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Глубокоуважаемые коллеги!

Готовясь представить читателю очередной выпуск нашего журнала и пересматривая его содержание, всякий раз ловлю себя на мысли о многогранности трансплантологии как области медицины и медицинской науки, охватывающей анализ клинического опыта и фундаментальные биомедицинские аспекты, биологические, технологические проблемы разработки и применения искусственных органов, прорывные исследования в области регенеративной медицины и многое другое. Но вопросы, связанные с донорством органов, безусловно, занимают в этом ряду особое место, в первую очередь в силу существующего во всем мире дефицита донорских органов для трансплантации.

Хочу обратить внимание заинтересованного читателя на публикуемые в настоящем выпуске статьи, посвященные технологиям машинной перфузии донорских органов. Для решения проблемы дефицита донорских органов и в нашей стране, и в мире разрабатывается и внедряется в клиническую практику технология ex vivo перфузии, которая позволяет работать с органами, не полностью отвечающими критериям пригодности для трансплантации. Предполагается, что использование технологии нормотермической ex vivo перфузии позволит восстановить функциональные возможности исходно скомпрометированных донорских органов и использовать для трансплантации доноров с расширенными критериями.

Очевидно, что решение этой актуальной и весьма амбициозной задачи требует разработки целого ряда научных направлений. Круг вопросов, подлежащих решению, включает, во-первых, создание и полный комплекс исследований перфузионного раствора, который позволит поддерживать и в случае успешного решения оптимизировать

ADVANCES IN MACHINE PERFUSION OF ISOLATED DONOR ORGANS

Dear colleagues,

As we get set to release the next issue of the Russian Journal of Transplantology and Artificial Organs and review its content, I have found myself consistently thinking about how versatile transplantology is as a field of medicine and medical science, covering the analysis of clinical experience and fundamental biomedical aspects; biological, technological problems of development and application of artificial organs; breakthrough research in regenerative medicine and much more. But the issues related to organ donation, of course, occupy a special place in this row, first of all, due to the existing global organ shortage crisis.

I would like to draw the attention of our readers to some of the papers in this upcoming journal issue that are devoted to machine perfusion of donor organs for transplantation. To tackle the organ shortage crisis both in our country and in the world, ex vivo organ perfusion is being developed and deployed into clinical practice. Ex vivo perfusion is used to rehabilitate organs that otherwise may not have been considered transplantable. It is assumed that the use of normothermic ex vivo perfusion would restore the functionality of initially compromised donor organs and allow for the use of expanded criteria donors for transplantation.

Obviously, the solution to this urgent and very ambitious challenge requires developing a number of scientific directions. The range of issues to be solved includes, first, creation and full complex of research on perfusate solution, which will allow to support, and in case of successful solution, optimize homeostasis

гомеостаз в трансплантате в условиях искусственного кровообращения в изолированном органе. Далее разработка, создание и исследования перфузионного комплекса – аппаратов и систем, обеспечивающих собственно процесс перфузии органа, поддержание и мониторинг показателей. Наконец, отдельное направление исследований – разработка протокола перфузии, включающего и собственно режим перфузии, и комплекс мер, направленных на восстановление донорского органа.

Работа, выполненная нами в НМИЦ ТИО им. В.И. Шумакова и публикуемая в настоящем выпуске, посвящена нормотермической ex vivo перфузии легких. Следует отметить, что из всех солидных органов наиболее низкий процент пригодных для трансплантации приходится именно на легкие. Совокупность факторов, сопутствующих смерти мозга, особенно влияет на качество донорских легких, более других органов восприимчивых к негативному воздействию этих факторов. В статье анализируются результаты успешного экспериментального исследования разработанного протокола перфузии донорских легких с использованием оригинального перфузионного раствора и отечественного аппаратного комплекса.

В статье А.В. Шабунина и соавт. представлен другой аспект проблемы – первый в России клинический опыт сочетанного применения автоматизированной системы компрессии грудной клетки и машинной оксигенированной перфузии почечных трансплантатов от донора с остановкой кровообращения.

Хочется выразить осторожный оптимизм относительно дальнейшего развития направления – перфузии донорских органов с восстановлением их функциональной полноценности и увеличением доступного числа донорских органов для трансплантации.

С особым удовольствием хочу пригласить к участию в работе очередного, XI Всероссийского съезда трансплантологов, который состоится 21–23 сентября 2022 года в ФГБУ «Национальный медицинский исследовательский центр трансплантологии и искусственных органов имени академика В.И. Шумакова» Минздрава России.

Предстоящий съезд отмечен памятными датами в истории трансплантологии: 55-летием первой в мире трансплантации сердца, 35-летием первой успешной трансплантации сердца в России и 25-летием начала программы родственной трансплантации печени детям.

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

in the graft under artificial circulation in the isolated organ. Further, development, creation and research of the perfusion complex – devices and systems that support the organ perfusion process itself – as well as maintenance and monitoring of perfusion indicators will all be needed. Finally, another area of research is the development of perfusion protocol, which includes both the perfusion regime itself and a set of measures aimed at rehabilitating a donor organ.

The work we performed at Shumakov National Medical Research Center of Transplantology and Artificial Organs and published in the present issue is devoted to normothermic ex vivo lung perfusion. It should be noted that of all solid organs, lungs have the lowest percentage suitable for transplant. A combination of factors associated with brain death especially affects the quality of donor lungs, which are more susceptible than other organs to the negative impact of these factors. The paper analyzes the results of a successful experimental study of the developed lung perfusion protocol using the original perfusate solution and Russian-made perfusion system.

A paper by Shabunin et al. presents another aspect of the problem – the first Russian clinical experience on combined use of automated chest compression system and hypothermic oxygenated machine perfusion for liver grafts donated after circulatory death.

Let me express my cautious optimism regarding further development of the field on donor organ perfusion, with restoration of its functionality and increase in the available number of donor organs suitable for transplantation.

It is with great pleasure that I invite you to participate in the 21st All-Russian Congress of Transplantologists, which will take place on September 21–23, 2022 at Shumakov National Medical Research Center of Transplantology and Artificial Organs in Moscow.

The forthcoming Congress is marked by commemorative dates in the history of transplantology: the 55th anniversary of the world's first heart transplantation, the 35th anniversary of the first successful heart transplantation in Russia and the 25th anniversary of the living related pediatric liver transplant program.

Sincerely,
S.V. Gautier
Editor-in-Chief,
Member, Russian Academy of Sciences

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THE "MICROBIOME" OF POST-LIVER TRANSPLANT COMPLICATIONS

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This paper reviews modern literature and presents a brief analysis of our own data on one of the most pressing issues in modern transplantology and, in particular, transplant hepatology – the role and place of gut-liver axis (GLA) in the early post-transplant period. **Objective:** to compare the correlation between gut microbiome palette and incidence of certain early postoperative complications in liver transplantation. **Materials and methods.** The study design is presented as a pilot, prospective, observational, double-blind study based on investigation of the composition of the microbiome residing in the large intestinal in patients that underwent orthotopic liver transplantation (OLTx). The primary cohort of patients consisted of 12 patients who underwent OLTx from a postmortem donor. To assess the gut microbiome palette, biomaterial was collected from all patients in the pre- and post-transplant period followed by next-generation sequencing. The study was conducted as primary study results registered under number NCT04281797. **Results.** In the preoperative period, differences close to statistically reliable in relation to *Actinobacteria* were observed in patients included in the liver transplant waiting list for cirrhosis (LC) and hepatocellular carcinoma (HCC) in cirrhosis. However, due to the pilot nature of the study, this study cohort was limited to an extremely small sample. In turn, in the post-transplant period, there was a statistically significant difference in the taxonomic range of *Actinobacteria* ($p < 0.05$) between the above groups, indicating a possible effect of liver transplantation on the gut microbiome. In addition, in the early post-transplant period, there was a marked difference in the microbiome palette between patients with and without acute cellular rejection. **Conclusion.** GLA and the gut microbiome play a critical role in many liver diseases, and may also have a significant impact on the post-transplant period. In this regard, further research in this direction will not only characterize the predictors and risk factors of bacterial infection and rejection episodes, but will also allow us to form a completely new approach to the treatment tactics for certain complications, including through formation of a microbiota-oriented pharmacotherapy.

Keywords: liver transplantation, bacterial complications, gut-liver axis, hepatocellular carcinoma, acute cellular rejection, sequencing, gut microbiota.

INTRODUCTION

The term gut-liver axis (GLA) in its present lexicon was introduced for the first time in 1978 by Volta et al. [1] of the University of Bologna, Italy, to denote a special relationship between the liver and intestines in relation to the production of IgA antibodies directed against intestinal microorganisms in liver cirrhosis [1]. Subsequently, GLA became referred to as an independent "virtual human organ" [2]. In 2010–20s, at numerous sessions of the European Association for the Study of the Liver (EASL), American Association for the Study of Liver Diseases (AASLD), Asian Pacific Association for the Study of the Liver (APASL), etc. the key role of GLA in the development and progression of non-alcoholic fatty liver disease (NAFLD) was clearly defined. Later this concept was applied to the recently formed and largely unstudied acute-on-chronic liver failure (ACLF), as well as the variability of its course depending on various factors associated with GLA [3–6]. Over time, the traditional concept of the physiological principles of GLA functioning, under the influence of new discoveries, began to undergo significant changes. So, in the spectrum of the concepts of immunobiological interaction regulation, GLA is now considered rather from the position of symbiotic two-vector dualism rather than the previously familiar monism theory, in which both organs work independently of each other.

In turn, GLA cannot exist without the gut microbiome palette. This fact was clearly demonstrated in a paper entitled 'Our "other" genome' published in Nature in 2010. It was then, in the context of international research, an active review of the etiological links and pathogenetic mechanisms of a number of infectious and noninfectious diseases began, taking into account

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new data on the human microbiome [7]. At the same time, the role of GLA has often been overestimated in a particular pathological process. For instance, from time to time, this concept has stimulated an extremely large number of expected and unexpected scientific findings and conclusions. Over time, increasing importance has been given to the intestinal microbiota, the functioning of the intestinal barrier, the innate immune response of the intestinal mucosa, the transfer of antigens from the liver to the intestine, the involvement of the liver itself in infectious patterns and, ultimately, metabolic damage [1, 8].

Objective: to compare the correlation between gut microbiome palette and incidence of certain early postoperative complications of liver transplantation.

MATERIALS AND METHODS

The study design is presented as a pilot, prospective, observational, double-blind study based on investigation of the microbiome composition of the large intestine in patients that underwent OLTx.

The study was conducted as primary study results registered under number NCT04281797.

The sample consisted of 12 patients who underwent OLTx for cirrhosis of various etiologies. All patients were hospitalized with cirrhosis and HCC in cirrhosis. One patient was hospitalized with autosomal dominant polycystic kidney disease (ADPKD) and autosomal dominant polycystic liver disease (ADPLD), which resulted in liver failure.

Meanwhile, 2 patients were excluded from the analysis because of concomitant enterocolitis. Patients with previous gastrointestinal surgical interventions and inflammatory bowel diseases were not included in the analysis due to proven changes in gut microbiome composition in this category of patients. The main characteristics of the patients are presented in Table.

Table

Main patient characteristics

Criteria	Number	Mean	Interval
Age		52.3	29–64
Gender			
– female	3		
– male	9		
Etiology			
– HCV	2		
– HBV			
– HCV + HBV	1		
– Cryptogenic	2		
– AIH	1		
– PBC	1		
– Wilson–Konovalov disease	1		
– Toxic	1		
– HCC + LC	2		
– ADPLD	1		
Child–Turcotte–Pugh			
– A	4	6	(5–7)
– B	5	8	(7–9)
– C	*3*	*10*	*(9–11)*
MELD		15	(6–30)
Ascites			
– absent	2		
– minimal	8		
– average	1		
– pronounced	1		
TIPS in pre-transplant period	2		
Immunosuppressive regimen			
TACROLIMUS + MMF + GKS	11		
TACROLIMUS + MMF + GKS + Azathioprine	1		
Advagraf + MMF + GKS + Sertikan	1		

In our opinion, the study showed several interesting results. In particular, on the ratio of microbiome between patients with cirrhosis and patients with HCC in cirrhosis. It should be noted that there was no statistically significant difference between patients of our own groups in the pre- and postoperative periods in terms of taxonomic typology. However, the difference in microbiome palette among patients with cirrhosis and patients with HCC in cirrhosis seems interesting. Although no significant differences in microbiome composition in the pre- and postoperative periods in each of these cohorts were detected, which in our opinion is directly related to the small sample of patients, values close to significant were achieved for a number of indicators (Fig. 1).

Moreover, the significance of differences in the gut microbiome composition in patients with HCC has been pointed out by several recent studies. Thus, according to Wang and Chen [9], "It is undoubted that gut microbiota play a critical role in the pathogenesis of HCC; this fact can be used not only as an early diagnosis of HCC, but also as a tool for improvement.

In addition, a seminal study by Ren et al. also points to the association between HCC and gut microbiota. In particular, the authors identified differences between patients with cirrhosis and HCC according to actinobacteria taxon [10]. This fact was confirmed by our study, which showed a statistically significant difference in this type between patients with cirrhosis and patients with HCC in cirrhosis (Fig. 2).

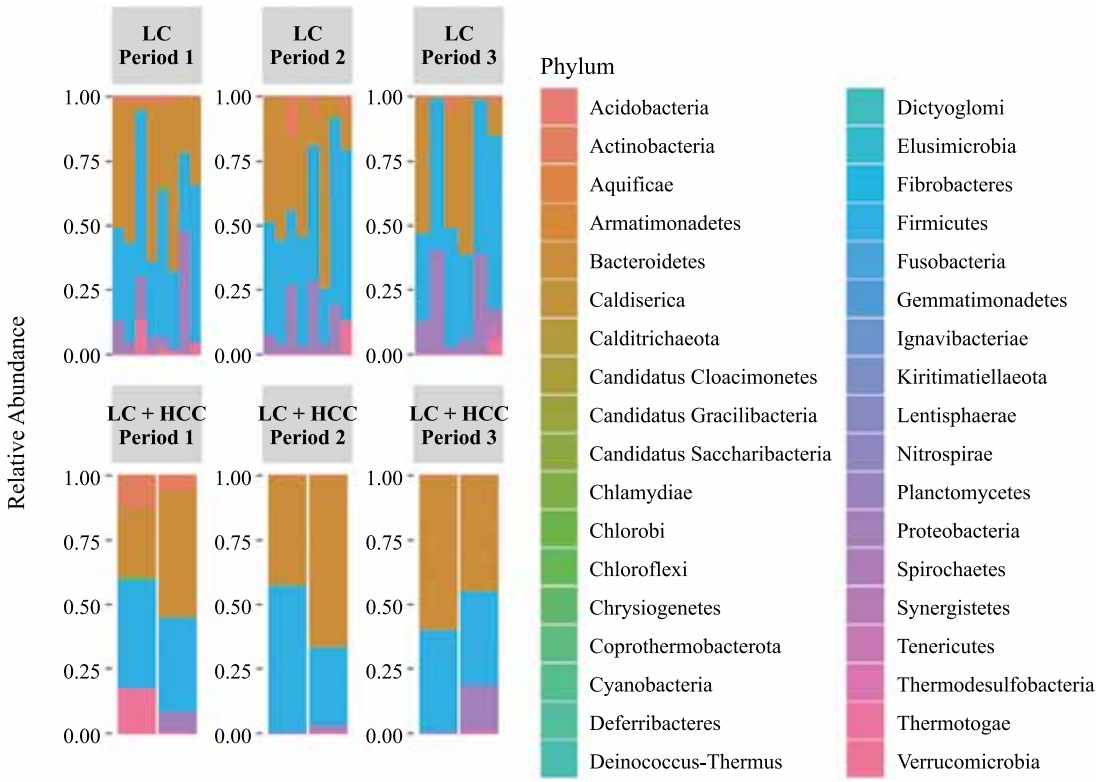
Furthermore, our pilot study identified significant differences in gut microbiota in patients with acute cellular rejection. For instance, we observed a pronounced change in the gut microbiome pattern in the postoperative period compared to the samples obtained before

transplantation. It should be noted that the mentioned taxonomic difference was not observed in patients who had no acute cellular rejection (ACR) (Fig. 3).

However, taking into account that ours is a pilot study that was based on a preliminarily small cohort of patients, we can assume that in the study of a large cohort of patients, the results will provide answers to many questions related to etiology and pathogenesis of ACR, thereby defining the "points of application of efforts" on the path to correcting this severe complication.

Meanwhile, we cannot consider our data to be sufficiently comparable with the above-mentioned studies

a



b

Phylum	.y.	group 1	group 2	p	p.adj	p.format	p.signif	method
Firmicutes	Abundance	LC	LC + HCC	0.8889	1	0.889	ns	Wilcoxon
Bacteroidetes	Abundance	LC	LC + HCC	0.7111	1	0.711	ns	Wilcoxon
Proteobacteria	Abundance	LC	LC + HCC	0.5333	1	0.533	ns	Wilcoxon
Verrucomicrobia	Abundance	LC	LC + HCC	0.8889	1	0.889	ns	Wilcoxon
Actinobacteria	Abundance	LC	LC + HCC	0.08889	0.44	0.089	ns	Wilcoxon

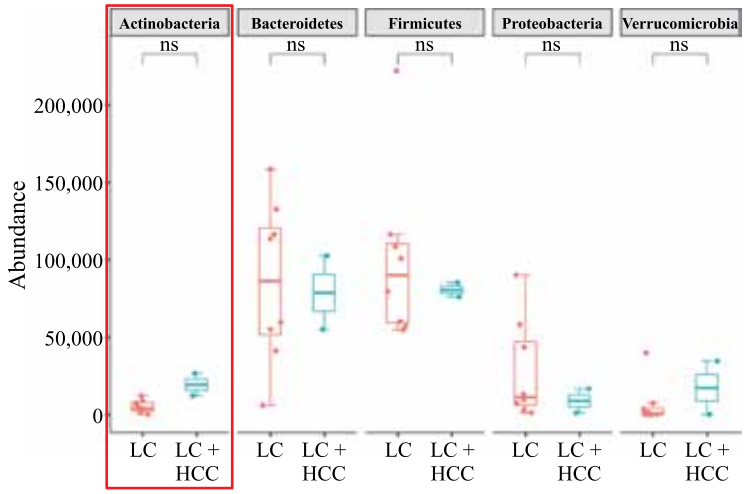


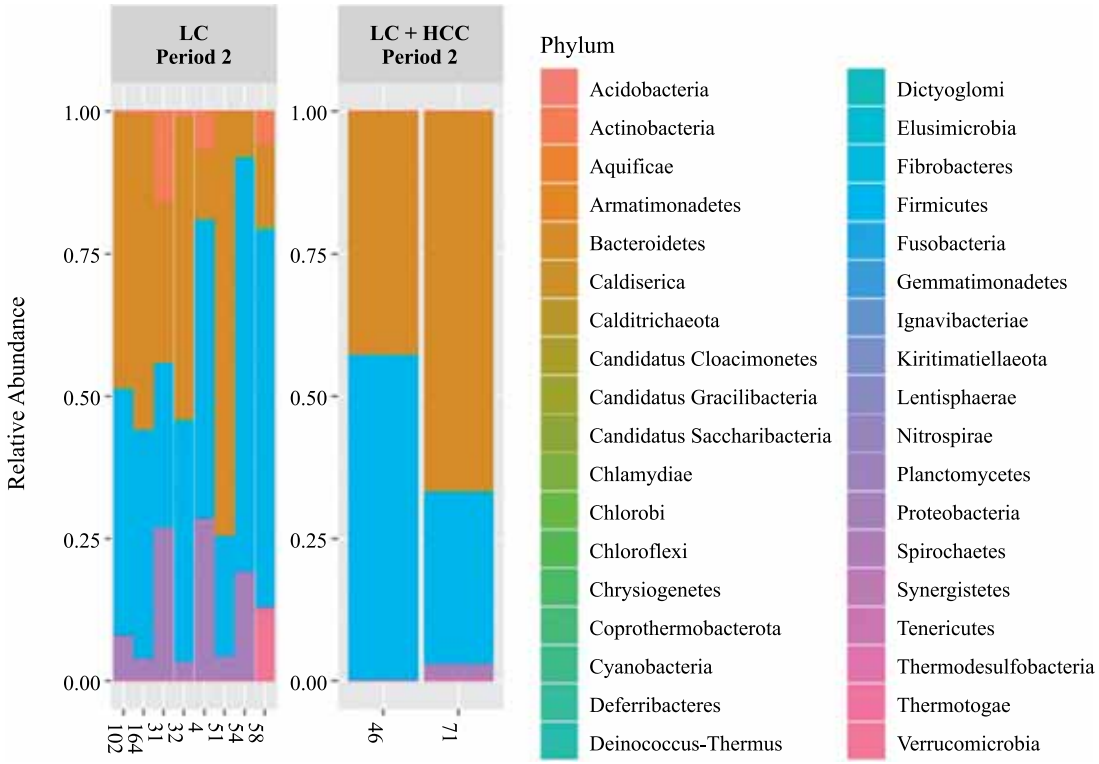
Fig. 1. Microbiome composition of different patient groups: a, total distribution of taxonomic types in patients with liver cirrhosis and with cirrhosis and HCC; b, total distribution of taxonomic types in patients with liver cirrhosis and with cirrhosis and HCC in pretransplant period. "Period 1", material collection before liver transplantation; "Period 2", material collection on the 3rd day after liver transplantation; "Period 3", material collection on the 10th day after liver transplantation)

due to the small sample size, absence of NAFLD cirrhosis and/or cirrhosis complicated by ACLF in the patient cohort in which the influence of microbiome and GLA has been studied and proven. However, the results we obtained can be considered promising and point to the extremely high importance of further research in this area both academically and practically.

DISCUSSION

The role of GLA in infectious complications

Infectious complications have been shown to be the leading cause of mortality after liver transplantation (LTx). Intra-abdominal infection, primary bacteremia and post-transplant pneumonia are the most common complications. The most frequently detected



Phylum	.y.	group 1	group 2	p	p.adj	p.format	p.signif	method
Firmicutes	Abundance	LC	LC + HCC	0.4	1	0.400	ns	Wilcoxon
Bacteroidetes	Abundance	LC	LC + HCC	0.5333	1	0.533	ns	Wilcoxon
Proteobacteria	Abundance	LC	LC + HCC	0.08889	0.36	0.089	ns	Wilcoxon
Verrucomicrobia	Abundance	LC	LC + HCC	0.3578	1	0.358	ns	Wilcoxon
Actinobacteria	Abundance	LC	LC + HCC	0.04444	0.22	0.044	*	Wilcoxon

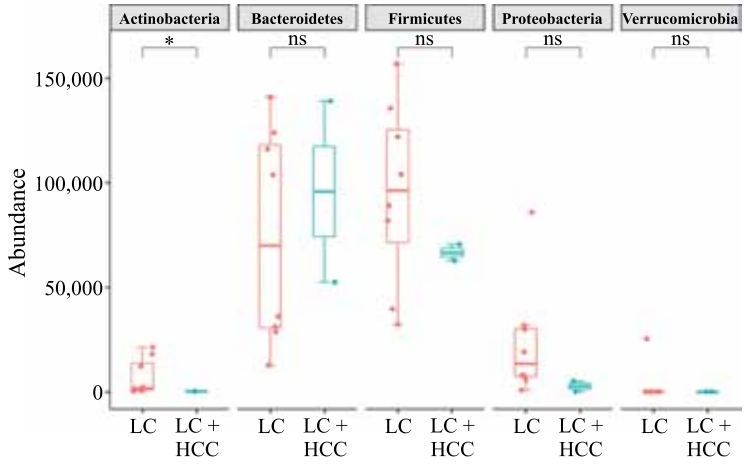


Fig. 2. Comparison of microbiota composition in patients with liver cirrhosis and liver cirrhosis. Statistically significant difference in actinobacteria is observed in patients who underwent liver transplantation due to liver cirrhosis a and liver cirrhosis + HCC. *, a statistically significant difference. “Period 2”, material collection after liver transplantation

microorganisms are staphylococci, enterococci and E. coli. In turn, high MELD, biliodigestive anastomosis, and pre-transplant infections are also known prognostic factors of this type of post-LTx complications [11–15]. In addition, colonization by multidrug-resistant bacteria and the severity of immunosuppressive therapy after LTx significantly aggravate the prognosis of overall survival and graft survival [13, 16–20]. Since the liver is constantly exposed to bacterial products of intestinal microbiome origin by means of anatomical and physiological connection between the intestine and liver, conditioned by portal blood inflow on one side and biliary tract on the other, which in their totality make up the GLA concept, it becomes clear that that GLA seems to play a significant role in these complications and risk factors [11, 12, 21]. This is increasingly supported by recent studies pointing to bacterial commensals and products of bacterial commensalism, such as pathogen-associated molecular patterns (PAMPs), which can move freely from the intestinal lumen into the liver against the background of a body compromised by pathological process, thereby triggering a cascade of immune and proinflammatory reactions [22, 23]. The altered balance of immune response regulation, known as cirrhosis-associated immune dysfunction, is well studied today in patients suffering from chronic diffuse liver disease. This alteration of adaptive immune processes decreases the body's ability to remove cytokines, bacteria and lipopolysaccharides from the general bloodstream, thereby negatively affecting the reparative characteristics of the body [12, 22, 24–26]. In the meantime, monocyte migration, chemotaxis and bacterial phagocytosis are significantly reduced in patients with cirrhosis compared to a healthy population; patients with ACLF have lower expression of antigen-presenting HLA-DR molecules on monocytes, which may lead to decreased monocyte activation and cytokine secretion. In experimental models, microbial translocation in mice induced type I interferon production, which led to interleukin-10 production by myeloid cells and subsequent loss of control over the infectious agent and higher mortality in the experimental animals [27–29]. At the same time, the number of works devoted to the problem of changes in immune response in a GLA context in patients and liver transplant recipients is very small, but the results of these studies will significantly increase the understanding of the role of intestinal microbiota in post-transplant complications. So, Wu et al, observed high levels of endotoxin and IL-6 expression in plasma among patients with liver cirrhosis, and the results of the study correlated with specific phenotypes of the gut microbiota in many parameters. In this study, LTx was used to restore gut microbiota,

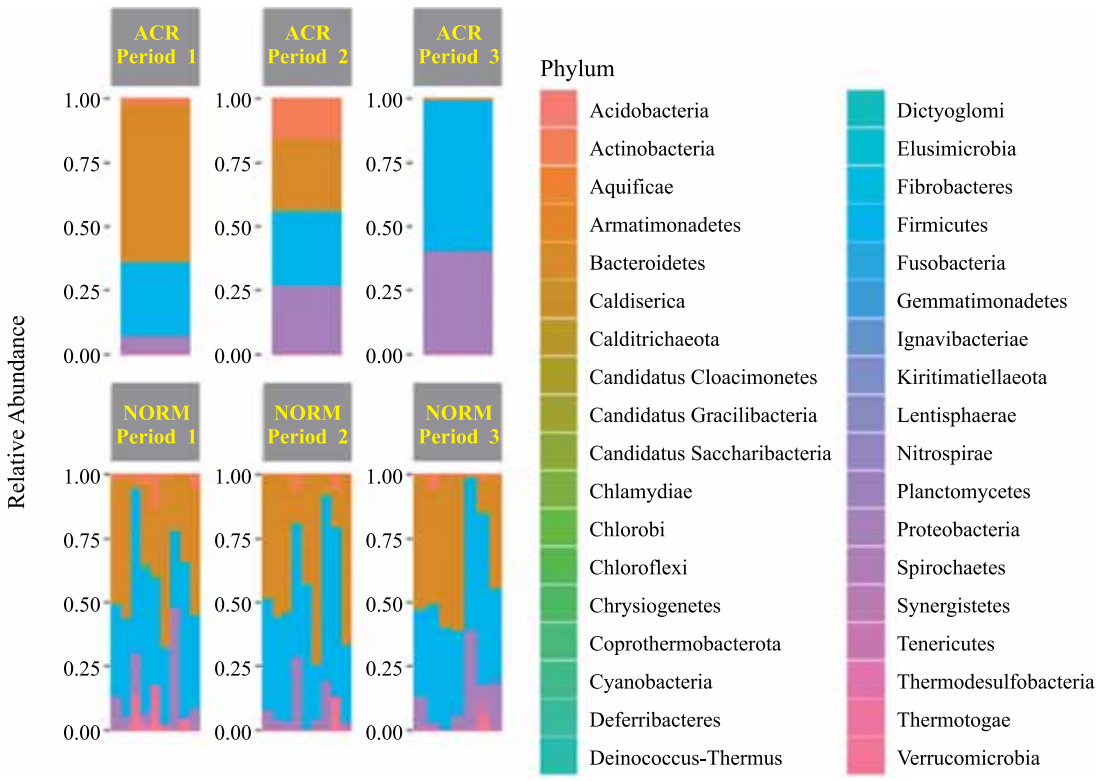


Fig. 3. Microbiota composition in patients with acute cellular rejection episode and a successful posttransplant period. "ACR", acute cellular rejection; "Period 1", material collection before liver transplantation; "Period 2", material collection on the 3rd day after liver transplantation; "Period 3", material collection on the 10th day after liver transplantation. There is a marked difference in the microbiome palette in patients before and after liver transplantation

as well as reduce plasma endotoxin levels in IL-6, which were directly associated with the incidence of post-LTx infectious complications [30]. In addition, in one of the most significant recent studies conducted at Kyoto University Hospital [31], the authors, in their prospective study, found a statistically significant difference in the microbiome map in patients who developed bacterial infection after undergoing LTx compared with the control group. Patients with resurgent bloodstream infections (BSI) had a significantly lower Shannon's diversity index (SDI) at the onset of bloodstream infection than in the pre-transplant period ($P = 0.026$). In the posttransplant period, SDI was also lower in BSI patients than in the non-BSI patients ($P = 0.040$).

Moreover, in the same study, the authors found a statistically significant difference with regard to SDI in ACR patients.

So, it can be assumed that microbiome restoration in the post-transplant period can significantly reduce the risks of infections by reducing microbial translocation and subsequent inflammation. In addition, given the works devoted to the immune response and immune regulation of processes occurring within the GLA, it is possible that a deeper understanding of the functioning of the "virtual organ" will also shed light on many unresolved issues associated with both the frequency of infectious complications and the frequency of liver transplant rejection.

Overall, although this is a promising field, there is currently little data on the modulation of immune response by the microbiome. The stratification of trigger mechanisms and systematization of risk factors is an urgent task facing modern transplantology [30, 32].

Pathogen-associated molecular patterns and immune response

Activation of toll-like receptors (TLRs), which are analogues of recognition receptors for various antigenic patterns in mammals, is important evidence of the close relationship between immune response and translocation of the intestinal microbiome within GLA [12, 30, 33–39]. According to Albilos et al., changes in the functionality of the gut microbiome appear to be more relevant to immune response activation than changes in its composition [12]. In turn, the infectious patterns of the intestinal microbiome are the so-called "PAMPs" which are products of microbial metabolism specifically produced only by pathogens, in this case bacteria and viruses; this term implies a large number of molecules such as lipopolysaccharides, lipids and nucleic acids [34, 37, 38]. In turn, 13 types of mammalian TLRs are currently known. In humans, there are 10 TLRs ranging from TLR1 to TLR10. TLR2, 4, 5, 9 play the greatest role in terms of GLA and microbiome influence on liver tissue [40]. TLRs are expressed in cells of the immune system, as well as in epithelial cells and fibroblasts. However, with respect to pathogen-associated molecular pattern recognition or TLR-PAMP recognition by TLRs, not all TLRs play the same role. For example, a significantly lower number of TLR2 was found in patients suffering from chronic progressive liver diseases compared to the healthy group, while the number of expressed TLR2 was significantly higher in patients suffering from chronic viral hepatitis and nonalcoholic steatohepatitis (NASH) [41]. As for TLR3, many authors point out their protective and anti-inflammatory role [40].

TLR4 selectively recognizes lipopolysaccharide (LPS), heat shock proteins, fibronectin or specific viral envelope proteins [42, 43]. This group of receptors is the most studied in terms of GLA. They have been noted to be significantly elevated in those patients suffering from chronic liver disease (CLD), whose portal blood had high levels of circulating LPS [40, 44–46]. In addition, the association between TLR4 and liver fibrosis has been demonstrated in a number of experimental models. For example, in experimental mice, TLR4-mediated MyD88-NF-κB activation enhances proinflammatory cytokine production, α-SMA, TIMP1 and TGF-β expression, and is associated with disturbances in the extracellular matrix architecture [40, 47].

In turn, TLR5 are less studied, but they are known to play a projective role in NASH pathogenesis. Meanwhile, peritoneal infiltration by flagellin, which is a ligand for TLR5, stimulates massive expression of interleukins, neutrophil and macrophage infiltration of the liver [48]. A large number of experimental studies have been devoted to TLR7, but the full range of their functions is still the subject of debate and discussion. However, their role in both NASH and other chronic progressive liver diseases is known. The presence of these diseases is always associated with a large number of expressed TLR7, as well as a large amount of production of SMA and type 1 collagen [49].

TLR9 appears to be of great importance in patients suffering from alcohol-related CLD, which has been proved by numerous experimental models. Their role in NASH development and progression has also been demonstrated [40].

PAMPs recognition by TLR usually leads to activation of the proinflammatory pathway signaling cascade, which initiates the activation of genes encoding the release of inflammatory cytokines and acute phase inflammation proteins [23, 34, 50–53]. This response mechanism is physiological and necessary for protection against pathogens, but its excessive or prolonged activation can cause functional and morphological changes, leading to a compensatory decrease in immune system activity during chronic pathogenic stimulation. Thus, chronic susceptibility to some infectious agents is formed [34]. For example, prolonged exposure to gram-negative bacteria presented by LPS can induce tolerance to this endotoxin, which is subsequently characterized

by impaired antigen presentation, decreased expression of proinflammatory mediators and overexpression of anti-inflammatory signal molecules [51, 53].

Apart from PAMPs, TLRs can recognize the so-called danger-associated molecular patterns (DAMPs), which originate from apoptotic destruction cells and also play an important role in immune-inflammatory response [43].

Thus, PAMPs translocation, including in the form of LPS and lipoteichoic acid as bacterial cell walls and DAMPs in the form of dead bacterial fragments, lead to initiation of the interaction of various cells of the immune system and production of inflammatory cytokines, followed a related response to their release into systemic circulation [12, 33, 34, 37, 54, 55].

Besides, the balance of proinflammatory and anti-inflammatory cytokines may shift the course of the underlying disease toward progression or regeneration in patients with chronic progressive liver disease [12, 33, 34, 55, 56–59].

Systemic inflammation in patients with CLD compared with healthy individuals is thought to be caused by translocation of PAMP and DAMP into the portal and systemic circulation through the compromised intestinal barrier [12, 23, 30, 34]. In this case, the physiologically slow blood flow in the liver sinusoids provides a close and complete interaction of intestinal molecules with parenchymal and nonparenchymal liver cells and, importantly, with immune cells [60]. Thus, induction of inflammatory response mediators formed due to active cytokine expression, plays an important role in activation of profibrotic and proinflammatory signals cascade, promoting further deterioration of CLDs [12, 17, 26, 33, 34, 54]. In response to the triggered immune cascade, T cells and additional macrophages originating from monocytes are recruited to the liver. Further, through TLR4 presented on the surface of macrophages, bacterial LPS is recognized leading to activation of tumor necrosis factor (TNF)-α synthesis [43, 51, 55]. Ultimately, PAMPs and/or DAMPs create a proinflammatory environment leading to hepatocyte injury, Ito cell activation, and liver fibrosis. It is this pathway that is of great importance today in the development of complications after LTx, particularly infectious complications, acute and chronic graft rejection, and post-transplant liver fibrosis [11, 19, 23, 54]. Furthermore, the importance of GLA and the intestinal microbiome in terms of immune response is further emphasized by studies demonstrating a link between HCC and chronic liver inflammation caused by microbial translocation, in particular by development of liver carcinoma in NASH [23, 61].

Hepatic regulation of gut microbiota

Intestinal microbiota and bacterial products influence liver function by influencing immune reactions occurring in the liver. The GLA concept also implies a reverse pathway, a pathway that regulates the microbiome colonizing the gut. This regulation fully reflects the bidirectionality of the GLA concept [12, 55, 57, 62]. Thus, the liver "delineates" the gut microbiota through IgA and bile release.

The latter is known to contain bile acids synthesized from cholesterol in the liver. These acids have a direct effect on gut microbes, causing membrane damage and disrupting the function of proteins, DNA, and bacteria. In turn, bile acids are metabolized in the intestine by the microbiota to form secondary bile acids that activate specific receptors, particularly the nuclear farnesoid X receptor (FXR) and the G protein-coupled bile acid receptor (GPBAR1), also called TGR5. These receptors regulate numerous immunological and metabolic pathways in the host, which may also indirectly influence the intestinal microbiota [8, 11, 12, 57].

In turn, bile acid composition can be indirectly regulated by the microbiota through the Myd88 signaling pathway, which changes the profile of bile acids [40, 63]. As a result of direct or indirect mechanisms of influence on the composition of bile acids, the intestinal microbiota composition may also change. So, for example, the number of Bacterioids may decrease and the number of Firmicutes may increase [7, 64]. As another example, the growth of Clostridium difficile can be suppressed by re-regulating secondary bile acid production. The liver is an important source of IgA production, which is transported to the intestine via the biliary tract. In turn, IgA is important for controlling the gut microbiota quantitatively, as well as protecting the intestinal mucosal layer [65, 66]. Impaired IgA production has been shown to result in a significant increase in the biomass of anaerobic microbes in the small intestine. In addition, it seems interesting that the transition to adult microbiota is also controlled by IgA, which has been proved by relevant studies [64, 67]. In particular, IgA-lacking mice show persistent colonization by gammaproteobacteria, which are normally present in neonates but are lost in adults [67]. Prolonged presence of these bacteria can induce pro-inflammatory cytokines in the colon and increase intestinal inflammation [68].

Since bile acids and the microbiome mutually influence each other, it is obvious that decreased secretion of bile acids into the intestine as observed, for example, in liver cirrhosis, promotes severe dysbiosis with the formation of multiple pathobionts [12, 65]. As liver cirrhosis progresses, changes in the microbiota lead to inflammatory intestinal phenomena, damage to the intestinal barrier and, as a consequence, initiation of inflammatory phenomena of the liver, which, in turn, further suppresses its secretion of bile acids. Moreover, decreased intestinal FXR signaling impairs the function of the intestinal barrier by reducing the thickness of the mucosa and antibac-

terial protein synthesis, thereby damaging the intestinal vascular barrier [8, 12].

Microbiome and graft rejection

To date, it is known that the immune system is a kind of "bridge" to maintain the symbiotic relationship between the microbiome and the host. As described above, the gut microbiota modulates the host immune system to a certain extent, and the immune system has an inverse effect on the gut microbiota composition [63, 69]. In turn, gut lymphoid tissue represented by T and B cells, antigen-presenting cells and many others play an important role in systemic and local immune responses [23, 41, 57]. The microbiota is also known to actively shape the host's systemic immune response [2, 57, 70, 71]. Dendritic cells migrate to mesenteric lymph nodes, where they present antigens to stimulate the production of effector T cells [23, 63]. These mechanisms play an important role after LTx, especially in hepatic ischemia-reperfusion injury (IRI) [23, 69]. At the same time, one should take into account the fact that hepatic IRI is always present to some extent after LTx [73, 74].

Thus, IRI leads to parenchymal metabolic disorders and hepatocyte death by releasing DAMPs, which signal through TLRs to activate innate immune cells (including Kupffer cells). Subsequent reperfusion enhances this pro-inflammatory innate immune response, which, if further preserved, can indirectly influence the adaptive immune response [74].

It is known that the severity of post-LTx IRI predicts early allograft dysfunction, the probability of complications, and long-term graft survival [75–80]. At the same time, the severity of IRI, according to a number of scientists, is of particular importance for studying the impact of the gut microbiome on innate immunity in the early post-transplant period [78, 81, 82]. For example, one study has shown that administration of probiotics, particularly bifidobacterium and lactobacillus, reduces the severity of IRI by reducing plasma endotoxin levels and restoring intestinal barrier function [82]. In addition, in rat experiments, preliminary ischemic preconditioning of the liver (short IRI periods to condition the tissue against prolonged periods of IRI) restores the intestinal microbial composition and reduces IRI, in particular increasing the number of lactobacillus, bifidobacterium and clostridiales, with a decrease in proteobacteria [82]. In addition, short-chain fatty acids (SCFAs) are powerful immunomodulators and can inhibit the activation of macrophages, a critical IRI mediator, with intravenous butyrate administration reducing the severity of IRI [73].

It has also been shown that FXR-mediated bile acid signaling affects the severity of IRI by restoring the bacterial composition involved in the synthesis of secondary bile acids. This fact can be considered as a potential idea of influencing the severity of IRI by means of obeticholic acid and lithocholic acid [83]. However, to date, we have not found any published evidence for this, nor have we found any large study devoted to this issue.

It has also been proven that the microbiome can influence adaptive immunity and changes in the gut microbiota are associated with ACR [10, 84]. Interestingly, early scientific works devoted to this issue did not confirm the link between the microbiota and ACR incidence. In our opinion, this is due to the limitation of these studies to the use of drugs directly affecting the microbiome characteristics. In particular, these studies focused on the relationship between gut decontamination, the use of prebiotics and probiotics, and their association with ACR [81]. At the same time, the relationship between microbiome and ACR was not considered from the perspective of GLA.

In turn, many modern experimental studies have been able to establish a significant association between the intestinal microbiome composition and ACR incidence [85–87]. For example, Ren et al. demonstrated a dramatic change in gut microbiome composition in rats that developed liver ACR compared to the group without ACR. This was assessed on days 3 and 7 after transplantation (the days that are most critical for ACR) [10]. Other original studies have shown an association between dysbiosis and ACR in LTx patients. Thus, changes in the following bacterial families were observed in patients with advanced ACR: Bacteroides, Enterobacteriaceae, Streptococcaceae and Bifidobacteriaceae, with a decrease in Enterococcus, Lactobacillus, Clostridium difficile, Ruminococcus and Peptostreptococcus.

In our own observation, two patients developed acute graft rejection in the immediate postoperative period. Of course, because of the small number of observations, it becomes impossible to perform a qualitative comparative statistical analysis. At the same time, as indicated today, an increasing number of theoretical and experimental studies point to the potential importance of the intestinal microbiome composition in liver ACR pathogenesis of a liver transplant [10, 83, 84]. In this regard, the obtained results of microbiome palette mapping in patients with ACR in a transplant seem promising to us.

Thus, the listed studies, including our own observations, indicate the possibility of changing treatment approaches in the management of LTx recipients. Prebiotic treatment options for ACR are considered promising, and their effect on ACR was evaluated in a meta-analysis of 3 randomized controlled trials. All of these included a study of the use of lactobacilli as probiotics in LTx patients [88–90]. Although there was some difference in the incidence of ACR, no statistical significance was noted by the authors. At the same time, it is known that there are presently a lot of works in this direction.

CONCLUSION

Considering all of the above, it becomes obvious that GLA plays a critical role in the course and progression of many liver diseases, and in some cases may act as the initial mechanism of etiological determinacy for certain diseases. In turn, it is also known that the intestinal microbiome is a key link in the functioning of this "virtual organ". So, the role of GLA in NAFLD and NASH has been proven. The contribution of GLA in fighting the condition of patients with ACLF is beyond doubt. Multi-author works point to the confirmed role of GLA in infectious complications in patients who underwent LTx [91–96]. In addition, the influence of GLA on some immune-inflammatory processes has been demonstrated. At the same time, the influence of the liver itself on the formation of the "architecture of the intestinal microbiome" is not in doubt today [97, 98]. In this regard, numerous scientific works of recent years have been devoted specifically to the study of the influence of GLA and the intestinal microbiota on certain processes in the body, including complications associated with the immune response and bacterial infection after LTx. These studies have become possible due to application of 16S rRNA profiling of the microbiome by means of next-generation sequencing (NGS), which is a group of methods for determining the nucleotide sequence of DNA and RNA to obtain a formal description of its primary structure [95, 96, 99, 100]. NGS methods make it possible to "read" several genome sites at once, in this case, the intestinal microbiome [101–105]. The resulting nucleotide pattern allows to determine the relationship between a particular microbiome and the frequency of certain postoperative complications. The pattern also allows for deepening the understanding of the pathophysiology of these complications. In turn, the results will facilitate not only the characterization of predictors and risk factors of bacterial infection and rejection episodes, but also the formation of a completely new approach to the treatment tactics for certain complications, including through formation of a microbiota-oriented pharmacotherapy.

The authors declare no conflict of interest.

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LIVER TRANSPLANT PROGRAM AT BOTKIN HOSPITAL. EXPERIENCE OF 100 SURGERIES

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Objective: to present an analysis of the results of 100 cadaveric liver transplants performed at Botkin Hospital from July 2018 to October 2021. **Materials and methods.** From July 2018 to October 2021, 100 orthotopic liver transplantation (LTx) from a deceased donor were performed at the surgical clinic of Botkin Hospital. The recipients were 58 males (58%) and 42 females (42%). The mean age of the recipients was 48.73 ± 8.56 (24–66) years, while their mean MELD was 19.54 ± 4.35 (15–33). The main indications for LTx were cirrhosis resulting from chronic viral hepatitis (CVH) C (52%), nutritional-toxic cirrhosis (20%), autoimmune liver and bile duct disease (18%), CVH B (7%), and hepatocellular carcinoma (HCC) (3%). During the period under study, 119 potential liver transplant donors were evaluated. The mean age of the donors was 44.2 ± 11.12 (21–63) years. Median levels of sodium, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin were 141 (138–146) mmol/L, 27 (20.7–47.4) units/L, 25 (17–41.5) units/L, and 9.65 (6.42–13.7) μmol/L, respectively. The median graft hepatic steatosis was 10% (5–15). LTx was performed using the piggyback technique (99/100 cases) and classic technique with inferior vena cava resection (1/100). End-to-end porto-portal vein anastomosis was performed (99/100 cases). Anastomosis of the donor organ's portal vein with the recipient's left gastric vein due to occlusive thrombosis of the recipient's portal vein was carried out (1/100). In all cases, a continuous end-to-end arterial anastomosis was formed. End-to-end choledochocholedochal anastomotic strictures (95/100) and end-to-side hepaticojejunostomy (5/100) were formed. **Results.** Median cold ischemia time was 312.5 minutes (280–380). Mean operative time was 488.91 ± 65.34 (95% CI: 475.9–501.9) minutes, median intraoperative blood loss was 1000 (600–1500) mL. Thirty-day mortality was 2% (Clavien–Dindo class V). Early postoperative complications (Clavien–Dindo class IIIa–IVa) developed in 12/100 patients (12%). Graft arterial thrombosis occurred in 3 cases (3%), biliary anastomotic strictures in 6 (6%), and subhepatic hematoma in 2 (2%). The average intensive care unit (ICU) bed day was 2.34 ± 1.67 (1–8). Total postoperative bed-day was 14.63 ± 5.35 (10–39). During case follow-up, a prolonged form of calcineurin inhibitor (CNI) was administered as immunosuppressive therapy in mono regimen (85 patients), in combination with mycophenolic acid derivatives (7), and in combination with everolimus (6). Of the 93 patients, 46 patients (49.46%) had the new coronavirus infection (COVID-19) before or after transplantation; in no case did COVID-19 lead to death. Six patients (13.04%) were hospitalized due to COVID-19. To date, 33/93 (25.48%) patients have been vaccinated, resulting in 75 (75%) liver transplant recipients immune to COVID-19. The overall 1-year survival rate was 95% and the 3-year survival rate was 91%. **Conclusion.** Introduction of LTx in multidisciplinary hospitals allows to, already at the start of the program, achieve immediate and long-term treatment outcomes (in decompensated diffuse liver disease) that are comparable to those of leading transplantation centers.

Keywords: liver transplantation, Botkin Hospital, survival rate.

INTRODUCTION

A little more than half a century has passed since the first successful LTx by the great surgeon Thomas Starzl (USA, 1967). Since then, LTx has become a standard procedure for patients with end-stage liver disease. To date, more than 80,000 LTx operations have been performed worldwide [1]. According to the 2020 European Liver Transplant Registry (ELTR), the 1-, 5-, 10- and 20-year patient survival are 90%, 72%, 61% and 40%, respectively [2]. Along with active development in surgical and anesthesiological techniques, similar results were achieved due to development and introduction of modern immunosuppressive drugs, preservative solutions, perfusion devices, etc. Despite shortages in donor organs, the indications for LTx are expanding, and the progress of medical science allows to identify more and more candidates for LTx. Therefore, development of organ donation is a priority for transplant surgeons around the world.

The Russian Federation is not among the world leaders in terms of donor activity and number of liver

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transplant surgeries per million population. However, the situation has significantly improved over the past 10 years. Every year, due to the enormous demand for this type of medical care, more and more LTx programs are opening in multidisciplinary hospitals in different regions of the country [3].

The first LTx at Botkin Hospital was performed in July 2018. Over the 3 years of existence of the transplant program, we have managed to implement an algorithm by which patients receive quality medical care at all stages: from examination and inclusion in the waiting list to discharge from the hospital with a functioning transplant and subsequent regular outpatient follow-up [4]. A large flow of patients with end-stage liver disease, active development of organ donation in Moscow, and coordinated work by a multidisciplinary team of specialists have all allowed us to cross the threshold of 100 operations as of October 2021. In this paper, we have summarized and analyzed the experience of 100 cadaveric liver transplants performed at Botkin Hospital from July 2018 to October 2021.

MATERIALS AND METHODS

From July 2018 to October 2021, 100 orthotopic liver transplants from a deceased donor were performed in the surgical clinic of Botkin Hospital. The recipients included 58 men (58%) and 42 women (42%). The mean age of the recipients was 48.73 ± 8.56 (24–66) years. The mean MELD of the recipients was 19.54 ± 4.35 (15–33). Among the etiological factors that led to cirrhosis, chronic viral hepatitis C prevailed, 52% (Fig. 1).

During the period described, 119 potential liver transplant donors were evaluated. To assess the suitability of a liver transplant, we evaluated the donor's laboratory parameters, ultrasound findings with the assessment of echogenicity of the liver parenchyma, visual assessment of the graft before and after cold perfusion, and emergency histological examination. Grafts with the presence of hepatic steatosis of more than 50% of the liver parenchyma were considered unsuitable for transplantation. Predictors of this condition were: elevated levels of AST, ALT, total bilirubin in the donor, increased echogenicity of the liver during ultrasound examination (Fig. 2), and jaundice coloration of the liver after cold perfusion (Fig. 3). The final decision on the suitability of the organ for transplantation was made on the basis of histological examination (Fig. 4). 100/119 liver grafts were found suitable for transplantation.

The mean age of the donors was 44.2 ± 11.12 (21–63) years; 85 donors (85%) had vasopressor support at the time of explantation. Median sodium, AST, ALT, and bilirubin levels were 141 (138–146) mmol/L, 27 (20.7–47.4) units/L, 25 (17–41.5) units/L, and 9.65 (6.42–13.7) mmol/L, respectively. The median time spent by the donor in the hospital was 48 (26–78.5) hours, and the median percentage of hepatic steatosis was 25 (15–30).

LTx was performed using the piggyback method (99/100 cases) and classic technique with inferior vena cava resection (1/100) due to HCC invasion. Separation of the end-to-side splenorenal anastomosis at the stage of hepatectomy (2/100 cases) and the side-to-side mesocaval anastomosis (1/100) were done. These anastomoses were previously formed to prevent portal hypertension complications. End-to-end porto-portal vein anastomosis (99/100 cases) was formed, in two of them against the background of partially recanalized thrombosis of the main portal vein trunk. Anastomosis of the donor organ's portal vein with the recipient's left gastric vein (1/100) was formed due to occlusive thrombosis of the recipient's portal vein. In 21 cases (21%), given the

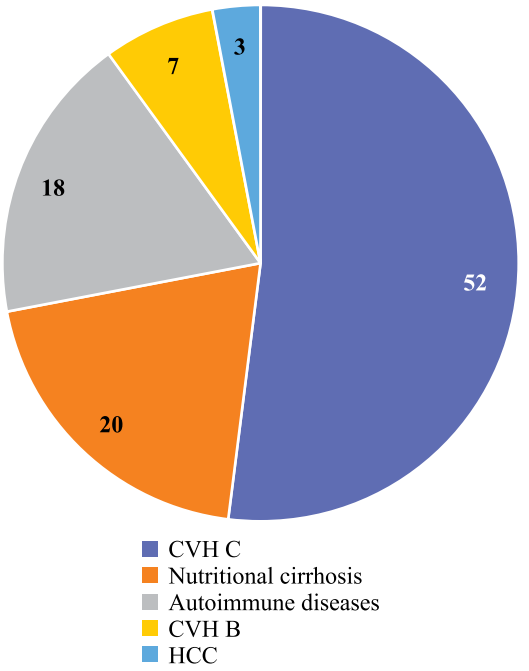


Fig. 1. Etiological structure of indications for LTx at Botkin Hospital (%)

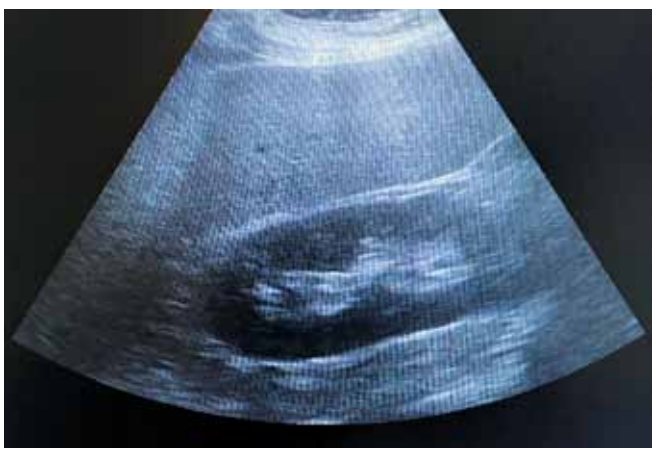


Fig. 2. Liver ultrasound in a potential donor. Increased liver echogenicity

specific arterial anatomy of the donor liver, a back-table vascular reconstruction was required (Figs. 5 and 6).

A continuous end-to-end arterial anastomosis was formed in all cases. Nodal end-to-end choledochocholedochal anastomosis was formed in 92 cases, continuous end-to-end choledochocholedochal anastomosis in 3 cases, nodal end-to-end hepaticojejunal anastomosis was formed in 5 cases in patients with autoimmune liver disease and common bile duct involvement.

RESULTS

Median cold ischemia time was 312.5 (280–380) minutes. Median operative time was 488.91 ± 65.34 (95% CI: 475.9–501.9) minutes, median intraoperative blood loss was 1000 (600–1500) mL. Thirty-day mortality was 2% (Clavien–Dindo class V). One death occurred from lightning-fast septic condition on postoperative day 3, another death resulted from acute heart failure on postoperative day 4. In addition, there were 12 complications (12%) that did not lead to death in the early postoperative period (Table). In 6 cases, the complications were managed by minimally invasive treatment, while relaparotomy was required in the other 6 cases (6%). So, there were postoperative complications in 14% of cases.

The average ICU bed-day was 2.34 ± 1.67 (1–8); total postoperative bed-day was 14.63 ± 5.35 (10–39). The long-term period had an immunological complication – acute humoral rejection with graft dysfunction, which was stopped by increasing immunosuppressive therapy, cascade plasmapheresis sessions with administration of immunoglobulins and rituximab.

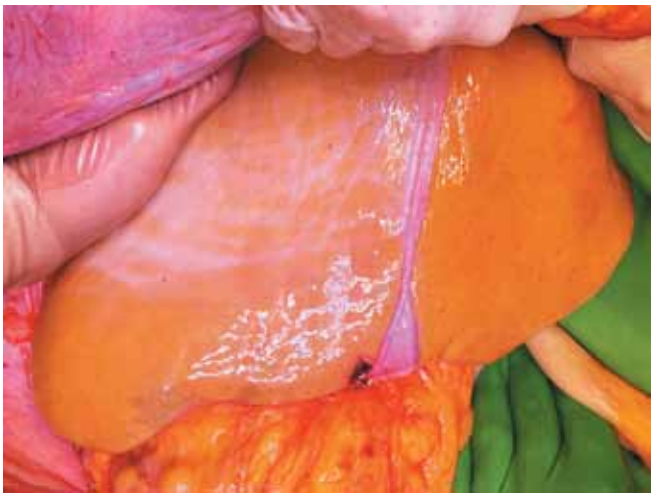


Fig. 3. Intraoperative photo. Liver after cold perfusion. Jaundice coloration as a sign of hepatic steatosis

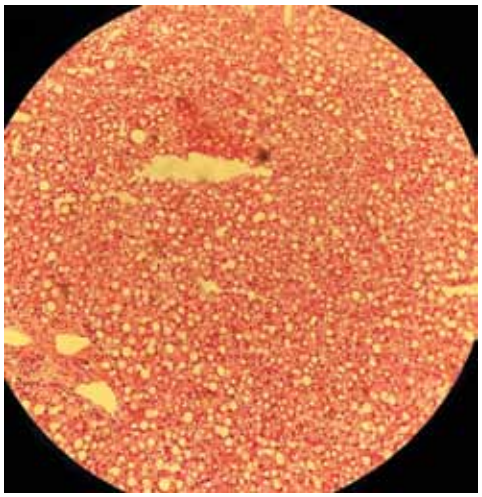


Fig. 4. Emergency histological examination. Over 80% hepatic steatosis. Organ unsuitable for transplantation

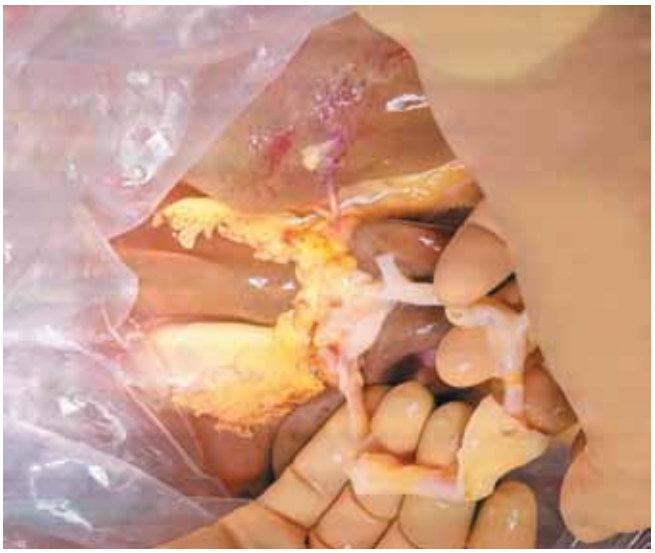


Fig. 5. Intraoperative photo, back-table stage. Graft arterial anatomy (Michels type III) – replacing the right hepatic artery originating from the superior mesenteric artery

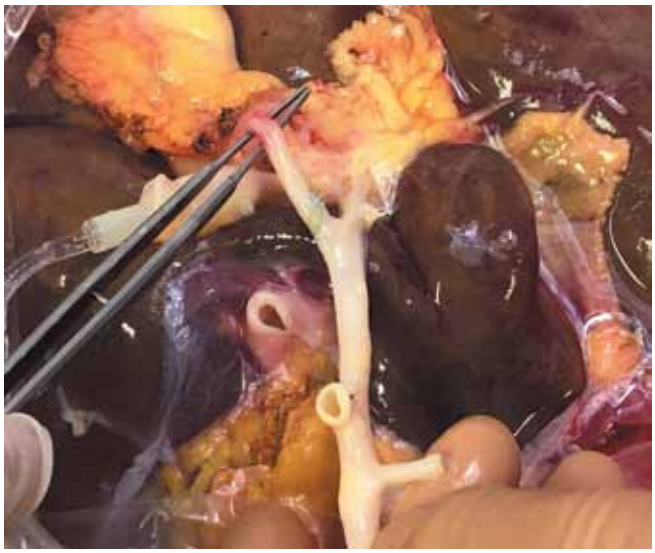


Fig. 6. Intraoperative photo, back-table stage. Vascular reconstruction of the hepatic graft. Anastomosis between the gastroduodenal artery and right hepatic artery

During case follow-up, a prolonged form of calcineurin inhibitor (CNI) was administered as immunosuppressive therapy in mono regimen (85 patients), in combination with mycophenolic acid derivatives (7), and in combination with everolimus (6).

In the long-term period, there were 5 deaths between month 3 and 21 after surgery. The overall 1-year and 3-year survival rates were 95% and 91%, respectively (Fig. 7).

Of the 93 patients under case follow-up, 46 recipients (49.46%) had COVID-19 before or after transplantation; in no case did COVID-19 lead to death after LTx. Six patients (13.04%) were hospitalized due to COVID-19. A total of 33/93 (25.48%) patients were vaccinated, of which 9/46 (19.5%) were vaccinated after the disease. The number of liver transplant recipients immune to COVID-19 was 75 (75%).

DISCUSSION

Liver transplantation is the gold standard of treatment for end-stage diffuse liver disease. Increasing the availability of transplantation care for this category of patients is an important step towards improving long-term treatment outcomes in this group of patients. Over the last few years, the number of effective donors in Moscow has doubled, reaching 20.9 per million population in 2020, which correlates with figures obtained by leading European countries. The opening of a new transplantation center at Botkin Hospital was a reasonable step for Moscow health care following the dramatic increase in the number of donor organs [5].

The multidisciplinary nature of the hospital was the key factor that made it possible to successfully start and actively develop the LTx program.

Table

Postoperative complications after orthotopic LTx at Botkin Hospital

Complication	Number, %	Clavien–Dindo	Correction method
Ascitic leak	1 (1%)	IIIa	Abdominal drainage
Arterial thrombosis	1 (1%)	IIIa	Endovascular balloon dilatation with stenting
Subhepatic hematoma	2 (2%)	IIIb	Relaparotomy, stopping bleeding
Ischemic cholangiopathy	1 (1%)	IIIb	Bilateral retrograde stenting with plastic stents
Hepaticojejunostomy stricture	1 (1%)	IIIb	Hepaticojejunostomy reconstruction
Biliary anastomotic stricture	3 (3%)	IIIb	Stricture stenting with a nitinol stent
Biliary anastomotic stricture	1 (3%)	IIIb	Hepaticojejunostomy formation
Arterial thrombosis	2 (2%)	IVa	Retransplantation
Heart failure	1 (1%)	V	
Sepsis	1 (1%)	V	
Total:	14 (14%)		

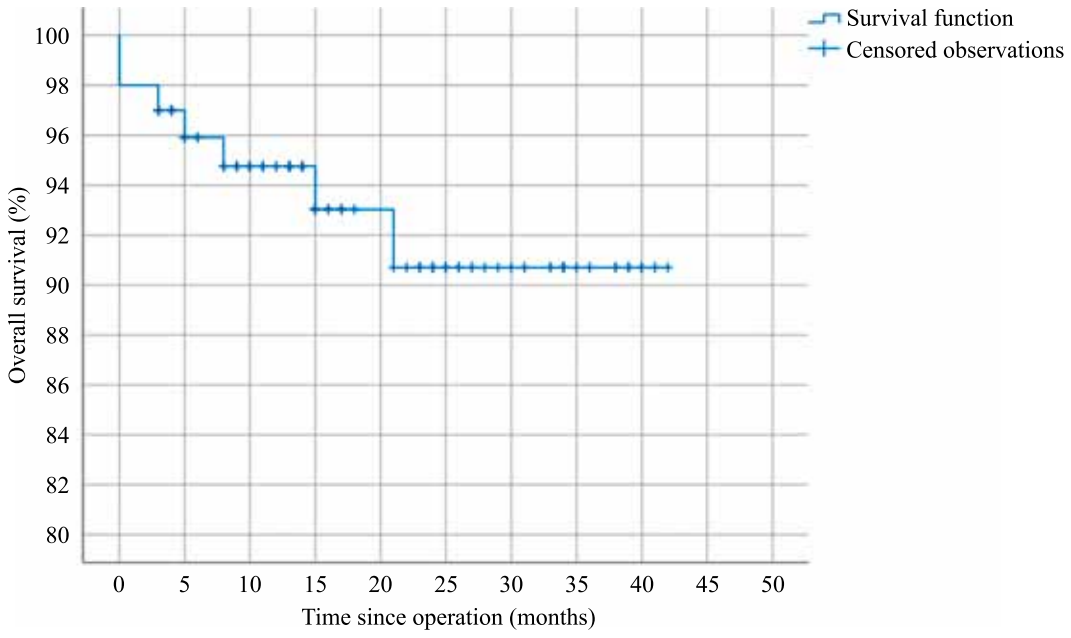


Fig. 7. Overall liver transplant survival rate

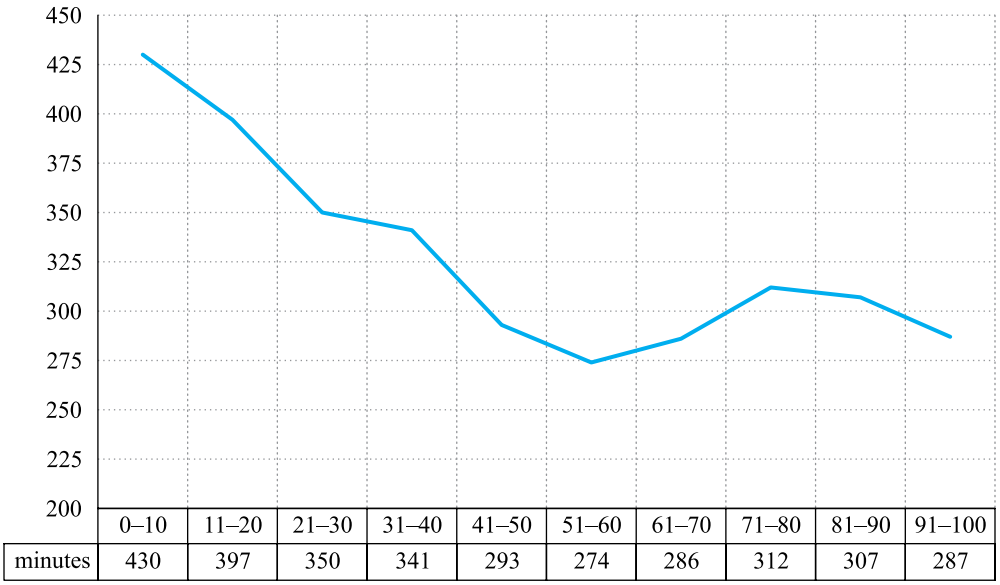
LTx is an emergency surgical intervention. At the time of surgery, transplant surgeons have sufficient information about the anatomy and functional state of the recipient's liver, but the team receives information about the anatomy and functional state of the liver graft only at the stage of explantation, and any deviations from the norm require informed, weighted, but quick decisions.

To assess the time of acquiring stable skills by the medical team providing liver transplantation medical care, we chose the cold ischemia time as an indicator, as it depends on the work of the organ explantation team, anesthesiologists, operating nurses, teams performing pre-transplant preparation of the liver transplant and hepatectomy (Fig. 8).

It can be seen from the graph that by the 40th operation, the average cold ischemia time had decreased to 293 minutes, which is slightly less than 5 hours, and then did not change significantly. This suggests that the surgical team acquires stable skills by the 40th operation.

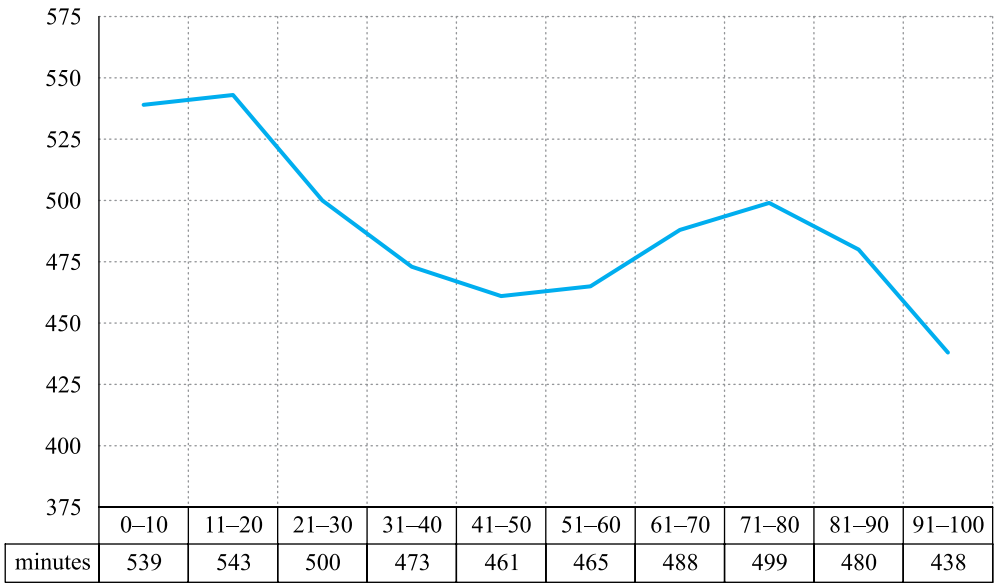
The surgical team directly encounters so many non-standard situations, which cannot be predicted at the preoperative stage: the severity of fibrous adhesions between the liver and the retrohepatic segment of the inferior vena cava, the level and diameter of the arterial junction, the nature of biliary reconstruction – biliary-biliary anastomosis, hepaticojejunal anastomosis.

We evaluated surgeons' learning curve for a new surgical intervention at the time of surgical intervention in the recipient (Fig. 9).



	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100
minutes	430	397	350	341	293	274	286	312	307	287

Fig. 8. Learning curve for liver transplant care teams



	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100
minutes	539	543	500	473	461	465	488	499	480	438

Fig. 9. Learning curve for transplant surgeons

The graph shows that by the 30th transplantation, the average time decreased to 473 minutes, then there was a second rise in the average operation time, which characterized the training of the second liver transplantation surgical team; it began to decrease also by the 30th operation. Thus, the learning curve of the liver transplantation surgical team was 30 operations.

Analysis of postoperative complications shows that the main part of them are complications of arterial and biliary anastomosis – 9/14 (64%). Development of the liver transplantation program in the multidisciplinary hospital allowed to stop most of these complications (5/9 (55.5%)) by minimally invasive treatment methods. The use of a coated nitinol stent with a short insertion time, a technique developed and first introduced in the Russian Federation in the surgical clinic of Botkin Hospital, demonstrated its safety and efficiency in the treatment of anastomotic bile duct strictures after orthotopic LTx [6]. The only experience of X-ray endovascular treatment of arterial thrombosis did not demonstrate its effectiveness in the long-term period regarding the condition of intrahepatic bile ducts. That is why in 2 patients where this complication reoccurred, LTx was performed with good long-term outcomes.

Case follow-up of liver transplant recipients is an important function of the transplant center. According to our data, liver transplant recipients compared to kidney recipients have a low risk of immunological complications in the long-term period. However, despite this, the transplantation center should have all the necessary laboratory, instrumental and drug support to stop graft rejection in this group of patients in order to prevent graft loss. A multidisciplinary hospital with the involvement of a multidisciplinary case conference certainly offers an advantage in the treatment of this type of complications as well.

Long-term survival rates are comparable to those in leading transplantation centers in Russia and the world [7–9], which is primarily due to the possibility of involving a multidisciplinary team for post-transplant management.

Our center has recently been focusing on educating patients before and after LTx on the importance of vaccination against COVID-19. Currently, 75% of our recipients are immune to SARS-Cov-2. None of our post-transplant recipients who have been vaccinated have had side effects. Meanwhile, the efficacy of various vaccines in liver transplant recipients needs to be investigated in the future. Currently, 88% of Botkin Hospital patients on the waiting list for cadaveric liver transplantation are immune to COVID-19.

CONCLUSION

Introduction of LTx in multidisciplinary hospitals allows to, already at the start of the program, achieve immediate and long-term treatment outcomes (in decompensated diffuse liver disease) that are similar to those of leading transplantation centers.

The authors declare no conflict of interest.

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POST-LIVER TRANSPLANT HBV INFECTION (REVIEW)

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Chronic hepatitis B virus (HBV) infection is common throughout the world. According to the World Health Organization, about 300 million people around the world are living with the HBV infection markers, with prevalence ranging from 0.4% to 8.5%, depending on the region. Untreated HBV infection results in severe liver disease, including cirrhosis and hepatocellular carcinoma (HCC), in at least one third of patients. While vaccination and new antiviral drugs are effective in preventing the severe consequences of HBV infection, liver transplantation remains the ultimate therapy for patients with HBV in cirrhosis. In patients with HBV replication, recurrence in the graft occurs in 100% of cases, which requires antiviral therapy combined with immunosuppressive therapy. According to the literature, de novo HBV infection after orthotopic liver transplantation (OLTx) in patients without replication and even in patients negative for hepatitis B surface antigen is between 1.7% and 5% [Castells L. et al., 2002]. After OLTx, liver recipients with baseline chronic HBV infection and patients with de novo HBV infection occurring after transplantation are indicated for long-term antiviral therapy.

Keywords: HBV infection, de novo, liver transplantation, nucleos(t)ide analogues, entecavir, tenofovir.

MAIN CHARACTERISTICS OF HBV INFECTION

HBV is the prototype member of a steadily growing family of viruses called hepadnaviruses. It is a partially double-stranded circular virion DNA (cDNA). According to the different genome sequence, there are 10 genotypes of HBV (A–J) [1]. The HBV genome basically encodes four types of antigens – HBsAg, HBcAg, HBeAg and HBxAg. The virus envelope consists of a double lipid bilayer and various proteins. The lipid bilayer contains the S antigen as well as the pre-S1 and pre-S2 antigens, which together make up the large, medium, and small protein forms on the envelope known collectively as hepatitis B surface antigen (HBsAg). Beneath the lipid bilayer is the viral capsid consisting of the bovine HBV antigen (HBcAg). The capsid contains circular, partially double-stranded DNA and DNA polymerase (encoded by the P gene). In addition, the serum contains a related nucleocapsid soluble E antigen called HBeAg. This antigen may be absent in some mutant strains. Gene X encodes a protein closely associated with the ability of HBV to cause virus-associated primary liver cancer [2].

HBV infection is widespread throughout the world. According to WHO, about 300 million people worldwide live with HBV, with a prevalence ranging from 0.4% to 8.5%, depending on the region.

The outcome of acute HBV infection depends on age. About 95% of infected infants, 20–30% of children infected at age 1–5 years, and less than 5% of adult patients develop chronic infection [3]. Untreated chronic HBV infection in at least one-third of patients leads to severe liver disease, including cirrhosis, hepatocellular carcinoma, and risk of hepatitis D virus (HDV) co/superinfection [4, 5]. The overall prevalence of HDV is about 0.98% (95% CI 0.61 to 1.42). In the HBsAg-positive population, HDV pooled prevalence was 14.57% (95% CI 12.93 to 16.27) [6].

While vaccination and new antiviral drugs are effective in preventing the severe consequences of HBV, liver transplantation (LTx) remains the ultimate therapy for patients with severe HBV-infected liver [7]. Besides, due to shortage of donor organs, in the clinical practice of some countries, especially in HBV endemic areas, organs from donors with HBV-infection markers are used for transplantation [8]. According to the literature, de novo HBV infection after OLTx is observed in 1.7–5% of cases [9]. After LTx, long-term antiviral therapy is indicated in patients with chronic hepatitis B and in patients with de novo HBV developing after LTx.

FEATURES OF HBV INFECTION AFTER LIVER TRANSPLANTATION

HBV reactivation after LTx is associated with various pre-transplant factors such as viral load at the time of transplantation, presence of HBeAg, and development of hepatocellular carcinoma. Various studies have demonstrated that certain HBV genotypes may be associated with a higher risk of recurrent infection. For example, genotype D has been shown to have this potential compared to genotype A [10]. If the viral load at the time of transplantation is above 10^5 copies/ml or 20,000 IU/ml,

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the patient is classified as being at high risk of recurrent HBV infection [10]. The virus cDNA is quite stable in infected cells and can persist in the latent state as a source for reactivation of the infection. It has long been known that hepatitis B virus cDNA can persist in the liver of patients decades after clinical and laboratory recovery from infection [11–13]. This persistence occurs despite an active immune response against the virus. In addition, clinical studies have demonstrated that therapy with nucleos(t)ide analogues can strongly suppress HBV DNA replication, but the decrease in the number of cDNA after one year of treatment was negligible [14]. Due to this peculiarity, HBV is rather difficult to eradicate, and its persistence, though at a low level, explains the reason for the possibility of hepatitis reactivation in any person infected with the virus, including after LTx.

Virus elimination occurs with the development of a sustained, polyclonal, multispecific CD4+ and CD8+ T cell response, as well as through B cell response and production of neutralizing anti-HBs antibodies. HBV-specific T cells both directly target infected cells for elimination through cytopathic mechanisms and suppress viral replication through interferon-mediated pathways [15, 16]. The neutralizing antibodies produced by activated B cells further limit the spread of HBV. Although these immune mechanisms are sufficient to control active HBV replication, they are probably not effective enough to destroy the entire pool of infected cells containing either "latent" HBV cDNA or low-replication HBV, which avoid exposure to HBV-specific immune cells [17]. Thus, these cells represent a reservoir of persistent HBV. Although the size and nature of this reservoir in individuals with serologic signs of HBV convalescence are unknown, it is clear that it is a source of HBV reactivation following disruption or suppression of immune control mechanisms. HBV reactivation after LTx is associated with suppression of the immune response by immunosuppressive drugs. Glucocorticoids suppress cell-mediated immunity by inhibiting the production of interleukins necessary for T and B cell proliferation [17]. Calcineurin inhibitors such as cyclosporine and tacrolimus suppress T cells by binding to immunophilin proteins and inhibiting interleukin production [18]. Thus, it is not surprising that after LTx and initiation of immunosuppressive therapy, the risk of potential reactivation of HBV infection increases.

DE NOVO HBV INFECTION AFTER LIVER TRANSPLANTATION

De novo HBV infection after LTx represents the development of infection in a patient without previous HBV markers and who has undergone surgical treatment for another liver disease. The source of HBV infection, as in the general population, can be transfusions of blood components, surgical interventions including dental surgery, sexual partners, etc. Also, in patients after LTx, a donor who is HBsAg negative but has HBc antibodies in serum and HBV cDNA in hepatocytes may be a source. After transplantation of such an organ to a recipient, the virus is reactivated against the background of immunosuppressive therapy, which leads to chronic inflammation of the graft [19].

LONG-TERM OUTCOMES OF LIVER TRANSPLANTATION IN HBV PATIENTS

Recurrent HBV infection after OLTx is an important factor that reduces graft and recipient survival, significantly worsening the long-term prognosis. Without prophylactic treatment, the HBV recurrence rate is very high, reaching 80–100%. Recurrence usually occurs between 6 and 12 months after LTx [20].

According to the European Liver Transplant Registry, 5,822 surgeries for Virus B related cirrhosis were performed from 1988 to 2016 [21]. This represents 5% of the total number of transplants during this period. Over the past 15 years, the role of HBV infection in cirrhosis requiring transplantation has decreased to 4%. The 1-, 5-, 10- and 15-year graft and patient survival rates were 82% and 86%, 72% and 76%, 66% and 70%, and 57% and 62%, respectively. Interestingly, the 1- and 5-year graft and patient survival rates after transplantation for alcoholic cirrhosis was similar to those of patients with baseline HBV, while the 10- and 15-year survival rates were significantly lower in patients with alcoholic cirrhosis – 55% and 59% and 40%, 43%, respectively. Graft and patient survival in patients with cirrhosis in HCV infection was lower than in patients with HCV infection (Table).

Table

Indications for liver transplantation and corresponding graft and recipient survival. European Liver Transplant Registry, data from January 2002 to December 2016 [21]

Reason for liver transplantation	Number of patients	% of total number of transplant surgeries	1-year graft and patient survival rates, %	5-year graft and patient survival rates, %	10-year graft and patient survival rates, %	15-year graft and patient survival rates, %
HBV	3826	4	82, 86	72, 76	66, 70	57, 62
HBV + HDV	1431	2	89, 93	84, 89	79, 83	75, 78
HCV	10495	12	78, 81	59, 64	46, 51	36, 40
Alcoholic cirrhosis	18135	20	83, 86	71, 75	55, 59	40, 43
Autoimmune diseases	2027	2	83, 88	74, 80	63, 72	45, 57

1,939 liver transplants were performed for Virus BD related cirrhosis, which accounted for 2% of the total number of operations. Transplant and patient survival rates were higher than those for hepatitis B monoinfection: The 1-, 5-, 10- and 15-year graft and patient survival rates were 89% and 93%, 84% and 89%, 79% and 83%, 75% and 78%, respectively. HBV accounted for 16% of etiologies of the underlying cirrhosis in HCC patients [21].

Patients without HBV replication after transplantation thanks to effective antiviral therapy have been shown to have a higher survival rate compared to recipients with persisting viremia. Providing effective antiviral therapy is essential to significantly improving long-term transplant outcomes in this category of patients [22, 23].

RISK FACTORS FOR PROGRESSION OF HBV AFTER LIVER TRANSPLANTATION

The following are risk factors for the development and progression of HBV after LTx [21]:

- Viral load at the time of LTx (more/less than 10^5 copies/mL of HBV DNA in serum)
- Presence/absence of HBeAg
- Presence/absence of resistance to antiviral medications
- Use of immunosuppressive drugs

The risk of reactivation is conventionally divided into high (if HBV infection reactivation rate is ≥10%), moderate (if risk of reactivation is 1–10%), and low (if risk of reactivation is <1%), depending on the type of immunosuppressive therapy and on the presence/absence of HBsAg, but positive anti-HBcAb. Treatment with calcineurin inhibitors is a moderate risk factor for reactivation (HBV reactivation rate of 1–10%) [24]. Low-dose corticosteroid therapy (prednisolone 10 mg orally daily for 4 weeks) can increase the risk of reactivation up to 10% in HBsAg-positive individuals. Medium-dose corticosteroids (10–20 mg orally daily) may increase the risk of seroconversion in HBsAg-negative and anti-HBc-positive individuals [25]. Therefore, these individuals require close monitoring. Routine screening for HBV infection, in the form of HBsAg and anti-HBs testing, is recommended for all patients at risk of HBV reactivation [26]. Prophylactic therapy with oral anti-HBV drugs is highly recommended for patients at high or intermediate risk of reactivation. For patients at low risk of reactivation, either proactive therapy or wait-and-see approach is recommended. Among HBsAg-negative and anti-HBc-positive patients, data on the risk of HBV reactivation and anticipatory therapy are very inconsistent in many situations. In general, the risk of HBV reactivation is much lower in HBsAg-negative and anti-HBc-positive patients than in HBsAg-positive patients. The greatest risk of reactivation requiring proactive therapy is associated with the use of B-cell depleting treatment regimens or transplantation. In most other cases in HBsAg-negative and anti-HBc-positive patients, close monitoring is recommended.

ANTIVIRAL THERAPY AFTER LIVER TRANSPLANTATION

In the late 1980s and early 1990s, there were studies showing that patients after LTx for HBV, without antiviral therapy, had a high risk of recurrent infection in the graft. Moreover, in patients without HBeAg replication and in the absence of HBeAg, the rate of recurrence is as high (50% to 75%) as in patients without these viral replication markers [27]. Liver recipients on immunosuppressive therapy and with persistent HBV developed aggressive chronic hepatitis, turning into cirrhosis or graft rejection within 1–2 years. In 1991, Davies et al. introduced the term “fibrosing cholestatic hepatitis” to describe a unique and fatal form of recurrent HBV infection. Histologically, fibrosing cholestatic hepatitis is characterized by balloon degeneration of hepatocytes, moderate or no inflammation, varying degrees of perisinusoidal fibrosis and cholestasis, and marked expression of HBsAg and HBcAg on immunohistochemistry [28]. Increased intracellular expression of HBV antigens is largely the result of immunosuppressive drugs, which weaken the immune response against infected liver cells and can directly stimulate viral replication. The 3-year survival rate of hepatitis B patients who underwent transplantation in the United States from 1987 to 1991 was only 55%, compared with 68–78% in patients who underwent LTx for other indications [29, 30]. A multicenter study in 1994 showed that LTx for HBV was associated with rapid graft infection and high mortality [31]. Therefore, the presence of HBsAg and HBeAg in patients was an absolute contraindication for LTx, and the presence of HBsAg without HBeAg as a relative contraindication [32].

Since the development of protocols for long-term prevention of human hepatitis B immune globulin (HBIg), which contains antibodies to hepatitis B surface antigen, in 1987, HBV-related liver disease has been included in the indications for OLTx in Europe [33]. However, the presence of hepatitis B was a relative contraindication for transplantation in the United States until the mid-1990s.

Interferon medications were once the basis of antiviral therapy for HBV infection before LTx, and were also used after LTx, as graft survival was very low without antiviral therapy [34]. Binding of type 1 interferon alfa to the interferon alpha receptor initiates a signal transduction pathway leading to the induction of multiple genes called interferon-stimulated genes. These genes encode multiple proteins that mediate the antiviral effects of interferon as well as its side effects. The number and severity of side effects, together with the injectable route of administration and the low efficacy of this therapy, are

the main reasons as to why interferon medications are hardly used today. In clinical trials, almost all patients had at least one adverse event. Serious adverse events were occurred in 10% of patients r treated with peginterferon alfa-2a and in 17% of patients treated with peginterferon alfa-2b. About 40% of patients needed dose adjustments due to adverse reactions. The most common reasons for dose modifications were neutropenia (27% for peginterferon alfa-2a and 18% for peginterferon alfa-2b) and thrombocytopenia (4% and 3%, respectively). About 14% and 10% of patients had to discontinue therapy because of adverse events [35]. The most common reasons for discontinuation of therapy were psychiatric (depression and irritability), systemic (e.g., fatigue, headache), or dyspepsia. Most patients experienced a flu-like syndrome, such as fatigue, fever, chills, myalgias, arthralgias, backache, headache, anorexia, nausea, diarrhea, impaired concentration, difficulty sleeping, weight loss, decreased libido, hair loss, and bone marrow suppression [36]. Interferon therapy also stimulates the body's immune response, increasing the risk of graft rejection, averaging 5%. After transplantation there are also hematological manifestations – cytopenia, anemia – requiring not only dose modification but also introduction of stimulants of hemopoiesis and leukopoiesis and even hemotransfusions (up to 50% of recipients) [37].

Human HBIg, which contains antibodies to hepatitis B surface antigen, is still used in many transplant centers. Some studies have shown that the combination of HBIg and direct-acting antivirals (DAAs) is effective in preventing HBV reactivation [38, 39]. In a meta-analysis including 1484 patients, a combination therapy of HBIg with nucleos(t)ide analogues was more effective in reducing HBV recurrence than monotherapy with DAAs, but the vast majority of included studies used lamivudine, adefovir or their combination [40].

At the same time, there is sufficient data showing that a monotherapy with DAAs has high efficacy in patients after LTx. A 53-month study of 362 patients who underwent LTx for cirrhosis resulting from HBV infection was conducted. None of the patients received HBIg. Half of the patients were placed on lamivudine (LAM), 39% received entecavir (ETV), and 12% received combination therapy (predominantly lamivudine + adefovir). The HBV recurrence rate at 3 years for LAM, ETV, and combination group was 17%, 0%, and 7%, respectively [41].

With the appearance of new nucleoside analogues (entecavir, tenofovir disoproxil fumarate, tenofovir alafenamide) with high resistance threshold, the concept of the need for lifelong use of HBIg to prevent HBV relapse, due to its high cost, lack of standard protocols and inconvenience in the long term (parenteral administration only), began to undergo significant changes: dose reduction, shortened course of administration, intraoperative administration only, which was not accompanied by an increased risk of HBV recurrence when coadministered with potent nucleoside analogues. At present, further studies on the possibility of completely excluding HBIg from antiviral therapy and preventing HBV recurrence after LTx are continuing [42–44].

Antiviral drugs for HBV infection can be divided into three classes:

- interferons
- Nucleoside reverse transcriptase inhibitors (lamivudine, telbivudine, entecavir)
- Nucleotide reverse transcriptase inhibitors (adefovir, tenofovir disoproxil fumarate, tenofovir alafenamide)

At present, interferons, as well as lamivudine, telbivudine and adefovir, are practically not used, especially after LTx, due to the high risk of side effects, resistance and low efficacy of these drugs. Several meta-analyses have shown the lamivudine to have a less favorable outcome for treatment and prevention of HBV reactivation than entecavir or tenofovir [45–47]. Tenofovir and entecavir are the most powerful antiviral drugs, characterized by a high genetic barrier to resistance and are used as monotherapy. The goal of antiviral therapy is to achieve and maintain a negative HBV DNA level.

Entecavir, an oral nucleotide analogue, is phosphorylated to form active triphosphate. By competing with its natural substrate, deoxyguanosine triphosphate, entecavir triphosphate inhibits all 3 functional activities of viral polymerase: 1) HBV polymerase priming, 2) reverse transcription of negative strand from pregenomic iRNA and 3) synthesis of positive strand HBV DNA. Entecavir triphosphate is a weak inhibitor of cellular DNA polymerases. The presence of mutations of HBV resistance to lamivudine increases the risk of entecavir resistance. Due to this, frequent monitoring of viral load in lamivudine-resistant patients and, if necessary, change of antiviral therapy are required. The drug is used in a 0.5–1 mg/day dose.

Tenofovir disoproxil fumarate is converted in the body to tenofovir, a nucleoside monophosphate (nucleotide) analogue of adenosine monophosphate. Tenofovir is subsequently converted to its active metabolite, tenofovir diphosphate. It is a nucleotide inhibitor of reverse transcriptase. The drug is used in a 300 mg/day dose.

Tenofovir alafenamide is a tenofovir phosphonoamidate prodrug (analog of 2'-deoxyinosine 5'-monophosphate). It penetrates primary hepatocytes by passive diffusion and is transported by hepatic capture transporters – organic anion transporting polypeptides. In primary hepatocytes, tenofovir alafenamide is primarily hydrolyzed by carboxylesterase-1 to form tenofovir. Intracellular tenofovir is subsequently phosphorylated to the pharmacologically active metabolite tenofovir diphosphate. Tenofovir diphosphate inhibits hepatitis B virus replication by introducing it into viral DNA via hepatitis B reverse transcriptase, resulting in DNA strand breakage. The drug is used in a 25 mg/day dose.

Another strategy for preventing HBV recurrence is the induction of active immunity through vaccination [48]. A study by Bienzle et al. showed the possibility of successful vaccination after LTx [49]. Vaccines targeting the preS1 domain, which can potentially overcome immune tolerance to HBV, have shown promising efficacy in developing an immune response in clinical trials. On the other hand, HBV vaccines may be more effective in preventing de novo hepatitis B infection in HBsAg-negative patients. In a study of 71 HBsAg-negative patients who received anti-HBc-positive grafts, de novo HBV infection did not develop in 54 patients who were vaccinated [50].

ADVERSE EVENTS THAT OCCUR WITH ANTIVIRAL THERAPY

The possibility of antiviral therapy with entecavir and tenofovir disoproxil fumarate for treatment of HBV infection in the post-transplantation period may be limited by resistance to a long-term drug, renal dysfunction against the background of combined administration with nephrotoxic drugs, especially with calcineurin inhibitors, as well as the presence of osteoporosis.

In patients with impaired renal function, the tenofovir disoproxil fumarate dose should be adjusted if creatinine clearance is <50 mL/min. In patients with 30–49 mL/min creatinine clearance, the interval between doses should be doubled. Patients with 10–29 mL/min creatinine clearance should use tenofovir disoproxil fumarate once or twice a week. For patients on hemodialysis, tenofovir disoproxil may be used after each hemodialysis session or every 7 days.

Studies investigating the efficacy and safety of tenofovir alafenamide in patients with chronic kidney disease (CKD) have shown the superiority of the drug over tenofovir disoproxil fumarate in influencing renal function and bone remodeling at weeks 48 and 96. A significant difference in glomerular filtration rate (GFR) reduction was demonstrated: 0.6 mL/min versus 5.4 mL/min in HBeAg-positive patients ($p < 0.0001$), 1.8 mL/min versus 4.8 mL/min in HBeAg-negative patients ($p = 0.004$). A significantly lower percentage reduction in bone mineral density in the hip was also reported compared with patients treated with tenofovir disoproxil fumarate (0.10% vs. 1.72% in HBeAg-positive patients ($p < 0.0001$) and 0.29% vs. 2.16% in HBeAg-negative patients ($p < 0.0001$) and spine (0.42% vs. 2.29% in HBeAg-positive patients, 0.88% vs. 2.51% in HBeAg-negative patients [51]. Studies have also been conducted on the use of tenofovir alafenamide in patients after LTx in the presence of chronic kidney disease (ID NCT02862548). There was demonstrated a significant increase in GFR in 48 weeks after the start of tenofovir alafenamide in patients after LTx taking calcineurin inhibitors, a decrease in alanine aminotransferase (ALT) levels compared with its activity on the background of tenofovir disoproxil fumarate [52].

CONCLUSION

The risk of HBV recurrence after liver transplantation in the absence of antiviral therapy is high, which is an important prognostic factor that reduces graft and patient survival. There is also a certain risk of de novo HBV infection after LTx requiring an antiviral therapy.

Based on analysis of published studies on HBV reactivation and de novo HBV infection in liver transplant patients, it can be stated that effective antiviral therapy is necessary to improve transplant outcomes and patient survival.

Given the lack of generally accepted protocols describing the treatment specifics for HBV infection developing after LTx, as well as the small number of studies on the use of DAAs after LTx, this study attempts to combine available data on the course of post-LTx HBV infection, effectiveness of antiviral therapy, and long-term outcomes (graft and patient survival rates).

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HISTORICAL ASPECTS AND CURRENT UNDERSTANDING OF AUTOIMMUNE HEPATITIS. WHEN IS LIVER TRANSPLANTATION INDICATED? (REVIEW)

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Autoimmune hepatitis (AIH) can occur at any age and is more common in women. The disease is a manifestation of autoimmune predisposition caused in genetically susceptible people exposed to certain environmental factors. The pathogenetic mechanism of AIH is not yet fully understood, but it involves an aggressive cellular immune response. The pathogenesis and severity of AIH also depend on various cytokines. This disease is characterized by elevated levels of transaminases – aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Liver histology plays a crucial role in confirming or supporting the clinical diagnosis of AIH. Diagnosis of AIH remains a challenge in clinical practice. AIH is one of the few liver diseases for which pharmacologic treatment has been shown to improve survival. Standard treatment is based on high-dose prednisone alone or prednisolone plus azathioprine. It leads to disease remission in 80%-90% of patients. Approximately 20% of patients do not respond to the standard steroid treatment and are treated with second-line immunosuppressive drugs: mycophenolate mofetil, budesonide, cyclosporine, tacrolimus, everolimus, and sirolimus. There have been reports on the use of infliximab and rituximab. In the natural course of AIH and resistance to therapy, there is a tendency for cirrhosis to develop and for the disease to progress to an end stage. These patients, as well as those diagnosed with fulminant liver failure, require liver transplantation.

Keywords: autoimmune hepatitis, AIH, pathogenesis, histology, treatment.

In 1950, the first description of hepatitis was published by Jan Waldenström. The disease was later to be called "autoimmune hepatitis" only in 1992. It has been previously referred to by various terms, most commonly "autoimmune chronic active hepatitis" [1]. Autoimmune hepatitis (AIH) is a rare liver disease [2–6] that occurs in children and adults of all ages and is characterized by progressive inflammatory hepatopathy [7]. Webb et al. [8] defined AIH as an uncommon idiopathic syndrome of immune-mediated destruction of hepatocytes, typically associated with autoantibodies. AIH can lead to acute liver failure [1], or the disease can become chronic and lead to an end-stage condition requiring liver transplantation [1, 2, 7].

Classification of autoimmune hepatitis. There are currently two main forms of AIH. Type 1 AIH is characterized by smooth muscle antibodies, antinuclear antibodies, or both, whereas Type 2 AIH is characterized by anti-liver/kidney microsomal antibodies, and anti-liver cytosol 1 antibodies, or both [4, 9]. Previously, there was a third form of AIH in which there are antibodies to soluble liver antigen (SLA-positive AIH). Later, it was found that SLA can be present in type 1 AIH and in cryptogenic cirrhosis. Immunoglobulin G4(IgG4)-related AIH recognized as a new disease [10].

Epidemiology of autoimmune hepatitis. AIH can occur at any age [7]. The average age of adult AIH patients is 58.6 years [11]. AIH incidence peaks around the age of 70 at diagnosis in both men and women. The incidence is lower at younger ages. In Japan, AIH incidence in both sexes peaks around age 60 [12]. However, AIH is more common in women than in men [4, 7, 13, 14]. Among the adult population, women are more frequently affected than men by a ratio from 3 : 1 to 8 : 1. A study by Abe et al. [11] found that the ratio of women to men suffering from AIH was 9 : 2. AIH mostly affects young women.

According to Werner et al. [15], AIH is a fairly uncommon disease in the Swedish population. Its incidence was 0.85 per 100,000 population, and 76% of the cases were females. Women had a peak after menopause, whereas men had a peak in the late teens. Autoantibodies indicative of AIH type 1 were found in 79% of cases. Almost half of the patients (49%) had other concomitant autoimmune diseases.

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L. Grønbæk et al. [16] identified AIH patients (n = 1721) from Danish nationwide health registries diagnosed from 1994 to 2012. The incidence rate was 1.68 per 100,000 population per year, and it doubled during the study period. Of the 1,318 patients who were biopsied at diagnosis, 28.3% had cirrhosis. In the first year after diagnosis, AIH patients had six-fold higher mortality than the general population; later, their mortality remained two-fold higher. Their 10-year cumulative mortality was 26.4% (95% CI 23.7 to 29.1). About 38.6% of deaths were liver-related.

In 2015, a nationwide survey of AIH patients in Japan (n = 1,682) diagnosed from 2009 to 2013 in 437 hospitals and clinics. The mean age at diagnosis was 60.0 years. Women (87.1%) were prevalent among the patients. Serum immunoglobulin G levels were high, peaking at 1.5–2.0 g/dL. Histological diagnosis of acute hepatitis, chronic hepatitis, and cirrhosis were seen in 11.7, 79.6, and 6.7% of patients respectively. In addition to elevated aminotransferase levels, the frequencies of emperipolesis and human leukocyte antigen (HLA)-DR2 positivity were higher in patients with acute hepatitis than in those with chronic hepatitis. Approximately 80% of patients were treated with corticosteroids, and in 97.7 % of them, their condition improved. Steroid pulse therapy was more frequently given to patients with acute hepatitis than to those with chronic hepatitis [12].

AIH prevalence and incidence are lower in the Asia-Pacific than in Europe and America. In Singapore and Brunei, the prevalence is 4–5 per 100,000 population, in Europe it is 10–20 : 100,000, and in Alaska it is as high as 43 : 100,000. European and American patients seem to have more severe disease, characterized with human leukocyte antigen-DR3 haplotype, younger age, more AIH-induced "cirrhosis" at diagnosis, higher elevated serum IgG levels [17].

Etiology of autoimmune hepatitis. The cause of AIH remains unknown [18], although both genetic and environmental factors are involved [3, 4, 7]. In other words, the disease is a manifestation of an autoimmune predisposition in genetically susceptible individuals exposed to likely environmental factors [19].

The liver is constantly exposed to a large number of different antigens: pathogenic infectious agents, toxins, tumor cells, food antigens and others. The liver's loss of tolerance to its own antigens can lead to AIH. The current paradigm states that the disease occurs in genetically susceptible subjects as a result of autoimmune processes caused by unknown factors, among which may be infections, chemicals and drugs. The disease etiology includes a clear association with: 1) HLA variants, 2) other non-HLA gene variants, 3) female sex, and 4) environment [8].

Risk factors for autoimmune hepatitis. Predictors of AIH are not clearly defined, but a genetic predisposition to AIH has been established for a relatively long time. AIH is not inherited in a Mendelian autosomal dominant, autosomal recessive, or sex-linked fashion. The mode of inheritance of the disorders is unknown and involves disruption of one or more genes working independently or together [20].

In AIH type 1, genetic predisposition is determined by a strong association with HLA antigens DRB1*0301 and DRB1*0401. In addition, the gene encoding cytotoxic T lymphocyte antigen-4 (CTLA-4) on chromosome 2q33 may also affect autoimmunity [21]. Similarly, both Europe and North America have a predisposition to AIH type 1 in individuals with HLA antigens DR3 (DRB1*0301) and DR4 (DRB1*0401). In a study of Japanese patients with type 1 AIH, all were found to have DRB1 alleles which encode histidine at position-13.

The predisposition to AIH type 2 is transmitted through HLA antigens DR7 (DRB1*0701) and DR3 (DRB1*0301). The disease is more aggressive and has a worse outcome in patients with DRB1*0701 antigens [22].

Not only genes of the major histocompatibility complex play an important role in autoimmune processes, but also genes involved in immune regulation and preservation of immune homeostasis, in particular those involved in apoptosis. According to K. Agarwal et al. [21], polymorphism of the Fas gene at position -670 does not influence susceptibility to AIH, but may affect the early development of cirrhosis. Cirrhosis at presentation was more common in patients with the adenosine/adenosine or adenosine/guanine genotypes than in those with the guanine/guanine genotype (29% versus 6%).

Pathogenesis of autoimmune hepatitis. The mechanism of the emergence and development of AIH is not fully understood, but it involves an aggressive cellular immune response [20]. Under the influence of yet unknown triggers, the mechanisms regulating immunity are violated. As a result, a pathological immune response, mediated by T-cells and directed against liver autoantigens, develops [23]. Immune reactions are inadequately controlled by damaged regulatory T cells [4]. Therefore, quantitative and functional defects in regulatory T cells play a crucial role in the onset and persistence of autoimmune liver injury in AIH [7, 8].

Various cytokines influence the pathogenesis and severity of AIH [24]. The complex interaction between proinflammatory cytokines and Th17 cytokines, as well as Treg IL-12p40 suppression are thought to play a central role in AIH pathogenesis. Serum IL-21 levels are significantly elevated in severe AIH cases compared to mild cases. Serum IL-21 levels positively correlate positively with total bilirubin levels and grading of necroinflammatory activity in liver biopsies [11].

Interleukin-33 (IL-33), which has proinflammatory activity, and its soluble ST2 (sST2) receptor, are involved in the pathogenesis of many autoimmune diseases. In the liver, IL-33 is secreted by hepatocytes and vascular

endothelial cells, including sinusoids. Their serum levels are significantly higher in AIH patients than in healthy individuals and in patients with other autoimmune diseases. Serum IL-33 and sST2 levels are significantly higher in acute-onset AIH than in chronic-onset AIH [18]. Serum IL-33 levels in patients with acute-onset AIH positively correlate with markers of hypergammaglobulinemia (IgG, IgM and IgA), liver injury (gamma glutamyltransferase and alkaline phosphatase) and proinflammatory cytokine levels (IL-17A and IL-4) [25]. Serum IL-33 and sST2 levels in AIH patients positively correlate with serum total bilirubin, ALT, and noninflammatory activity, but negatively correlate with serum albumin and prothrombin time. In AIH patients responding to prednisolone treatment, serum IL-33 and sST2 levels are significantly reduced after treatment. Interestingly, high serum IL-33 levels were associated with a significantly higher risk of recurrence [18]. The authors came to the following conclusions: 1) IL-33 and sST2 play an important role in the pathogenesis and severity of AIH; 2) they may be a promising target for AIH therapy.

Biochemical changes. AIH is characterized by elevated levels of transaminases [3–5]: AST and ALT [14]. Regardless of age, gender, or ethnicity, AIH can be suspected in patients with unexplained elevated liver enzymes and/or cirrhosis. Serum aminotransferase levels in AIH patients vary widely, and autoantibodies are not consistently present [26].

Immunological manifestations. In AIH, organ-specific and nonorgan-specific autoantibodies are present in the blood serum, and there is increased IgG levels [3–6, 14, 23]. According to Kim et al. [13], antinuclear antibodies, smooth muscle antibodies and hepatic/renal microsomal antibodies were present in 94.2%, 23.0% and 2.9% of AIH patients, respectively.

In acute presentation, in contrast to chronic AIH, there are often atypical immunoserological manifestations [27]. Thus, IgG levels may remain within the normal range. According to Lohse and Mieli-Vergani [28], 5% to 10% of patients with AIH have normal IgG levels at the time of diagnosis. In another study [26], 39% (27/70) of AIH patients also had normal IgG levels. According to the authors, this suggests that many AIH patients have atypical manifestations of the disease. In these patients, AIH can only be diagnosed if, in addition to a high autoantibody titer, there is a histological pattern "typical" of the disease. Therefore, close collaboration between hepatologists and pathologists is crucial for the accuracy of AIH diagnosis [27].

In AIH, T helper cells (T(H)0) are activated. In the presence of interleukin 12 (IL-12) or IL-4, T(H)0 lymphocytes can differentiate into T(H)1 cells, which play a leading role in macrophage activation. Increased HLA class I expression makes liver cells vulnerable to attack by CD8 T cells and induces expression of HLA class II hepatocytes. In addition, T(H)1 cells can differentiate into T(H)2 cells that produce IL-4, IL-10, and IL-13. These cytokines promote antibody production by B-lymphocytes. Recognition of autoantigens is tightly controlled by regulatory mechanisms, such as CD4+CD25 regulatory T cells. Thus, AIH is characterized by a quantitative and functional disruption of regulatory T cells, leading to preservation of effector immune responses followed by persistent liver destruction [29, 30].

AIH increases the number of follicular helper T (Tfh) cells expressing interleukin IL-21 in peripheral blood. IL-21 member of the type-I cytokine family. This interleukin exerts various effects on the immune system, including B cell activation, plasma cell differentiation, and immunoglobulin production. The level of IL-21 was found to be significantly elevated in the serum of patients with AIH compared with other liver diseases and controls ($P < 0.0001$). Moreover, the higher the level was, the more severe AIH was ($P < 0.05$). In addition, serum IL-21 levels correlated positively with total serum bilirubin levels ($p < 0.05$), grading of necroinflammatory activity in AIH patients ($p < 0.005$) and negatively correlated with serum albumin levels ($p < 0.05$). In patients with biochemical remission of AIH, serum IL-21 levels remained elevated and correlated positively with serum IgG levels ($p < 0.01$). significantly higher than that in healthy volunteers [11]. The authors conclude that IL-21 may play an important role in the pathogenesis of AIH, and may represent a promising target for AIH therapy.

Autoimmune hepatitis is associated with a predominance of T helper 1 (Th1) expression and a decrease in the number and function of regulatory T cells (Tregs). The role of circulating activated Tfh and plasma cells in the pathogenesis of AIH is associated with hypergammaglobulinemia [31].

Pathomorphology. Liver histology is critical in diagnosing AIH, especially when using simplified IAIHG criteria [32]. According to some investigators [33], biopsy for AIH can be excluded in patients with other clinical criteria for the disease. However, liver biopsy currently remains mandatory for AIH diagnosis [34]. In addition, liver biopsies are performed to monitor the effectiveness of therapy and to determine further treatment strategy.

The most typical, but non-specific pathohistological finding in AIH is the presence of borderline hepatitis (also called interface hepatitis), in which there is inflammation not only of the portal tract, but also of the periportal parenchyma, with its infiltration by lymphocytes, plasma cells and macrophages [3, 4, 6, 14, 18, 23]. The lymphocytic inflammatory infiltrate contains a large number of CD4+ T cells [8]. The high content of plasma cells in the inflammatory infiltrate is also one of the main histological indicators of AIH. In severe and progressive disease, centrilobular lesions and necrosis as well as bridge necroses are present.

In a study by Sandler et al. [35], 96% (79/82) of AIH patients had morphological signs of borderline hepatitis

with infiltrates consisting of lymphocytes and plasma cells; in addition, emperiopoiesis was diagnosed in 60% (49/82) and rosette formation in 23% (19/82).

Necrosis of hepatocytes leads to liver fibrosis [8]. Liver fibrosis and cirrhosis can occur even in the subacute course of the disease [28]. At diagnosis, almost 30% of patients already have cirrhosis [36], and in a study by Abe et al. [11] – only 18.2%. In AIH, corticosteroid treatment leads to partial restoration of liver morphology in 53–57% of patients. Fibrosis progression is slowed or prevented in 79% of patients. If it is not possible to completely suppress inflammatory activity within 12 months, cirrhosis continues to progress in 54% of patients, and results in death or requirement for liver transplantation in 15% [37]. Despite treatment, almost half of patients (46%) still have histological activity of AIH amid improved biochemical parameters [38].

In acute presentation of AIH, in contrast to chronic AIH, there are often atypical histological manifestations [27, 34]. Chronic AIH is histologically characterized by borderline hepatitis, plasma cell infiltration and centrilobular necrosis. Acute AIH is not significantly different histologically from chronic AIH. However, histological active findings such as lobular inflammation, macrophages and focal necrosis or single cell necrosis were significantly more frequent in patients with acute presentation of AIH, whereas portal fibrosis was significantly more frequent in patients with chronic AIH [27]. Based on pathohistological findings, the authors believe that almost all cases of acute presentation of AIH might be exacerbations of non-symptomatic pre-existing chronic AIH.

The diagnostic criteria commonly used for classical chronic AIH are generally applicable to acute exacerbation, but acute-onset AIH may present with additional pathological features – centrilobular necrosis. However, centrilobular necrosis is also a feature of drug-induced liver injury, and there are no known histological characteristics to differentiate drug-induced liver injury from acute-onset AIH. Moreover, immune-mediated drug-induced liver injury makes diagnosis even more difficult [34].

Importantly, immunohistochemical studies have revealed high expression of IL-33 in liver slices from AIH patients. IL-33 expression in AIH is concentrated in the inflammation areas and is observed in the sinusoidal endothelial cells and other vessels, but was not detected in intrahepatic bile ducts [18].

Immunohistochemical phenotyping of inflammatory cells in the liver shows a predominance of T cells. Among them, the majority were CD4 helper/inducer cells, and the number of CD8 cytotoxic/suppressor cells was negligible. In addition, natural killers, monocytes/macrophages, and B-lymphocytes were present in the infiltrates [8, 29].

The simplified score is a reliable and simple tool for diagnosing AIH. However, both systems cannot unmask autoimmune hepatitis component efficiently in AIH patients with concurrent autoimmune or non-autoimmune liver diseases [39]. According to the authors, their study also strongly reiterates the importance of liver biopsy when examining patients.

Autoimmune hepatitis and malignancies. Patients with AIH have a high risk of malignant tumors due to immunological abnormalities, use of immunosuppressive agents and chronic inflammation. Grønbæk et al. [16] found that the 10-year cumulative risk of hepatocellular carcinoma in AIH was 0.7%. Male gender and cirrhosis were associated with high mortality and development of hepatocellular carcinoma. 3.6% of deaths were from hepatocellular carcinoma. Even higher rates of malignant tumors in AIH are given by Arinaga-Hino et al. [40]. In their study, of 256 patients suffering from AIH, 27 (10.5%) developed malignancies; 11 (4.3%) with hepatobiliary cancer and 16 (6.3%) with extrahepatic malignancies. The risk factors for hepatobiliary cancer at the diagnosis of AIH were low levels of alanine aminotransferase ($P = 0.0226$), low platelet counts ($P < 0.0001$), and cirrhosis ($P = 0.0004$). The risk factor for extrahepatic malignancy was relapse of AIH ($P = 0.0485$).

Diagnosis. In 1993, the International Autoimmune Hepatitis Group (IAIHG) codified diagnostic criteria to identify patients with having either probable or definite AIH for research purposes [41]. In 1999, the IAIHG revised the descriptive diagnostic criteria to optimize AIH diagnosis in individuals with atypical manifestations of the disease as well as to improve the accuracy of excluding cholestatic autoimmune liver diseases (primary biliary cirrhosis and primary sclerosing cholangitis). As a result of the revision, the specificity of the criteria was improved to 90%. The revised criteria also showed very good efficacy in patients with few or atypical signs of AIH [42]. However, the diagnostic criteria for AIH remained complex, with 13 components and 29 possible classes, which limited their application in routine clinical practice. Therefore, a simplified scoring system for diagnosing AIH in routine clinical practice was developed in 2008 [43, 44]. These criteria consist of only four available parameters: liver histology, autoantibody titers, IgG level, and exclusion of viral hepatitis (Table). Out of a total of eight points, a probable diagnosis of AIH is made at six points, and a definite diagnosis of AIH is made at seven or eight points. The simplified criteria were originally defined and validated in a retrospective cohort study involving 11 international centers from the Americas, Europe, and Asia [44]. In this study, response to immunosuppressive therapy was also mandatorily included in all AIH patients. Subsequently, the AIH diagnostic simplified system was used in numerous other studies [32, 39, 45–51].

Table

Simplified diagnostic criteria for AIH (according to Hennes et al. [44])

Variable	Cutoff	Points
ANA or SMA	≥1 : 40	1
ANA or SMA	≥1 : 80	2
or LKM	≥1 : 40	
or SLA	Positive	
IgG	> Upper normal limit	1
	>1.10 times upper normal limit	2
Liver histology	Compatible with AIH	1
	Typical AIH	2
Absence of viral hepatitis	Yes	2

Note. ANA, antinuclear antibodies; SMA, smooth muscle cell antibodies; LKM, liver-kidney microsomal antibodies; SLA, soluble liver/liver-pancreas antibodies. ≥6: probable AIH; ≥7: definite AIH.

Hennes et al. [44] (2008) reported 88% sensitivity and 97% specificity for the diagnosis of probable AIH (≥6 points) and 81% sensitivity and 99% specificity for the diagnosis of definite AIH (≥7 points). Several other studies have confirmed the sensitivity and specificity of a simplified scoring system for the diagnosis of AIH in American [37], Mexican [52], and Korean [13] patients. In these studies, sensitivity and specificity of detecting a probable AIH ranged from 65% to 95% and from 90% to 98%, respectively, while sensitivity and specificity of detecting a definite AIH ranged from 15% to 87% and 99% to 100%, respectively. Using simplified criteria, H. Wobser et al. [26] determined the overall sensitivity and specificity of detecting a probable AIH (score ≥6) to be 96% and 97%, respectively. For diagnosis of definite AIH (scores ≥7), the sensitivity and specificity were 43% and 100%.

In a study by Qiu et al. [32], the simplified criteria had sensitivity and specificity of 90% and 95%, respectively, for the diagnosis of probable AIH in Chinese patients. This compares well with the more stringent revised original criteria, which had sensitivity and specificity of 100% and 93%, respectively, for probable AIH. In addition, the predictability of the revised original criteria and simplified criteria were 96% and 94% for probable AIH, and 88% and 87% for definite AIH, respectively. The authors concluded that the simplified criteria are highly sensitive and specific for the diagnosis of AIH in Chinese patients.

AIH may have cholestatic features that are outside the codified diagnostic criteria. Patients with AIH may have antimitochondrial antibodies and coincidental bile duct injury or loss (2%–13% of patients), focal biliary strictures and dilations based on cholangiography (2%–11%), or histologic changes in bile duct injury or loss in the absence of other features (5%–11%). These findings probably represent atypical manifestations of AIH or variants of primary biliary cirrhosis (PBC) or primary sclerosing cholangitis (PSC), depending on the predominant findings. Serum levels of alkaline phosphatase and γ-glutamyl transferase, histologic features of bile duct injury, and findings from cholangiography are associated with responsiveness to corticosteroid therapy and individualized alternative treatments [37].

Rapid diagnosis and initiation of immunosuppressive treatment are necessary for both acute exacerbation and acute-onset to prevent fatal liver failure [34]. However, the diagnosis of acute AIH with atypical features remains a difficult challenge; authors believe that the revised original scoring system has shown better results in patients with acute-onset AIH than the simplified system [46]. Li et al. [47] also note that the revised scoring system has better performance in diagnosing AIH patients than the simplified scoring system. Many chronic liver diseases can coexist with AIH [53, 54]. Therefore, correct and timely diagnosis of AIH remains a challenging problem in clinical practice [26].

Overlap syndrome. The so-called "overlap syndrome" has long been recognized, in which there are signs of two autoimmune liver diseases, for example, AIH and PBC or PSC [55]. Patients with a combination of AIH and primary biliary cirrhosis suffered from a more aggressive form of PBC [28]. Combined therapy with ursodeoxycholic acid and low-dose immunosuppressive drugs was effective in these patients.

Overlap between AIH and PSC is rare, especially with the new scoring system. Of 147 patients with PSC, the simplified scoring system identified two patients with probable AIH, demonstrating the high specificity of this system [56].

Differential diagnosis. To make a diagnosis of AIH, PBC and PSC must first be excluded, and then such diseases as chronic viral hepatitis, Wilson–Konovalov disease, Alpha-1 antitrypsin deficiency, hemochromatosis, drug-induced hepatitis, alcoholic hepatitis, nonalcoholic fatty liver disease, etc. It is particularly important to distinguish AIH from other forms of chronic hepatitis because most patients respond to anti-inflammatory and/or immunosuppressive therapy [57].

Clinic. AIH is an inflammatory liver disease with a wide range of clinical manifestations [58], ranging from subclinical to fulminant hepatitis [6] or from isolated acute or chronic hypertransaminasemia to acute liver failure [4, 59, 60]. A study by Kim et al. [13] AIH patients reported that 30.6% were asymptomatic, 22.7% were cirrhotic, and 4.3% displayed hepatic decompensation. In most cases, AIH with acute presentation is merely acute exacerbation of classical chronic AIH, but pure acute-onset AIH without previous symptoms of chronic liver disease is also encountered [34]. In acute presentation, in contrast to chronic AIH, there are often atypical clinical manifestations [12, 61].

AIH has diverse clinical phenotypes and outcomes in ethnic groups within a country and between countries,

and these differences may reflect genetic predispositions, indigenous etiological agents, pharmacogenomic mechanisms and socioeconomic reasons. In the United States, African-American patients have cirrhosis more commonly, treatment failure more frequently and higher mortality than white American patients. Survival is poorest in Asian-American patients. AIH in other countries is frequently associated with genetic predispositions that may favor susceptibility to indigenous etiological agents. Acute-on-chronic liver disease increases mortality and socioeconomic and cultural factors affect prognosis. Ethnic-based deviations from classical phenotypes can complicate the diagnosis and treatment of AIH in non-white American populations [62].

Therapy. AIH is one of the few liver diseases for which pharmacologic treatment has been shown to improve survival [3, 57, 58]. Non-specific immunosuppression is the current standard therapy [8], which is prescribed immediately after diagnosis [4, 63] and which prevents rapid deterioration and promotes remission and long-term survival [64]. The treatment not only can prolong patients' lives, but also improve their quality of life and avoid liver transplantation [57]. Response to steroid treatment is considered as an additional criterion in the diagnosis of AIH [3, 26]. Lack of response to steroids is grounds for revision of the diagnosis [23].

Standard treatment regimens include high-dose prednisolone alone or prednisolone plus azathioprine [19, 23]. Positive effects with steroid treatment are observed in 75%–90% of patients [3, 23]. However, approximately 20% of patients do not respond to steroid treatment, and second-line immunosuppressive medications are used for their treatment. These drugs are also used in patients who cannot tolerate standard therapy. Second-line drugs include mycophenolate mofetil, budesonide, cyclosporine, tacrolimus, everolimus, and sirolimus. However, there have been no randomized controlled trials of the efficacy of second-line drugs in the treatment of AIH. Mycophenolate mofetil is the most widely used second-line drug; it is particularly effective in patients with azathioprine intolerance. Experience with infliximab and rituximab has been published. However, there is a high risk of infectious complications when treated with these drugs [5, 23].

Treatment of AIH with various immunosuppressive drugs is aimed at minimizing liver inflammation [65–67], which reduces the risk of fibrosis progression and cirrhosis development, hence reducing the need for liver transplantation [68].

Current studies aimed at restoring the regulatory function of T cells in vitro to acquire tolerance in vivo have shown promising results [4]. Further elucidation of the cellular and molecular pathways involved in the pathogenesis of AIH is likely to lead to the discovery of new, adaptable and better tolerated therapies [8; 23].

In the natural course of AIH, there is a tendency for liver cirrhosis [69] and progression to end-stage disease [19]. Resistance to therapy also leads to end-stage liver disease. These patients, as well as those found to have fulminant liver failure at diagnosis, require liver transplantation [2, 64, 70].

Outcomes. Without treatment, the prognosis is poor [5, 23], often leading to cirrhosis, liver failure, and patient death [36, 71–73]. The presence of cirrhosis at diagnosis of AIH, lack of response to initial immunosuppressive therapy or elevated international normalized ratio were associated with poor outcome and requirement for liver transplantation [7]. Otherwise, most deaths were associated with liver failure, shock, or gastrointestinal bleeding [71]. In contrast, Ngu et al. [74] (2013) suggest that histological cirrhosis at diagnosis is not associated with poor prognosis and does not influence the response to initial immunosuppressive treatment. According to these authors, incomplete normalization of ALT at 6 months, low serum albumin concentration at diagnosis, and age at presentation of ≤20 years or >60 years were significant independent predictors of liver-related death or requirement for liver transplantation.

CONCLUSION

The term "autoimmune hepatitis" was coined in 1992. There are currently two main forms of AIH. Type 1 AIH is characterized by smooth muscle antibodies, antinuclear antibodies, or both, whereas Type 2 AIH is characterized by anti-liver/kidney microsomal antibodies, and anti-liver cytosol 1 antibodies, or both. Autoimmune hepatitis can occur at any age and is more common in women than in men. The disease is a manifestation of an autoimmune predisposition caused in genetically susceptible people exposed to certain environmental factors. The pathogenetic mechanisms of AIH are not yet fully understood, but it involves an aggressive cellular immune response. The main role is attributed to defects in regulatory T cells. Various cytokines also influence the pathogenesis and severity of AIH. This disease is characterized by elevated levels of transaminases: AST and ALT.

Liver histology plays a crucial role in confirming or supporting the clinical diagnosis of AIH. The most typical, but non-specific pathohistological finding in AIH is the presence of borderline hepatitis, in which there is inflammation not only of the portal tract, but also of the periportal parenchyma, with its infiltration by lymphocytes, plasma cells and macrophages. The lymphocytic inflammatory infiltrate contains a large number of CD4+ T cells. The high content of plasma cells in the inflammatory infiltrate is one of the main histological indicators of AIH. In severe and progressive disease, centrilobular lesions and necrosis as well as bridging necrosis are present. Hepatocyte necrosis leads to liver fibrosis and cirrhosis. Acute presentation

of AIH often has atypical histological manifestations in the form of centrilobular necrosis. Immunohistochemical studies revealed high expression of IL-33 in areas of inflammation, which is observed in the sinusoidal endothelial cells and other vessels. Phenotyping of inflammatory cells in the liver showed a predominance of CD4 helper/inducer cells, while the number of CD8 cytotoxic/suppressor cells was insignificant. Besides, natural killer cells, monocytes/macrophages, and B-lymphocytes were present in the infiltrates. Patients with AIH had a high risk of malignancies due to immunological disorders, use of immunosuppressive agents and chronic inflammation. AIH may have cholestatic features that are outside the codified diagnostic criteria. Rapid diagnosis and initiation of immunosuppressive treatment are necessary for both acute exacerbation and acute onset to prevent fatal liver failure. However, the diagnosis of AIH remains a challenging problem in clinical practice.

AIH is one of the few liver diseases for which pharmacologic treatment has been shown to improve survival. Standard treatment regimens include high-dose prednisone alone or prednisolone plus azathioprine. Approximately 20% of patients do not respond to steroid treatment and are treated with second-line immunosuppressive drugs: mycophenolate mofetil, budesonide, cyclosporine, tacrolimus, everolimus, and sirolimus. In the natural course of AIH and resistance to therapy, there is a tendency for cirrhosis to develop and for the disease to progress to an end stage. These patients, as well as those with fulminant liver failure at diagnosis, require liver transplantation.

The authors declare no conflict of interest.

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THE ROLE OF FRAILTY IN SELECTING PATIENTS FOR HEART TRANSPLANTATION

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The role of frailty in cardiovascular disease is becoming increasingly recognized. Up to 79% of patients with heart failure are frail. Frailty is associated with reduced quality of life and poor prognosis. This review summarizes the available literature on frailty and its key role in waitlisting patients for heart transplantation.

Keywords: heart failure, heart transplantation, frailty.

Over the past decade, patients with end-stage heart failure (HF) requiring heart transplantation (HTx) have significantly increased in number all over the world [1, 2]. In the Russian Federation, the prevalence of New York Heart Association (NYHA) classes I–IV HF is 7% of the general population (about 7.9 million people); 2.1% (2.4 million people) have end-stage HF (NYHA classes III–IV) [3]. Analysis of heart transplants performed at Shumakov National Medical Research Center of Transplantology and Artificial Organs from 1986 to 2018 have shown that the number of heart transplants performed annually is clearly rising. For example, 194 heart transplant surgeries were performed in 2018 alone [4]. Although HTx remains the only effective method of radical treatment for end-stage HF, and the criteria for inclusion in the waiting list (WL) have significantly expanded during the last decade, the possibility of performing it in high-risk patients remains a subject of active discussion among specialists in cardiothoracic transplantology [5]. So, along with the indications for inclusion in heart transplant WL, there are absolute and relative contraindications to this type of surgical treatment (Table 1).

As can be seen from the table, a number of comorbidities previously considered as absolute contraindications for HTx are now considered as relative ones, which aggravates the contingent of patients coming to transplant centers for end-stage HF. In this regard, the revision of WL inclusion criteria, taking into account a comprehensive assessment of the severity of comorbidity and its impact on the body as a whole, becomes an urgent task. The use of frailty assessment criteria as one of the factors that determine whether a patient should be included on the heart transplant WL is widely discussed [8]. English-language literature uses the term "frailty" as such a criterion, which has no clear analogue in Russian literature and is often used in the context of malaise, fatigue, cachexia and general asthenia and their influence on the early and long-term postoperative prognosis in heart recipients.

The objective of our review was to summarize the currently available data on frailty in potential heart recipients and its impact on survival after HTx.

Frailty is characterized by decreased endurance, depressed physiological functions and reduced body reserves, which in turn is accompanied by increased susceptibility to various pathogenic factors and stressors, leading to decompensation of the underlying disease and/or concomitant pathology, increased frequency of hospitalizations and worsened patient survival prognosis [6, 7].

In the guidelines of the International Society of Heart and Lung Transplantation revised and published in 2016, frailty syndrome and its importance as a prognostic marker of the outcomes of upcoming surgical treatment was included for the first time in the criteria for selection of patients for HTx [1].

In February 2018, a consensus conference was held in Phoenix (Arizona), the main purpose of which was to standardize nomenclature in the assessment of frailty, to determine the main methods of diagnosis of this syndrome, and to assess the significance of the syndrome in persons in need of solid organ transplantation [8]. Thus, the relevance of this problem is beyond doubt and requires further research in this area.

PATHOPHYSIOLOGY OF FRAILTY

Currently, there is no consensus on the pathophysiological mechanisms of frailty, which is due to its multifactorial nature. One of the factors of this syndrome is chronic inflammatory response characterized by long-term steady increase in the level of cytokines, IL-6,

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tumor necrosis factor-α (TNF-α), interferon-γ (INF-γ) and C-reactive protein (CRP). Endocrine dysfunction is important, with decreased levels of insulin-like growth factor-1 (IGF-1) and 25-hydroxy vitamin D [9].

The combination of chronic inflammatory response with endocrine dysfunction, as well as a number of other factors can cause changes in the human body that are characteristic of chronological aging processes. Such changes include apoptosis, mitochondrial dysfunction, DNA damage, stem cell depletion, immune aging and pronounced inflammatory response in reaction to the effects of stressors [10, 11]. In natural aging, the disruption of body homeostasis against the background of stressors does not entail severe consequences and is easily restored by the body's own physiological reserves. On the contrary, disruption of homeostasis in frailty syndrome is of an unregulated nature, which is manifested by severe functional abnormalities in the body in response to minor stressors and its inability to quickly restore its normal physiological state.

A peculiarity of frailty is that it can have a negative impact on several organs and systems of the patient's body at once, including the central nervous system, immune, endocrine and musculoskeletal systems. The central nervous system is affected due to dystrophic changes in the brain, clinically manifested as cognitive disorders [12, 13].

Sunita R Jha et al. assessed the presence of physical frailty in 156 patients (109 men, 47 women), aged 53 ± 13 years, diagnosed with HF and left ventricular ejection fraction of 27% ± 14%. All the patients underwent physical frailty assessment using the Fried Frailty Phenotype (FFP). Cognition was assessed with the Montreal Cognitive Assessment (MoCA), and depression with the Depression in Medical Illness questionnaire. Thus, to predict long-term outcomes, the authors assessed the value of 4 composite frailty measures: physical frailty (PF ≥ 3 of 5 = frailty), "cognitive frailty" (CogF ≥ 3 of 6 = frail), "depressive frailty" (DepF ≥ 3 of 6 = frail), and "cognitive-depressive frailty" (ComF ≥ 3 of 7 = frail) in predicting outcomes.

During follow-up, 28 patients died before any surgical treatment for heart failure (ventricular assist device implantation and/or HTx). The one-year survival rate among patients with normal or mildly reduced test scores was 81% ± 5% vs 58% ± 10% ($p < 0.02$) in the frail cohorts.

Table 1

Indications and contraindications for inclusion in heart transplant waiting list

Absolute indications	1. Hemodynamic disorders against the background of heart failure: – Refractory cardiogenic shock – Documented dependence on intravenous inotropic support to maintain adequate organ perfusion 2. Peak VO_2 less than 14 mL/kg/minute with achievement of anaerobic metabolism or less than 12 mL/kg/minute with the use of β-blockers 3. Severe symptoms of ischemia that consistently limit routine activity and are not amenable to myocardial revascularization 4. Recurrent symptomatic ventricular arrhythmias refractory to all therapeutic and surgical modalities
Absolute contraindications	1. Systemic disease with life expectancy <2 years: – Active neoplasm (if preexisting, evaluation with an oncologist is necessary to stratify the risk of recurrence and establish a time to wait after remission) · – Systemic disease with multi-organ involvement (systemic lupus erythematosus, amyloidosis, sarcoidosis) – Severe chronic obstructive pulmonary disease (FEV1 <1 L · – Renal or hepatic severe dysfunction, if associated renal or liver transplant is not feasible 2. Irreversible pulmonary hypertension – Pulmonary artery systolic pressure >50 mmHg · – Transpulmonary gradient >12 mmHg – Pulmonary vascular resistance >3 Wood units despite treatment and nitric oxide challenge
Relative contraindications	1. Age >70 years (carefully selected patients may be considered) 2. Diabetes with end-organ damage (except non-proliferative retinopathy) or persistent poor glycemic control (HbA1c >7.5%) despite treatment 3. Active infection, except VAD infection. Patients with HIV, hepatitis, Chagas disease and tuberculosis can be considered under strict eligibility criteria 4. Severe peripheral arterial or cerebrovascular disease, if revascularization before HTx is not possible 5. Other serious comorbidities with poor prognosis, such as neuromuscular diseases 6. Obesity: BMI >35 kg/m^2 7. Cachexia: BMI <18 kg/m^2 8. Current tobacco, alcohol or drug abuse 9. Insufficient social support 10. Elevated panel-reactive antibody test defined as >10%

The authors showed that frail patients had a worse prognosis of survival in both the preoperative and postoperative periods [14].

Wilson et al. conducted a retrospective cohort analysis of 144 patients in need of lung transplantation and evaluated the effect of frailty on the post-transplant survival of recipients. The authors showed that pre-transplant frailty was an independent predictor of decreased survival after lung transplantation [15].

In postoperative management, heart recipients need lifelong administration of immunosuppressive drugs to prevent acute rejection and graft dysfunction. In this regard, preservation of cognitive functions in patients requiring HTx is important to ensure adequate long-term administration of life-sustaining medications [16].

Sarcopenia is another manifestation of frailty. It is caused by constantly elevated levels of inflammatory cytokines, decreased levels of anabolic hormones, micronutrient deficiencies, lack of physical activity, and disruption in the normal functioning of the central nervous and endocrine systems. Thus, disruption of homeostasis mechanisms that maintain the normal balance between muscle cell preservation and catabolism leads to loss of muscle mass and skeletal muscular dystrophy. Reduced physical activity and lack of appetite triggers a vicious cycle of further reduction in muscle mass and reduces the quantity of amino acids the body needs during stress [17]. The main associations between frailty and comorbidity are shown in Figure.

As can be seen from Figure 1, a long history of cardiovascular disease leading to subclinical failure of other organs and systems of the body, such as heart failure, also influence the development of frailty syndrome [18].

ASSESSING THE SEVERITY OF FRAILTY

The frailty assessment scale was first proposed by Linda P. Fried, and its effectiveness was confirmed in the Cardiovascular Health Study. According to the FFP scale, the presence of three or more criteria can indicate the development of the clinical phenotype of frailty syndrome (Table 2) [19, 20].

Table 2

Predictors of frailty

Criteria	Comments
Weight loss	Weight loss of >4.5 kg within the past year
Muscle loss	>20% decrease in muscle strength measured by dynamometry adjusted for age, sex, and body mass index
Fatigue	Decreased exercise tolerance
Slowness	Slow walking speed given gender and height
Low levels of physical activity	Lowest kilocalorie expenditure in the past week as measured by Minnesota Leisure Activity Scale

Muscle strength and gait speed are quantitative criteria for the FFP scale and provide a more objective assessment of physical frailty than the other three measures [21]. The presence of three or more criteria assessed with this scale indicates the presence of frailty in a patient. The FFP scale scores have been derived and used to assess disease prognosis and mortality among HF inpatients of the general patient population [22–24].

In patients with chronic heart failure, fluid retention in the body can lead to weight fluctuations and make it difficult to assess true weight loss. In this situation, decreased serum albumin levels are a more accurate marker of weight loss due to patient malnutrition.

The Frailty Index provides a more accurate quantitative assessment of the severity of the syndrome to the FFP scale. The Frailty Index is calculated using a questionnaire based on 30 to 70 different indicators, including the presence of various comorbidities, changes in laboratory values, and functional deficits [25].

Another method of assessing the presence of frailty is the Short Physical Performance Battery (SPPB), which measures a patient's physical characteristics. This test includes assessing gait speed, the number of times a chair is lifted, and holding tandem balance for a certain amount

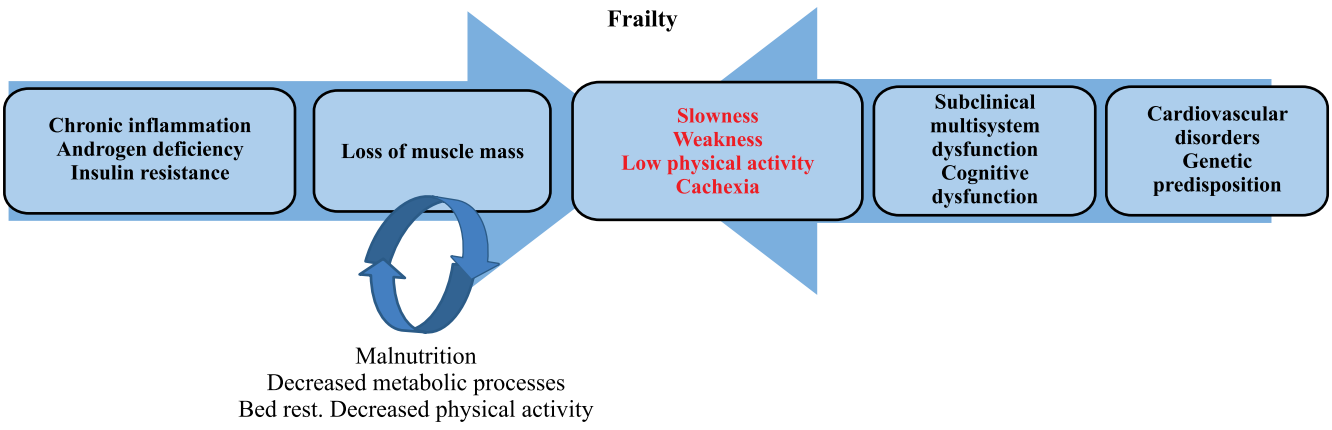

Fig. Association between frailty and comorbidity

of time. Each indicator is scored from 1 to 4; a total score of less than 5 indicates that the person is frail [26–29].

FRAILTY IN PATIENTS WITH HEART FAILURE

In patients with HF, frailty is a predictor of adverse events regardless of commonly known cardiovascular risk factors [30, 31].

In their work Volpato S. et al. showed that among patients hospitalized for decompensated HF and assessed by the SPPB scale, low score at admission was associated with longer hospital stay, and low SPPB score at discharge was associated with unfavorable prognosis of repeated hospitalizations and mortality [32]. Similar data were obtained in a FRAIL-HF study, where it was shown that frail patients hospitalized for decompensated HF had significantly worse 1-year survival prognosis than the control group [33].

In a study by Jha SR et al, frailty was diagnosed in 120 patients who needed and/or were on the heart transplant WL. The diagnosis was made on the basis of data obtained from the FFP scale, markers of heart failure severity, and the severity of cognitive impairment assessed by MoCA. The authors showed that frailty was diagnosed in one-third of the waitlisted patients, and this syndrome was associated with increased annual mortality, which was 50% in patients with this syndrome compared with 20% in the comparison group [34–36].

A group of authors led by Peter S. Macdonald conducted a retrospective analysis of 140 patients who underwent orthotopic HTx. Of the 140 recipients, 43 were frail (F) six months or more before transplantation; the remaining 97 were non-frail (NF). Post-transplant survival rates for the NF cohort at 1 and 12 months were 97% and 95% (95% CI), respectively. In contrast, post-transplant survival rates for the F cohort at the same time points were 86% and 74% ($p < 0.0008$ vs NF cohort), respectively. The authors concluded that frailty in heart recipients was independently associated with post-transplant mortality with a hazard ratio of 3.8 (95% CI: 1.4–10.5). Intensive care unit and hospital length of stay were significantly longer in the F cohort than in the NF cohort ($p < 0.05$) [37].

Today, the question about the criteria for frailty reversibility after radical surgical treatment for HF by implantation of long-term mechanical circulatory support systems or HTx remains open. How do we distinguish between reversible and irreversible frailty? What is the role of "pre-rehabilitation" to reduce the risk of adverse prognosis after cardiac surgery for patients with reversible frailty? Is implantation of long-term mechanical circulatory support systems as a "bridge to heart transplantation" in this category of patients for the purpose of rehabilitation and preparation for subsequent transplantation reasonable? [38].

Currently, there is no simple test that can accurately assess the reversibility of frailty against the background of radical correction of HF. Relatively young patients with a clinical picture of severe HF in the absence of concomitant pathology have a favorable prognosis of reversibility of functional reserves of the body against the background of surgical treatment. The age category of recipients with severe comorbid pathology contributing to frailty has the least favorable prognosis due to lack of complete recovery of the body against implantation of long-term mechanical circulatory support systems or HTx [39].

Maurer et al. evaluated the regression of weakness syndrome in 29 elderly patients (mean age 71 years) who had a left ventricular assist device (LVAD) implanted in them. The authors showed that despite the improvement in clinical condition 6 months after LVAD implantation, 53% of the patients still had clinical manifestations of frailty. So, they concluded that frailty cannot be completely reversible in this age group [40].

Data available in the literature are currently insufficient to answer the question of whether it is reasonable to include frail patients on the heart transplant WL. Frailty is associated with significantly higher postoperative mortality, but this conclusion is based on a single observation and requires further research [34].

CONCLUSION

According to the literature, frailty is an independent predictor of poor survival in end-stage HF requiring implantation of long-term mechanical circulatory support systems or heart transplantation [41, 42]. However, due to the absence of a unified algorithm for diagnosing this condition, it is not possible to make unequivocal conclusions about the severity and reversibility of this syndrome in patients with HF, which requires further research [1, 43].

The authors declare no conflict of interest.

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HEART TRANSPLANTATION AND COVID-19 IN THE EARLY POSTOPERATIVE PERIOD IN HYPERTROPHIC CARDIOMYOPATHY: A CLINICAL CASE

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Hypertrophic cardiomyopathy (HCM) is a disease that is usually unresponsive to conservative pathogenetic therapy. It does not have clearly developed surgical correction algorithms. Heart transplantation (HTx) is the sole therapeutic option when drug therapy is ineffective and surgical reduction of hypertrophic myocardium is not feasible. There are only sporadic reports in the literature about HTx for HCM. The novel coronavirus disease 2019 (COVID-19) pandemic has significantly affected the work of cardiac surgical units and, in particular, organ transplantation activities. This paper presents a clinical case of an HCM patient who underwent HTx, complicated by COVID-19 infection in the early postoperative period.

Keywords: hypertrophic cardiomyopathy, heart transplantation, COVID-19.

INTRODUCTION

HCM is a genetically determined myocardial disease characterized by severe left ventricular (LV) hypertrophy, less often by right ventricular hypertrophy, which cannot be explained exclusively by increased pressure load. It occurs in the absence of another cardiac or systemic disease, metabolic or multiorgan syndrome, associated with LV hypertrophy. More often, hypertrophy has asymmetric character due to thickened interventricular septum (IVS) [1].

HCM has an estimated prevalence of 1 in 500 to 1 in 200 people. One manifestation of the disease is sudden cardiac death (SCD), with an incidence of 1% per year [2–4].

HCM has been considered a "sarcomere disease" [5], caused by mutations in certain contractile protein genes. The morphological reflection of these processes is the development of cardiomyocyte disarray and hypertrophy, as well as interstitial fibrosis [6].

HCM pathophysiology is determined by a complex of interrelated factors, including obstruction syndrome, myocardial ischemia, diastolic LV dysfunction, presence of mitral regurgitation and arrhythmias. Obstruction is noted in certain left ventricular sections (left ventricular outflow tract (LVOT), middle section with papillary muscles and apical section). The main mechanism of obstruction is myocardial hypertrophy, which leads to LV cavity narrowing in different areas. In the LVOT, basal narrowing of hypertrophied IVS occurs; while the most frequent variant – hypertrophy of LV free wall, middle section of IVS and papillary muscles – occurs in middle sections of the LV, while apex hypertrophy occurs in the apical variant [7]. Another important mechanism in obstruction creation is considered to be systolic anterior motion (SAM) of the mitral valve. SAM is caused by the contact of anterior leaflet with the IVS in early systole as a result of accelerated blood flow through narrowed outflow tract, creating high-ejecting flow that pulls the mitral leaflet into LVOT [8].

To date, here are the existing HCM treatment methods: drug therapy, endovascular interventions and surgical methods of hypertrophic myocardial reduction. Conservative therapy includes drugs with negative inotropic action, aimed at reducing obstruction in LVOT (beta-blockers, verapamil, disopyramide) [9].

For patients with drug-refractory HCM, surgical treatment is decisive and is possible with the use of interventional methods of treatment (alcohol septal ablation) or with septal myoectomy under artificial circulation [10, 11]. The efficacy of the operation is quite high, with a decrease in pressure gradient in the LV cavity and, as a consequence, improvement in symptomatology and general condition of patients [12].

However, orthotopic heart transplantation (HTx) remains the only treatment modality for a small number

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of HCM patients who are not amenable to optimal drug therapy and are not candidates for conventional surgical treatment [13, 14].

This case report demonstrates the features of treatment of a patient with a transplanted donor heart and COVID-19 that developed in the early postoperative period.

CLINICAL CASE

Patient R., male, 35 years old, since 2018 started experiencing shortness of breath, chest tightness during minor physical exertion and associated the complaints with being overweight.

In 2019, while at work, he felt chest compression and lost consciousness. The ambulance crew recorded an ECG with rhythm disturbance and ischemic changes (atrial flutter rhythm, ST elevation to 1–2 mm in leads I, aVL, and V3–4, and ST depression to 2 mm in leads II, III, and aVF). After care (heparin 4000 units intravenously, clopidogrel 300 mg orally, acetylsalicylic acid 250 mg orally), the patient was taken to the hospital with acute coronary syndrome (ACS). Coronary angiogram (CAG) revealed no application points for percutaneous coronary intervention (PCI) – thrombotic occlusion was visualized in the distal third of the circumflex artery with the diameter of the artery in this section being less than 1.5 mm; the other arteries were without organic lesions). Spontaneous thrombolysis probably occurred.

Further examination of the patient in the current hospitalization resulted in the final diagnosis of HCM with LV outflow tract obstruction. Genetic testing to confirm the diagnosis was not performed. The diagnosis was made by specific results of instrumental diagnostic methods characteristic of HCM, as well as by excluding pathological conditions that could lead to LV hypertrophy (primary and secondary arterial hypertension, aortic stenosis).

EchoCG showed marked LV myocardial hypertrophy with predominant IVS thickening (3.8 cm) with LVOT obstruction (maximum pressure gradient, 19.4 mmHg MPG at rest, 40 mmHg MPG in Valsalva maneuver). Left ventricular end-diastolic diameter (LVEDD) – 72, left ventricular ejection fraction (LVEF) – 57%. LV enlargement. SAM of the mitral valve. Grade 1 mitral regurgitation (MR).

Myocardial perfusion scintigraphy showed scintigraphic signs of HCM with predominant IVS lesion. Regional contractility parameters: moderate septal hypokinesis. No signs of postinfarction cardiosclerosis (PICS) were detected.

Contrast-enhanced cardiac MRI showed asymmetric pronounced left ventricular myocardial hypertrophy with predominant thickening of the basal and middle IVS segments, left ventricular middle lower segment, with LVOT obstruction signs (Fig. 1).

In addition, to confirm the diagnosis, a morphological examination of the myocardium was performed as shown in Fig. 2. The patient was prescribed drug therapy with beta-1 blockers (bisoprolol 5 mg per day).

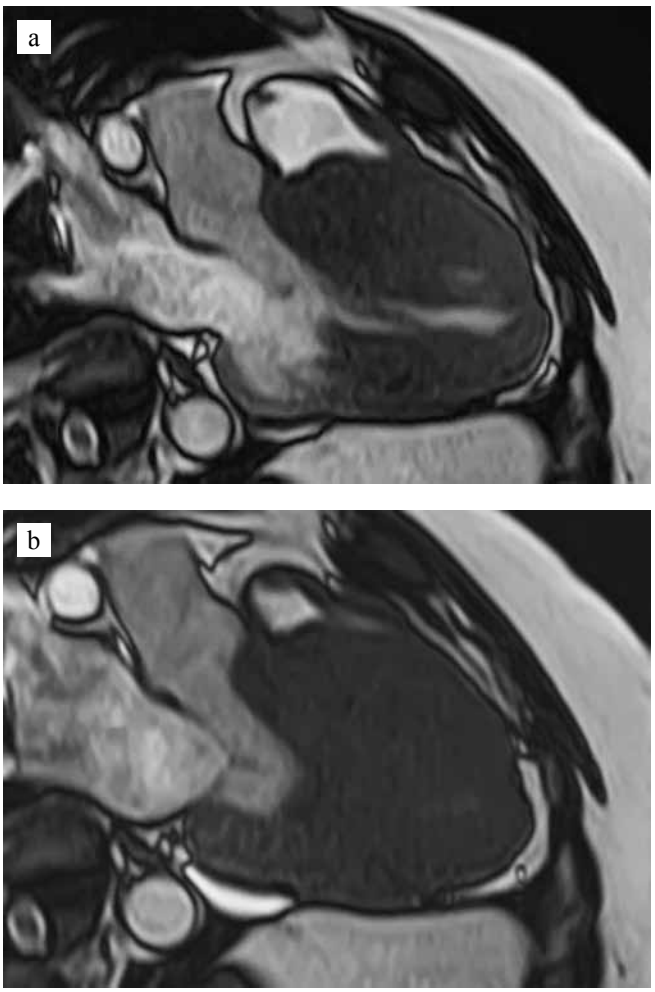

Fig. 1. Contrast-enhanced cardiac MRI. a, diastole; b, systole

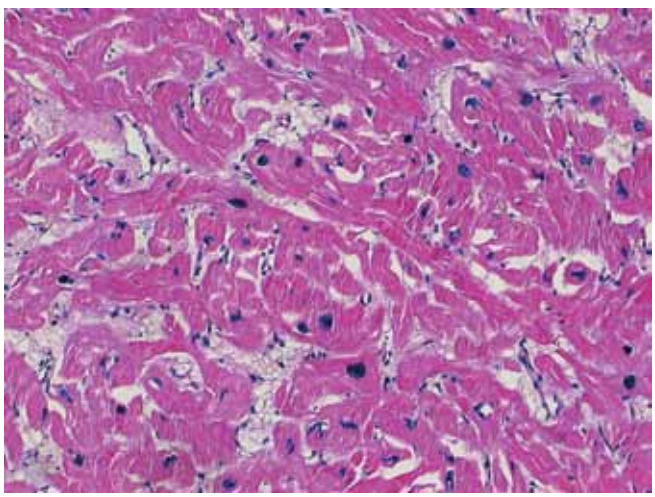

Fig. 2. Morphological analysis of the myocardium. Myocardial disarray: chaotic multidirectional course of muscle fibers, pronounced hypertrophy of some cardiomyocytes combined with atrophy of others, intermuscular fibrosis and stromal lipomatosis. H&E staining, 200× magnification

The patient was then referred to Bakulev National Medical Research Center of Cardiovascular Surgery for examination. Here, surgical correction via septal myoectomy was considered inappropriate due to the extreme thickness of the IVS. The patient was discharged with recommendations for orthotopic HTx. Given the risk of sudden arrhythmic death, a dual-chamber implantable cardioverter-defibrillator (ICD) was implanted. The SCD stratification on the HCM Risk-SCD scale was more than 6%, which was the indication for ICD implantation. The scale considers parameters such as family history of SCD, syncope, unstable ventricular tachycardia, maximum LV wall thickness, age, LV diameter and LVOT pressure gradient.

In October 2020, due to the emergence of a heart donor, the patient was admitted to the cardiac surgical unit for HTx with complaints of severe weakness, shortness of breath when walking up to 100 m, and retrosternal pain during physical exercise.

Upon admission, the patient's condition was moderate, he was fully conscious, body temperature 36.7 °C. Respiratory rate 16 per minute, breathing independently, SpO_2 99%. Blood pressure 138/74 mm Hg, heart rate 64 per minute, pacemaker rhythm (ICD), no pulse deficit. A PCR test for COVID-19 was performed (21/10/20) – SARS-CoV-2 RNA was not detected.

According to echocardiography, at the moment of admission, pronounced asymmetric LV myocardial hypertrophy with predominant IVS thickening (39 mm) and LVOT obstruction with 21 mmHg MPG at rest, 40 mmHg MPG in Valsalva maneuver. LVEDD 70. LVEF 55%. Enlargement of both atria. Minor mitral and aortic regurgitation.

On the day of admission (October 21, 2020), the patient underwent orthotopic HTx under artificial circulation and pharmacological cold cardioplegia.

HTx was performed using a bicaval technique. After median sternotomy and longitudinal dissection of the pericardium, the body was heparinized. A heart-lung machine was connected according to the scheme: aorta – superior vena cava (SVC) – inferior vena cava (IVC). The vena cava was squeezed and a transverse clamp was applied to the aorta. The aorta and the pulmonary artery were crossed above the commissures of the semilunar valves, the vena cava at the level of their confluence with the right atrium. A platform with the mouths of the pulmonary veins was made from the left atrium. The patient's heart was extracted, and the donor heart implanted. Implantation started with left atrial anastomosis. Then, anastomosis of the IVC and SVC was performed. The implantation was completed by forming aortic and pulmonary artery anastomoses. The operation was completed by preventing air embolism, removing the clamp, decannulating the heart-lung machine, installing drains, electrodes and layer-by-layer wound suturing.

The postoperative period from October 21, 2020 to November 8, 2020 was uneventful. The patient was fully conscious on day 1 after surgery. He was extubated on day 2. Breathing was spontaneous (unassisted) and adequate. Hemodynamics was stable, cardiotonic support was disconnected on day 6. On day 7, the patient was transferred from the intensive care unit to the general ward of the cardiac surgery department.

On day 19 (November 9, 2020), the patient's temperature increased to 38.0 °C; he had general weakness, increased sweating, chills, slight shortness of breath, cough with little sputum, he could still taste and smell. It should be noted that further slight increase in temperature to subfebrile values (37.0–37.7 °C) was associated with the treatment with immunosuppressive drugs (tacrolimus, mycophenolic acid), whose use was necessary according to the management protocol for patients after solid organ transplantation. His condition was satisfactory, he was fully conscious. Respiratory rate was 16 per minute, breathing was unassisted, SpO_2 99%. Blood pressure 120/74 mm Hg, heart rate 82 per minute, sinus rhythm.

Due to the spread of COVID-19, a swab was taken from the nose and throat for PCR to exclude COVID-19 infection. The result turned out positive. A chest CT scan was performed (November 11, 2020), which revealed infiltrative hypoventricular changes in the lower lobes on both sides, focal changes in the lungs, with a high probability corresponding to viral pneumonia, CT-2.

After the patient had tested positive to COVID-19, a case conference of physicians was held, which resulted to a decision to add the following to the management protocol for patients after solid organ transplantation: anticovid convalescent plasma (4 times 200 mL each), monoclonal antibodies against human IL-6 receptor (olokizumab 0.4 mL once, tocilizumab 400 mg once).

Based on the results of clinical and instrumental data, a telemedical consultation was conducted with leading specialists in the management of patients after HTx from Shumakov National Medical Research Center of Transplantology and Artificial Organs. It was recommended to reduce immunosuppressive therapy (withdrawal of mycophenolic acid preparations, reduction of methylprednisolone dose), to continue tacrolimus therapy, add broad spectrum antibiotics (azithromycin/ceftaroline/levofloxacin) and to start low molecular weight heparins (enoxaparin sodium). All recommendations were implemented.

In the laboratory data, the concentration of C-reactive protein increased during the first days of the disease and then decreased. Other inflammatory markers (leukocytes, sedimentation rate) were within the reference values (Fig. 3).

The patient was dynamically followed up for changes in the lungs by chest CT scan. In the lungs, on both sides, we detected ground-glass type lung tissue thickening

zones, which increased as the disease progressed from small foci to diffuse nature. The degree of lung lesions progressed from CT-2 to CT-4. In addition, consolidation zones in the lower lobes of both lungs persisted for a long time (Fig. 4).

On day 23 (November 13, 2020), there was a decrease in oxygen saturation (SpO_2 88%). The patient was transferred to humidified oxygen with 95% SpO_2 saturation. The dynamics of changes in SpO_2 is shown in Fig. 5.

On day 29 (November 19, 2020), a repeated telemedicine consultation with Shumakov National Medical

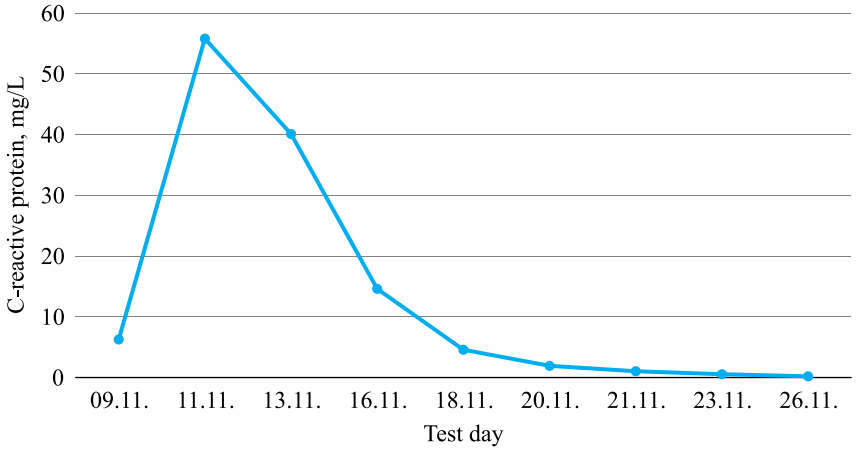

Fig. 3. Changes in C-reactive protein

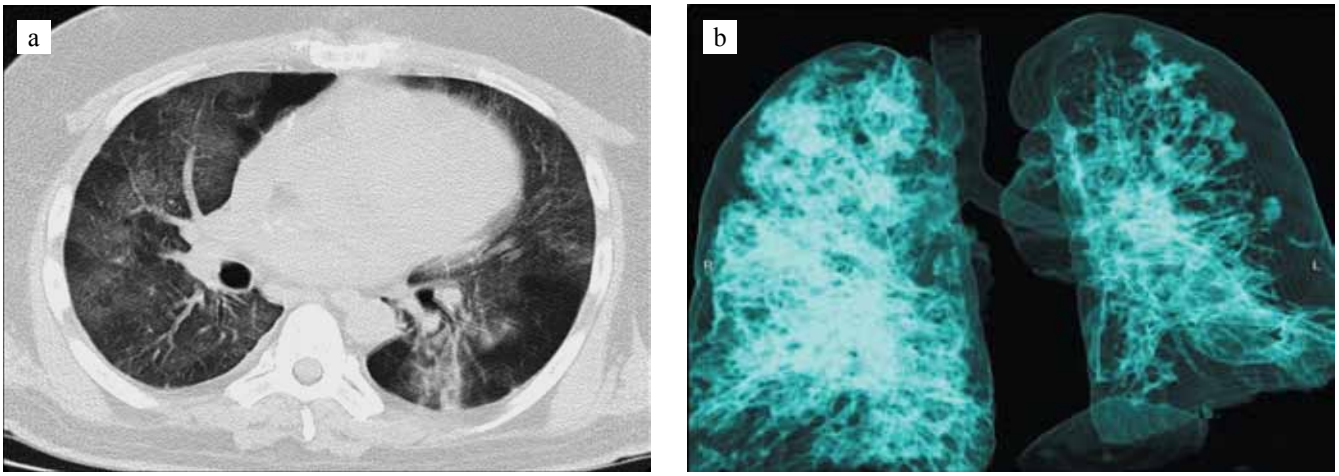

Fig. 4. a, Axial chest CT image (18/11/20); b, Chest CT image in pulmonary view (18/11/20)

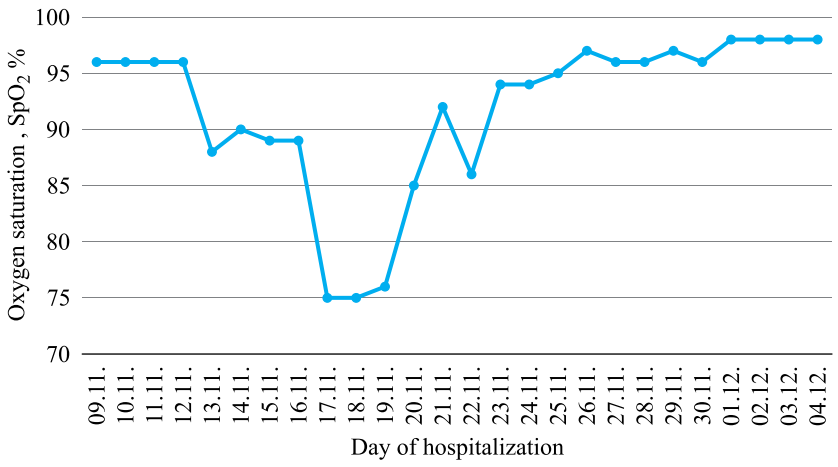

Fig. 5. Changes in oxygen saturation (SpO_2, %)

Research Center of Transplantology and Artificial Organs was conducted. It was recommended to continue therapy in the same volume.

During treatment, the patient was stable, temperature was within the normal range with episodes of return to subfebrile numbers, minor shortness of breath persisted, oxygen saturation rose to normal units, hemodynamics was stable (BP 125/75, pulse 75, sinus rhythm).

A second swab was taken from the nose and throat for PCR test to check for COVID-19: no SARS-CoV-2 RNA was detected.

Chest CT scan (December 2, 2020) before the patient's discharge showed positive dynamics, pathological changes in the resolution stage.

Echocardiography (December 4, 2020) before the patient's discharge showed no zones of impaired local myocardial contractility in the left ventricle. EF 56%. Cardiac chambers were not dilated. Moderate pulmonary hypertension (pulmonary artery systolic pressure 44 mm Hg). Minor mitral and tricuspid regurgitation.

Myocardial transplant biopsy (December 2, 2020) conducted before discharge showed mild cellular rejection without signs of humoral crisis (Fig. 6).

The patient was discharged with improved condition. Body temperature and oxygen saturations were normal, there were no complaints.

At present, a year later, the patient is in satisfactory condition.

DISCUSSION AND CONCLUSION

The COVID-19 pandemic has presented an unprecedented challenge to the global health and public health system. The epidemiologic situation has led to limitations in access to routine surgical care, as well as limitations in the ability to perform solid organ transplantation procedures due to the regrouping of hospital resources.

This, in turn, leads to the so-called secondary effects in the form of delayed patient care, including patients with heart failure of various etiologies. Therefore, the adaptation of high-tech surgery centers to work under the current epidemiological situation is an extremely important task.

The given clinical case allows us to conclude that HTx can be successfully performed in patients with hypertrophic obstructive cardiomyopathy when other methods of radical correction cannot be applied due to clinical and anatomical features. The COVID-19 infection is a disease with a significant risk of fatal complications, especially in patients with organ transplants and immunosuppressive therapy. However, even in the early postoperative period, timely and multidisciplinary management of this complex category of patients allows to achieve a good clinical outcome.

The authors declare no conflict of interest.

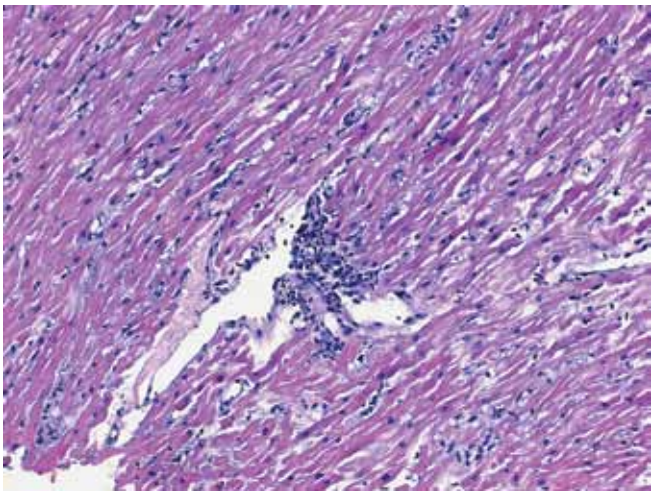

Fig. 6. Morphological examination of myocardial transplant. Myocardial transplant has a mild cellular rejection without signs of humoral crisis, RI pAMR0: a single focal perivascular lymphocytic infiltrate without cardiomyocyte damage in the interstitium. H&E staining, 200× magnification

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IMPACT OF THE GROWTH HORMONE AND IGF-1 ON GRAFT FUNCTION AND IMMUNE RESPONSE IN PEDIATRIC LIVER RECIPIENTS

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Growth hormone (GH) and insulin-like growth factor 1 (IGF-1) are the most important regulators of growth, regeneration and metabolism. The influence of GH and IGF-1 on pediatric liver transplant outcomes is mediated through growth and body weight regulation, specific effects on hepatocyte function and immune system activity. In recent years, the blood levels of these factors and life expectancy, both in healthy individuals and liver recipients, have been shown to be correlated. In pediatric liver recipients, neurohumoral regulation of graft function and other functions of the growing organism, has not been studied enough. The results of studies on the levels and dynamics of GH and IGF-1 in the blood of liver recipients can serve as a basis for assessing the state of graft using new minimally invasive methods and identifying therapeutic targets for personalized therapy. This review summarizes the current understanding of the significance of GH/IGF-1 hormones in hepatobiliary diseases and pediatric liver transplantation (LTx).

Keywords: biomarker, pediatric liver transplantation, liver disease, liver fibrosis.

GH and IGF-1 are the key links in neurohumoral regulation of metabolism. They can affect tissues through different intracellular signaling pathways. GH stimulates IGF-1 synthesis, which, in turn, influences GH production by the principle of negative feedback, inhibiting its synthesis [1].

GH is synthesized in the anterior pituitary lobe and secreted into the blood, with maximum blood concentrations every 3–5 hours. The nature of GH secretion differs in men and women and depends on age. The highest level of the hormone in the blood is observed during fetal development. With age, the baseline level, frequency, and amplitude of hormone secretion peaks decrease. The range of reference values of GH in the blood of children aged 1–3 years is 2–10 ng/mL, and in adults it is 1–5 ng/mL [2].

IGF-1 is a polypeptide hormone produced by many tissues. More than 90% of IGF-1 circulating in the systemic circulation is synthesized by hepatocytes [3]. Plasma IGF-1 levels, in contrast to GH, practically do not change during a day. The range of reference values of plasma IGF-1 levels in children aged 1–3 years is 5–300 ng/mL [3]. The maximum plasma IGF-1 level in children is observed during puberty and gradually decreases over the years.

The significance of growth hormone and IGF-1 in pediatric liver transplantation (LTx) may be related to their role in regulation of growth and body weight, their influence on hepatocyte function and immune system activity [4, 5].

The physiological effects of GH and IGF-1 on cells are mediated through transmembrane receptors found on the surface of many cell types, including hepatocytes and lymphocytes [6]. The effect of GH and IGF-1 is largely determined by the level of receptor expression, which depends on cell type and may change under the influence of various factors. The GH/IGF-1 effect depends on GH and IGF-1 production, on one hand, and on IGF-1-binding proteins, proteases that degrade the IGF-1-binding protein complex, and GH and IGF-1 receptors, on the other hand [7].

Growth regulation is one of the main functions that GH and IGF-1 have in common. In addition, both in children and adults, GH plays an important role in metabolic regulation. IGF-1 is the main mediator of anabolic and mitogenic effects of GH in peripheral tissues and is a key factor in regulation of body weight. On the other hand, they have different effects on glucose and lipid metabolism: GH increases the blood glucose level and promotes lipolysis, while IGF-1 has the opposite effects [8, 9].

GROWTH HORMONE AND INSULIN-LIKE GROWTH FACTOR 1 IN LIVER DISEASES

IGF-1 synthesis by hepatocytes is impaired in liver disease leading to increased GH secretion. Despite high

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serum GH levels in patients with chronic liver disease (CLD), low IGF-1 levels can also result from hepatocyte resistance to GH [10, 11]. It is supposed that metabolic disorders frequently found in patients with liver diseases – insulin resistance, malnutrition, osteopenia, etc. – are associated with impaired neurohumoral regulation caused by IGF-1 deficiency [12–14].

The degree of decrease in IGF-1 levels in patients with CLD correlates with the severity of hepatocyte dysfunction [15, 16]. Administration of recombinant IGF-1 leads to the arrest of liver fibrous degeneration [17, 18]. In an experiment on animals with nonalcoholic steatohepatitis (NASH), treatment with recombinant IGF-1 has been shown to improve liver function in cirrhosis. It was also found in the experiment that the degree of hepatic ischemia/reperfusion injury is less when IGF-1 levels are higher [19, 20]. Some experimental studies have suggested the possibility of using IGF-1 in clinical practice. It has been shown that recombinant IGF-1 in patients with cirrhosis increases serum albumin levels and improves energy metabolism [21].

GH levels are significantly higher in adult patients with cirrhosis than in healthy individuals. This is associated with impaired IGF-1 synthesis by the liver in the end stage of hepatobiliary diseases [9]. GH levels in recipients decrease to normal values in adults as early as on day 7 after LTx, while IGF-1 levels increase [12].

Our studies have shown that in young children with severe hepatobiliary diseases, as in adults, GH levels are elevated and IGF-1 is reduced, which is combined with stunted growth and weight retardation. The degree of increased GH levels in these children is not correlated with IGF-1 levels and anthropometric indices, but is associated with the Pediatric End-stage Liver Disease (PELD) score for liver disease severity and liver fibrosis severity. After LTx, GH and IGF-1 levels in children are comparable with the levels of these hormones in healthy children and significantly correlate with the growth of recipient children [22].

Previously, it was believed that IGF-1 does not directly affect hepatocyte function because in a healthy liver, a small number of IGF-1 receptors are expressed on the surface of hepatocytes. However, further studies have shown that in some liver diseases, there is increased expression of these receptors [4]. In acute viral hepatitis and chronic hepatitis B and C, expression of IGF-1 receptors on hepatocytes is higher than in a healthy liver. There is also increased IGF-1 levels, which is believed to accelerate regeneration of damaged hepatocytes [23].

The antifibrotic effect of IGF-1 is realized both directly through the GH/IGF-1 system and indirectly through regulation of other profibrogenic factors [24, 25]. Stellate cells play a key role in liver fibrosis. Their activation, caused by chronic trauma, oxidative stress, increased inflammatory cytokines and lipopolysaccharides, leads to their transformation into fibroblasts [26]. It has been shown that IGF-1 can inactivate hepatic stellate cells and induce their aging, thus limiting fibrosis [27]. The above results indicate that decreased IGF-1 production in the liver is not only the result of liver dysfunction, but also plays an important role in fibrosis.

It is known that life expectancy is closely related to the GH/IGF-1 system, which may be of some importance for the development of techniques for predicting recipient and graft survival [28, 29].

In an experiment, it was shown that IGF-1 can improve survival rates in rats with acute liver failure induced by D-galactosamine and lipopolysaccharide administration. Prophylactic administration of IGF-1 to animals prevented an increase in bilirubin levels and transaminase activity [18].

Clinical studies have established an association between GH and IGF-1 levels in adult LTx recipients with 3-month and 3-year survival [28]. Our studies also showed an association between GH levels and 6-month survival in pediatric liver recipients [30].

THE ROLE OF GH/IGF-1 IN REGULATION OF IMMUNE RESPONSE

The role of the GH/IGF-1 system in the regulation of immune response has been the topic of many studies in recent decades [6, 31]. It has been shown that mutual regulation of the neuroendocrine and immune systems is ensured by the presence of common ligands and receptors, as a result of which neuroendocrine hormones have immunoregulatory functions, and cytokines affect neuronal functions. In addition, cells of the immune system can synthesize and secrete neuroendocrine hormones such as adrenocorticotropin, GH, prolactin, thyrotropin and others, and a wide range of cytokines can be produced by microglia cells in the central nervous system [32].

Cells of the immune system contain GH receptors, which has a direct effect on all major immune cell types, thereby influencing the immune response. In turn, cytokines produced by cells of the immune system specifically affect GH secretion by the pituitary gland. It has been shown that interleukins (IL-2, IL-6, IL-11, IL-1) and ciliary neurotrophic factor stimulate GH secretion, whereas transforming growth factor beta (TGF-b) and tumor necrosis factor alpha (TNF-α) can inhibit its secretion [33].

GH is necessary for immune system development and maintenance of cell-mediated and humoral responses. It affects hematopoiesis by stimulating neutrophil differentiation, increases erythropoiesis and bone marrow cell proliferation, and enhances thymocyte proliferation and export [34]. GH stimulates the production of cytokines – IL-1, IL-2, IL-6, interferon-c, TGF-b, and TNF-α [35].

In addition, GH prevents lymphocyte apoptosis by increasing NO production, reducing the synthesis of caspases involved in apoptosis, and promoting tubulin

polymerization, which stabilizes the microtubule network [36]. Some experimental studies have shown that GH protects immune cells against the immunosuppressive effect of glucocorticoids. Injection of GH into rats after dexamethasone administration or surgical stress improved the immune response [31].

The effects of IGF-1 on the immune system are manifold and are associated with the regulation of cell proliferation, differentiation, and metabolism. It has been established that the functional activity of IGF-1 and IGF-1 receptors on T cells is enhanced during T cell activation, proliferation, chemotaxis, and apoptosis [37]. IGF-1 also stimulates natural killer (NK) activity [38]. On one hand, IGF-1 exhibits the properties of a nonspecific immunomodulator by stimulating lymphopoiesis, immunoglobulin synthesis, and T cell differentiation; on the other hand, it has a selective inhibitory effect on IL-2-dependent lymphocyte growth, and also causes proliferation of regulatory T cells, preventing autoimmune diseases in mice [5, 39].

The presence of the IGF-1 receptor and binding protein in myeloid cells suggests the influence of IGF-1 on hematopoiesis and inflammation. IGF-1 can act as a proinflammatory factor by stimulating proinflammatory cytokines and chemokines, such as TNF-a and IL-8, and can also have an anti-inflammatory effect by stimulating IL-10 secretion and inhibiting Th1-mediated cellular immune responses in activated T cells [6, 40].

IGF-1 can affect the pathogenesis of immune diseases by regulating the activity of immune cells through endocrine, paracrine, and autocrine mechanisms. Experimental and clinical studies have shown that IGF-1 reduces immune response in autoimmune diseases [5]. In autoimmune diseases such as Graves' disease, rheumatoid arthritis, some inflammatory bowel diseases, and type 1 diabetes, the IGF-1 levels are decreased, which is accompanied by immunosuppression [41]. Elevated IGF-1 levels occur in some cancer types in which tumor cells express the hormone and its receptors, thus increasing immunosuppression and tumor growth [42, 43].

Some effects of IGF-1 and tacrolimus have been found to be realized via common calcineurin-dependent cellular pathways [44, 45]. In experimental studies, it has been shown that intravenous and oral administration of tacrolimus in rats leads to increased IGF-1 levels and also enhances biliary excretion, which is regulated by both factors separately or together [46]. Our studies have shown that IGF-1 levels directly correlate with the tacrolimus dose administered in paediatric patients one year after LTx. This allows us to consider it as a potential biomarker of immunosuppression efficiency [47]. However, the mechanisms of this relationship are not clear and further research is needed to understand them.

CONCLUSION

GH and IGF-1 levels not only depend on liver function but also largely determine its condition and can be considered as indicators of liver function in patients with hepatobiliary diseases. Changes in these hormone levels after LTx can serve as an objective indicator of the degree of normalization of the synthetic function of graft and recovery of neurohumoral regulation in paediatric liver recipients.

Further study on interrelations between the GH/IGF-1 hormonal system and other factors influencing the liver graft function will allow to estimate more precisely the possibilities of using GH and IGF-1 both to verify graft condition and to improve therapy in pediatric liver transplant recipients.

The authors declare no conflict of interest.

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TESTING OF THE pHEMA HYDROGEL AS AN IMPLANTATION MATERIAL FOR REPLACEMENT OF OSTEOCHONDRAL DEFECTS IN ANIMALS

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Objective: to evaluate the features of reparative chondrogenesis and osteogenesis in animal experiments with the implantation of porous poly(2-hydroxyethyl methacrylate) (pHEMA) hydrogel into osteochondral defects. **Materials and methods.** Cylindrical pHEMA implants (5 mm in diameter) were synthesized by radical polymerization. The implants were subjected to light microscopy and mechanical tests to characterize the structure and viscoelastic properties of the material. In experimental group #1, four pHEMA specimens were implanted into formed defects in the distal femoral epiphysis of rabbits. In experimental group #2, allogeneic chondrocytes were applied to the surface of four specimens before implantation. In the control series, four defects were not replaced with implants. Tissue regeneration was investigated by morphological and morphometric methods 30 days after operation. **Results.** The pHEMA implants were heterogeneous specimens with irregularly shaped pores – up to 30 × 10 μm at the surface and 300 × 120 μm inside. With >10% static compressive stress, the Young's modulus was 54.7 kPa. For dynamic stress, increased frequency of compression-relaxation cycles from 0.01 Hz to 20.0 Hz led to increased storage modulus from 20 kPa to 38 kPa on average, and increased loss modulus from 2 kPa to 10 kPa. Indicators of semi-quantitative assessment of local inflammatory response to pHEMA implantation had the following values in points: pHEMA, 4.7 ± 0.3; pHEMA with allogeneic chondrocytes, 6.0 ± 1.0; control, 4.3 ± 0.3. The ratio of connective, bone, and cartilage tissues proper in the regenerates had the following respective values: pHEMA, 79%, 20%, 1%; pHEMA with chondrocytes, 82%, 16%, 2%; control, 9%, 74%, 17%. **Conclusion.** In a short-term experiment, pHEMA implants did not trigger a pronounced inflammatory response in the surrounding tissues and can be classified as biocompatible materials. However, the tested implants had low conductivity with respect to bone and cartilage cells, which can be improved by stabilizing the pore size and increasing the rigidity when synthesizing the material.

Keywords: osteochondral defects, implants, pHEMA hydrogel, physical properties, biocompatibility, cartilage tissue, bone tissue.

INTRODUCTION

Focal osteochondral lesions are commonly found in 61–63% of patients during arthroscopy [1]. The spontaneous repair ability of the articular cartilage tissue is very limited, and their presence provokes deforming osteoarthritis and reduces patients' quality of life [1]. On this basis, treatment of osteochondral injuries of the joint is an urgent task today.

To stimulate the regeneration of articular cartilage, a number of techniques, which have shown satisfactory medium-term treatment outcomes, are used. They include mosaicplasty [2], autochondrogenesis (AMIC) induced on a cell culture scaffold [3], autologous chondrocyte transplantation (ACI) [4], including those associated with collagen matrix (MACT/MACI) [5], and introduction of mesenchymal stromal cells [6]. At the same time, there is yet no method of treatment that provides organ-specific restoration of hyaline cartilage and complete long-term clinical remission [7]. This fact forces us to look for new ways to replace cartilage defects, including new cell transplantation matrices.

A wide range of materials for cellular matrices is known, among which hydrogels occupy a significant niche. A gel is a polymer swollen in a solvent; its composition can contain up to 99% liquid. Synthetic hydrogels are considered to be biomimetics of biological tissues, since with an appropriate chemical composition

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and certain physical and/or chemical influences, they demonstrate similar mechanical properties to living tissues [8, 9].

In this study, the pHEMA hydrogel was used as the material for osteochondral implants. It is known that pHEMA is nontoxic, inert, and biocompatible [10]. Through controlled synthesis, the physical properties of pHEMA can be approximated to those of biological tissues. In particular, the viscoelastic properties of pHEMA, its oxygen permeability, and its solvent content can match the characteristics of the extracellular matrix [11].

The biocompatibility of pHEMA and its physicochemical properties has made it useful in the manufacture of contact lenses, artificial corneas, drug delivery systems, and matrices for controlled stem cell differentiation [12]. In orthopedics, the possibility of using pHEMA for fabrication of artificial cartilage, nucleus pulposus of the intervertebral disc prosthesis, and its use as a mechanical vibration damper in the fabrication of a total intervertebral disc endoprosthesis is discussed [11, 13].

In terms of application of synthetic materials in medicine, pHEMA hydrogels have an advantage associated with the possibility of creating various morphological structures in the form of pores during synthesis [14]. The porous structure of pHEMA makes it possible to introduce various bioactive compounds, including those with antimicrobial activity [15]. Besides, porous synthetic hydrogels have proven themselves as three-dimensional cell culture matrices used in replacement therapy and regenerative medicine [12].

Based on the above, the aim of this study was to investigate cartilage and bone tissue regeneration during the filling of osteochondral defects in the femoral epiphysis with pHEMA porous hydrogel implants in in vivo animal experiments. The results of assessment of the structure and viscoelastic properties of the pHEMA implants, as well as data on the course of tissue reparative processes after implantation of hydrogels without cells and with allogeneic chondrocytes are presented.

MATERIALS AND METHODS

Synthesis of pHEMA-based implants

Synthetic pHEMA hydrogels were obtained by radical polymerization in an aqueous solution of monomer (hydroxyethyl methacrylate, HEMA) at 70 °C in the presence of a crosslinking agent, N,N'-methylenediacrylamide (MDAA). The monomer concentration was 2 mol/dm^3 (2 M) and the MDAA concentration was 0.02 mol/dm^3 (0.02 M). This ensured the formation of a mesh polymer structure in which the molar ratio of the mesh nodes to the links in the linear fragments was 1 : 100. We used 3 mM ammonium persulfate as a polymerization initiator. Polymerization was carried out in cylindrical polyethylene molds for one hour, after which the samples were removed from the molds and washed in distilled water for two weeks with daily water changes.

As a result, pHEMA specimens, ~5 mm in diameter and 120–150 mm long, were obtained, after which they were shortened with a scalpel to the implant size (~5.5 mm). To validate the elastic properties of pHEMA, specimens with a larger diameter (~9 mm) were synthesized.

pHEMA mechanical testing

The viscoelastic properties of the gels were evaluated on special mechanical testing equipment, and is described in detail in our earlier publications [16, 17]. Briefly, the mechanical testing setup contained precision force and displacement transducers, and a linear electromagnetic motor to give the specimens arbitrarily shaped deformations. Cylindrical pHEMA specimens, ~9 mm in diameter and ~5 mm in height, were placed into a cuvette filled with a solution in which the gel was pre-swollen. One end of the sample was rigidly attached to the force transducer and the other end to the motor lever.

To obtain the stress-strain relation, the specimens were given stepwise compressive strains with a 50 μm step and the value of the elastic force arising in the specimen was recorded. To determine the rheological characteristics of the material, the storage modulus (G') and loss modulus (G''), sinusoidal compressive strains with an amplitude of ~3% of the initial height of the specimen and frequency 0.01 to 20 Hz were applied to the specimens. The method of setting and analyzing the effects of low-amplitude periodic deformations is widely used to determine the viscoelastic properties of materials, including pHEMA [18].

Experimental animals and study groups

Sexually mature Soviet Chinchilla male rabbits (age 5 months, weight 2.8–3.5 kg), with animal certificate No. 2020/2KSh dated April 12, 2020, were used in the work. Management, surgical interventions, and euthanasia were performed in accordance with the requirements and principles of biomedical research involving vertebrate animals set forth in the European Convention (1986) and its protocol of 1998. The experimental protocol was approved by the Ethical Committee of the Ural State Medical University (protocol No. 2 of February 28, 2020).

In 6 rabbits, standard large osteochondral defects were created in the articular patellar surface of the metaphysis of both femurs (n = 12). Such defects did not result in complete tissue replacement during the spontaneous reparative process, which corresponds to the literature [19]. Throughout the experiment, no animal death or purulent complications were observed. The animals were agile, their support ability and the correct position

of the limbs were preserved. One rabbit was used as a chondrocyte donor.

The animals were divided into 3 groups of 2 rabbits each. In the control group (CG), the defects were not replaced with implants (n = 4). In the experimental group #1 (EG-1), the defects were replaced with pHEMA implants (n = 4); in experimental group #2 (EG-2), the defects were filled with pHEMA implants with allogeneic chondrocytes adhered to them (n = 4). Thirty days after surgery, all animals were removed from the experiment, after which the femur metaphysis were examined.

Preparation of pHEMA implants with adherent chondrocytes

Chondrocyte culture was obtained from the cartilage tissue of the rabbit knee joint used as a donor. Chondrocytes were isolated from the tissue by dissociation with collagenase-I. For this purpose, the cartilage tissue of the articular surfaces of the hip and knee joints was crushed and incubated at 37 °C in 0.3 mg/ml collagenase solution for 90 minutes. The solution was then replaced with a fresh one in 0.5 mg/ml collagenase concentration; incubation was performed for 16 hours at 37 °C with this solution.

Dissociated cells were grown as a monolayer culture in culture vials using specialized chondrocyte growth medium kit (Cell Applications, Inc.) in a CO_2 incubator (37 °C, 5% CO_2, 100% relative humidity). Once the monolayer reached 70% confluence, the cells were transplanted to a second passage. Cells were removed from the plastic using a 0.25% trypsin solution with EDTA. Chondrocytes from the second passage were used for seeding on the implant. The cell phenotype was confirmed using Alcian blue stain and nuclear red stain.

Chondrocytes were seeded on one of the flat surfaces of high-density pHEMA implants (300,000 cells/cm^2 implant surface). For this purpose, sterile hydrogels were placed vertically in a Petri dish, close to each other, and filled with a chondrocytic medium above the level of their upper edge. Chondrocytes were removed from the plastic with trypsin and resuspended in the chondrocytic medium. The obtained suspension was applied to the implant-coating medium, after which the cells were deposited on the implant surface. The implants were incubated for one day in a CO_2 incubator, and then used for implantation in rabbits.

To confirm chondrocyte adhesion on the implant surface, some samples were fixed and stained with pyrazolone yellow (cytoplasm) and DAPI (nuclei) according to the technique described earlier [17]. Fluorescence microscopy of the stained samples confirmed the adhesion of chondrocytes on the hydrogel surface. At the same time, the attached cells were irregularly distributed on the gel surface, with the formation of dense multilayer clusters.

Surgical procedure for pHEMA implantation

Surgical interventions were performed under general anesthesia (intramuscularly – Rometar 2% – 8 mg/kg (Rometar 2% Spofa, Prague, Czech Republic), isoletil – 6 mg/kg (Zoletil-100, VirbacSanteAnimale). Medial access with dissection of the patellar-retaining ligament was used to perform arthrotomy with surgical dislocation of the patella laterally. A cylindrical osteochondral defect (Fig. 1, a) was formed on the anterior surface of the distal femoral epiphysis in the area of the patellofemoral junction surface with a 5.0-mm diameter drill with a stopper.

Two transverse canals were formed in the distal femoral epiphysis with a 1.5-mm diameter wire through the lateral cortical bone walls at the defect level and 4 mm proximal to the defect. Implants, 5 mm in diameter and 5.5 mm in height, were inserted into the defects (Fig. 1, b) and fixed to the defect walls with transverse sutures through the transverse canals (Fig. 1, c, d). The surgical wound was sutured in layers to restore the integrity of the ligament holding the patella.

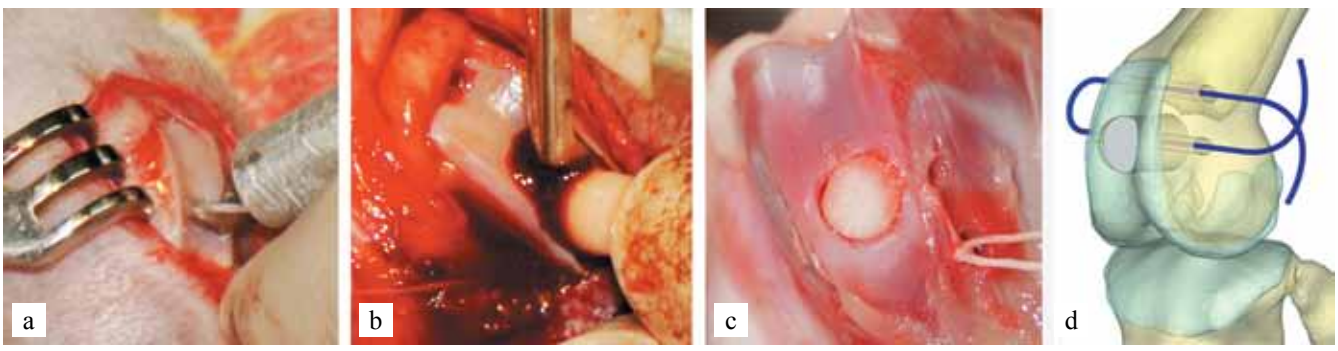

Fig. 1. Stages of implant insertion in a rabbit's femoral epiphysis. a, formation of a standard defect with a drill with a stopper; b, implant insertion; c, the view of the articular surface after implant placement; d, diagram showing implant localization and its fixation with transosseous sutures

Methodology of the morphological study

The material (distal femoral epiphysis) for morphological study was obtained immediately after the animals had been removed from the experiment. Femoral fragments were fixed in 10% neutral buffered formalin (BioOptica). Bone tissue was decalcified, and part of the bone material was embedded in paraffin. Under standard paraffin wiring protocol, the pHEMA material lost up to 1/2 of its volume, which deformed the delicate newly formed tissue surrounding the implants and disrupted the topographic unity of the preparation. Therefore, a part of bone fragments was poured into gelatin and subjected to cryotomy. Epimetaphyseal slices were made in the sagittal plane.

Hematoxylin and eosin were used as visual stains. Van Gieson's stain was used to identify the connective tissue components. Micros MS300 light microscope was used for descriptive morphology. Digitization of preparations and morphometric studies were performed on 3DHISTECH PANNORAMIC Midi scanning microscope using Pannoramic Viewer software.

Morphometric assessment of the regenerate included determination of the height of the supra-implantation regenerate, thickness of the peri-implantation capsule, depth of regenerating tissue sprouting into the implant pores, area and ratio of tissue components in the peri-implantation area, counting of chondrocytes and their isogenic groups in the newly formed cartilage tissue.

The local biological effect of the implants was determined by a semi-quantitative assessment of the inflammatory reaction [20]. Inflammatory response was characterized by the presence of necrosis zones, the number of pro-inflammatory cells – polymorphonuclear leukocytes, mast cells, lymphocytes, macrophages, plasma cells and giant multinucleated cells in the field of view under ×400 magnification.

Statistical analysis

The results are presented as $X \pm m$, where X is the arithmetic mean and m is the error of the arithmetic mean. The nonparametric Mann–Whitney U Test was used to assess differences between two independent samples. The acceptable level of statistical significance was $p \leq 0.05$. Statistical analysis of the data was performed using licensed software Statistica 6.0.

RESULTS

Features of the architecture of pHEMA implants

Synthetic pHEMA hydrogels have a number of structural features that distinguish them from other gels used for biomedical purposes, in particular, from the widespread polyacrylamide hydrogels. Unlike the latter, pHEMA hydrogels are a heterogeneous macroporous system [18]. The HEMA monomer is well soluble in water, while the pHEMA polymer has limited water solubility. Therefore, during hydrogel synthesis, the initially homogeneous monomer solution undergoes phase separation as polymerization proceeds. At the same time, the formed irregular polymer mesh separates from the aqueous medium and forms a macroporous structure, which is schematically shown in Fig. 2, a.

The structure of pHEMA contains large, irregularly shaped pores filled with liquid. The pore walls are formed by the irregular grid structure of pHEMA, which, in turn, also contains water and is permeable to dissolved salts and simple compounds (sugars, amino acids, etc.). The heterogeneous nature of pHEMA is manifested, in particular, by the fact that its samples are not transparent but are milky-white due to light scattering on the walls of macropores in the gel structure (Fig. 1, b, c).

Fig. 2 shows as an example the photos of cryosections of pHEMA implants recorded before and after the experiment (Fig. 2, b, c). It is clearly seen that in the depth

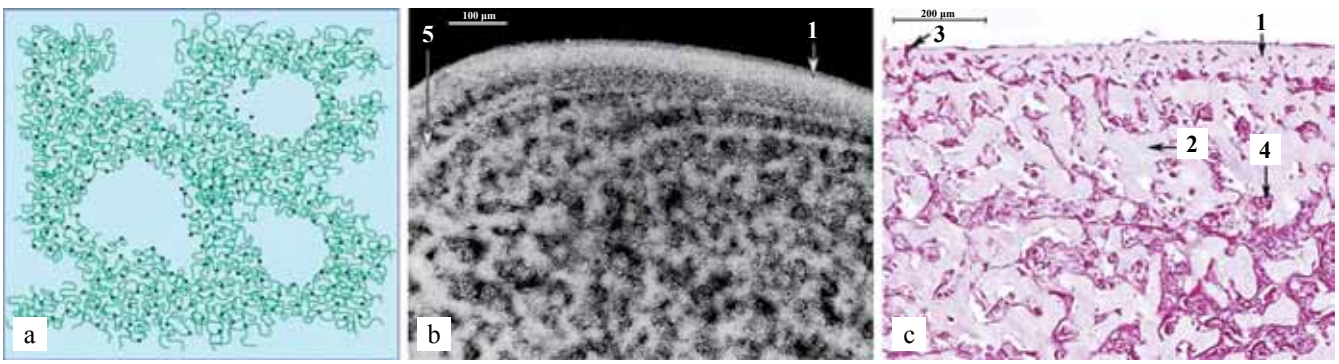

Fig. 2. a, diagram showing the molecular structure of synthetic pHEMA hydrogel. Lines are the linear fragments of polymer chains, dots represent mesh nodes; b, c, – implant imaging examples, light microscopy. b, pHEMA cryosection before experiment (100× magnification, color rendering inverted); c, pHEMA cryosection 30 days after implantation (200× magnification, H&E stain). 1, dense surface layer; 2, pHEMA; 3, pore communicating with the surface; 4, pores inside the implant with loose irregular connective tissue and osteoid; 5, concentric layers inside the implant containing small pores

of the samples, the pores (dark areas in Fig. 2, b, in Fig. 2, c the pores are filled with loose connective tissue) have an irregular shape, they do not exceed 300 μm in size on the long axis and 120 μm on the short axis. The surface dense layer of the implants, 20–150 microns wide, has much smaller pores (up to 30 μm on the long axis, up to 10 μm on the short axis), with a small number of pores communicating with the surface.

In general, the internal architecture of the implant can be characterized as a highly hydrated macroporous polymeric material with a high degree of structural heterogeneity.

Viscoelastic properties of pHEMA

Fig. 3 shows the results of mechanical tests of pHEMA samples in static (n = 5) and dynamic (n = 6) loading modes. The stress-strain dependences were obtained for the gel compression deformation with a 50 μm step (Fig. 3, a). It can be seen that mechanical stress in the gel increases with increasing strain. In general, the dependence is not linear, and qualitatively resembles the one for biological soft tissues [21]. On the curve, there are two areas in which the relationship is linear and is described by linear regression equation: the first – up to 5–6% deformation, the second – at deformations greater than 10%. The coefficient in the first term of the equation, defined as the slope of the tangent to the curve, corresponds to Young's modulus, whose value for the first and second parts of the curve is 19.6 kPa and 54.7 kPa, respectively.

Fig. 3, b illustrates the results of the mechanical test analysis of pHEMA in the dynamic load regime. The frequency-dependent values for the storage modulus (G') as a measure of gel elasticity and the loss modulus (G") as a characteristic of the object's viscosity are shown. It can be seen that at extremely low frequencies, pHEMA behaves more like an elastic body. The storage modulus is close to the Young's modulus determined in the static mode over the first range of small deformations. As the frequency of compression-relaxation cycles of gels increases from 0.01 Hz to 20.0 Hz, the storage modulus increases from 20 kPa to 38 kPa on average, and the loss modulus increases from 2 kPa to 10 kPa. That is, the contribution of the viscous component to the mechanical response of the gel to deformation begins to increase. In general, approximately the same dynamics of the moduli with a significant increase in the strain frequency indicates a good stabilization of the bonds in the structure of the pHEMA polymer network. This implies a high degree of gel wear resistance in the selected load range.

Macroscopic examination of osteochondral tissues

Thirty days after surgery in the CG, the articular surfaces of the distal femoral epiphysis showed irregularities and thickening in the areas near the defects (Fig. 4, a).

In EG-1 and EG-2, the spatial arrangement of the implants was maintained throughout the experiment. In EG-1, the implants were covered with thin regenerates with small gaping areas, and there were roller-like thickenings on the dorsal surface of the condyles (Fig. 4, b). The greatest changes in the articular surface were found in EG-2 – signs of regenerative hypertrophy in the form of valvular outgrowths throughout the dorsal surface of the distal epiphysis. The regenerates over the implants are thin with perforations (Fig. 4, c).

Microscopic examination of regenerates

The articular surfaces in the peri-implantation area had pronounced degenerative changes in the superficial and intermediate cartilage zones (Fig. 5), as well as signs of reactive-productive repair. In particular, animals of all series showed thickening on articular surfaces formed by bone trabeculae with signs of active osteogenesis, structured hyaline cartilage with fibrillated matrix, and broad perichondrium.

The structure of the articular surface regenerate differed in the animals of the experimental and control groups. In CG, the regenerate was formed by a broad

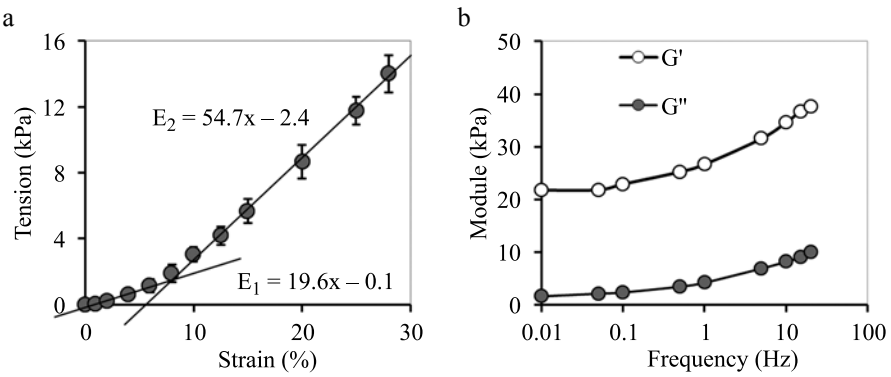

Fig. 3. Viscoelastic properties of pHEMA: a, relationship between mechanical stress in the gel and its deformation; b, storage modulus (G') and loss modulus (G'') versus angular frequency (representative graphs)

layer of partially structured hyaline-like cartilage with a thin layer of dense irregular connective tissue similar to perichondrium (Fig. 5, a). At the subchondral level and deeper, the cavity was filled with newly formed bone tissue; the subchondral plate was not restored. Repair processes were not completed.

In EG-1 and EG-2, the regenerates on the articular surface were formed by dense irregular connective tissue with loci of loose connective tissue and signs of active angiogenesis. Cartilage tissue regeneration occurred only at the edges of the implantation cavity; its main source of development was probably chondrocyte precursor cells from the subchondral bone tissue. In EG-1, the regenerate was formed by dense irregular connective tissue with high cellularity and relatively loosely arranged fibers (Fig. 5, b); in EG-2 (Fig. 5, c), it was represented by connective tissue with denser collagen fibers, which indicates its greater maturity. The area of the regenerate (the area corresponding to the cartilage projection before the defect was formed) in EG-1 and EG-2 was 2.9 and 2.3 times smaller than in CG ($p < 0.05$), respectively.

In EG-1 and EG-2, throughout the contact with the implantation bed, the implants were surrounded by connective-tissue capsules that included separate loci of chondrogenesis and osteogenesis. In EG-1 and EG-2, the capsules were formed by dense irregular connective tissue (Fig. 6, a, b). The capsule thickness in EG-1 and EG-2 (193 ± 77 μm and 180 ± 84 μm, respectively) and their areas did not differ significantly in the experimental group. In the peri-implant area between the newly formed bone trabeculae, pHEMA fragments were detected, indicating partial implant failure in vivo (Fig. 6, a).

The ratios of connective tissue proper (dense and loose), bone tissue, and cartilage tissue within the regenerates had the following respective values: pHEMA, 79%, 20%, 1%; pHEMA with chondrocytes, 82%, 16%, 2%; control, 9%, 74%, 17%. Cartilage tissue in EG-1 and EG-2 was represented by small non-vascular fields with single and small isogenic groups of chondrocytes (EG-2 showed a tendency to increase) with high cell density and little matrix volume, which indicated cartilage immaturity and distinguished the experimental groups from the control.

In both experimental groups, the loose connective tissue fibers "grew" into the open pores of the implant communicating with the surface. The entire depth of the pore spaces was predominantly filled with loose connective tissue with newly formed capillaries and small

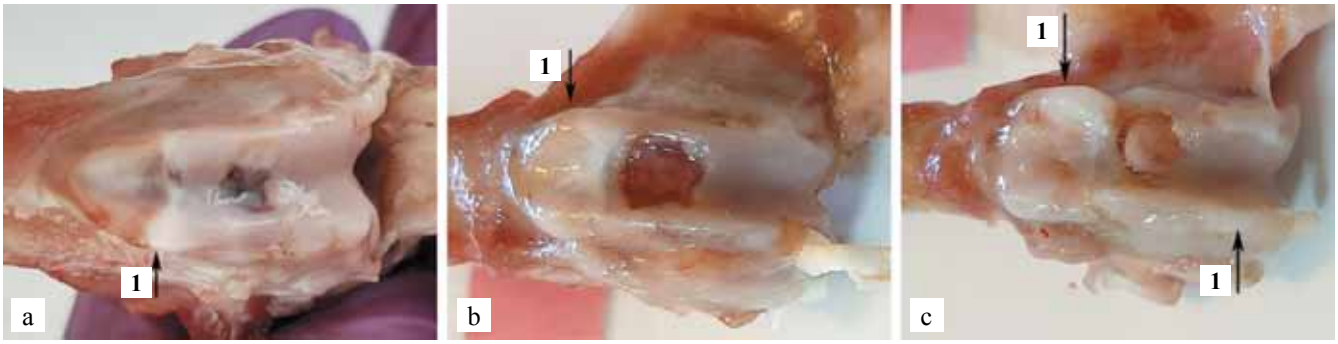

Fig. 4. Macro specimens of distal femoral epiphyses of rabbits, 30 days after surgery. a, defect not filled with implant; b, defect filled with pHEMA implant; c, defect filled with pHEMA implant with adherent allogeneic chondrocytes. 1, roller-shaped bone crest thickenings

Fig. 5. Regeneration of the articular surface (distal femoral epiphysis in rabbits) in the border area of the defect cavity, 30 days after surgery. H&E stain. Light microscopy, 100× magnification. a, control group; b, experimental group 1; c, experimental group 2. 1, articular cartilage; 2, cavity boundary; 3, newly formed reticulofibrous bone tissue; 4, newly formed cartilage tissue; 5, connective tissue proper on the implant surface; 6, implant

areas of bone tissue (Fig. 6, c, d). The pores of the lateral implant surfaces contacting the cavity bed contained foci of osteogenesis.

Microscopic assessment of local inflammatory response

A semi-quantitative evaluation of the local biological effects of the pHEMA implants revealed that the inflammatory response scores in the experimental groups were slightly higher than in the control (CG, 4.33 ± 0.33; EG-1, 4.7 ± 0.33; EG-2, 6.0 ± 1.0), but the differences were not statistically reliable. The inflammatory response scores were elevated mainly due to increased number of macrophages in the surrounding tissues in both groups and mast cells detected in EG-2 peri-implantation tissues. No giant multinucleated cells were detected in the surrounding tissues.

Thus, the pHEMA material in vivo did not cause a pronounced inflammatory reaction in the surrounding tissues under the conditions of this experiment, taking into account the cumulative assessment of the selected criteria.

DISCUSSION

In this work, the reparative processes of osteochondral tissues were investigated when the osteochondral defect was filled with an implant based on porous pHEMA. The choice of pHEMA was based on the literature data according to which this gel has good biocompatibility [10–13].

In vivo experiment detected single reticulofibrous bone trabeculae "sprouting" into the implant surface pores 30 days after implant introduction. This indicates the possibility of osseointegration of the material in a longer postoperative follow-up period. When pHEMA with chondrocytes was used, there was a slight increase

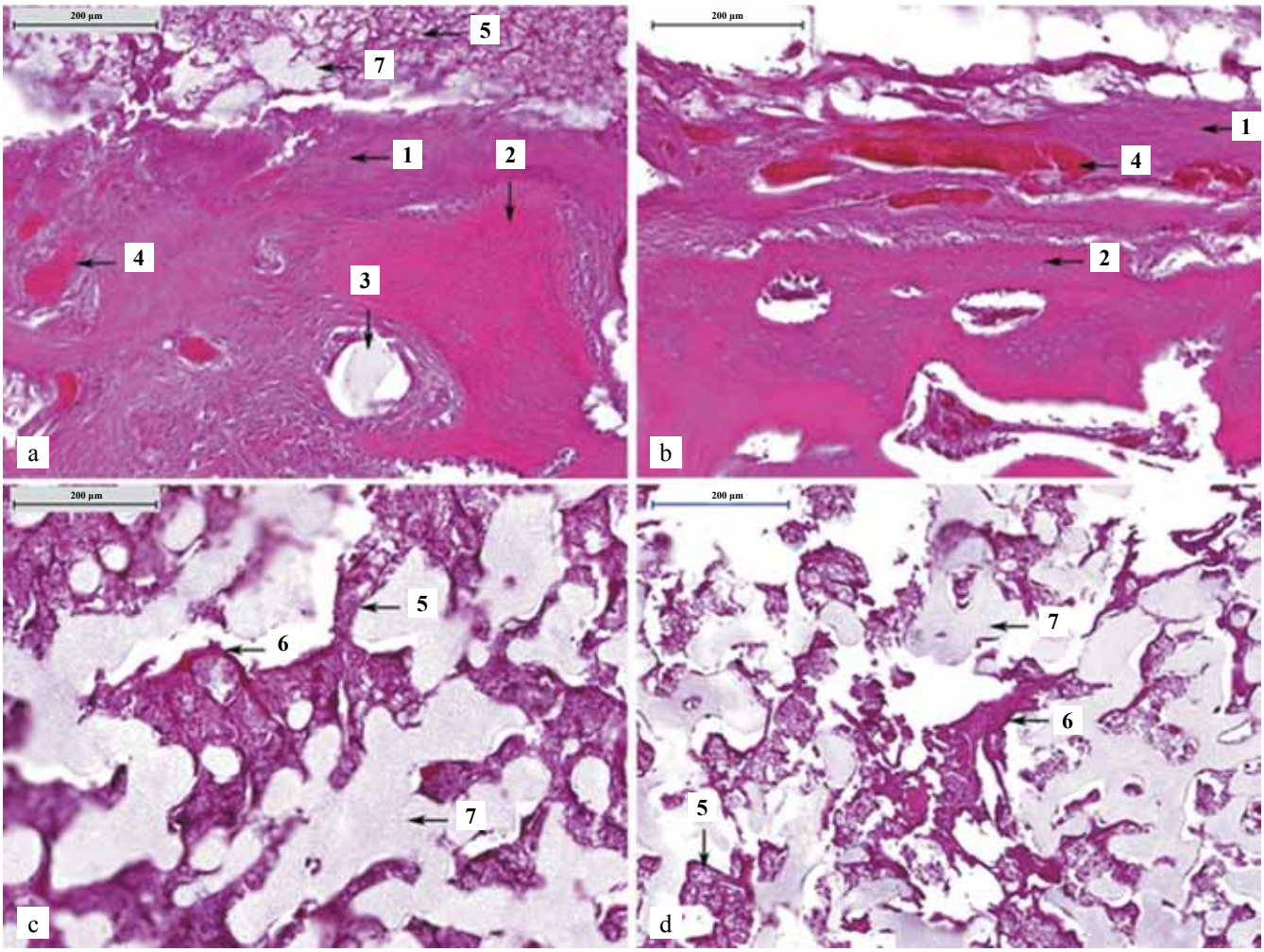

Fig. 6. Regenerates of osteochondral defects of the distal femoral epiphysis of rabbits, 30 days after implantation. H&E stain. Light microscopy, 200× magnification. a, c, experimental group 1; b, d, experimental group 2; a, b, implant cavity bed; c, d, implant. 1, connective tissue capsule in the implant cavity bed; 2, bone tissue inside the capsule; 3, pHEMA fragments inside the newly formed bone tissue; 4, vascular hyperemia; 5, connective tissue proper in the implant pores; 6, bone tissue in the implant pores; 7, pHEMA implant

in the area of chondrogenesis regions as compared to implants without cells.

At the same time, the totality of the results indicates poor integration of osteochondral tissues with the polymeric matrix of pHEMA. In terms of tissue response to the implant introduced, the polymeric matrix used should be referred to the category of biotolerant materials. The body response is characterized by formation of connective tissue capsule isolating the polymer matrix from the tissues and introduction of predominantly connective tissue into the implant pores. Thus, pHEMA implants showed minimal conductive properties with respect to osteochondral tissues when embedded into the osteoarticular defect.

The result obtained may be related to a number of physicochemical properties of the bioengineered matrix used [22]. It is known that the architecture of the pore space plays a key role in the delivery systems of cellular structures. Thus, maximum migration of chondrocytes [23] and osteoblasts [24] inside three-dimensional matrices is observed in the presence of 250–325 μm pores communicating with the surface. On the contrary, for fibroblasts, the highest level of adhesion and migration into the gel-based framework was observed at <100–160 μm pore sizes [25].

In the nomenclature of colloidal systems, pHEMA belongs to the category of macroporous polymers, i.e., it has a >1 μm pore size. In this study, pHEMA implants were used, which, according to optical microscopy, had irregularly shaped pores reaching a size of 300 μm × 120 μm. It should be noted that due to the presence of liquid phase in the gel, it is difficult to judge to what extent the studied implant cryosections (see Fig. 2, b, c) reflect the native structure of the material. At the same time, the obtained facts allow us to speak about the high degree of heterogeneity of pore size distribution in the samples. Indeed, the pores immediately near the implant surface were fundamentally smaller than the pores in the depth of the sample.

It can be hypothesized that the formation of a dense, finely porous pHEMA layer is associated with the features of polymer synthesis in polyethylene molds directly under the implant size (5 mm in diameter). However, the assumption that the mold walls have an influence over polymerization of the material in the boundary layer does not yet have a clear physicochemical substantiation.

Thus, the surface structure of hydrogels favored the migration of fibroblasts into pHEMA but did not promote cartilage or bone tissue migration. Consequently, it can be assumed that the implant sprouting of predominantly loose fibrous connective tissue in relation to insignificant volumes of osteochondral tissue is mainly caused by insufficient number of large pores on the surface of the used pHEMA implants.

It should be added that a possible reason for the extremely slow sprouting of osteochondral tissues into the implant may be the insufficient rigidity of the pHEMA platform used. Mechanical tests carried out on the material show that the elastic modulus of hydrogels was about an order of magnitude lower than those that ensure good chondrocyte proliferation on pHEMA [26]. At the same time, the established patterns of the dynamics of mechanical moduli with increasing strain rate (see Fig. 3, a), as well as the stress-strain relationship (see Fig. 3, b) were qualitatively consistent with the results of similar tests of canine intervertebral discs [27].

The aggregate data of histological examination 30 days after surgery implies that the level of tissue response to implantation of gels, including those with adhesive allogeneic chondrocytes, can be recognized as moderate against the background of the reaction caused by surgical trauma. The established facts do not contradict the data of other studies in which the absence of immune rejection of transplanted allogeneic chondrocytes in rabbits was noted, including, probably, due to their immunosuppressive activity [28].

In general, the information obtained in this work are consistent with the known facts about the influence of the physicochemical properties and surface structure of pHEMA on the biological activity of cells in in vitro experiments. This fact allows us to formulate the ways to optimize pHEMA synthesis to increase the biocompatibility of these hydrogels when used as implants for osteochondral defects in vivo.

CONCLUSION

This work is one of a limited number of in vivo studies that was aimed at investigating the possible implantation of pHEMA hydrogels to create engineering products with physical properties and structure close to native tissue. The hydrogels used in this work did not cause severe rejection response when implanted into rabbit femoral metaphysis, but sprouted predominantly fibrous connective tissue with an almost complete absence of osteochondral tissue in them.

The compatibility of the hydrogel with osteochondral tissue can be improved by creating a large-pore structure not only inside, but primarily at the surface of pHEMA. The results presented in the work suggest that reduction of heterogeneity in pore size distribution in the sample can be achieved by synthesizing pHEMA in a volume significantly larger than that required for implant fabrication. This will minimize the difference in pore size at the surface and inside the polymer.

In addition, attention is drawn to the literature data, where the increase in biocompatibility of hydrogel-based implants by means of modifying the polymer structure with solid fibers or particles is considered [29]. In particular, in a number of works [16, 17, 30], we showed

that inclusion of magnetic iron oxide nanoparticles into polyacrylamide gel composition leads to a significant increase in adhesion and cell proliferation on the surface of ferrogels in in vitro experiments. Thus, synthesis of pHEMA ferrogel samples with optimized pore sizes, as well as assessment of the tissue compatibility of these magnetic composites, is the direction of our future work.

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The authors declare no conflict of interest.

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CRYOGENICALLY STRUCTURED GELATIN-BASED HYDROGEL AS A RESORBABLE MACROPOROUS MATRIX FOR BIOMEDICAL TECHNOLOGIES

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Objective: to investigate the biological properties of a matrix made of cryogenically structured hydrogel in the form of a macroporous gelatin sponge, as well as the possibility of creating cell-engineered constructs (CECs) on its basis. **Materials and methods.** The main components of the cryogenically structured hydrogel were gelatin (type A) obtained from porcine skin collagen, N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide, (EDC) and urea (all from Sigma-Aldrich, USA). Surface morphology was examined using scanning electron microscopy (SEM). The degree of swelling in water of the samples was determined by gravimetric method. Cytotoxicity was studied on NIH3T3, a fibroblast cell line isolated from a mouse, and on human adipose-derived mesenchymal stem/stromal cells (hAMSCs) using IncuCyte ZOOM (EssenBioscience, USA). The metabolic activity of hAMSCs was assessed using PrestoBlue™ reagents (Invitrogen™, USA). To create CECs, we used hAMSCs, human hepatocellular carcinoma cell line HepG2 or human umbilical vein endothelial cell lines EA.hy926. Albumin content in the culture medium was determined by enzyme immunoassay. Ammonia metabolism rate was assessed after 90 minutes of incubation with 1 mM ammonium chloride (Sigma-Aldrich, USA) diluted in a culture medium on day 15 of the experiment. **Results.** Obtaining a cryogenically structured hydrogel scaffold in the form of macroporous gelatin sponge included freezing an aqueous solution of a gelatin+urea mixture, removal of polycrystals of frozen solvent by lyophilization, extraction of urea with ethanol and treatment of the cryostructurate with an ethanol solution of EDC. Scanning electron microscopy identified three types of pores on the carrier surface: large (109 ± 17 μm), medium (39 ± 10 μm), and small (16 ± 6 μm). The degree of swelling in water of the matrix samples was 3.8 ± 0.2 g H_2O per 1 g of dry polymer. The macroporous gelatin sponge as a part of CEC was found to have the ability to support adhesion and proliferation of hAMSCs, EA.hy926 and HepG2 for 28, 15 and 9 days, respectively. Albumin secretion and ammonia metabolism when HepG2 cells were cultured on the gelatin sponge were detected. **Conclusion.** The use of a matrix made from macroporous cryogenically structured gelatin-based hydrogel for tissue engineering products is shown to be promising using a cell-engineered liver construct as a case.

Keywords: cryogenically structured hydrogel, gelatin, macroporous sponge, tissue engineering, liver.

INTRODUCTION

According to projections for the coming years, acute shortages in donor organs will only worsen. This is already stimulating a search for alternative ways to compensate or replace the functions of damaged vital organs. For these purposes, along with the use of medical methods and artificial organs, technologies based on implantation of cell-engineered constructs (CECs), including matrix carriers loaded with stem and/or specialized cells, appear promising [1].

Independent studies by a number of scientific groups have shown that the creation of CECs based on resorbable biopolymer matrices makes it possible to provide a microenvironment close to the natural extracellular matrix to facilitate cell adhesion, proliferation, differentiation and functional activity [2, 3]. Gelatin, a denaturation product of collagen, is most commonly used to form matrices in the form of sponges, meshes, and hydrogels [4, 5], is not only less immunogenic, but also, like collagen, contains the Arg-Gly-Asp (RGD) amino acid sequence, which determines its adhesive properties [6, 7]. Drug and cell carriers based on gelatin or in combination with other natural or synthetic polymers are widely used in various forms, including capsules and microcapsules, micro- and nanoparticles, micro- and nanofibers, and hydrogels [8–10].

When creating CECs, the preferred matrix forms are macroporous systems, including solid polymer scaffolds

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and sponges [11]. An open network of interconnected macropores (100 to 350 μm) ensures unhindered cell penetration and transport of oxygen, vital products and nutrients [12]. The functional properties of such matrices in CECs depend not only on the porosity, size and degree of interconnectedness of the pores [13], but also on the type of cells seeded on them. For example, for chondrocytes [14] and osteoblasts [15], the best results in adhesion and migration for the inner surface of the hydrogel matrix were achieved in the presence of open 250–325 μm pores, and for fibroblasts – with open pores of no more than 100–160 μm [16, 17].

One of the technological approaches to the formation of 3D carriers in the form of sponges is cryogenic structuring of polymer systems [18–21]. When the formation of covalent or non-covalent knots of three-dimensional mesh occurs in a frozen sample, this process itself is called cryotropic gelation, and the resulting polymeric objects are called cryogels; if there is no gelation, the final products (usually after removal of the frozen solvent) are polymeric objects called cryostructurates [22]. Macroporosity of both cryogels and cryostructurates is their characteristic morphological feature; it is formed by polycrystals of the frozen solvent acting as a porogen [23].

The objective of this work was to investigate the biological properties of a scaffold made of cryogenically structured hydrogel in the form of a macroporous gelatin sponge, as well as the possibility of creating CECs on its basis.

MATERIALS AND METHODS

Obtaining a gelatin-based cryogenically structured hydrogel

Gelatin (type A) obtained from porcine skin collagen, EDC (all from Sigma-Aldrich Inc., USA), urea (high purity) and 96% ethanol (Reachem, Russia) were used without additional purification to obtain macroporous gelatin sponges [24].

Dry gelatin was dispersed in a calculated deionized water volume and then dissolved with stirring at 60 °C. Urea was dissolved in a prepared 6% polymer solution to obtain a 1 mol/L concentration. This solution was then poured into 2 mL plastic Petri dishes (40 mm diameter), which were placed on a strictly horizontal metal plate in liquid cryostat chamber F-32 (Julabo, Germany) with a predetermined negative temperature of –20 °C. Samples were frozen and incubated for 18 hours and then freeze-dried using an ALPHA 1-2 LD plus freeze-drying machine (Martin Christ, Germany). Dry discs were washed with ethanol to dissolve and remove urea until urea was absent in the washing liquid, and then transferred to 0.05 M ethanol solution of EDC, where they were incubated with periodic stirring for 48 hours, and then discs were washed 3 times 30 minutes each with pure ethanol, under which layer obtained samples were stored at 4 °C.

Physicochemical properties and microstructure of cryogenically structured gelatin carrier

The degree of swelling in water of samples of macroporous gelatin matrices was determined by gravimetric method. For this purpose, free liquid was removed from the spongy sample swollen in water under a 145 g load on a glass filter under vacuum (water-jet pump) for 5 minutes. The resulting sample was weighed and then dried (air thermostat SNOL 24/200, AB Utenos Elektrotechnika, Lithuania) at 105 °C until constant weight was achieved.

The degree of swelling (S – swelling), which is an indicator of cross-linking density of 3D polymer mesh of the material, was calculated by the formula:

$$S = \frac{m_{wt} - m_{dr}}{m_{dr}} \text{ (g } H_2O\text{/g polymer)},$$

where m_{wt} is the mass of wet sample, and m_{dr} is the mass of dried sample.

The morphology of the surface and the nearest subsurface of the samples was studied by SEM using lanthanide staining. The processing protocol included an initial wash, exposure in BioREE-A contrasting solution (Glaucon LLC, Russia) for 45 minutes, and a final wash with distilled water. After that, excess moisture was removed from the sample surface using an air brush and placed on the slide of an EVO LS10 microscope (Zeiss, Germany). Observations were performed in low vacuum (EP, 70 Pa) at an accelerating voltage of 20–25 kV. The images were captured with a backscattered electron detector (BSE mode). The pore size of the cryostructured carrier was determined by measuring 90 randomly selected pores on SEM images using Image J software (National Institutes of Health, USA).

Cell cultures

Cultures of NIH3T3 (ATCC®CRL-1658™) EA.hy926 (ATCC®CRL-2922™) from the American Type Culture Collection (ATCC) were stored in liquid nitrogen at –196 °C before use. After thawing, NIH3T3 and EA.hy926 were seeded into 25 cm^2 standard culture vials (CELLSTAR® Greiner Bio-One, Germany) and cultured in appropriate complete cell culture medium DMEM with high glucose content (PanEco, Russia) supplemented with 10% bovine serum (CS, Biosera, Germany) or fetal bovine serum (HyClone, USA), antibiotic-antimycotic Anti-Anti (Gibco® by Life Technologies™, USA) and 2 mM alanyl-glutamine (PanEco, Russia), respectively, in a CO_2 incubator under standard conditions: 37 °C, in a humid atmosphere containing (5 ± 1)% CO_2.

A hAMSCs culture was obtained at Shumakov National Medical Research Center of Transplantology and Artificial Organs according to the previously developed technique [25]. The HepG2 cell culture was taken from the collection of cell cultures of Shumakov National Medical Research Center of Transplantology and Artificial Organs. Prior to use, hAMSCs and HepG2 were stored in liquid nitrogen at –196 °C. After thawing, hAMSCs and HepG2 were seeded into standard 25 cm^2 culture vials (CELLSTAR® Greiner Bio-One, Germany) and cultured in complete cell culture medium DMEM/F12 (PanEco, Russia) supplemented with 10% fetal bovine serum (HyClone, USA), 10 μg/mL human basic fibroblast growth factor (FGF-2, Peprotech, AF-100-18B, USA), antibiotic-antimycotic Anti-Anti (Gibco® by Life Technologies™, USA), 1 mM HEPES (Gibco® by Life Technologies™, SC) and 2 mM alanyl-glutamine (PanEco, Russia) in a CO_2 incubator under standard conditions: 37 °C, in a humid atmosphere containing (5 ± 1) % CO_2; hAMSCs (passages 5–6) were used in the experiments.

Before the experiment, cells were removed from the surface of the culture plate using dissociation reagent TrypLE™ Express Enzyme (Gibco® by Life Technologies™, UK) and a suspension with the required cell concentration was prepared.

The initial number of cells in the suspension was determined on an automated cell counter (TC20™ Automated Cell Counter, BIORAD, Singapore) with simultaneous viability analysis by trypan blue dye exclusion (BIORAD, # 145-0013, Singapore).

Medium cytotoxicity assessment

To determine the cytotoxicity of gelatin sponge samples, NIH3T3 mouse fibroblasts line were seeded into flat-bottomed 6-well culture plates (CELLSTAR® Greiner Bio-One, Germany) at a concentration of 5 × 10^5 cells per well and incubated for 24 hours at 37 °C in a humid atmosphere containing (5 ± 1)% CO_2 until a (80 ± 10) % monolayer was formed. Then gelatin sponge samples were placed on the surface of the cell monolayer in the form of disks 6 mm in diameter and 2 mm thick, thoroughly washed from ethanol residues with two portions of sterile distilled water and left for 24 hours in complete cell culture medium (CCCM) at 37 °C. The CCCM served as the negative control sample, while single-element aqueous zinc standard 10 mg/mL (Sigma-Aldrich, USA) served as a positive control sample.

For a more detailed assessment of growth dynamics, additional plates in which cells were incubated from the moment of introduction in the presence of sponge samples using IncuCyte ZOOM system (EssenBioscience, USA), which makes it possible to automatically estimate the monolayer density in automatic mode every 2 hours throughout the experiment with simultaneous construction of growth curves. The experiment lasted for 90 hours.

Assessment of cell adhesion and proliferation support

For a comparative study of the influence of hydrogel matrices on hAMSCs growth parameters, we used gelatin sponge samples (cylinders 6 mm in diameter and 2 mm thick) and biopolymer-based microheterogeneous collagen-containing hydrogel (BMCH, BIOMIR Service JSC, Russia) (0.2 mL) with the following characteristics: average microparticle size 145.79 ± 0.09 μm; elastic modulus 1170 ± 12 Pa; viscosity modulus 62.9 ± 7.9 Pa; resorption time – up to 9 months. BMCH has been shown to be effective as a matrix for creating various medical and biological products [1, 26].

To assess the ability of the test samples to support adhesion and proliferation of hAMSCs cultures, 1 mL of cell suspension with a 1 × 10^5 cells/ml concentration was dropwise applied to the surface of the sample presaturated with CCCM for 24 hours at 37 °C. The samples were placed in 50 mL centrifuge tubes and left in a CO_2 incubator for cell attachment for 1 hour, after which the level of CCCM in the tubes was brought to 5 mL and cultivation was continued under standard conditions. Test tube lids were loosely closed to maintain gas exchange. On days 1, 3, 6, 9, and 14, three portions of CCCM were taken for the metabolic activity test with PrestoBlue™ HS Cell Viability Reagent (Invitrogen™ by Thermo Fisher Scientific, USA) according to the protocol recommended by the manufacturer. Spectrophotometric analysis was performed using a Spark 10 M microplate reader (Tecan, Austria) with Spark Control™ Magellan V1.2.20 software at 570 nm and 600 nm wavelengths. Optical absorbance measurements were used to calculate the metabolic activity coefficient (K) using the formula:

$$K = \frac{117.216 \times Abs_{570} - 80.586 \times Abs_{600}}{155.677 \times Abs_{600} - 14.652 \times Abs_{570}} \times 100\%,$$

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

The number of cells corresponding to the value of the obtained coefficient K was determined from the calibration graph, which was plotted using the values of metabolic activity coefficients corresponding to the known numbers of cells.

CECs based on cryogenically structured gelatin matrix and different cell types

To create CECs based on macroporous gelatin sponges and hAMSCs, HepG2 or EA.hy926 cells, suspensions of corresponding cultures with a 1 × 10^6 kl/ml concentration were prepared. Sponge samples in the form of discs, 1 cm^2 in area and 2 mm thick, were immersed in the suspension and processed for 1 hour using a laboratory

shaker in orbital stirring mode at 40 rpm to improve cell penetration deep into the spongy structure of the sample. The resulting CECs were cultured under standard conditions for 9, 15, and 28 days using HepG2, EA.hy926, and hAMSCs, respectively.

The pattern of cell distribution over the sample volume, viability, morphology, and proliferative activity were assessed by in vivo microscopy with fluorescent dyes Live/Dead® Viability/Cytotoxicity Kit (Molecular Probes® by Life Technologies™, USA) according to the protocol recommended by the manufacturer.

Functional properties of HepG2 cells when cultured on macroporous gelatin sponge

HepG2 cells (5 × 105 kl) were plated on a 10 × 10 × 2 mm fragment of gelatin sponge. The resulting CECs were cultured in CCCM under standard conditions for 15 days. On day 15, albumin content in the culture medium was determined by enzyme immunoassay using Human Albumin ELISA Kit (Invitrogen™ by Thermo Fisher Scientific, USA). As a control, we used culture medium from cells that were cultured on plastic in the same quantity.

Ammonia metabolism rate was determined after 90 minutes of incubation with 1 mM ammonium chloride (Sigma-Aldrich, USA) diluted in culture medium on day 15 of the experiment. The amount of urea in the medium was estimated on a KonelabPrime 60i biochemical analyzer (ThermoFisher Scientific, Finland).

Significance of differences was determined by Student's t-test (standard software package Microsoft Excel 2007). Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Fig. 1, a shows the appearance of a sample of gelatin-based cryogenically structured hydrogel in the form of a macroporous sponge. Scanning electron microscopy allowed us to distinguish three types of pores on the surface of the carrier: large (109 ± 17 μm), medium (39 ± 10 μm), and small (16 ± 6 μm) (Fig. 1, b).

Note that large pores are able to ensure migration of cells into the sponge thickness, while the importance of medium- and small-sized pores lies in supporting the efficient mass transfer of nutrients and gases.

Cytotoxicity of cryogenically structured gelatin matrix

Assessment of the cytotoxicity of the gelatin sponges obtained in this work by direct contact revealed no negative effect on the development of NIH3T3 cells. No changes in cell morphology or a decrease in cell proliferation were found both during the first hours and after 72 hours of cell-carrier contact.

The obtained data confirm the cell growth curves when cultured on the culture plate in the presence of gelatin sponge, demonstrating an increase in confluence of cell monolayer in all variants of the experiment with the dynamics characteristic of this cell culture (Fig. 2). Note that to correctly compare the data of two curves shown in Fig. 2, we need to introduce a correction factor of 1.12 for the experimental variant that takes into account the well area (9.6 cm^2) occupied by the sample (1 cm^2) and excluded in the automatic analysis of images. Taking into account the correction, there were no significant differences in the confluence of the cell monolayer on the plateau in the experiment (the confluence of the monolayer was 94 ± 5% without sample and 85 ± 6% with sample).

The ability of the cryogenically structured gelatin sponge to support cell adhesion and proliferation, confirmed on NIH3T3 cells, allowed us to proceed to creation of CEC – cultivation of human cells (hAMSCs, EA.hy926 and HepG2) on the cryogenically structured gelatin sponge.

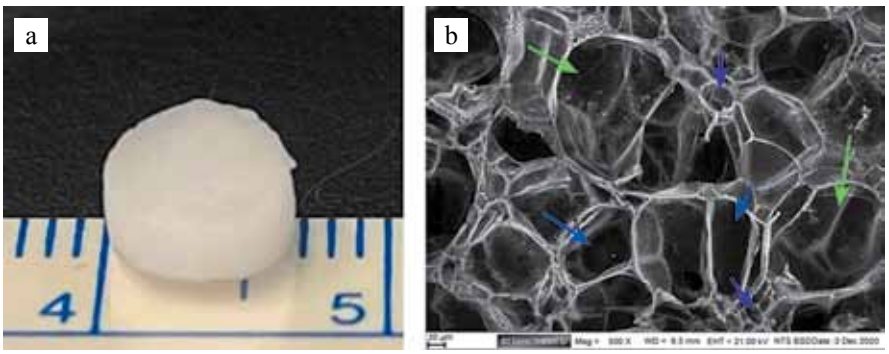

Fig. 1. Gelatin-based macroporous sponge morphology. a, View of the carrier; b, Microphotograph of the surface structure. SEM using BioREE lanthanoid contrasting. Scale bar 20 μm. Green arrows, large pores; blue arrows, medium-sized pores; purple arrows, small pores

Metabolic activity of hAMSCs during culturing

Mesenchymal stem cells (MSCs) perform various biological functions, which determines their relevance in tissue engineering. Firstly, MSCs can differentiate into various directions, including chondrogenic, osteogenic, adipogenic, myogenic and neurogenic differentiations [27]. Secondly, MSCs secretion has a positive effect on the therapy of various diseases [28].

The number of hAMSCs for the study of metabolic activity when cultured on a cryogenically structured gelatin sponge and BMCH was 100,000 cells/mL. The growth curves show that cell adhesion on the surface is only 20–25% of the applied amount (Fig. 3). In the case of the gelatin matrix, a lag phase was observed, which was necessary for cell adaptation and plating, after which active proliferation began after 3 days, and by day 6 of the experiment, the number of proliferating cells had increased 4–5-fold to 100,000 cells. Then, after a slight plateau up to 9 days, there was further logarithmic growth of the cell population up to the end of the experiment – 14 days. The absence of an increase in proliferative activity during 6–9 days on the cryogenically structured gelatin matrix and continuation of the logarithmic growth of the population afterwards, apparently, are related to cell colonization of the most accessible macropore surface and cell migration into the sample volume. When cultured with BMCH, cell adaptation is much faster, as evidenced by the absence of a pronounced lag phase. Intensive proliferation is observed almost from the beginning of the experiment and already by day 3, the number of cells is twice as high as that of gelatin sponge. Also, more rapid and active cell growth was observed, a plateau being reached by day 9. The maximum cell number per sample reached about 280,000 cells for BMCH on day 9 and about 220,000 cells for the cryogenically structured gelatin hydrogel on day 14 of the experiment. The decrease in the number of cells by day 14 of cultivation with BMCH as compared to day 9 indicates the onset of the cell death phase, probably related to the lack of a free carrier surface for cell colonization. In general, hAMSCs, when cultured on the investigated hydrogels, showed growth dynamics typical of this cell type on the culture plate [29].

Thus, a more intensive proliferative activity of hAMSCs is observed when they are cultured on BMCH, while cell mass growth is slower in the presence of a gelatin matrix. However, at day 14, the number of hAMSCs with metabolic activity is higher in the case of gelatin sponge than for BMCH.

Cultivation of cells of different types on a cryogenically structured gelatin sponge

It was shown that mesenchymal, epithelial and endothelial cells exhibit a high level of adhesion to the matrix surface, actively proliferate and repopulate the carrier surface when applied in an amount of 500,000 per 1 cm^2. The use of "stacking", i.e., shifting the focus point of the microscope objective deep into the sample at a depth of about 100 μm, followed by software image processing, showed that the cells spread into the internal volume of the sponge as well.

The most prolonged cell growth was observed in the case of culturing hAMSCs on a gelatin sponge (Fig. 4), and by day 28, dense 3D structures with a high cell density were formed in the matrix volume.

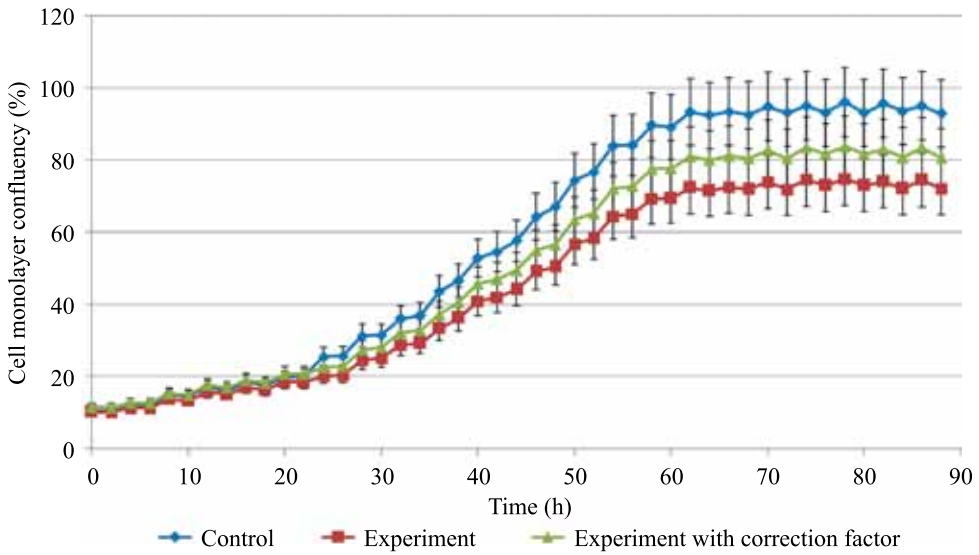

Fig. 2. Growth curve of NIH3T3 on culture plate in the presence of cryogenically structured gelatin sponge (experiment) and without cryogenically structured gelatin sponge (control)

As seen in Fig. 4, b, ethidium homodimer-1, in addition to dead cells, stained the matrix red, allowing the pore walls to be visualized.

HepG2 cells, which are usually used as an in vitro model of hepatocytes, also actively proliferated on the matrix (Fig. 5).

On day 3 of the experiment, there was active cell proliferation and spread over the area of the carrier. On day 7, formation of cell clusters occurred in the samples, and by day 9, the surface of the gelatin sponge macropores was almost completely populated with cells (see Fig. 5).

One of the conditions for creation of tissue equivalents is matrix vascularization [30]. Immortalized cell lines, including EA.hy926 line, demonstrating similarity to primary endothelial cells, are widely used to create models of capillary system in tissue-engineered constructs in vitro [31]. When EA.hy926 was cultured on a macroporous gelatin matrix, it was rapidly and uniformly populated with cells (Fig. 6).

By day 15 of cultivation, dense cell structures were formed on the surface with cells sprouting into the sponge volume. At the same time, the proportion of living cells prevailed over dead ones.

Assessment of the functional properties of HepG2 when cultured on a cryogenically structured gelatin carrier

The presence of functional properties of the created CEC were analyzed by albumin synthesis and urea production. The Table shows the results of the assessment

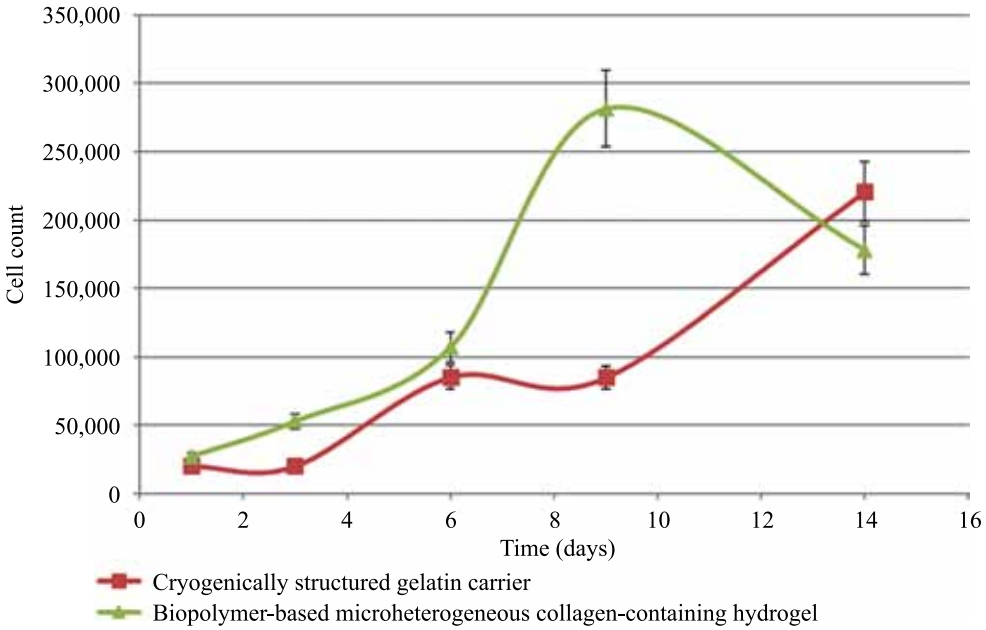

Fig. 3. Growth curves of hAMSCs in a cryogenically structured gelatin sponge and a collagen-containing biopolymer-based hydrogel

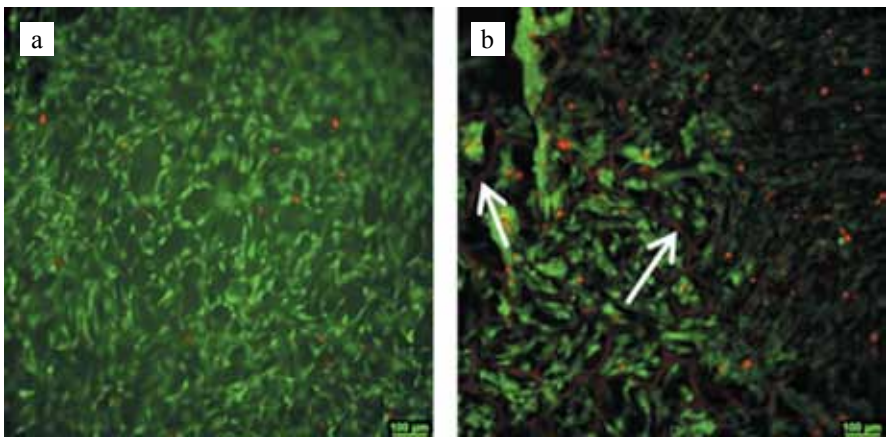

Fig. 4. Growth of hAMSCs in a cryogenically structured gelatin sponge: a, 9 days in culture; b, 28 days. Live/Dead™ staining, live cells are stained green, dead cells are stained red. Arrows show the walls of the sponge pores. Scale bar 100 μm

of albumin synthesis by HepG2 cells in suspension and in the CEC.

The data obtained indicate that HepG2 seeded on a gelatin sponge can maintain its secretory function and ammonia metabolism at 15 days of cultivation at a higher level than as a cell suspension.

CONCLUSION

The biological properties of a gelatin-based cryogenically structured hydrogel as a resorbable macroporous sponge were studied.

The absence of cytotoxicity and the presence of functional properties of the samples in vitro were proved on NIH3T3, hAMSCs, EA.hy926 and HepG2 cultures. Using the example of a liver CEC, creation of tissue-engineered products using a matrix of macroporous gelatin-based cryogenically structured hydrogel were shown to have some prospects.

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The authors declare no conflict of interest.

Table

Albumin content and urea level in the culture medium samples on day 15 of HepG2 culturing in a suspension (control) and in cryogenically structured gelatin sponge (experiment)

	Albumin, mmol/mL	Urea, mmol/L
Cultivation in suspension	997 ± 139	1.1 ± 0.1
Cultivation in a cryogenically structured gelatin sponge	1560 ± 312	1.8 ± 0.4

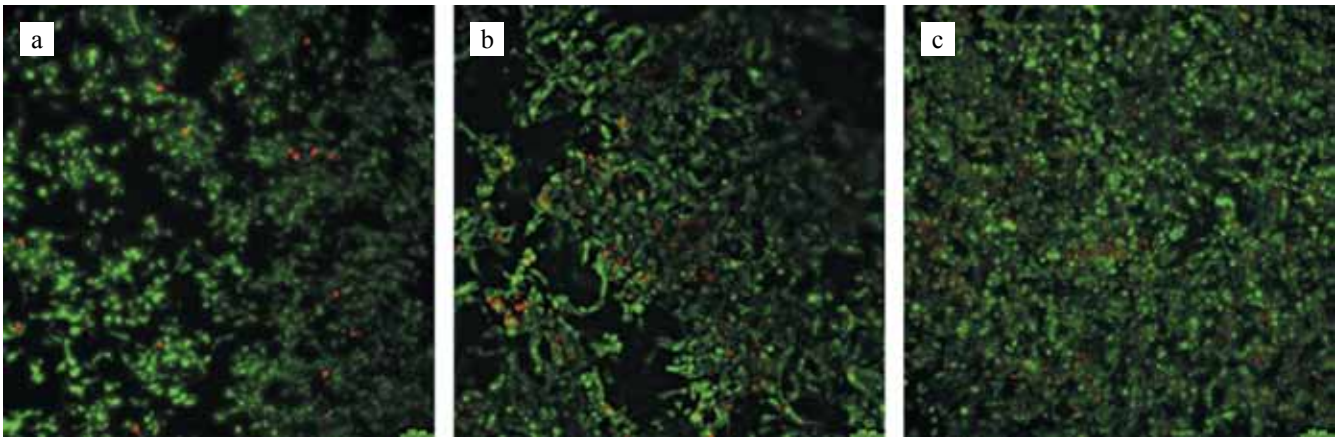

Fig. 5. Growth of HepG2 in a cryogenically structured gelatin sponge: a, 3 days in culture; b, 7 days; C, 9 days. Live/Dead™ staining, live cells are stained green, dead cells are stained red. Scale bar 100 μm

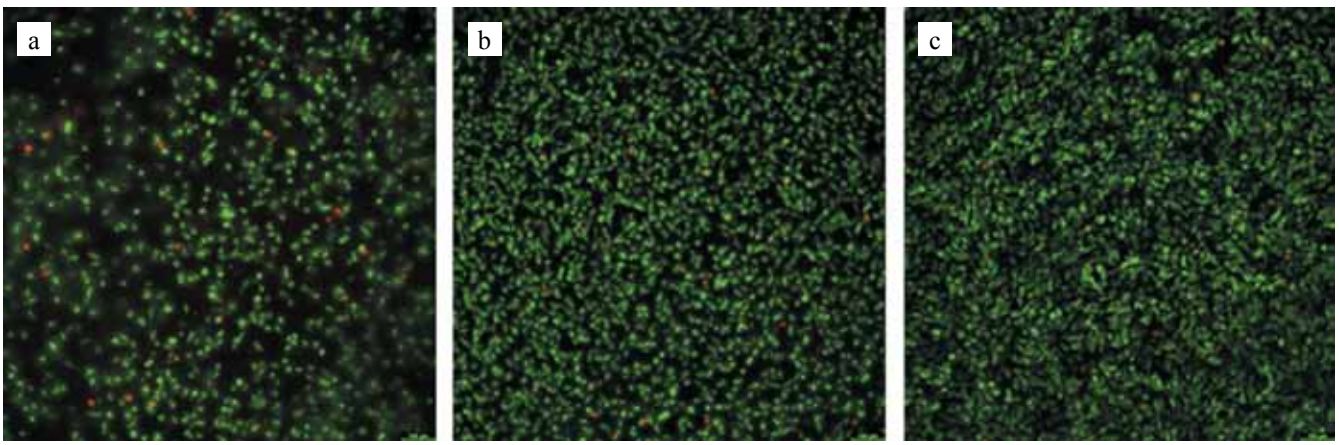

Fig. 6. Growth of EA.hy926 in a cryogenically structured gelatin sponge. a, 2 days in culture; b, 7 days; c, 15 days. Live/Dead™ staining, live cells are stained green, dead cells are stained red. Scale bar 100 μm

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NORMOTHERMIC *EX VIVO* PERFUSION OF ISOLATED LUNGS IN AN EXPERIMENT USING A RUSSIAN-MADE PERFUSION SYSTEM

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According to global health statistics, respiratory diseases, together with infectious complications and hereditary lung diseases, rank as the third leading cause of death. Today, lung transplantation (LTx) is a well-recognized modality of treatment for end-stage chronic lung disease. However, the number of LTx surgeries performed is much lower than other solid organs. This is due to the high requirements for the potential donor and characteristics of the lung graft, reflecting the efficiency of gas exchange function. Non-compliance with the selection criteria leads to deselection of donors, which, according to various estimates, occurs in 80–85% of cases. One of the ways to increase the number of lung transplant surgeries is to restore them to the level of optimal gas exchange parameters, which can be achieved and objectively assessed during normothermic ex vivo lung perfusion (EVLP). EVLP is becoming increasingly common at leading transplantation centers in Europe and North America. This has significantly increased the number of transplant surgeries as a result of using lungs procured from suboptimal donors and rehabilitated via EVLP. In our pilot study, the developed Russian-made mechanical circulatory support system showed that performing normothermic EVLP for isolated lungs under experimental conditions is feasible. Basic and optimized perfusion protocols have fully shown that they are reliable and efficient.

Keywords: lung transplantation, donation, ex-vivo perfusion.

INTRODUCTION

Respiratory diseases, including those with complications, as well as hereditary lung diseases, are a socially significant problem all over the world as they lead to end-stage pulmonary disease [1]. Lung transplantation (LTx) is currently the most effective radical way of treating patients with severe respiratory disease resulting from lung diseases of various etiologies [1–3]. However, the use of LTx is limited by the extremely low percentage of donor organs suitable for transplantation [4]. Donor lungs are extremely susceptible to complications of brain death. It is accompanied by a high incidence of non-specific changes, such as neurogenic edema, which makes lungs unsuitable for transplantation and, as a consequence, leads to deselection of organs from LTx [5]. A combination of factors affecting the quality of a lung graft decreases the number of suitable donor lungs to 15–20%, while liver and kidney transplants, on average, are used in 69% and 90% of cases, respectively [3, 6, 7, 8]. The shortage of effective lung donors increases the 1- and 2-year waitlist mortality rates by 20–40% [9].

One of the ways to solve the problem of donor organ shortage is the use of expanded criteria donors (ECDs) and the use of ex-vivo graft perfusion in order to restore the functional activity (rehabilitation) of the donor organ [8].

The relatively recent beginning of application of the above method in world practice, as well as the limited number of studies, make urgent and necessary the development of this direction in national programs in order to improve the effectiveness of transplantation care. This necessitates experimental work on the development and improvement of the ex vivo lung perfusion (EVLP) technique.

The objective of this study is to perform and evaluate the effectiveness of a pilot isolated closed-circuit ex vivo perfusion using a proprietary extracorporeal perfusion complex under experimental conditions.

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MATERIALS AND METHODS

Isolated lungs obtained from a 45 kg Romanov sheep were used in the experimental study. The experimental work program was approved by the Biosafety and Bioethics Committee. The work was carried out in compliance with the rules of the European Convention for the treatment of laboratory animals and the 2010/63/EU Directive [14, 15].

The experiment included stages of anesthesia of the experimental animal, lung explantation, static hypothermic storage, initiation of ex vivo lung perfusion with fixation of main parameters.

Donor anesthesia stage

On the day of the experiment, 60 minutes before surgery, the animals were sedated in the pen with a zolazepam solution at 15 mg/kg dose. During sedation, the animal was taken to the operating room, the operating field was shaved, which corresponded to the anatomical landmarks between the cricoid on the neck of the experimental animal and the anterior projection of the Tuffier's line, and vascular access points were determined. The external ear vein was catheterized with a 20 G catheter.

The animal was positioned on the operating table in the supine position. Under aseptic conditions, a 7 Fr double-lumen central venous catheter was placed into the left external jugular vein at the level of the cricoid.

Then, under aseptic conditions, the common carotid artery was catheterized with a 5 Fr catheter for invasive blood pressure (BP) monitoring. Monitoring was done via the Efficia CM Philips™ monitoring system.

Premedication was performed under invasive monitoring of BP, central venous pressure (CVP) and ECG: lornoxicam 8 mg, metoclopramide 10 mg, chloropyramine 20 mg. Injection anesthesia included intravenous atropine 1 mg, methylprednisolone 500 mg, and zolazepam 10 mg/kg.

Tracheal intubation was performed by direct laryngoscopy. Mechanical ventilation was performed using an anaesthetic machine in volume control mode at 8–10 mL/kg, peak inspiratory pressure did not exceed 25 cmH_2O, positive end-expiratory pressure (PEEP) did not exceed 5 cmH_2O, respiratory rate (RR) was 25 breaths per minute. Anaesthetic depth was controlled using an isoflurane vaporizer.

Optimal anesthesia for explantation surgery was achieved at 2.52.5 vaporizer level – 3% vol. Optimal hemodynamic parameters were: BP 110/80 mmHg, SpO_2 99–100, heart rate 90 bpm.

Donor lung procurement procedure

Surgical access was achieved through median sternotomy. The pericardium was opened longitudinally, and the aorta and pulmonary artery were divided bluntly. After administering sodium heparin at 300 units/kg dose, the aorta and the pulmonary artery were sutured. The aorta was cannulated with a 7 Fr catheter to collect donor blood. The pulmonary artery was cannulated with a 20 Fr straight cannula. The first step was autologous blood harvesting in a hemocontainer with citrate preservative. After blood harvesting was completed, prostaglandin E1 solution (alprostadil) 20 μg was injected into the pulmonary artery. In order to decompress the left heart, the left auricle was incised longitudinally. Then Celsior™ solution (Ganzyme, France) was injected through the pulmonary artery at 4 °C temperature in 2 liters. Upon completion of the perfusion of the preservative solution, we proceeded to lung explantation. For the convenience of procurement, the lungs were taken together with the heart as a single complex, and a machine suture was applied to the trachea 5–6 cm from the bifurcation. On completion of the explantation, the lungs were placed in a sterile bag, followed by static hypothermic preservation in a thermocontainer for 2 hours.

Assembly of the EVLP perfusion circuit

In the experiment, the perfusion circuit had a closed structure. The circuit consisted of a cardiotomy reservoir and membrane oxygenator. A hydrocirculatory heat exchanger element, a deoxygenating mixture consisting of N_2 86%, CO_2 8%, O_2 6%, and an oxygen-air mixture were connected to the oxygenator. A centrifugal blood pump "Biosoft-M LLC" with a hydrophilic head was installed in the trunk system between the cardiotomy reservoir and oxygenator. The line after the oxygenator was connected to a cannula installed in the pulmonary artery. Outflow into the oxygenator was performed actively through a cannula placed in the left atrium. The pressure in the trunk system was measured by placing three invasive sensors: the first was placed after the oxygenator to measure pressure in the proximal perfusion circuit, the second was placed directly in the pulmonary artery cannula to measure perfusion pressure in the pulmonary artery, the third sensor measured pressure in the cannula placed in the left atrium. The graft was positioned in a sterile container communicating with the cardiotomy reservoir. The graft was perfused through the pulmonary artery; perfusate was drained actively through a cannula inserted into the left atrium through the left ventricular wall. The schematic design of the circuit is shown in Fig. 1.

We used our own perfusion solution based on human albumin as perfusate. The perfusate was 1.5 liters in volume. Erythrocyte mass was prepared by centrifugation of whole deleukocyted blood for 15 minutes at 3,500 rpm. Meropenem 1000 mg, methylprednisolone 1000 mg, short-acting insulin 4 U, 40% glucose solution 5 mL were all added to the perfusate. Target hematocrit level was 15%.

The experimental protocol was developed on the basis of the ex vivo lung perfusion protocol proposed by M. Cypel et al. (Toronto, Canada) in 2009 [20]. Ex vivo perfusion lasted for 360 minutes.

EVLP initiation

The initial perfusion temperature was 20 °C, the target pulmonary artery pressure should not exceed 15 mmHg. The perfusion rate was adjusted based on pulmonary artery and left atrial pressures at the start of perfusion and was 150–200 ml/min. Left atrial pressure was regulated by the cardiotomy reservoir positioning height, the optimal range was 3–5 mm Hg. Gas-air mixture flow, where FiO_2 <0.5, was set corresponding to the target minimum pO_2 values >100 mmHg. A deoxygenating mixture with a 1 : 1 flow rate to the perfusion rate was required to achieve a pCO_2 of 40 to 50 mmHg.

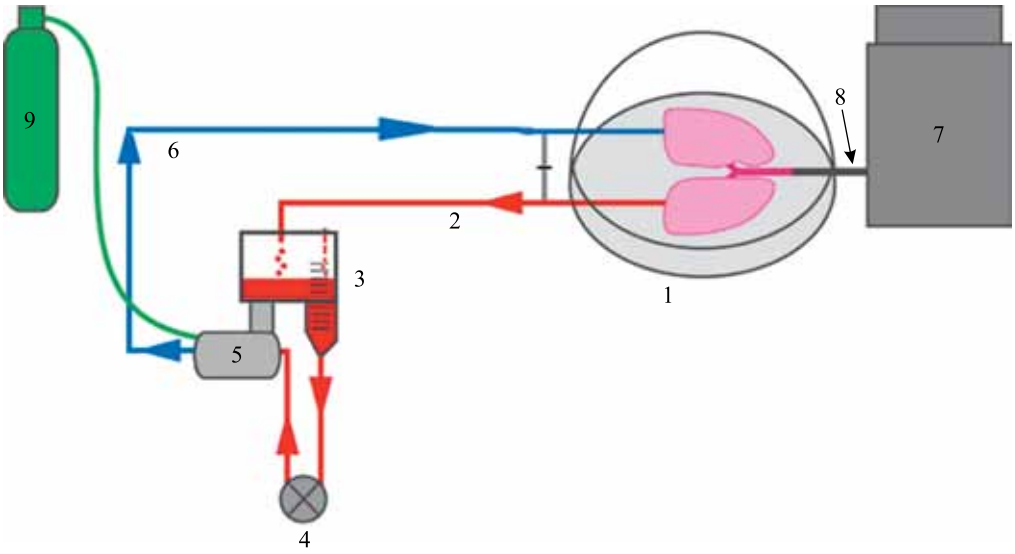

Fig. 1. Schematic layout of perfusion circuit. 1, Open organ chamber; 2, left atrial line; 3, cardiotomy reservoir; 4, centrifugal pump; 5, oxygenator; 6, venous line to the pulmonary artery; 7, ventilator; 8, air line; 9, balloon with deoxygenating mixture connected to oxygenator

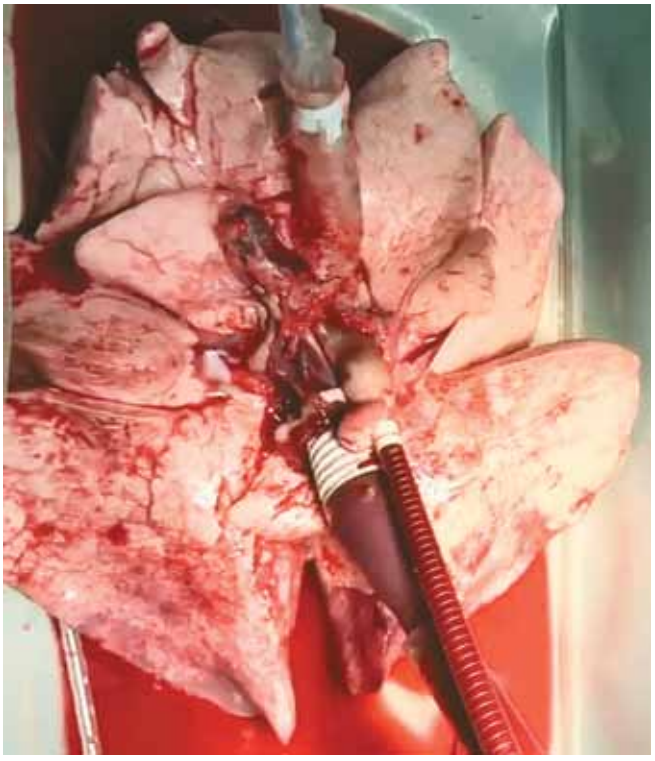

Fig. 2. View of the lung graft during perfusion

The ionic and gas composition of the perfusion solution was monitored using an ABL 800 gas analyzer. Target perfusion volume was 40% of the calculated cardiac output. When all parameters were stabilized, perfusion rate was increased to 800 mL/min for 20 minutes, the perfusate was warmed to 32 °C.

Upon reaching the target temperature of 34 °C, mechanical ventilation was initiated. The ventilation parameters were composed of an inspiratory volume of 7 mL/kg, PEEP 5 cmH_2O, RR 10 breaths per minute. Fraction of inspired oxygen was FiO_2 <0.5. The gas composition of the perfusate was monitored. Over the next 20 minutes, the target temperature of 37 °C was reached, the volumetric perfusion rate increased by 40% of cardiac output (based on 70 ml/kg of experimental animal weight) to 1200 mL/min. Perfusion lasted for 360 minutes. General view of the lung graft at the time of perfusion is shown in Fig. 2.

Assessment of graft function during EVLP procedure

After warming the graft to 37 °C and stabilizing the gas and ionic composition parameters of the perfus-

ate, with an oxygen fraction at 50% inspiration (FiO_2 = 0.50), we performed instrumental, manual and laboratory evaluations. The surgeon palpated and visually assessed the homogeneity of lung parenchyma and the absence of infiltrative changes in it, and atelectasis was resolved. Throughout the entire period of perfusion, pulmonary artery and left atrium pressures were measured directly. The data were displayed on the monitor in real time and recorded every 30 minutes. The main parameters were pulmonary artery pressure (PAP, mm Hg) and pulmonary vascular resistance (PVR, Wood units/m^2), which was calculated using the formula.

$$PVR = \frac{PAP - LAP}{PaF},$$

were PVR is pulmonary vascular resistance, PAP – pulmonary artery pressure (mmHg), LAP – left atrial pressure (mmHg), PaF – perfusion volume (L/min).

PVR was expressed in Wood units; to calculate in dyn·s/cm^5 units, the result of the equation was multiplied by 80.

In order to analyze pulmonary oxygenation function, two blood portions were taken from the venous cannula (pulmonary artery) and arterial cannula (left atrium). The samples were analyzed on an ABL 800 blood gas analyzer (Radiometer Medical ApS, Denmark). We used the PaO_2/FiO_2 equation (ratio of arterial oxygen partial pressure to fraction of inspired oxygen) to calculate oxygenation index. Obtained data were plotted against time points corresponding to the graft assessment periods.

Upon completion of perfusion, lung parenchyma fragments were fixed in 10% neutral buffered formalin solution (pH 7.4) for at least 24 hours. Isopropyl alcohol and petroleum ether were used to fix the material into paraffin blocks. 5 μm-thick paraffin sections were stained with hematoxylin and eosin. Microscopic analysis was performed using a light microscope with a 10× eyepiece lens and objective lenses 4, 10, 40, and 100. Photographs were taken with a digital camera.

The obtained sections were examined by a pathologist for vascular thrombosis, hemorrhages, interstitial and alveolar edema, and cellular infiltration.

RESULTS

The PaO_2/FiO_2 ratio prior to donor lung explantation was 240 mmHg. Throughout the entire ex vivo perfusion procedure, the respiratory index had positive growth dynamics. After 360 minutes of perfusion, the oxygenation index was 430 mm Hg, which is a good indicator of lung respiratory function recovery (Fig. 3).

Pulmonary vascular resistance

Throughout the ex vivo perfusion procedure, PVR witnessed a steady decrease. At the beginning, PVR was 800 dyn·s/cm^5; however, at the end of perfusion, PVR was 320 dyn·s/cm^5; the dynamics of PVR changes are shown in Fig. 4.

Dynamic compliance

Changes in the dynamic compliance index from 20 to 46 mL/cmH_2O at the end of perfusion indicates its adequacy and is an indirect criterion for the absence of pulmonary parenchymal edema. It is this index that objectively represents lung recruitability and reflects airiness and extensibility of pulmonary parenchyma. These changes in the parameters of dynamic compliance of the lung transplant are not an unambiguous criterion of donor organ's suitability, but they indicate preservation of lung parenchyma (Fig. 5).

Morphological study data

Histological examination of lung samples after perfusion showed structural integrity of the tissue and no signs of edema. In most sections, the alveoli were well inflated. The pulmonary alveolus and the peribronchovascular interstitium were slightly thickened (Fig. 6).

The lung fragments had a preserved structure. The lung parenchyma was not pathologically altered in all

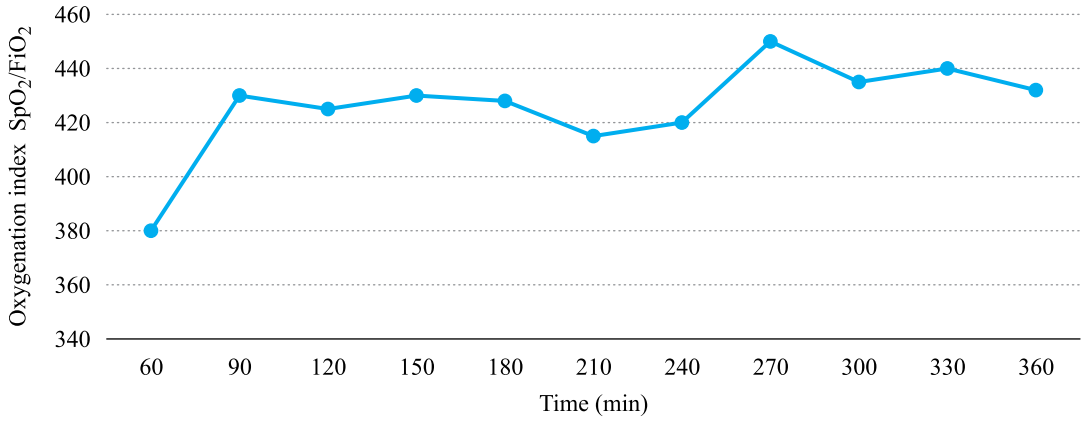

Fig. 3. Oxygenation index dynamics

groups of specimens; well-inflated alveoli were noted in most sections. Microatelectasis was heterogeneously distributed in both groups and occurred only in separate sections. The pulmonary alveolus and the peribronchovascular interstitium were slightly thickened.

DISCUSSION

Pathophysiological processes resulting from brain death cause intracorporeal organ damage, making it largely difficult to objectively assess the true functional

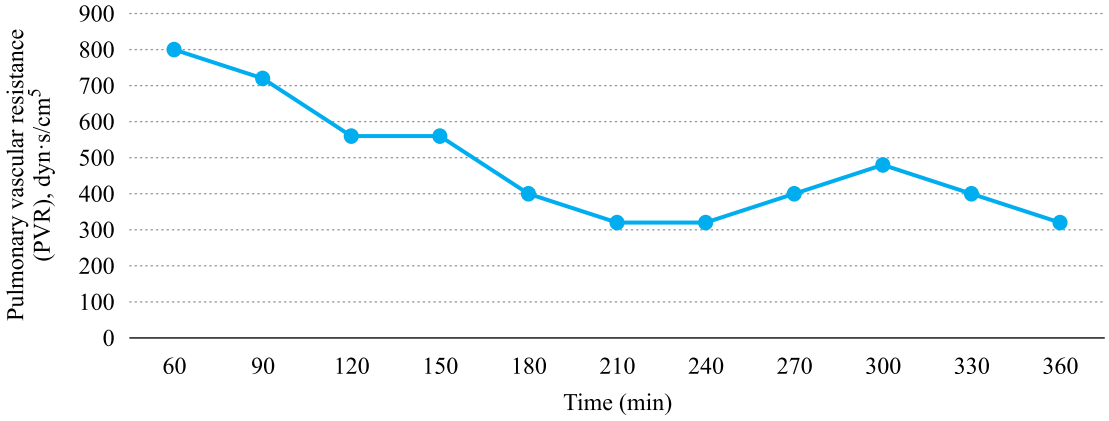

Fig. 4. Pulmonary vascular resistance dynamics

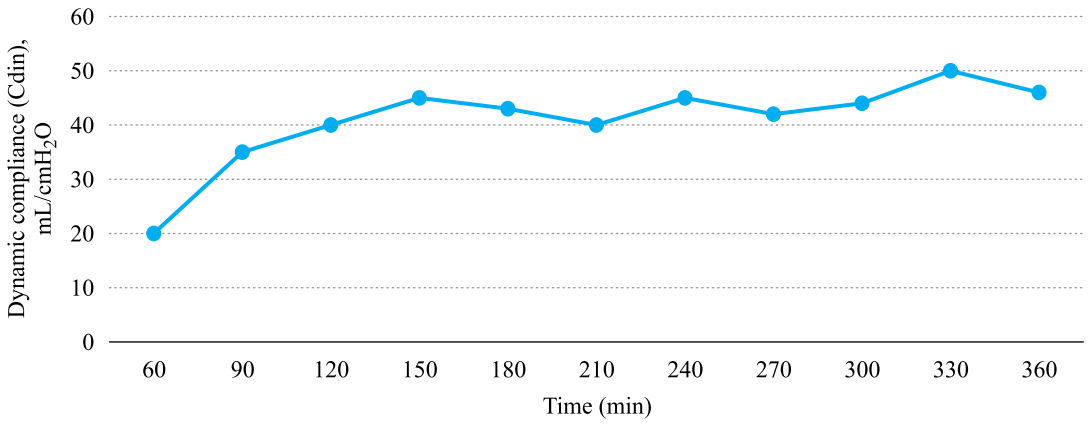

Fig. 5. Dynamic compliance dynamics

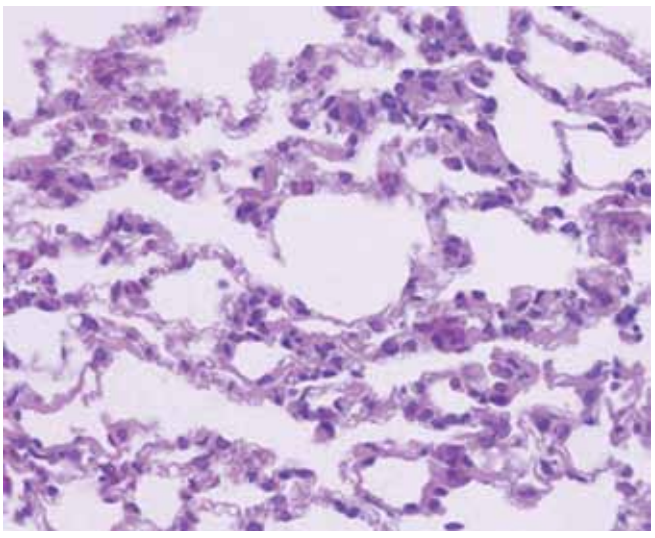

Fig. 6. Histological view of a lung specimen taken at the end of perfusion

state of donor lungs. Some reasons for organ deselection before transplantation (pulmonary edema, low gas exchange rates, presence of a large amount of purulent bronchial secretion, etc.) can be corrected and reassessed using extracorporeal lung perfusion techniques [15, 17, 18].

In order to restore and evaluate compromised lungs obtained from suboptimal donors, normothermic EVLP is used in clinical practice of the leading world transplant centers [15]. This technique allows mitigating the effects of damaging pathophysiological factors of the donor on the graft, rehabilitating and reassessing the lung graft [5, 16–20].

The emergence of EVLP has opened new horizons in lung transplantation worldwide. In 2006, a research team of Stig Steen et al. (Lund University Hospital, Sweden) reported the first results of successful transplantation

of one lung after EVLP procedure [23]. In 2009, Cypel et al. (Toronto, Canada) presented their own protocol, which later became the most widespread due to the best results and due to the possibility of a prolonged perfusion, lasting for more than 12 hours [6]. The EVLP procedure allowed for major expansion of the donor lung pool, thereby increasing the number of transplantations. The study was based on a perfusion protocol developed by Cypel et al. for lung evaluation and rehabilitation. The study was able to demonstrate the effectiveness of EVLP procedure on the first compact domestically manufactured circulatory assist device in an experiment on a ram model. The experimental conditions were as close to clinical practice as possible.

As of today, there are no registered normothermic organ perfusion devices in the Russian Federation. Developing a domestic perfusion equipment is a priority amid the current donor lung shortage. The blood circulation auxiliary device developed by Biosoft-M is small in size, has high functionality and ease of operation compared with its foreign counterparts. The closed-type perfusion protocol we have chosen is optimal and promising, allowing for prolonged perfusion of donor lungs. Prolonged perfusion gives a better chance of recovery of the function of expanded criteria donor lungs [6, 21]. According to the Toronto protocol, the time required to adequately assess the recovery of lung transplant function is from 4 to 12 hours [6, 22]. In the experiment, we were able to achieve a satisfactory oxygenation index, which was 430 mmHg at the end of perfusion, and PVR reduction, which indicates adequate perfusion. The absence of pulmonary parenchymal edema and pathological changes, according to histological study results, indicates the effectiveness and safety of the technique [19, 20].

CONCLUSION

Normothermic EVLP can be performed using a device developed for auxiliary blood circulation and adapted for normothermic EVLP purposes. The presented protocol was shown to be effective and has good prospects for further use and improvement.

The authors declare no conflict of interest.

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ASYSTOLE KIDNEY DONATION USING AUTOMATED CHEST COMPRESSION SYSTEM AND HYPOTHERMIC OXYGENATED MACHINE PERFUSION (FIRST EXPERIENCE IN THE RUSSIAN FEDERATION)

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Objective: to demonstrate, using a clinical case, the first successful experience in a combined use of an automated chest compression device (ACCD) and hypothermic oxygenated machine perfusion (HOPE) for kidney transplantation from a donor with irreversible cardiopulmonary arrest. **Materials and methods.** In the presented clinical case, ACCD was successfully used in a donor who was pronounced dead following an irreversible cardiopulmonary arrest. This allowed to minimize the primary warm ischemia time. Kidney graft HOPE for 585 minutes reduced the static cold storage time to 165 minutes. **Results.** In the uneventful postoperative period, there was immediate kidney graft function. This allowed for rapid rehabilitation and discharge from hospital. **Conclusion.** Introduction of ACCD and HOPE will increase the number of donor organs, mainly kidneys intended for transplantation.

Keywords: kidney transplantation, automated chest compression device, organ donation, hypothermic oxygenated machine perfusion.

INTRODUCTION

Donation after circulatory death (DCD) is a well-established practice in many countries. Currently, 18 of 35 countries in Europe use DCD donors, of which 8 use only DCD donors after sudden cardiac arrest, the so-called uncontrolled asystole donors [1]. At the same time, the potential use of such donors is huge. For example, it has been estimated that the use of DCD donors after cardiac arrest in the U.S. can attract up to 22,000 additional potential organs per year [2]. A significant increase in cases of primary nonfunctioning grafts and grafts with delayed function is the reason for limited use of organs from DCD donors [3–5]. Moreover, the success of kidney grafts donated after circulatory death depends not only on the technical execution and perioperative management of the recipient, but also on the effective logistics of interaction between donor centers and kidney transplant centers [6]. Modern logistics of DCD is quite complicated, requiring high organization of the donor process, use of additional modern features – ACCDs, hypo- or normothermic perfusion of organs in situ, as well as machine perfusion of donor organs at the final stage [7]. The mentioned limitations, logistical and technological difficulties lead to the fact that only 17 effective DCD donors were registered in the Russian Federation in 2020 [8].

The development of modern protocols for post-ischemic rehabilitation of kidney grafts using the latest technology will expand the pool of DCD donors with sudden cardiac arrest.

CLINICAL CASE

Effective donor: male, 43 years old, diagnosed with penetrating head injury, severe cerebral contusion, and acute subdural hematoma on the left frontoparietal temporal region with 150 cm^3 volume. Decompressive skull trepanation was carried out, the hematoma was removed on November 29, 2021. Alcohol intoxication. The length of in-hospital stay from the moment of admission to the date of death was 27 hours. In the postoperative period, there were hypotension and sinus tachycardia with 121 bpm heart rate, which required adequate volemic support and intravenous norepinephrine administration at 890 ng/kg/min dose. The patient's level of consciousness corresponded to 3 points on the Glasgow Coma Scale (GCS). However, there was positive tracheal reflex, which made it impossible to diagnose brain death. By the end of day 1 of stay at the hospital, the patient's hemodynamic indicators were getting worse – hypotension was increasing, thus, vasopressor support increased to 1700 ng/kg/min. The patient suffered a cardiac arrest on November 30, 2021, at 4 pm; ACCD resuscitation was initiated. The resuscitation lasted 30 minutes with-

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out any effect, subsequently he was pronounced dead. After death, the ACCD was continued to provide minimal perfusion of organs and tissues as an anti-ischemic protection. Mechanical ventilation with an FIO_2 of 100% was also initiated. Against the background of the ACCD, surgical access to the right femoral vessels was performed, the femoral artery and vein were isolated and taken on a holder. The femoral artery lumen was opened and a double-balloon triple-lumen catheter (DBTL catheter) with 22 Fr lumen diameter was inserted into the femoral vein; a 24 Fr venous cannula was inserted into the femoral vein lumen to drain the perfusate. Cold isolated perfusion of the abdominal organs with Custodiol preservative solution was initiated. At the start of cold perfusion, mechanical ventilation and automatic chest compression were terminated. Operation of the ACCD lasted for a total of 58 minutes, which included 30 minutes of resuscitation. The ACCD operation and appearance of the kidneys at the end of in situ perfusion are shown in Figs. 1 and 2, respectively.

Kidney graft: *The kidney graft was delivered in a transport container by a team from Moscow Organ Donation Center to Botkin Hospital at 7 pm on November 30, 2021. At 7:15 p.m., the graft was removed from the container, and perfusate was taken for microbiological examination. On examination, the left kidney graft was of medium size, homogeneous color, without tumor-like masses. The only renal artery extending from the aorta and the only renal vein were identified. The proximal renal artery was mobilized, no atherosclerotic plaques were detected at the orifice. The doctors decided to initiate HOPE. Centrifugal blood pump Maquet Jostra was prepared. The renal artery was cannulated (Fig. 3), HOPE was initiated at 7:45 pm (static cold storage time was 135 minutes).*

Renal graft temperature before perfusion was 8.9 °C, perfusion solution pressure in the renal artery was 40 mmHg, perfusion solution volume rate was 200 mL/min, and vascular resistance index was 0.2. HOPE time was 585 minutes. During this period, renal graft temperature did not increase above 6 °C (Fig. 4).

Perforating solution pressure during the procedure was maintained at 40 mmHg. Volumetric flow rate at the end of the procedure was 250 mL/min, vascular resistance index was 0.16. Partial pressure of oxygen in perfusion ranged from 337 to 591.

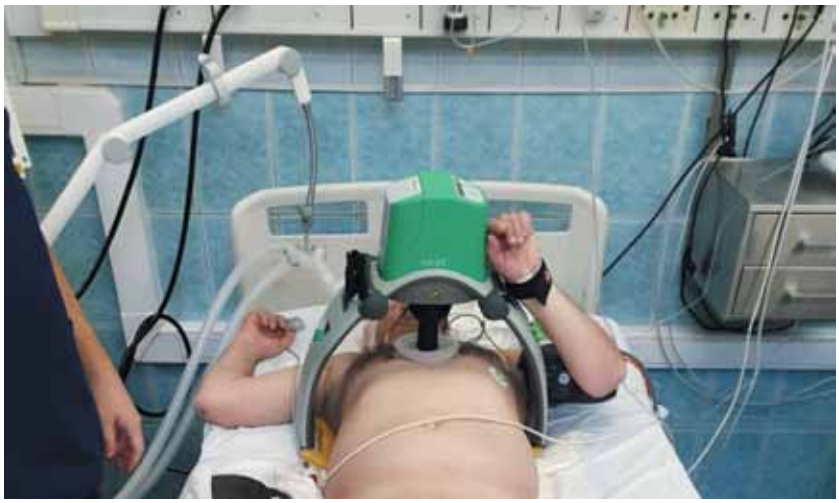

Fig. 1. ACCD operation after the patient had been declared dead

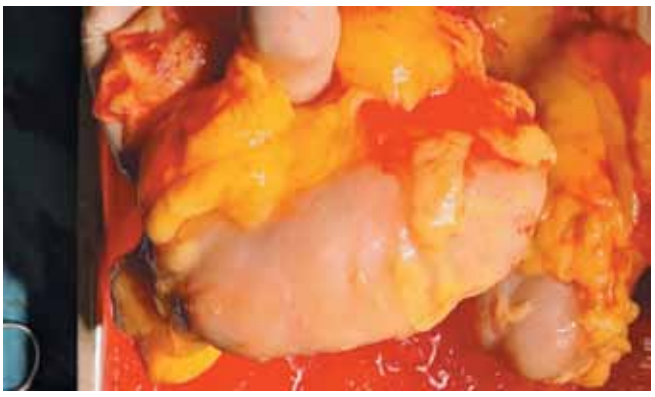

Fig. 2. Donor kidney after in situ cold perfusion

After the skin incision in the recipient has begun, machine perfusion was suspended. Pre-transplantation preparation of the renal graft was 30 minutes (total static cold storage time was 165 minutes).

Renal transplant recipient: *After cross-matching and donor typing, donor-recipient pairing was performed at 10 pm – recipient B, 41 years old, suffering from chronic kidney disease as a result of IgA nephropathy. He had been under long-term hemodialysis since June 2018 (A, B, Dr match). The patient had been waitlisted for 19 months. He was admitted at Botkin Hospital at 01:00 am. On December 01, 2021, during preoperative examination, he was diagnosed with high blood potas-*

sium (up to 6.9 mmol/l), which required preoperative hemodialysis for 3 hours.

The surgical intervention was initiated on December 1, 2021, at 5:30 am according to standard technique. After blood flow was initiated, the graft acquired physiological turgor, evenly turned pink, and urine flow through the ureter was noted (Fig. 5).

After suturing the anterior abdominal wall muscles, intraoperative ultrasound examination was performed, which revealed satisfactory arterial (resistance index 0.77) and venous blood flow (Fig. 6).

The postoperative period was uneventful. There was immediate kidney graft function. Creatinine levels normalised on postoperative day 6. After the immunosuppressive dose had been selected and the internal ureteral stent removed, the patient was discharged for outpatient follow-up.

DISCUSSION

Over the past 5 years, the number of effective donors per million population in Moscow has doubled (11.4 in 2016, 21.4 in 2020). This was mainly due to an increase in the number of brain-dead donors. Improvements in organ donation allowed to open new kidney transplantation centers in the metropolitan area [9].

Meanwhile, there was no active use of DCD during this period of time. This is due to the fact that prolonged primary warm ischemia of renal grafts obtained from donors after cardiac arrest and then prolonged static cold storage significantly worsen the immediate and long-term outcomes of kidney transplantation. Therefore, shortage of donor organs prompts researchers to search for a solution to minimize warm and cold ischemia time and widen their use in transplantation practice.

Modern automated chest compression systems have proven themselves well in pre-hospital and hospital phases as simple and reliable devices that can be used

Fig. 3. HOPE under the supervision of a transplant surgeon

to provide quality and long-term maintenance of sufficient blood supply to vital organs, allowing patients to be delivered to the hospital. This system has attracted the attention of many donor programs around the world, including the Moscow City Organ Donation Center because of its ability to maintain blood supply for a long time. The use of this system in DCD minimizes primary warm ischemia time of renal grafts, allows for cannulation of the great vessels and initiation of cold perfusion under technologically acceptable conditions.

After completion of in situ perfusion of the organ in the donor's body, the organ enters the stage of static cold

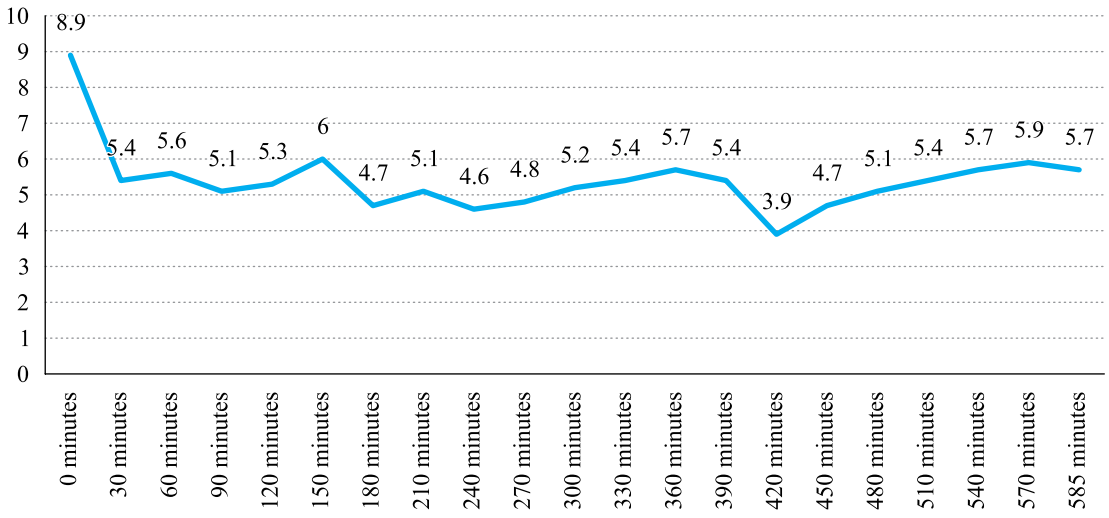

Fig. 4. Dynamics of renal graft temperature during HOPE

storage and remains in this state until it is incorporated into the recipient's bloodstream. During cold storage, the donor is subjected to virological and genetic examination, which can take 6 or more hours. Thus, the total static cold storage time is most often between 12 and 18 hours, and in some cases, it may exceed this. As is known, nearly 95% of cellular adenosine triphosphate (ATP) is depleted within 4 hours of static cold storage, and the latter switch to anaerobic metabolism [10]. This leads to accumulation of reactive oxygen species, development of intracellular acidosis, decrease in Na,K-ATPase, eventually causing apoptosis.

The introduction of HOPE – a technology that allows the delivery of oxygen required to maintain minimal metabolic processes over a long period of time – into wide clinical practice at Botkin Hospital may allow mitigating these negative aspects of the use of kidneys donated after circulatory death.

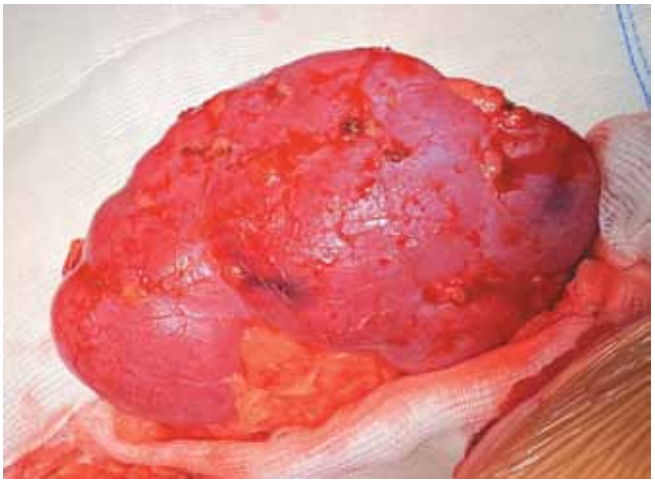

Fig. 5. Renal graft after reperfusion

Our first experience has demonstrated the positive aspects of the combined use of modern technologies to mitigate renal ischemia injury. ACCD allowed to reduce the negative effect of primary warm ischemia, while the use of HOPE reduced the static cold storage time to 165 minutes and facilitated post-ischemic rehabilitation. The immediate kidney graft function, uneventful postoperative period and early rehabilitation of the recipient convinced the staff at the Organ Donation Center and Botkin Hospital Transplantation Center to continue to carefully analyze the new practice of asystolic kidney donation.

The success of kidney transplantation depends on the work of the medical staff from the donor conditioning stage to perioperative management of the renal graft recipient. The multidisciplinary approach allows us to minimize the possible risks of kidney transplantation, which is also a strong reason by kidney transplant recipients to choose this approach over other renal replacement therapy methods. The introduction of modern techniques at each of the stages only helps in the implementation of this task.

CONCLUSION

The combined use of ACCDs and HOPE when using organs donated after circulatory death allows to minimize primary warm ischemia time and static cold storage time, thereby mitigating their negative effects on renal graft function. Further application of this protocol allows us to reasonably expect an increase in the number of donor organs for transplantation purposes.

The authors declare no conflict of interest.

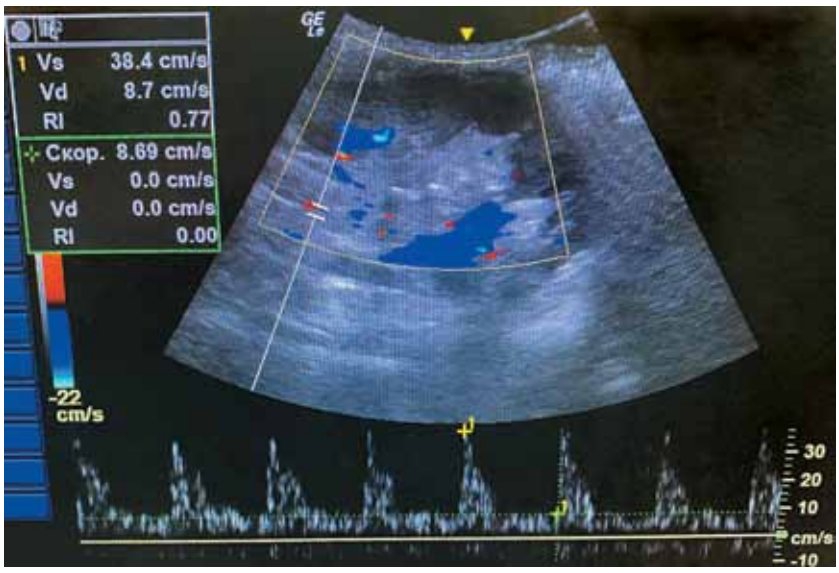

Fig. 6. Intraoperative kidney graft ultrasound

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WAYS OF IMPROVING THE LEGAL REGULATION OF HUMAN ORGAN AND TISSUE TRANSPLANTATION IN THE RUSSIAN FEDERATION

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Over the past 10 years, significant breakthroughs have been achieved in Russian transplantology in the field of regulatory legal framework. During this period, the powers of government authorities in the field of healthcare on organization of transplant care and organ donation have been defined, and sources and mechanisms for target financing of medical activities related to organ donation for transplantation purposes have been identified. The procedure for providing medical care under surgery (human organ and/or tissue transplantation) has been adopted, and a state registry system for donor organs, donors and recipients has been created. Measures on organ donation and transplantation in the Russian Federation have been approved within the "Healthcare Development", a framework of the state program of the Russian Federation. The Shumakov National Medical Research Center of Transplantology and Artificial Organs (Shumakov Center) has also been identified as the core institution that coordinates the activities of the entire transplant industry in the Russian Federation. Transplant medical care is currently being provided by specialist physicians trained in human organ and tissue transplantation, in collaboration with other specialist physicians. The Nomenclature of Specialties of Specialists with Higher Medical and Pharmaceutical Education, approved by the Russian Ministry of Health via Order No. 700n of October 7, 2015, does not contain a separate specialty related to human organ and tissue transplantation activities, and this is quite justified. However, in order to improve the legal regulation of transplantation activities, it is necessary to unify the requirements for specialists providing medical care in human organ/tissue transplantation. This can be achieved by developing uniform approaches to the definition of labor functions in the professional standards of specialist doctors involved in transplantation.

Keywords: organ donation, organ transplantation, tissue transplantation, donation and transplantation law, donation and transplantation procedure, donation and transplantation licensing, medical personnel, nomenclature of medical specialties, nomenclature of medical services, professional standards of specialist physicians, organ and tissue transplantation, surgery, transplant team.

According to the Constitution of the Russian Federation, a person, their rights and freedoms are the supreme value, and the recognition, observance and protection of human and civil rights and freedoms are the duties of the state; everyone has the right to life and the right to health protection and medical care. Among the inalienable human rights is also the right to physical integrity, enshrined in Article 22 of the Constitution of the Russian Federation, which excludes unlawful influence on a person in both physical and mental sense [1], and the concept of "physical integrity" covers not only the lifetime existence of the human body, but also creates the necessary prerequisites for the legal protection of the body of a deceased person. This applies equally to the right to state protection of personal dignity, as well as to the human right, derived from these constitutional rights to a dignified handling of the human body after death.

When performing organ and/or tissue transplantation, the task is to achieve a balance of constitutionally significant values and protected rights that do not violate the rights of any of them, which are determined by the legal regulation in this sphere, taking into account, among other things, moral, social and other aspects.

In the Soviet Union, organ transplantation started developing in the 1960s. However, until 1985, only kidney transplantations were performed in the Soviet Union; the possibility of transplantation of other organs arose after the government allowed the leading, most trained

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institutions to diagnose brain death. For the subsequent 20 years (until 2006), development of various types of organ transplantation was not systemic, but was a priority for individual clinics.

In modern Russia, Law No. 4180-1 dated December 22, 1992 "On Transplantation of Human Organs and Tissues" (hereinafter referred to as "Transplantation Law") was adopted in 1992 [2].

According to the Transplantation Law, human organ and tissue transplantation (hOTT) is a means of saving life and restoring the health of citizens and may be carried out under compliance with the laws of the Russian Federation and human rights in accordance with humane principles proclaimed by the international community, with the interests of the individual prevailing over the interests of society or science. On this basis, Article 1 of the Transplantation Law stipulates that hOTT from a living donor or a dead person is used only if other medical means cannot guarantee preservation of life of the patient (recipient) or restoration of their health. Removal of organs and tissues from a living donor is permitted only if their health, according to a Concilium of physicians, will not cause significant harm and may only take place with the consent of a living donor; human organs and tissues may not be bought or sold; the purchase and sale of such organs and tissues, as well as advertising of these actions carry criminal liability in accordance with the laws of the Russian Federation.

The task of saving lives and restoring the health of citizens through transplantation remains an extremely urgent problem and is addressed taking into account not only scientific achievements in this sphere but also by improving legal regulation.

In the period from 2006 to 2021, there has been a long-term positive trend in the development of transplantology in the Russian Federation. It has been characterized by an annual increase in the number of organ and tissue transplants from 662 to 2,348, an expansion in the geography of transplantation care and organ donation from 20 to 35 federal subjects of the Russian Federation, an increase from 31 to 63 in the number of medical organizations performing organ transplants and an increase in the number of patients with transplanted organs under observation and receiving lifelong drug therapy with immunosuppressants increased from 4,007 to 21,012. In Russia, post-transplant survival rates are comparable (not worse) than in Europe and the USA.

From 2019 to 2021, medical care accessibility through human organ transplantation was improved within the framework of the departmental target program "Organ Donation and Transplantation in the Russian Federation" [3], and from January 2022 – in accordance with the Complex of process measures "Organization of Organ Donation and Transplantation in the Russian Federation", approved by the Russian Ministry of Health on December 28, 2021, which is part of "Healthcare Development", a state program of the Russian Federation.

Within the framework of the mentioned Complex, tasks on improving the regulation of medical activities related to human organ donation for transplantation, increasing the number of human organ transplants performed, increasing the volume of medical activity associated with human organ donation for transplantation, and raising public awareness of the social significance of human organ donation are all being solved. For instance, by 2024 there are plans to increase to 73 the number of medical institutions allowed to harvest, procure and transplant human organs and tissues and which have submitted information about donor organs, organ donors, patients (recipients) to the information system of the Russian Ministry of Health. The number of donor organs for transplantation in regional medical organizations should be 1,520.

In accordance with Article 4 of the Transplantation Law, the Russian Ministry of Health, together with the Russian Academy of Sciences, approves the current list of health care institutions allowed to harvest and procure human organs and tissues and the list of health care institutions allowed to carry out human organ and tissue transplant surgeries. At present, the list of health care institutions is approved by order No. 515n/1, a joint order of the Russian Ministry of Health and Russian Academy of Sciences dated May 25, 2021 [4].

The Russian Ministry of Health has created a state registry for donor organs, donors and recipients [5]. On its basis, a personalized transplantation register, *Transplantology*, a federal state information system, has been developed and implemented at regional transplantation centers since 2018, which includes, in addition to the System of registration of donor organs, donors and recipients, a register of non-consent for organ removal after death for transplantation and the Unified Waiting List for organ transplantation.

According to Federal Law No. 323-FZ of November 21, 2011 "On the Fundamentals of Protecting the Health of Citizens in the Russian Federation" (hereinafter referred to as "Federal Law No. 323-FZ"), the basic principles of health protection include the priority of patient interests in the provision of medical care, accessibility and quality of medical care, as well as unacceptability of refusal of medical care [6]. According to Federal Law No. 323-FZ, transplantation is a type of medical professional activity and that the implementation of measures to organize hOTT medical activities, including organ and tissue donation for transplantation is under the powers of the federal executive body responsible for developing and implementing state policy and legal regulation in the field of healthcare.

The content of Article 47 of Federal Law No. 323-FZ providing for registration of donor organs and tissues, as well as persons in need of hOTT is significantly expanded and clarified by Federal Law No. 271-FZ of 13 July 2015, which established legal grounds for registering donor human organs and tissues, organ and tissue

donors, patients (recipients) and procedures for financing relevant activities [7].

For the first time, a list of human organs – objects of transplantation – and the list of health care institutions that are allowed to perform organ transplantation were established by the Russian Ministry of Health via Order No. 448 and by the Russian Academy of Medical Sciences via Order No. 106 of December 13, 2001 [8].

Pursuant to Article 2 of the Transplantation Law, the Russian Ministry of Health and the Russian Academy of Medical Sciences approved the list of transplantation objects, including 25 names of transplantation objects, via joint order No. 306n/3 of June 4, 2015 [9].

The concept of medical activity enshrines that it is a professional activity on the provision of medical care, medical evaluation, medical examinations and medical check-up, sanitary and anti-epidemic (preventive) measures and professional activities associated with hOTT, handling of donor blood and (or) its components for medical purposes (Article 2 of Federal Law No. 323-FZ).

According to Article 12 of the Federal Law No. 99-FZ of May 4, 2011 "On Licensing of Certain Types of Activities", medical activity is subject to licensing [10].

In order to obtain a medical activity license, the applicant must meet the established license requirements.

The specified licensing requirements are contained in the Medical Activity Licensing Regulation (except for the specified activities carried out by medical institutions and other organizations that are part of the private healthcare system, on the territory of the Skolkovo Innovation Center, approved by Resolution No. 852 of the Government of the Russian Federation dated June 1, 2021 (hereinafter referred to as "Licensing Regulation") [11].

Apart from the general requirements for all applicants for a medical activity license, for entities intending to provide hOTT services, there is a requirement for organizational and legal status – hOTT is performed exclusively at state and municipal healthcare institutions. This correlates with the provisions enshrined in Article 4 of the Transplantation Law.

Separately, it should be noted that the list of services constituting medical activity provided for in the Annex to the Licensing Regulation contains several types of human organ and tissue transplantation services: removal and storage of human organs and tissues for transplantation; bone marrow and hematopoietic stem cell transplantation; hematopoietic stem cell and bone marrow transportation; human organ and tissue transportation for transplantation; surgery (organ and tissue transplantation).

So, in order for a state/municipal institution to be allowed to provide medical care for organ transplantation and to perform medical activities related to organ donation for transplantation, the institution must meet the following requirements: obtain a license for the relevant services, be included in the lists of healthcare institutions that perform organ and tissue harvesting, procurement and transplantation, which are approved by the Russian Ministry of Health together with the Russian Academy of Medical Sciences [4].

In addition, one of the main requirements when applying for a medical activity license is compliance with the Medical Care Procedure approved by the authorized federal executive body and mandatory for all medical organizations on the territory of the Russian Federation (Article 37 of Federal Law No. 323-FZ).

The Medical Care Procedure for Surgery (human organ and tissue transplantation) was approved by Order No. 567n of October 31, 2012 of the Russian Ministry of Health (hereinafter referred to as "Procedure") [12]. The Procedure stipulates that medical care for hOTT shall be provided in the form of specialized, including high-tech, medical care, under inpatient settings.

Transplant medical care is provided depending on the type and object of transplantation at surgical wards, including pediatric surgical wards, cardiac surgical wards, surgical (thoracic) wards, and urological wards.

The Procedure also establishes rules for activities of a surgical ward performing hOTT, recommended staffing standards for such surgical ward and standard of additional equipment for a medical institution where there is such a surgical ward.

At the same time, the imperfections of the Procedure are conspicuous, for example, lack of specification of the scope of regulation of the procedure (with indication of transplantation objects to which the Procedure applies).

The Procedure also does not contain provisions regulating human donor organ and (or) tissue transportation for transplantation. It should be noted that human organ and tissue transportation services for transplantation are independent, separately licensed services included into the list of services constituting medical activity envisaged by the Annex to the Licensing Regulation, and they contain several types of services.

In this regard, work is currently underway to update the Procedure to ensure its compliance with developing transplantation technologies.

The recommended staffing standards for a surgical hOTT ward contain subsections depending on the type and object of transplantation.

For example, a surgical (urological) ward that performs kidney and pancreas transplantation includes the following positions: a surgeon (urologist); a nephrologist (general practitioner); a pediatrician (in the case of provision of kidney transplant medical care for minors); an endocrinologist (in the case of provision of medical care for kidney and pancreas transplantation for patients suffering from diabetes).

A pediatric surgical (urological) ward that performs kidney transplantation shall include the following positions: pediatric surgeon (pediatric urologist and andrologist); surgeon (urologist); pediatrician (pediatric nephrologist); general practitioner.

A surgical ward that performs liver transplantation must be staffed with the following positions: a surgeon; a gastroenterologist (a general practitioner); a pediatrician (in the case of provision of medical care for liver transplantation for minors).

A pediatric surgical ward that performs liver transplantation must be staffed with the following positions: a pediatric surgeon; a surgeon; a pediatrician (a gastroenterologist); a general practitioner.

A cardiac surgical ward that performs heart transplantation must be staffed with the following positions: a cardiovascular surgeon, a cardiologist, a thoracic surgeon (in the case of provision of heart-lung transplant medical care), a pulmonologist, a pediatrician (in the case of provision of heart transplant medical care for minors).

A thoracic surgical ward that performs lung transplantation must be staffed with the following positions: a thoracic surgeon; a pulmonologist; a pediatrician (in the case of provision of lung transplant medical care for minors).

Analytical activities and organizational and methodological management of regional and district medical institutions at federal subjects of the Russian Federation in the field of surgery (organ transplantation) are performed by the Shumakov Center. It is included in the network of national medical research centers and is classified as the highest (fourth) level of medical organizations [13, 14].

In the course of the analytical activities of the Shumakov Center, the following activities are carried out: analysis of implementation of clinical guidelines at medical institutions of the federal subjects of the Russian Federation; collection and analysis of information on the state of organization of medical care at the federal subjects of the Russian Federation; assessment of the uniqueness of drugs included in the list of vital and essential drugs within the framework of the existing clinical practice on their use and the possibility of replacing these drugs; analysis of the availability in federal subjects of the Russian Federation of drugs most in demand in practice and used in basic treatment regimens, the presence of defects and their causes; analysis and assessment, taking into account medical care standards and clinical guidelines of the needs of federal subjects of the Russian Federation for drugs included in the list of vital and essential drugs for medical use; formation and updating of the list of priority research areas in the field of health care, including the development of personalized approaches in medicine; development of methodological guidelines on creation of conditions for provision of paid medical services to foreign citizens; analysis of staffing of medical institutions at federal subjects of the Russian Federation and the need for training (retraining) of medical workers; analysis of professional standards in health care and training programs of medical and pharmaceutical education.

In addition, the Shumakov Center provides organizational and methodological support for the following activities: introduction and development of medical information systems that ensure the implementation of quality management and quality control of medical care in surgery (organ transplantation) at medical institutions participating in the territorial program of state guarantees of free medical care for citizens, including through information interaction between medical information systems; analysis and evaluation of organization of medical care at the federal subjects of the Russian Federation through field visits to these federal subjects and remotely using medical information systems with the development of guidelines for improving the provision of medical care in surgery (organ transplantation) at the federal subjects of the Russian Federation and quarterly monitoring of the implementation of these guidelines; holding consultations (telemedicine Concilium) among medical institutions at federal subjects of the Russian Federation; development of interactive electronic educational modules for medical workers.

At present, the Russian Transplant Society has developed and is under expert review seven draft clinical guidelines: liver fragment living donation (Z52.6, adults); heart transplantation, presence of a transplanted heart, heart transplant death and rejection (Z94.1, T86.2, I42, I25.3, I25.5, I50, children/adults); kidney living donation (Z52.4, adults); pancreas transplantation, presence of transplanted pancreas, pancreas graft death and rejection (Z94.8, T86.8, E10, E10.2, N18.5, adults); lung transplantation, heart-lung transplantation, presence of a transplanted lung, presence of a transplanted heart-lung complex, lung graft death and rejection, heart-lung graft death and rejection (J43.9, J44.9, J47, J84, J98, J98.4, J99.1, E84.0, E84.9, I27.0, I27.8, I27.9, I28, Z94.2, Z94.3, T86.3, T86.8, children/adults); liver transplantation, transplanted liver, liver transplant death and rejection (Z94.4, T86.4, children/adults); kidney transplantation, presence of a transplanted kidney, kidney transplant death and rejection (Z94.0; T86.1, children/adults).

After approval of these clinical guidelines in accordance with the established procedure, appropriate medical care standards will be developed and approved on their basis This would contribute to the creation of uniform conditions and requirements in the provision of medical care throughout the Russian Federation.

In turn, medical care standards are developed on the basis of the Nomenclature of Medical Services [15]. The current Nomenclature contains only 15 medical services related to hOTT. At the same time, there are differences in the terms used in the names of medical services and there are no medical services for some transplantation objects.

Thus, work is being done to further detail the Nomenclature of Medical Services, including medical services specifying the anatomical area of transplantation, clarifying the technology of transplantation operations.

The Nomenclature of Specialties of Specialists with Higher Medical and Pharmaceutical Education, approved

by the Russian Ministry of Health via Order No. 700n of October 7, 2015, does not contain a separate specialty related to hOTT activities [16]. Transplant medical care is provided by medical specialists trained in hOTT, in collaboration with other medical specialists. Transplantology specialists (surgeons, nephrologists, cardiovascular surgeons, urologists, therapists, pediatricians, gastroenterologists, cardiologists, doctors of laboratory, functional and radiology diagnostics, health organizers and others) are united by a professional community – the Russian Transplant Society.

The various requirements contained in the professional standards for the above-mentioned medical specialists come under notice.

For example, in professional standard "Surgeon" (Order No. 743n of the Russian Ministry of Labor dated November 26, 2018), the following labor functions are specified: "transplantation of the musculocutaneous complex", " simultaneous transplantation of the musculocutaneous complex", and "autotransplantation of the musculocutaneous complex" [17]. Professional standard "Nephrologist" (Order No. 712n of the Russian Ministry of Labor dated November 20, 2018) presents a generalized labor function "providing medical care to patients in the field of nephrology, including kidney transplant recipients" [18].

At the same time, professional standards "Pediatric surgeon" (Order No. 134n of the Russian Ministry of Labor dated March 14, 2018), "Urologist" (Order No. 137n of the Russian Ministry of Labor dated March 14, 2018), "Pediatric urologist/andrologist" (Order No. 4n of the Russian Ministry of Labor dated January 13, 2021), "General practitioner (district physician)" (Order No. 293n of the Russian Ministry of Labor dated March 21, 2017), "District pediatrician" (Order No. 306n of the Russian Ministry of Labor dated March 27, 2017), "Endocrinologist" (Order of the Russian Ministry of Labor of March 14, 2018 No. 132n), "Gastroenterologist" (Order No. 139n of the Russian Ministry of Labor dated March 11, 2019), "Cardiovascular surgeon" (Order No. 143n of the Russian Ministry of Labor dated March 14, 2018), "Cardiologist" (Order No. 140n of the Russian Ministry of Labor dated March 14, 2018), "Thoracic surgeon" (Order No. 140n of the Russian Ministry of Labor dated March 11, 2019), and "Pulmonologist" (Order No. 154n of the Russian Ministry of Labor dated March 19, 2019), have no labor functions that contain references to "transplants," "organ and tissue transplantation" [19–29].

When providing medical care under the program of state guarantees of free medical assistance to citizens and territorial programs of state guarantees of free medical care to citizens, medical activities associated with human organ and tissue donation for transplantation, including measures for medical examination of the donor, ensuring the safety of donor organs and tissues until they are harvested from the donor, harvest of donor organs and tissues, storage and transportation of donor organs and tissues (Article 80 of Federal Law No. 323-FZ). At the same time, financial support for medical activities related to human organ donation for the purpose of transplantation comes from allocations from the national budget and from the budgets of federal subjects of the Russian Federation (Article 83 of Federal Law No. 323-FZ).

In order to develop the transplant service in the Russian Federation, it would be advisable to introduce a differentiated approach to the rules of allocation of federal budget funds for transplant medical care, including the use of financial incentives, for example, introduction of a factor that increases the amount of subsidy provided to the budget of a federal subject of the Russian Federation that provides high-tech transplant medical care.

The limited number of institutions (Shumakov Center, Evdokimov Moscow State University of Medicine and Dentistry and Moscow Regional Clinical Research Institute (Moscow Oblast) that provide postgraduate training for specialists in organ donation and transplantation is conspicuous.

In order to increase the number of hOTT medical care specialists, additional study is required on the introduction of teaching the basics of transplantology in the secondary and higher medical and pharmaceutical educational institutions in order to identify the interest of future specialists in this field.

Given the multidisciplinary approach, it is necessary to note the importance of forming a register of transplantation teams – specialized structural units of medical institutions that perform organ and/or tissue transplantation, including specialist physicians. The composition of such teams should depend on the object of transplantation, the individual characteristics of the organ recipient, and include medical specialists who have received postgraduate training in organ donation and transplantation, an anesthesiologist/resuscitator, medical specialists who provide medical care to recipients with a transplanted organ (nephrologist, gastroenterologist).

Based on the analysis carried out, we can conclude that it is necessary to continuously improve not only the legal regulation of transplantation activities, but also practical implementation of existing transplantology regulations and programs.

As priority measures for improving the organization of transplantology today, it is expedient to:

- Expand the network of medical institutions that perform hOTT;
- Promote further development of interregional collaboration between these medical institutions for the exchange of unclaimed donor organs that are suitable for transplantation;
- Intensify sharing of information on the possibilities of personalized transplantation *Transplantology* with regional transplant centers;

– Form unified approaches to the definition of labor functions in the professional standards of specialist physicians providing hOTT medical care;
– Harmonize the Nomenclature of Medical Services taking into account the list of transplant objects;
– Update the Medical Care Procedure for Surgery (human organ and tissue transplantation) taking into account the developing trends in the field of transplantation.

The authors declare no conflict of interest.

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ON THE POSSIBILITY OF THERAPEUTIC ACTION AFTER TRANSDERMAL PATCH APPLICATION

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Background. As scientific knowledge about the peculiarities of the structure and functional properties of the skin increased, it became clearer that during transdermal administration, drug may accumulate in the deep layers of the dermis and subsequently get diffused into the bloodstream even after the transdermal therapeutic system (TTS), also called transdermal patch, had been removed. **Objective:** to quantify active drug substances remaining in an animal skin after TTS application. **Materials and methods.** Two previously developed transdermal patches containing Russian-made drug substances were chosen for the study: aminodihydrophthalazinedione sodium (immunomodulator) and bis(1-vinylimidazole-N) zinc diacetate (antidote for carbon monoxide). The study was performed on male Chinchilla rabbits weighing 2.5–3 kg. Five series of experiments were performed for each substance: immediately after removal of the patch, 4 hours later, at week 1, 2 and 3 after removal. High-performance liquid chromatography and atomic absorption spectroscopy methods were used to quantify residual drug substances left in the skin. **Results.** In the skin flap that was in contact with the aminodihydrophthalazinedione sodium TTS for 24 hours, 0.516 mg of the drug was detected immediately after removal of the patch. Over the next two weeks, the drug substance in the skin decreased with the immunomodulator significantly reducing to 0.41 mg in the first 4 hours. In the skin flap that had been in contact with zinc bis(1-vinylimidazole-N) diacetate for 24 hours, about 1 mg of the drug was present immediately after patch removal. Four hours after removal of the transdermal patch, the quantity of active substance in the skin remained practically unchanged. At week 1 and 2, the quantity of the antidote decreased slightly to ~0.7 mg and ~0.25 mg, respectively. **Conclusion.** For transdermal application of aminodihydrophthalazinedione sodium, the skin can act as a drug depot and prolong the effect of this drug even after the transdermal patch had been removed. No such effect was found in the case of bis(1-vinylimidazole-N) zinc diacetate, which is apparently due to the different solubility of the drugs in the biotissue.

Keywords: transdermal therapeutic system, residual drug, skin.

INTRODUCTION

One of the advantages of transdermal therapeutic systems (TTS) over traditional methods of drug administration is the immediate termination of the drug after removing the TTS from the patient's skin, thus preventing the development of several side effects of the drug and avoiding drug overdose [1, 2].

However, as scientific knowledge about the peculiarities of the structure and functional properties of the skin increased, it became clearer that during percutaneous administration, drugs can accumulate in the deep layers of the dermis and subsequently diffuse into the bloodstream even after the patch had been removed. For example, fentanyl, as a lipophilic drug, continues to be absorbed into the subcutaneous fatty tissue and remains in it almost 24 hours after removal of the patch from the patient's skin [3]. We did not find any other studies on the effects of TTS in the open press. It should be noted that the data obtained by studying the quantity of residual drug substance in the skin after removal of the transdermal patch can make significant changes in the TTS regimen.

The purpose of this work was to quantify active drug substances remaining in an animal skin after TTS application.

MATERIALS AND METHODS

The following two previously developed TTSs [4, 5] containing Russian-made drug substances were chosen for the study of the quantity of drug substances in the skin after removal of the transdermal patch:

1. Aminodihydrophthalazinedione sodium (trade name Galavit, SELVIM LLC). Molecular mass is 206 Da.
2. Bis(1-vinylimidazole-N) zinc diacetate (trade name Acizol, Favorsky Irkutsk Institute of Chemistry). Molecular weight 372 Da.

Since both drugs are hydrophilic, they were introduced into the patch as part of water-in-oil emulsion compositions. This approach is related to the fact that the

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intercellular space of the epidermal barrier, represented by a complex lipid mixture, is the main route of percutaneous penetration for most compounds [6].

Auxiliary substances and materials approved for medical use were used in the manufacture of TTS laboratory samples.

Microemulsion compositions with drug substances included the following components: purified water (FS 42-2620-97), 0.9% sodium chloride solution (Escom, Russia), sodium dodecyl sulfate (AppliChem Panreac, Spain), apricot kernel oil (Desert Whale Jojoba Company Ltd, USA), alpha-tocopheryl acetate (BASF SE, Germany), docusate sodium (Sigma, USA), and emulsifier Decaglyn PR-20 (Nikko Chemicals Co., Ltd., Japan). Foam tape 9773 (3M, USA), sorbent base PALV-01 (Palma Group of Companies LLC, Russia), and Scotchpak 9730 film (3M, USA) were used to create the transdermal patch.

The following reagents were also used: ethylenediaminetetraacetic acid (EDTA) (Sigma, USA), acetylcysteine (Sigma, USA), papain (Sigma, USA), government standard sample of aqueous solution of zinc ions (GSO 7837-2000), syringe filters (Agilent, cellulose acetate 0.45 μm, 25 mm).

Equipment used in the work: Dispergator (Heidolph DIAX 900, Germany); ultrasonic homogenizer (Heilscher UIS250V, Germany); analytical scales (GH-200 AND, Japan); centrifuge (Hettich Rotina 38R, Germany); liquid chromatograph (Agilent 1200, USA) equipped with a UV detector, autosampler, degasser and column thermostat; hotplate magnetic stirrer (IKA RT10, Germany); atomic absorption analyzer (Analyst A100, Perkin Elmer).

Research methodology

The study was conducted on male Chinchilla rabbits weighing 2.5–3 kg.

The animals were obtained from the breeding nursery of Krolinfo LLC. The producer provided a veterinary certificate of the last health check. All experimental animals were specially bred and had not previously participated in studies. They were quarantined for 14 days. All manipulations with the animals were performed according to the rules adopted in the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (ETS 123) Strasbourg, 1986).

Laboratory samples of the patch were attached on pre-shaved areas of the skin of the rabbit back at the base of the neck.

Five series of experiments were performed for each drug substance: investigation of the drug content in the skin immediately after removal of the transdermal patch, four hours, one, two, and three weeks after removal of the patch. After the rabbits were removed from the experiment using Zoletil 100 (Virbus Sante Animale, France) and Rometar (Bioveta, Czech Republic), a skin flap was taken from the place of the back where the patch was applied.

Methodology for quantifying the residual drug substances in the skin

The subcutaneous fat area (SFA) was separated from the dermis, and everything was crushed. Dissolution of the skin and subcutaneous fat was performed separately at 600 °C and under constant stirring in a 0.2 M phosphate buffer solution with the addition of EDTA, acetylcysteine, and papain.

The quantity of bis(1-vinylimidazole-N) zinc diacetate in the solution was determined by atomic absorption spectroscopy. Since the acyzol molecule contains zinc ion ($C_{14}H_{18}N_4O_4Zn$), we used the state standard reference sample GSO 7837-2000 of aqueous solution of zinc ions to construct the calibration curve. The conversion coefficient of zinc concentration to drug concentration was 5.7.

The quantity of aminodihydrophthalazinedione sodium in the solution was determined using the high-performance liquid chromatography technique we developed earlier [7].

A 600 μL skin solution was added to a 2.0 mL centrifuge microtube, 200 μL of 50% aqueous trifluoroacetic acid solution (by volume) was added. The mixture was stirred for 2 min and centrifuged at 6,000 rpm for 10 min. 500 μL of supernatant was transferred to a 1.5 mL microvial, 55 μL of 50% potassium hydroxide solution (by weight) was added, and the mixture was stirred.

Chromatographic determination was performed under the following conditions: chromatographic column: Mediterranea Sea 18 25 × 0.46 cm, 5 μm (Teknokroma Analitica SA, Spain) with an 8 × 4 mm pre-column filled with the same sorbent. Mobile phase: acetonitrile – 0.015% aqueous solution (by volume) of trifluoroacetic acid, pH = 2.5 (15 : 85). The mobile phase was prefiltered and degassed in a vacuum filtration device. Rate of flow of the mobile phase: 0.8 mL/min. Elution mode: isocratic. Column thermostat temperature: 25 °C. Sample volume injected: 10 μL. Detection wavelength: 221 nm. Retention time: Approx. 11.7 min. Chromatographic time: 16 min. Lower limit of aminodihydrophthalazinedione sodium quantification: 50 ng/ml. Linearity range of the technique: 50–2000 ng/mL.

Quantification of bis(1-vinylimidazole-N) zinc diacetate left in the patch after use

After removal of the transdermal patch, the TTS samples were cut into several pieces, placed in a 250 mL conical flask, and filled with 150 mL of distilled water. Extraction of drug substances was done from the TTS was done in a boiling water bath for 1 hour. This extraction was repeated 2 more times. Then the solution was

filtered through a paper filter into a 1000 mL volumetric flask and brought to the mark with distilled water. Then the obtained solution was diluted in a 1 : 25 ratio. The quantity of bis(1-vinylimidazole-N) zinc diacetate was determined in the solutions by spectrophotometric method at 225 ± 2 nm maximum absorption spectrum, using the following formula:

$$x = \frac{D_x \times m \times 25}{D_0},$$

where D_x is optical density of the test solution, D_0 is optical density of the control sample, m (in grams) is the mass of bis(1-vinylimidazole-N) zinc diacetate taken to prepare the control sample, 25 is the dilution factor.

To prepare a control sample, 0.015 g of bis(1-vinylimidazole-N) zinc diacetate was placed in a 1000 mL volumetric flask and diluted with distilled water to the mark.

Quantification of aminodihydrophthalazinedione sodium left in the patch after use

After removal of the transdermal patch, the TTS samples were cut into several pieces and placed in a 250 mL conical flask. They were filled with 0.5% alcohol-aqueous (1 : 1) sodium dodecyl sulfate solution (150 mL). The drug substance was released into the solution at 600 °C and constantly stirred on a hotplate magnetic stirrer for 1 hour and 45 minutes. Then the solution was filtered through a paper filter into a 500 mL volumetric flask. This extraction was repeated 1 more time. Then the volume in the flask was brought to the mark with 0.5% alcohol-aqueous (1 : 1) sodium dodecyl sulfate solution. The quantity of aminodihydrophthalazinedione sodium in the solutions was determined by spectrophotometric method at an absorption maximum of 294 ± 2 nm using formula:

$$x = \frac{D_x \times 10 \times 500}{D_0},$$

where D_x is optical density of the test solution, D_0 is optical density of the control sample, 10 is concentration (in mg/mL) of aminodihydrophthalazinedione sodium control solution, 500 is the volume (in mL) of the test solution.

Statistical processing of the results was done using Microsoft Office Excel 2010 software.

RESULTS

TTS application in each series of experiments lasted for 24 hours. The quantity of drug substance in the patches was 100 mg and 20 mg for the antidote for carbon monoxide and the immunomodulator, respectively.

The quantity of drug substance remaining in the patch after detachment was examined for both test substances.

Thus, the quantity of immunomodulator in TTS after application was 8.4 ± 2.8 mg. Consequently, ~11.6 mg of the drug entered the skin from the patch during 24 hours of the experiment. According to the results of the study of pharmacokinetics of aminodihydrophthalazinedione sodium in the blood of a rabbit during percutaneous administration, it was found that the time to reach a plateau in drug concentration on the pharmacokinetic curve was about 4 hours [7].

We made the assumption that the skin can accumulate the active substance that will continue to flow into the bloodstream even after removal of the transdermal patch from the skin and can compensate for the temporary delay in the onset of action of the transdermal dosage form. So, the time required to reach a constant concentration of the drug in the blood should be taken into account when replacing the patch in case of long-term use.

Table 1 shows the quantity of immunomodulator contained in the skin and subcutaneous fat of rabbits at different times after patch removal.

Table 1

Quantity of residual aminodihydrophthalazinedione sodium in the skin and subcutaneous tissue of rabbits at different times after removal of the transdermal patch

Object of study	Quantity of drug substance after patch removal (mg)			
	Immediately (n = 3)	At 4 hours (n = 3)	At week 1 (n = 3)	At week 2 (n = 2)
Skin	0.51 ± 0.02	0.10 ± 0.002	0.013 ± 0.005	0.0021 ± 0.0004
ST	0.006 ± 0.003	0.005 ± 0.002	0.005 ± 0.001	0.0013 ± 0.0005

As shown in Table 1, 0.516 mg of aminodihydrophthalazinedione sodium was present in the skin flap that had been in contact with the TTS for 24 hours immediately after its detachment. Over the next two weeks, there was a decrease in the quantity of the drug substance in the skin, with a significant decrease to 0.41 mg occurring in the first 4 hours. This value may be therapeutically significant in the case of transdermal administration, given the small daily dose of Galavit® (25 mg, orally) [8].

The results obtained during the study of the content of the immunomodulator in the skin should be taken into account when developing a regimen for the aminodihydrophthalazinedione sodium TTS.

A similar series of experiments was performed for TTS with the antidote for carbon monoxide. The quantity of active substance in the bis(1-vinylimidazole-N) zinc diacetate TTS after application in a rabbit was 28.1 ± 4.3 mg. Thus, about 70 mg entered the skin from the dosage form. About 1 mg active substance bis(1-vinylimidazole-N) zinc diacetate was present in the skin flap

that had been in contact with the patch for 24 hours immediately after its detachment (Table 2).

Table 2

Quantity of residual bis(1-vinylimidazole-N) zinc diacetate in the skin and subcutaneous tissue of rabbits at different times after removal of the transdermal patch

Object of study	Quantity of drug substance after patch removal (mg)			
	Immediately (n = 3)	At 4 hours (n = 3)	At week 1 (n = 3)	At week 2 (n = 2)
Skin	0.92 ± 0.01	0.89 ± 0.03	0.63 ± 0.02	0.19 ± 0.05
ST	0.06 ± 0.03	0.06 ± 0.02	0.05 ± 0.08	0.04 ± 0.03

Four hours after TTS removal, the quantity of the drug substance in the skin and subcutaneous fatty tissue remained virtually unchanged, in contrast to the result obtained at the same time point in skin examination after application of the immunomodulator TTS. At week 1 and 2, the quantity of the antidote decreased slightly to ~0.7 mg and ~0.25 mg, respectively. Thus, the quantity of active ingredient eliminated from the skin per week, 0.3–0.4 mg, is negligible compared to the required daily dose of the drug (120 mg orally) and may not have a significant therapeutic effect [9].

Three weeks after removal of the transdermal therapeutic system for both drugs, the quantity of active substance in the skin was at the lower limit of sensitivity of the quantitative methods used.

CONCLUSION

This work examined the residual amounts of the immunomodulator and antidote for carbon monoxide in animal skin and in transdermal therapeutic systems 24 hours after application. For transdermal application of aminodihydrophthalazinedione sodium, the skin can be a drug depot and prolong the effect of this drug even after the transdermal patch had been removed, compensating for the temporary delay in the onset of the next patch. No such effect was found in the case of bis(1-vinylimidazole-N) zinc diacetate, which is apparently due to the different solubility of the studied drugs in the biotissue.

So, when conducting preclinical studies of transdermal delivery systems with the aim of developing the dosage form application scheme, possible accumulation of drug substance in the skin layers in concentrations that have a therapeutic effect should be taken into account.

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EFFICACY OF SURGICAL TECHNIQUES FOR MORBID OBESITY AND THEIR POTENTIALS IN END-STAGE RENAL DISEASE IN PREPARATION FOR KIDNEY TRANSPLANTATION

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Obesity is a modern "epidemic" not only in the general population but also among patients with end-stage renal disease (ESRD) who require kidney transplantation (KTx). The objective of this literature review is to analyze global studies on surgical methods of treating morbid obesity and their potentials in ESRD patients in preparation for KTx.

Keywords: morbid obesity, kidney transplantation, bariatric surgery.

The World Health Organization defines obesity as abnormal or excessive fat accumulation that presents a risk to health. It is classified based on the body mass index (BMI), the ratio of body weight to height: 30.0 to 34.9 kg/m^2 (class I obesity), 35.0 to 39.9 kg/m^2 (class 2 obesity), and ≥40 kg/m^2 (class 3 obesity). Over the past three decades, the number of overweight (BMI ≥25 kg/m^2) and obese (BMI ≥30 kg/m^2) adults worldwide has increased substantially [1]. The BMI classification, although an imperfect tool for defining obesity, is currently the most widely used in clinical practice [2]. BMI's limitation is due to the fact that important demographic data of patients are not considered, such as age and ethnicity, percentage and composition (subcutaneous or visceral) of adipose tissue and muscle mass [3, 4]. Despite these limitations, it is likely that BMI will continue to be used as part of the diagnosis in kidney transplant candidate selection. It is easily calculated from weight and height, can be easily recorded and tracked over time, is well established in clinical practice, and is by far the most widely used anthropometric measure of body weight [5].

In fact, obesity is an independent risk factor for chronic kidney disease (CKD). Arterial hypertension and diabetes mellitus, the two most frequent comorbidities associated with obesity, may be a major cause of kidney failure and pose a major challenge for candidate selection, waiting list management and prediction of pre- and post-transplant outcomes [6, 7]. The relationship between increased body weight and ESRD is complex and paradoxical. Given the evidence of extremely adverse effects of obesity on various pathological processes, it seems paradoxical that obesity is persistently associated with lower mortality in patients with severe CKD and ESRD. At least some of the beneficial effects associated with increased BMI have been shown to be down to the presence of higher muscle mass. However, there is evidence to suggest that increased adipose tissue, especially subcutaneous (nonvisceral) tissue, may also be associated with better patient outcomes. In this regard, dietary protein-energy restriction efforts may lead to increased mortality, which should be considered in the management of potential kidney transplant recipients.

BMI ≥35 is generally considered to be a relative contraindication to KTx because of adverse outcomes, including postoperative complications, higher rates of new-onset diabetes after transplantation (NODAT), delayed graft function and/or receipt of a primary nonfunctioning graft [8]. Obese patients on hemodialysis are excluded from the waiting list despite therapeutic possibility of reducing body weight; there is limited possibility of performing a kidney transplant and living a full life [9].

SHORT-TERM AND LONG-TERM CLINICAL AND SURGICAL OUTCOMES OF KIDNEY TRANSPLANTATION IN OVERWEIGHT PATIENTS

KTx improves survival in obese recipients compared to treatment with long-term hemodialysis. However, overweight in renal transplant recipients is accompanied by increased incidence of delayed function and acute rejection, risk of graft loss, surgical complications and prolonged hospitalization [2, 10].

In a 2014 meta-analysis, **Nicoletto et al.** analyzed the results of studies on obese and nonobese patients who underwent KTx and evaluated the following outcomes – delayed graft function, acute rejection, graft and patient survival at 1 or 5 years after transplantation, and death by cardiovascular disease. Twenty-one studies involv-

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ing 9,296 patients were analyzed. It was concluded that pre-transplant obesity was associated with a relative risk of delayed graft function. However, no association was found between obesity and acute graft rejection [11]. The authors report that possible explanations for this distribution may be related to major changes and advances in immunosuppressive therapy along with improved surgical and clinical management of obese patients and prevention of their complications (e.g., hypertension, cardiovascular disease, diabetes, etc.).

In another meta-analysis, **Lafranca et al.** included 56 studies and 5,526 patients who were divided into those with high BMI (>30 kg/m^2) and low BMI (<30 kg/m^2). The main outcomes analyzed were survival (patient survival, graft survival, mortality), kidney function outcomes (delayed graft function and acute rejection) and metabolic conditions (new-onset post-transplant diabetes and hypertension). Other outcomes were related to infection and surgery (length of surgery, length of hospital stay, wound infection, incisional hernia, wound dilation, and other side effects). This latter group is of particular interest because the study showed more surgical complications in obese patients than in non-obese patients [2]. Renal transplant recipients with a BMI >30 kg/m^2 had worse 3-year graft and patient survival. The deleterious effect of higher BMI on renal function was also manifested in the fact that the incidence of delayed graft function and acute rejection was higher in patients with a higher BMI [12]. The incidence of new onset diabetes and high blood pressure was higher in obese patients. Finally, with regard to surgical outcomes, patients with low BMI show significantly fewer complications; the only exceptions are lymphocele and hematomas – perhaps because these two conditions are not necessarily dependent on BMI, as the authors themselves observed. Nevertheless, despite worse results in patients with high BMI, transplantation remains the most effective approach in patients with CKD, but weight loss before transplantation should be recommended [2].

Naik et al. conducted a retrospective analysis in 2016 to investigate the effect of obesity on allograft survival in first-time kidney transplant recipients [13]. The results showed an independent stepwise association between higher BMI and cumulative incidence of dysfunction and overall graft loss. The authors suggested that despite the evidence suggesting that transplantation has a positive effect in patients with high BMI, surgical and clinical management tactics should be adopted with caution. The 1-year follow-up showed no worsening of outcomes in obese patients compared with overweight and non-obese patients. Another study also showed no difference in rates of new-onset diabetes or allograft loss, although the glomerular filtration rate was lower in overweight and obese patients at 3 and 6 months after transplantation [14].

In obesity, surgical intervention is longer and warm ischemia time increases, which is a risk factor for delayed graft function [15]. Obesity is closely related to high sympathetic nervous system activity, which leads to renal vasoconstriction [16]. Moreover, rapid administration of calcineurin inhibitors after transplantation, possibly at higher doses in overweight or obese patients, can aggravate vasoconstriction and further impair graft perfusion, increasing the risk of delayed function. Another possible explanation is the association between obesity and increased prothrombotic activity and endothelial dysfunction [17]. Body fat mass, in particular central obesity, is associated with higher levels of thrombin formation [18], which is a risk factor for venous thromboembolism [19]. Increased prothrombotic activity and endothelial dysfunction may contribute to the risk of graft microthrombosis, which itself may play an important role in delayed graft function [20].

In the last decade, studies have shown that robot-assisted KTx can be performed in patients with extremely high BMI. **Garcia-Roca et al.** reported that 52.8% of procedures among transplant candidates with a BMI of 45 kg/m^2 were performed using a robotic technique [21]. This procedure is costly, but initial results show less postoperative pain and fewer wound complications, such as surgical site infections and hernia. These results may be particularly beneficial for obese patients with regard to overall costs and rehospitalization.

Thus, a higher BMI creates more problems in terms of perioperative, short-term and long-term outcomes in patients requiring renal transplantation, especially with regard to increased risk of delayed graft function and graft loss. There are probably three reasons for the increased risk: immunosuppression, a subclinical proinflammatory state well known in patients with high BMI, and a higher incidence of associated cardiovascular disease.

CURRENT APPROACHES TO SURGICAL TREATMENT OF MORBID OBESITY, INCLUDING IN CKD PATIENTS

Increased incidence of complications and suboptimal outcomes in obese and morbidly obese kidney transplant recipients has led many transplant centers to reject patients with a BMI of 30 to 40 kg/m^2 [22]. In this situation, weight loss becomes unavoidable to be eligible for KTx. However, regardless of the rules followed by each clinic, weight loss prior to transplantation should be strongly recommended in order to speed up listing and improve surgical and renal outcomes in obese and CKD patients [23]. To achieve this result, there are two main strategies: the conservative one, which mainly involves diet and exercise, and the more aggressive one, which involves surgical intervention. The conservative approach has been preferred for many years because of its lower cost and less traumatic nature. Kidney transplant candidates

were advised to see a nutritionist as soon as possible with regular monitoring of body weight variation. Dietary recommendations were highly individualized and included dietary and exercise plans to achieve specific goals. A possible initial therapy strategy for weight loss consisted of a recommendation to reduce body weight by about 10% of baseline, with a weight loss of 1 to 2 kg per month [24]. Behavioral interventions targeting both diet and physical activity show small but significant benefits in maintaining weight loss. However, a significant number of patients fail to reach their target weight either because of poor compliance or inadequate therapy plans [25].

The first problem to be faced with this conservative approach is the high level of exclusion in the follow-up of obese patients committed to diet and exercise. Another major concern is that, despite an encouraging initial response in terms of weight loss, long-term outcomes are still a matter of debate since weight gain occurs at different rates in different patients.

In this sense, bariatric surgery has proven to be a highly effective method for weight loss compared to therapeutic weight loss methods [26]. It has been found that these surgeries can be performed safely, including in dialysis patients [27]. In an effort to overcome morbid obesity as a barrier to KTx, a two-stage approach is being developed for such kidney transplant candidates. ESRD patients suitable for KTx but having BMI >30 kg/m^2, undergo bariatric surgery first. After persistent weight loss, the patients are reassessed and then placed on the KTx waiting list.

In gastric surgery, many surgical methods of treatment have been developed and implemented, with resection methods occupying a leading spot [28].

Gastric bypass anastomosis was developed in the late 1970s, which was later transformed into Roux-en-Y anastomosis. This procedure was found to produce a weight loss equivalent to the first technique, but with a much lower risk of complications. Sleeve gastrectomy was for a long time only an integral part of biliopancreatic bypass surgery as modified by Hess-Marceau. In the early 2000s, **M. Gagner et al.** (USA) decided to perform biliopancreatic bypass in two stages in severe overweight patients: the first was "Sleeve gastrectomy", and already after weight loss and improvement in patients' condition, they planned to perform the second stage "Intestinal stage" [29]. It turned out that for some patients, the first stage was quite sufficient to achieve the desired weight loss [29]. The surgical intervention is performed using laparoscopic access, which reduces trauma and promotes early postoperative rehabilitation of the patient. Over time, laparoscopic sleeve gastrectomy (LSG) became adapted as a stand-alone procedure for weight loss. Currently, it is the most commonly performed bariatric procedure in the world [30, 31].

Long-term follow-up results have demonstrated its similar efficacy in weight loss, allowing patients to lose 80% of excess body weight within the first year after surgery [32–34], in the resolution of comorbidities, and in mortality and morbidity rates compared to Roux-en-Y gastric bypass (RYGB), recognized as the gold standard of bariatric surgery.

Thus, bariatric surgeries can be divided into three categories:

1. Malabsorptive surgeries. These procedures create an artificial anatomical change that bypasses part of the small intestine with the effect of reducing the amount of nutrients and calories a person absorbs. Biliopancreatic diversion with or without duodenal switch is a typical type of malabsorptive procedure.
2. Restrictive surgeries. The goal of these procedures is to reduce the amount of food consumed by reversible or irreversible, fixed or adjustable resizing of the stomach, leaving less room for food and creating a quick sense of fullness in patients. The main restrictive procedures are placing an adjustable laparoscopic gastric band, performing an LSG, and placing an intragastric balloon [35].
3. Mixed operations. These interventions include both restrictive and malabsorptive techniques (usually gastric size reduction and bypass anastomosis of the small intestine, respectively) [36]. A typical mixed procedure is the RYGB.

Bariatric surgeries can be performed from a traditional surgical approach, using laparoscopy or robotics.

All of the above approaches have advantages and disadvantages. Suffice it to emphasize that a pure malabsorption procedure is associated with important pharmacokinetic consequences, since the integrity of the intestinal tract is important for both nutrients and drug absorption. Simple malabsorptive surgery should hardly be considered in the pre-transplant evaluation of obese patients [37]. However, the results are mixed. Some restrictive procedures, such as laparoscopic gastric banding [38], possibly related to a higher likelihood of gastric band erosion and displacement in immunocompromised patients, have also been reported [39]. Although various bariatric approaches to post-transplant patient management have been reported [40]; two types are the most common: LSG and RYGB. In terms of frequency of performance in Russia, longitudinal gastric resection has taken the leading position among bariatric operations [41].

Thomas et al. published a single-center retrospective analysis on the clinical outcomes of the RYGB technique in 33 CKD patients before KTx with a mean BMI of 43.5 ± 0.7 kg/m^2 [42]. The authors found that 87% of patients using RYGB achieved a BMI <35 kg/m^2, perioperative mortality was 0%, and improved metabolism in diabetes and hypertension. These achievements made it possible to perform kidney transplantation in patients.

However, post-transplant outcomes showed that biopsy-proven acute rejection occurred significantly higher among RYGB vs control patients, and this is consistent with the fact that these patients had a lower trough calcineurin inhibitors. This may be related to the RYGB mechanism: in reducing the absorption capacity of the intestinal tract, RYGB also adversely affects the bioavailability of immunosuppressants [43]. The problem related to pharmacokinetics is not present in the other main type of bariatric surgery for kidney transplant candidates, namely LSG, because it is a restrictive procedure mainly affecting the size of the stomach.

In 2018, **Kim et al.** published a retrospective analysis from a single center comparing pre- and post-transplant outcomes in patients after sleeve resection. Post-LSG kidney recipients were compared with similar-BMI recipients who did not undergo LSG [44]. Among post-LSG patients, mean BMI was 41.5 kg/m^2 at initial encounter, which decreased to 32.3 prior to KTx and persisted further; the rate of 30-day rehospitalization, complications and mortality after LSG was 0%. In addition to weight loss, some other positive effects of bariatric surgery are also evident, especially for high blood pressure. Observations have shown that after kidney transplantation, patients who underwent LSG had lower rates of new-onset diabetes mellitus, delayed graft function and other common complications in obese transplant patients [44]. In addition, the overall postoperative period of these patients did not differ significantly from that of control group patients.

The incidence of serious post-LSG complications ranges from 0% to 6% [45–47]. Early complications include leakage from the resection site, bleeding, symptomatic stenosis, pulmonary embolism, including a particular risk of portomesenteric venous thrombosis and dehydration. Late complications include stricture, weight gain, and malnutrition [45, 47, 48].

Thus, morbidly obese patients represent a multidisciplinary problem and were until recently considered inoperable because of such limitations. Research findings suggest that bariatric surgical procedures appear to be effective in reversing the effects of morbid obesity prior to KTx and that they may improve access to the surgical field. Thus, LSG is recommended as a feasible procedure and the procedure of first choice for transplant candidates with high BMI.

AVAILABILITY AND EFFICACY OF TRANSPLANTATION CARE FOR CKD PATIENTS AFTER SURGICAL TREATMENT OF OBESITY

Meta-analyses have confirmed that bariatric surgery has higher effectiveness than nonsurgical therapy in achieving sustained weight loss in obese patients in the general population, and including potential renal transplant recipients [49, 50]. In 1996, **Marterre et al.** first described an open gastric bypass anastomosis in three morbidly obese kidney transplant recipients 6–8 years following KTx. The authors reported a significant reduction in body weight, hypertension, post-transplant diabetes mellitus and hyperlipidemia [51]. Since then, successful KTx after weight loss surgery has been directly associated with improved survival and quality of life compared with dialysis [52]. Morbid obesity still remains a significant obstacle to KTx because of suboptimal postoperative outcomes. According to the findings of **Segev et al.**, obese patients were less likely to receive a transplant from a deceased donor after being placed on the waiting list, and they stayed on the waiting list longer [53]. **Gill et al.** published a retrospective analysis of 702,456 CKD patients aged 18–70 years (captured in the US Renal Data System between 1995 and 2007), where they found that obesity affects many interrelated aspects of transplant practice, including candidate selection, prediction of pre- and post-transplant outcomes, and waiting list management [54].

Recently, using laparoscopic gastric resection, patients with CKD have been able to achieve significant weight loss and become eligible for transplantation. **Kim Y. et al.** reported significant improvements in type 2 diabetes mellitus, hypertension, delayed graft function and new-onset diabetes after transplantation in patients with laparoscopic gastric resection compared with kidney recipients without it [44]. Improvements in comorbid conditions such as diabetes, hypertension, and renal function have been reported in three studies [32, 55, 56].

Dziodzio et al. published a review of bariatric surgery in CKD patients before transplantation and found only 8 retrospective studies involving 154 patients. These authors documented weight loss in all published series (weight loss range 21–68%) and noted that gastric bypass was the most effective procedure (weight loss rate 64.3 versus 48.9% after laparoscopic gastric resection). The overall mortality rate was 4.2% for patients with gastric bypass and 3.9% for patients with laparoscopic gastric resection [32].

According to **Hoogeveen EK et al.**, ESRD patients with morbid obesity after LSG before kidney transplantation have improved post-transplant outcomes [58].

CONCLUSION

Obesity in the general population has reached pandemic proportions in recent decades, and as a consequence, this is affecting growth in the population of CKD patients requiring KTx who are simultaneously obese. There is enough evidence in the literature to argue that obesity is a risk factor for surgical complications but not a contraindication for KTx. Outcomes can be greatly improved by multidisciplinary and multimodal treatment strategies. Current techniques with minimally invasive techniques, mainly using robotic and laparoscopic tech-

niques, can dramatically reduce the incidence of surgical complications with comparable graft and survival rates for patients with a non-obese population.

Bariatric surgery is a modern method of treating obesity and related conditions, but its use in patients with severe CKD remains limited because of the risk of severe postoperative complications [4]. However, rapid and persistent loss of excess body weight can significantly reduce blood pressure, compensate for blood sugar levels, which will have an impact on the effectiveness of renal replacement therapy procedures, reducing the frequency and severity of diabetes mellitus [59, 60]. This will lead to earlier inclusion of ESRD patients in the waiting list and will increase the post-KTx survival rate due to better kidney transplant function and lower percentage of graft rejection. In this regard, surgical treatment of obesity should be considered as an intermediate stage of preparation for KTx [33]. One of the minimally invasive methods of treatment for morbid obesity in ESRD patients can be LSG, the results of which have demonstrated effectiveness and safety in abdominal surgery, although nutrient deficiency remains a problem in this situation [61]. In general, these surgeries do not appear to have an adverse effect on absorption of immunosuppressive drugs [62].

Thus, studies on the use of laparoscopic gastric reduction in ESRD patients are important and the study of this method will further increase the availability of transplant care for overweight patients who previously had relative contraindications to surgical interventions.

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SUCCESSFUL SURGICAL CORRECTION OF ASCENDING AORTIC DISSECTION IN A KIDNEY TRANSPLANT PATIENT

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Cardiovascular disease is the leading cause of death in patients with a transplanted kidney and in graft loss. We present the first clinical case of successful surgical correction of ascending aortic dissection (DeBakey type I) in a young patient with a functioning kidney graft. The patient underwent the first cadaveric kidney transplantation (KTx), which was complicated by acute humoral rejection and suboptimal graft function. High blood pressure, anemia, elevated blood levels of triglycerides, phosphorus, parathyroid hormone, and uric acid were recorded. A repeat KTx was performed five years later; the patient's condition and kidney function were satisfactory. Three years later,the patient started experiencing severe pain along the thoracic and lumbar spine; his blood creatinine level was 408 μmol/L. Computed tomography and echocardiography diagnosed DeBakey type I aortic dissection (AD) with critical narrowing of the true aortic lumen at certain levels, dissection of aortic branches. Aortic resection surgery with prosthetic replacement of the ascending aorta according to David procedure with reimplantation of coronary artery orifices according to Kouchoukos technique, prosthetic replacement of the aortic arch with debranching of brachiocephalic artery and left common carotid artery were successfully performed as planned under endotracheal anesthesia, cardiopulmonary bypass and selective pharmacological cold cardioplegia. The peculiarities of the course, possible causes and outcomes of surgical correction of thoracic AD in the patient are discussed.

Keywords: kidney transplantation, vascular calcification, thoracic aortic dissection (DeBakey type I), prosthetic ascending aortic replacement.

INTRODUCTION

KTx is generally recognized as the best modality in renal replacement therapy to achieve satisfactory medical and social rehabilitation, improve the quality of life and increase the life expectancy of patients suffering from end-stage renal disease. Currently, about 1 million renal transplant recipients are registered in the world; they are more than 10,000 in Russia, and the figure is going up annually [1–3]. Cardiovascular diseases remain one of the leading causes of high mortality in kidney transplant patients and early kidney graft loss [4, 5].

Ascending AD (Stanford type A, DeBakey type I) occupies a special place in the structure of cardiovascular diseases. It is a rare, but very serious and dangerous condition, characterized by a high incidence of early fatal complications and mortality. There are very few population data on the prevalence of ascending AD and patient mortality from this disease. The disease is more common in older men, the leading risk factor is chronic high blood pressure [6, 7].

There has been information on proximal AD in patients with chronic kidney disease (CKD) in sporadic publications. It has been previously reported that AD has been found to be the most common cause of sudden cardiac death in dialysis patients [8]. A recent analysis by German researchers involving 14,911 patients presenting with acute type A aortic dissection for surgical intervention between 2006 and 2014 showed that 2,871 (19.3%) of them had CKD [6]. A just-published US study, which used a national database (US Renal Data System database), followed up 461 dialysis patients with type A aortic dissection from 1987 to 2015 [9].

There is even less information on surgical treatment outcomes, short- and long-term survival of patients with end-stage renal disease who underwent proximal aortic grafting, since there are very few such patients in large studies based on the Society of Thoracic Surgeons (STS) data [7, 10, 11]. Thus, according to Lee T.C. et al. [12], early mortality after surgical intervention for acute type A aortic dissection was 17.4%. International registry, which did not include patients with CKD, has documented a decrease in in-hospital surgical mortality from 25 to 18% in type A aortic dissection during the last two decades [13]. However, in the cohort of dialysis patients who underwent surgery for type A aortic dissection, perioperative mortality was 24.3% and 10-year mortality was 87.9 ± 2.2%. Age equal to or over 65 years, congestive heart failure and diabetes mellitus leading to end-stage renal disease were independent risk factors worsening the long-term outcomes of proximal aortic reconstruction [9].

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In our review of open access publications, we found no studies or descriptions of individual cases of ascending AD in patients with CKD who underwent renal transplantation. We present the first such clinical case: a young patient with a functioning renal transplant was diagnosed with DeBakey type I ascending AD; he underwent successful surgical correction.

CLINICAL CASE STUDY

First kidney transplantation

Patient A., 33 years old, has been observed at the kidney transplant department of Vladimirsky Moscow Regional Clinical Research Institute in Moscow since October, 2012. According to the patient, the family has no history of sudden death, thoracic aortic dissection/aneurysm. He has been overweight since adolescence. At the age of 22, proteinuria and increased blood pressure up to 170/100 mmHg were detected after a sore throat, and chronic glomerulonephritis was diagnosed (without histological confirmation). In the next three years, renal function deteriorated to the point of end-stage kidney failure. In the fall of 2012, the patient developed arteriovenous fistula on the left forearm and hemodialysis treatment was initiated. In the spring of the following year, a blood group and HLA-antigen-compatible cadaveric donor kidney was transplanted into the left iliac region. Early postoperative period had no complications, renal graft function was immediate. The patient was discharged three weeks later with the following readings: height 180 cm, weight 109 kg (BMI 33.6), blood pressure 125/80 mm Hg, creatinine 150 μmol/L, estimated glomerular filtration rate (eGFR) 53 mL/min, hemoglobin 101 g/L, albumin 40 g/L, cholesterol 4.5 mmol/L, glucose 5.6 mmol/L, triglycerides 3.0 mmol/L, uric acid 311 μmol/L, phosphorus 0.78 mmol/L, daily proteinuria 0.7 g. Three months later, he underwent surgery to close the arteriovenous fistula.

Deterioration of kidney graft function and increase in blood pressure to 140–150/100 mm Hg were observed since spring 2014. For this reason, repeated inpatient treatment was carried out: pulse methylprednisolone therapy, filtration plasmapheresis courses, nephroprotective and antihypertensive therapy. Renal graft function remained unstable (Table 1). Renal transplant biopsies were performed: report of May 21, 2014 – acute humoral rejection (BANFF2A); report of January 28, 2016 – focal global and segmental glomerulosclerosis with elements of collapsing nephritis, transplant glomerulonephritis (IgA nephropathy); report of October 17, 2017 – IgA nephropathy combined with chronic transplant glomerulopathy (predominance of C4d expression in the periphery of capillary loops). Blood pressure was maintained within the 130–150/80–90 mm Hg interval against the background of combined antihypertensive therapy. Dynamic laboratory examination revealed non-medically correctable slight anemia, as well as shifts in lipid and mineral-bone metabolism (Table 1).

Dynamic echocardiography (EchoCG) visualized dense dilated aorta, bigger left atrium and left ventricular myocardial hypertrophy (Table 2).

Repeat kidney transplantation

In April 2018, an arteriovenous fistula was formed in the lower third of the right forearm due to the progressive deterioration of the renal graft function; hemodialysis was resumed. Two months later, a cadaveric kidney was transplanted to the right iliac region and the first graft was removed. Kidney graft function was delayed, two hemodialysis sessions were performed. The patient's condition was satisfactory. A month later he was discharged for outpatient follow-up. Blood hemoglobin 90 g/L, creatinine 200 μmol/L, (eGFR 37 mL/min), uric acid 550 μmol/L, other biochemical parameters were within the reference interval, daily proteinuria

Table 1

Results of laboratory examination of patient A. after the first KTx

Indicator	First KTx on 26/03/2013						
	November 2013	May 2014	July 2015	January 2016	August 2017	November 2017	January 2018
Urea, mmol/L	9.2	10.7	10.3	20.0	12.2	20.9	13.7
Creatinine, μmol/L	140	170	240	210	270	310	420
eGFR, mL/min	58	46	30	35	26	22	15
Proteinuria, g/day.	0.6	1.5	0.6	2.7	4.6	8.0	10.8
Tacrolimus, ng/mL	7.1	6.4	6.6	6.1	5.3	4.9	8.2
Hemoglobin, g/L	107	99	104	103	108	98	89
Cholesterol, mmol/L	5.8	5.5	5.3	5.3	4.7	3.5	5.1
Triglycerides, mmol/L	2.5	3.2	2.7	3.1	2.6	2.9	2.2
Total calcium, mmol/L	2.53	2.69	2.33	2.42	2.49	2.23	2.26
Phosphorus, mmol/L	1.01	1.53	1.54	1.57	1.86	1.79	1.75
Uric acid, μmol/L	311	401	388	397	511	543	555
Parathyroid hormone, pg/mL	–	116	–	500	477	–	489

1.1 g. EchoCG: moderate left ventricular myocardial hypertrophy; left atrium enlargement; dilation and sclerotic changes in the ascending aorta (Table 5). Morphological examination of the removed graft: graft glomerulonephritis (IgA nephropathy); chronic graft arteriopathy; hyaline arteriolopathy; fibrous calcified atherosclerotic plaques in the renal artery.

In the next 2.5 years, the patient's condition remained satisfactory, renal graft function was stable, and there were no metabolic disorders against the background of medical correction (Table 3).

Diagnosis and surgical correction of DeBakey type I aortic dissection

The patient noted that his condition started deteriorating from early March 2021: increasing weakness, shortness of breath with little physical activity, lower appetite and 8 kg weight loss; from early April, he experienced severe and gradually increasing pain along the thoracic and lumbar spine with irradiation into the abdominal cavity, resistant to analgesics. The patient was admitted to the kidney transplant department of Vladimirsky Moscow Regional Clinical Research Institute on April 9, 2021 with 408 μmol/L creatinine.

Table 2

EchoCG results for patient A. before and after the first KTx

Heart chambers	First KTx on 26/03/2013				
	12/11/2012	12/04/2013	25/01/2015	1/07/2015	20/10/2017
Aorta	Dense	Dense	Dense	Dense	Dense
Aortic root diameter, cm	3.9	4.2	4.0	3.6	3.9
Ascending aortic diameter, cm	3.6	3.6	3.4	3.9	3.5
Aortic valve	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
Regurgitation	no	no	no	no	no
Left atrium, cm	4.3	4.2	4.0	3.9	4.1
Left ventricle					
EDD, cm	5.5	5.3	5.4	5.3	5.4
ESD, cm	3.8	3.1	3.2	3.3	3.2
EDV, mL	151	132	143	135	143
ESV, mL	61	37	40	62	40
EF, %	60	72	72	67	72
Right atrium, cm	3.7	2.1	2.1	2.1	2.1
Right ventricle, cm	3.7	2.8	2.8	2.8	2.8
Pulmonary artery, cm	2.7	1.9	2.7	2.8	2.5
Interventricular septum, cm	1.4	1.3	1.3	1.3	1.4
Mitral, tricuspid and pulmonary valves	Unchanged	Unchanged	Unchanged	Unchanged	Unchanged
Regurgitation	gr. 0–1 (+)	gr. 0–1 (+)	gr. 0–1 (+)	gr. 0–1 (+)	gr. 0–1 (+)

Note. EDD, end-diastolic dimension; ESD, end-systolic dimension; EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction.

Table 3

Results of laboratory examination of patient A. after repeat KTx

Indicator	Repeat KTx on 04/07/2013			
	16/10/2018	12/08/2019	25/05/2020	10/03/2021
Urea, mmol/L	15.4	10.2	11.7	10.2
Creatinine, μmol/L	276	180	203	198
eGFR, mL/min	25	42	36	36
Proteinuria, g/day.	0.7	0.5	0	0.3
Tacrolimus, ng/mL	4.8	5.7	5.6	6.4
Hemoglobin, g/L	97	99	103	101
Cholesterol, mmol/L	4.2	5.4	4.4	4.1
Triglycerides, mmol/L	2.2	1.9	1.6	1.5
Total calcium, mmol/L	2.31	2.47	2.43	2.38
Phosphorus, mmol/L	1.91	1.47	1.23	1.16
Uric acid, μmol/L	550	401	381	366
Parathyroid hormone, pg/mL	193	153	147	–

On examination, he was in a state of moderate severity. Fully conscious and correct physique. Height 180 cm, weight 90 kg (BMI 27.8). The skin and visible mucous membranes were without features. There were no edemas. Body temperature was 36.7 °C. Respiratory and cardiovascular systems had no visible pathology. Respiratory rate was 19 breaths per minute. Heart rate was 80 per min. Blood pressure was 140/80 mm Hg. Systolic murmur over the apex of the heart. Clean and moist tongue. The abdomen was soft, painful in the epigastric and mesogastric regions. No peritoneal symptoms. Audible peristaltic murmurs. Normal stool. The liver and spleen were not palpated. Free urination. Kidney graft in the right iliac region, not enlarged, elastic, painless.

Laboratory examination revealed moderate anemia and drastically reduced renal graft function (Table 4).

The reasons for the severe pain and acute deterioration in graft function were not clear. The patient was referred for abdominal and thoracic computed tomography (CT). X-ray CT scan of the aorta with intravenous bolus contrast (Iomeron 400–99mm) was performed on a multislice CT scanner (256 slices) with ECG synchronization. Cardiomegaly (CTR 53%) was detected. There was fluid in the pericardial cavity with 10 mm layer thickness. The coronary arteries branch off projectionally from their respective sinuses. The aortic root diameter is 44 mm, the ascending aortic diameter is 44 mm, the aortic arch diameter is 37 mm, and the descending aortic diameter is 32 mm. DeBakey type I AD is detected (Fig. 1). The proximal border of the dissection is reliably visualized approximately 46 mm above the annulus level. The dissection extends further into the aorta throughout. There are no signs of intramural hematoma. The true lumen is narrow, about 6 mm wide at the level of origin of visceral branches. In the infrarenal region, there is local narrowing to a filiform level, then again about 6 mm wide. False lumen – maximum width of up to 33 mm in the ascending part and 28 mm in the descending part. No thrombotic masses are detected in the false lumen. The brachiocephalic arteries depart from the aortic arch typically, the left artery departs at the border of the true and false lumens. Transition of AD to the mouths of the left common carotid and subclavian arteries is not excluded. The right common subclavian artery branches depart from the true aortic lumen; the right common, external and internal subclavian arteries contrast homogeneously. The left common subclavian artery branches off from the true and false lumens, its dissection is visualized, its true lumen is narrowed to a critical level. The left internal subclavian artery branches off from the true lumen, the left external one – probably from the true and false lumens, its dissection and narrowing to the threadlike level of the true lumen are visualized, fenestration is detected in the distal part. Abdominal aortic branches: gastric and splenic trunk dissection and splenic artery are visualized; common hepatic artery has no signs of dissection. The superior and inferior mesenteric arteries and the right renal artery branch off from the true lumen, the left renal artery branches off from the false lumen. The artery to the renal graft is visualized from the right external iliac artery with irregular narrowing due to combined plaque (~50%). Conclusion.

Table 4

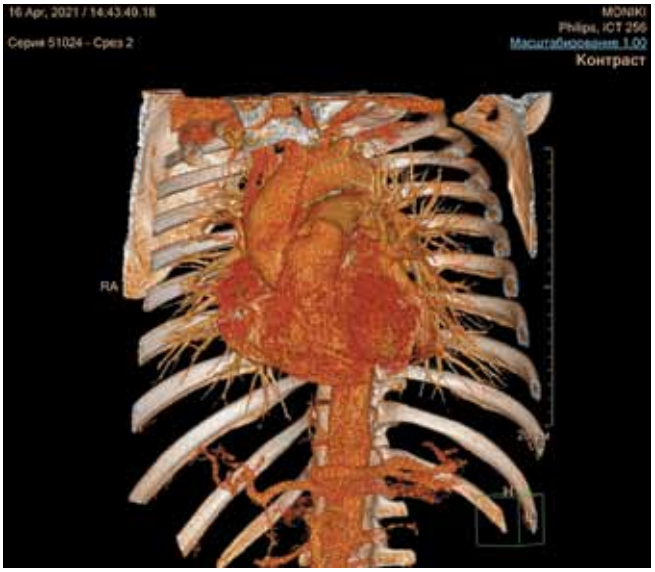
Results of laboratory examination of patient A. before and after surgical correction of DeBakey type I AD

Indicator	Prosthetic replacement of thoracic aorta on 1/06/2021		
	11/04/2021	26/05/2021	28/06/2021
Hemoglobin, g/L	91	105	98
Leukocytes, $\times 10^9$/L	7.8	12.2	8.4
Platelets, $\times 10^9$/L	480	338	370
Total bilirubin, μmol/L	7.3	13.7	16.2
Total protein, g/L	65	70	71
Albumin, g/L	39	32	39
Urea, mmol/L	25.6	15.1	11.1
Creatinine, μmol/L	462	218	168
eGFR, ml/min	14	32	46
Glucose, mmol/L	4.8	4.1	4.7
Potassium, mmol/L	4.5	4.8	4.6
Sodium, mmol/L	137	139	141
Total calcium, mmol/L	2.24	2.52	2.47
ALT, u/L	10	19	23
AST, u/L	15	37	30
Total cholesterol, mmol/L	2.7	2.8	2.9
Proteinuria, g/day	2.28	0.9	0.5
Tacrolimus, ng/mL	7.9	6.7	5.1
SARS-CoV-2 RNA	Not detected	Not detected	–

DeBakey type I AD with critical narrowing of the true aortic lumen at some levels, dissection of aortic branches. Variant of visceral branches originating from the aorta. Narrowing of the artery to the graft. Cardiomegaly.

Ascending AD was confirmed by echocardiography: visualized were dissecting aortic aneurysm (above the sinotubular junction to the visible part of the descending aorta), type 2 aortic insufficiency, dilated left heart chambers, left ventricular myocardial hypertrophy, moderate enlargement of the right heart chambers, dilated pulmonary artery trunk and branches (Table 5).

So, after CT and EchoCG findings, the patient was diagnosed with DeBakey type I AD with critical narrowing of the true aortic lumen at certain levels, dissection of aortic branches. The case conference decided that the patient be transferred to the cardiac surgery department for surgical treatment – aortic prosthesis. On June 1, 2021, under endotracheal anesthesia, cardiopulmonary bypass and selective pharmacological cold cardioplegia (Custodiol solution), the patient underwent aortic resection surgery with prosthetic replacement of the ascending aorta with VASCUTEK Gelweave 30 mm according to David procedure, with reimplantation of coronary artery orifices according to Kouchoukos technique, prosthetic replacement of the aortic arch with multibranched vascular graft VASCUTEK Gelweave 28 mm with debranching of the brachiocephalic artery and left common carotid artery. The surgery lasted for 375 minutes, cardiopulmonary pass and circulatory arrest of internal organs lasted for 197 and 20 minutes respectively. Intraoperative hypothermia was 30–26 °C, cerebral perfusion was bispherical. Histological examination of removed aortic segment: a fragment of muscular-elastic artery, lipid spots in the intima, fibrous atherosclerotic plaques; dissection at the medial layer level with formation of false lumen; focal fragmentation of elastic fibers in the area of dissection.



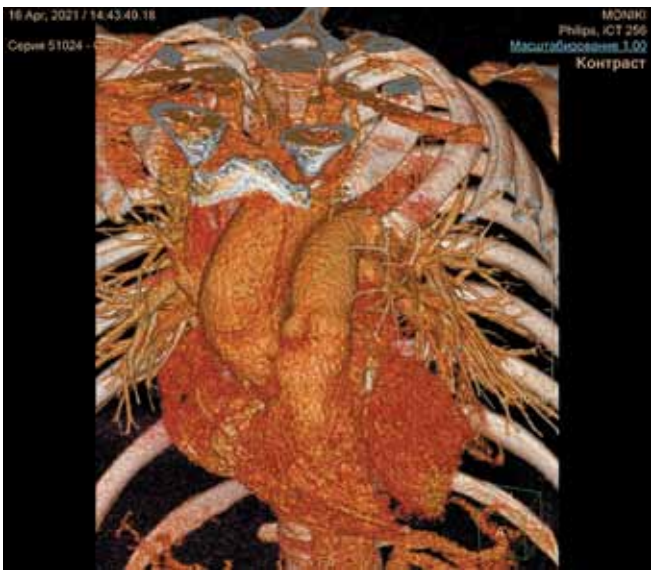


Fig. 1. Preoperative computerized tomography images in kidney transplant recipient with type I aortic dissection

After the operation, the patient was transferred to the intensive care unit, and the next morning, artificial ventilation was stopped (postoperative artificial ventilation lasted for 14 hours). The patient's condition remained stable. On day 7 after the operation, the chest X-ray and CT scans revealed pericarditis and bilateral hydrothorax. Drainage of the right pleural cavity removed 600 mL of hemorrhagic fluid. Due to hemorrhagic discharge continuing through the drainage, the patient underwent urgent resternotomy, revision and sanitation of the pericardial cavity (100 mL of liquid blood and 300 mL of blood clots were removed) and pleural cavities (150 mL in the left, 100 mL of serous hemorrhagic fluid in the right); the source of bleeding was not identified. On day 15 after operation, the patient was transferred in a satisfactory condition to the cardiac surgical department. Control transthoracic EchoCG three weeks after the operation showed decreased size of the left heart and aortic regurgitation (Table 5). Non-contrast-enhanced thoracic and abdominal CT scans: the prosthetic ascending aorta area is homogeneous; a dissection extending to the descending aorta is identified, the left common and external iliac arteries, without dynamics as compared with the previous study, was determined from the aortic arch level (Fig. 2). Kidney graft function remained stable (Table 4). One month after surgery, the patient was discharged for outpatient follow-up with a recommendation for dynamic monitoring and subsequent surgical correction of the descending AD.

The patient was examined on month 3 after surgery. Results of laboratory blood tests: Hemoglobin 115 g/L, urea 10.7 mmol/L, creatinine 190 μmol/L (eGFR 40 mL/min), uric acid 539 μmol/L, albumin 44 g/L, total bilirubin 16.3 μmol/L, total cholesterol 3.1 mmol/L, triglycerides 1.4 mmol/L, C-reactive protein 2.8 mg/L, phosphorus 1.53 mmol/L, other electrolytes and enzymes within

the reference interval, tacrolimus 6.1 ng/mL, no proteinuria. Thoracic and abdominal CT scans: the prosthetic ascending aorta area is homogeneous; with contrast enhancement, the release of the drug beyond the prosthetic aorta is not determined; dissection from the aortic arch level persists, spreading further to the descending aorta; the left common and external iliac arteries, without dynamics as compared with the previous study (Fig. 3). Recommendation of the cardiac surgeon: re-examination and hospitalization for surgical endovascular correction of the abdominal AD in 3–4 months' time.

DISCUSSION

Analyzing the patient's medical history, we paid attention to some peculiarities of its course. In our observation, thoracic AD developed in the young man, whereas such patients are much older both in the general population and in the population with end-stage renal disease. For instance, in the German study mentioned earlier, the mean age of patients with thoracic AD was 58.3 years, and the mean age of dialysis patients who underwent ascending aortic grafting was 64 years (53–73) [6, 9]. It is known that arterial hypertension is the most common predisposing factor for thoracic AD, but in our patient, it was hardly the only factor responsible for this condition. The patient also had no family history of sudden death and thoracic AD, detected in 20–40% of such cases, although he was not subjected to genetic testing [14, 15].

It appears that vascular calcification – intimal (atherosclerosis) and medial (arteriosclerosis) – was the main pathophysiological process leading to dissection of the aorta and its branches in this patient. Simultaneous coexistence of both variants of vascular calcification in patients with CKD before and after KTx is real, but a more characteristic variant, a "specific feature" is the development of medial calcinosis. Factors that procalcify the arterial wall in CKD include male gender, high blood pressure, obesity, long-term dialysis treatment, metabolic disorders, shifts in mineral and bone metabolism, local and systemic inflammation, oxidative stress, uremic toxin

Table 5

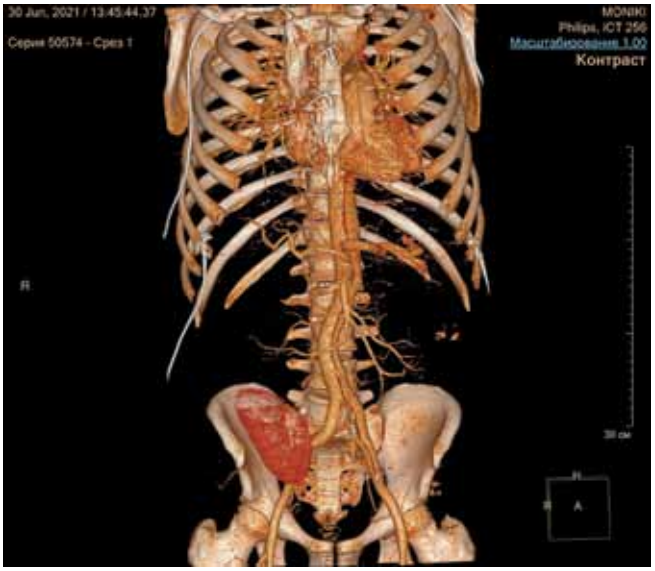
EchoCG results for patient A. before and after surgical correction of DeBakey type I AD

Heart chambers	Prosthetic replacement of thoracic aorta on 1/06/2021		
	12/09/2018	27/04/2021	23/06/2021
Aorta	dense		
Aortic root diameter	4.2 cm	4.5 cm	2.5 cm
Sinotubular junction diameter	–		
Ascending aortic diameter	4.0 cm	3.5 cm 4.7 cm (false lumen 3.0 cm)	3.0 cm (prosthetic part)
Aortic arch diameter	–	3.4 cm	
Descending aortic diameter	–	3.6 cm. Above the sinotubular junction, dissection of the aortic wall up to the visible part of the descending part is detected; a double contour is not clearly visualized in the area of origin of the left carotid artery	
Aortic valve			
Annulus diameter	–	24 mm	24 mm
Regurgitation	gr. 0–1 (+)	gr. 2 (+)	gr. 1–1.5 (+)
Left atrium	4.4 cm, volume 69 mL	4.2 cm, volume 74 mL	3.7 cm
Left ventricle	Hypertrophy	Hypertrophy	Hypertrophy 17 mm
EDD	5.6 cm	5.4 cm	4.7 cm
ESD	3.7 cm	3.1 cm	3.8 cm
EDV	152 mL	141 mL	127 mL
ESV	58 mL	40 mL	45 mL
EF	61%	71%	61%
Right atrium	44 mL	65 mL	46 mL
Right ventricle	31 mm	37 mm	30 mm
Pulmonary artery	Trunk 26 mm	Trunk 34 mm, branches 20 mm	Trunk 28 mm
Interventricular septum	13 mm; LVPW 12 mm	18 mm; LVPW 15 mm	17 mm; LVPW 16 mm
Mitral, tricuspid and pulmonary valves	Unchanged	Unchanged	Unchanged
Regurgitation	gr. 0–1 (+)	gr. 1–1.5 (+)	gr. 0–1 (+)

Note. EDD, end-diastolic dimension; ESD, end-systolic dimension; EDV, end-diastolic volume; ESV, end-systolic volume; EF, ejection fraction; LVPW, left ventricular posterior wall.

accumulation and many others [16–19]. Interesting data on the possible role of uric acid in vascular calcification has been obtained [20]. Vascular calcification formed at the phase of end-stage renal failure is irreversible. After successful KTx, despite partial or complete resolution of the metabolic disorders inherent in CKD, recipients continue to be exposed to some procalcifying stimuli that contribute to progression of pre-existing vascular calcification or its development de novo [18, 21]. In particular, new post-transplantation risk factors for vascular calcification include immunosuppressive therapy with direct and mediated effect on the vascular wall through carbohydrate and lipid metabolism. Another potential factor in the post-transplant period is the often-diagnosed magnesium deficiency associated with inhibition of its tubular reabsorption under the influence of calcineurin inhibitors [22]. Finally, reduced renal graft function is a significant risk factor for vascular calcification [23, 24]. Maréchal S. et al. [25] observed 197 renal transplant recipients for 4.4 years and found a 4%-per-year increase in aortic calcification. In their study, risk factors for its development/progression were aortic calcification before KTx, high pulse pressure, older age, serum phosphorus, male gender, statin and aspirin therapy. A group of Italian researchers, performing multiple regression analysis, found the following predictors for aortic calcification in kidney transplant patients: body mass index, serum magnesium, age, systolic blood pressure, proteinuria, and dialysis duration [23].

Everything described above is applicable to our patient – after KTx, he had all of the above, as well as a number of risk factors atherosclerosis and arteriosclerosis. The duration of chronic renal failure, including the pre-dialysis phase and dialysis therapy, was short; there



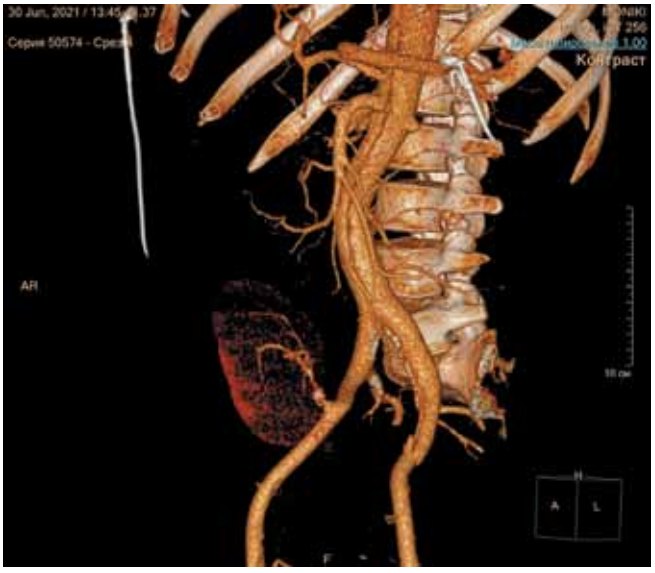
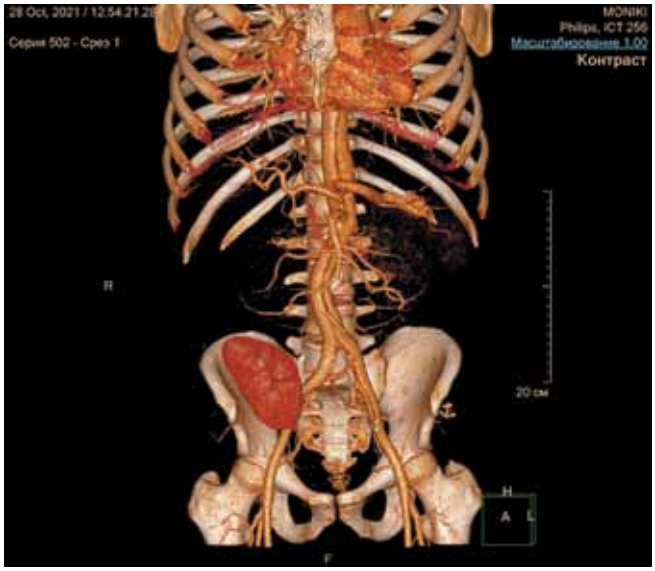


Fig. 2. Postoperative computerized tomography images in kidney transplant recipient with type I aortic dissection



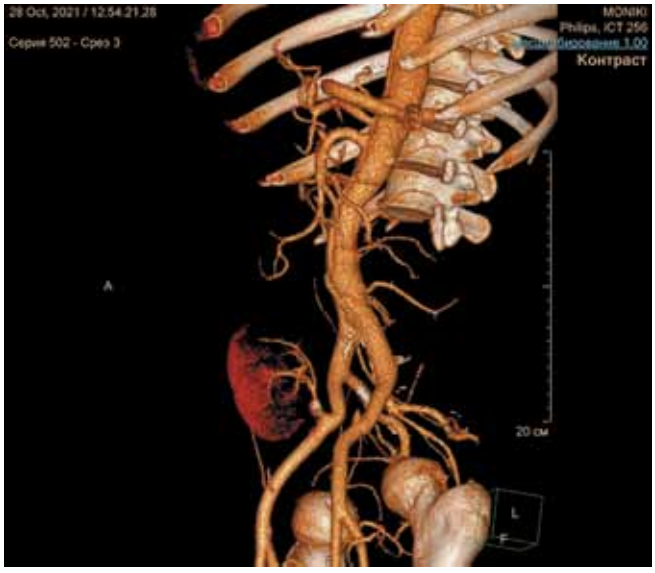


Fig. 3. Computerized tomography images in kidney transplant recipient with type I aortic dissection 3 months after intervention

is no information about the presence of vascular calcification during that period. But it is known that the patient remained overweight for several years before and after KTx. There were high levels of triglycerides in the blood immediately after the first KTx and then for five years until the second KTx. Acute humoral rejection diagnosed one year after the operation, which required pulse methylprednisolone therapy, further reduction in kidney graft function and persistent increasing proteinuria contributed to the aggravation of arterial hypertension and appearance, in addition to hypertriglyceridemia, other metabolic anomalies – hyperphosphatemia, hyperuricemia, and increased parathyroid hormone levels. It is thought that hyperphosphatemia, which was observed for several years after the first KTx, is of the greatest importance in the development/progression of aortic calcification. Currently, high phosphorus levels are considered the main, unique inducer of vascular (medial) calcification in CKD, which is either passively deposited from extracellular fluid into the vascular wall in the form of calcium phosphate, or promotes the acquisition of an osteoblast-like phenotype by vascular smooth muscle cells [26]. All disorders diagnosed in this patient (obesity, long-term suboptimal renal graft function, multiple endocrine and metabolic disorders) most likely, were the pathogenetic mechanisms contributing to the progression, if the process appeared from the onset of CKD, or to the formation of de novo calcification of the aorta and its branches, which, in turn, caused their dissection. The existence of widespread atherosclerosis in the patient was convincingly confirmed by histological examination of the renal artery of the first graft, the removed section of the thoracic aorta, as well as by CT scan of the renal artery of the second graft. Known structural features of the root and ascending aortic wall could also be important for the formation of dissection in this area [27].

The outcome of surgical treatment of thoracic AD in our patient deserves a separate discussion. Surgery is presently the optimal solution for proximal AD, significantly reducing mortality [7]. Aortic root reimplantation surgery with preservation of the native aortic valve was proposed by David T.E. and Feindel C.M. 30 years ago. It now represents a generally accepted and widely used surgical option for proximal aortic aneurysm/dissection, which avoids many early and long-term postoperative complications [28, 29]. In the general population, perioperative mortality and long-term survival rates in proximal aortic grafting are quite optimistic [30, 31].

A group of German surgeons, having summarized more than 25 years of experience of such surgery in 732 patients with proximal aortic root aneurysm and dissection, convincingly demonstrated its high safety and low risk of perioperative complications and mortality. The authors also confirmed the excellent long-term outcomes of David's procedure and stable aortic valve function in the majority of patients [32].

In dialysis patients, both perioperative and long-term mortality in proximal aortic grafting were very high, which, apparently, is due to kidney failure and chronic dialysis with associated various shifts in homeostasis [9]. However, high perioperative and long-term mortality should not serve as a reason to delay urgent lifesaving intervention for acute type A aortic dissection in a patient with end-stage renal disease. There is no information about the immediate and long-term outcomes of surgical treatment of AD in kidney transplant recipients. It is known that, in general, patient survival after KTx is much better than in the dialysis population, and if a dialysis patient underwent KTx after aortic grafting, his life expectancy was longer than that of those who remained on dialysis [9].

In our patient, a complex operation to replace the ascending aorta and aortic arch, despite early postoperative complications (right-sided hydrothorax, pericarditis), which required repeated surgical intervention, ended successfully. Apparently, young age, relatively small aortic diameter, absence of severe heart failure, diabetes mellitus and serious homeostatic disorders with a satisfactorily functioning re-transplanted kidney contributed to a good surgical outcome.

CONCLUSION

Patients with CKD represent a high-risk population for cardiovascular disease. Thoracic aortic dissection/aneurysm is a rare vascular condition that nevertheless requires special clinical vigilance due to high incidence of fatal complications. Vascular calcification, which is common in CKD, is a predisposing factor for thoracic aortic dissection/aneurysm. Surgical intervention – aortic root grafting – is the treatment of choice for proximal AD, although immediate and long-term outcomes in patients with end-stage renal disease are not as optimistic as in the general population.

This present clinical case has demonstrated the successful outcome of scheduled surgical correction of proximal AD in a patient with end-stage renal disease, who has undergone two kidney transplants. The presence of comorbidity in CKD patients before and after renal transplantation requires a cautious approach to the management tactics for proximal AD, proper, careful selection and preparation of such patients, especially for a scheduled grafting.

The authors declare no conflict of interest.

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