria donors in deceased donor kidney transplantation – An Asian perspective. *Indian journal of urology: IJU: journal of the Urological Society of India*. 2020; 36 (2): 89.
5. *Gondos A, Döhler B, Brenner H, Opelz G*. Kidney graft survival in Europe and the United States: strikingly different long-term outcomes. *Transplantation*. 2013; 95 (2): 267–274.
6. *Saat TC, van den Akker EK, IJzermans JN, Dor FJ, de Bruin RW*. Improving the outcome of kidney transplantation by ameliorating renal ischemia reperfusion injury: lost in translation? *Journal of translational medicine*. 2016; 14 (1): 1–9.
7. *Kaminski J, Delpech PO, Kaaki-Hosni S, Promeyrat X, Hauet T, Hannaert P*. Oxygen consumption by warm ischemia-injured porcine kidneys in hypothermic static and machine preservation. *Journal of Surgical Research*. 2019; 242: 78–86.
8. *Cannon RM, Franklin GA*. Machine perfusion for improving outcomes following renal transplant: current perspectives. *Transplant Research and Risk Management*. 2016; 8: 1–7.

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