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Глубокоуважаемые коллеги!

Представляя первый в 2021 году выпуск журнала, хотелось бы, как всегда, наметить планы и определить перспективы развития на предстоящий год.

2020 год был особенным – мы все оказались перед необходимостью жить и работать в условиях беспрецедентной по масштабу пандемии, вызванной новой коронавирусной инфекцией SARS-Cov-2. 2020 год создал новую реальность, которая сохранится, вероятно, и после окончания кризиса. Одна из ее характеристик – быстрое развитие и внедрение цифровизации, применение интерактивных технологий, что позволило получить неоценимый дополнительный опыт.

Большая часть мероприятий – научных, учебных, организационных – проводилась в дистанционном режиме. Так, в интерактивном дистанционном формате 5–7 октября прошел X съезд трансплантологов с участием специалистов из ведущих федеральных центров, из научных и клинических учреждений регионального уровня, а также дальнего и ближнего зарубежья. Организаторы, научный комитет приложили все усилия, чтобы это мероприятие, несмотря на непривычный формат, было полноценным и информативным для всех его участников. Материалы съезда традиционно опубликованы в Приложении к нашему журналу.

Ставшие привычными формы дистанционной работы, которые распространились на проведение научно-практических форумов, заседаний ученых советов и даже диссертационных советов, отчасти останутся в нашей жизни. Однако ничто не может заменить живое общение. Рассчитываем, что очередной, V конг-

2021: A NEW REALITY AND CONVENTIONAL PRIORITIES

Dear colleagues!

As we present the first issue of the Journal in 2021, we would like, as always, to outline our plans and perspectives for the coming year.

The year 2020 was an extraordinary one. We all found ourselves having to live and work under an unprecedented pandemic caused by the new coronavirus infection SARS-CoV-2. The year 2020 brought up a new reality that is very likely to persist beyond the end of the crisis. One of its realities is the rapid development and adoption of digitalization and the use of interactive technology, which has provided invaluable additional experience.

Most events, scientific, educational, or organizational events, were held remotely. For instance, on October 5-7, the 10th Transplant Congress was held in an interactive remote format with the participation of specialists from leading federal centres, from regional research and clinical institutions, and from far and near abroad. The organizers and the scientific committee made every effort to ensure that this event, despite its unusual format, was complete and informative for all participants. The materials of the Congress are traditionally published in the Appendix to our journal.

Working remotely, which has become a commonplace and has spread to scientific and practical forums, meetings of academic councils and even dissertation councils, will partly remain in our lives. However, nothing can replace real-world communication. We hope that the next 5th Organ Trans-

ресс «Трансплантация и донорство органов» в сентябре 2021 года пройдет в Москве уже в очном режиме.

Для «Вестника трансплантологии и искусственных органов» стратегическим приоритетом является обеспечение стандартов качества научных публикаций. В течение нескольких предшествующих лет были сделаны важные шаги по пути совершенствования нашего журнала как полноценного и авторитетного инструмента научного процесса. Безусловно, и индексация в международных информационных системах Scopus и WoS, и издание полнотекстовой англоязычной версии, и расширение тематики публикаций немало способствовали увеличению известности и популярности журнала. Однако необходимо осознать, что главным является содержательная ценность: насколько публикуемые статьи востребованы научным сообществом и в нашей стране, и в мире; насколько состоятелен уровень исследований; насколько качество и форма представления материалов отвечают современным стандартам.

Именно эти вопросы будут главными для нас в 2021 году; они же должны составить предмет усилий и авторов, и редакторов в долгосрочной перспективе.

С уважением,
академик РАН С.В. Готье

plantation and Donation Congress coming up in September 2021 will be held in Moscow in a face-to-face format.

The strategic priority of the Russian Journal of Transplantology and Artificial Organs is to ensure quality standards for research publications. Over the past few years, important steps have been taken to improve our journal as a full-fledged and reputable tool for the scientific process. Indexing our journal in Scopus and WoS, publication of a full-text English version, and expansion of research topics have very greatly boosted the fame and popularity of the Journal. However, we must realize that the main thing here is the content value. We must ask ourselves the following questions: To what extent are the published papers in demand among the scientific community in Russia and in the world? How sound is the level of research? Does the quality and form of presentation of materials meet modern standards?

These issues will play a central role in our work in 2021. They should also be the long-term focus of authors and editors alike.

Sincerely,
S.V. Gautier,
Member, Russian Academy of Sciences

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LIVER STEATOSIS IN BRAIN DEATH DONORS

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Objective: to study the frequency of fatty hepatosis in liver biopsies of consecutive brain death donors before cold preservation. **Materials and methods.** Liver biopsies (before cold preservation) of 300 consecutive donors with brain death were studied. Histological preparations were stained with hematoxylin and eosin, and tricolor Masson staining was performed. **Results.** The frequency of different degrees of fat hepatosis in men and women did not differ significantly (>0.05). Fat dystrophy of hepatocytes was absent in more than half of the cases (n = 182; 60.7%). A slight degree of fatty degeneration was diagnosed in 57 (19,0%) donors. In total, 239 (79.7%) donor livers were absolutely suitable for transplantation. Moderate degree of steatosis, which is associated with early biliary complications, was detected in 18 (6.0%) cases, and severe degree, which is a contraindication to the use of the organ for transplantation, was detected in 43 (14.3%) cases. **Conclusion.** Before cold preservation, liver from brain death donors is relatively rarely unsuitable for transplantation.

Keywords: donor liver, biopsy, steatosis.

INTRODUCTION

Donor liver steatosis is one of the important morphological criteria to determine the suitability of an organ for transplantation. With an increase in the number of patients with diabetes and obesity, non-alcoholic fatty liver disease is becoming more common, affecting a quarter of adults worldwide [1]. This disease is manifested either as simple steatosis or non-alcoholic steatohepatitis [2]. Currently, donor liver with steatosis has been used for transplantation. The use of steatous liver is associated with both a lack of donor organs and an increase in the prevalence of fatty liver disease in the general population [3, 4].

Increase in the degree of macrovesicular steatosis of the donor liver above 30% is well known to associate with the risk of increased reperfusion damage, thus increasing the incidence of primary non-functioning graft and its lower survival [1, 5, 6]. However, there is more and more evidence that, with careful selection of recipients, donor liver with moderate and severe macrovesicular steatosis can be successfully used for transplantation [1, 5, 6]. After liver transplantation with steatosis, a decrease in its degree is observed [5].

However, donor liver with steatosis is more susceptible to ischemic damage during cold preservation [7]. Such organs are poorly restored after transplantation. Ischemic and reperfusion injury disrupts microcirculation due to disruption of the vascular endothelial lining [8], increases oxidative damage to mitochondria, and also increases neutrophil aggregation and leads to an imbalance in cytokine release [6]. All this increases the risk of organ dysfunction after transplantation [7].

Therapeutic approaches to expand the use of steatosis donor liver transplantation are currently being intensively studied. Preconditioning of the donor liver can reduce the accumulation of xanthine and suppress the activity of xanthine oxidase, which increases with steatosis during cold ischemia, and thereby protect it from damage. Several pharmacological agents have also been shown to be effective in protecting donor liver with steatosis from ischemic and reperfusion damage.

The present study was aimed at investigating the nature and degree of steatosis in the liver of brain death donors before its cold preservation.

MATERIALS AND METHODS

The liver biopsies of 300 consecutive brain death donors were histologically examined. The biopsies were performed prior to cold preservation of the donor liver. Biopsies were fixed in 10% neutral formalin and embedded in paraffin. From paraffin blocks, histological sections with a thickness of 4–5 μm were prepared, which were stained with hematoxylin and eosin, as well as by Masson's method. The preparations were studied in the bright field of a Leica DM 6000B microscope. Statistical processing of the results was carried out using the statis-

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tical software package Statistica 7.0 (StatSoft, USA) and MS Office EXCEL (Microsoft, USA). The significance of the differences was assessed by the Student's test. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Hepatic steatosis was qualitatively assessed on the basis of determining the size of fatty vacuoles in the cytoplasm of hepatocytes. Microvesicular, medium-vesicular, and macrovesicular steatosis were distinguished. Many researchers [9] do not differentiate medium droplet steatosis. However, in our opinion, it is advisable to do this, since in case of medium-drop fatty hepatosis, fatty degeneration of hepatocytes occurs, but they remain viable and after the cessation of exposure to damaging factors (often after liver transplantation), their complete repair occurs.

The presence of only small vesicles in the liver in the cytoplasm of hepatocytes, even if their diffuse distribution took place, in our opinion, is not fatty hepatosis, but is a simple fatty infiltration, which has a transient nature and, most often, alimentary origin. According to the literature [10], the function of hepatocytes is preserved.

In macrovesicular steatosis, large fatty vesicles occupy almost the entire space of the cytoplasm, pushing the nucleus to the periphery of hepatocytes. They are in a state of parabiosis, and, more often than not, their death occurs.

The severity of hepatic steatosis is semi-quantitatively estimated by the percentage of hepatocytes containing lipids [11]. Usually, with steatosis, vesicles of various sizes (small, medium and large) are present in the cytoplasm of hepatocytes in the liver. Therefore, we determined the severity of fatty hepatosis by the number of hepatocytes in which medium- or macrovesicular fatty degeneration prevailed.

The presence of fatty vesicles in less than 5% of hepatocytes, we, like other researchers [11], did not refer to the category of steatous liver. Mild fatty hepatosis was diagnosed on the condition that from 5% to 30% of hepatocytes contained medium-vesicular and/or macrovesicular fatty vacuoles. Such a liver is quite suitable for transplantation, provided that there are no other pathological processes in it. The case is presented of our own observation. Donor D., woman of 62 (biopsy No. 7010-18 of 09.08.19). Death occurred due to acute cerebrovascular accident of the hemorrhagic type. Some biochemical parameters at the time of biopsy were as follows: bilirubin 11, 7; ALT/AST = 21/23. The liver is of average size, yellow color, with rounded edges. Histology revealed coarse fatty degeneration in less than 30% of hepatocytes (Fig. 1). Conclusion: mild fatty hepatosis, no fibrosis (F0).

Unfortunately, a mild degree of fatty hepatosis can be combined with another pathology, which casts doubt on the possibility of its transplantation. For example, donor S., a man of 46 (biopsy No. 7277-79 of 16.08.19), whose death occurred due to acute cerebrovascular accident of the hemorrhagic type (bilirubin 38.9; ALT/AST = 16.8/13.3); the liver of average size, gray-brown color, with sharp edges. Histology revealed macrovesicular fatty degeneration in less than 30% of hepatocytes mainly located in the periportal zones. However, in addition, many hepatocytes had sandy nuclei, which is a manifestation of severe dystrophy. The portal tracts were fibrosed with multiple septa (Figs. 2, 3). Based on this, the following conclusion was made: mild fatty hepatosis, hepatocyte dystrophy, moderate liver fibrosis (F2).

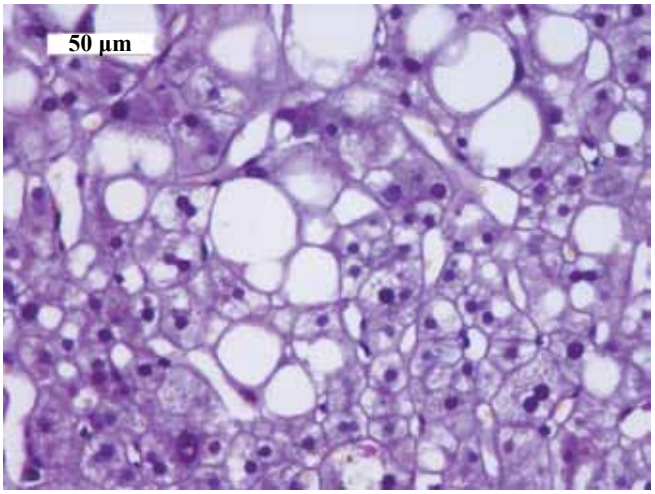

Fig. 1. Donor D., female, 62. In the picture: a small group of macrovesicular fatty dystrophy of hepatocytes. Masson's trichrome stain. Microscope, ×40

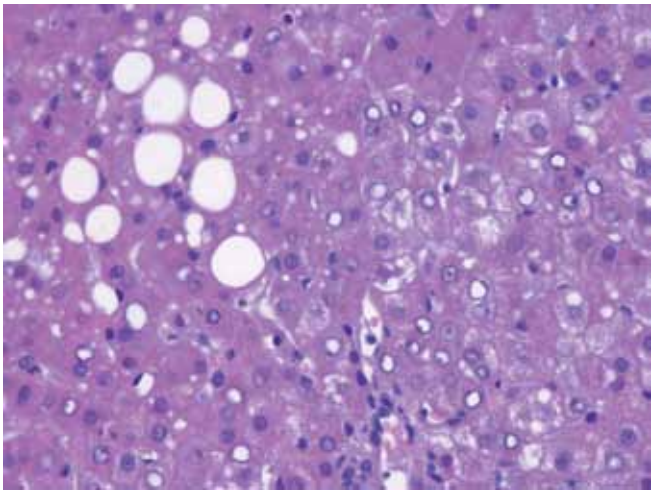

Fig. 2. Donor S., male, 46. In the picture: a small group of macrovesicular fatty dystrophy of hepatocytes. A large number of hepatocytes with sand poisons. Masson's trichrome stain. Microscope, ×40

Moderate (medium) degree of fatty hepatosis was diagnosed in the presence of 30% to 60% of hepatocytes with fatty vacuoles. Moderate, especially moderate vesicular, steatosis is a relative contraindication for liver transplantation. Donor P., male of 56 (biopsy No. 5644-45 of 03.10.17). Death of the brain as a result of closed traumatic brain injury. Total bilirubin 41.1, AST/ALT = 18.2/47.0. The liver of normal size, yellow-gray color, with moderate swelling, rounded edges. Histology revealed large-drop fatty degeneration in less than 60% of hepatocytes (Figs. 4, 5). Mild liver fibrosis. Conclusion. Moderate degree of coarse fatty hepatosis. F1.

Moderate steatosis is a relative contraindication for liver transplantation unless other lesions are present in the liver biopsy. Here is one of our observations, in which there was a combination of a moderate degree of medium vesicular steatosis with a severe degree of ischemic damage. Donor R., male of 33 (biopsy No. 1217-19 of 02/08/19). Brain death occurred as a result of a closed traumatic brain injury. Total bilirubin –20.2. AST/ALT = 2200/1990. The results of histological examination showed a violation of the beam and lobular structure of the liver. Large foci (up to 50% of the area of the preparation) of severe balloon dystrophy and necrosis of hepatocytes. Fatty degeneration of other hepatocytes (Figs. 6, 7). Conclusion: severe ischemic liver damage; moderate degree of medium vesicular fatty hepatosis; no fibrosis (F0).

If the number of hepatocytes with obesity was 60% or more, then such a liver was classified as severe fatty

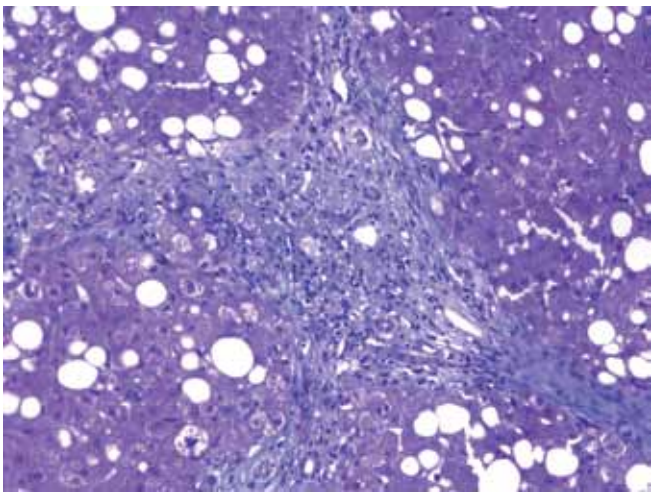

Fig. 3. The same specimen. In the picture: sclerosed portal tract with septa. In periportal hepatocytes – polymorphic fatty dystrophy. Masson's trichrome stain. Microscope, ×40

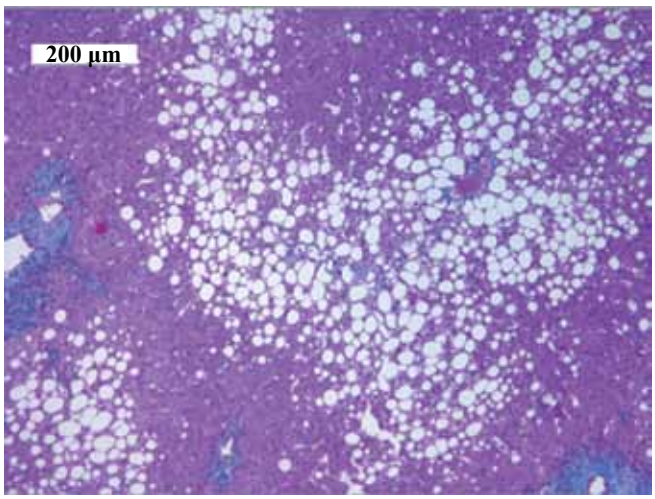

Fig. 4. Polymorphic fatty dystrophy in less than 60% of hepatocytes. Masson's trichrome stain. Microscope, ×10

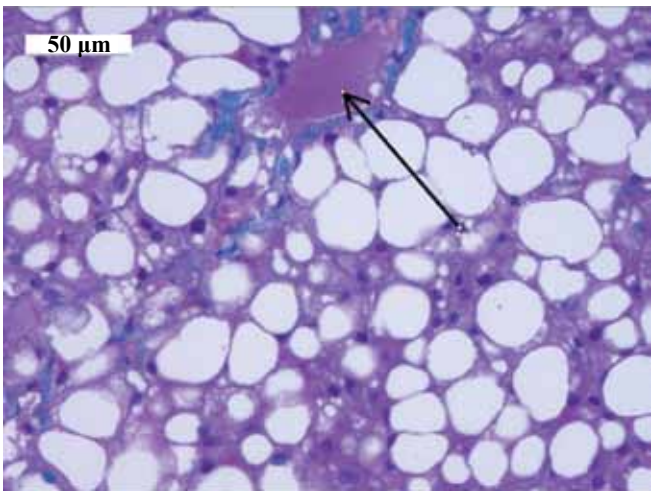

Fig. 5. The same specimen. Polymorphic fatty dystrophy of hepatocytes under high magnification of the microscope. The arrow indicates the central vein. Microscope, ×40

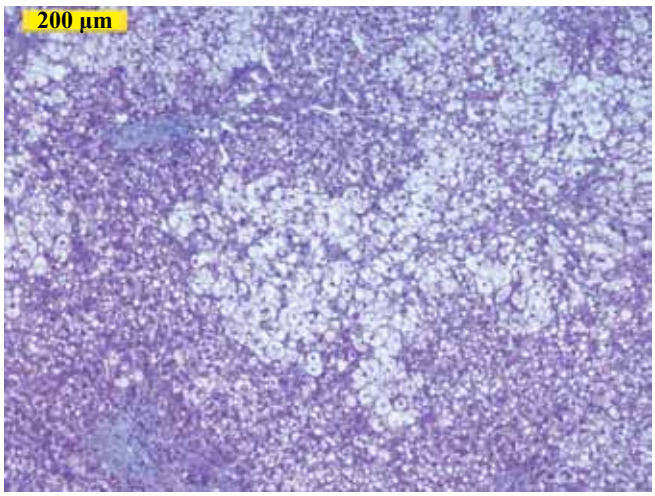

Fig. 6. The focus of balloon dystrophy of hepatocytes, on the periphery of which medium-drop fatty dystrophy is less than 60% of hepatocytes. Masson's trichrome stain. Microscope, ×10

hepatosis. Donor L., male of 50 (biopsy No. 7019-21 of 08/09/19). Brain death occurred due to acute hemorrhagic disorders of cerebral circulation. Basic clinical data (at the time of biopsy): ALT/AST = 47/89, bilirubin 20.3. The enlarged liver, of yellow color, with sharp edges. Histology revealed polymorphic fatty degeneration of about 80% of hepatocytes (Fig. 8). Conclusion: severe fatty hepatosis. F0.

With a severe degree of fatty hepatosis, in some cases, fatty hepatosis developed into steatohepatitis. The case of these observations is presented. Donor V., male of 65 (Biopsy No. 7244-52 of 22.08.19). Acute cerebral circulation disorder of hemorrhagic type. Brain death. Bilirubin 46.3, ALT/AST = 50/43. The liver is enlarged, of gray-yellow color. Histology revealed large droplet fatty degeneration in more than 60% of hepatocytes. The rest of the hepatocytes were with hydropic protein dystrophy. The portal tracts are sclerosed with the formation of septa, moderate proliferation of the bile ducts and with pronounced polymorphic leukocyte (mainly mononuclear) infiltration (Fig. 9). Figs. 10 and 11 show the pathology at high magnification. Conclusion: steatohepatitis, moderate liver fibrosis (F2).

The obtained results of the study of various degrees of fatty hepatosis in donors with brain death before cold storage are shown in Fig. 12. Fatty degeneration of hepatocytes was absent in more than half of the observations (n = 182; 60.7%). Mild fatty degeneration, diagnosed in 57 (19.0%) donors, is not a contraindication for liver transplantation. So, initially 239 (79.7%) of 300 donor livers were suitable for transplantation. Moderate degree (II), which is associated with early biliary complications, was identified in 18 (6.0%) cases. Severe degree (III), which is a contraindication to using the organ for trans-

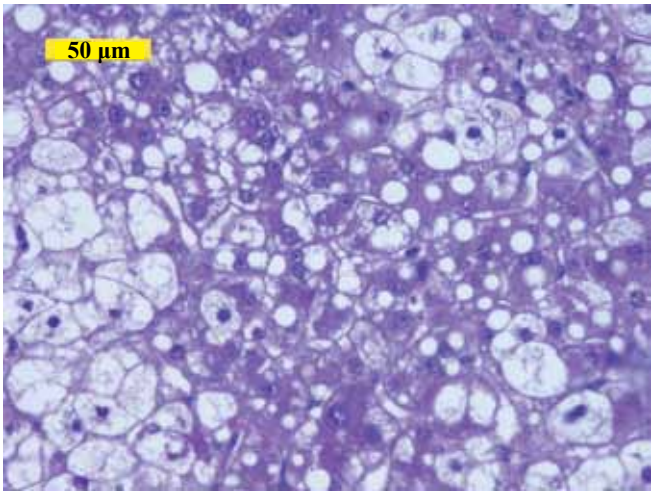

Fig. 7. The same specimen. Microscope, ×40

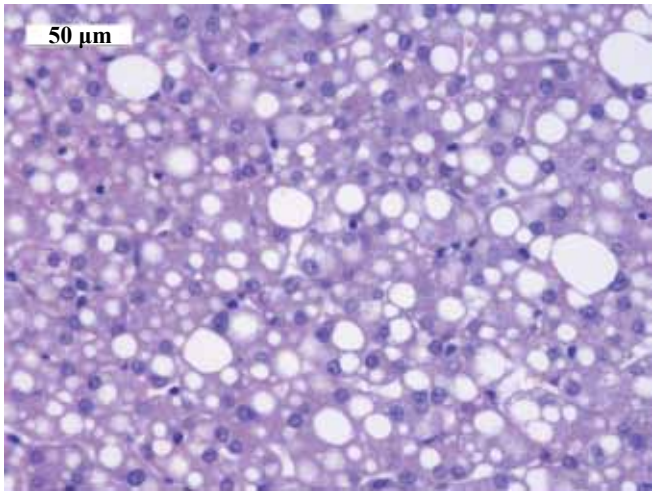

Fig. 8. Polymorphic fatty dystrophy of about 80% of hepatocytes. Masson's trichrome stain. Microscope, ×40

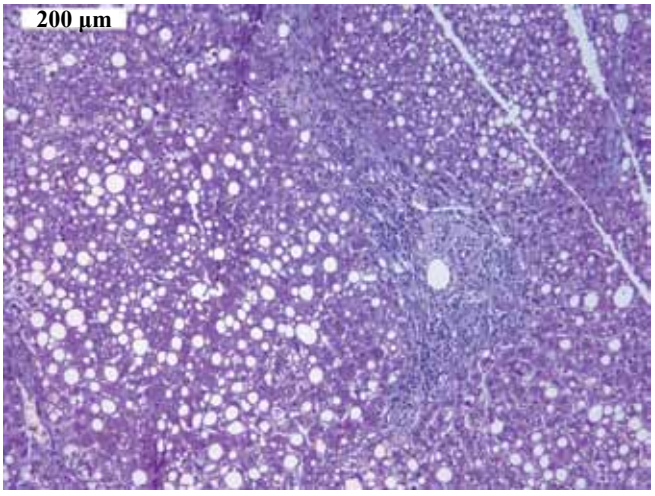

Fig. 9. Polymorphic, mostly middle vesicle fat dystrophy of more than 60% of hepatocytes. Sclerosis of the portal tract with septa. Masson's trichrome stain. Microscope, ×10

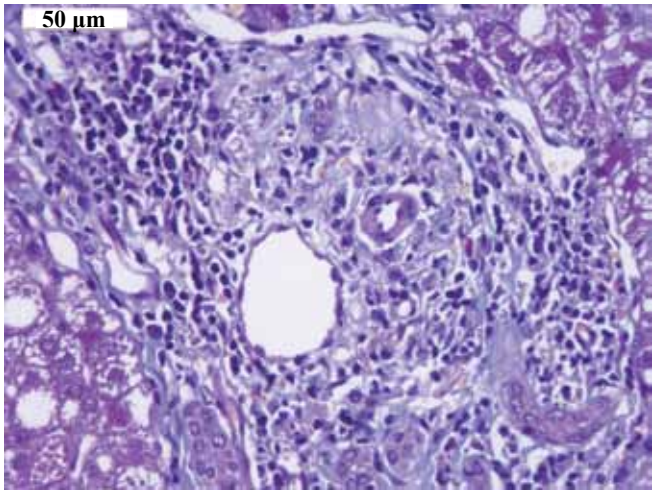

Fig. 10. In the sclerosed portal tract, there is a dense inflammatory infiltration by mononuclear cells. Masson's trichrome stain. Microscope, ×40

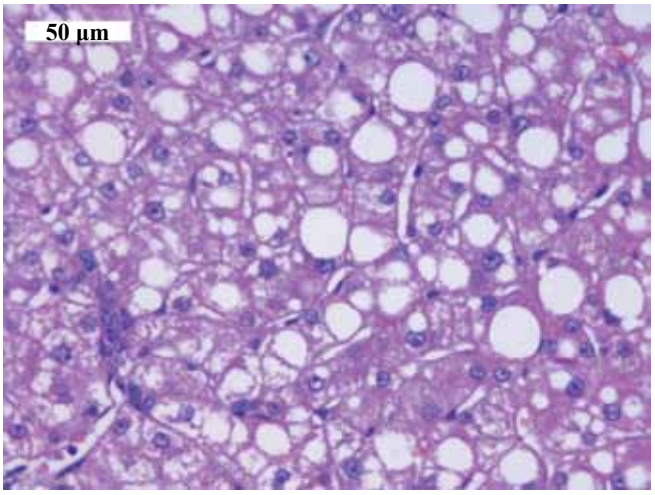

Fig. 11. Polymorphic, mainly medium-vesicular, fat dystrophy of more than 60% of hepatocytes. Masson's trichrome stain. Microscope, ×40

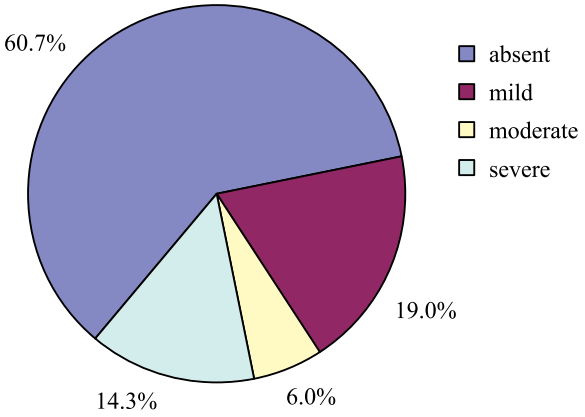

Fig. 12. Various degrees of steatosis in livers of brain death donors before cold preservation

plantation, was diagnosed in 43 (14.3%) donors. The number of donors who belong to donors with extended criteria we attributed the liver with moderate and severe fatty hepatosis (n = 61; 20.3%). The incidence of various degrees of fatty hepatosis in men and women did not differ significantly (>0.05). Therefore, the groups were not divided by gender.

With the increasing demand for donor organs, a higher number of donor livers with steatosis are used in liver transplantation [12]. Recent studies have shown that transplantation of a donor liver with steatosis does not significantly increase the risk of poor outcomes of the transplantation [6]. J.A. Steggerda et al. (2020) [13] raised the threshold limits for the degree of macrovesicular steatosis to 50%. The steatosis degree sharply decreases after liver transplantation [14], which is an additional argument in favor of the possibility of using a donor liver for transplantation with moderate to severe steatosis [6].

CONCLUSION

1. Of the consecutive 300 donors, 239 (79.7%) had no fatty hepatosis or had mild hepatosis, which is not a contraindication for liver transplantation.
2. A relative contraindication to the use of a donor liver for transplantation was in 18 (5%) donors with a moderate degree of steatosis. An absolute contraindication for transplantation is complicated steatosis with inflammation and fibrosis (steatohepatitis).
3. Severe fatty hepatosis was diagnosed in 43 (14.3%) donors. Transplantation of such a liver is permissible in emergencies (urgent surgery) when there is a high risk of near death of the recipient and no more acceptable donor livers.

The authors declare no conflict of interest.

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OUTCOMES OF LIVER TRANSPLANTATION IN THE ERA OF MODERN ANTIVIRAL THERAPY FOR HEPATITIS C

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The emergence of direct-acting antivirals (DAAs) has become the basis for a new potential treatment for chronic hepatitis C (CHC) in patients with decompensated cirrhosis, who previously had no other alternative than liver transplantation (LT). However, optimal timing of antiviral therapy (AVT) remains an issue. **Objective:** to present a spectrum of clinical outcomes in LT waitlisted patients with HCV-related cirrhosis, who received and did not receive DAA therapy. **Materials and methods.** Enrolled for the study were 49 waitlisted patients with HCV-related end-stage liver diseases. The patients were divided into 2 groups: Group 1 included 40 patients who received DAA therapy before LT, while Group 2 consisted of 9 patients who did not receive antiviral treatment while on the LT waiting list. **Results.** The sample was represented in most cases by patients who had MELD/Na score <20. Only six had MELD/Na score >20, but <25. At the time of analysis, 38 patients had reached 12 weeks post AVT. Of these, 35 (92.1%) had sustained virologic response (SVR). Of these, 51.4% (n = 18) of cases showed decreased MELD/Na. There were no changes in 22.9% (n = 8). Increased MELD/Na was noted in 25.7% (n = 9). In 42.8% (n = 15) of cases, sustained elimination of HCV infection led to delisting. Among patients without SVR, increased MELD/Na was observed in all cases (n = 3). In the non-AVT group, one patient showed improved liver function (11.1%); in the rest, MELD/Na either remained stable or continued to increase – 44.5% (n = 4). A comparison of the frequency of deaths depending on AVT showed statistically significant differences ($p < 0.001$, $V = 0.728$). Among the non-AVT patients, the likelihood of waitlist death increased 66.5 times (95% CI: 7.99–554). **Conclusion:** DAA therapy carries significant advantages for waitlisted patients with MELD/Na score <25.

Keywords: waiting list, liver transplantation, antiviral therapy, direct-acting antivirals.

INTRODUCTION

For decades, chronic hepatitis C (CHC) has remained the most common indication for orthotopic liver transplantation worldwide [1]. The emergence of direct-acting antivirals (DAAs) for the treatment of hepatitis C virus (HCV) infection has revolutionized the field of liver transplantation (LT). The main achievements in modern antiviral therapy (AVT) regimens are high efficacy and a favorable safety profile for both patients with decompensated cirrhosis (Child–Turcotte–Pugh (CTP) classes B and C) and those in the post-transplant period [2]. However, a new subject of discussion now centers on the choice of optimal timing of DAA therapy in LT waitlisted patients [3].

Every year, new evidence emerges indicating that sustained virologic response (SVR) in patients with decompensated cirrhosis (CTP classes B and C) can lead to stabilization or relative compensation of liver function, which suggests that post-transplant outcomes can be improved and the need for liver transplantation may be reduced in this large patient cohort [4].

However, CHC treatment in LT candidates is recommended if the MELD (Model for End-stage Liver Disease) score does not exceed 20. The choice of AVT regimens in this patient cohort is limited by contraindications for protease inhibitors. In the Russian Federation, clinicians are limited to three AVT regimens: sofosbuvir/daclatasvir, sofosbuvir/ledipasvir, and sofosbuvir/velpatasvir with or without ribavirin. While for liver recipients, there are no limits to therapeutic possibilities within DAAs regimens [5].

There is also contradictory evidence on antiviral treatment in patients with hepatocellular carcinoma (HCC). Since for this group of patients, the indications for orthotopic liver transplantation (OLT) are often not associated with the functional state of the liver, achievement of SVR will therefore not affect prognosis. Moreover, there are opinions that DAAs promote HCC progression and recurrent tumor process in the postoperative period [6, 7].

An important factor affecting the efficiency of a transplant center is the state of the donor resource. Often, a shortage in donor organs can lead to higher waiting times for liver transplantation and more critical decompensation and deaths before operation. Therefore, a successful AVT can become an integral tool for improving waitlist survival [8].

While discussions continue, clinical practice is expanding our knowledge of the impact of AVT on liver

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tissue functionality and developing individual approaches to navigate these competing issues.

MATERIALS AND METHODS

From March 2016 to April 2020, indications for OLT were established in 153 patients at the transplant center of Vladimirsky Moscow Regional Research Clinical Institute. Enrolled for the study were 49 (32%) OLT waitlisted patients with CHC-related end-stage liver diseases. The patients were divided into 2 groups: Group 1 included 40 patients who received DAA therapy in the LT waiting list, while Group 2 consisted of 9 patients who did not receive antiviral treatment while in the waiting list. In the study group, the following outcomes were observed: liver transplantation, delisting, dropout (delisting due to contraindications to transplantation), death.

The studied medical documentation fully met the requirements of the study: it contained the necessary information about patient's physical status, data from laboratory and imaging examinations to assess the dynamics of liver function and complications of cirrhosis during the observation period in the waiting list and in the post-transplant period.

The dynamics of liver function was assessed by calculating the MELD-Na score at inclusion in the OLT waiting list and at the time of outcome. The difference between these indicators shows the dynamics of the state and is represented by Δ MELD-Na. Patients were delisted at our center with a stable MELD-Na <15 for 6 months.

The AVT on the waiting list was performed according to current guidelines for the treatment of CHC in patients with cirrhosis. For decompensated liver cirrhosis (CTP classes B and C), the treatment regimen was sofosbuvir + daclatasvir, and three patients with compensated cirrhosis and HCC were prescribed protease inhibitor regimens (ombitasvir/ritonavir/paritaprevir + dasabuvir or glecaprevir/pibrentasvir). The following virologic response criteria have been adopted to assess the efficacy of antiviral treatment:

– SVR – the HCV RNA PCR test came out negative 12 weeks after the end of AVT;
– recurrence – HCV RNA PCR test came out positive 12 weeks after the end of AVT [9].

Since all waitlisted patients had different periods up to the moment of outcome, we used the person-years index to standardize the indicators of certain events in the study groups. This indicator was introduced by the professional community of specialists in the field of organ donation and organ donor transplantation and is used in the Scientific Registry of Transplant Recipients (SRTR), USA to assess waiting list outcomes. The person-years index was calculated by dividing the number of days spent by each candidate on the waiting list by 365.25 (average number of days in a year). The coefficient of the outcome of interest was determined by dividing the number of cases by the sum of person-years in the study group and multiplied by 100 (expressed in units – the number of cases per 100 person-years) [10].

$$\text{Person-years index} = \frac{\text{Waiting list time}}{365.25}$$

$$\text{Outcome coefficient} = \frac{\text{Number of outcome cases}}{\text{Sum of "person-years" indices in the study group}} \times 100$$

This makes it possible to compare the true waitlist mortality at different periods of the program and between transplant centers, regardless of the absolute number of patients standing on the list and the waiting time of each candidate. So, if there were 100 waitlisted candidates between January 1 to December 31, 25 of them were observed in the list for 90 days from this period, while 75 were observed for 80 days, then the sum of the person-years indices will be (90/365.25) × 25 + (180/365.25) × 75 = 43.12. If at the same time, 30 patients died, then the outcome rate would be 30/43.12 × 100 = 69.5 deaths per 100 person-years. In other words, this indicator characterizes outcomes on the waiting list, where 100 people were observed within one year.

Data analysis was carried out using statistical software Statistica 13 and the Jamovi program (The jamovi project, 2020). To characterize the studied cohort for all statistical parameters, descriptive statistics were used, which was determined by the type of statistical parameter. Indicators with normal distribution are represented by the following values: mean value of the sample and standard deviation. To describe quantitative parameters with abnormal distribution, the median, 25 and 75 quartiles, were used. To assess the normal distribution of quantitative data, the Shapiro–Wilk test, skewness and kurtosis indicators were used. Frequency and percentage were used when describing qualitative parameters or quantitative characteristics that take only a very small number of values.

Statistical comparison of mean values of quantitative continuous variables between two independent groups was carried out using Student's t-test (for indicators distributed approximately normally). The Mann–Whitney U test and the Kruskal–Wallis test were used to compare independent populations in cases where there were no signs of normal data distribution or to compare by ordinal indicator. The equality of variances was assessed using the Levene's test. Analysis of variance was also used to compare several independent populations.

Spearman rank-order correlation was used to study the relationship between the phenomena represented by quantitative data, whose distribution differed from the normal. Contingency tables were constructed to compare

groups by a binary trait expressing clinical outcome. Fisher's exact test was used to compare the distribution of qualitative variables. Assessment of the strength of the connection between the signs was carried out using the Cramer V criterion. Standardized residuals were calculated for each cell in the contingency table to determine the contribution of different populations to the formation of the factor relationship indicator. Version 4.0.0 of the R program was used to create a graph to visualize the strength of interrelationship between the populations of the contingency table. The survival function of patients was assessed using the Kaplan–Meier method and compared statistically using the log rank test, which implies predicting the risk of death for patients on the waiting list depending on their AVT status. Risk is viewed as a function of time. Differences in indicators were considered statistically significant at $p < 0.05$ significance level.

RESULTS

The analysis included 49 patients with CHC-related cirrhosis waitlisted for liver transplantation. The median age was 52 [46; 59]. A comparison of the AVT and non-AVT patient cohorts revealed no significant differences in gender composition, in the initial stage of cirrhosis decompensation and in the time of onset of the outcome after being listed (Table 1). All deaths and dropouts were due to critical decompensated cirrhosis.

Characteristics of patients who received DAA therapy

At inclusion in the waiting list, 36 patients had MELD-Na score <20, while for 4 candidates (10%, 4/40), the score ranged between 21 and 25. At the time of analysis, 38 patients had reached the endpoint of antiviral treatment efficacy evaluation – 12 weeks after the end of AVT (Fig. 1). Of these, 92.1% (n = 35/38) had SVR. In 45.7% (n = 16/35) of cases, persistent elimination of HCV infection was accompanied by significant improvement in liver function with subsequent disappearance of indications for liver transplantation (delisting). The median follow-up time after delisting at the time of analysis was 36 [27; 41] months. In 15 patients (94%), compensated liver function was preserved, only one

Table 1

Comparison of the initial characteristics of the study groups

Indicators	Group 1. AVT + n = 40	Group 2. AVT – n = 9	Intergroup differences (p)
Women/men	17/23	2/7	0.451
Median age (years)	50 [45.5; 58.3] (36–69)	56 [55; 60] (46–67)	0.093
Initial MELD/Na score	16 [13; 18]	15 [14; 17]	0.876
Person-years median	0.5 [0.25; 0.83]	0.25 [0.25; 0.5]	0.514
HCC: yes/no	6/33	1/7	1.0
GFR	59 [47; 79]	59 [43; 72]	0.959

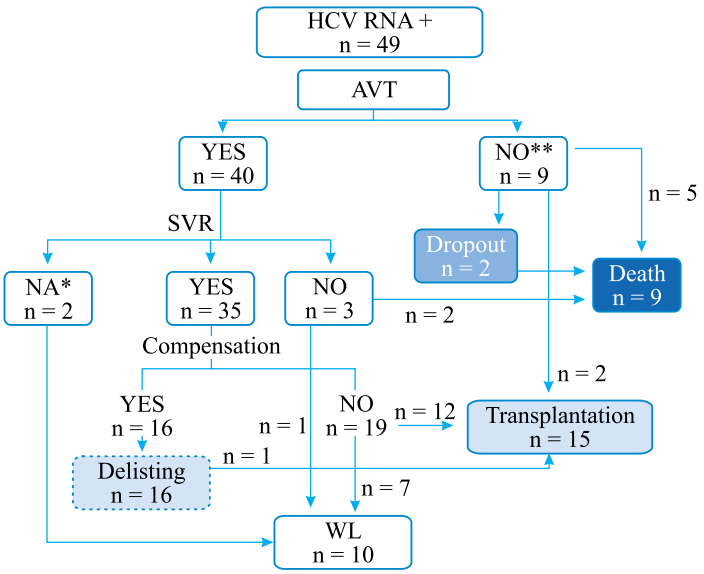

Fig. 1. Outcomes in waitlisted patients with or without AVT DAA.
* – patients underwent a course of antiviral treatment, but have not reached the deadline for evaluating the effectiveness of the SVR therapy yet; ** – therapy was not prescribed because DAAs were not available

patient (6%, 1/16), six months after delisting, had clinical progression of cirrhosis, manifested by edematous-ascitic syndrome and hepatic encephalopathy of grade 2. In this regard, after re-listing, the patient underwent liver transplantation. Favorable results were also observed among the remaining patients who achieved SVR: 12 patients (34.3%, 12/35) had liver transplantation and 7 patients (20%, 7/35) without significant progression of the disease are on the OLT waiting list. Persons who received AVT on the waiting list and achieved SVR had no recurrence of HCV infection in the post-transplant period. Statistical analysis showed no relationship between delisting and demographics and between delisting and baseline MELD-Na. However, a statistically significant positive correlation was found between Δ MELD-Na and the patient's age (Spearman's rs = 0.419, p = 0.015), that is, the older the patient was, the more often there was higher MELD-Na score, despite successful treatment outcomes.

Four of the patients who received AVT were diagnosed with HCC (10%, 6/40), with tumor spread based on the Milan criteria. Simultaneously with etiotropic treatment, the patients underwent locoregional therapy based on indications. At the time of analysis, one patient had not reached the endpoint of the AVT efficacy assessment and in one case (20%, 1/5), there was a relapse of HCV infection. Four patients (80%, 4/5) had SVR: three of them underwent liver transplantation and one patient is on the waiting list.

Let us separately consider the outcomes in three patients (7%, n = 3/38) who had a relapse after DAA therapy. Two of them were placed on the waiting list for decompensated cirrhosis with baseline MELD-Na score of 14 and 17. They were 52 and 41 years old at the time of inclusion on the waiting list. The first patient died as a result of occlusive portal vein thrombosis with subsequent development of acute liver failure and type 1 hepatorenal syndrome. The other patient continues to be followed up on the waiting list. The third case of post-AVT relapse was reported in a patient with HCC. During the follow-up period, oncological process did not progress. However, the patient died due to complications after an episode of bleeding esophageal varices. To exclude factors that could affect outcomes in patients who did not achieve SVR, we compared the groups in terms of time of outcome, person-years index level, baseline MELD-Na scores, age, gender, and Δ MELD-Na. However, no statistically significant differences were found.

Characteristics of patients who did not receive DAA therapy on the OLT waiting list

At the time of listing, two patients (22%, 2/9) had MELD-Na scores of 21 and 24. In other cases, the MELD-Na score did not exceed 20. One patient (11%, 1/9) was diagnosed with HCC on the background of cirrhosis. Prevalence of the process was within the Milan criteria. Patients were not given etiotropic treatment due to the unavailability of suitable AVT regimens. The observed difference in the outcome spectrum in these patients in comparison with group 1 turned out to be interesting: the majority of patients had an adverse outcome – only two patients from this group survived. All adverse outcome cases (death and dropout) were due to complications of cirrhosis.

Analysis of differences in outcomes between patient cohorts with and without AVT

When studying the dynamics of the functional state of the liver in the waiting list, which was determined by the change in the MELD-Na score, patients with SVR in 51.4% (n = 18/35) cases had a decrease in this indicator. There were no changes in 22.9% (n = 8/35), while an increase in MELD-Na score was noted in 25.7% (n = 9/35). All patients without SVR (n = 3) had an increased MELD-Na score. In the non-AVT group, only one patient had liver function compensation (11.1%, n = 1/9), progression or stable MELD-Na score was the same – 44.5% (n = 4/9). In the group of patients with SVR, the median decrease in the MELD-Na score was –4 [–7; –2] (–11…–1), and the median increase in MELD-Na was +3 [1; 4] (1–7). A more significant increase in the MELD-Na score was observed in persons who did not reach SVR: median +5 [3.5; 18] (2–30), which was comparable to the indicators for patients who did not receive AVT: median +6 [5.75; 7.5] (5–12).

Curious were the results of the dynamics of the functional status of the liver in patients with baseline MELD-Na score >25 (n = 6). Of these, four patients received AVT, and SVR was observed in all cases. None of them showed disease progression, and the median decrease in MELD-Na was –10 [–10.5; –9.5]. According to two medical records in the non-AVT group, disease progression with Δ MELD-Na +6 was observed in one case; in the other case, the patient's condition was stable. A statistically significant relationship between a decrease in MELD-Na and the presence of SVR and age was determined. Gender, presence of HCC, and baseline MELD-Na score were found to have no influence on the dynamics of the functional status of the liver (Table 2).

Despite the fact that the difference in time to outcome in the two groups was statistically insignificant, the death rate in the group of patients who did not receive AVT (24 cases per 100 person-years) was significantly higher than that for patients with AVT (7 cases per 100 person-years). Thus, the emergence of DAAs had a significant positive impact on the efficiency of the transplant center.

Statistically significant differences ($p < 0.001$) were obtained by comparing the death rate depending on AVT. With no AVT, the chances of dying on the waiting list increased 66.5 times (95% of CI: 7.99–554). There was

a strong association between the compared signs (V = 0.728). Fig. 1 shows the relationship between DAA treatment in the waiting list and the presence and absence of death. In our study, we found a strong positive correlation between death and absence of AVT in waitlisted patients (st. res = 4.1, $p < 0.05$) and a strong negative correlation between death and favorable outcome (st. res = –1.97, $p < 0.05$). During AVT, there was a strong negative correlation with death (st. res = –1.97, $p < 0.05$). Thus, in the absence of antiviral therapy, the patient is more likely to die than to survive.

Analysis of the probability of death by Kaplan–Meier method using the log rank test also found statistically significant differences in survival between groups ($p < 0.001$). Moreover, in the group of patients treated with antiviral therapy, we observed a long "plateau" period in patient survival 7 months after listing, which may be a manifestation of persistent stabilization of liver function in patients who had successful AVT outcomes.

DISCUSSION

Thanks to successful therapy in patients with advanced liver disease, we see the benefits of AVT in terms of further prognosis and reduced need for liver transplantation. According to published data, successful treatment of CHC improved liver function in the short term in 60% of patients. This was accompanied by decreased MELD scores, while 17% had no changes, and 23%, on the contrary, recorded an increase in MELD scores [11]. The results described are very close to the data obtained

Table 2

Characteristics of patients with different MELD/Na dynamics

Indicators	Reduction MELD/Na n = 20	No reduction MELD/Na n = 29	Intergroup differences (p)
Male	14	16	0.454
Median age (years)	46.5 [41.8; 51.3]	56 [50.0; 60.0]	**0.002**
Initial MELD/Na score	16.0 [15.5; 18.3]	15.0 [13.0; 17.0]	0.127
HCC: yes/no	1/19	6/23	0.216
GFR	59.0 [59.0; 66.8] (47.0–79.0)	59.0 [56.5; 68.0] (48.0–77.0)	0.512
SVR: yes/no	18/0	17/12	0.024

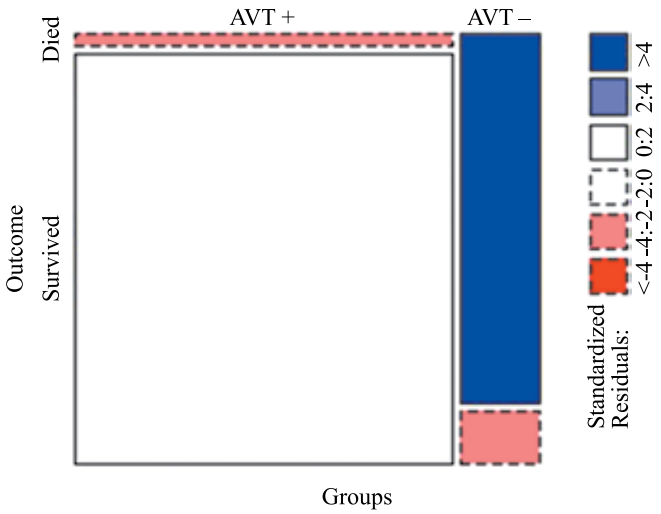

Fig. 2. Impact of AVT in the waiting list with and without fatal outcomes. Standardized Residuals. This graphical representation of contingency tables (made in the R program) allows to evaluate the contribution of different factor combinations to the formation of the relationship indicator. The sizes of the rectangles show the proportions of observations, while the color is for the value of the standardized residual, which reflects the statistical significance of the deviation of the indicator from the expected value. A standardized residual of more than 1.96 indicates a statistically significant positive relationship between the indicators, while values less than –1.96 indicate a negative relationship

in our study: 51.4%, 22.9% and 25.7%, respectively. The decrease in MELD after effective AVT in decompensated cirrhosis in large studies varies with a mean of –2, while in a small proportion of patients, the MELD continued to deteriorate with a median of +1 [12]. These changes were more pronounced in our sample: the median decrease in the MELD-Na score was 4, and the median increase in MELD-Na was +3. Some difference in the results may be due to the use of a more accurate MELD-Na index.

It was noted that 15 patients (42%) with decompensated cirrhosis were delisted for liver transplantation due to persistent clinical improvement after achieving SVR. The delisted patients showed either complete regression or improvement in hepatic decompensation. At the time of writing this paper, the median follow-up time after delisting was 36 months. According to a study by the European Liver and Intestine Transplant Association (ELITA), 20.4% of patients were delisted after effective AVT and 33% were inactivated from the transplant list [13]. These results provide grounds for optimism on the reduction in the need for liver transplantation in almost one third of patients in this large population.

Improvement in liver function and quality of life can be achieved after successful therapy, but not in all patients. Predictors of improvement or inability to compensate have been identified earlier, but at present they

are not reliable enough to be widely applied in clinical practice. According to analysis of data drawn from the Scientific Registry of Liver Transplant Recipients of the USA, the following algorithm was proposed for CHC treatment in liver transplant candidates: for patients with MELD score <20, DAA therapy is carried out with the aim of possible delisting and prevention of reinfection in the post-transplant period, with a MELD score of 20 to 27 and a GFR >30 mL/min/1.73 m^2, the decision to use AVT should be individualized, depending on the availability of OLT and presence of related conditions, with a MELD score >27 and/or GFR <30 mL/min/1.73 m^2, AVT should be postponed until the post-transplant period [14]. At our center, we found no clear correlation between baseline MELD and the likelihood of delisting. This is primarily due to the fact that our sample consists mostly of patients with MELD <20, only six had MELD >20, while not exceeding 25. Also, there were no significant GFR deviations at the time of listing and the start of AVT. However, out of 4 patients with a MELD score of 20 to 25 who received AVT, 2 (50%) showed sustained clinical improvement for which they were delisted. Besides, the recommendation to prioritize liver transplantation in a patient with high MELD, followed by AVT, is valid for countries with a national MELD-based organ allocation system, where waiting times can be reduced to hours and days if clinically necessary. Given the shortage of donor organs in our country Russia, the use of such a resource as AVT can reduce the risks of waitlist mortality for this complex patient category. In this regard, we also believe that DAA therapy cannot be limited by the recommended MELD threshold of 20 and should be considered individually.

When examining the effect of DAAs on outcomes in patients with HCC, we did not observe any significant deterioration among this patient cohort. Also, data from recent meta-analyzes report that a high level of HCC progression was associated with predominant use of DAAs in elderly people with concomitant disorders and/or significant complications of cirrhosis [15–17]. This gives grounds to assert that the presence of HCC is not a determining factor for not assigning DAAs. The solution to the issue must be comprehensive with an assessment of the prevalence of the tumor process, the functional state of the liver and further prognosis.

Since there were not enough patients with graft reinfection at our center, we cannot draw conclusions on the outcome of AVT in CHC patients in the post-transplant period. According to published data from clinical practice, the frequency of SVR in patients undergoing DAA therapy varies from 93% to 100%. At the same time, DAA therapy is characterized by a good safety profile. Only one study reported mild liver transplant rejection in 2.7% of cases (n = 1), which was stopped by high-dose pulse glucocorticoid therapy with positive clinical effect, without further functional graft disorders

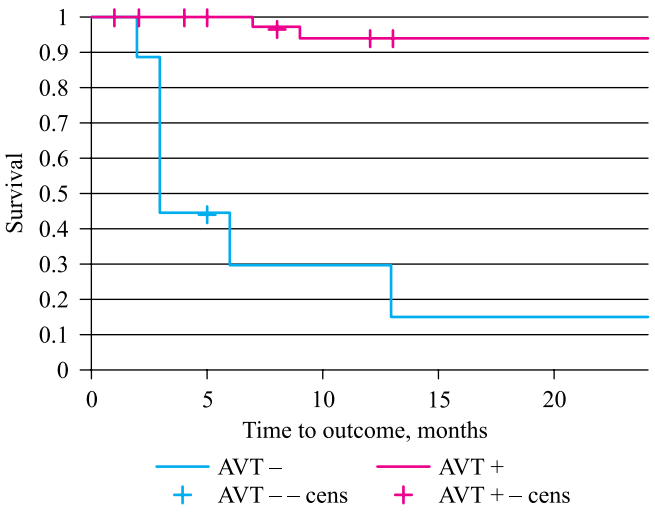

Fig. 3. Kaplan–Meier waitlist survival analysis. Censored were cases of being dropped from the waiting list due to liver transplantation

[18–20]. However, recurrence of HCV infection after liver transplantation is associated with graft dysfunction. There is evidence that lack of effective AVT in the post-transplant period leads to cirrhosis in about one third of patients within 5 years after OLT [21]. In our center, all patients who received AVT before liver transplantation did not have a recurrence of viral hepatitis C in the graft. This may become an additional factor that can improve long-term outcomes in liver transplantation.

It should be noted that most patients with decompensated cirrhosis may have OLT limitations due to issues with availability of donor organs or presence of relative contraindications for OLT. Therefore, these patients should be considered for CHC treatment with the hope that successful DAA therapy may have benefits at varying degrees. At our center, the death rate decreased by almost 3.5 times based on the calculation of the number of cases per 100 person-years.

CONCLUSION

For several years since the first interferon-free AVT regimens appeared, dozens of patients have been able to receive treatment at our center. We see a significant advantage of safe and effective treatments for OLT candidates who face immediate complication risks under persistent infection. Most modern scientific research is aimed at identifying predictors of disease regression after DAA therapy. However, the positive effects of therapy, such as reduced inflammatory activity, slower disease progression and prevention of graft reinfection, are no less relevant under organ shortages. From our study, we conclude that for all waitlisted candidates with HCV infection, DAA therapy in the preoperative period is preferable if there are no contraindications for the treatment.

The authors declare no conflict of interest.

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BENEFITS OF VACUUM-ASSISTED CLOSURE THERAPY OVER STANDARD TREATMENTS FOR INFECTED AND CHRONIC NON-HEALING WOUNDS AFTER KIDNEY TRANSPLANTATION

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Objective: to evaluate the effectiveness of vacuum-assisted closure (VAC) therapy in comparison with standard treatments for infected and chronic non-healing wounds after kidney transplantation. **Materials and methods.** From June 2018 to November 2019, 75 kidney transplants from deceased donors were performed at the Transplantation Ward of Botkin City Clinical Hospital. There were 47 men (62.6%) and 28 women (37.4%). Standard surgical technique was used. Immunosuppressive therapy was carried out according to a three-component scheme with anti-CD25 monoclonal antibody induction (basiliximab) intraoperatively and on day 4. All patients received antibiotic therapy with protected third-generation cephalosporins for 7 days after surgery. Postoperative complications were evaluated according to the Clavien–Dindo classification. Standard methods, including daily dressings using modern dressing materials (group I) and VAC therapy (group II) were used for treating infected and chronic non-healing wounds. **Results.** 30-day mortality in the postoperative period was zero. Postoperative complications were recorded in 11 patients (14.6%), of which 7 had postoperative wound complications. Group I included 3 patients (1 with a *Klebsiella pneumonia*-infected wound and 2 with chronic non-healing wounds and no microflora growth). Group 2 had 4 patients (3 with infected wounds (*Esherichia coli* – 1, *Klebsiella pneumonia* – 2) and 1 with chronic non-healing wound). Complete cleansing of wound, absence of bacterial growth according to the microbiological examination, and maturation of granulations according to histological examination were considered as the criteria upon which a wound could be sutured in both groups of patients. The average time between the start of treatment and secondary suturing in group 1 patients was 33.11 ± 5.43 (28–37) and 15.01 ± 3.15 (13–17) days in group 1 and group 2 respectively. **Conclusion.** VAC therapy in patients with wound complications resulting from kidney transplantation, in comparison with standard treatment, can achieve rapid wound cleansing, acute inflammation relief and accelerated maturation of mature granulation tissue, thereby improving treatment outcomes in this category of patients.

Keywords: kidney transplantation, wound infection, VAC therapy.

INTRODUCTION

Healthcare-associated infection is the most common complication in medicine [1]. Postoperative wound infection is the most common type of infectious complications in surgical patients [2]. This complication increases the cost of medical care, and also increases postoperative mortality [3].

In the Russian Federation, 1361 kidney transplant surgeries were performed in 2018, which accounted for 62% of all organ transplants [4]. The incidence of postoperative wound infection following kidney transplantation is 10–15% [5, 6]. Among the risk factors for this complication are 1) recipient-related factors: age, obesity, diabetes, smoking and malnutrition; 2) surgical factors: surgical technique, wound closure method; 3) factors specific to this surgical intervention, such as: immunosuppressive therapy, delayed graft function, need for dialysis after surgery [7].

Therapy of infected wounds after kidney transplantation comes with significant difficulties due to the need for immunosuppressive therapy, which, on one hand, complicates conservative treatment of the infection, and on the other hand, reduces the reparative processes in the wound [8]. All this leads to the fact that it often takes more than 5 weeks to treat such complications [9].

One of the modern methods of treating infected wounds in surgery is application of negative pressure (VAC-therapy), which is associated with a short wound-healing period [10]. The experience of using this technique in patients who have undergone kidney transplantation is extremely limited [11], which requires further studies of the effectiveness of VAC therapy in this category of patients.

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MATERIALS AND METHODS

From June 2018 to November 2019, 75 kidney transplants from a deceased donor were performed at Botkin City Clinical Hospital. There were 47 men (62.6%) and 28 women (37.4%). The average age was 46.01 ± 11.33 (20–70 years old). The average body mass index (BMI) was 26.09 ± 4.47 (17–36). Donor characteristics are presented in Table 1.

Table 1

Characteristics of deceased kidney donors

Average age, years	46.41 ± 10.05 (22–65)
Pressor support Yes No	 71 4
Average BMI	27.12 ± 4.81 (20–43)
Time spent in hospital, hour	64.25 ± 63.52 (13–480)
Average donor creatinine, μmol/L	93.46 ± 27.47 (41–180)

The standard surgical technique was used. The urethral and central venous catheters were removed on day 7 after transplantation. All patients received antibiotic therapy with protected third-generation cephalosporins for 7 days after surgery. The following immunosuppressive therapy scheme was used: anti-CD25 monoclonal antibodies (basiliximab), intraoperatively and on postoperative day 4, tacrolimus with a 8–10 ng/ml target concentration, mycophenolic acid at 1000 mg twice a day and prednisolone at 30 mg per day. The ureteral stent was removed in the operating room under aseptic conditions on days 14 or 21. Postoperative sutures were removed on day 21. Postoperative complications were assessed according to the Clavien–Dindo classification. Delayed renal graft function was defined as the need for dialysis in the first 5 days after surgery. Long-term non-healing postoperative wound was defined as dehiscence of the wound edges after suture removal.

RESULTS

The characteristics of the surgical interventions performed are presented in Table 2.

Table 2

Characteristics of surgical interventions for cadaveric kidney transplantation

Average cold ischemia time, min	594.58 ± 193.95 (133–1180)
Average blood loss, mL	104.32 ± 51.12 (30–300)
Average bed-days in ICU	1.56 ± 0.85 (0–4)
Average total bed-days	16.57 ± 13.23 (8–101)
Graft function: immediate delayed	 53 22
Postoperative complications, abs/(%)	11 (14.6%)
Postoperative mortality	0

There was no 30-day mortality in the early postoperative period. Postoperative complications were registered in 11 patients (14.6%), 7 of whom had complications from postoperative wound: suppuration (4 patients, 5.3%), long-term non-healing wound (3 patients, 4%), lymphocele (3 patients, 4%), and urosepsis (1 patient, 1.3%). According to the Clavien–Dindo classification: class II (3 complications, 4%), IIIA (1 complication, 1.3%), IIIB (6 complications, 8%), and IVa (1 complication, 1.3%). Suppuration of postoperative wound was recorded within 28 to 42 days after the operation. In 3 patients (1 patient with an infected wound (*Klebsiella pneumonia* in microbiological examination (Fig. 1)) and 2 patients with long-term non-healing wounds (no growth in microbiological examination)), standard treatment methods were used, including daily dressings using modern dressing materials to create a humid environment, administration of antibiotic therapy in accordance with the antibiotic chart, reducing immunosuppressive therapy.

After the wound was completely cleansed, there was no bacterial growth, which was confirmed by microbiological examination, and granulation matured, which was confirmed by histological examination of edges of the wound, the wound was then closed (Fig. 2). The patients described above constituted Group I of the study.

The second group included 4 patients (3 patients with infected wounds (1 patient with *Esherichia coli*, 2 patients with *Klebsiella pneumonia*) (Fig. 3) and 1 patient with a long-term non-healing wound).

The treatment strategy for this group of patients was to install a VAC system with 90 mm Hg constant pressure (Fig. 4).

Dressings were performed every week. In the absence of systemic inflammatory response syndrome, antibiotic therapy was not prescribed, and immunosuppressive

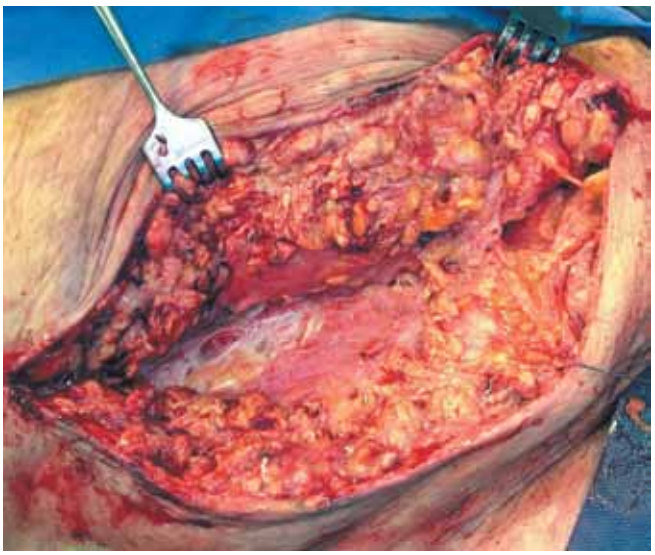

Fig. 1. Infected postoperative wound after kidney transplantation (18 days after transplantation)

therapy was reduced. The wound closure criteria were similar to those of the first group (Fig. 5).

An important objective criterion for determining the time of wound closure is the histological examination of the edges of the wound with the determination of granulation tissue maturation. During the primary and subsequent surgical interventions, sampling was performed from the superficial and deep edges of the wound with dynamic monitoring of the reparative process (Fig. 6).

During primary surgical interventions for infected postoperative wounds, the edges of the wound presented as gangrenous-like acute inflammation with a scab on the wound surface. Inflammatory infiltration was present in the lower layers with the presence of numerous segmented granulocytes. The vascular walls were pareticly dilated with necrosis of the muscular layer (Fig. 7).

On day 5–7 after VAC therapy, formation of young granulation tissue on the wound surface with lymphoid infiltration along the interfatty connective tissue layers was noted. Inflammatory infiltrates were represented

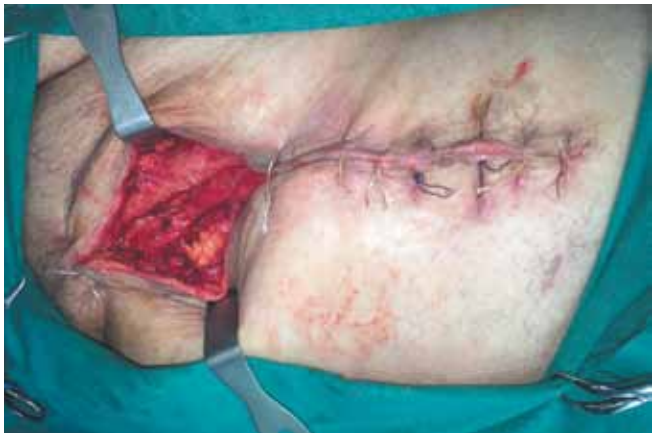

Fig. 2. Outcome of infected wound treatment. Secondary sutures (31 days of treatment)

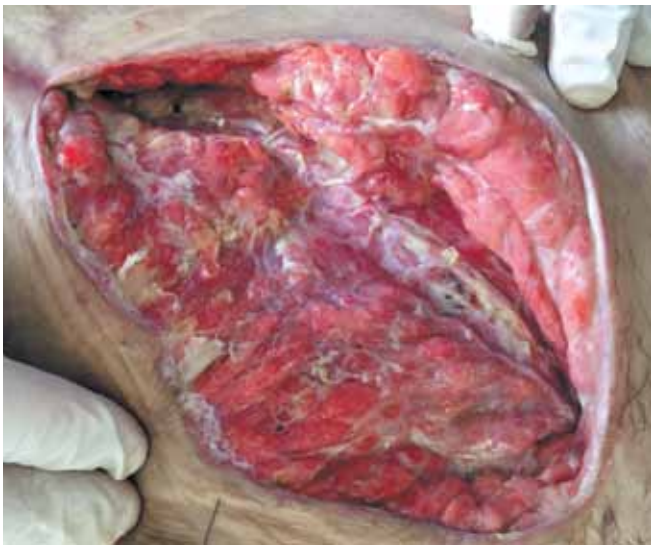

Fig. 3. Infected postoperative wound after kidney transplantation (14 days after surgery)

Fig. 4. Application of VAC system on an infected postoperative wound

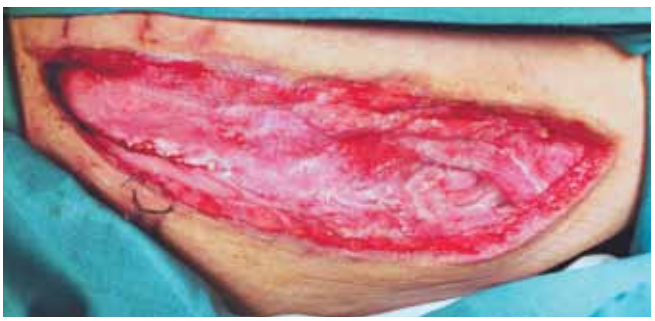

Fig. 5. Complete cleansing of the postoperative wound after VAC therapy (13 days after treatment)

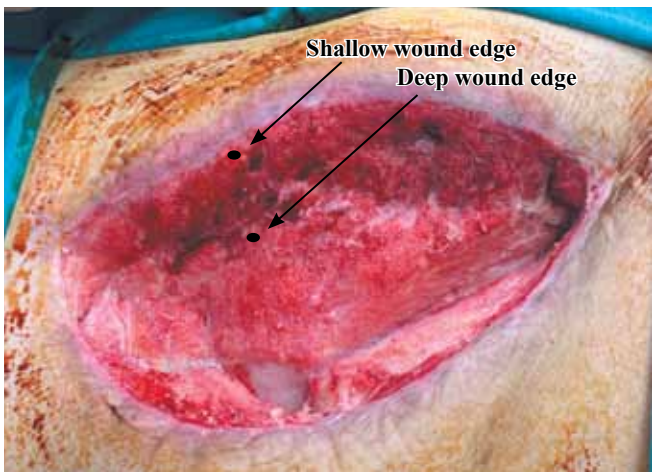

Fig. 6. External view of the wound. Locations from where material was taken for histological examination are marked

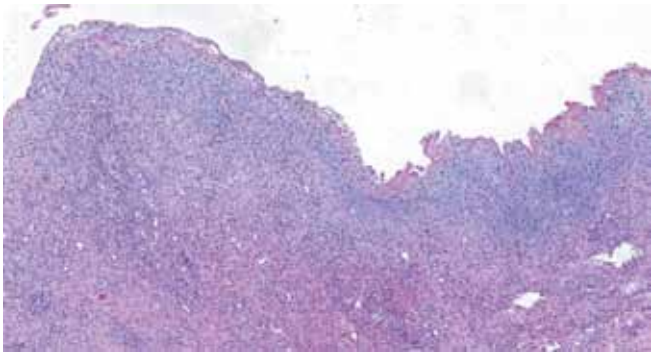

Fig. 7. Muscle fibers are not visible, the scab is represented by an inflammatory shaft in the form of a homonized structureless mass. No granulation tissue was formed. H&E stain

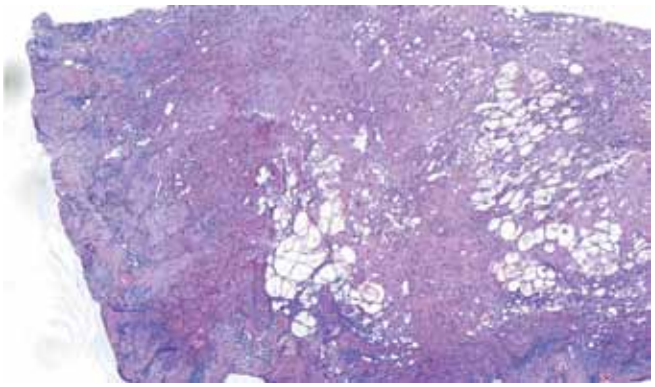

Fig. 8. Young granulation fields can be seen around adipose tissue "islets"

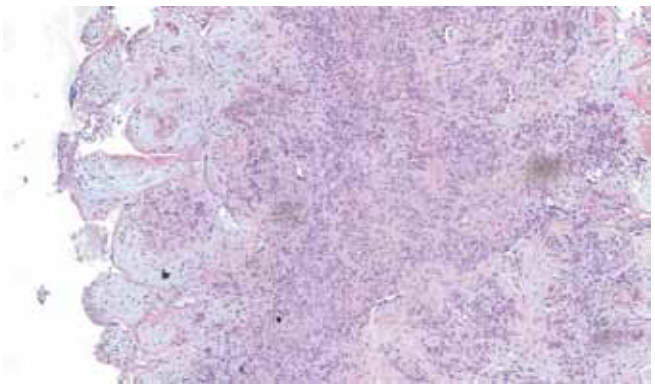

Fig. 9. Mature granulation tissue. No inflammation. Formation of rough papillary structures on the wound surface

predominantly by mononuclear cells. Myofibroblasts, sinusoidal capillaries surrounded by delicate intercellular substance with metachromasia were seen in the "young" granulation tissue (Fig. 8).

In the later stages, mature granulation tissue with thicker vessels and collagen fibrosis was formed. There was maturation zoning in the form of rough papillae on the wound surface. Inflammatory infiltration was scanty, single mature lymphocytes were visible (Fig. 9).

The average times between the beginning of treatment and the application of secondary sutures in group 1 and group 2 were 33.11 ± 5.43 (28–37) and 15.01 ± 3.15 (13–17) days, respectively.

DISCUSSION

In the experience of Botkin City Clinical Hospital, wound complications are the most frequent type of complications after kidney transplantation, and accounts for 9.3%. Development of this type of complication increases the patient's hospital stay by an average of 31.67 ± 5.43 days, thereby increasing treatment costs. The need for prolonged antibacterial therapy due to an open wound surface also carries a potential threat of complications, the most frequent of which is pseudomembranous colitis. All this necessitates the introduction of modern wound treatment methods into clinical transplantology.

The use of the VAC system in patients with wound complications after kidney transplantation, compared to standard daily dressings, made it possible to achieve wound cleansing at significantly early periods (15.01 ± 3.15 vs 33.11 ± 5.43, $p < 0.05$) and maturation of granulation wound, which was confirmed by histological examination. Other important advantages of VAC therapy are early de-escalation of antibiotic therapy, as well as patient convenience compared to daily dressings.

Direct adjacency of vascular anastomosis or neocystoureteroanastomosis to the wound bed, and active diffuse tissue bleeding are contraindications to the use of the technique.

Based on the data obtained, we have developed our own protocol for the use of VAC therapy in patients with wound complications after kidney transplantation: during the first intervention, a wound culture is performed, the wound is debritched with removal of non-viable tissues, the VAC system is installed with 100 mm Hg constant pressure. In the absence of intoxication syndrome, the dressing is changed every 7 days. With repeated dressings, the edges of the wound are taken for microbiological and histological examination. The criteria for application of secondary sutures are: absence of bacterial growth in the wound, absence of systemic inflammatory reaction syndrome, presence of mature granulation tissue in the superficial and deep edges of the wound.

CONCLUSION

VAC therapy in patients with wound complications after kidney transplantation, in comparison to standard treatment methods, allows to achieve rapid wound cleansing, relief of acute inflammation and acceleration of maturation of mature granulation tissue, thereby improving treatment outcomes in this patient cohort.

The authors declare no conflict of interest.

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IMPLANTATION OF A CARDIAC CONTRACTILITY MODULATOR IN CHRONIC HEART FAILURE AND ATRIAL FIBRILLATION: RESULTS OF A 6-MONTH FOLLOW-UP OF ONE HUNDRED PATIENTS

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Objective: to study the effect of cardiac contractility modulation (CCM) in patients with chronic heart failure (CHF) and atrial fibrillation (AF). **Materials and methods.** In a group of 100 patients with CHF and AF, the following studies were performed before implantation of the CCM and after 6 months of follow-up: 12-channel ECG, transthoracic Echocardiography, 6-minute walk test, determination of the level of pro-natriuretic N-terminal peptide (NT-proBNP), and a questionnaire based on the Minnesota quality of life questionnaire for patients with CHF (MHFLQ). All patients received long-term optimal medication therapy for CHF before surgery. **Results.** The results show a positive effect of the use of MCC in patients with CHF and AF on reverse LV remodeling, functional class of CHF, and levels of NT-pro-BNP regardless of the form of AF. **Conclusion.** The use of MCC may be a promising treatment method in addition to optimal medication therapy in patients with CHF and AF.

Keywords: heart failure, atrial fibrillation, modulation of heart contractility, left ventricular ejection fraction, quality of life.

INTRODUCTION

Chronic heart failure (CHF) and atrial fibrillation (AF) are common cardiovascular diseases that often complicate each other's course and have a significant impact on prognosis in both cases. AF is the most common arrhythmia that occurs in CHF, with an average prevalence of 30 to 50% [1–5]. Having common risk factors, AF and CHF often coexist or can accelerate / exacerbate each other's course, which leads to a significant increase in mortality, which is higher with a combination of diseases than with any condition alone [6, 7]. According to the large ACALM registry, where 929,552 patients were analyzed, 31,695 (3.4%) had AF without CHF, 20,768 (2.2%) had CHF in sinus rhythm, and 10,992 (1.2%) had CHF with AF [7]. Patients with CHF in AF had the highest all-cause mortality (70.8%), followed by patients with CHF in sinus rhythm (64.1%), and in patients with AF alone, mortality was lower at 45.1% ($p < 0.0001$). Patients who developed new-onset AF, CHF, or both had significantly higher mortality rates (58.5%, 70.7%, and 74.8%, respectively) compared with those who already had these conditions long-term (48.5%, 63.7% and 67.2%, respectively, $p < 0.0001$).

Despite a significant number of studies aimed at studying CHF and AF, it is still unclear which treatment approaches can affect the prognosis and delay the development of the end stage of CHF in this group of patients [8]. Patients with CHF and AF with disease progression are potential recipients for heart transplantation. Currently, there are several therapeutic approaches in the treatment of patients with AF and CHF. These are pharmacological tactics of frequency and rhythm control for AF, the increasing importance of catheter ablation, as well as optimization of cardiac resynchronization therapy (CRT), and, of course, optimization of CHF therapy in this group of patients. Pharmacological control of rhythm in patients with AF and CHF did not lead to an improvement in severe outcomes such as death from cardiovascular disease [9]. Studies of the use of AF catheter ablation have shown improvements in symptoms, exercise tolerance, quality of life and increased left ventricular ejection fraction (LVEF) in AF patients with CHF [10], as well as reduced all-cause mortality and hospitalizations for worsening CHF after catheter ablation of AF in patients with low LV ejection fraction [11]. According to the 2020 European guidelines for atrial fibrillation, AF catheter ablation may be considered on a case-by-case

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basis in patients with CHF with low LVEF (CHF/lLVEF) to improve survival and reduce hospitalization, and for patients with a high likelihood of tachy-induced cardiomyopathy, regardless of symptom severity recommended with class IB [12]. Atrioventricular node ablation with biventricular pacemaker placement is considered for patients with persistent AF and systolic dysfunction who have a rapid ventricular rate refractory to pharmacological therapy [13, 14]. Thus, the limited efficacy of drug therapy, catheter ablation, and CRT in patients with CHF and AF currently requires a search for new treatments in this category of patients. Today, patients with AF and CHF, who, on the background of optimal drug therapy, who retain the clinical picture of CHF and do not have indications for CRT and catheter ablation, can be offered such a type of treatment as implantation of a new generation cardiac contractility modulator (CCM) (Optimizer® Smart). This is an electrophysiological method of treatment, which is based on the application of a biphasic electrical impulse in the absolutely refractory period of the cardiomyocyte depolarization (CMC) phase, 30 ms after the QRS complex is detected [15]. The effect of CCM differs from other implantable devices (CRT, cardioverter-defibrillator) in that it does not affect the heart rate. As a result of CCM work, the contraction of the heart muscle improves, exercise tolerance increases, and the quality of life of patients increases [15]. The expert consensus on CHF considers this method of treatment as possible in patients with LVEF 25–45%, QRS complex <130 ms, without specifying the presence or absence of AF [16]. A new method of treatment, CCM implantation in such a severe category of patients with CHF and AF, may make it possible to postpone and/or even avoid heart transplantation.

The paper presents the results of a follow-up of CHF and AF patients with implanted Optimizer® Smart devices for 6 months. The aim of the study is to evaluate the efficacy of CCM in patients with CHF and various forms of AF.

MATERIALS AND METHODS

The study included 100 patients who signed informed consent and met the following inclusion criteria: documented clinically manifested CHF/lLVEF (20–40%), II–III FC according to the classification of the New York Heart Association (NYHA) during at least 3 months before screening in combination with AF, optimal CHF therapy according to current recommendations, stable condition for the last 30 days or more. The exclusion criteria were: the patient's refusal to participate in the study; being on the active list of heart transplantation or after heart transplantation, terminal CHF; acute diseases that, in the opinion of the investigator, could adversely affect the safety and/or effectiveness of treatment; reversible causes of CHF; recent major surgery or trauma; recent cardiac events, including myocardial infarction, percutaneous coronary intervention, or heart surgery within the previous 3 months; decompensation CHF; acute myocarditis; hypertrophic obstructive cardiomyopathy; angina pectoris IV FC or CHF IV FC (NYHA); mechanical tricuspid valve prosthesis; obstruction of vascular access; medical conditions that limit life expectancy to 1 year. The implantation of CCM Optimizer® Smart devices was performed in 2018–2019.

The CCM electrodes were inserted through the subclavian vein, and the CCM was implanted on the right side of the chest. Two ventricular electrodes with active fixation – Ingevity (Boston Scientific) – were positioned in the projection of the interventricular septum, mainly in its lower and middle third. Upper – RV (right ventricular) and lower – LS (local sense) electrodes were also tested intraoperatively using an analyzer (Medtronic). The sensitivity (R-waves), stimulation thresholds, and resistance were measured standard for the implantation of a pacemaker (pacemaker). After obtaining satisfactory parameters, a test was carried out using the Optimizer programmer. All patients were given special chargers to charge the CCM system from the mains weekly for 40–50 minutes. According to the study protocol, all patients before device implantation and after 2 and 6 months of follow-up underwent the following studies: 12-channel ECG (electrocardiogram), transthoracic echocardiography (EchoCG), 6-minute walk test, NT-proBNP level determination, questionnaire according to the Minnesota quality questionnaire life of patients with CHF (MH-FLQ). A 6-minute walk test was used to objectively assess the CHF FC.

Transthoracic echocardiography was performed on an expert-level ultrasound machine (Vivid E9, GE, Norway) with M5Sc-D matrix ultrasound transducer. with the patient in LLP, with ECG synchronization and standard echocardiographic positions in B, M, PW, CW, tissue myocardial Doppler sonography. The study data were saved in digital format for offline analysis. The image was then processed with EchoPac workstation (version 6.1, General Electric Medical Health). According to transthoracic echocardiography, the following standard parameters were assessed: anteroposterior LA size, maximum LA volume, maximum LA volume index, end-diastolic and systolic LV dimensions, antero-posterior and basal RV dimensions, PP area, myocardial mass and LV myocardial mass index, end LV diastolic and systolic volumes with LVEF (biplane Simpson) determination.

NT-proBNP concentration was determined with Cobas 411 (Roche Diagnostics, Switzerland) automatic analyzer.

The data were statistically analyzed with Excel 2010 and STATISTICA 10 (StatSoft Inc., USA). Qualitative values are presented as absolute values and percentages. The following methods of statistical analysis were used: two-sided F-Fisher's test and U-Mann–Whitney test. Correlation analysis was performed with Spearman's

rank test. The sample parameters given in the table are presented as M (sd) and Me [Lq; Uq], where M, the mean; sd, standard deviation, Median, Lq; Uq, interquartile range. $P < 0.05$ was taken as the minimum level of significance. After installation of the device, all patients were observed on an outpatient basis and all studies were carried out at baseline and after 6 months of observation.

RESULTS

The clinical and demographic characteristics of the patients are given in Table 1. Of the 100 patients included in the study, 83% were male. Age was 60 [56.0; 66.0] years, the duration of CHF at the time of inclusion was more than 1 year and the duration of the disease was 24 [18; 44] months. Of the entire cohort of patients with CHF, 41 had FC II (41%), 59 – FC III (59%). The analysis included patients with both paroxysmal – 51 (51%) and permanent forms of AF – 49 (49%), AF duration was 24 [12; 48] months.

All patients included in the study, prior to CCM implantation, received optimal CHF drug therapy (angiotensin converting enzyme inhibitors / angiotensin II receptor blockers / angiotensin receptor blockers and neprilisin inhibitors, beta-blockers, mineralocorticoid antagonists as mineralocorticoid receptor antagonists, loop diuretics) and have been compensating for CHF events for at least 30 days (Table 2).

There were no registered intraoperative complications during the implantation of the CCM system. It should be noted that 5 out of 100 patients felt discomfort in the form of pulsation with minimal parameters (complaints arose a day after the operation, when patients were activated, the dislocation of the electrodes was excluded by a control check of the parameters with a programmer and x-ray of the chest organs), so these required disconnec-

Table 1

Clinical and demographic characteristics of the patients

Parameter	Value
Age, years	60 [56.0; 66.0]
Male / Female, n (%)	83 (83) / 17 (17)
Ischemic / non-ischemic CHF genesis, n (%)	54 (54%) / 46 (46%)
CHF FC (NYHA), n (%)	II FC-41 (41%) / III FC-59 (59%)
LVEF, %	33 [28; 37]
CHF duration, months	24 [18; 44]
AF duration, months	24 [12; 48]
AF paroxysmal form, n (%)	50 (50%)
AF permanent form, n (%)	50 (50%)
Type 2 diabetes mellitus, n (%)	30 (30%)
BMI, kg/m^2	29 [27; 33]
ICD / CRT-D / ECP, n (%)	24 (24%) / 1 (1%) / 3 (3%)

Note. ICD, implantable cardioverter-defibrillators; ECP, electric cardiac pacemaker.

Table 2

Patients drug therapy during follow-up

Drug	% prescr.	Average dosage, mg
Angiotensin-converting enzyme inhibitors	43	
Perindopril / Enalapril	35 / 8	5 ± 2.5 / 27.5 ± 5
Angiotensin II receptor blockers	25	
Candesaran / Losartan / Valsartan	5 / 18 / 2	8 ± 4 / 50 ± 25 / 160 ± 160
Angiotensin Receptor and Neprilisin Inhibitors Sakubitril / Valsartan	32	200 ± 100
Beta-blockers	100	
Bisoprolol / Carvedilol / Metoprolol	85 / 5 / 10	7.5 ± 2.5 / 50 ± 25 / 200 ± 50
Amiodarone	13	200
Digoxin	15	0.25
Mineralocorticoid receptor antagonists	100	
Eplerenone / Spironolactone	17.5 / 82.5	50 ± 12.5 / 25 ± 12.5
Diuretics	100	
Torasemide / Furosemide	65 / 35	10 ± 5 / 40 ± 20
Anticoagulants	100	
Apixaban / Rivaroxaban / Dabigatran / Warfarin	30 / 45 / 15 / 10	10 / 20 / 300 / 25 ± 12.5

tion of one of the ventricular electrodes. In one case, an electrode dislocation was detected during a patient visit 2 months after CCM implantation, which required re-hospitalization and correction of the electrode position. One patient developed a complication in the form of suppuration of the CCM bed, which required removal of the system after 1 month after implantation. Three out of five patients whose electrodes had previously been disconnected were able to turn on the second ventricular electrode after 2 months. All other patients responded to the device satisfactorily.

Six months after CCM implantation, in 99 patients, the percentage of therapeutic stimulation was 93.7 [82.7; 98.2] (according to the recommendation of the device manufacturer, the optimal percentage of applied therapy is more than 70), with the time of applied therapy per day – 7 h [7; 8].

The most frequent reason for the increase in the time of the applied therapy was an insufficient percentage of stimulation due to a high heart rate (the threshold for the device's operation is limited to a heart rate of 110 beats/min). In this regard, careful monitoring of heart rate is required with a permanent form of AF.

CHF FC analysis showed a statistically significant decrease in CHF FC in the entire cohort of patients 6 months after CCM implantation: from 3.0 [2.0; 3.0] to 2.0 [2.0; 2.0] ($p < 0.0001$) and as a percentage, there was a decrease in FC to II in 84% of patients, in 10% of patients the FC level decreased to III, in the remaining 6% it remained unchanged.

After 6-month follow-up after implantation of the CCM system, all patients showed a statistically significant increase in exercise tolerance, which was objectively demonstrated by an increase in the distance traveled (m) according to the results of the 6-minute walk test and amounted to 6 months later. 340 [300; 400] compared with the initial data (330 [283; 384]) ($p < 0.0008$).

By MLHFQ, there was a significant decrease in the number of points from 40 [33; 45] to 28 [24; 29] ($p < 0.005$) after 6 months with CCM therapy.

To objectively assess the course of CHF against the background of 6 months of CCM therapy, the concentration of the NT-proBNP marker was analyzed and a tendency towards a decrease in this indicator from 1180 [482.8; 3123] to 1108 [403.2; 2000] pg/ml ($p = 0.07$).

To assess the reverse myocardial remodeling, transthoracic echocardiography was performed. The main EchoCG parameters of patients in dynamics are given in Table 3.

After 6 months, against the background of CCM implantation in patients, LVEF increased statistically significantly from 33 [28; 37]% to 38 [32; 37]% ($p = 0.000001$). In addition, by 6 months of treatment, ESD and EDD LV indicators also achieved statically significant results (Table 3). For volumetric parameters, LV significantly decreased ESV, while for EDV, there was a tendency to decrease. The same dynamics was observed in relation to LA volume.

Further, a comparative analysis of echocardiographic parameters and the level of NT-pro BNP in the group of patients with permanent ($n = 50$) and paroxysmal AF ($n = 50$) and implanted CCM was carried out; it should be noted that initially patients with permanent AF had a higher level of NT-pro BNP (1599 [820.1; 3334] and 927 [302; 2428], $p = 0.002$) and significantly larger LA sizes (linear size 49 [44; 52] and 44 [40; 46] $p = 0,000001$, volume LA 132 [110; 160] and 88 [74; 99], $p = 0.000001$). For the rest of the parameters, no statistically significant differences were found. The data are given in Table 4.

In addition, a comparative analysis was conducted of echocardiographic parameters depending on the form of AF during 6 months of CCM therapy. It should be noted that, regardless of the AF form, there was a statistically significant increase in LVEF, a decrease in the linear dimensions of LV and LV ESV, as well as a trend towards a decrease in LV EDV in the group of paroxysmal AFs, which did not reach static significance. The results are shown in Table 5.

DISCUSSION

The possibilities of using CCM therapy have become wider due to the advent of a new generation of devices that allow implanting two ventricular electrodes without atrial detection, and, accordingly, conducting CCM therapy if patients have AF. Thus, for patients with a persisting clinic of heart failure and a narrow QRS and

Table 3

Echocardiography dynamics at CCM therapy after 6 months

Parameter	Initial	6 months	*p*
LVEF,%	33 [28; 37]	38 [32; 37]	0.000001
LV at end diastole dimension (EDD) , mm	66 [62; 71]	63 [59; 69]	0.00001
LV at end systole dimension (ESD), mm	55 [49; 61]	51 [45; 58]	0.00008
LV at end-diastolic volume (EDV), ml	202 [173; 250]	196 [160; 237]	0.06
LV at end systole volume (ESV), ml	137 [110; 182]	115 [94; 160]	0.0001
LA, mm	47 [43; 5.1]	46 [42; 50]	0.55
LA volume, ml	108 [87; 140]	95 [70; 128]	0.08

Note. LA – left atrium.

AF complex against the background of optimal drug therapy, it became realistic to use this method of treatment – CCM implantation. Currently, there are very few works in the world literature devoted to the study of the effect of CCM in patients with CHF and AF, and there are insufficient data and large studies that would show the effect of CCM on reverse myocardial remodeling in this category of patients [17–19]. The results of our work demonstrate a positive effect of CCM in patients with CHF and AF on clinical status, NT-proBNP level, echocardiographic parameters of left ventricular remodeling. It should be noted that already after 6 months of treatment, a statistically significant increase in LVEF was observed during CCM therapy, regardless of the form of AF. Thus, the improvement in the contractile function of the LV myocardium makes it possible to judge the processes of reverse remodeling in patients with CHF both with paroxysmal and permanent AF with implanted CCM devices. According to a meta-analysis of randomized clinical trials that assessed the short-term effect and safety after device implantation, it was shown that the use of CCM in patients with sinus rhythm and CHF led to an improvement in the quality of life but did not show a statistically significant difference in the LVEF, the 6-minute test. walking, hospitalization for CHF and all other causes, and mortality from all causes [20]. In 2019, for the first time, the results of a long-term prospective 3-year follow-up of patients with CHF, sinus rhythm and CCM (CCM-REG) were obtained [21]. This registry included a total of 140 patients with 25% ≤ LVEF ≤ 45% receiving CCM therapy, but LVEF was assessed in only 51 patients. A significant increase in LVEF was in the subgroup with LVEF 35–45% (initially 38.2 ± 2.4% and up to 41.0 ± 7.2% after 6 months (n = 19, p = 0.081). Taking into account the above, detailed and the targeted assessment of echocardiographic parameters and assessment of the clinical status of patients with CHF and AF in patients with implanted CCM, there has been no study published to date.

Thus, the data obtained in the present study for the first time showed a positive effect of CCM therapy on the clinical course of the disease and myocardial remodeling processes in combination with CHF and AF.

CONCLUSION

Despite substantial advances in the treatment of patients with CHF and AF, the problem of increasing the duration and quality of life in such a complex group of patients remains very urgent, due to the extremely poor prognosis and imminent heart transplantation. The introduction of CCM therapy into complex treatment in patients with CHF and AF, according to our results, allows us to assert a significant improvement in the quality of life, a significant positive effect on LV remodeling, and provides an opportunity to postpone heart transplantation. Obviously, this promising treatment method requires further research on its clinical and prognostic significance in patients with CHF and AF, as well as to

Table 4

Comparative characteristics of echocardiographic parameters and NT-pro BNP values in the group of permanent and paroxysmal AF

Parameter	Permanent AF (n = 50)	Paroxysmal AF (n = 50)	*p*
LVEF,%	32 [28; 36]	35 [28; 38]	0.3
LV EDD, mm	69 [62; 72]	66 [62; 70]	0.2
LV ESD, mm	56 [49; 61]	53 [49; 61]	0.4
LV EDV, ml	201 [173; 241]	214 [170; 271]	0.5
LV ESV, ml	135 [109; 172]	138 [110; 195]	0.6
LA, mm	49 [44; 52]	44 [40; 46]	0.000001
LA V, ml	132 [110; 160]	88 [74; 99]	0.000001
NT-proBNP, pg/ml	1599 [820.1; 3334]	927 [302; 2428]	0.002

Table 5

Dynamics of echocardiographic parameters in patients with permanent and paroxysmal AF during treatment

AF	Permanent AF Group (n = 50)			Paroxysmal AF Group (n = 50)		
Parameter	initial	6 months	*p*	initial	6 months	*p*
LVEF	32 [28; 36]	37 [32; 41]	0.000004	35 [28; 38]	38 [30; 43]	0.000001
LV EDD, mm	69 [62; 72]	65 [58; 72]	0.001	66 [62; 70]	63 [60; 69]	0.001
LV ESD, mm	56 [49; 61]	52 [44; 60]	0.002	53 [49; 61]	51 [46; 57]	0.01
LV EDV, mm	201 [173; 241]	196 [153; 237]	0.44	214 [170; 271]	191 [161; 237]	0.09
LV ESV, mm	135 [109; 172]	130 [90; 160]	0.04	138 [110; 195]	111 [94; 140]	0.0009
LA, mm	49 [44; 52]	49 [46; 53]	0.8	44 [40; 46]	42 [40; 46]	0.4
LA V, ml	132 [110; 160]	127 [100; 150]	0.2	88 [74; 99]	77 [65; 97]	0.2

assess the safety, complication rate, number of hospitalizations, and survival of this group of patients on the background of CCM therapy.

The authors declare no conflict of interest.

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A BILE DUCT STENT BROKEN DURING REPEAT PREGNANCY IN A POST-LIVER TRANSPLANT PATIENT

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A young female patient who developed anastomotic biliary stricture following an orthotopic liver transplantation was observed. A self-expandable metallic stent was placed to correct the stricture. At the 8th month of her repeat pregnancy, the stent broke asymptomatically into half. Fortunately, the second childbirth, like the first one, had no complications. Eighteen months later, due to obstruction of fragments by sludge and gallstones, re-stenting was performed with a coated biliary stent. Four years and five months later, recurrent jaundice occurred due to occlusion of the second stent. This was addressed by surgical removal of both stents. Two years after surgery, the bile ducts remain completely patent. We found only two cases in literature on a similar extremely rare biliary stenting complication. It has been suggested that stent deformation may be related to pregnancy. The feasibility of using stenting in benign biliary strictures in some clinical situations is discussed.

Keywords: orthotopic liver transplantation, biliary stricture, biliary stenting, broken stent, pregnancy.

Biliary strictures occur in 10–15% of patients following orthotopic liver transplantation (OLTx) [1, 2]. Early anastomotic strictures are associated with technical errors in the operation, and late ones are related to excessive development of scar tissue in the anastomosis area. Modern methods of interventional radiology and endoscopy allow to eliminate this complication minimally and invasively in a number of cases.

We have observed successful correction of anastomotic biliary stricture that developed after OLTx. To correct it, several endobiliary and surgical interventions were required.

CASE

A 24-year-old female patient was diagnosed in 2005 with liver cirrhosis (HCV, HBV), Child–Pugh B, portal hypertension, parenchymal jaundice. In May 2006, she was placed on the waiting list, and in September 2006 OLTx was performed from a cadaveric donor. Biliary reconstruction consisted of anastomosis between the donor's common bile duct and the recipient's hepatic duct end-to-end on a T-shaped drain. She was discharged on day 25 with satisfactory graft function.

In January 2007, 5 months after OLTx, an increase in cholestasis markers was noted. Ultrasound examination of the liver revealed dilatation of the lobular bile ducts up to 8 mm, narrowing in the anastomosis area. Bile duct stricture was diagnosed. An attempt at endoscopic catheterization was unsuccessful. Percutaneous transhepatic cholangiography confirmed the presence of a 10 mm long and 2 mm diameter stricture in the anastomosis area (Fig., a), cholangio drainage was installed.

The patient insisted on removing the drainage because she was planning to become pregnant and then give birth in the near future. We performed dilatation and stenting of the anastomosis area with an uncoated 8 × 60 mm metal stent (S.M.A.R.T® CONTROL, Cordis) with removal of the external-internal drainage (Fig., b, c). Cholestasis phenomena were arrested, bile ducts were markedly reduced in diameter. She was discharged on day 6.

Pregnancy in August 2007, successful Caesarean section delivery in April 2008 (boy). In October 2010, she had another pregnancy, also without complications. A routine ultrasound scan at 8 months revealed an angular deformation in the middle of the stent (60°) with a slight detachment of its fragments. Given the long gestational age, absence of jaundice and complete bile duct patency, it was decided not to perform surgery. In June 2011, she had another cesarean section delivery (girl). At routine examination in October 2011, there were no signs of impaired bile duct patency.

In October 2012, 4 years and 9 months after stenting, obstructive jaundice developed. Ultrasound examination revealed bile duct dilation, stent deformation with impaired patency. X-ray examination confirmed that the stent was broken in half. An attempt at endoscopic removal was unsuccessful; a papillosphincterotomy was performed, and several gallstones were removed. Percutaneous cholangiography revealed that the proximal (closer to the hilum) fragment of the stent deformed and

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blocked the bile ducts with the formation of sludge and multiple gallstones (Fig., d). On the second attempt, all the stones were successfully washed away, an external-internal drainage was installed.

In the next 6 months, we performed 5 balloon plasty procedures on the bile ducts with no success: the ducts were straightened on an 8 mm balloon; but after removing the instrument, angular deformity was formed again; biliary manometric test [3] showed preservation of biliary hypertension above the obstruction. Given the patient's extremely negative attitude to the operation and ineffectiveness of bilioplasty, it was decided to re-stent. A 8 × 60 mm covered biliary stent Wallflex (Boston) was placed so as to match the fragments and eliminate

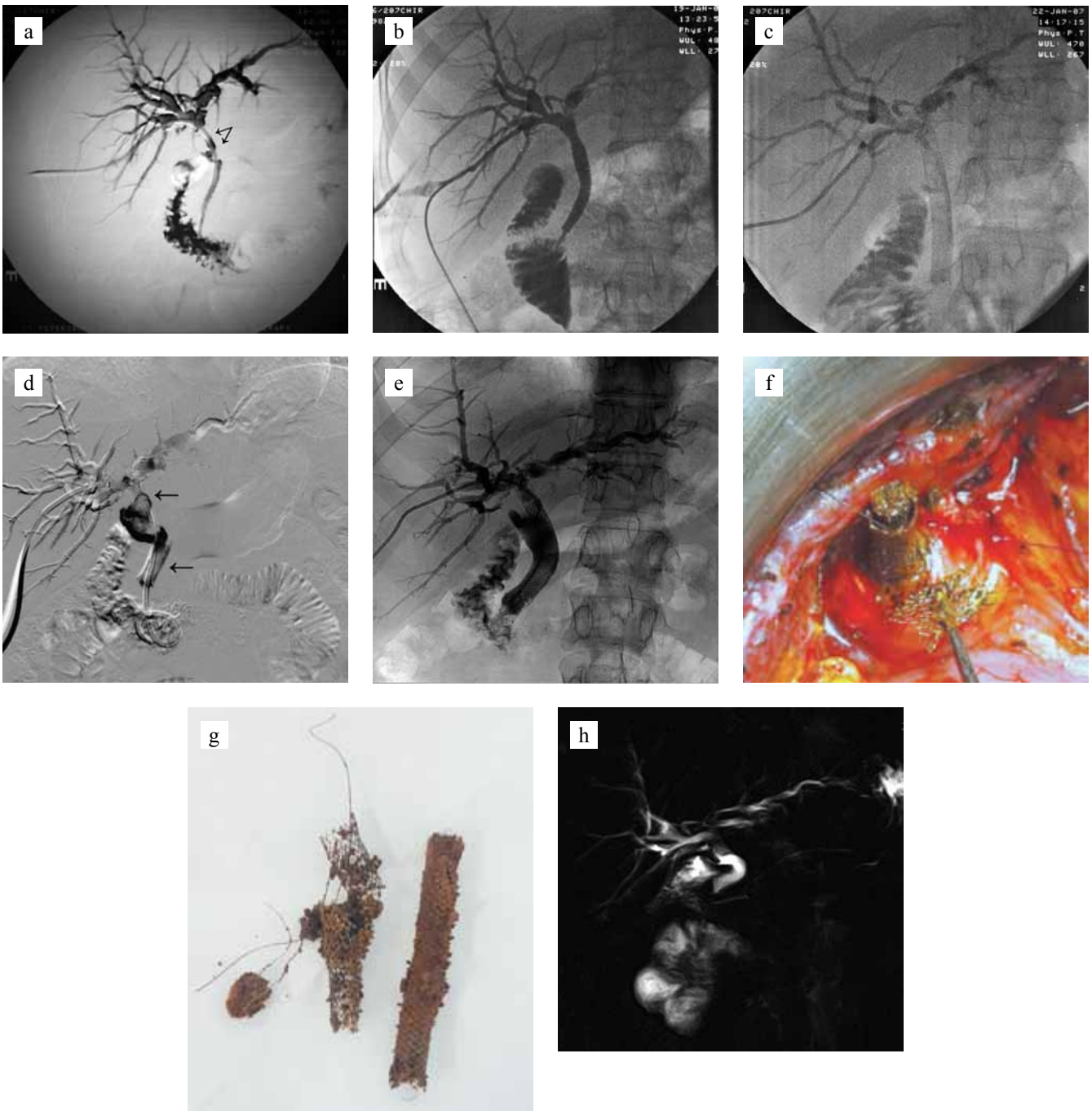

Fig. Radiographic images and intra/postoperative photographs of a patient with post-transplant biliary stricture: a) percutaneous transhepatic cholangiography showing 2-cm anastomotic biliary stricture (arrows); b) after placing a bare nitinol stent S.M.A.R.T. CONTROL, contrast agent flows freely into the duodenum; c) position of stent in the bile ducts; d) fragments of broken stent (arrows), obturated by sludge and stones, cause bile duct obstruction; e) covered biliary stent Wallflex placed coaxially through the broken fragments: normal biliary drainage restored; f) intraoperative photo: a fragment of the nitinol stent captured with forceps, biliary stent occluded by sludge and stones; g) photograph of removed stents; h) magnetic resonance cholangiography 2 years after surgery: bile ducts are completely patent

bile flow obstruction (Fig., e). Hyperbilirubinemia was controlled, the drainage was removed 2 months later, after confirmation of good bile duct patency.

Recurrent jaundice occurred in January 2018, 4 years and 7 months after restenting. Magnetic resonance imaging revealed the cause to be stent occlusion by sludge and gallstones. Open surgery – laparotomy, choledochotomy, stone and stent removal (Fig., f, g) – was performed 2 weeks later. It was decided to leave the distal part of the first stent because it had completely epithelialized and did not interfere with duct patency, and attempts to remove it could significantly complicate the course of the intervention. The operation was completed with leaving the control bile drainage, which was removed after 3 months.

Control examinations (the last one was in May 2020) showed unobstructed duct patency (Fig., h). The patient was feeling well and was receiving 125 mg of Sandimmune on maintenance therapy.

DISCUSSION

Significant anastomotic biliary stricture developing after OLTx is a life-threatening complication that must be corrected. Treatment methods include endoscopic (mostly) or percutaneous transhepatic bilioplasty with or without stenting, surgical reconstruction [1–2, 4–6]. If these methods are unsuccessful, liver resection together with the stricture or even retransplantation may be required.

Treatment of stricture in our patient can be roughly divided into three stages. The first one was percutaneous drainage with insertion of bare metal stents in 2007. Most authors preferred to treat strictures either with balloon plasty, at the same time noting the disadvantages of such approach in the form of treatment duration (6 months and more) and lack of prognostic criteria of success [1–2]. Primary surgical reconstruction comes with a high risk of complications, especially against the background of significant immunosuppressive therapy, and also not always a positive final outcome [1–2, 4]. Stenting rapidly addressed the problem without surgical risk, but the risk of stent occlusion after some time should have been considered [5–7].

Taking into account these factors and the patient's persistent desire to get rid of the problem as soon as possible, we chose stenting, having warned the patient that additional interventions might be required in the future. The jaundice was quickly stopped. The patient successfully gave birth to two children. The stent worked adequately for 4 years and 3 months before it failed.

The second stage of treatment was required due to recurrent jaundice. As a rule, complications from the stent are caused by its "clogging" with sludge/stones or by "neointimal" sprouting. Cases of stent failure have been described in hepatobiliary tumors deforming the stent as the malignant process progresses.

A broken metal biliary stent in a benign stricture is an extremely rare event. We found only two detailed observations in the literature [8, 9]. K. Kawakubo et al. [8] described a patient with benign biliary stricture after liver resection, which was successfully eliminated with an uncovered metal stent. After 4 years, signs of recurrent cholangitis appeared, which was the reason for hospitalization. Endoscopic retrograde cholangiopancreatography (ERCP) demonstrated that the stent was torn in half and the distal of the stent part was floating in the dilated common bile duct. After balloon dilatation of the papilla, the stent fragment was successfully removed with endoscopic forceps.

I. Zuber-Jerger and F. Kullmann [9] observed a 93-year-old patient in whom, after unsuccessful treatment of cholangitis stricture with repetitive plastic stenting, a self-expandable metal stent was also endoscopically installed. One year later, the patient developed abdominal pain, fever, and jaundice. Duodenoscopy showed an incomplete fracture of the stent. It had migrated into the duodenum. This fragment was removed using an Nd:YAG laser, after which a second stent was placed.

In our case, the stent did not migrate from the bile ducts. It can be assumed that the possible cause of its breaking was ductal deformation during two pregnancies. This reason is also supported by the timing of stent fragmentation: the 8th month of the second pregnancy, when the enlarged uterus presses on the liver as much as possible and pushes it to the subcostal area. This mechanism has not yet been considered in literature.

As for treatment, we considered all possible options. An attempt at endoscopic access was unsuccessful. Given the technical possibility of repeated prosthetic replacement, and the appearance of modern covered stents designed specifically for the biliary tract by that time, we chose this option, considering that in case of failure, open surgery will not be ruled out. The patient fully understood the risks of both interventions and preferred a less aggressive approach. The stent was installed coaxially through the fragments such that the fragments of the first stent were almost perfectly aligned, the construction remained passable for 4 years and 7 months.

The third stage consisted in surgical removal of the stents occluded by sludge. Fortunately, there was no need to extend the operation to complex liver reconstruction or resection. There were no signs of bile ducts dysfunction for more than 2 years.

On the whole, the patient's treatment should be considered successful. On one hand, three procedures were required to completely cure the biliary stricture, but two of them were minimally invasive and allowed to provide a good, full-fledged quality of life for 9 years. Unfortunately, attempts at endoscopic stent removal were unsuccessful. The patient underwent a small open surgery without any complications. The total follow-up period

was 13.5 years. Both children, 12 and 9 years old, are growing and developing normally.

The above observation suggests that the use of standard methods (bilioplasty, installation of a temporary stent with endoscopic removal after a few months) is not always possible and effective in a particular clinical situation. Most researchers recommend refraining from stenting benign strictures, but do not deny that there are situations where this method can be justified [1, 2, 6, 7, 10]. We fully share this point of view: there are correct recommendations, and there is a specific clinical situation. The most important thing is the final treatment outcome. An extremely important factor is having a multidisciplinary medical team that would determine the options and sequence of treatment procedures [1, 2].

Introduction of biosoluble stents is expected to open up a certain perspective in the treatment of such patients in the future. The first outcomes of their use look very promising [11, 12].

The authors declare no conflict of interest.

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REGENERATIVE AND HEPATOSPECIFIC ACTIVITY OF TOTAL RNA FROM XENOGENIC BONE MARROW CELLS

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Objective: to study the peculiarities of the induction effect of total RNA (tRNA) from xenogenic bone marrow cells (BMCs) on regeneration processes in the recipient's native liver with extensive liver resection using an adoptive transfer model. **Materials and methods.** The study was carried out on an adoptive transfer model using male Wistar rats (n = 20) and guinea pigs (n = 17). The donors were rats (n = 10). 12 hours after extensive liver resection (70-75%), tRNA was isolated from BMCs and injected into intact (non-operated) recipients intraperitoneally at a dose of 30 μg/100 g of weight. The induction effect of the tRNA on operated rats was studied in 3 groups of recipients: Group 1 (control, n = 5) – administration of saline to guinea pigs; Group 2 (control, n = 10) – administration of tRNA from a donor rat to a recipient rat (allogeneic transfer); Group 3 (experiment, n = 12) – administration of tRNA from a donor rat to a recipient guinea pig (xenogeneic transfer). In histological preparations of recipient livers, after 48, 72 hours and 7 days, we studied the mitotic activity of hepatocytes and the features of the microscopic picture of the liver. The significance of differences in the compared groups was assessed using the parametric Student's t-test. **Results.** The ability of BMC tRNA to tissue-specifically activate regenerative and immune responses in the liver after extensive resection was found to depend on the donor and recipient species identity. Introduction of allogeneic donor tRNA in the recipient's liver resulted in predominant enhancement in hepatocyte mitotic activity ($p < 0.05$). The use of xenogeneic donor tRNA leads to enhanced activity of only immuno-inflammatory reactions in the recipient's liver, such as sinusoidal cell activation, lymphocytic infiltration into sinusoids, and portal tract infiltration by inflammatory cells. **Conclusion.** To induce regenerative processes in the liver, tRNA obtained from allogeneic BMCs should be used.

Keywords: bone marrow cells, total RNA, xenogeneity, adoptive transfer, liver, resection, regeneration.

The therapeutic potential of RNA specimens from animal parenchymatous organs, which were used to activate regenerative processes in homologous damaged organs, was the subject of in-depth studies at the end of the last century [1–3]. Currently, owing to the development of the theory of stem cells and the improvement in application of cell technologies in medicine, research on regulation of regenerative processes in damaged organs has focused on studying the prospects of using total RNA (tRNA) from lymphoid bone marrow cells (peripheral blood lymphocytes, thymus, spleen and bone marrow cells). It has been shown that tRNA isolated from these cells, similarly to immune system cells, is able to actively participate in the regulation of physiological and repair regenerative processes in organs and tissues of various histotypes [4–7], and therefore can be used as a universal means of regenerative therapy. When injected into a recipient's body, various tissue RNAs and, moreover, RNAs of immune system cells, which include bone marrow cells, do not only regulate repair morphogenesis processes in damaged organs. They are also capable of inducing immune responses that can weaken or even distort the degree of regenerative processes. For example, when using xenogenic donor material, which, in terms of economy and availability, is one of the most preferred sources for obtaining tRNA specimens for medicine [8].

The aim of this study is to study the effect of tRNA derived from rat bone marrow cells after extensive liver resection on induction of regenerative processes in a guinea pig liver, using an adoptive transfer model.

MATERIALS AND METHODS

The work was performed on male Wistar rats weighing 250–300 g (n = 20) and guinea pigs weighing 350–450 g (n = 17). The adoptive transfer model was used to study the features of the effect of xenogeneic tRNA on regenerative processes in the liver [9]. Earlier, using this model, we proved that tRNA from bone marrow cells (BMCs) of an allogeneic donor effectively performs targeted delivery of regenerative signals to the damaged liver of an allogeneic recipient [7]. To prove the ability of tRNA from xenogeneic BMCs in liver damage to

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transfer regenerative information, we used an experimental model of extensive liver resection (70–75%), which is known to be accompanied by activation of hypertrophic regeneration mechanisms with pronounced mitotic activity in the remaining part of the organ [10]. Rats with partial hepatectomy constituted the donor group (n = 10). Bone marrow was taken from donor rats 12 hours after liver resection (this interval is required for the appearance of morphogenetically active cells in the bone marrow) and mononuclear (hematopoietic) BMCs fraction was isolated from it, which was then used to obtain tRNA. Total RNA from the mononuclear fraction of BMCs was isolated by the method developed by biotechnology company Evrogen (Russia) using the ExtractRNA reagent, which made it possible to obtain from 105.5 to 127.7 μg of total RNA from each 3.5×10^7. The ability to accumulate and transfer regenerative signals specifically to the liver when using tRNA from the mononuclear fraction of BMCs was assessed in rats based on severity of induction of the proliferative activity of liver hepatocytes in intact recipients 48 hours, 72 hours, and 7 days after they were injected with donor material (tRNA from rats with liver resection) at a dose of 30 μg/100 g of animal weight.

The recipients were divided into 3 groups: group 1 consisted of control injection of saline to guinea pig (n = 5); group 2 included administration of tRNA from a donor rat to a recipient rat (n = 10); group 3 was made up of administration of tRNA from donor rat to recipient guinea pig (n = 12). At the indicated time after tRNA administration, liver pieces were taken from recipients, histological specimens were prepared from them, followed by hematoxylin and eosin staining. The number of mitotically dividing cells was determined in 30 fields of view (Leica DMLS microscope, Germany) with subsequent calculation of the mitotic index (MI) in ppm (‰). The significance of differences in the mitotic activity of hepatocytes in the compared groups was assessed using the parametric Student's t-test ($p < 0.05$).

RESULTS AND DISCUSSION

It was found that in control group 1, where the recipients (guinea pigs) were injected with saline, mitotic activity of hepatocytes did not significantly differ from the original values at all the studied periods (48 hours, 72 hours, and 7 days). MI values did not exceed 0.02 ± 0.01‰ (0–2 mitosis per 30 fields of view), Fig. 1. There were no signs of cellular infiltration in the liver tissue of guinea pigs in this group at all the follow-up periods.

However, in control group 2, where activated tRNA from BMCs of rats was injected not into guinea pigs but into healthy allogeneic recipient rats intraperitoneally, a significant increase in mitotic activity of hepatocytes was noted at 48 and 72 hours after adoptive transfer. MI values at these periods were 0.7 ± 0.2 respectively ($p < 0.05$). Mitoses were detected in 5–7 out of the 30 studied fields of view compared to the original level (0–2 mitosis per 30 fields of view), Fig. 2.

By day 7, the mitotic activity of hepatocytes in this group had returned to the original values. It is important to note that in control group 2, at 48 and 72 hours, not only was there an increase in mitotic activity in the liver tissue of recipient rats, but also a weakly marked increase in cellular infiltration, indicating the appearance of hepatospecific (tissue-specific) immune signals in a healthy allogeneic recipient.

The results obtained in control group 2 showed that tRNA from BMCs is a carrier of both regenerative (proliferative) and immune signals induced by extensive liver resection in the donor's body. A study of the effect of adoptive transfer in experimental group 3, where activated

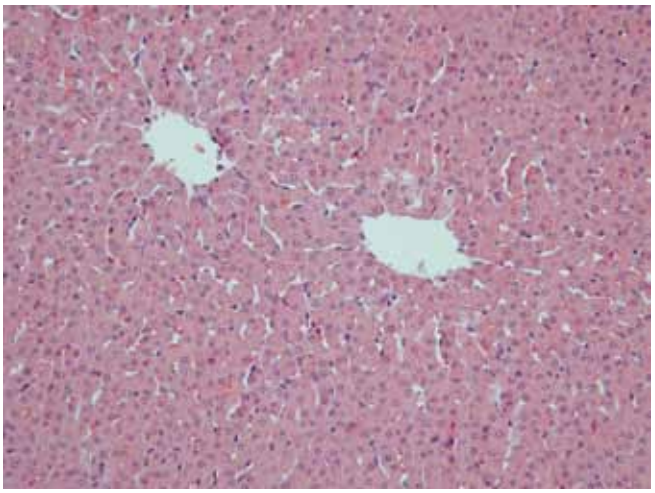

Fig. 1. Histological picture of the liver of a healthy guinea pig after administration of saline (control). No signs of hepatocyte proliferative activity and sinusoidal cell activation. H&E stain. 200× magnification

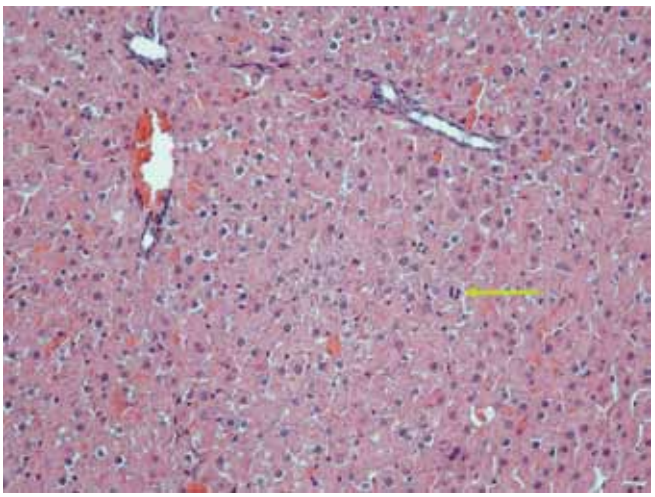

Fig. 2. Histological picture of the liver of a healthy rat 48 hours after administration of tRNA obtained from a rat with extensive liver resection (allogeneic adoptive transfer). Signs of hepatocyte proliferative activity (mitosis indicated by arrow). H&E stain. 200× magnification

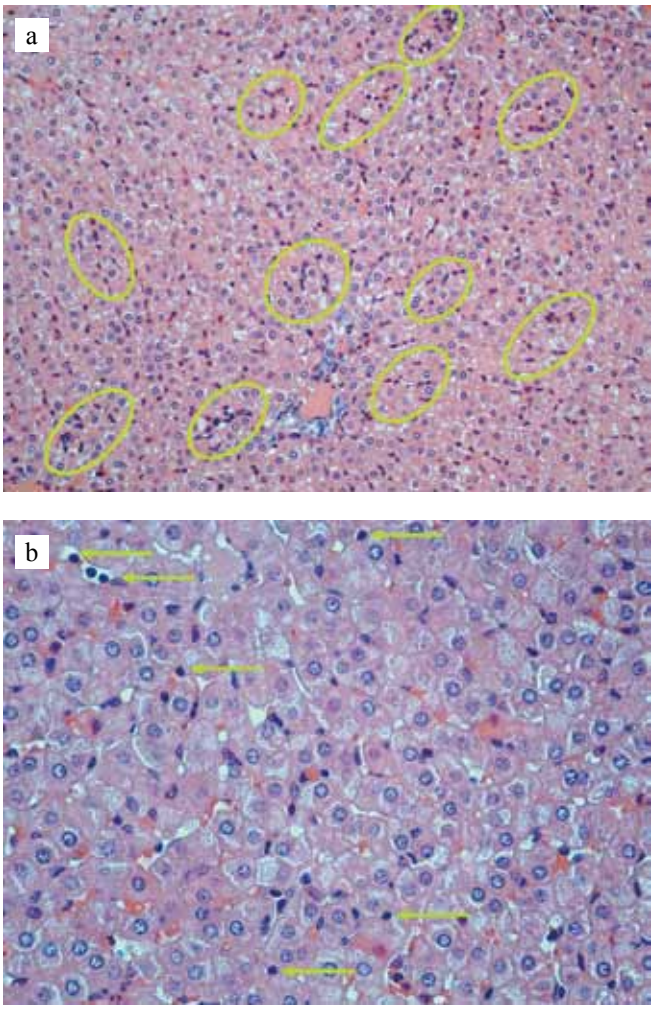

Fig. 3. Histological picture of the liver of a healthy guinea pig at day after 7 days of administration of tRNA obtained from a rat with extensive liver resection (xenogenic adoptive transfer): a) signs of pronounced sinusoidal cell activation (indicated by an oval). H&E stain. 200× magnification; b) lymphocytes in the sinusoidal lumen (indicated by arrows). H&E stain. 400× magnification

tRNA from BMCs of rats was injected intraperitoneally into intact xenogeneic recipients, showed that the histological picture of guinea pig liver significantly differed from the histological picture of the liver of allogeneic recipients (rats) in group 2.

It was established that for the mitotic activity of hepatocytes at the same observation periods (48 hours, 72 hours, and 7 days), MI values did not significantly differ from the initial level; they remained within 0.02 ± 0.01‰. Besides, histological preparations of the guinea pig liver at all studied periods showed diffuse activation of the liver sinusoidal cells, the presence of lymphocytes in sinusoids, as well as minor signs of infiltration of the liver portal tracts by inflammatory cells, which was especially pronounced at day 7 (Fig. 3, a, b).

Thus, it has been shown that xenogenic tRNA in a recipient's body during adoptive transfer does not induce mitotic and proliferative activity of hepatocytes but enhances hepatospecific immune response. Lymphoid cells, especially peripheral blood lymphocytes, are known to be carriers of regenerative signals in the body [4, 9], which are capable of targeted delivery of homologous and xenogenic RNA to cells [11]. The absence of a regulatory effect of activated tRNA on the mitotic activity of hepatocytes in group 3, apparently, can be associated with the fact that after contact with xenogeneic immune RNA, the recipient's lymphoid cells acquire new immunoregulatory properties and, upon contact with target organ cells, change the functional state of the RNA molecules of these cells [4]. Consequently, under the influence of xenogenic tRNA delivered to the cells, numerous regulatory protein-noncoding RNAs of the recipient liver cells become unable to exert regulatory effect on mRNA and activate translation and/or transcription of protein-coding genes at the level of the genome of these cells [6].

The mechanisms underlying the changes in the immunoregulatory properties of lymphoid cells in the recipient's body after their contact with immune RNA are not yet clear. However, incorporation of RNA into lymphoid cells should undoubtedly be one of the important factors for their subsequent activation.

In group 3 with introduction of xenogeneic tRNA in the recipient's liver, we detected activation of not only lymphocytes but also liver sinusoidal cells (due to the common mesenchymal origin with lymphocytes): Kupffer cells, endotheliocytes lining the liver sinusoids, perisinusoidal cells (Ito cells/stellate cells) and others.

Excessive activation of liver cells is what can explain the fact that when modeling liver damage by chronic CCl4 inoculation, induction of regenerative processes in the liver of mice using xenogeneic tRNA of rat liver is accompanied by a twofold increase in the amount of interlobular connective tissue and collagen at month 2 in comparison to the control [12]. Decreased number of necrosis foci in the liver was also noted. The authors believe that lower animal death may be associated not so much with increased mitotic activity of hepatocytes by this period, but with accelerated replacement of necrotizing liver cells by connective tissue and decreased intoxication.

When modeling adoptive transfer using xenogenic tRNA in the recipient's liver, the activity of liver cells of mesenchymal origin (sinusoidal cells) also significantly increases, as well as infiltration of the liver portal tracts by inflammatory cells, in the absence of activation of the mitotic activity of hepatocytes (see Fig. 2).

There is an opinion [13] that adequate exchange of regenerative information in the body is provided by production of two types of exosomes by immune cells: immune RNAs, which are involved in stimulating innate and acquired immunity mechanisms, and non-immune ones, through which RNA performs a remote synchronization of cell proliferation and differentiation processes. Based on the studies carried out, it can be concluded that

xenogeneic tRNA from BMCs stimulates predominantly immune regeneration mechanisms in the recipient's liver through activation of the inflammatory process. On the contrary, allogeneic (syngeneic) RNA predominantly enhances the mitotic and proliferative activity of parenchymal cells. These differences in the induction of regenerative processes in organs when using allogeneic (or syngeneic) and xenogeneic tRNA allow us to recognize that production and use of allogeneic tRNA preparations from BMCs is more effective, promising, and preferable than with xenogeneic tRNA preparations.

CONCLUSIONS

1. The adoptive transfer model allows to identify specific mechanisms for triggering the regenerative process when using allogeneic and xenogenic tRNA from BMCs.
2. The intrinsic ability of tRNA to hepatospecifically regulate regenerative and immune responses in the liver is expressed by predominant intensification of mitotic (proliferative) activity of hepatocytes when using allogeneic tRNA, and by intensification of immune-inflammatory reactions in the liver when using xenogeneic tRNA.
3. When choosing a source for tRNA isolation and application in the clinic, preference should be given to allogeneic sources of lymphoid cells, which effectively accelerate the processes of regenerative morphogenesis of cells of the damaged organ (liver).

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IN VIVO ASSESSMENT OF THE BIOCOMPATIBLE PROPERTIES OF RESORBABLE POROUS MATERIALS FOR PLEURAL IMPLANTATION

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Correcting the pleural cavity space or filling large residual cavities (up to 500–700 cm^3), arising as a result of extensive combined resections of the lung or extrapleural pneumolysis in tuberculosis and other lung diseases, still remains a challenging issue. The surgical methods used to correct the pleural cavity space are traumatic in nature. Moreover, various biological and synthetic materials used are not effective enough. **Objective:** to conduct an in vivo study of the biocompatible properties of laboratory samples of porous materials based on polylactide (PLA) and polycaprolactone (PCL) as potential materials for pleural implants development, as part of the general problem of developing a resorbable porous implant for intra- and extrapleural implantation and in situ formation of a "biological filling" to correct the volume of the pleural cavity. **Materials and methods.** In vivo subcutaneous implantation was performed in Wistar rats. The experiment involved the following samples: No. 1 – 3.0%; No. 2 – 4.0%; No. 3 – 1.7%. The ratio of the polymers in the solution was, respectively: 3/1, 1/3 and 1/1 PLA/PCL. Highly porous implants were obtained by lyophilization. The porosity of the samples ranged from 96.0% to 98.3%. The Young's modulus was from 100 to 1800 kPa. In the control group, a Mentor silicone implant shell was used. The explantation time was 1, 2, 3, 4, 5, 8, 12, 14 weeks. Histological, histochemical and immunohistochemical studies of explants and surrounding local tissues were conducted. **Results.** Reaction of local tissues to the implantation of three types of samples of different composition from PLA/PCL, accompanied by material resorption processes, replacement by fibrous tissue, vascularization and encapsulation, without perifocal inflammation and reactive changes, indicates the biocompatibility of the materials studied. In control samples with silicone implant, a long-lasting perifocal reaction from eosinophilic leukocytes was revealed, which prevents us from excluding the possibility of an allergic reaction to the implant material in the surrounding tissues. **Conclusion.** In vivo experiments on the small animals show the biosafety and high biocompatibility of laboratory samples of bioresorbable highly porous matrices based on polylactide and polycaprolatcon as potential materials for development of pleural implants. Further studies with scaling of laboratory samples and a detailed study of the dynamics of biodegradation of porous matrices in vivo in large animals are required. The need for further improvement in laboratory samples of bioresorbable pleural implants is associated with giving the porous matrices antibacterial, bioactive and X-ray contrast properties.

Keywords: polylactide (PLA), polycaprolactone (PCL), bioresorbable materials, pleural implant, biocompatibility, extrapleural implantation, interpleural implantation, local tissue response to implants.

INTRODUCTION

In thoracic surgery, particularly in pulmonary tuberculosis surgery, there has long been the problem of correcting the pleural cavity volume, or filling large residual cavities (up to 500–700 cm^3) resulting from surgical intervention – extensive combined lung resections or extrapleural pneumolysis. The surgical methods used to correct the pleural cavity volume, such as one-stage or delayed thoracoplasty, used so far, are traumatic, accompanied by chest deformity and severe postoperative pain syndrome [1–4].

Over the entire period of existence of extrapleural pneumolysis surgery and correction of the pleural cavity volume after combined lung resections, about a hundred different methods have been proposed using various

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biological and synthetic materials. However, all of them have proved to be insufficiently effective [5–10].

Implantable biological materials, such as collagen, collagen sponges, structured collagen, fibrinogen, gelatin, hyaluronic acid, etc., have fast resorption timeframe that is insufficient for manifestation of a collapsosurgical effect.

Among synthetic materials, polyurethane foam, fiberglass, polymethylmethacrylate, polystyrene, silicone, etc. have been used. In recent years, silicone prostheses and expanders, designed for reconstructive and plastic breast surgery, have proven to be the best [11–14].

In the last decade, there has been a trend towards wider clinical use of resorbable implants in various surgical specialties. For instance, in maxillofacial surgery, neurosurgery, traumatology, orthopedics, and dentistry, resorbable implants based on polymers and copolymers of glycolic and lactic acids are replacing titanium metal implants. More complex polymer compositions combining different synthetic and biological polymers, as well as various bioactive preparations are also used.

One of the advantages of resorbable implants is that once the healing effect is achieved, the implant does not require additional surgery to remove it. After a certain time, the implant undergoes bioresorption; polymer degradation products are harmless to the body. It should be noted that currently in Russia there are no registered implants for targeted use in thoracic surgery, particularly in pulmonary tuberculosis surgery.

The main requirements for the properties of a pleural implant are: low specific gravity, compliance of the Young's modulus of the implant with the elasticity modulus of the soft tissues of the chest, hydrophobicity of the main volume in combination with hydrophilicity of the surface layer, controllability of implant resorption time, ability to be replaced in the process of resorption by its own tissue, ability to neovascularization.

Synthetic polymers, polylactide and polycaprolactone, are part of various resorbable materials and implants used in maxillofacial surgery, traumatology and orthopedics, as well as in endovascular surgery. Despite the active use of fibers and molded products based on these polymers in medicine, there are few studies on porous materials [15–17]. Also, unlike the known porous materials based on proteins and polysaccharides, medical products made of polylactide and polycaprolactone are resorbed at a significantly lower rate, which is important for their long-term functioning as a pleural filling.

Objective: to conduct an in vivo study of the biocompatible properties of laboratory samples of bioresorbable highly porous matrices based on polylactide (PLA) and polycaprolactone (PCL) as potential materials for the development of pleural implants.

CHARACTERISTICS OF THE STUDY OBJECTS

The following were chosen as starting materials: poly(L-)lactide (PLA) 4032D "*Nature Works*" with 200 kDa average molecular weight (Mw) and ~2 polydispersity index (PDI); polycaprolactone (PCL) No. 440744 "*Sigma Aldrich*" with 80 kDa number average molecular weight (Mn) and ~2 polydispersity index (PDI).

At the department of nanobiomaterials and structures, Kurchatov Institute, laboratory samples of porous materials of three compositions were prepared at on the basis of the developed technology by freeze-drying of frozen solutions of a PLA/PCL polymer mixture in 1,4-dioxane: sample No. 1, containing 3 wt.% of PLA/PCL mixture in initial solution with a 3/1 polymer ratio; sample No. 2, containing 4 wt.% PLA/PCL with a 1/3 ratio and sample No. 3, containing 1.7 wt.% of a mixture of PLA/PCL polymers in an initial solution with a 1/1 ratio (Fig. 1, a, b).

Regardless of the composition, all three samples have a branched structure with interpenetrating pores. A typical photomicrograph of a cut of the material is shown in Fig. 1, c. The average pore sizes are 100–150 μm.

The mechanical properties of spongy materials significantly depend on both the porosity of the materials and the polymer composition. Due to the fact that the glass

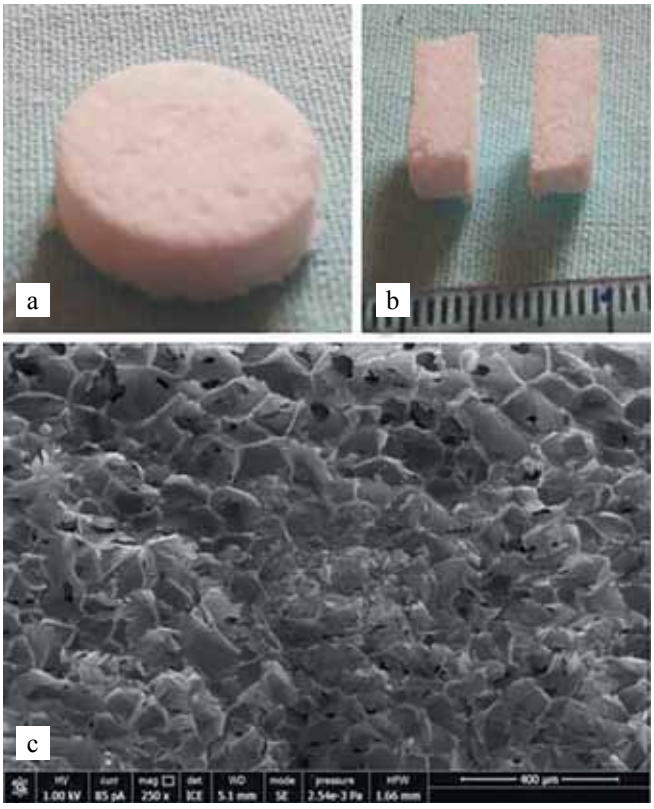

Fig. 1. Appearance and electron microscopy of the PLA/PCL sample: a) appearance of the laboratory sample 1.7% PLA/PCL 1/1; b) cross-section of the laboratory sample 1.7% PLA/PCL 1/1; c) scanning electron microscopy of a slice of a spongy material based on polylactide obtained by cryolyophilization. Accelerating voltage 1 kV

transition temperature of polycaprolactone lies around –60 °C, adding it decreases the elastic modulus of the material. Thus, the mechanical properties can be tuned over a wide range of values. For the spongy materials discussed in this paper, the elastic moduli are 1800 ± 250 kPa (Sample 1), 1240 ± 320 kPa (Sample 2), and 97.7 ± 9.5 kPa (Sample 3). The samples were sterilized by radiation with 1.5 Mrad maximum dose.

At the next stage, in vitro studies of hemolytic, cytotoxic, and matrix properties as well as a study of biodegradation of laboratory samples in a model environment were carried out at the department of biomedical technologies and tissue engineering, Shumakov National Medical Research Center of Transplantology and Artificial Organs.

Hemolytic properties were examined on an extract obtained from experimental samples of sponges using rabbit red blood cell mass. Data (percentage of hemolysis is less than 2) obtained from the study suggests that the extract of experimental samples of sponges from PLA and PCL is free of hemolytically active substances, while the product itself has no hemolytic effect and meets the requirements for medical devices according to GOST ISO 10993-4-2011 "Research on devices that interact with blood".

Cytotoxicity studies were performed on a test-culture of NIH/3T3 mouse fibroblast cells. Matrix properties were examined on a culture of human adipose tissue-derived mesenchymal stem cells. Results from these studies suggest that the experimental samples of PLA and PCL sponges have no cytotoxic effect. However, the surface of the sponges studied does not have sufficient matrix properties, poorly supports cell adhesion, and does not provide the necessary cell proliferation conditions. Hydrophilization of the sponge surface may be a promising approach for improving the matrix properties of a material. In order to verify this assumption, some samples were selected for hydrophilization of their surface with gelatin. With the subsequent re-experiment on the cultivation of human adipose tissue-derived mesenchymal stem cells. In this case, the matrix properties of the sponges improved significantly.

In the biodegradation study, samples of porous matrix were incubated statically at 37 °C in 20 mL of 0.025 M phosphate-buffered saline containing nipagin and nipazole at 0.06% and 0.02% concentrations, respectively. Weight loss due to degradation was recorded gravimetrically on an analytical balance. The buffered saline solution was replaced with a fresh one every 2 weeks. The PLA/PCL porous samples were found to be resistant to biodegradation in phosphate-buffered saline at 37 °C for 26 weeks. This suggests that the volume and shape of laboratory samples can be maintained in planned long-term in vivo studies.

IN VIVO BIOCOMPATIBILITY ASSESSMENT METHODS

In the study of biocompatibility in vivo, we used subcutaneous implantation of samples into Wistar rats (total number of animals n = 8). All studies on laboratory animals were carried out in strict accordance with the laws of the Russian Federation (the Rules of Laboratory Practice, approved under Order No. 708 of the Ministry of Health of Russia, dated August 23, 2010, as well as the GOST R ISO 10993-2-2009 standard "Medical Devices. Assessment of the Biological Action of Medical Devices. Part 2. Requirements for Animal Welfare") and in compliance with the biotic principles approved by the European Convention for the Protection of Vertebrate Animals, 2005.

The implanted samples were disc-shaped porous materials with 5 mm diameter and 3 mm thickness.

The experimental research methodology was as follows. After 1.0 mL of ketamine was injected intramuscularly on the previously epilated and treated skin of the back, an incision was made along the midline with a length of about 4 cm. Then soft tissues were bluntly dissected in the corners of the wound and in different directions, soft tissues were stratified up to the muscle fascia, forming four implant beds, one placed in each bed, and a fragment of the silicone implant capsule was placed in the fourth bed. The distance between the implanted samples was about 4 cm. After the implant has been placed, each bed was isolated by suturing with an atraumatic non-absorbable 4/0 prolene thread.

The animals were taken out of the experiment by an overdose of ether anesthesia. The explanation periods were: 1, 2, 3, 4, 5, 8, 12, 14 weeks (one animal per explantation point). At autopsy, the macroscopic picture of the implantation area and the condition of the implants themselves were assessed, after which the tissues of the implanted area were taken for further morphological examination.

MORPHOLOGICAL EXAMINATION

Tissue materials were fixed in 10% neutral formalin, performed based on a standard technique. Histological sections, 3–5 microns thick, were prepared. The following histological stains were used: hematoxylin and eosin for observational microscopy of implants with adjacent soft tissues and skin; histochemistry – elastica Van Gieson's stain to detect fibrotic processes, fibrous structures; Brachet stain to detect plasma cells in the infiltrate. In addition, an immunohistochemistry with the *CD34* antibody was performed to assess implant vascularization.

RESULTS AND DISCUSSION

Macroscopic assessment of implants and local tissues showed that during the first two weeks, external examination of animal skin in the area of implantation of resorbable porous materials (samples No. 1, 2 and 3 – PLA/PCL) showed no visual signs of the presence of implants in the subcutaneous fascia. On palpation, the implants were well defined, their consistency was assessed as soft-elastic. The skin over the implants was mobile, with no signs of inflammation.

At week 6–14, there was a gradual thickening of the soft tissues of the implantation area with transformation from soft-elastic to dense-elastic consistency. At the same time, in the studied areas, mobility of the skin and soft tissues in the studied areas was preserved, and the implant contours were more clearly defined.

In the silicone implant area, the changes are not very pronounced, apparently due to its thickness (1 mm); there are practically no changes in the dynamics of palpation at different study periods; soft tissue mobility is preserved.

During explantation at all implantation periods, examination of the surrounding tissues (muscles, subcutaneous fascia, skin) showed no macroscopic signs of inflammation. The surface of muscles and fascia was smooth, shiny, elastic consistency, that is, there were no signs of inflammation. The dynamics of macroscopic changes in the implant itself consists in gradual impregnation from the periphery to the center with tissue fluid and a change in the structure of the implant substance: if the porous structure of the implant was well differentiated in the early stages, then by the end of the observation, the implants were completely replaced by grayish fibrous tissue; capillaries were visually traced both on the surface and in the implant thickness (Fig. 2, a, b). The silicone implant did not change outwardly in dynamics, it retained its structure – colorless, transparent, with a smooth shiny surface (Fig. 2, c).

Thus, macroscopic examination revealed that during the experiment, the inflammatory response of the surrounding tissues to implantation of samples (samples No. 1, 2 and 3 – PLA/PCL) was practically identical, weakly expressed and practically disappears within a month; the implant material underwent changes, and silicone implants were almost identical at different explantation periods.

Microscopic examination of samples of bioresorbable highly porous matrix (samples No. 1, 2 and 3 – PLA/PCL) showed that a week after implantation, there was a slight edema of the adjacent muscles and fascia around them, mild or moderate diffuse focal infiltration, and the density of the infiltrate near the implant was higher. The cellular composition of the inflammatory infiltrate was polymorphic. It was dominated by macrophages, in some places with an admixture of lymphocytes, single plasma cells, and eosinophilic leukocytes (Fig. 3). Eosinophilic leukocytes were sporadic and were found only in separate fields of view (1–5 cells at 400× magnification). Within a month, the nature of the infiltrate changed to mononuclear and was represented by macrophages, lymphocytes, and plasma cells. Severity of inflammatory infiltration was regarded as minimal.

The dynamics of changes in the material of the implanted samples in response to the reaction of the surrounding tissues to foreign material did not depend on their composition. A week later, there was mild granulomatous reaction around all the samples; multinucleated

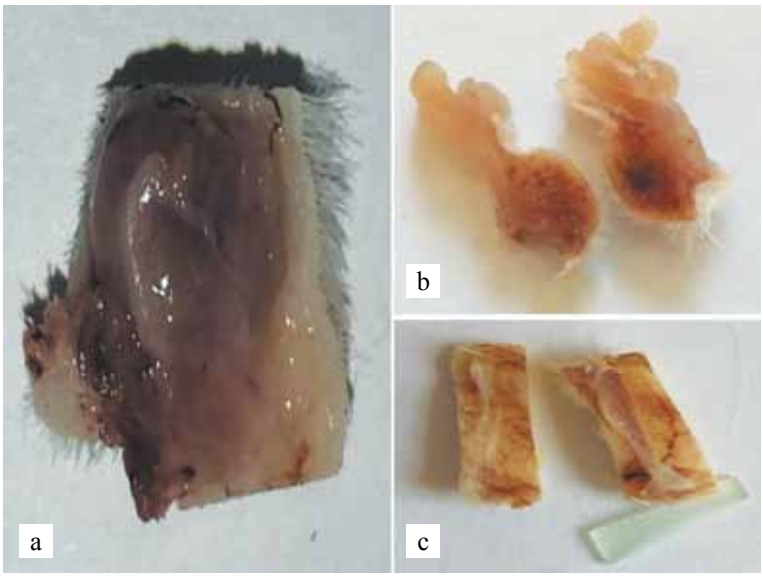

Fig. 2. Macro-preparations of PLA/PCL subcutaneous implants: a) subcutaneous implant 3.0% 3/1 PLA/PCL, 9 weeks, with capillaries on the surface; b) subcutaneous implant 3.0% 3/1 PLA/PCL, 14 weeks, replacement of the implant material by grayish fibrous tissue; c) silicone implant, 14 weeks, unchanged implant structure

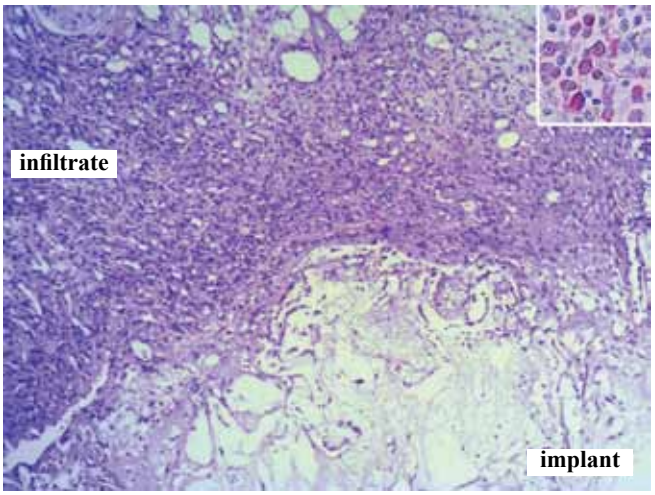

Fig. 3. Perifocal exudative reaction in the soft tissues surrounding the PLA/PCL implant (inset – plasma cells in the infiltrate). H&E stain. 100× magnification; inset – Brasch staining. 400× magnification

foreign body giant cells (FBGCs) with signs of phagocytosis of the implant material were present (Fig. 4, a, b). No capsule formation around the implants was detected. There were also no signs of implant vascularization at this period.

Two weeks after implantation, in the samples of bioresorbable highly porous matrix (samples No. 1, 2, and 3 – PLA/PCL), single small capillaries were detected in areas of the forming granulation tissue, which were located between foreign body granulomas along the implant periphery. Immunohistochemistry with the CD34 antibody showed positive expression in the basement membrane of small vessels located in the implant thickness (Fig. 5), which confirmed the presence of vascularization signs.

Four weeks after implantation, there was a subtotal granulomatous reaction with a large number of FBGCs, with partial replacement of implants with fibrous tissue,

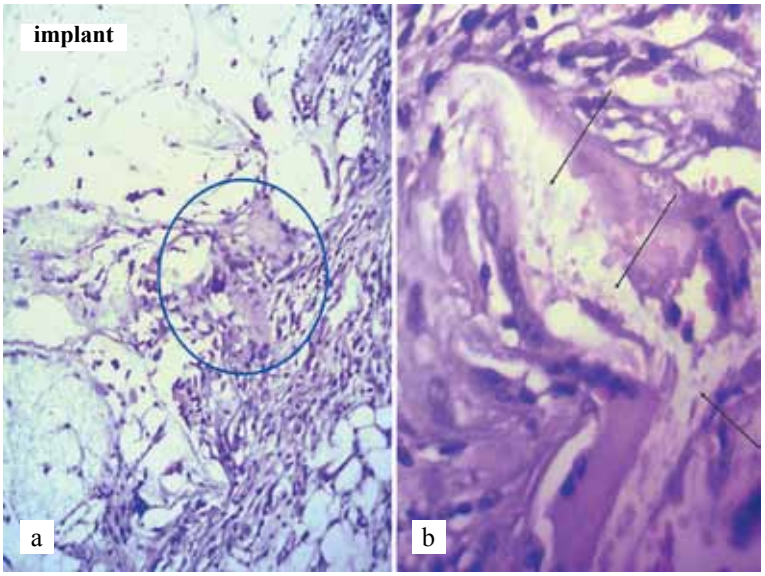

Fig. 4. Morphological picture 1 week after PLA/PCL implantation: a) forming foreign body granuloma inflammation on the periphery of the PLA/PCL implant (circled). H&E stain. 200× magnification; b) giant foreign body cell with signs of phagocytosis of the PLA/PCL implant material (phagocytosed material is indicated by arrows). H&E stain. 1000× magnification

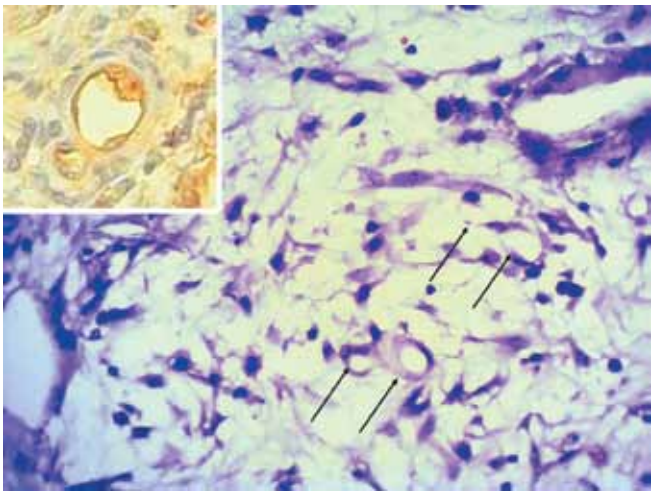

Fig. 5. Morphological picture 2 weeks after PLA/PCL implantation. Initial signs of vascularization of PLA/PCL implant material, formation of single capillaries (capillaries are indicated by arrows). H&E stain. 400× magnification; inset – capillary in the implant (immunohistochemical study with CD34 antibody)

which was confirmed by histochemical examination with Van Gieson stain. There were vascularization phenomena (beginning of vascularization from week 2) and formation of a thin, in some places loose connective tissue capsule around the implant (Fig. 6, a, b, c, d).

After 8 weeks, the implants were almost completely replaced by fibrous tissue; the Van Gieson stain revealed both the presence of diffusely located collagen fibers with small, preserved areas of granulomatous inflammation (such as foreign bodies) between them, and micro areas of focal fibrosis. Microparticles of phagocytosed implant material were found in the FBGC cytoplasm. There was vascularization of the whole implant volume with detection of sinusoidal capillaries and thin-walled vessels, with the presence of erythrocytes in their lumen. There was a thin fibrous capsule formed around the implants, well defined by both observational microscopy and van Gieson stain.

After 12 weeks, the implants were completely replaced with fibrous tissue with a clear formation of multidirectional bundles of collagen fibers with a small amount of FBGCs interfascicularly, with signs of phagocytosis of the sample material located between the collagen fibers. Signs of vascularization were well expressed in the form of full-blooded sinusoidal capillaries and vessels. There was no perifocal inflammatory response in the soft tissues.

After 14 weeks, there was a complete replacement of the bioresorbable highly porous matrix implants (samples No. 1, 2 and 3 – PLA/PCL) with fibrous tissue, with almost complete absence of granulomatous inflammation. Single foreign body giant cells remained between collagen fibers. The implants were completely vascularized throughout the entire thickness. A fibrous capsule was formed in all samples. No inflammation or calcification was observed in the samples and adjacent soft tissues (Fig. 7, a, b, c; Fig. 8).

With silicone implantation (a fragment of the silicone breast implant shell), the fibrous capsule was well defined *after two weeks*. Note that a small diffuse cellular infiltration with predominance of eosinophilic leukocytes (up to 50 eosinophilic leukocytes per field of view at 400× magnification) was detected in the adjacent fascia of the silicone implant, with an eosinophilic reaction of approximately the same severity in the infiltrate at early stages of implantation (one week).

After 8 weeks (2 months), the structure of the implant material was completely preserved; there was no replacement with fibrous tissue, there was practically no granulomatous reaction (only isolated very small fuzzy

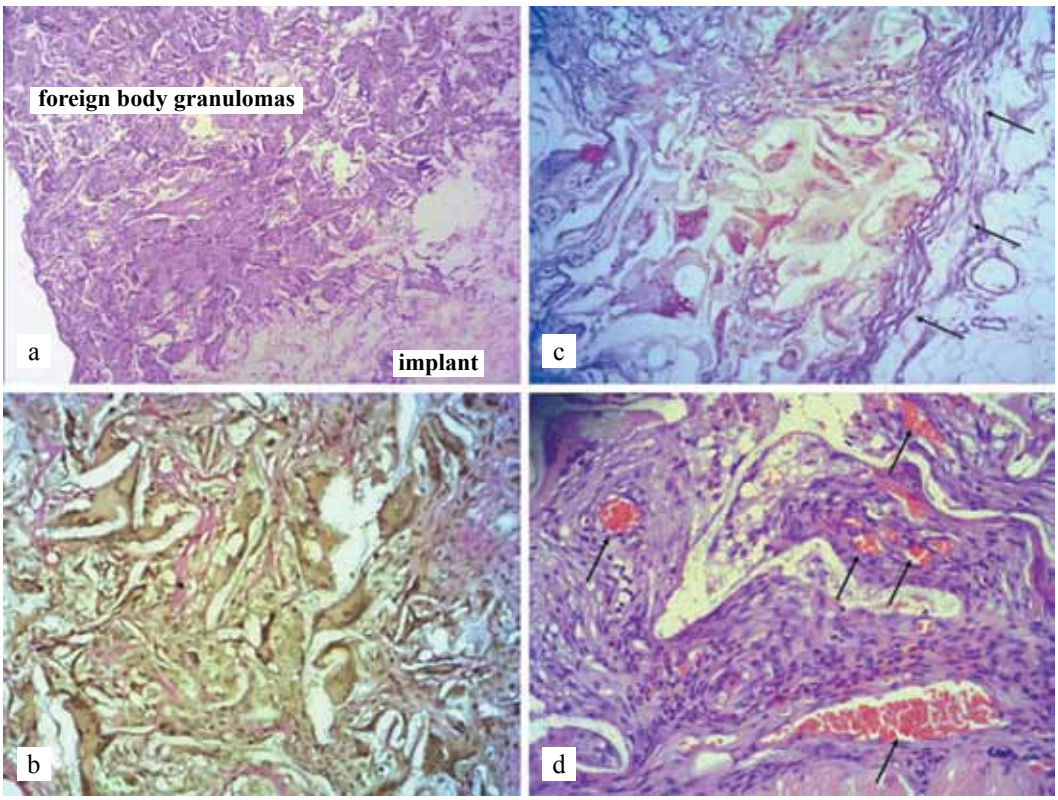

Fig. 6. Morphological picture 4 weeks after PLA/PCL implantation: a) subtotal replacement of the PLA/PCL implant by foreign body granulomas. H&E stain. 200× magnification; b) partial replacement of the PLA/PCL implant by connective tissue; red-stained fibrous fibers located between the foreign body granulomas. Van Gieson's stain. 400× magnification; c) forming capsule around the PLA/PCL implant (indicated by arrows). Van Gieson's stain. 400× magnification; d) vascularization of the PLA/PCL implant, sinusoidal capillaries and vessels (indicated by arrows). H&E stain. 400× magnification

macrophage granulomas were detected); no signs of vascularization were found.

After 14 weeks (3.5 months), the capsule was formed, the implant was still completely intact. There was no foreign body granulomatous reaction, no signs of vascularization, or replacement of the implant with fibrous tissue. There were no reactive inflammatory and exudative phenomena in the adjacent soft tissues (muscles, fatty tissue, fascia, skin with subcutaneous fatty tissue) (Fig. 9, a, b, c).

Thus, reaction of local tissues to the implantation of three types of samples of different composition from

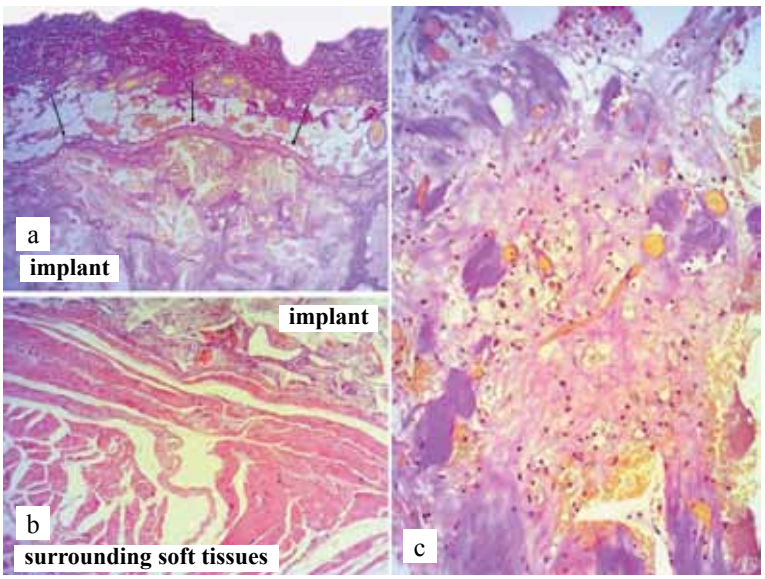

Fig. 7. Morphological picture 14 weeks after PLA/PCL implantation: a) capsule formed around the PLA/PCL implant (indicated by arrows). Van Gieson's stain. 100× magnification; b) absence of inflammatory infiltrate in the soft tissues surrounding the PLA/PCL implant. H&E stain. 100× magnification; c) replacement of PLA/PCL implants by fibrous tissue with almost complete absence of foreign body granuloma inflammation. Van Gieson's stain. 200× magnification

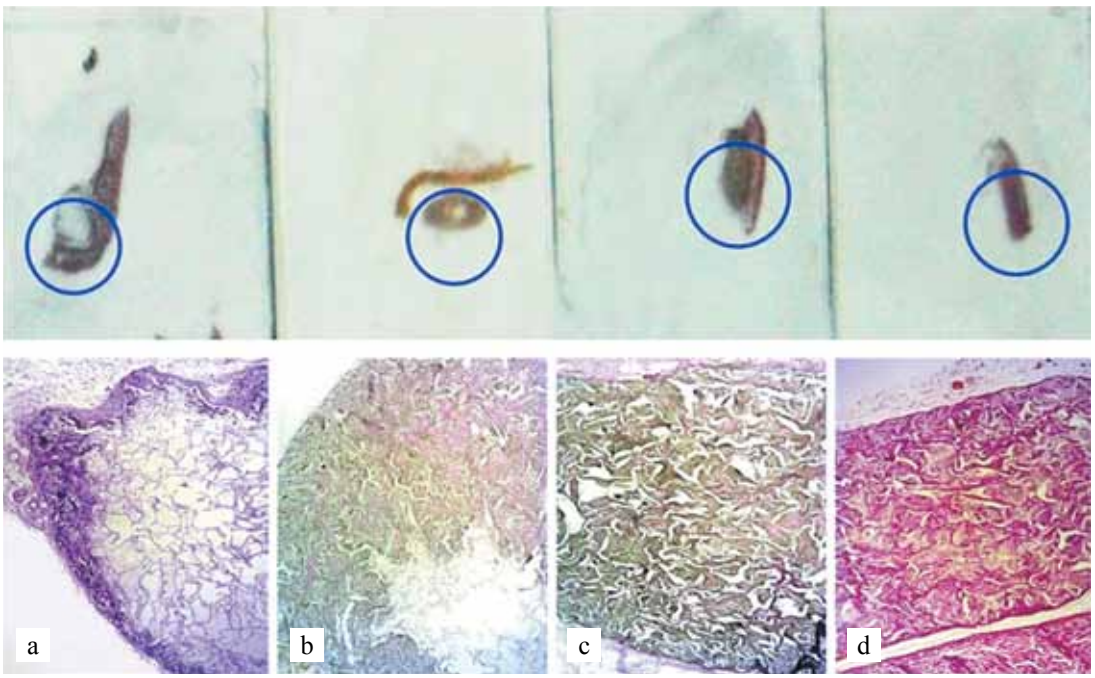

Fig. 8. Dynamics of morphological changes in PLA/PCL implants in the period from 1 to 14 weeks. Upper row – macro preparations, implant with surrounding soft tissues. Bottom row – microscopic preparations (1 week – H&E stain; other preparations – Van Gieson's stain. 100× magnification): a) 1 week – marginal granulomatous reaction of foreign bodies in the implant; b) 4 weeks – subtotal replacement of the implant by foreign body granulomas; c) 8–12 weeks – complete replacement of the implant by foreign body granulomas with fibrous tissue formation; 14 weeks – replacement of the implant by fibrous tissue with single foreign body granulomas

PLA/PCL, accompanied by processes of material resorption, its replacement by fibrous tissue, vascularization, and encapsulation, without perifocal inflammatory process and reactive changes, indicates that the studied materials are biocompatible.

When using a silicone implant, the structure of its material remains unchanged, granulomatous resorption reaction is practically not formed and is detected along the periphery of the sample for several weeks. Fibrosis of the implant was not detected, but there was a delimitation of its well-formed fibrous capsule, which began to form a week earlier than in the rest of the experimental samples. There was pronounced reaction of eosinophilic leucocytes in the adjoining soft tissues for a long time, which may indicate an allergic effect of the implant material on the macroorganism tissues.

FINDINGS:

1. A comprehensive morphological study of bioresorbable highly porous matrix samples (samples No. 1, 2 and 3 – PLA/PCL) using histochemical and immunohistochemical techniques showed no differences in cellular and tissue reactions of porous matrices with different PLA/PCL ratios.
2. During the first month (from 2–3 weeks of implantation), there started the formation of a capsule around the implant (PLA/PCL), development of a foreign body granulomatous reaction in the peripheral parts of the implant, spreading into the thickness of the implanted material, with signs of pronounced phagocytosis, which indicated cellular biodegradation of the implant material.
3. Starting from week 2 after implantation, implant vascularization was noted, which was confirmed via immunohistochemistry. The study of implants over time showed their gradual replacement with fibrous tissue at week 12–14 and a good neovascularization.
4. Reaction of surrounding tissues was identical in all samples (PLA/PCL), was poorly expressed, manifested by focal edema and small focal cellular lymphoid or lymphoid-eosinophilic infiltration. Perifocal reactive changes disappeared within a month after implantation.
5. In control samples with a silicone implant, there was no bioresorption of the material and no neovascularization process was detected. There was a long-lasting rather pronounced perifocal reaction from eosinophilic leukocytes, which does not exclude the possibility of an allergic effect of the implant material on adjacent tissues.

CONCLUSION

The in vivo experimental work conducted on small animals showed the biosafety and high biocompatibility of laboratory samples of a bioresorbable highly porous matrix as potential materials for pleural implants. Further studies with the scaling of laboratory samples, as well as a detailed study of the dynamics of biodegradation of porous matrices in vivo in large animals are required. Further improvement in laboratory samples of bioresorbable highly porous implants is associated with imparting

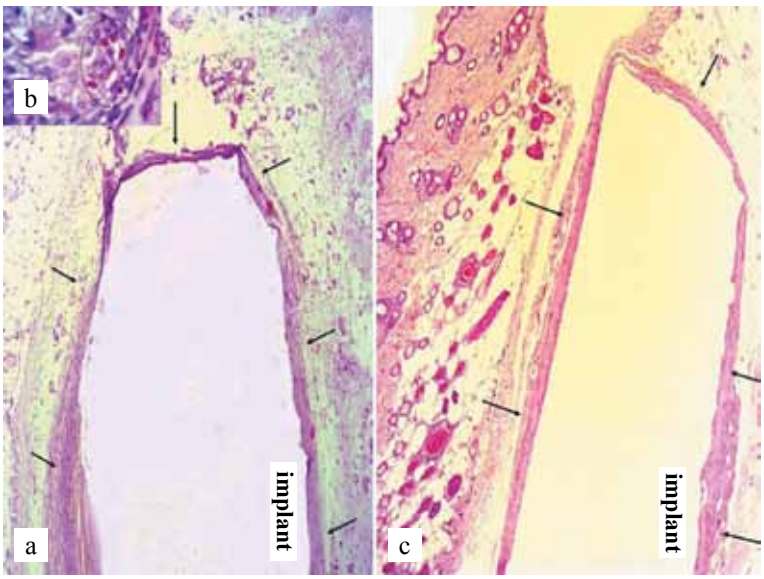

Fig. 9. Morphological picture 4 and 8 weeks after implantation of a fragment of the silicone capsule of Mentor breast implant: a) silicone implant, 4 weeks, intact, with a thin fibrous capsule (indicated by arrows). H&E stain. 100× magnification; b) in the soft tissues surrounding the silicone implant, eosinophilic reaction (eosinophilic leukocytes are surrounded by a round frame). H&E stain. 1000× magnification; c) silicone implant, 8 weeks, intact, with formed fibrous capsule (indicated by arrows). H&E stain. 100× magnification

antibacterial, bioactive and X-ray contrast properties to porous matrices.

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The authors declare no conflict of interest.

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MATERIALS FOR CREATING TISSUE-ENGINEERED CONSTRUCTS USING 3D BIOPRINTING: CARTILAGINOUS AND SOFT TISSUE RESTORATION

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3D Bioprinting is a dynamically developing technology for tissue engineering and regenerative medicine. The main advantage of this technique is its ability to reproduce a given scaffold geometry and structure both in terms of the shape of the tissue-engineered construct and the distribution of its components. The key factor in bioprinting is bio ink, a cell-laden biocompatible material that mimics extracellular matrix. To meet all the requirements, the bio ink must include not only the main material, but also other components ensuring cell proliferation, differentiation and scaffold performance as a whole. The purpose of this review is to describe the most common materials applicable in bioprinting, consider their properties, prospects and limitations in cartilage restoration.

Keywords: regenerative medicine, tissue engineering, cartilage tissue, biomaterials, hydrogel, 3D bioprinting, scaffold.

INTRODUCTION

The cartilaginous tissue of the musculoskeletal system is exposed to great mechanical stress, is easily damaged, and due to the lack of blood and lymphatic vessels in it, it is slow to recover. Cartilage defects are often caused by trauma, age-related metabolic disorders, congenital diseases, and a number of other factors, in particular, endocrine pathologies and malignant neoplasms. Restoration of damaged cartilage remains a major medical problem, and modern tissue engineering can provide new solutions to it.

In recent years, 3D bioprinting has become increasingly common in tissue engineering. The advantage of the technology lies in the ability to form tissue-engineered constructs (scaffolds) with a given geometry and structure. Among the main methods of 3D bioprinting are extrusion, inkjet and laser. The most used technology today is extrusion-based 3D bioprinting. One of its main advantages is the ability to produce high cell-density constructs and the use of several components in printing [1–4], which became possible thanks to the emergence of 3D bioprinters with multiple printheads (dispensers).

A special class of biomaterials, bio-inks, is used to manufacture scaffolds through bioprinting. The concept "bio-ink" was first used in 2003 [5] and currently means a solution or hydrogel with cells [4, 6]. Bio-ink components are classified based on their role in scaffold creation [7, 8]. So sacrificial (support) materials are needed to support the construct during printing until the base material is completely polymerized, in particular when channels and cavities are formed in the scaffold. Other groups are the structural components (give the scaffold additional rigidity, modify porosity, etc.). And finally, functional components, which provide conditions for cell proliferation, differentiation, and synthetic activity.

The development of materials suitable for use as bio-ink is a special challenge. These materials must be suitable for both the printing process and for subsequent maturation of the scaffold with incorporated cells. For these purposes, a number of natural biomaterials have already been tested, including alginate [9–16], gelatin [17–23], collagen [24–30], hyaluronic acid (HA) [17, 31–34], silk fibroin [20–22], chitosan [31, 35, 36] and agarose [37, 38]. Synthetic materials such as polycaprolactone [9, 22, 39–42] and polylactide [43–45] are also widely used.

The main role of a biomaterial in tissue regeneration is to support cell function. Thus, materials for creating a scaffold must provide transport of gases, nutrients, and regulatory factors in order to make cell survival, proliferation, and differentiation possible. Besides, they must undergo biological degradation at a controlled rate close to the rate of regeneration of the tissue being replaced and be non-toxic and non-immunogenic. Finally, they should not only serve as a supporting structure for cells, but also provide mechanical strength of the tissue const-

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ruct as a whole and make its fixation in the implantation zone possible.

An ideal example of such a material is natural extracellular matrix (ECM), whose basic properties should be mimicked by scaffolds. The ECM microenvironment provides not only physical support for cell adhesion, but also signals regulating the life cycle, metabolism, and their differentiated state. The ECM is the main source and conductor of biochemical and biomechanical signals to ensure the organization and functioning of the tissue as a whole [46]. ECM is a multicomponent system of matrix macromolecules, composition and structures specific to each tissue type. The main ECM components are fiber-forming proteins such as collagens, elastin, fibronectin, laminins, glycoproteins, proteoglycans, and glycosaminoglycans [47]. In most tissues, the main fibril-forming component of the ECM is type I collagen, and in cartilaginous tissue, type II collagen [47].

In the aspect of 3D bioprinting, most natural polymers have insufficient mechanical properties and weak degradation. In contrast, synthetic polymers have good mechanical properties, but do not contain macromolecules normally found in living tissues. Therefore, various combinations of these materials are promising. Synthetic polymers are often added to gels in the form of granules or microfibers. At the same time, many authors have noted that simultaneous printing with natural and synthetic polymers is difficult due to the incompatibility of optimal temperatures: the printing temperature is in the range of 100 to 240 °C for synthetic polymers, and 4 to 30 °C for biogels [9, 15, 25].

The objective of this review is to highlight biomaterials and their combinations used primarily for cartilage repair. Meanwhile, the presented materials can be used for repair and regeneration of most soft tissues.

1. MAIN NATURAL COMPONENTS OF BIO-INK

Natural polymers such as agarose, alginate, hyaluronic acid, gelatin, collagen, fibroin, and chitosan are the most common as the main component of bio-ink, due to a certain similarity with ECM.

Agarose. It is a polysaccharide derived from red and brown algae, which consists of alternating residues of beta-D-galactopyranose and 3,6-anhydro-alpha-L-galactopyranose. It is widely used in molecular biology and tissue engineering due to its reversible gelling properties. In this case, the sol-gel and gel-sol transition temperature, as in the case of most hydrogels, depends not only on the concentration of the initial solution, but also on the molecular weight of the polymer [48]. Disadvantages of agarose-based bio-ink include a lack of conditions for maintaining cell growth [49, 50] and a low biodegradation rate [48]. Therefore, agarose is recommended to be used only as a sacrificial material, for example, for creating microchannels during scaffold vascularization [38].

Alginate. It is a polysaccharide derived from brown algae. It consists of guluronic and mannuronic acids [51]. This polymer supports cell growth well [52] and is relatively inexpensive. The material is readily soluble in water and polymerizes with divalent cations such as calcium and barium, as a result of ion exchange reactions [10, 44]. However, the biocompatibility of alginate is lower than that of natural polymers of animal origin, such as gelatin [53]. Alginate hydrogels degrade by releasing cross-linking gel cations or by decomposing the main chain through glycoside bond hydrolysis [54]. The main disadvantage of alginate is considered to be its low biomechanical properties, which complicate the printing process [16].

Chitosan. Chitosan is a natural polysaccharide derived from alkaline N-deacetylated arthropod chitin [55]. Chitin microfibrils are the main structural components in the exoskeleton of crustaceans and insects. It is also a part of the cell walls of fungi and yeast [56]. The hydrophilic structure of chitosan promotes adhesion and proliferation of almost all cell types [57]. The degradation rate of chitosan in comparison with natural polymers of animal origin, such as collagen, gelatin, and fibrin, is relatively low [57] and depends on both the degree of its deacetylation and its molecular weight [58]. In general, the half-degradation time in the body exceeds 30 days [59]. It is also known that this polymer is biocompatible, has antimicrobial properties, low toxicity, and immunogenicity, and, consequently, is of interest as a scaffold material [60–62].

Hyaluronic acid. Hyaluronic acid (HA) is a non-sulfated glycosaminoglycan consisting of repeating disaccharide fragments of N-acetyl-d-glucosamine and d-glucuronic acid [63]. It is found in almost all types of connective tissue [64]. In the body, it supports a number of biological processes such as cell growth, migration, and differentiation [65]. It is obtained by extraction from animal tissues (typically rooster combs) or biotechnologically as a product of the synthesis of modified bacteria of the genus *Streptococcus* or *Pasteurella* [64]. Due to the high content of carboxyl and hydroxyl groups, HA is a highly hydrophilic compound; therefore, it is capable of forming a gel-like structure in aqueous solutions as a result of intermolecular interaction of linear macromolecules [63]. However, as a 3D printing material, HA has limitations due to its weak mechanical properties, slow gelation, and very short biodegradation period [66, 67]. Therefore, in bio-inks, it is usually used in combination with other materials, such as alginate [68], gelatin [33], and collagen [34].

Collagen. Collagen is the main structural protein in most connective tissue types, maintaining the biological and structural integrity of ECM. Collagen has low immunogenicity, good biocompatibility, biodegradability, and regulatory functions in relation to cell adhesion, migration, and differentiation [69]. At 37 °C, it forms a

hydrogel with a triple helix structure [70]. Collagen is characterized by relatively low mechanical properties; but due to its high biocompatibility, it is one of the most frequently used scaffold components [26–29]. However, most of the commercial collagen preparations are immunogenic, which requires the use of its highly purified variants for tissue engineering.

Gelatin. This protein is a product of collagen denaturation and does not differ from the latter in terms of its amino acid composition [20]. Gelatin can be obtained from bones, tendons, or skin of animals by acidic or basic hydrolysis [71]. Despite its chemical composition similar to collagen, it lacks antigenic and immunogenic properties [72]. In vivo degradation time of gelatin crosslinked with glutaraldehyde, according to some data, is about 3 weeks [73]. Gelatin is often used in bioprinting as the main component or in combination with other biomaterials [20, 22, 33]. The most widespread are its modified forms, such as gelatin methacrylate (GelMA), which polymerizes quickly enough under the influence of UV, allowing full use of 3D-printing capabilities [17, 33, 37].

Silk fibroin. It is a natural macromolecular protein polymer with good biocompatibility and mechanical properties suitable for printing, and biodegradability [74]. Fibroin protein forms layers of antiparallel beta sheets [75]. Fibroin molecular composition and structure can vary depending on the silk source. For instance, silk formed by silkworm consists of two main proteins – sericin and fibroin. Fibroin is the structural center of silk, and sericin is the surrounding sticky component [75]. Gelation of silk fibroin can be induced in its aqueous solutions by high temperature, lowering of pH, sonication, and freezing; its electrogelation with formation of the β-structure conformation, which physically crosslinks and stabilizes the gel, has also been described [74]. Modification of silk fibroin with methacrylate has also been obtained [76]. Silk is degraded in vivo by proteolytic enzymes slowly (usually over a year) [77] and has good mechanical properties in terms of bioprinting [78].

The materials described above are actively used in biomedical research worldwide, as evidenced by the analysis of publications available in the PubMed database (Fig. 1). It should be noted that the bulk of the experimental work on scaffolds for cartilage replacement was performed using collagen: it has been very frequently used in the first 15 years of the 21st century. However, the situation has changed in the last 5 years: authors give preference to alternative variants of the main component of tissue-engineered constructs, specifically chitosan and fibroin (Fig. 2). One should also pay attention to the decrease in the frequency of agarose use in recent years, which may be related to its weak matrix properties for cells and extremely low rate of biodegradation. A similar trend may become characteristic of HA and alginate in the next 5 years. In general, it can be noted that the materials presented in Fig. 2 (with the exception of agarose) have been used with approximately the same frequency in the last 5 years – from 6.3 to 8.3% of the total number of studies.

2. MULTICOMPONENT BIO-INK

Obviously, the use of only one material as a bio-ink cannot provide all the mechanical and functional properties that are required to complete tissue-engineered constructs (TECs); so in recent years, scaffolds have been formed using a combination of several materials.

A silk fibroin and gelatin combination is quite often used [20, 21, 22, 79]. Silk fibroin acts as a structural material providing the mechanical properties of the gel and its biodegradation, while gelatin gives the viscosity (required for bioprinting) to the initial solution and elasticity to the scaffold after polymerization. In terms of ease of extrusion, gel strength in combination with its cytocompatibility, the following ratio of components of various silk and gelatin grades showed good results:

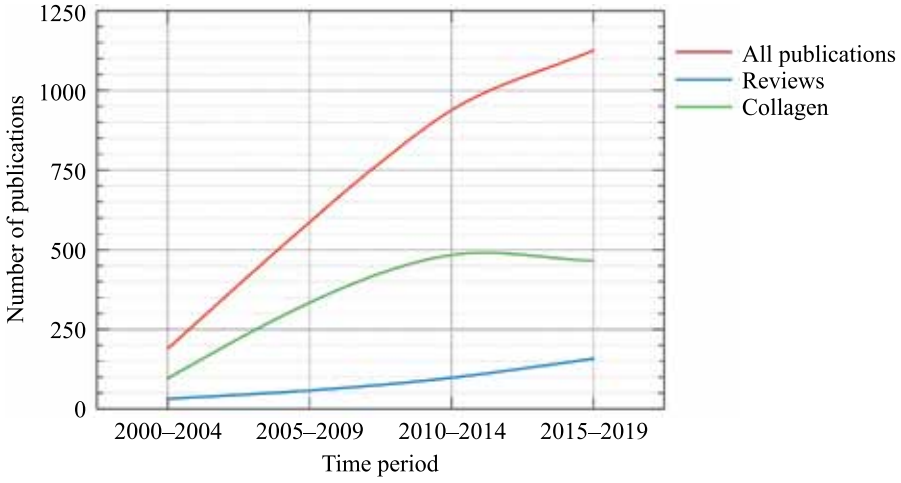

Fig. 1. Number of publications (from 2000 to 2019) on creation of tissue-engineered constructs for cartilage replacement

Bombyx mori 1.5%, Philosophamia ricini 1.5% and gelatin 7% [22]. The authors noted that more than 9% gelatin content and more than 2% silk content created very high viscosity and excessive printing pressure. Gelatin content below 5% provided insufficient viscosity, while silk fibroin content less than 1% resulted in very slow gelation. The silk to gelatin ratio 1:2 (6.9%) provided optimal mechanical properties (in terms of compression modulus), degradation rate, and microenvironment for cell proliferation, differentiation, and formation of cartilage tissue [20]. In changing the percentage ratio of silk fibroin in a gelatin-based hydrogel (30%) and nano-hydroxyapatite-based hydrogel (3%), Wu et al. found that 10% silk fibroin provides better mechanical properties to the scaffold (tensile modulus was 10.6 MPa) [21]. With increased silk fibroin content, the number of hydrogen bonds between molecules and, as a consequence, the degree of crosslinking of fibrils increased; biodegradation rate in this case naturally decreased. It should be noted that, according to Ke et al. [39], native human cartilage has a 14.7 MPa modulus of elasticity, which is close to the values obtained in the above work.

Combinations of gelatin with HA were investigated by Sakai et al. [17, 33]. The authors showed that GelMA and methacrylated HA content determined the behavior of cells in the scaffold. Thus, in scaffolds with a gelatin content of 1% and 2% versus 3% and 5%, a more pronounced suppression of cell growth was observed. In these works, only modified versions of gelatin and HA were used. Addition of methacrylate groups made the material suitable for rapid cross-linking, and, despite a rather low gelatin content, created a hydrogel structure stable at physiological temperatures, close in mechanical properties to those of native hyaline cartilage. A combination of thiolated HA with methacrylated collagen was investigated in a similar way [34]. The optimal for bioprinting, according to the authors, is a collagen/HA ratio of 3:1 with 6% and 2% content, respectively. Although other formulations (2:1 and 4:1) showed similar mechanical properties and were able to maintain cell viability in the same way as the 3:1 gel ratio. However, with such component ratios, the gels also exhibited certain drawbacks. For example, at a 4:1 ratio, formation of collagen bundles in the solution was observed already at room temperature, which, according to the authors, was associated with excessive collagen concentration. The 2:1 formulation, on the other hand, was characterized by insufficient amount of this material for cell interaction.

The chitosan-collagen pair is a frequently tested combination [24, 25]. An in vitro study showed the biocompatibility of scaffolds made with these materials: they supported the adhesion of mature chondrocytes, their spread over the surface and within TECs, providing a high level of their viability. In addition, it was shown that the amount of chitosan in the scaffold composition is the parameter directly affecting the pore size and its morphology [24]. Inclusion of hyaluronic acid in chitosan scaffold enhanced ECM cartilage production, chondrocyte proliferation, and cell adhesion to the scaffold surfaces [31].

Alginate-based scaffolds remain one of the most accessible and studied options [9, 37]. Research by Daly et al. [37] showed that alginate and agarose hydrogels supported hyaline-like cartilage formation to a greater extent than GelMA, as evidenced by the pronounced staining of newly formed tissues for type II collagen. On the other hand, GelMA promoted the formation of fibrous cartilage to a greater extent, as evidenced by the detection of higher amounts of type I collagen in the scaffolds. High levels of cell viability (~80%) were retained in all scaffolds after printing when the above components were used as bioinks. GelMA showed the best printability in this work, creating structures with greater accuracy than alginate and agarose bio-ink. Algi-

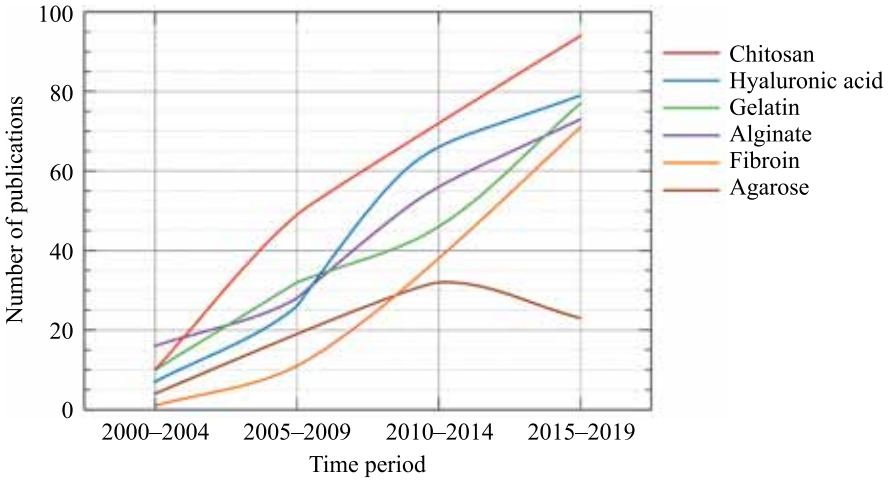

Fig. 2. Number of publications (experimental studies only, from 2000 to 2019) on the use of a range of biomaterials for creation of cartilage scaffolds

nate- and collagen-based TECs showed a homogeneous distribution of chondrocytes, increased expression of cartilage-specific genes, namely Acan, Sox9, and Col2a1, and decreased Col1a1 expression, proving that the chondrocyte phenotype is preserved [9].

Decellularized ECM can be used as bio-ink components, providing a natural microenvironment for cells. The advantages of such a component include the presence of biochemical signals of the original native ECM, correct protein proportions, and ability to selectively retain the adhesion and proliferation of cells of a particular tissue or organ. In a recently published paper by Basok et al., a microdispersed tissue-specific matrix was obtained from decellularized porcine articular cartilage, which retained the morphofunctional properties of ECM [80, 81]. The authors showed that such a matrix is capable of supporting the adhesion, proliferation, and chondrogenic differentiation of mesenchymal stromal cells.

3. MATERIALS THAT REINFORCE SCAFFOLD STIFFNESS

3.1. Scaffold materials

Scaffold materials serve to stiffen the construct. Moreover, they must be biocompatible, or at least bioinert and have a low degradation rate in the body. Synthetic polymers such as polycaprolactone (PCL), polylactide (PLA), polyglycolic acid (PGA), copolymer of lactic and glycolic acids (PLGA) are commonly used as scaffold materials [39, 40, 82, 83].

PCL is the most commonly used polymer for 3D porous scaffolds. It is a linear aliphatic polyester obtained by ring-opening polymerization of ε-caprolactone [84]. It is biodegradable, but more stable than PLA, since it is semi-crystalline and hydrophobic [85, 86]. Pati et al. [82] used PCL to support decellularized adipose tissue encrusted with mesenchymal stem cells. The volume of the structure remained constant for a long time due to the fact that the PCL scaffold retained its structure during the tissue remodeling process. Shim et al. [87] also used PCL support to create a scaffold with atelocollagen and supramolecular HA for the reconstruction of osteochondral defects in rabbit knee joints. PCL has already received FDA approval for clinical use [88].

PLA is a thermoplastic complex polyester that is derived from corn, sugarcane, wheat, or rice, making it affordable and inexpensive [89]. PGA is a synthetic polymer of glycolic acid [90]. PGA is more crystalline than PLA because it does not contain a methyl side chain; however, PLA is more hydrophobic [91].

Another scaffold material is the synthetic copolymer PLGA (usually 75% lactic acid and 25% glycolic acid) [92]. It is also a biocompatible material that degrades to non-toxic products (H_2O and CO_2 [93]). Like PCL, PLGA has already received FDA approval for clinical use [88].

The main disadvantage of the above-described synthetic polymer materials in terms of 3D printing is the need to maintain a high temperature when printing them (from 100 to 230 °C), which makes it difficult to use them together with hydrogels with cells. One of the options for creating composite scaffolds is the two-stage printing tactic: first, plastic, and then hydrogel. For instance, in a recent study, Kaye et al. [83] used a system with two dispensers – for printing separately PCL and alginate/collagen hydrogel with chondrocytes: hydrogel was printed into PCL channels after the latter had cooled. Thus, a tracheal tissue construct was obtained, which was implanted in New Zealand rabbits. The authors showed that such a scaffold induces cartilage formation while maintaining its integrity. It should be noted that the authors separated the hydrogel with chondrocytes from tracheal lumen with an intermediate membrane. In the absence of such separation, there was a tendency for inflammation, cartilage growth limitation and stenosis. PCL and hydrogel were used in another work on tracheal scaffold fabrication [39]. The authors obtained scaffolds that had mechanical properties similar to native tracheal cartilage and smooth muscle tissue. Izadifar et al. [10] formed constructs from a cell-containing alginate hydrogel in channels created between PCL strands in each layer. This approach demonstrated the possibility of creating a scaffold with the required geometry and high level of cell survival. The work of Romanazzo et al. was similar in printing method. [40]. Cell viability in the resulting scaffolds varied from 70 to 90% [10, 40].

Other possibilities for optimizing the mechanical properties of scaffolds produced by 3D printing are also described. For example, the addition of various nanoparticles (nanosilicates, halloysite nanotubes, nanocellulose, graphene) to TECs increases their rigidity and biological activity [16, 94–96]. For instance, the addition of alginate, methylcellulose, and halloysite nanotubes to a hydrogel at 20 mg/mL to 40 mg/mL concentration increased the tensile strength proportionally twofold (from 164 to 381 kPa), and the compressive stress 1.5 times (from 426 to 648 kPa) [16].

The mechanical properties of bio-ink with different proportions of chitosan, gelatin, and hyaluronic acid increased with the addition of graphene [94, 97, 98]. It has been shown that a 0.06% graphene content is most conducive to the formation of a porous scaffold structure, as well as a high value of the compression modulus. It should be noted that dependence of the mechanical properties of the scaffold on graphene content turned out to be nonlinear. Graphene can also be used in powder form: Sayyar et al. [97] showed that the addition of 0.5% graphene increased the tensile strength and elastic modulus of methacrylated chitosan by more than 67% and 40%, respectively, and also improved the adhesion and proliferation of L929 fibroblasts. Xavier et al. studied GelMA-based bio-ink with the addition

of 2% nanosilicate [98]. Nanosilicate (in proportion to its concentration) increased the mechanical strength of the scaffold, and nanosilicate-laponite (decomposes into magnesium, orthosilicic acid and lithium readily removed by the body) facilitated the process of removing the scaffold biodegradation products.

Cellulose and methylcellulose are commonly used options for enhancing the stiffness of a bio-ink scaffold [11–13, 95, 96, 99]. Müller et al. [99] used commercial bio-ink based on sodium alginate and nanocellulose for cartilage 3D printing. Addition of nanocellulose improved the bioprinting quality. However, this component had a negative effect on cell proliferation. These data were confirmed in the publication on the use of nanocellulose hydrogels for auricular cartilage: the average cell viability after biofabrication did not exceed 68.5–76.9%. [95]. Adding methylcellulose to the hydrogels increased the scaffold elasticity and stability, as well as microporosity [13]. In addition, this proved to be one of the optimal approaches to achieve a higher elasticity of the hydrogel coming out of the printing needle, which opens up the possibility of printing large multilayer constructs [96].

Addition of PCL and PLA microfibers to printing hydrogels can be an additional option to improve the rigidity of finished constructs. For example, PCL microfibers have been successfully used by Daly et al. [34]. Narayanan et al. used bio-ink with PLA nanofibers (0.5%) in the design of meniscus tissues [44]. It can also be noted that PCL granules form clusters of cells around themselves, promoting their survival and proliferation in the scaffold [100].

3.2. Sacrificial components

The use of sacrificial components in scaffold formation is one of the key bioprinting techniques today. A combination of the base hydrogel with incorporated cells and the sacrificial material during printing allows both to provide temporary support of the base hydrogel until its complete polymerization, and to form niches and channels imitating blood vessels responsible for the access of gases and nutrients [30, 38, 101–106]. The main requirements for the sacrificial material are complete utilization from the scaffold within a specified timeframe and the absence of cytotoxicity of its degradation products. Various materials used for this purpose have been described in the literature. Lee et al. [105] used gelatin to form channels with a lumen of up to 1 mm in the collagen scaffold. Bertassoni et al. [38] developed a similar strategy for building vasculature using agarose gel. A number of studies have used the commercial product Pluronic F-127 as a sacrificial material [30, 101, 103]. In particular, using this component, it was possible to form macropores in a scaffold of nanofiber collagen [30]. Fitzsimmons et al. found that Pluronic F-127 has an advantage over gelatin as a sacrificial material for the creation of vascularized tissues due to the uniformity of the filament during printing and a higher compression modulus [101]. The use of filaments made of polyvinyl alcohol [102] and alginate [104] as a sacrificial material has been described. In addition to filaments, the sacrificial material can be in the form of microspheres, providing the scaffold with controlled microporosity [106, 107].

4. MECHANISMS OF POLYMERIZATION IN 3D PRINTING

Most of the materials used for bioprinting are initially in the state of solutions or suspensions, and must undergo the polymerization (cross-linking) stage (in order to form an elastic gel in the scaffold), which, depending on the experiment design, scaffold architecture and geometry, begins before printing, during printing or after formation of each layer. Controlled cross-linking of different materials is provided by different physical and chemical influences – light, temperature, ion concentration, pH, etc.

The most physiological for collagen is the "temperature" type of polymerization, which spontaneously occurs when the solution temperature rises to 20 °C [30]. In these cases, extrusion is performed with cold solution (+4 °C to +8 °C), and the platform on which the printing takes place is heated to 25–35 °C [108]. Collagen polymerization can also be induced by lowering the pH of the solution [109], but this can negatively affect cell viability in the formed scaffold [108, 110].

One of the new options for controlled polymerization of collagen with other materials is the use of genipin [1, 24, 25, 111, 112]. The genipin crosslinking mechanism is due to several nucleophilic substitution reactions involving different sites of collagen molecules [25]. In particular, it has been shown that to obtain optimal mechanical, structural and biological properties of a scaffold for replacing cartilage defects based on collagen and chitosan, a 1% genipin content is recommended [25]. After cross-linking, collagen and chitosan form a macroporous layer in which chondrocytes remain viable, mainly in the areas adjacent to the pores [24]. Genipin cross-linking is also possible for formation of gelatin- and silk fibroin-based scaffolds [20]. It is important to note that genipin is used due to шеы stable but long-term polymerization process of up to 1 hour [25, 111], which to some extent limits the use of this approach in the formation of large-sized scaffolds. In addition, some studies have shown delayed adverse effects of genipin, particularly in the degradation of the basic scaffold material [113]. An alternative to genipin is tannic acid, whose crosslinking mechanism is due to the formation of numerous hydrogen bonds between the two materials [112]. In Yeo et al., the optimum concentration of tannic acid for crosslinking was 2% [112]. However, Lee et al. observed improved mechanical properties even at 0.5% content [1].

Alginate solutions are characterized by the ability for ionotropic gelation under the action of such cations as Ca^{2+}, Ba^{2+}, Cu^{2+}, Al^{3+}, which act as crosslinking agents, interacting with the carboxyl groups of guluronate blocks of polysaccharide molecules; mannuronate blocks remain free in this case [14]. Calcium chloride is most often used as a crosslinking agent in alginate-based hydrogels [11, 12, 15].

In recent years, photocrosslinkable biomaterials have become increasingly common. This approach has several advantages over other crosslinking methods since it makes it easy to control printing by adjusting the rate and degree of cure of the resulting construct. Many natural biomaterials, such as gelatin [18, 19], silk fibroin [76], and collagen [114], are polymerized by acrylation under a UV lamp at 365 nm wavelength. Drzewiecki et al. demonstrated the use of photocuring of collagen methacrylamide as a fibrill-forming bio-ink for scaffold fabrication [114]. Photocrosslinking of HA methacrylate has been described by Onofrillo et al. when creating a cartilage scaffold [19]. Similarly, a gel based on silk fibroin methacrylate (SilMA) was prepared and studied, which, according to the authors, is biocompatible, biodegradable and has suitable biological and mechanical strength [76]. In contrast to genipin polymerization, in photocuring of methacrylate, polymerization of a single layer is completed in 5 minutes. However, some authors note that the disadvantage of acrylation is that the scaffolds have reduced biocompatibility, since unreacted acrylic groups are cytotoxic and, moreover, can cause local inflammatory reactions in vivo [115]. The frequently used photoinitiator Irgacure 2959, which is a source of free radicals required for polymerization reaction, has the same disadvantage [116]. Reactions with phenolic residues in natural biomaterials are another way to initiate cross-linking formation. For example, the mechanical properties of a hydrogel made from gelatin and HA modified with phenolic hydroxyl groups can be controlled by changing the concentrations of tris(bipyridine) ruthenium(II) dioxide and sodium-ammonium persulfate and the light irradiation time [17]. Riboflavin can also be used as a photoinitiator for collagen, which under a UV lamb causes the formation of covalent cross-links between amino acid groups in collagen chains [117]. The main advantage of riboflavin is that it is usually present in the body and, unlike other photoinitiators, is not cytotoxic. Riboflavin-induced photopolymerization of collagen hydrogel containing fibrochondrocytes did not change the scaffold shape, while increasing the expression levels of type II collagen and aggrecan genes in cells [70]. The optimal riboflavin level, increasing the elastic modulus, was 0.01% [70]. The broad utility of riboflavin has been shown by Batchelor et al. [118]. It should be noted that the use of riboflavin allows relatively rapid polymerization (from 10 seconds to 5 minutes) under visible light [70, 117].

For scaffolds made from a silk-gelatin mixture, physical cross-linking can be performed under the influence of ultrasound [119], which induces "crystallization" of β-structures of fibroin as a result of increased molecular vibration, hydration of hydrophobic domains, and short-term increase in local temperature. "Cross-linking" of fibroin (and gelatin) can also be achieved by self-assembly using two different types of fibroin [22].

One of the options for maintaining a balance between printability and stiffness of the resulting construct is to use double polymerization of the material. The first stage involves selecting the viscosity of bio-ink (in the "gel-solution" boundary state) suitable for the printing process, and the second stage involves increasing the stiffness/elasticity (transition to the gel state) necessary to maintain the geometry immediately after after each layer is printed. Such an approach has been described in detail by Skardal A. et al. for the polymerization of acrylates and alkynes in the case of creating scaffolds based on collagen, HA, and gelatin [120]. Kajave et al. addressed the issues related to insufficient mechanical properties and rapid degradation of scaffolds obtained in this way, which is inherent in all TECs obtained using low concentrations of collagen [26]. The authors showed that sequential application of UV and genipin (0.5 mM) significantly improves the elasticity of scaffolds and increases their degradation time in the body both with incorporated cells and in cell-free variants.

5. COMMERCIAL INKS FOR 3D PRINTING

In recent years, commercial bio-ink preparations have appeared on the biotechnology market. For example, CELLINK (Sweden) developed bio-ink based on alginate, collagen, gelatin and chitosan – CELLINK's GelX series based on methacrylated gelatin and CELLINK Bioink based on nanofibrous cellulose and alginate, which can be modified with RGD peptides, tricalcium phosphate, laminins, and fibrinogen [121]. Their suitability for bioprinting has been demonstrated in a number of recent studies [11, 12, 95, 99]. Israeli company CollPlant chemically modified recombinant human collagen to create bio-ink (rhCollagen BioInk) suitable for a variety of printing technologies, including extrusion, inkjet printing, laser induced direct transfer and stereolithography. Advanced BioMatrix (USA) developed LifeInk 200 and LifeInk 240 bioinks for extrusion printing based on collagen, matacrylated collagen, gelatin, and HA, as well as thiolated HA [122]. The company also produces bioprinting ink. Biogelx produced synthetic bioinks that form a nanofiber network mimicking the extracellular matrix. These bio-inks can support cell growth and proliferation, signal transmission, and have rheological properties suitable for bioprinting [123]. It is worth noting that the cost of such bio-ink is quite high.

In addition to materials presently adapted for 3D bioprinting, a whole range of commercial cartilage re-

pair products are currently in clinical trials. They are either off-the-shelf scaffolds or hydrogels that polymerize rapidly at the implantation site. Among them are NOVOCART 3D, RevaFlex and MACI. RevaFlex is a tissue-engineered cartilage implant for knee cartilage repair and regeneration, containing allogeneic juvenile chondrocytes [124]. NOVOCART 3D is positioned as a personalized implant based on patient-derived chondrocytes, which are cultured on collagen scaffolds [125]. Similar is MACI, which contains autologous chondrocytes cultured on porcine collagen membrane and is designed to repair knee cartilage damage [126].

CONCLUSION

Publications of the last 5 years devoted to the use of various biomaterials in 3D-bioprinting of cartilaginous and soft tissues have been analyzed. We have discussed the advantages and disadvantages of the basic components of scaffolds, approaches to scaffold polymerization, including the types and features of the use of cross-linking agents, the ways of improving the properties of bio-ink, in particular by using additional components responsible for stiffness, porosity and other basic scaffold properties. Trends towards changes in the frequency of use of a number of materials have been analyzed. In general, despite a wide variety of basic biomaterials and a range of additional components used in the creation of TECs for replacement of cartilage and soft tissue defects, the search for new options for complete replacement of ECM continues.

The authors declare no conflict of interest.

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EVALUATION OF CALCIFICATION RESISTANCE OF XENOPERICARDIUM TREATED WITH POLYHYDROXY COMPOUNDS

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Calcification of biomaterials used in prosthetic heart valves has been a challenging issue in cardiovascular surgery. The objective of this work is to compare the efficiency of polyvinyl alcohol (PVA) and tannic acid (TA) modification of xenomaterials, pre-stabilized with glutaraldehyde (GA) and ethylene glycol diglycidyl ether (EGDE), in reducing calcification. Analysis of mechanical properties evaluated under uniaxial tension, showed a significant increase in the tensile strength of the test samples compared to the control (unmodified) samples ($p < 0.05$). Additional treatment of GA-fixed tissue with PVA and TA significantly reduced the amount of calcium in the samples implanted into rats for a 60-day follow-up ($p < 0.05$). The level of calcification of samples pre-stabilized with EGDE and treated with PVA and TA did not differ from the control group ($p = 0.063$). Cumulative analysis of the study results demonstrated that the GA-fixed biomaterial modified with PVA and TA can reduce calcium-binding activity and increase strength. This indicates the prospects for clinical application of the proposed treatment methods. This being said, the issue of long-term body response requires further study of the long-term stability of the modified biomaterial under physiologic blood flow conditions.

Keywords: xenopericardium, polyvinyl alcohol, tannic acid, ethylene glycol diglycidyl ether, glutaraldehyde, calcification.

INTRODUCTION

At present, valvular heart disease is tackled by replacing the failed valve with a mechanical or biological prosthesis [1, 2]. Biological substitutes made from animal xenotissues, in contrast to mechanical devices, are characterized by hemodynamics comparable to the native valve and high hemocompatibility [3]. At the same time, xenogeneic material is prone to degradation under enzymatic, chemical and physical effects of the human body environment (both blood and surrounding tissues). For this reason, biological tissue is pretreated with chemical agents that stabilize the structure in order to minimize the body's immune response [4]. The principle of action of chemical stabilizers lies in their interaction with amino groups of collagen, the main protein of the extracellular matrix of the biomaterial, and, as a consequence, formation of additional cross-links in the collagen molecular structure [5, 6]. The most common stabilizing agent in world practice is glutaraldehyde (GA) [7]; however, there are also alternative preservative compounds, such as ethylene glycol diglycidyl ether (EGDE) [8]. Despite the complex treatment of biological prosthesis xenomaterial and the use of modern post-treatments (anticalcium, antithrombotic), it is not possible to achieve complete freedom from dysfunctions comparable to mechanical prostheses – 20–30 years in most cases. In this regard, such valves require replacement after a certain time – their service life is limited, first of all, by the calcification and structural degradation of the biological tissue. There are several hypotheses in publications explaining the mechanism of this process, among which a preservative plays an important role [9]. However, mineralization (calcification) is most likely caused by a complex of events, representing a multifactorial process [10]. To combat the calcification of biological heart valve prostheses, various methods have been proposed, including the use of a new preservative [11], "masking" of free aldehyde groups of glutaraldehyde [12], surface modification with aminodiphosphonates [13], preliminary tissue decellularization to remove alpha-galactose residues, as an element provoking the immune response and calcification [12], filling voids in the collagen space to eliminate potential sites for development of passive mineralization [14], etc. Some of the proposed solutions have already been put into industrial practice, but the problem of calcification has not yet been completely eliminated.

Having analyzed the literature data, we identified a promising area of research – modification of stabilized xenogeneic tissues with bulk polyhydroxy compounds. Such treatment is expected to reduce the calcium-binding activity of the material due to formation of an additional hydrophilic layer, masking of the active groups of the preservative (GA), and filling of voids in the xenotissue

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structure [14, 15]. Two polyhydroxy compounds were chosen as modifying agents: polyvinyl alcohol (PVA, linear structure) and tannic acid (TA, bulky, branched structure).

RESEARCH METHODOLOGY

The object of the study was bovine (cattle) pericardium preserved according to standard methods – 5% EGDE and 0.625% GA. Tannic acid (ACS reagent, Sigma Aldrich, USA) and polyvinyl alcohol Mw 67,000, 88% degree of hydrolysis of acetate group (Sigma-Aldrich, USA) were used as reagents for modifying the xenopericardium in order to reduce calcium-binding activity. The material was modified in two ways: in 5% PVA solution in isotonic saline solution and in 3% TA solution in isotonic saline solution. Exposure lasted for 24 hours at 37 °C and constant stirring in hydrochloric acid as a reaction catalyst.

To confirm the presence of additional hydroxyl groups introduced by the described method in the structure of modified materials, tissue samples (n = 2, for each group) were dried and examined by infrared spectroscopy on an Infralum FT-801 IR Fourier spectrometer (Russia). The analysis was carried out using diffuse reflectance infrared spectra.

Changes in its mechanical properties – ultimate tensile strength, elongation to rupture, and Young's modulus – were used as a criterion for the effectiveness of these xenopericardium treatment methods. For this purpose, samples of the original and modified biomaterial (n = 5, for each study group) were evaluated under uniaxial tension on universal testing machine Zwick/Roell (Zwick GmbH, Germany). Samples were prepared using a specially shaped knife (B083, corresponding to ISO 37: 2017) on a ZCP 020 punch press (Zwick GmbH & Co. KG, Germany). The ultimate tensile strength of biological tissue was determined by the maximum tensile stress before the onset of destruction of the sample (MPa). Elastic-deformation properties were assessed by relative elongation, corrected taking into account the nature of destruction of the samples (%) and Young's modulus (MPa), which was determined in the ranges of small deformations corresponding to the range of physiological load. To measure the thickness of the samples, we used a thickness gauge, TR (ZAO Krasny Instrumentalshchik, Russia) with a ±0.01 mm margin of error (clamping force ≤1.5 N) was used.

The effectiveness of reducing calcium-binding activity was assessed by in vitro and in vivo tests. To determine in vitro the resistance of the original and modified material to calcification, biotissue samples were kept in a solution simulating the physiological environment of the human body. For this purpose, 5 × 5 mm pericardial flaps (n = 5) were placed individually in a 2 mL solution containing sterile medium for growing human and animal cell cultures (DMEM, Sigma-Aldrich, USA), fetal bovine serum (FBS, Sigma-Aldrich, USA), calcium chloride and sodium monohydrogen phosphate. Calcification level was determined after week 2 and 3 of incubation at 37 °C in a CO_2 incubator, the carbon dioxide concentration being 5%. Cryosections of incubated samples, pre-stained with alizarin red S, were examined by light microscopy on an AXIO Imager A1 device (Carl Zeiss, Germany).

Material resistance to calcification was also evaluated in vivo by subcutaneous implantation in male Wistar rats (n = 5) (weight 55–70 g). The follow-up period was 2 months. All interventions were performed under general anesthesia in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS N 123), with the Russian Ministry of Health order No. 199n dated April 1, 2016 "On Approval of the Rules of Good Laboratory Practice" and with Interstate Standard GOST 33044-2014. After the prescribed period, the animals were withdrawn from the study by an overdose of anesthesia. Samples of the biomaterial were explanted, the surrounding tissues were removed and dried to constant weight, after which they were exposed to 50% perchloric acid under heating in order to complete hydrolysis until a clear solution was obtained. Samples diluted with distilled water were examined for calcium content on an icap 6300 atomic emission spectrometer with inductively coupled plasma (Thermo Scientific, USA).

Quantitative data was analyzed using statistical methods in medical and biological information processing software STATISTICA 6.0 (StatSoft, Inc., USA). Using the Kolmogorov–Smirnov test, the distribution model in the samples was determined as nonparametric, as a result of which the results цуку presented as medians (M), quartiles (25% and 75%), minimum and maximum values. The Mann–Whitney U test was used to assess the statistical significance of the differences between the two independent groups; the significance of differences was recorded at a $p < 0.05$ significance level.

RESULTS

Evaluation of mechanical properties

The experiment showed that the ultimate tensile strength of the modified samples in both studied parallels (EGDE- and GA-preserved xenopericardium) was significantly higher than the control values ($p < 0.05$) (Table). In the case of PVA modification, this indicator exceeded the control value by 2.7 and 1.6 times for the EGDE and GA pericardium, respectively. The tensile strength of the TA-treated xenopericardium was 2.4 times higher for EGDE-conditioned material and 1.8 times higher for GA-stabilized material.

In terms of elongation and Young's modulus, no significant differences were found between the groups,

except for the Young's modulus of the EGDE-prefixed and polyvinyl alcohol-modified specimens.

Infrared spectroscopy

The diffuse reflectance spectra obtained for the modified samples (PVA- and TA-treated xenopericardium) revealed changes in the character (width and shape) of the band caused by stretching vibrations of the interacting O–H– bonds in the 3200–3600 cm^{-1} range [16] (Fig. 1). The region of the spectrum obtained for each of the studied samples up to 1800 cm^{-1} contains characteristic bands related to the protein structures of the pericardial tissue, in particular, collagen. As a rule, amide I bands (~1700 cm^{-1}) arise from C=O stretching vibrations associated with N–H bending vibrations. Amide II bands (~1575 cm^{-1}) arise from N–H bending vibrations with C–N stretching vibrations [17]. The bands of C–O–C vibrations caused by the presence of epoxy groups, lie in the 800–900 cm^{-1} range [18]. According to reports, the presence of a carbonyl group determines the appearance of a band in the infrared spectrum of the compound in

Table

Mechanical properties of modified and control materials

Name	Strength, MPa	Relative elongation, %	Young's modulus, MPa
Control (GA)	12.34 (7.75; 9.38; 14.00; 14.63)#	54.12 (45.73; 48.30; 58.75; 60.83)	1.69 (1.12; 1.25; 2.01; 2.13)
Control (EGDE)	3.48 (3.12; 3.14; 4.06; 4.96)*	54.51 (47.38; 47.98; 64.16; 65.79)	1.720 (1.67; 1.68; 1.80; 1.82)
GA + TA	21.67 (20.64; 20.92; 23.11; 23.79)*	61.18 (57.94; 58.78; 64.72; 65.89)	1.66 (1.47; 1.49; 1.72; 1.76)
EGDE + TA	8.48 (4.40; 6.23; 8.75; 9.00)#	48.23 (39.89; 43.35; 62.26; 62.89)	1.63 (1.47; 1.53; 2.00; 2.04)
GA + PVA	19.35 (16.04; 16.36; 24.58; 26.72)*	57.00 (47.87; 49.29; 65.38; 73.25)	1.56 (1.06; 1.29; 1.60; 1.63)
EGDE + PVA	9.55 (5.07; 6.89; 10.50; 10.65)#	58.34 (42.49; 48.80; 65.06; 66.81)	2.04 (1.94; 1.97; 2.38; 2.50)#

* – statistically significantly different from the GA control ($p < 0.05$), # – statistically significantly different from the EGDE control ($p < 0.05$).

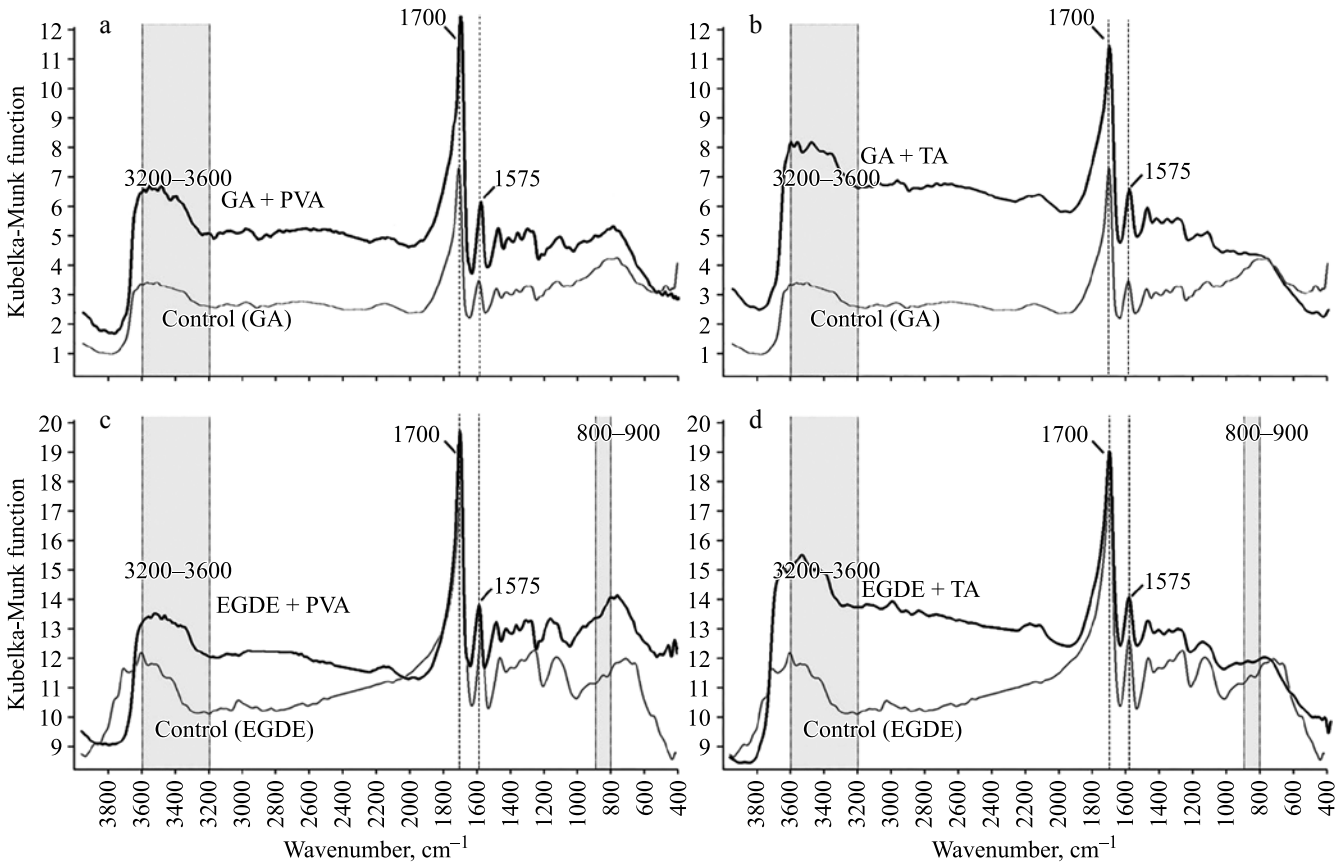

Fig. 1. Diffuse reflectance IR spectra of biomaterial stabilized with EDGE and GA, and modified with PVA and TA: a) comparison of the spectra of GA-pericardium treated with PVA with the control; b) comparison of the spectra of GA-pericardium treated with TA with the control; c) comparison of the spectra of EDGE pericardium treated with PVA with the control; d) comparison of the spectra of EDGE pericardium treated with TA with the control

the 1660–1770 cm^{-1} region [19]. In our case, this band obviously overlaps with the amide I band.

In vitro calcification assessment

Microscopic analysis of slices stained for the presence of calcium and obtained after incubation of samples in solution to simulate accelerated calcification in vitro showed an increase in the resistance to mineralization in all PVA- and TA-modified materials compared with the control groups (Fig. 2). There were no visual differences in the amount of calcium in the PVA- and TA-treated samples. However, a greater propensity to form calcifications was noted for the entire parallel of GA pre-stabilized tissues (Fig. 2).

In vivo calcification

Assessment of calcium content in xenopericardium samples implanted in rats revealed, first of all, a statistically significantly higher level of calcification for the GA pre-stabilized material, $p < 0.05$ (Fig. 3). At the same time, additional treatment of GA-fixed tissue with polyhydroxy compounds (PVA and TA) made it possible to significantly reduce calcium content in the samples, $p < 0.05$. The level of calcification of samples pre-stabilized

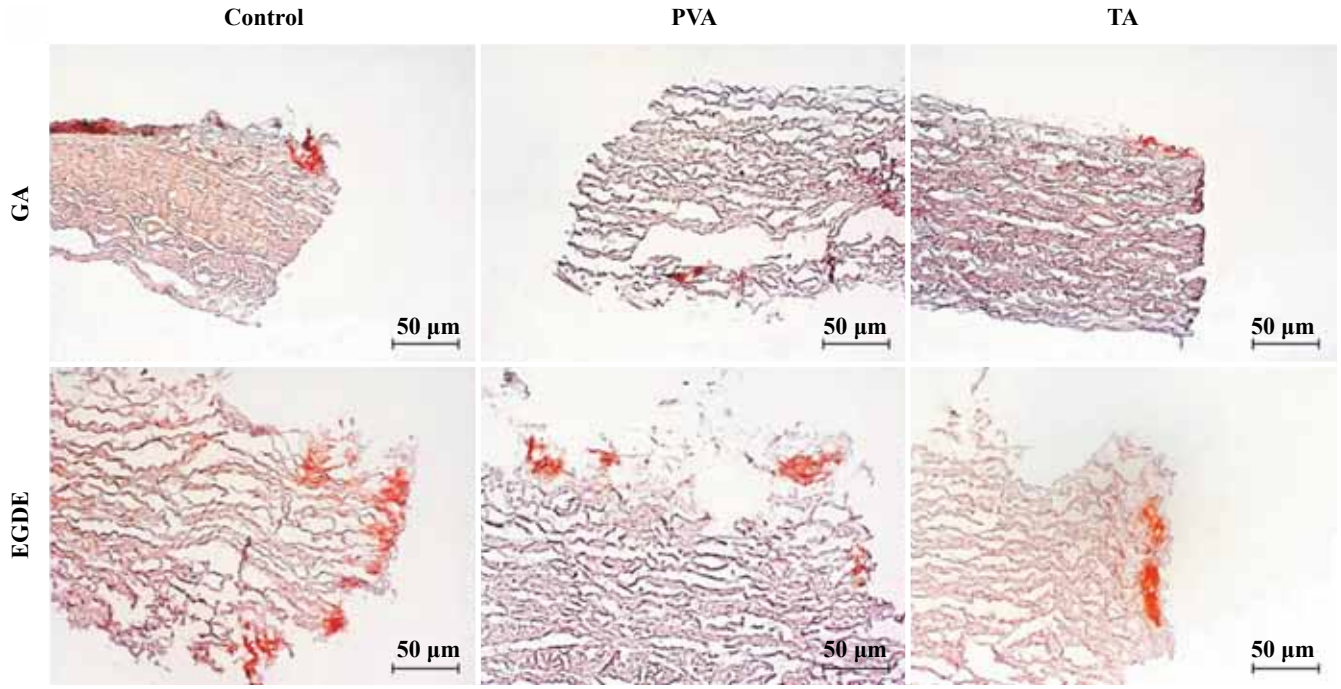

Fig. 2. Histological sections of biomaterial samples, stabilized with EDGE and GA, and modified with PVA and TA. Evaluation of in vitro calcification. 200× magnification

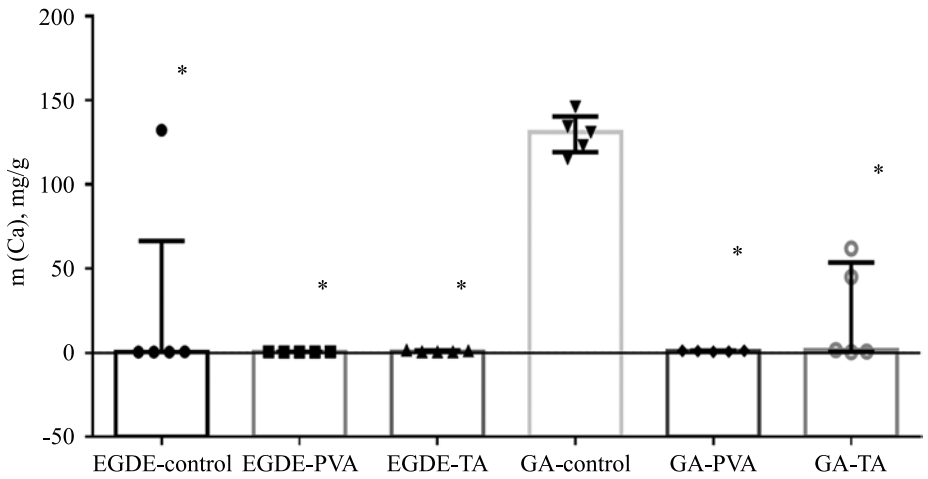

Fig. 3. Calcium content in samples implanted in rats for 60 days
* – statistically significantly different from the GA control group.

with EGDE and treated with PVA and TA did not differ from the control group (p = 0.063).

DISCUSSION OF RESULTS

Modification of xenopericardium with polyhydroxy compounds, pre-stabilized with chemical agents, is based on the principle of interaction of free groups of the preservative with the hydroxyl groups of the modifier. In addition to covalent chemical bonds, polyvinyl alcohol (I) and tannic acid (II) can form less stable hydrogen bonds with amino acid functional groups of collagen molecules, and can be physically absorbed in the voids of the biomaterial and on its surface. Fig. 4 and 5 illustrate the chemical processes occurring during modification using a reaction with polyvinyl alcohol as an example.

Due to the peculiarities of the chemical structure of the main chain and the presence of hydroxyl groups, PVA is a non-toxic and highly hydrophilic compound. Films and hydrogels made on its basis are used, inter alia, in experiments to create a polymeric heart valve [20]. Tannic acid, in turn, has been studied earlier as an individual substance that stabilizes biological tissue [21] and as an additional component in pericardial fixation with glutaric aldehyde [22]. Works performed established a

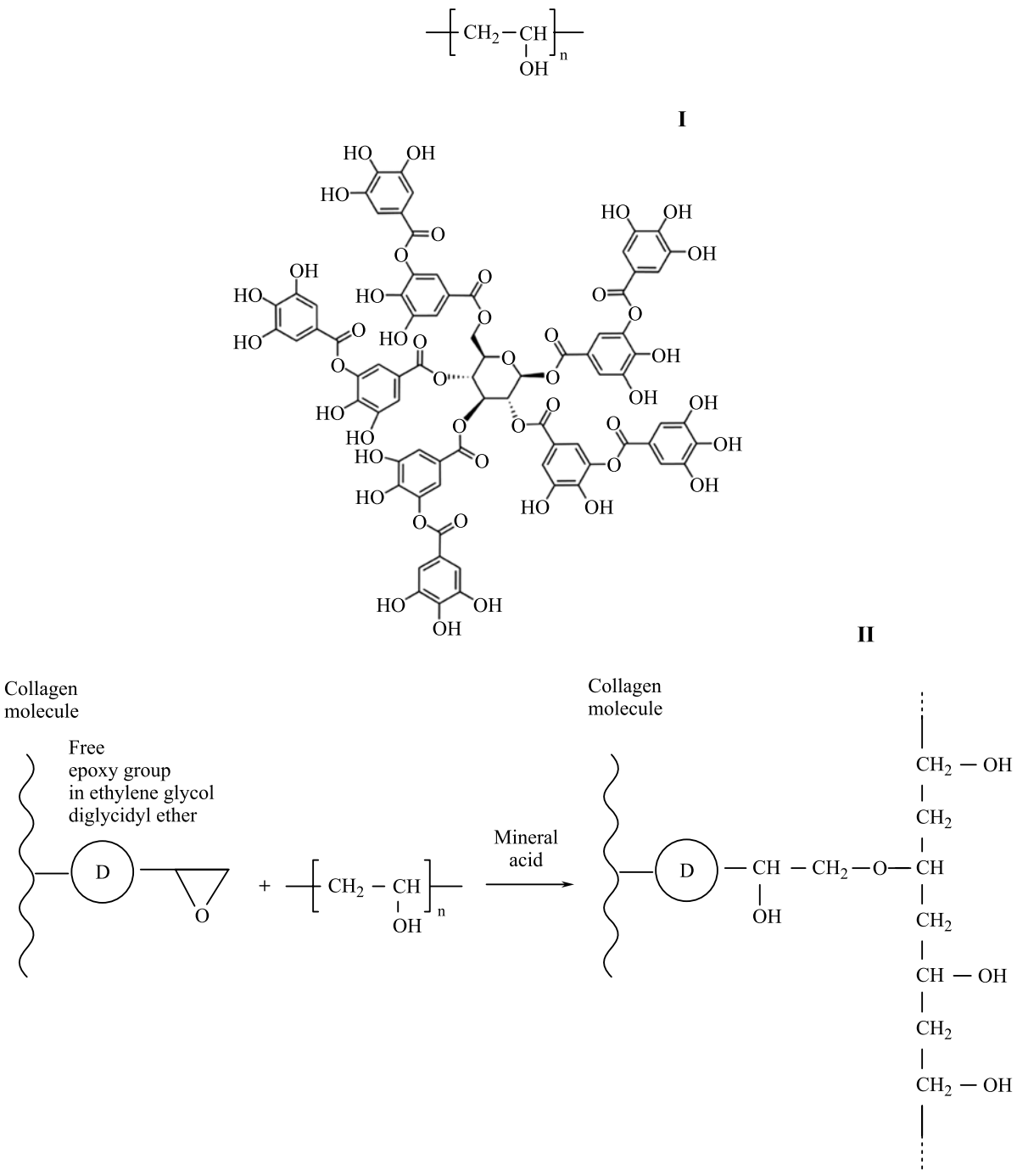

Fig. 4. Interaction between epoxy groups in EDGE-stabilized xenopericardium and hydroxyl groups in polyvinyl alcohol

reduced body's immune response (experiment with rats) and reduced calcium-binding activity of the biomaterial, as well as preserved collagen fibers of biological tissue [21, 22].

Mechanical properties

The experimentally obtained strength values of the samples of the control groups are generally similar to the literature data [23], where, among other things, a higher strength of materials stabilized with glutaraldehyde compared to EGDE was noted, which correlates with the results obtained by us earlier.

Increased strength characteristics of PVA- and TA-modified pericardium may indicate the formation of additional interfibrillar cross-links in collagen [24]. At the same time, the absence of statistically significant differences in strengths between the TA and PVA groups, although it does not allow us to state unequivocally, indicates that these compounds are chemically equivalent. However, the presented chemical interaction scheme reflects only the assumed reaction mechanism; probably, in addition to the proposed explanation, other paths are possible, which is associated with the complexity of the systems under study. Notwithstanding, in the absence of a critical effect on Young's modulus and elasticity of the material modification, one can assert that there is no negative effect on the pericardial structure of the reagents used and the reaction conditions.

Infrared spectroscopy

The change in the nature of the band in the spectral region caused by the O–H stretching vibrations of the interacting hydroxyl groups may be due to the breaking of bonds of unreacted epoxy groups of the preservative, resulting in the formation of new hydroxyl groups, new covalent and hydrogen bonds in the case of EGDE-preserved samples. In this case, direct grafting of polyhydroxyl compounds (PVA and TA) to the surface of the biological tissue makes the main contribution to increased band width. The ambiguity in interpretation of IR spectra is due to the complexity of the composition and structure of the biological structures of the material under study. At the same time, the absence of significant changes in the location of the characteristic bands of the spectrum indicates the preservation of the tissue structure, in particular, tertiary collagen and elastin molecules. Based on the foregoing, it can be argued that modification has no negative effect on the architectonics of biological material.

In vitro calcification

The amount of calcium phosphate precipitate in the in vitro model system is limited and depends on the initial concentrations of the working solution. In this case, a relatively unlimited amount of salt is formed on the implant surface in the blood stream. Moreover, calcium phosphate precipitation in the in vitro system is random compared to pathological calcification. Consequently, the nature of the crystalline mineral phase in the in vitro system is hardly predictable and may differ from in vivo. Besides, there is strong evidence supporting the role of cells in calcification [25]. However, the proposed in vitro model can be used to explain the formation of passive deposits that do not imply cellular involvement [26].

Polyhydroxyl coating formed on the surface of biological materials modified with polyvinyl alcohol and tannic acid is a highly hydrophilic surface, which, under physiological conditions, has a neutral reaction in the case of PVA and weakly acidic for TA. Due to the neutralization of free groups of preservatives capable of provoking calcium accumulation in the tissue, and also due to the filling of voids in the structure of the material [27], such a modification probably partially limits the penetration of calcium and phosphate ions into the tissue, since the existing spaces are potential calcification nucleation centers [14].

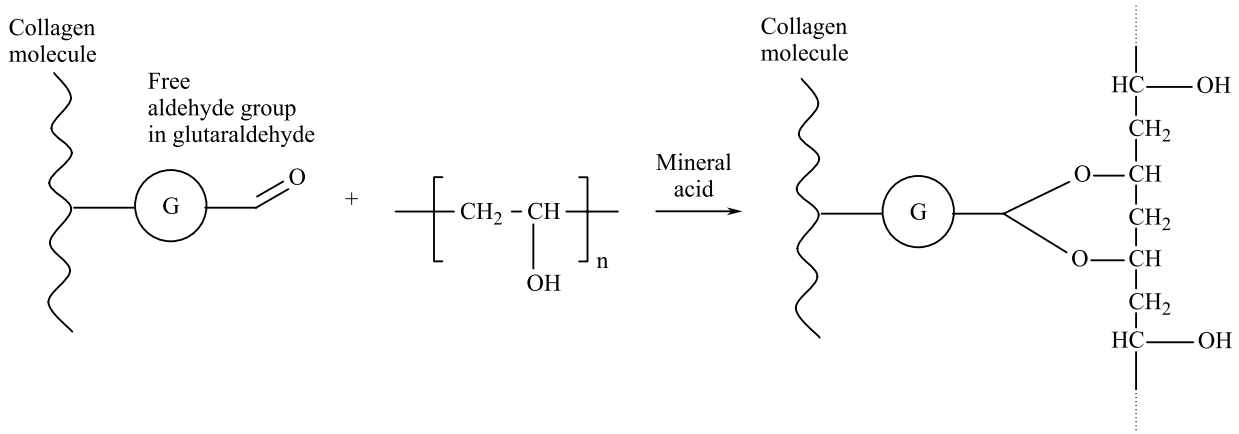

Fig. 5. Hemiacetal formation as a result of interaction between the aldehyde group in glutaraldehyde and two nearest hydroxyl groups in polyvinyl alcohol

In vivo calcification

A decrease in the level of GA-preserved pericardium calcification as a result of PVA and TA modification is probably associated with decreased toxicity of the aldehyde groups of the preservative, and, as a consequence, with reduced inflammatory response. Inflammation is known to precede the development of implanted tissue mineralization [28]. In addition, tannic acid has been reported to be able to bind elastin, which is also a promoting factor in calcification [29]. No similar information about polyvinyl alcohol has been found in publications; so, this issue requires experimental confirmation. Meanwhile, the calcium content in the EGDE group was lower than the same value for the GA control group. However, the presented implantation model for rats does not give a complete picture, since long-term clinical outcomes indicate calcification of biological prostheses of the heart valve preserved by EGDE [8]. For this reason, modification is necessary, and the methods we have proposed in this work may be quite promising as they demonstrate the calcium-binding resistance of the treated tissues.

CONCLUSION

A cumulative analysis of the study results demonstrated the ability of the modified PVA and TA tissues, pre-preserved with GA and EGDE, to reduce calcium-binding activity and increase strength, which indicates the prospects for clinical application of the proposed treatment methods. At the same time, the issue of long-term body response requires further study of the long-term stability of the modified biomaterial under physiological blood flow conditions.

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The authors declare no conflict of interest.

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VALVE-SPARING OPERATIONS ON THE AORTIC VALVE AND THE ASCENDING AORTA: RADICAL CORRECTION OF CONGENITAL AND ACQUIRED HEART DISEASES. IMMEDIATE OUTCOMES

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This paper presents the immediate outcomes of valve-sparing operations on the aortic valve and ascending aorta in radical correction of congenital and acquired heart disease. **Materials and methods.** The study enrolled 50 patients with aortic insufficiency who were operated upon at Shumakov National Medical Research Center of Transplantology and Artificial Organs from 2011 to 2019. The mean age was 48 ± 16 years, 64% of them were men (n = 32). The study included patients with tricuspid (n = 36, 72%) and bicuspid (n = 14, 28%) aortic valves. Aortic valve reimplantation was performed in 32 (64%) patients, aortic root remodeling - in 1 (2%). 17 (34%) patients had no aortic root reconstruction or remodeling. Aortic valve reimplantation was done in 4 (8%) cases in combination with coronary artery bypass grafting, and in 4 (8%) with mitral and tricuspid valve repair. **Results.** Thirty-day mortality was 0%. In 1 case (2%), a permanent pacemaker was installed due to complete atrioventricular block. There were no neurological and coronary events, and cases of endocarditis. In all patients (100%), aortic valve insufficiency after surgical correction did not exceed grade 1 according to echocardiographic follow-up examination. On aortic valve mean and peak gradients were 8 ± 6 and 15 ± 7 mm Hg, respectively. **Findings.** Type I and II valve-sparing reconstructive surgery (for bicuspid and tricuspid aortic valves) is an excellent alternative to prosthetic repair with great postoperative outcomes, low valve-associated complications and low mortality.

Keywords: valve-sparing surgery, aortic valve reconstruction, aortic insufficiency, aortic regurgitation.

According to European studies, dystrophic diseases constitute the main group of patients with aortic insufficiency – about two-thirds of all observations (Iung B., 2003). Among them there is a significant group of patients with elastic uncalcified tricuspid or bicuspid valves, with aortic insufficiency type 1 (aortic root enlargement with normal leaflet mobility) or type 2 (leaflet prolapse) (Lancellotti P., 2010; le Polain de Waroux J.B., 2007; Lansac E., 2008).

Congenital supraaortic stenosis is the rarest obstructive lesion of the left ventricle. Using the Doty technology, an inverted Y-shaped incision is performed in the ascending aorta down to the noncoronary sinus and the right coronary sinus to the left of the right coronary artery ostium. In some cases, an incision in the right coronary sinus is made to the right of the right coronary artery ostium if the coronary artery is too close to the commissure between the left and right aortic valve leaflets. Extended aortoplasty aims at a more symmetrical enlargement of the aortic root by suturing an inverted Y-shaped patch into the non-coronary sinus and right coronary sinus. Despite small number of observations, many authors consider this technique to be preferable.

In 2017, Tirone David presented his long-term results of aortic valve reimplantation over the past 20 years. In-hospital mortality rate was 1%, survival rate was 72%, and the freedom from reintervention was 96% (David T.E., 2017). In the same year, reconstructive valve-sparing surgeries were included in the European Guidelines for the Management of Valvular Heart Disease as an alternative to aortic valve replacement (class IC) (Baumgartner V., 2017; Karciauskas D., 2019). To date, the choice of access for aortic root reconstruction does not affect long-term outcomes, and a minimally invasive approach has excellent early and mid-term outcomes compared to the conventional midline approach (Charchyan E.R., 2020).

There are many questions surrounding bicuspid valve reconstruction. In 2019, American colleagues analysed 770 publications and 92 major papers, selecting 26 studies. The results showed low hospital mortality, high 5-year survival, and low risk of reintervention. The authors also noted that strengthening of the annulus fibrosus improves long-term outcomes, while calcification and fibrosis of the leaflets, on the contrary, increase the risk of re-intervention (Arnaoutakis G.J., 2019; Boodhwani

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M., 2009). Insufficient height of leaflet coaptation less than 9 mm at intraoperative assessment may also be a predictor of recurrent aortic insufficiency >2+, less 9 mm at intraoperative assessment (Karciauskas D., 2019).

Objective: to evaluate the immediate outcomes of valve-sparing reconstructions in aortic valve insufficiency.

MATERIALS AND METHODS

The study enrolled 50 patients with aortic insufficiency, who were operated at our center from 2011 to 2019. The mean age was 48 ± 16 years, 64% men (n = 32). The study included patients with tricuspid (n = 36, 72%) and bicuspid (n = 14, 28%) aortic valves. Five patients (10%) were diagnosed with Marfan syndrome. Before surgery, all patients underwent standard examinations (electrocardiography, echocardiography, chest X-rays and contrast-enhanced spiral CT, as well as examinations to exclude concomitant conditions). Preoperative characteristics of the patients are presented in Table 1.

Table 1

Preoperative patient characteristics (n = 50)

Characteristics	Value
Male	32 (64%)
Age, y	48 ± 16
Arterial hypertension	38 (76%)
Hemodynamically significant coronary artery stenosis	12 (24%)
Stanford type A aortic dissection	8 (16%)
Ascending aortic aneurysm	35 (70%)
Aortic arch aneurysm	2 (4%)
Mitral valve disease	12 (24%)
Tricuspidal valve disease	7 (14%)
Marfan syndrome	5 (10%)
New York Heart Association (NYHA) Functional Classification	
Class 1	0
Class 2	10 (20%)
Class 3	32 (64%)
Class IV	17 (34%)
Aortic valve insufficiency	
<2 degrees	17 (34%)
2 degrees	12 (24%)
≥3 degrees	21 (42%)
Bicuspid aortic valve	14 (28%)
Tricuspid aortic valve	36 (72%)
Ejection fraction, %	60 ± 7
Left ventricular mass index (LVMI)	226.1 ± 44.5

Malformation was corrected through a median sternotomy in 47 patients (94%); the rest of the patients had the malformation corrected through an upper median mininotomy. Cannulation was performed in the aorta and right atrium; the left ventricle was drained through the right superior pulmonary vein. For combined pathology, two cannulas were used in the inferior and superior vena cava. Myocardial protection was performed by selective administration of cold cardioplegic solution. In isolated lesions, blood hyperkalemic cardioplegia was performed according to the Calafiori technique; custodiol was used for cases of combined pathology. The volume and frequency of administration varied depending on patient characteristics and extent of surgery performed.

CASE STUDY

On January 9, 2019, a 31-year-old female patient G. was admitted to the cardiac surgery department with the following diagnosis: "Marfan syndrome. Debakey type 1 dissecting aortic aneurysm, subacute stage. Aortic valve regurgitation grade 3. Mitral valve regurgitation grade 2, tricuspid valve regurgitation grade 2. Pulmonary hypertension group 2; Atrial fibrillation, paroxysmal form. Circulatory insufficiency IIb, functional class IV.

The patient's medical history shows that the patient has been suffering from arterial hypertension for a long time, the maximum reaching 180/100 mmHg. In 2019, an echocardiographic examination conducted at her residence revealed an aortic aneurysm with dilatation of the ascending aorta to 6.7 cm.

At the time of examination, her condition was of moderate severity. Marfanoid appearance, scoliosis. Acrocyanosis, pasty legs and feet. BP = 130/60 mmHg, heart rate = 74 beats per minute. Muffled heart sounds, arrhythmic. A systolic murmur was heard in the second intercostal space on the right and a diastolic murmur on the apex. ECG showed atrial fibrillation, normoform, left axis deviation (LAD).

EchoCG showed aortic valve fibrous ring 2.5 cm, at the level of Valsalva sinus 6.0 cm, ascending aorta 7.5 cm, arch 2.9 cm. The left atrium is 5.5 cm (anteroposterior size), 5.3 × 6.6 cm (from apical access). The right ventricle is 3.6 cm. The left ventricle: EDD 6.8 cm, EDV 238 mL, ESD 4.8 cm, ESV 106 mL, stroke volume (SV) 132 mL. Ejection fraction (EF) 56% (according to Teichholz). Interventricular septum (IVS) 1.2 cm, left ventricular (LV) posterior wall 1.25 cm. Left ventricular mass (LVM) 495.1 g. Left ventricular mass index (LVMI) 257.3 g/m^2. Aortic valve regurgitation grade 3, mitral valve regurgitation grade 2, tricuspid valve regurgitation grade 2. Pulmonary artery pressure 50 mmHg.

Chest spiral CT findings: aortic annulus 3.5 cm, ascending diameter 8.3 cm, 5.2 cm at the Valsalva sinus level, aortic arch diameter 2.9 cm. LV EDD 7.7 cm (Fig. 1).

Ultrasound examination of the brachiocephalic arteries and the lower limb arteries revealed no hemodynamically significant stenoses. Coronary angiography: right type, without hemodynamically significant stenoses.

On January 10, 2019, ascending aorta replacement by David procedure and aortic arch replacement with a multi-branch prosthesis under cardiopulmonary

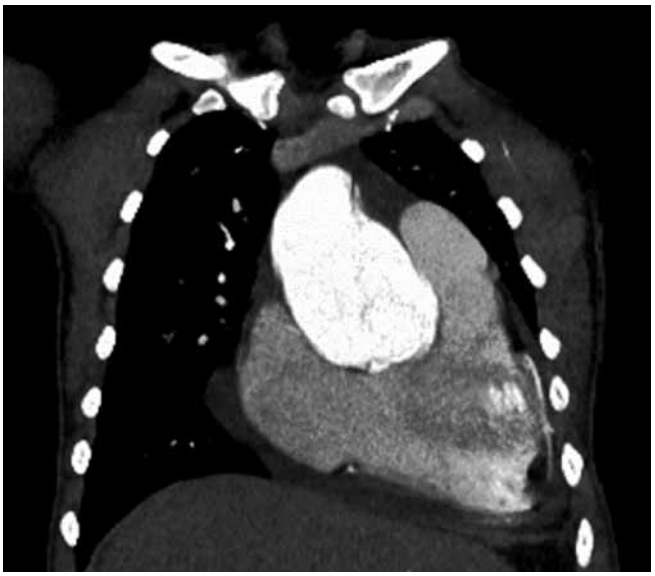

Fig. 1. Contrast-enhanced chest CT scan, lumen of the ascending aorta

bypass (CPB), circulatory arrest and selective antegrade cerebral perfusion (SACP) were performed.

Intraoperatively, the heart was enlarged owing to left ventricular hypertrophy, there was systolic-diastolic tremor over the aorta, the pulmonary artery was not tense. The ascending aorta is dilated to 8 cm. Aneurysmal dilation of the aorta ends at the union of the ascending aorta with the aortic arch. Further, the aorta is about 2 cm in diameter.

The aortic arch and vena cava were cannulated, with the latter being bypassed (Fig. 2). CPB was initiated with hypothermia at 24.8 °C, myocardial protection with chilled cardioplegic solution "Custodiol" (3 litres) into the coronary artery orifices. Heart drainage through the right superior pulmonary vein.

After clamping the aorta, longitudinal aortotomy was performed. On examination, the aortic wall was degeneratively changed, thinned (Fig. 3). The dissection began in the Valsalva sinuses of the non-coronary and right coronary artery with a detachment of the right coronary artery orifice and went beyond the aortic forceps. The aortic wall was dissected circularly with multiple intimal ruptures in the ascending section. The aortic valve was tricuspid, the cusps with marginal thickening, sagging into the left ventricular cavity in the place with detached commissures. The aortic annulus was about 3 cm (Fig. 4).

The ascending aorta was excised with the isolation of the aortic commissures and coronary artery orifices

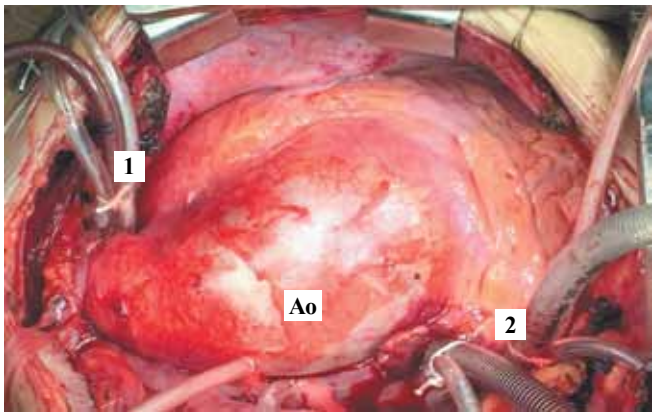

Fig. 2. Cannulation of the aortic arch (1) and vena cava (2).

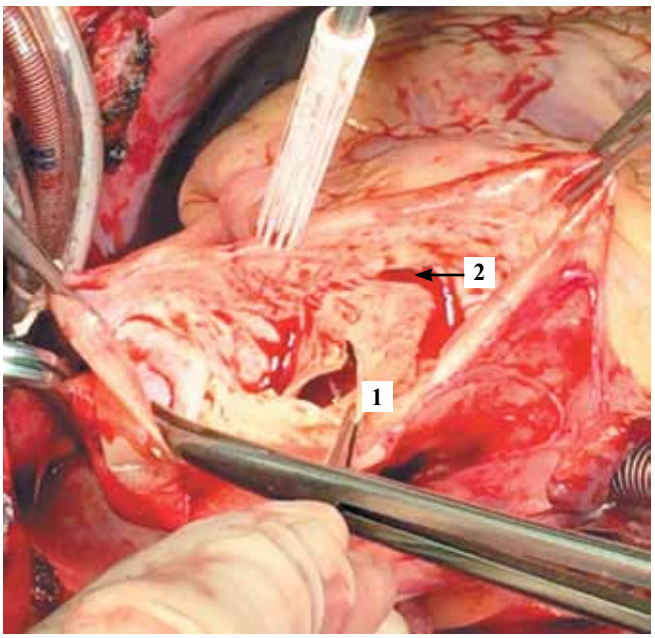

Fig. 3. Longitudinal aortotomy. Aortic valve (1), right coronary arterial orifice (2)

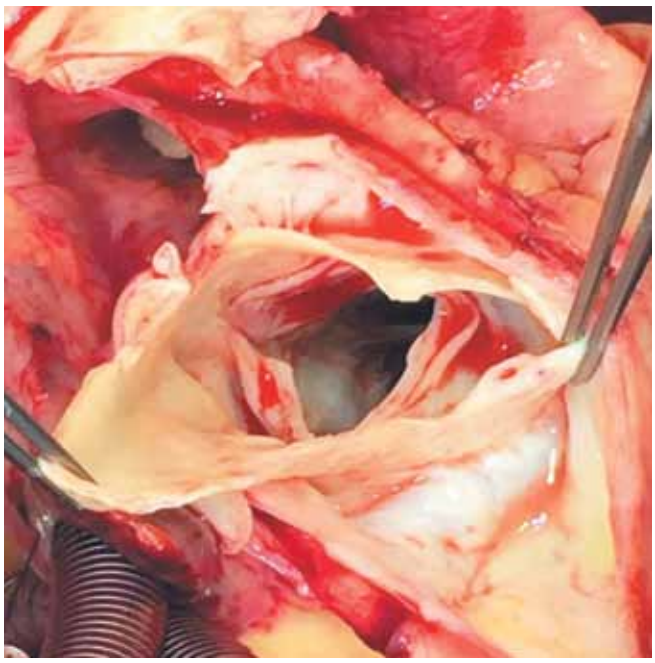

Fig. 4. Aortic root

on the sites. The aortic annulus was stitched with 12 U-shaped sutures on the spacers from the ventricular side. The aortic root was reimplanted into the Gelweave™ Valsalva vascular prosthesis. Multiple nodal sutures were used to perform plication of the right coronary and non-coronary leaflets (Fig. 5).

Against the background of hypothermia at 25 °C, cardiopulmonary bypass was stopped and bilateral SACP through the brachiocephalic trunk and the left common carotid artery was started. The clamp was removed from the aorta. The aortic wall at the arch level was also 2/3 dissected with intimal ruptures in several places and separation of the brachiocephalic trunk and left common carotid artery orifices. The aortic arch was excised. The diameter of the descending thoracic aorta was 18 mm, which prevented lowering of the "elephant trunk" into the descending aorta. A continuous twisted suture formed a distal anastomosis of a multi-branch prosthesis with the descending aorta with a "sandwich" angioplasty of the aortic wall. Cannulation of the additional prosthesis branch. Distal perfusion was started.

An anastomosis of the distal branch of the prosthesis with the left subclavian artery was formed with prolene 5-0 continuous twisted suture. Blood flow through the subclavian artery was initiated.

Interprosthetic anastomosis (ascending aorta and multi-branch prosthesis) was formed with prolene 5-0 continuous twisted suture. Clamp was removed from the aorta; coronary blood flow was restored.

Anastomosis of the middle branch of the prosthesis with the left carotid artery (LCA) was formed with prolene 5-0 continuous twisted suture. Blood flow was started on the LCA. Anastomosis of the proximal branch of the prosthesis with the brachiocephalic trunk was formed with prolene 5-0 continuous twisted suture. Blood flow was started along the brachiocephalic trunk (Fig. 6).

After warming, cardiac activity was restored with the help of two defibrillator shocks, almost immediately with sinus rhythm. At the end of the surgery, there was satisfactory hemodynamics against the background of moderate doses of catecholamines (Dopamine 3 μg/kg/min and Dobutamine 2 μg/kg/min).

CPB was ended, the vena cava and aorta were decannulated. Perfusion branch was ligated with suturing. An electrode was sutured to the right ventricular myocardium. Hemostasis. The pericardial and anterior mediastinal cavities were drained, the pericardium was sutured, and the sternum was tightened with 7 wire sutures. Postoperative wound was sutured tightly in layers. Iodine. Aseptic sticker.

CPB time 271 min, myocardial ischemia 216 min, circulatory arrest 40 min, SACP 110 min. Intraoperative blood loss 1000 mL. Total duration of mechanical ventilation was 11 hours 29 minutes. Length of stay in the intensive care unit was 1 day. During the first 24 hours, 300 mL of serous-hemorrhagic discharge were received via backup drains.

The postoperative period was uneventful. According to EchoCG, the aortic annulus was 2.4 cm, the ascending aorta was 3.4 cm, the arch was 3.0 cm. The left atrium was 4.4 × 5.2 cm (from the apical access). Left ventricle: EDD 5.9 cm, EDV 174 mL, ESD 4.2 cm, ESV 78 mL, SV 96 mL. EF 57% (according to Teichholz). IVS 1.2 cm, LV posterior wall 1.2 cm. LVM 375.7 g. LVMI 195.2 g/m^2. Aortic valve regurgitation grade 1, mitral valve regurgitation grade 2, tricuspid valve regurgitation grade 1. Pulmonary artery pressure 26 mmHg. A chest spiral CT scan with 3D reconstruction was performed in the postoperative period (Fig. 7).

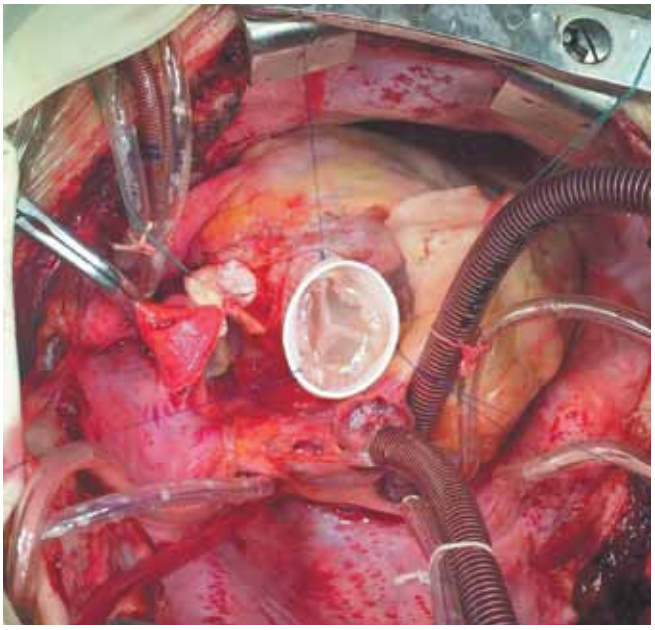

Fig. 5. Valve sparing aortic root replacement, David procedure

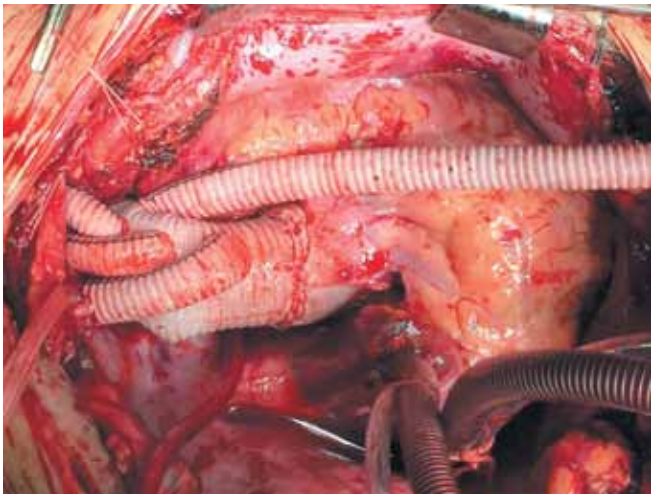

Fig. 6. Aortic arch replacement using a trifurcated branched graft

Histological examination showed dysplastic changes in the aorta.

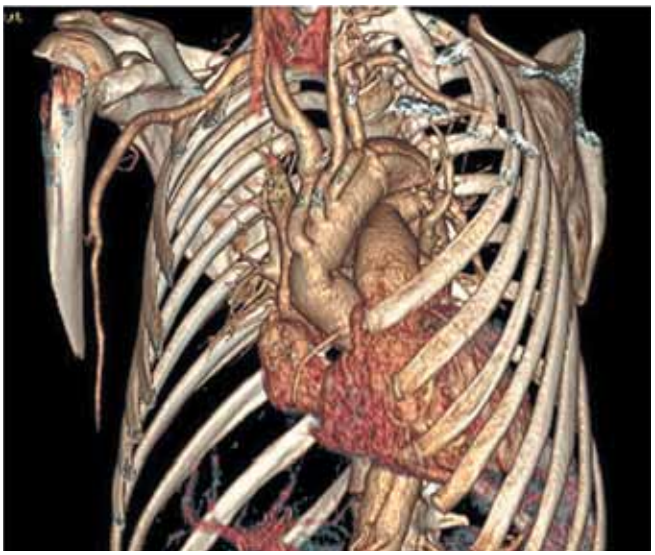

Fig. 7. 3D volume rendered CT reconstruction

Table 2

Surgical correction and postoperative indicators

Characteristics	Value
David I	**23 (46%)**
with mitral and tricuspid valve repair	1 (2%)
with mitral and tricuspid valve repair and CABG surgery	1 (2%)
with elephant trunk prosthesis	1 (2%)
Florida Sleeve	**9 (18%)**
Standard	7 (14%)
with mitral and tricuspid valve repair	1 (2%)
with CABG surgery	1 (2%)
Yacoub	**1 (2%)**
Aortic valve repair without root procedure	**17 (34%)**
Supracoronary intervention with sinotubular narrowing	**6 (12%)**
with CABG surgery	3 (6%)
Aortic valve leaflet repair	**10 (20%)**
with mitral and tricuspid valve repair	3 (6%)
with CABG surgery	1 (2%)
with subaortic diaphragm resection	1 (2%)
Doty	**1 (2%)**
CPB time, min	128 ± 31
Aortic cross-clamp time, min	103 ± 31
Body temperature during artificial circulation, °C	32 ± 2
Need for catecholamines	32 (64%)
Adrenalin	3 (6%)
Norepinephrine	2 (4%)
Early extubation in the operating room	33 (66%)
Intensive care unit stay, days	2 ± 1
Postoperative wound duration, days	10 ± 4
Permanent pacemaker implantation	1 (2%)
30-day in-hospital mortality	0

The patient was discharged on day 17 after surgery in a satisfactory condition under the supervision of a cardiologist and surgeon at her residence.

IMMEDIATE OUTCOMES

Surgical correction and postoperative indicators are presented in Table 2.

The David I procedure was performed in 23 patients (46%), Florida Sleeve in 9 (18%), and isolated aortic valve leaflet plasty without aortic root replacement in 11 (22%). Central leaflet plication was performed in 18 patients (36%).

CPB had to be reconnected in 2 cases (4%) due to eruption of the suture of the right coronary artery orifice.

The 30-day mortality was zero. In 1 case (2%), a permanent pacemaker was installed due to complete atrioventricular block. There were no neurological and coronary events, as well as cases of endocarditis.

Echocardiographic examination revealed that in all patients (100%), aortic insufficiency after surgical correction did not exceed grade 1. On the aortic valve, the mean and peak gradients were 8 ± 6 and 15 ± 7 mm Hg, respectively.

CONCLUSION

Valve-sparing reconstructive surgery, preserving the native leaflets, is a very good alternative to aortic valve replacement, featuring excellent postoperative outcomes. Indications include intact types 1 and 2 leaflets, both the bicuspid and the tricuspid aortic valves.

The authors declare no conflict of interest.

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PROGNOSTIC VALUE OF TROPONIN I AFTER CORONARY ARTERY BYPASS GRAFTING (AMIRI-CABG STUDY)

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In 2017, the European Society of Cardiology outlined the importance of the problem of diagnosing myocardial ischemia-reperfusion injury following coronary artery bypass grafting. Myocardial injury can be accompanied by a critical decline in the cardiac index and an increase in cardiac troponin I plasma levels. The prognostic value troponin I elevation after coronary artery bypass grafting is poorly understood. **Objective:** to determine the prognostic value of troponin I plasma levels in relation to a fall in the cardiac index after coronary artery bypass grafting (CABG). **Task:** To determine the probability the cardiac index falling below 2.2 for troponin I levels in the first hours, and on days 1, 2, 3, 4 after CABG. **Materials and methods.** The single-center, non-randomized prospective study, running from 2016 to 2019, included 336 patients admitted for elective surgical treatment of coronary artery disease. The CABG patients were divided into three observation groups: off-pump (n = 175), on-pump (n = 128), and pump-assisted (n = 33). Troponin I levels were measured in the first hours, and on days 1, 2, 3, 4 after surgery using the Pathfast Compact immunoassay analyzer. Cardiac index was measured by invasive method. **Results.** In patients with a cardiac index higher than 2.2, troponin I level did not exceed 0.5 ng/mL in the off-pump group, 6 ng/mL in the on-pump group, and 3.5 ng/mL in the pump-assisted group. Patients with cardiac index lower than 2.2 have comparable troponin I levels in all groups – 21 ng/mL. Troponin I thresholds on day 1 after surgery, which, when exceeded, was associated with the likelihood of the cardiac index falling below 2.2, was 3.78 ng/mL in the off-pump group, 9.67 ng/mL in the on-pump group and 17.06 ng/mL in the pump-assisted group. **Conclusion.** After off-pump CABG, clinically significant myocardial injury should be expected at lower troponin I levels (3.78 ng/mL) than after on-pump CABG (9.67 ng/mL) and pump-assisted CABG (14.7 ng/mL).

Keywords: beating coronary artery bypass grafting, off-pump, on-pump, pump-assisted, ischemia-reperfusion, myocardial injury, troponin I, troponin I prognostic value.

INTRODUCTION

CABG surgery is a recognized and effective treatment for coronary heart disease in multivessel coronary artery disease. Despite the effectiveness and widespread use of CABG, myocardial ischemia-reperfusion injury associated with coronary artery surgery remains an unresolved problem [1, 2]. For instance, it is reported that 30-day mortality after a CABG surgery ranges from 1 to 3% despite advances in medical technology [3]. Existing methods of cardioprotection, such as cardioplegia, do not always provide sufficient myocardial protection [2]. A solution to this problem is being sought in different directions, e.g., development of more advanced cardioplegic solutions [4–8]. Another possible approach may be off-pump CABG [9]. However, the risk of incomplete revascularization has been reported in off-pump CABG operations [10]. The possibility of stimulating myocardial regeneration with the help of cell therapy, which can level out the regular ischemia-reperfusion injury caused by surgery, is being considered [11–14]. The possibility of myocardial ischemic preconditioning, which should reduce myocardial injury area has been studied [15]. Another approach may consist of timely diagnosis of the severity of perioperative myocardial ischemia-reperfusion injury during a CABG surgery [16]. Timely and accurate diagnosis of ischemia-reperfusion injury after a CABG surgery and an accurate assessment of its severity and clinical significance remain unresolved challenges [16]. Myocardial ischemia-reperfusion injury after a CABG surgery can be accompanied by a significantly decreased cardiac index (CI) and death, and therefore is of great clinical importance [16]. Rapid diagnosis of the severity of perioperative myocardial ischemia-reperfusion injury is a relevant approach. A working group of the European Society of Cardiology distinguishes perioperative myocardial injury and type 5 myocardial infarction [16]. Criteria for the diagnosis of type 5 myocardial infarction have been proposed, which include an elevation of cardiac troponin values of more than 10 times the 99th percentile upper reference limit

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value during the first 48 hours following CABG surgery in patients with normal preoperative cardiac troponin levels together with either a new Q wave and/or newly detected left bundle branch block by electrocardiogram (ECG) and/or angiographic documented new occlusion of the coronary artery and/or shunt and/or newly detected new areas of myocardial death by imaging and/or new areas of myocardial kinetics disturbance [16]. It is reported that the upper reference limit value for the increase in cardiac troponin differs depending on the manufacturer of the diagnostic kit and each clinic should determine its own threshold cardiac troponin level; available data are contradictory [16]. Thus, clarification of the predictive value of an increase in cardiac troponin levels associated with increased risk of a clinically significant decrease in CI may improve CABG outcomes.

Objective: to determine the prognostic value of troponin I plasma levels in relation to a fall in CI following a CABG surgery.

Task: to determine the probability of the CI falling below 2.2 for troponin I levels in the first hours, at days 1, 2, 3, 4 after coronary bypass surgery.

MATERIALS AND METHODS

The study included patients with coronary heart disease admitted at the Pavlov First St. Petersburg State Medical University in St. Petersburg for planned surgical treatment in the period from 2016 to 2019. Inclusion criteria for the study included presence of ischemic heart disease (exertional angina pectoris functional class 3–4) with proven multivessel coronary artery disease, patient's consent. Exclusion criteria: patient refusal, presence of heart valve pathology, acute coronary syndrome.

Of the patients with coronary heart disease admitted at the Research Institute of Surgery and Emergency Medicine, Pavlov First St. Petersburg State Medical University from 2016 to 2019, 336 people who met the inclusion criteria were included in the study.

Study type: the prospective non-randomized, single-center study approved by the local ethics committee and confirmed by the Academic Council of Pavlov First St. Petersburg State Medical University is registered in the international register of clinical trials U.S. National Library of Medicine, ClinicalTrials.gov Identifier: NCT03050489 "Assessment of myocardial ischemic-reperfusion injury during off- and on-pump CABG (AMIRI-CABG)".

The patients were divided into the following observation groups: off-pump CABG (n = 175), on-pump CABG (with aortic clamping, n = 128), pump-assisted CABG (without aortic clamping, n = 33). On-pump CABG surgeries were performed routinely, according to the standard technique, through a median sternotomy. A HL-20 heart-lung machine from Maquet and a single-use circuit of a heart-lung machine with the Affinity Fusion oxygenator from Medtronic were used. Extracorporeal cardiopulmonary bypass circuit was connected according to the aorta-right atrium-inferior vena cava scheme (one two-stage cannula). Filling the apparatus: mannitol 15% – 200 ml, gelofusin – 500 ml, sterofundin – 500 ml, $NaHCO_3$ 5% – 50 ml, tranexam – 20 ml, antibiotic 2 g, heparin 2 mL, insulin 10 U, dexamethasone 24 mg. Cardiopulmonary bypass was performed either in hypothermia at 32.0 °C temperature with aortic clamping (on-pump CABG), or under normothermal conditions (36.6 °C) without aortic clamping (pump-assisted CABG). In pump-assisted CABG, tissue stabilizer Medtronic Octopus was used during shunt placement.

If aortic clamping was necessary, antegrade and retrograde administration of blood cardioplegia (2–6 °C) with the addition of glucose (5% – 250 mL), potassium chloride (10% – 30 mL), magnesium sulfate (25% – 20 mL), and lidocaine (10% – 2 mL) was used to stop cardiac activity and protect the myocardium. Off-pump surgeries were performed routinely, according to the standard technique, through a median sternotomy. During shunt placement, tissue stabilizer Medtronic Octopus was used.

Regardless of coronary bypass type, 100% of patients included in the study underwent mammary-coronary anastomosis to the anterior interventricular artery. Ischemia-reperfusion injury was assessed by determining the troponin I levels before CABG, after CABG, and also on days 1, 2, 3, 4 after CABG. Troponin I levels were determined using immunoanalyzer Pathfast Compact. A CI $\leq$2.2, measured by an invasive technique was the criterion for postoperative heart failure [17, 18].

STATISTICAL DATA PROCESSING

All parameters studied were tested for normal distribution (Shapiro–Wilk test, Kolmogorov–Smirnov test). Incidence of complications was compared using Fisher's exact test. Construction of logistic regression, ROC-curve, calculation of the probability of postoperative heart failure was performed using the SAS Enterprise Guide 9.4 software. Troponin I thresholds were determined using the Youden index. Graphs were plotted in SAS Enterprise Guide 9.4.

RESULTS

The severity of coronary lesions and comorbidities were comparable in all observation groups (Table).

Baseline troponin I levels were normal in all the groups, <0.05 ng/ml. In the AMIRI-CABG study, troponin I thresholds, conditionally distinguishing between regular ischemia-reperfusion injury and type 5 myocardial infarction, was determined at 12.4 ng/ml within 24 to 48 hours after CABG surgery. According to different researchers, cardiac troponin threshold ranges from 9 ng/ml to 25 ng/ml [19].

Incidence of myocardial infarction (new ST elevation of more than 2 mm in two or more leads, new Q wave, new left bundle branch block) was 1.1% (n = 2),

0.78% (n = 1), and 0% in the off-pump, on-pump, and pump-assisted groups, respectively. The differences were statistically insignificant, Fisher's exact test p = 1.0. The presented data were obtained on a sample of n = 336 patients, of whom myocardial infarction signs were observed in three people by ECG. The incidence of a fall in CI below 2.2 was 8.57% (n = 15) in the off-pump CABG group, 11.72% (n = 15) in the on-pump group, and 3.03 % (n = 1) pump-assisted group. Differences in the observation groups were statistically insignificant (Fisher's exact test). The mean troponin I level on day 1 after CABG in the off-pump and on-pump groups in patients whose CI fell below 2.2 was comparable – 21 ng/mL. Troponin I levels in hemodynamically stable patients with a CI >2.2 varied significantly across the groups (Fig. 1).

Table

Basic patient characteristics

	Group 1: off-pump CABG, n = 175 (181)*	Group 2: on-pump CABG with aortic clamping, n = 128	Group 3: off-pump CABG without aortic clamping, n = 33 (27)**	p
Age, years, av. ± SD	63.5 ± 7.3	63.5 ± 7.13	64.3 ± 8.9	p > 0.05
Gender				
male	78.3 %	74.2%	74.1%	0.7
female	21.7 %	25.8 %	25.9%	
Syntax Score II	41.35 [32.7–50.8]	42.25 [31.1–49.9]	43.5 [34.5–53.6]	0.6
Euroscore II	1.03 [0.7–1.5]	0.97 [0.6–1.6]	0.79 [0.6–1.4]	0.3
Charlson/Deyo Index	5 [4–7]	5 [4–6]	5 [4–6]	0.1
EF (Simpson method) before surgery (%)	62.0 [55.0–67.0]	62 [59–66]	63 [55–65]	0.9
Preoperative troponin I levels, ng/mL				
Median Lower and upper quartile	0.008 [0.003–0.018]	0.014 [0.007–0.025]	0.014 [0.005–0.06]	0.09
Main characteristics of surgical intervention				
Surgery duration, min	290 [250–330]	330 [300–363]	335 [290–355]	
Average number of shunts	3	3	3	

Note. Charlson/Deyo Index – comorbidity index, allows taking into account comorbidities, EDVI – end-diastolic volume index, ESVI – end-systolic volume index, av. – average, SD – standard deviation, EF – ejection fraction, LCA trunk – left coronary arterial trunk, AIB – anterior interventricular branch, DB – diagonal branch, CB – circumflex branch, OMB – obtuse marginal branch, RCA – right coronary artery, PIB – posterior interventricular branch. Median [upper quartile – lower quartile] values are indicated. * The number of surgeries that began without CPB is indicated in brackets; conversion was performed in 6 cases. ** The number of operations planned with concurrent CPB is indicated in brackets.

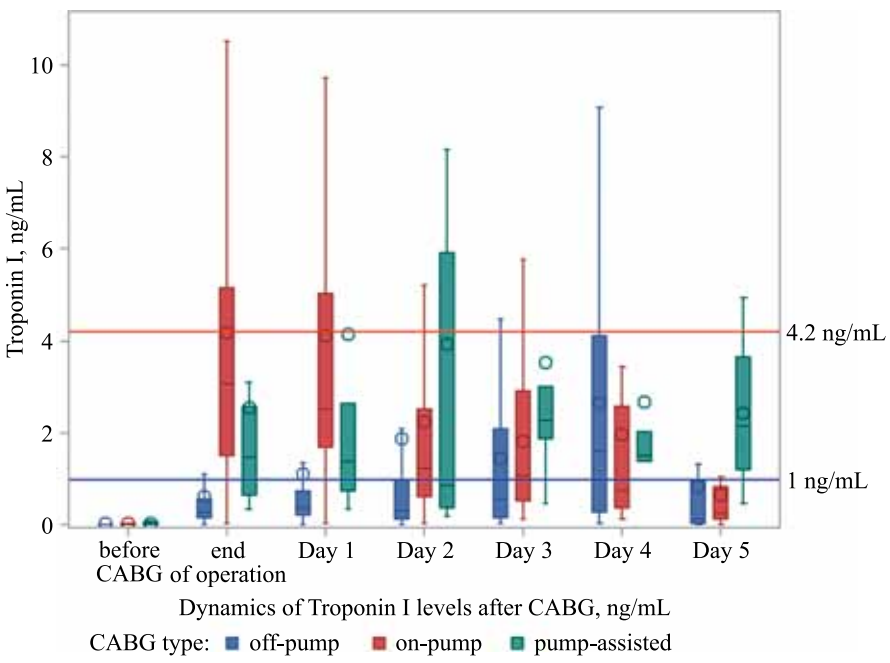

Fig. 1. Troponin I levels after CABG in patients with CI >2.2

The predictive value of increased troponin I level varied among the groups. For example, at 5.1 ng/mL after surgery, the probability of the CI falling below 2.2 was 12% in the off-pump CABG group and 7% in the on-pump CABG group (Fig. 2).

The predictive value of increased troponin I level on day 1 after surgery was also different among the observation groups. At 17.9 ng/mL after surgery, the probability of the CI falling below 2.2 was 71%, 56%, and 10% in the off-pump, on-pump and pump-assisted CABG groups, respectively (Fig. 3).

At 14.7 ng/mL troponin I level on day 2 after surgery, the probability of the CI falling below 2.2 was 38.5%, 67.8%, and 20% in the off-pump, on-pump and pump-assisted CABG groups, respectively (Fig. 4).

At 2.6 ng/mL troponin I level on day 3 after surgery, the probability of the CI falling below 2.2 was 13.2% and 24.3% in the off-pump and on-pump groups, respectively (Fig. 5).

The probability of the CI falling below 2.2 in the pump-assisted CABG group on day 3 after surgery could not be calculated due to unreliable logistic regression.

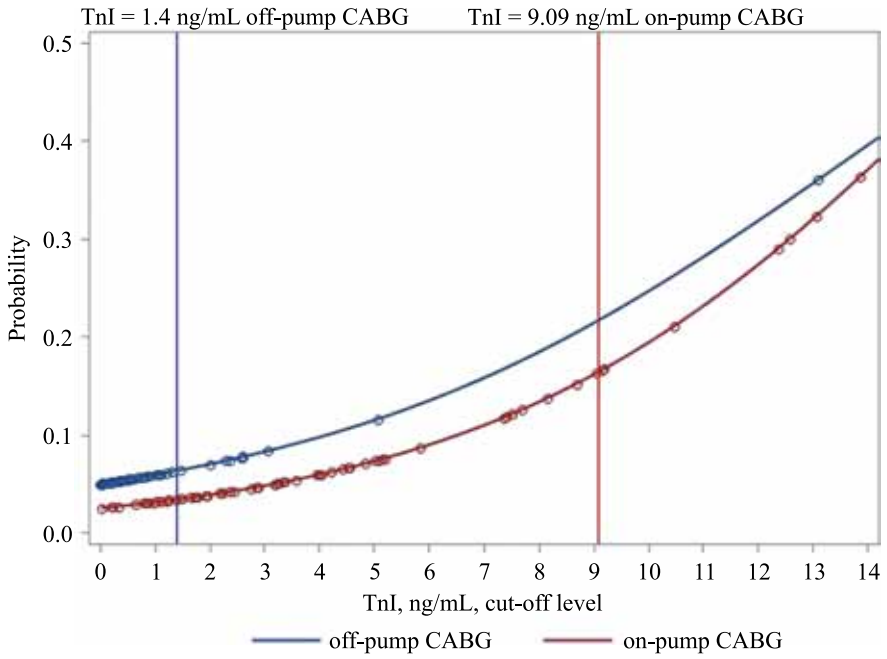

Fig. 2. Probability of CI falling below 2.2 after CABG at different troponin I levels at the end of surgery. The vertical lines indicate troponin I thresholds above which the probability of the CI falling below 2.2 should be considered as clinically significant

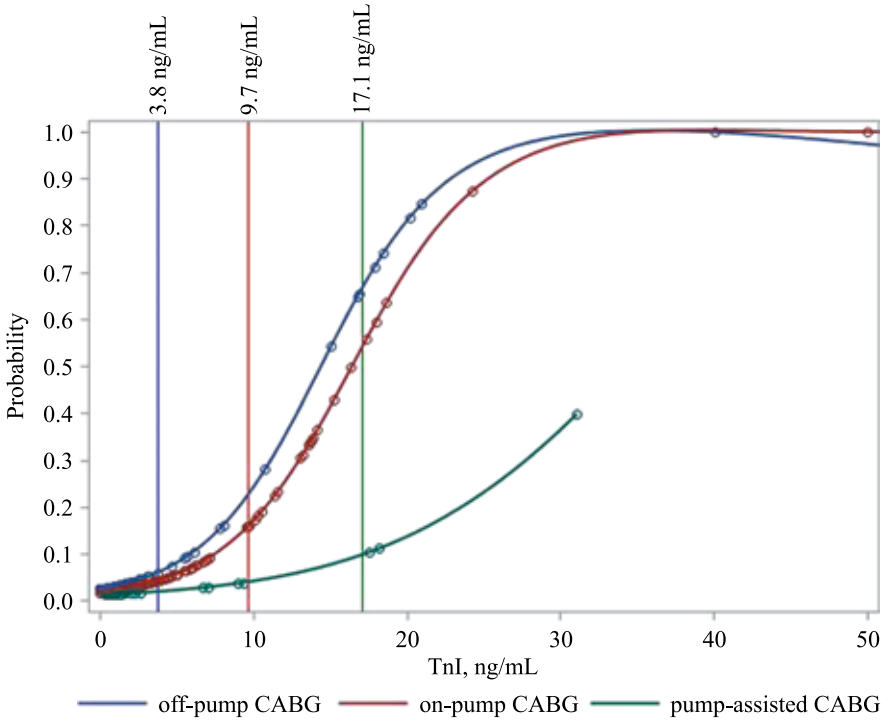

Fig. 3. Probability of CI falling below 2.2 on day 1 after CABG at different troponin I levels. The vertical lines indicate troponin I thresholds above which the probability of the CI falling below 2.2 should be considered as clinically significant

At 2.6 ng/mL troponin I level on day 4 after surgery, the probability of the CI falling below 2.2 was 18.9% and 29.4% in the off-pump and on-pump groups, respectively (Fig. 6).

The probability of the CI falling below 2.2 in the pump-assisted CABG group on day 4 after surgery could not be calculated due to unreliable logistic regression.

DISCUSSION

Coronary artery bypass grafting is accompanied by a regular myocardial ischemia-reperfusion injury [2]. It is assumed that off-pump CABG is associated with less myocardial ischemia-reperfusion injury. However, the absence of CPB cannot completely exclude clinically significant myocardial damage. Moreover, the risk of incomplete revascularization in off-pump CABG interventions has been reported [10].

In 2017, the European Society of Cardiology published the conclusion reached by its working group on the assessment of myocardial ischemia-reperfusion injury and diagnosis of type 5 myocardial infarction. In the same year, international register ClinicalTrial.gov (U.S. National Library of Medicine) registered a study by the Department of Faculty Surgery of the Research Institute of Surgery and Emergency Medicine, Pavlov First St. Petersburg State Medical University, dedicated

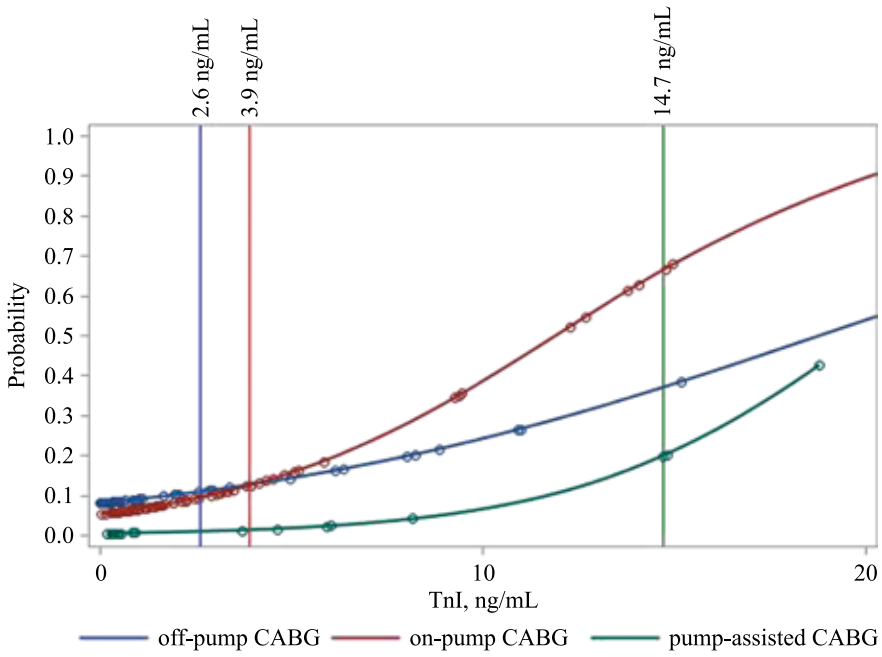

Fig. 4. Probability of CI falling below 2.2 on day 2 after CABG at different troponin I levels. The vertical lines indicate troponin I thresholds above which the probability of the CI falling below 2.2 should be considered as clinically significant

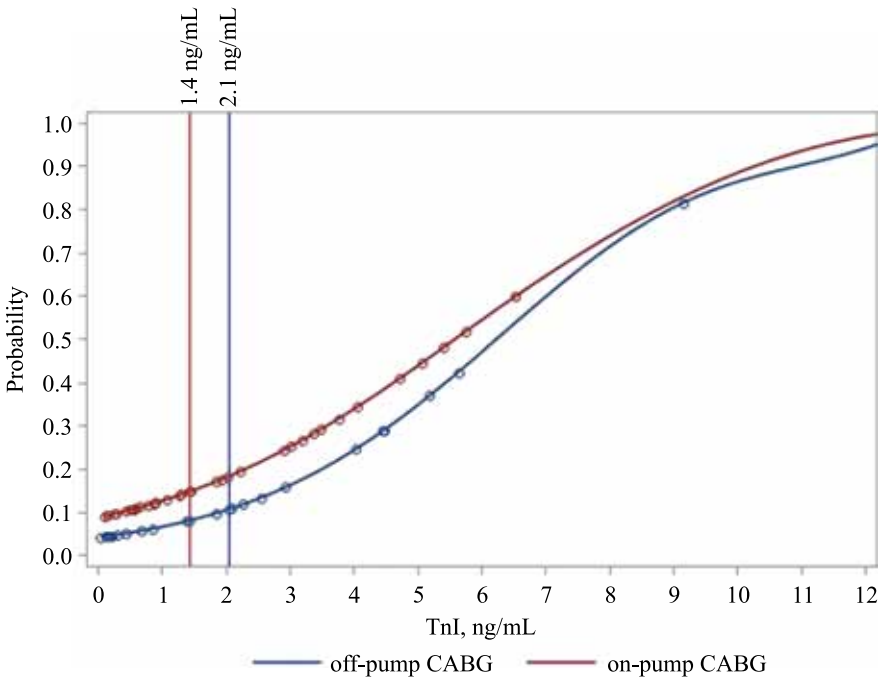

Fig. 5. Probability of CI falling below 2.2 on day 3 after CABG at different troponin I levels. The vertical lines indicate troponin I thresholds above which the probability of the CI falling below 2.2 should be considered as clinically significant

to the assessment of myocardial ischemia-reperfusion injury after different types of CABG – Assessment of Myocardial Ischemic-Reperfusion Injury During Off- and On-Pump CABG (AMIRI-CABG ClinicalTrials.gov Identifier: NCT03050489). Thus, a comparison of myocardial ischemia-reperfusion injury after different types of CABG was, for the first time, performed based on new and more precise criteria proposed by the European Society of Cardiology.

The AMIRI-CABG study established that any increase in troponin I levels after coronary artery bypass surgery above the manufacturer's recommended threshold of 0.05 ng/mL is associated with an increased risk of the CI falling below 2.2.

Troponin I levels exceeded the norm in 100% of patients with a CI exceeding 2.2 in all observation groups. However, the same cardiac troponin levels after a beating heart CABG surgery and under on-pump support is associated with a different probability of the CI falling below 2.2. So, Fig. 3 shows that at 3.8 ng/mL troponin I level, the probability of the CI falling below 2.2 was 6%, 4%, and 2% in the off-pump, on-pump, and pump-assisted CABG groups, respectively. Troponin I thresholds of a clinically significant probability of the CI falling below 2.2 were 3.8 ng/mL, 9.67 ng/mL and 17.1 ng/mL in the off-pump, on-pump, and pump-assisted groups, respectively (Figs. 2–6). This suggests that the prognostic value of an increase in troponin I after an off-pump, on-pump and pump-assisted CABG surgeries is different. We did not find any current studies comparing the prognostic value of troponin I with an accurate calculation of the likelihood of the CI falling below 2.2 after different types of CABG according to the current diagnostic criteria for myocardial ischemia-reperfusion injury following a CABG surgery, proposed by the European Society of Cardiology in 2017. The European Society of Cardiology points out the limited accuracy of isolated elevation in cardiac troponin, used without taking into account imaging and clinical data, in determining the degree of myocardial ischemia-reperfusion injury following a CABG surgery [16]. Thus, the AMIRI-CABG study suggests that the prognostic value of an increase in troponin I after off-pump, on-pump, and pump-assisted CABG surgeries differs significantly. Clinically significant myocardial injury after an off-pump CABG surgery should be expected at lower troponin I levels than after an on-pump CABG. In patients with a CI >2.2, troponin I levels did not exceed 0.5 ng/mL, 6 ng/mL, and 3.5 ng/mL in the off-pump, on-pump, and pump-assisted groups, respectively.

The AMIRI-CABG study has level II evidence. Further studies on the predictive value of cardiac troponin elevation after CABG are needed due to the inherent limitations of single-center and non-randomized studies.

CONCLUSION

After off-pump CABG surgery, clinically significant myocardial injury should be expected at lower troponin I levels (3.78 ng/mL) than after on-pump CABG surgery (9.67 ng/mL) and pump-assisted CABG surgery (14.7 ng/mL). Pump-assisted CABG may be associated with a significantly lower risk of the cardiac index falling. In patients with a cardiac index higher than 2.2, troponin I levels did not exceed 0.5 ng/mL, 6 ng/mL, and

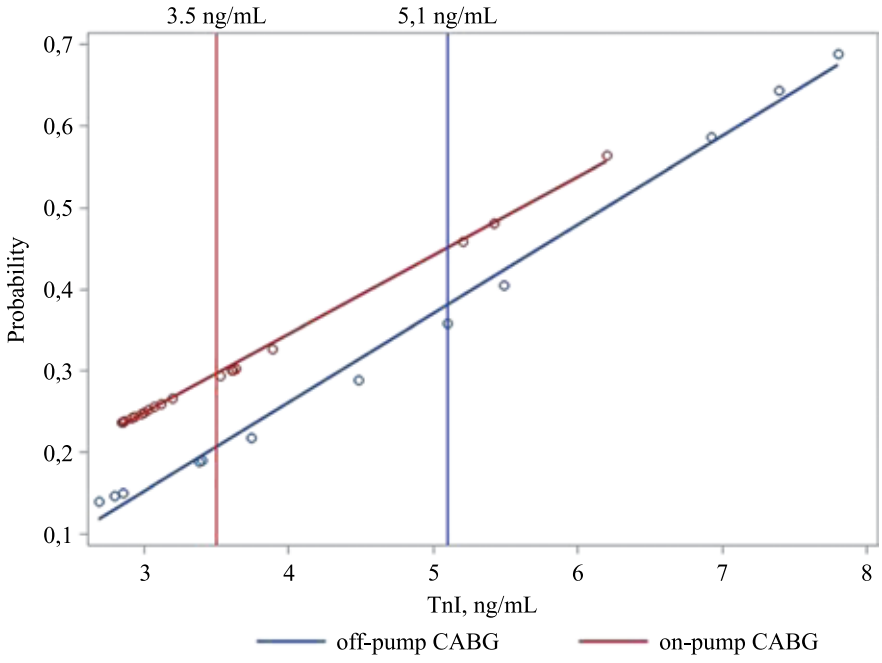

Fig. 6. Probability of CI falling below 2.2 on day 4 after CABG at different troponin I thresholds. The vertical lines indicate troponin I thresholds above which the probability of the CI falling below 2.2 should be considered as clinically significant

3.5 ng/mL in the off-pump, on-pump, and pump-assisted CABG groups, respectively.

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The authors declare no conflict of interest.

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RE-INTERVENTIONS AFTER THE ROSS PROCEDURE: REASONS, TECHNICAL APPROACHES, IMMEDIATE OUTCOMES

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Re-interventions after pulmonary autograft aortic valve replacement (Ross procedure) may be associated with dysfunction of the neoaortic, neopulmonary, or both operated valves. Late dysfunction, other than infective endocarditis, is associated with underlying conditions, technical errors, and unsuitable pulmonary trunk replacement materials. Re-interventions are technically complex, while tactical approaches have not been definitively formulated. **Objective:** to analyze re-interventions in patients after Ross procedure, technical approaches and immediate outcomes. **Material and methods.** Between 2001 and 2019, 14 patients were reoperated upon within 2 days to 21 years after primary Ross procedure. Early prosthetic endocarditis (2) and technical errors (1) were the reasons for early postoperative re-intervention. Neoaortic valve insufficiency (7), including pulmonary valve dysfunction (2), pulmonary valve degeneration (2), pulmonary prosthetic valve endocarditis (1), aortic, pulmonary and mitral valve endocarditis (1) were the reasons for late postoperative re-intervention. Based on the lesion volume, neoaortic valve replacement (3), neoaortic root replacement (6), including pulmonary valve/trunk replacement (8), and pulmonary trunk stenting (2) were performed. **Results.** In-hospital mortality was 7.1%. One patient died of early endocarditis after primary procedure. The postoperative period for the remaining patients was uneventful. Microscopic examination of the neoaorta revealed fragmentation of elastic fibers and rearrangement of tissue histoarchitectonics. In the pulmonary position, the aortic allograft and stentless xenograft had severe calcification and valve stenosis. **Conclusions.** Neoaortic valve insufficiency associated with cusp prolapse and neoaortic root dilatation may be the reasons for re-interventions after the Ross procedure. The second reason for re-interventions is valve graft dysfunction in the pulmonary trunk position. Elective reoperations on the neoaortic root and/or lung graft, despite the large volume, can be performed with low mortality and morbidity. Aortic allografts and xenografts for reconstruction of the right ventricular outflow tract (RVOT) is unjustified due to early and more severe dysfunction compared to pulmonary allograft.

Keywords: Ross procedure, autograft, allograft, aortic valve, reoperation.

INTRODUCTION

When replacing the aortic valve (AV) in young patients, surgeons are faced with the challenge of choosing a prosthesis. Biological prostheses have limited durability; mechanical prostheses seriously change the patient's lifestyle, binding him/her to lifelong anticoagulant therapy, which, in a number of patients, does not prevent thromboembolic and hemorrhagic complications [1–5]. In addition, in children with small-diameter implanted prosthesis, a "prosthesis–patient" mismatch develops over time, with the formation of high transvalvular gradients and the need for reimplantation of a larger valve [6]. An alternative to mechanical prosthesis is the aortic valve prosthesis with a pulmonary autograft (Ross procedure). Pulmonary autograft provides long-term stability of outcomes, low probability of dysfunction and reintervention, excellent hemodynamic parameters even with a narrow fibrous ring (FR) and high quality of life for patients; it does not require anticoagulants, is able to grow as the body grows, which is important for children [7, 9]. The restrain among surgeons towards Ross procedure is due to the more complicated implantation technique, as well as a possible need for reintervention for neo-aortic valve dysfunction and/or right ventricular outflow tract (RVOT) prostheses.

MATERIALS AND METHODS

The Department of Emergency Surgery for Acquired Heart Diseases, Bakulev National Medical Research Center for Cardiovascular Surgery performed 80 Ross procedures from November 2001 to March 2019. During this period, 14 repeated interventions were performed again after the Ross procedure. Eight patients had been primarily operated on at other institutions and 6 in our series of 80 operations (7.5%). Of those reoperated, there were 11 men, mean age was 22.5 years (8–47). From medical history and extracts from previous medical records, the main cause of primary surgery was congenital bicuspid aortic valve – 13, including active infective endocarditis (IE) of the AV in 3 patients. By lesion morphology, most patients (13) initially had aortic insufficiency

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(AI), while 1 patient had isolated aortic stenosis (AoS). In 13 cases, a pulmonary autograph was implanted using the free root technique with reimplantation of the coronary artery ostia, while in 1 patient, it was done using the subcoronary technique. A cryopreserved pulmonary allograft was used to restore the integrity of the RVOT in 8 patients, aortic allograft in 3 cases and stentless xenograft in 3 patients (2 xenoaortic, 1 – xenopericardial). The mean time from primary surgery to re-surgery for all reasons was 8 ± 1.9 years (Table 1).

Table 1

Clinical characteristics of patients during the first surgery

Age at first operation (years)	22.5 ± 4 (8–47)
≤18 years	8
Gender	
Men	11 (79%)
Women	3 (21%)
Hemodynamic changes in the AV during the first operation	
Stenosis	1
Insufficiency	13
Etiology of AV disorder at the first operation	
Bicuspid AV / tricuspid AV	13/1
Infective AV endocarditis, primary	1
Infective AV endocarditis, secondary	2
RVOT prosthesis	
Lung allograft	8
Aortic allograft	3
Stentless xenograft	3
Autograft implantation technique	
Subcoronary technique	1 (7%)
Free root	13 (93%)

In preparation for surgery, all patients underwent a comprehensive examination, including echocardiography, contrast-enhanced multislice CT (MSCT), and three-dimensional reconstruction of the heart and blood vessels. The diameter of the aorta, pulmonary conduit at different levels, anastomotic zones, the degree of adherence of heart structures to the sternum were determined, which allowed to plan surgical support and safe access. All patients over 40 years of age underwent coronary angiography.

Reintervention technique

12 operations were performed under complete sternotomy, hypothermic (26–28 °C) cardiopulmonary bypass and pharmaco-cold cardioplegia. Central cannulation of the aorta and both vena cavae was used in 10 patients. In two cases, we first cannulated and initiated cardiopulmonary bypass through the femoral vessels, then the arterial cannula was moved into the ascending aorta. Right heart cardiolysis was performed, the aorta and pulmonary trunk were isolated. A conventional technique was used to replace the aortic and pulmonary valves. For aortic root replacement, the pulmonary autograft wall was dissected up to the annulus fibrosus with mobilization of the coronary artery (CA) ostia. A dacron conduit with a mechanical prosthesis and direct implantation of the CA ostia into the conduit wall was used. In the case of pulmonary valve IE and/or pulmonary trunk calcification, the latter was completely excised, and a valve-containing conduit (dacron with mechanical prosthesis or pulmonary allograft) was implanted.

Endovascular intervention for the correction of degenerative pulmonary artery (PA) conduit stenosis in two patients was performed in the X-ray operating room and consisted of PA trunk stenting.

Reasons for re-interventions in the early postoperative period

Three re-interventions were performed in the early postoperative period. One patient showed signs of myocardial ischemia on ECG on day 2 after the primary operation. Coronary angiography revealed left coronary artery (LCA) torsion in the area of implantation into the autograft. On emergency re-intervention, the anastomosis was dissolved and re-applied, and coronary artery bypass grafting of the anterior interventricular branch was performed preventively. The second patient had prolonged fever in the early postoperative period without the effect of antibiotic therapy. EchoCG revealed vegetations on the pulmonary allograft. The patient underwent a pulmonary allograft replacement but died from intractable systemic infection and erosive bleeding from the aortic wall. In the third patient, who was operated on in the active stage of infective endocarditis of the aortic valve with an annulus fibrosus abscess, early prosthetic endocarditis of the pulmonary autograft and pulmonary allograft one month after the Ross procedure was the indication for re-intervention. The patient underwent aortic root replacement with a synthetic valve-containing conduit and pulmonary artery replacement with a pulmonary allograft.

Reasons and volume of re-interventions in the long-term period

Grade 3 neoaortic valve regurgitation was detected in 3 patients. The cause of regurgitation was prolapse of one or all three leaflets without neoaortic root dilatation. The mean time from primary surgery to re-intervention was 10.3 years (9–12 years).

Neo-aortic rook dilatation ≥45 mm with marked AV regurgitation was an indication for re-intervention in 4 patients (Fig. 1, a, b). The mean time from primary surgery to re-intervention was 12.2 years (5–21 years).

The reason for prosthesis replacement in the RVOT in 7 patients with autograft valve failure was moderate lung allograft dysfunction (3 patients), aortic allograft stenosis at the level of the proximal and distal anasto-

mosis (2 patients), calcification and stenosis of stentless aortic xenograft (1 patient) (Table 2).

In 2 patients, the indication for repeated surgical intervention was late prosthetic valve IE. In one case, three years after the Ross procedure, there was an isolated pulmonary allograft lesion. In the second case, the indication for re-intervention was autograft dissection and active IE of the neoaortic, aortic allograft in RVOT and mitral valves 14 years after surgery (Fig. 2).

Two patients with stentless xenografts in the RVOT position and no neoaortic valve dysfunction underwent stenting of narrowed proximal and distal xenograft anastomoses (Fig. 3). As a result of stenting, there was a decrease in right ventricular (RV) pressure, systolic pressure gradients between the RV and PA, and a more than 75% increase in the diameter of the stented segment.

In 4 patients with neoaortic dilatation and neoaortic valve regurgitation, repeated surgery included aortic root replacement with a synthetic valve-containing conduit with mechanical prosthesis (Bentall–De Bono procedure) and valve or pulmonary artery trunk replacement. Implantation of a mechanical prosthesis in AV and RVOT positions was performed in 2 patients. In two cases with severe stenosis and calcification of the prosthesis in RVOT, a synthetic conduit with a mechanical prosthesis was also used for its replacement (Table 3).

In late neo-aortic valve and aortic root replacement, regardless of the function of the neopulmonary valve, we adhered to the tactics of its replacement. Implantation of mechanical prostheses in the position of the aortic and pulmonary valves is the method of choice in our department.

A year after the primary Ross procedure with neoaortic root dilatation with its pronounced insufficiency and enlargement of the proximal aortic arch without pul-

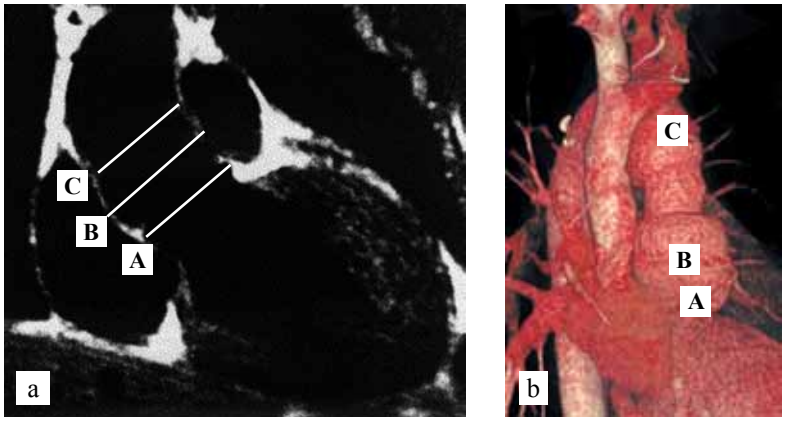

Fig. 1. Cardiac computed tomography: a) patient F., 21 years after surgery (A – aortic annulus – 50 mm, B – sinuses of Valsalva – 55 mm, C – sinotubular junction – 49 mm); b) patient A., 5 years after surgery (A – aortic annulus – 26 mm, B – sinuses of Valsalva – 47 mm, C – ascending aorta – 37 mm)

Table 2

Hemodynamic parameters of RVOT prostheses in patients with autograft and prosthesis replacement in RVOT

	Pulmonary allograft	Aortic allograft	Aortic xenograft
Peak pressure gradient, mm Hg.	19	25	40
Mean pressure gradient, mm Hg.	11	12	18
Regurgitation, degree	<1	<2	3
Diameter at the proximal anastomosis level, mm	21	15	16
Diameter at the distal anastomosis level, mm	26	19	21

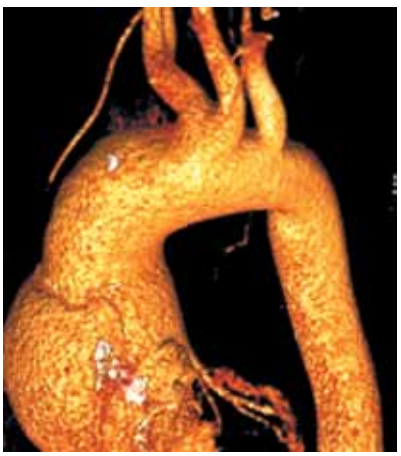

Fig. 2. MSCT of patient R., 14 years after surgery. (34 mm diameter of the aortic annulus, 80 mm at the level of the sinuses of Valsalva, 64 mm at the level of the pulmonary artery trunk)

monary allograft dysfunction in RVOT, in connection with a planned pregnancy, a 31-year-old female patient underwent replacement of the ascending aorta and part of the arch with a synthetic conduit with a stented bioprosthesis without intervention on the prosthesis (pulmonary allograft) in RVOT.

RESULTS

Cardiopulmonary bypass lasted for an average of 278 (160–429) min. The average time of aortic clamping was 156 min (120–265). Intraoperative blood loss was 400 mL (350–550). ICU length of stay was 1.9 ± 0.53 days. Mechanical ventilation lasted for 19 ± 5.9 hours. Length of in-hospital stay was 21 ± 3.1 days.

At the hospital stage, there was 1 death resulting from erosive bleeding from the aorta and unresponsive generalized infection.

The early postoperative period was uneventful for 13 patients. In the late postoperative period (7 ± 3.2 years), patients with mechanical prosthetic valves in the aortic valve position and in the pulmonary artery position (n = 10) followed anticoagulant therapy with target INR values from 2.0 to 3.5. There were no prosthetic thrombosis and no thromboembolic complications. All discharged patients are alive and active. The patients who underwent stenting are dynamically monitored. Given the absence of a valve in the stent, the right ventricular function is assessed in a targeted manner. No thrombosis, stent fracture or restenosis were observed for up to 2 years. Hemodynamic and volumetric parameters of the right ventricle are satisfactory.

Histological picture of explanted prostheses

Histological examination of all explanted bioprosthetic valves was carried out. The pulmonary autograft was characterized by the following changes: in the leaves there are areas of disorganization and fragmentation of elastic fibers, destruction of smooth muscle cells with focal basophilia of the main substance and fibrosis (Fig. 4). In the autograft wall, fibrosis of the middle membrane develops with an increased number of small capillary-type blood vessels in the outer. In some cases, formation of atherosclerotic plaques and acute inflammation areas was found in the autograft.

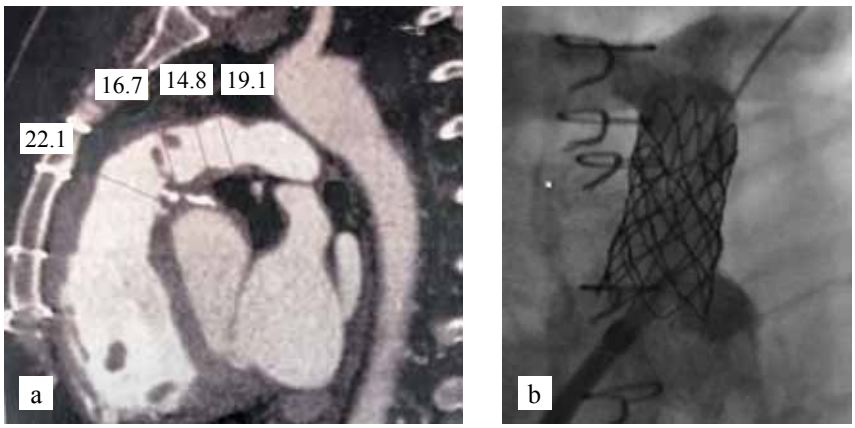

Fig. 3. Patient T., 11 years after Ross procedure: a) MSCT of the RVOT prosthesis (walls are calcified, stenosis in the anastomosis projection); b) angiogram of the implanted stent in the RVOT prosthesis position (xenograft)

Table 3

Types of re-interventions performed

Bentall–De Bono Procedure	PVR (with pulmonary allograft)	2
	PVR (with mechanical prosthesis)	2
	PVR (with conduit)	1
	Pulmonary valve revision	1
AVR + PVR (mechanical prosthesis)		2
AVR + PVR conduit (mechanical prosthesis)		1
PVR with pulmonary allograft		2
RVOT stenting		2
AIV CABG		1

* PVR (pulmonary valve replacement), AVR (aortic valve replacement), RVOT (right ventricular outflow tract), AIV CABG (coronary artery bypass grafting of the anterior interventricular artery).

The histological pictures of the explanted pulmonary and aortic allografts differ. The pulmonary allograft is represented by a thinner wall, absence of cells, and proper arrangement of collagen and elastic fibers.

The aortic allograft is characterized by a denser wall, with petrification areas that create high gradients at the level of the valve, distal and proximal anastomoses (Fig. 5).

Stentless xenografts are characterized by extensive petrificates with the development of tissue ossification (Fig. 6).

DISCUSSION

Excellent long-term survival, low risk of thromboembolic and hemorrhagic complications are the main advantages of the Ross procedure [1–5] (Table 4).

However, the operation remains technically more complicated than the standard aortic valve replacement with stented prosthesis. The correctness of the anastomosis between the autograft and the left ventricular outflow tract (LVOT), anastomoses with the coronary arteries, as well as the duration of aortic clamping and cardiopulmonary bypass play a role in immediate mortality and survival. Even Donald Ross noted that with increasing experience, the initial problems of compression, kinking,

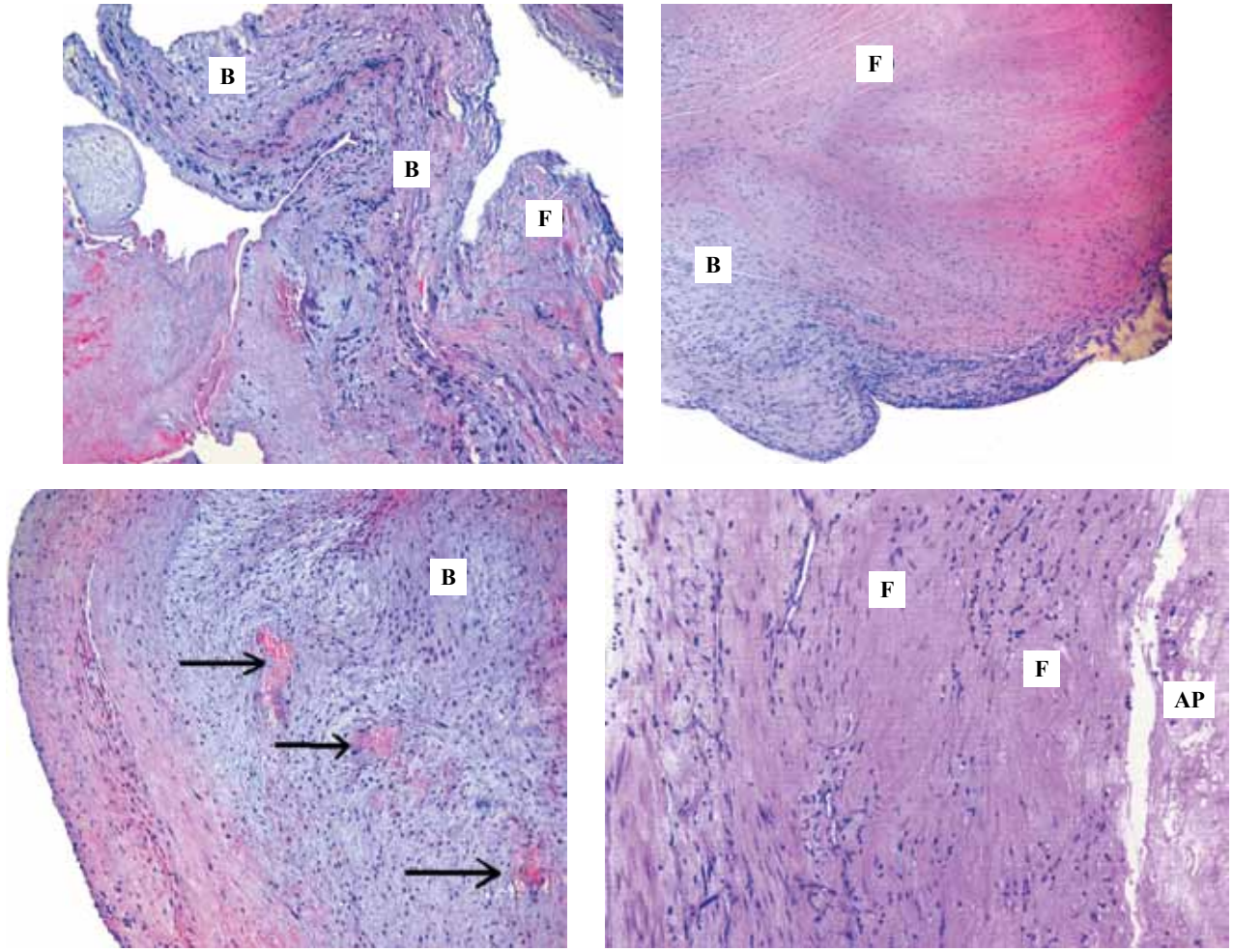

Fig. 4. Autograft leaflets. Micrograph. H&E stain. 100× magnification. In the valves of the pulmonary autografts, there is a picture of focal basophilia (B), fibrosis (F). Area of tissue destruction and eosinophilia (indicated by an arrow). Atherosclerotic plaque (AP)

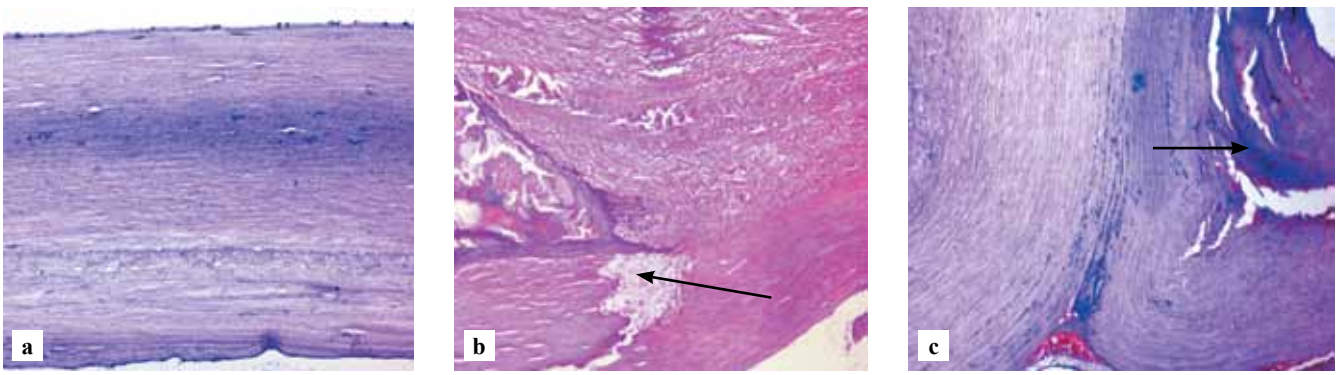

Fig. 5. Wall of grafts in the pulmonary artery position. Micrograph. H&E stain. 100× magnification: a) pulmonary allograft, built from collagen and elastic fibers; b–c) aortic allograft with a tissue petrification focus (indicated by an arrow)

torsion of the coronary arteries and complete heart block have been largely overcome [10]. We had one case of torsion of the LCA ostium, which was diagnosed on time and eliminated.

Re-interventions are characterized by prolonged aortic torsion, blood loss, high risk of injury to the structures of the heart and coronary arteries, and should be provided with adequate anesthetic and perfusion support, performed in a specialized center with a wide arsenal of techniques and means to eliminate sudden fatal complications.

Pulmonary autograft dilatation is one of the reasons for neoaortic valve reinterventions. Dilation of the sinotubular junction causes tension in the neovalve cusps with the development of central regurgitation. We found this phenomenon in 5 patients, one of whom even developed autograft wall dissection. Studies show that autograft dilatation occurs regardless of the implantation method and is due to the inability of the pulmonary trunk and valve to adapt to systemic arterial pressure [10, 11]. The process of remodeling has been demonstrated in explanted pulmonary autografts that have been subjected to systemic circulation for more than a decade [12]. Histological examination of the explants revealed the destruction of elastic fibers, smooth muscle cells with replacement of the extracellular matrix with connective tissue. Similar results have been obtained in the study of our material. For the prevention of neoaortic dilatation, some authors suggest the use of autologous tissues or synthetic materials, which serve as an external sheath for an autograft [5, 13, 14].

To prevent neoaortic dilatation, Magdi Yacoub suggests implanting the autograft subannularly for proximal support with the aortic annulus fibrosus, and perform the

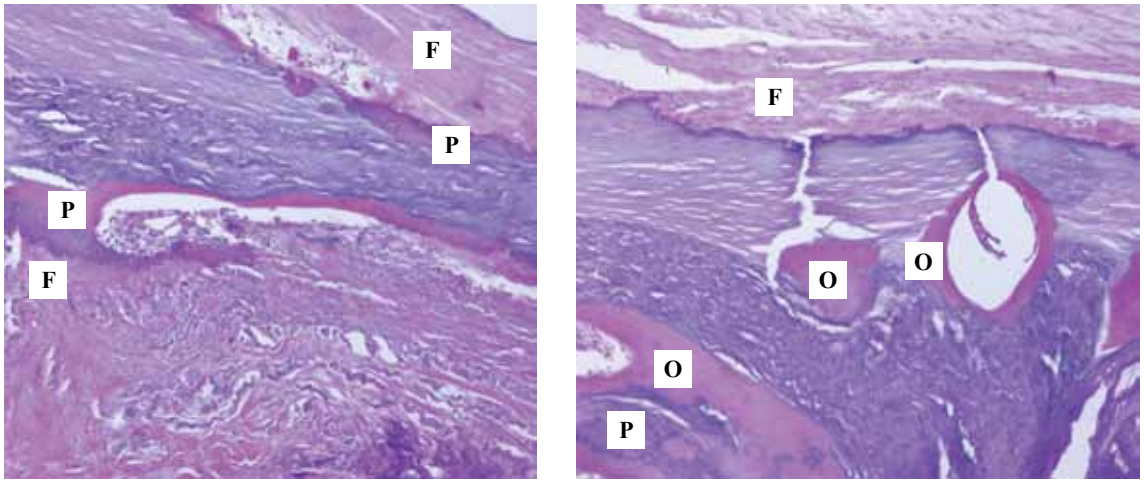

Fig. 6. Xenograft wall in the pulmonary artery position. Micrograph. H&E stain. 100× magnification. Xenograft with areas of fibrosis (F), pertrification (P), tissue ossification (O)

Table 4

Freedom from re-interventions on the neoaortic valve and RVOT prostheses in the long-term postoperative period

Author	Number of cases	Autograft	RVOT prostheses
Bogers A.J., 2004	123	89% (10 years)	91% (10 years)
Kouchoukos N.T., 2007	119	75% (10 years)	86% (10 years)
Elkins R.C., 2008	489	90% (10 years) 83 % (16 years)	90% (10 years) 82 % (16 years)
Mokhles M.M., 2012	161	84% (10 years) 51% (18 years)	90% (10 years) 81% (18 years)
Da Costa F., 2014	414	90.7% (15 years)	92.5% (15 years)
Weimar T., 2014	645	91.6% (12 years)	95% (12 years)
Martin E., 2017	310	96% (10 years) 90% (15 years) 76% (20 years)	96.6% (10 years) 92.1% (15 years) 82.3% (20 years)
Sharifulin R., 2018	793	91.4 (10 years)	91.4% (10 years)
Sievers H.H., 2018	630	96.4% (10 years) 89.8% (20 years)	96.5% (10 years) 91.0% (20 years)
David T.E., 2018	212	83.2% (20 years)	91.8% (20 years)

distal anastomosis at or slightly above the sinotubular ridge [15].

One of the factors for neoaortic dilatation and stratification may consist of a technical error when a long pulmonary artery autograft is used without strengthening the proximal and distal anastomoses areas. In our practice, to support an autograft implanted using the free root technique, we bring the diameters of the annulus fibrosus and the native aorta into full compliance, create the distal anastomosis 1 cm above the sinotubular ridge of the autograft, and also stabilize the proximal and distal anastomosis using synthetic strips (PTFE, Teflon).

A number of researchers believe that the possible predictors of neoaortic dilatation are male gender, mismatch in the size of the aortic root and pulmonary autograft, aortic annulus larger than 25 mm, and aortic insufficiency. T. David suggests reducing the diameter of the aortic annulus and ascending aorta to the size of the pulmonary artery to prevent dilatation. However, this does not always prevent long-term dysfunction in patients with congenital AV abnormalities. Pulmonary autograft dilatation was observed in 9 of 10 patients, all had a wide aortic annulus (≥27 mm) before surgery. Before 15 years, there were no neoaortic reinterventions in patients with an aortic annulus less than 27 mm and in women. The author concluded that women with AoS are ideal candidates for surgery; secondly, a dilated aortic annulus is a marker of connective tissue dysplasia, which may also be present on the pulmonary valve, which can cause premature neoaortic dysfunction [16, 17]. Similar figures are reported by Elkins and colleagues. In this study, the Ross procedure was performed in 487 patients, 197 of whom were younger than 18 years of age. At 16 years after surgery, 164 patients with AI were 59% free of neo-aortic dysfunction, which was significantly less than in 304 patients with AoS, in whom this indicator was 82%. The risk of autograft dysfunction in men was 3 times higher than in women. Annulus reduction and fixation was performed using synthetic material or autopericardium (FR >27 mm) in 96 patients with primary AI, for whom actuarial freedom from autograft valve failure was 87% at 10 years [7]. Weimar T. and colleagues have shown that reinterventions are performed 6 times more often in men than in women. Multivariate analysis showed that AI and aortic annulus of at least 26 mm are predictors of reinterventions [18].

We consider congenital AV defect with AI and ascending aorta aneurysm as one of the contraindications for Ross's procedure.

AI development with autograft cusp prolapse in our material was observed in 3 cases. On histological examination of the autograft cusps, we found basophilia of the main substance, reflecting the processes of intercellular synthesis. On one hand, these changes may be due to a nonspecific response of the connective tissue structures of the pulmonary valve to systemic arterial pressure; on the other hand, they may be associated with connective tissue dysplasia of the valve apparatus in patients with aortic defect. The ischemic nature of degeneration cannot be ruled out, since blood supply to the wall and cusps is disrupted at the time of transplantation. Basophilia of the main substance leads to thickening, prolapse of the cusps and valve dysfunction. Besides, pulmonary autograft can undergo the same changes (atherosclerosis, infective endocarditis) as the native AV.

Most authors use cryopreserved pulmonary allografts for RVOT reconstruction during the Ross procedure. However, some surgeons allow the use of stentless aortic allografts, xenoaortic, xenopericardial conduits, and engineered PTFE conduits. In our material, aortic allograft and stentless xenograft were implanted in 6 patients. We do not use aortic allograft for the right heart because it degenerates much more often than pulmonary allograft. According to James Albert e. al., the freedom from dysfunction 5 years after surgery for aortic allograft in RVOT was 76% compared to 94% for pulmonary allograft [19]. Similar results were presented by Yankah A. When comparing the function of aortic and pulmonary allografts, freedom from degeneration was 18% and 75%, freedom from dysfunction was 62% and 93%, respectively [20]. Perhaps this is related to the thicker wall of aortic allografts, which, when remodeled and replaced by connective tissue, creates a narrower lumen and higher gradients on RVOT.

The use of xenografts for pulmonary artery reconstruction in young patients is undesirable. Xenograft degeneration develops 10 times more often than pulmonary allograft degeneration [21–24]. In adult patients, xenograft dysfunction occurs less frequently, and they can be used in the absence of allografts. Moreover, in this era of rapid development in percutaneous technologies, endovascular intervention in dysfunction can become a low-traumatic temporary solution to the problem. In two of our patients with pulmonary xenograft calcification and stenosis, the use of stents significantly reduced the systolic gradient and led to clinical improvement.

Reinterventions due to IE were performed in 4 patients, 2 patients at the hospital stage, 1 in the midterm (IE allograft in RVOT after 3 years), 1 in the long term after surgery (neoarticular dissection and IE after 14 years).

Infective endocarditis in AV with abscess formation and aortic root destruction presents difficulties in surgical treatment and is associated with high mortality rates [25, 26]. In the case of IE with infection spreading to paravalvular structures (FR, mitral-aortic contact), allografts or autografts are preferred [25–29]. On the other hand, it has been shown that the rate of recurrent infection in patients with active IE does not depend on the type of prosthesis used, but on the radicality of removal of infected tissues [26, 27, 30, 31]. As our experience also shows, in case of extensive destruction of the aortic root structures or mediastinal infection, even considering

structural dysfunction in the late postoperative period, allografts should be preferred since structural degeneration is a much less complicated problem than recurrent IE.

The choice of a pulmonary valve prosthesis remains important in reintervention after the Ross procedure. This issue is overlooked by most authors. Any biological valve can undergo late degeneration. If the Ross procedure makes it possible to avoid anticoagulants, then during the second operation, a mechanical prosthesis is most often implanted in the aortic position and warfarin therapy is administered. In our opinion, keeping any valve graft in the pulmonary position carries the risk of another reoperation. Therefore, mechanical bicuspid prostheses were implanted in 4 patients in the aortic and pulmonary valve positions. Follow-up showed normal prosthetic function at standard INR (2.0–3.5).

CONCLUSION

Autograft dysfunction in the late postoperative period is a consequence of cusp prolapse and/or with autograft remodeling and dilation at different levels. To increase the autograft lifespan, it is necessary to ensure a match between the diameter of the pulmonary trunk and the aorta, which is achieved using the free root technique; using external support for the proximal and distal anastomoses. Planned repeated neoaortic root and/or pulmonary graft surgery, despite the large volume, can be performed with low mortality and complication rates. Implantation of aortic allografts and xenografts in young patients for RVOT reconstruction is unjustified due to the development of earlier and more severe dysfunction in comparison to pulmonary allografts. The use of an endovascular aid for isolated dysfunction of RVOT prostheses allows delaying the reintervention. It is necessary to strive towards making the repeat operation the "last" one; implantation of mechanical prosthetic valves in the aortic and pulmonary valve position is the most justified.

The authors declare no conflict of interest.

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IMPLANTATION OF A LONG BIOLOGICAL PATCH IN CLASSICAL CAROTID ENDARTERECTOMY FOR EXTENDED ATHEROSCLEROTIC LESIONS. LONG-TERM OUTCOMES

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Objective: to analyze the in-hospital and long-term outcomes of classical carotid endarterectomy (CEE) in extended atherosclerotic lesions in comparison with the outcomes of this operation in local atherosclerotic plaque (AP). **Materials and Methods.** This study, which lasted from January 2010 to December 2020, included 148 patients with extended AP and hemodynamically significant internal carotid artery (ICA) stenosis. The term "extended" was understood as a hemodynamically significant lesion ≥5 cm long. These patients made up Group 1. Group 2 was formed over the same period of time from 632 patients with hemodynamically significant stenosis <5 cm long. In both cohorts, CEE with repair of the reconstruction zone with a diepoxide-treated xenopericardial patch was performed. Long-term follow-up was 71.4 ± 45.6 months. **Results.** The groups were comparable in terms of frequency of in-hospital complications: death (group 1: 0.67%, n = 1; group 2: 0.5%, n = 3; p = 0.74; OR = 1.42; 95% CI 0.14–13.6), myocardial infarction (MI) (group 1: 0.67%, n = 1; group 2: 0.5%, n = 3; p = 0.74; OR = 1.42; 95% CI 0.14–13.6), ischemic stroke (group 1: 0%; group 2: 0.5%, n = 3; p = 0.91; OR = 0.6; 95% CI 0.03–11.8), combined endpoint (death + MI + stroke) (group 1: 1.35%, n = 2; group 2: 1.4%, n = 9; p = 0.74; OR = 0.94; 95% CI 0.2–4.43). The groups were also comparable in terms of frequency of long-term complications: death (group 1: 2.0%, n = 3; group 2: 2.05%, n = 13; p = 0.76; OR = 0.98; 95% CI 0.27–3.5), MI (group 1: 2.7%, n = 4; group 2: 2.4%, n = 15; p = 0.95; OR = 1.14; 95% CI 0.37–3.49), ischemic stroke (group 1: 5.4%, n = 8; group 2: 5.2%, n = 33; p = 0.9; OR = 1.03; 95% CI 0.46–2.29), ICA occlusion and restenosis (group 1: 12.8%, n = 19; group 2: 13.3%, n = 84; p = 0.99; OR = 0.96; 95% CI 0.56–1.63), combined endpoint (death + MI + stroke) (group 1: 10.1%, n = 15; group 2: 9.6%, n = 61; p = 0.98; OR = 1.05; 95% CI 0.58–1.91). Analysis of survival graphs revealed no significant intergroup differences for all types of complications (lethal outcome: p = 0.56; MI: p = 0.73; stroke/mini-stroke: p = 0.89; ICA restenosis/occlusion: p = 0.82; combined end point: p = 0.71). Their increase was uniform in both groups. However, more than half of all ICA restenoses and occlusions were visualized in the first 6 months after CEE. **Conclusion.** Implantation of a long patch (≥5 cm) is not characterized by increased incidence of restenosis and all adverse cardiovascular events during in-hospital and long-term follow-up.

Keywords: carotid endarterectomy, classical carotid endarterectomy, patch, restenosis, extended lesion, extended atherosclerotic plaque, temporary shunt, neointimal hyperplasia.

INTRODUCTION

Carotid endarterectomy (CEA) has long been a routine operation in vascular hospitals [1–5]. At the same time, despite the entire arsenal of reconstruction methods, existing national guidelines consider only two types of CEA as the reference ones: 1. Eversion; 2. Classical with patch angioplasty of the reconstructed zone [1]. In this case, only the preferences of the operating surgeon and the experience of the medical institution are sufficient for choosing the intervention technique for each individual patient [1]. However, if with a standard lesion volume

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both methods have proven to be effective and safe, then in the presence of an extended atherosclerotic plaque (EAP), traditional reconstruction techniques cannot always be justified [6–8]. The counterparts of surgery allowing to achieve a confident effect of revascularization in these conditions, include internal carotid artery (ICA) replacement, formation of new bifurcation, autoarterial reconstruction, and ICA autotransplantation [6, 7, 9, 10]. But in some cases, the operating team may have more conservative views on the choice of CEA type, giving preference to the classical technique. In addition, a prosthesis is not always available, and the autovein can be preserved for future coronary revascularization. However, it is known that the arterial surface after endarterectomy is characterized by severe inflammatory process, the risk of parietal thrombosis and a high probability of progression of neointimal hyperplasia, which may cause early loss of the vessel lumen [11]. But there have been no large, randomized studies on this issue, and, as a result, this conclusion can be considered as an opinion rather than a postulate [1]. Thus, the long-term patency of the reconstructed zone after implantation of a long patch in the presence of extended EAP remains unclear. The answer to this question can be obtained only by comparing the long-term outcomes of such operations with the outcomes of traditional classical CEA in local atherosclerotic lesions.

The aim of this study was to analyze the in-hospital and long-term outcomes of classical CEA in extended atherosclerotic lesions in comparison with the outcomes of this intervention in local EAP.

MATERIALS AND METHODS

This cohort, comparative, retrospective, open-label study from January 2010 to December 2020 enrolled 148 patients with extended EAP and hemodynamically significant ICA stenosis. The term "extended" (in view of the absence of definition and gradation of EAP sizes in the current guidelines) was understood as a hemodynamically significant lesion of the common and internal carotid arteries ≥5 cm in length (since the standard patch size does not exceed 5 cm) (Fig. 1). These patients were included in group 1. Group 2 was formed over the same period from 632 patients with hemodynamically significant stenosis <5 cm in length. In both cohorts, classical CEA with diepoxide-treated xenopericardial patch angioplasty of the reconstructed zone was performed. Classical CEA was chosen based on national guidelines, according to which the decision in favor of a particular type of revascularization is made, relying on the preferences and experience of the operating surgeon.

Inclusion criteria were: 1) Indications for CEA according to current guidelines; exclusion criteria were: 1) Contraindications to CEA according to current guidelines; 2) The presence of pathology limiting patient follow-up in the long-term.

Risk stratification of postoperative complications and comorbid background severity were assessed using the EuroSCORE II scale. Severity of coronary atherosclerosis was measured using the SYNTAX Score interactive calculator (www.syntaxscore.com). This calculator provides the following gradation based on severity of lesion: low lesion level (≤22 points), intermediate (23–32) and high (≥33).

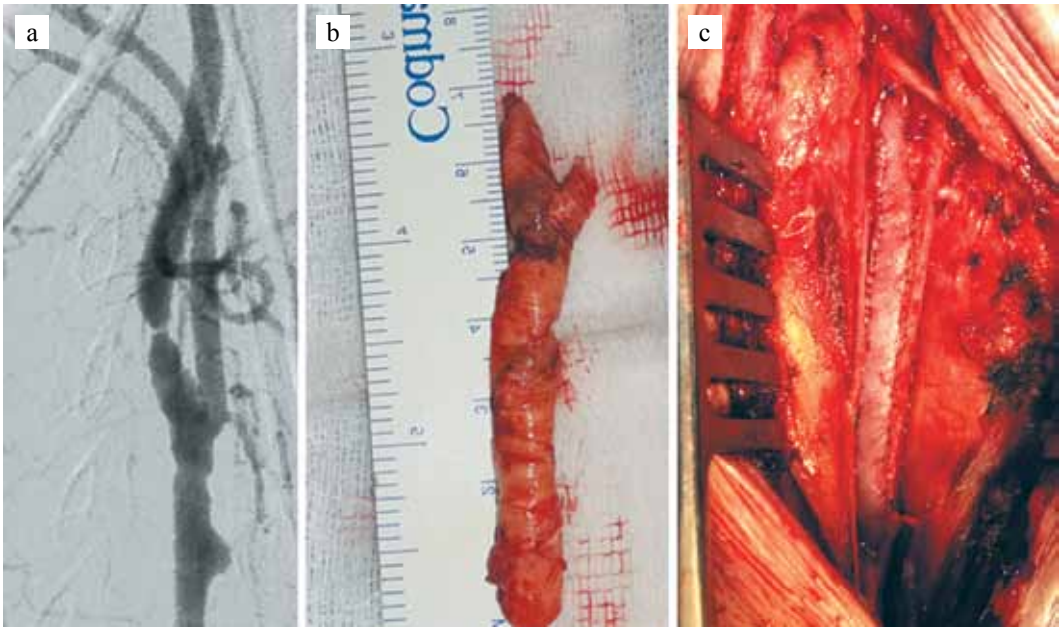


Fig. 1. A clinical example of the presence of an extended atherosclerotic lesion: a) right carotid bifurcation CT angiography (90% ICA stenosis); b) removed atherosclerotic plaque, 7 cm long; c) diepoxide-treated xenopericardial implanted patch

Brachiocephalic atherosclerosis was visualized using transcranial doppler ultrasound (TCDU), color triplex scanning of the brachiocephalic arteries (CTSBA) (using a 7–7.5 MHz linear transducer) on MySono U6-RUS devices (Samsung Electronics), Philips Affiniti 30. Multispiral CT (MSCT) angiography was performed if significant stenosis is detected by CTSBA, there is increased blood flow rate according to TCDU, and there is unstable EAP in the ICA. The degree of stenosis was determined according to the NASCET classification.

The compensatory capabilities of cerebral blood flow during CEA were assessed as follows. When the level of systolic blood pressure (SBP) ≤160 mmHg, the latter was increased pharmacologically to 190–200 mm Hg. Then 5 5,000 IU of heparin were injected intravenously, the arteries were clamped. Invasive measurement of ICA retrograde pressure was performed. When the blood pressure was less than 60% of the systemic blood pressure, a temporary shunt was used.

The control points were understood as the development of unfavorable cardiovascular events like death, myocardial infarction (MI), acute cerebrovascular accident/transient ischemic attack (ACVA/TIA), thrombosis of the reconstructed area, bleeding type 3b and higher on the Bleeding Academic Research Consortium (BARC) scale, composite endpoint (death + ACVA/TIA + hemorrhagic transformation + MI), ICA restenosis, and ICA occlusion. The reconstruction area was visualized using TCDU on day 3 after surgery. Information about the condition of patients in the long-term period was obtained by phone survey and by inviting patients to the clinic for examination by a cardiovascular surgeon, cardiologist, neurologist, control imaging of the reconstructed area using TCDU and, if necessary, MSCT angiography. The long-term follow-up period was 71.4 ± 45.6 months.

The study was performed in accordance with the principles of Good Clinical Practice and Declaration of Helsinki.

Distribution type was determined using the Kolmogorov–Smirnov test. Groups were compared using Mann–Whitney U test and Pearson chi-square with Yates's correction. Survival charts were plotted using the Kaplan–Meier analysis. The graphs were compared using the Logrank test. Differences were assessed as significant at $p < 0.05$. Study results were processed using the Graph Pad Prism software package (www.graphpad.com).

The groups were comparable in all clinical and anamnestic parameters. The overwhelming majority were male and the elderly. One in five had a history of MI and percutaneous coronary intervention (PCI). Multifocal atherosclerosis (MFA) with hemodynamically significant stenoses in three arterial basins was diagnosed in a quarter of cases. In more than half of the cases, stenosis was symptomatic. According to the EuroSCORE II interactive calculator, the severity of the comorbid background in the presented sample corresponded to the average level (Table 1).

RESULTS

Brachiocephalic angiography showed that the groups were comparable in most parameters. The degree of ICA stenosis most often exceeded 80%; unstable EAP was visualized in every fifth patient. Moreover, the extent of lesion was statistically greater in Group 1. The severity

Table 1

Comparative clinical and anamnestic characteristics of patient groups

Indicator	Group 1 (lesion ≥5 cm) n = 148	Group 2 (lesion <5 cm) n = 632	p	OR	95% CI
Age, M ± m, years	65.2 ± 5.3	64.8 ± 5.1	0.35	–	–
Male, n (%)	94 (63.5)	406 (64.2)	0.94	0.96	0.66–1.40
NYHA FC 1–2, n (%)	61 (41.2)	249 (39.4)	0.75	1.07	0.74–1.55
PICS, n (%)	27 (18.2)	117 (18.5)	0.96	0.98	0.61–1.56
COPD, n (%)	4 (2.7)	13 (2.05)	0.86	1.32	0.42–4.11
MFA with hemodynamically significant lesions of three arterial basins, n (%)	36 (24.3)	155 (24.5)	0.95	0.98	0.65–1.50
DM, n (%)	15 (10.1)	71 (11.2)	0.81	0.89	0.49–1.60
CKD, n (%)	5 (3.4)	20 (3.2)	0.89	1.07	0.39–2.90
LVEF, M ± m, %	59.1 ± 3.7	58.8 ± 4.2	0.11	–	–
Left ventricular aneurysm, n (%)	1 (0.7)	3 (0.5)	0.74	1.42	0.14–13.82
EuroSCORE II, M ± m	2.6 ± 0.4	2.5 ± 0.3	0.26	–	–
History of PCI, n (%)	35 (23.6)	147 (23.2)	0.99	1.02	0.67–1.55
History of CABG, n (%)	2 (1.35)	9 (1.4)	0.74	0.94	0.20–4.43
History of stroke/mini stroke, n (%)	89 (60.1)	394 (62.3)	0.68	0.91	0.63–1.31

Note. NYHA FC – New York Heart Association Functional Classification, PICS – Postinfarction cardiosclerosis, DM – diabetes mellitus, AH – arterial hypertension, COPD – chronic obstructive pulmonary disease, CKD – Chronic kidney disease, MFA – multifocal atherosclerosis, LVEF – left ventricular ejection fraction, PCI – percutaneous coronary intervention, CABG – coronary artery bypass grafting.

of coronary bed stenosis, as calculated by the SYNTAX score interactive calculator, was mild (Table 2).

There should be a special focus on perioperative characteristics. Expectedly, in terms of clamping time, group 1 showed the highest values in terms of clamping time (Fig. 2). This indicator in 9 cases exceeded 60 minutes (maximum 73 minutes). In a situation where the clamping time exceeded 50 minutes and it was impossible to start blood flow, we installed a temporary shunt (despite satisfactory retrograde pressure values initially at baseline), which made it possible to avoid the development of intraoperative ischemic stroke. This trend was reflected in the fact that temporary shunt was used statistically more often in Group 1 (Table 2).

The groups did not differ in the incidence of postoperative complications. In Group 1, death occurred on day 9 after operation. The EAP in the ICA spread above the hypoglossal nerve, necessitating transection of the glossopharyngeal plexus to isolate the ICA. The patient had contralateral dysphagia as an outcome of ischemic stroke. Intervention from the ipsilateral side resulted in total dysphagia. In the postoperative period, against the background of inability to swallow food, the patient was inserted with a gastrostomy tube. Nevertheless, on day 9, the patient tried to swallow cooked oat cereal on his own, which resulted in airway obstruction and prolonged asphyxia with cardiac arrest.

The causes of death in Group 2 were: hemispheric ischemic stroke resulting from intimal detachment distal to the reconstructed zone with further ICA thrombosis (1 patient) and hemorrhagic transformation (two patients).

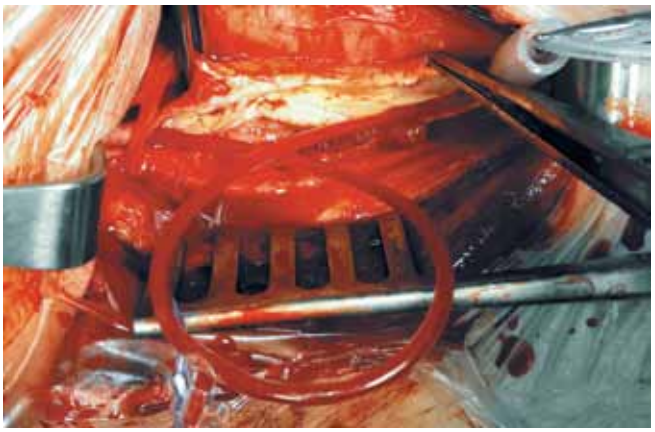


Fig. 2. Installed temporary shunt in a patient with extended atherosclerotic lesion

All intraoperative nonfatal ischemic stroke (n = 3) was observed only in Group 2 in patients who had a temporary shunt installed. Distal embolism was the likely cause. The cause of MI in both groups was stent thrombosis in the coronary artery, which required repeated unplanned revascularization with a successful outcome (Table 3).

In the long-term follow-up period, there were no significant intergroup differences in the incidence of complications. All ACVA/TIAs occurred against the back-

Table 2

Angiographic and perioperative characteristics

Indicator	Group 1 (lesion ≥5 cm)	Group 2 (lesion <5 cm)	p	OR	95% CI
	n = 148	n = 632			
% ICA stenosis	81.3 ± 5.1	82.6 ± 6.3	0.24	–	–
Unstable AP, n (%)	34 (22.9)	148 (23.4)	0.99	0.97	0.63–1.49
AP length, M ± m	7.1 ± 1.2	3.3 ± 1.1	0.001	–	–
SYNTAX score with a history of myocardial revascularization, M ± m	15.1 ± 3.5	16.2 ± 3.2	0.38	–	–
ICA clamping time, min	51.5 ± 6.6	27.0 ± 2.7	0.03	–	–
TS placement	23 (15.5)	57 (9.0)	0.02	1.85	1.10–3.12

Note. ICA – internal carotid artery, ECA – external carotid artery, AP – atherosclerotic plaque, TS – temporary shunt.

Table 3

In-hospital outcomes

Indicator	Group 1 (lesion ≥5 cm)	Group 2 (lesion <5 cm)	p	OR	95% CI
	n = 148	n = 632			
Death, n (%)	1 (0.67)	3 (0.5)	0.74	1.42	0.14–13.6
MI (non-fatal), n (%)	1 (0.67)	3 (0.5)	0.74	1.42	0.14–13.6
Stroke/mini stroke (non-fatal), n (%)	0	3 (0.5)	0.91	0.60	0.03–11.8
BARC type 3b bleeding and higher, n (%)	1 (0.67)	4 (0.63)	0.60	1.06	0.11–9.63
ICA thrombosis, n (%)	0	1 (0.15)	0.42	1.41	0.05–35.0
Combined end point, n (%)	2 (1.35)	9 (1.4)	0.74	0.94	0.20–4.43

Note. MI – Myocardial infarction, ICA – internal carotid artery.

ground of development of hemodynamically significant ICA restenosis 9.5 ± 3.7 months after CEA. In all cases, unplanned cerebral revascularization in the volume of reCEA was performed (group 1: 40%, n = 6; group 2: 36.7%, n = 25; p = 0.95; OR = 1.14; 95% CI = 0.36–3.60) or CAS (group 1: 60%, n = 9; group 2: 63.2%, n = 43; p = 0.95; OR = 0.87; 95% CI = 0.27–2.74) (Table 4).

Analysis of survival charts also revealed no significant intergroup differences for all types of complications (death: p = 0.56; MI: p = 0.73; ACVA/TIA: p = 0.89; ICA restenosis/occlusion: p = 0.82; composite endpoint: p = 0.71). They increased uniformly in both groups (Figs. 3–7). However, more than half of all ICA restenoses and occlusions were visualized in the first six months after CEA. Histological examination after the implemented reCEA showed that neointimal hyperplasia was the cause of lumen loss (Fig. 6).

DISCUSSION

This study has shown that implantation of a long patch in extended atherosclerotic lesions does not exceed the risks of developing restenosis in the reconstructed area relative to classical CEA with local EAP. However, it should be noted that in the long-term period in the whole sample, there was increased incidence of lumen loss, reaching 13.2% (n = 103). And it was precisely this condition that became decisive in the formation of secondary ischemic events in the brain, which, in some cases, led to death. This trend is widely known and has already been discussed many times in large studies and meta-analyzes [12–16]. Several authors have argued that

Table 4

Long-term follow-up complications

Indicator	Group 1 (lesion ≥5 cm) n = 148	Group 2 (lesion <5 cm) n = 632	p	OR	95% CI
Death, n (%)	3 (2.0)	13 (2.05)	0.76	0.98	0.27–3.50
MI (non-fatal), n (%)	4 (2.7)	15 (2.4)	0.95	1.14	0.37–3.49
Stroke/mini stroke (non-fatal), n (%)	8 (5.4)	33 (5.2)	0.90	1.03	0.46–2.29
ICA restenosis >60%, n (%)	15 (10.1)	68 (10.7)	0.94	0.93	0.51–1.68
Repeated unplanned cerebral revascularization (reCEA, CAS), n (%)	15 (10.1)	68 (10.7)	0.94	0.93	0.51–1.68
ICA occlusion, n (%)	4 (2.7)	16 (2.5)	0.86	1.06	0.35–3.24
Total occlusions and restenosis, n (%)	19 (12.8)	84 (13.3)	0.99	0.96	0.56–1.63
Combined end point, n (%)	15 (10.1)	61 (9.6)	0.98	1.05	0.58–1.91

Note. MI – myocardial infarction, ICA – internal carotid artery, reCEA – repeated carotid endarterectomy, CAS – carotid angioplasty and stenting.

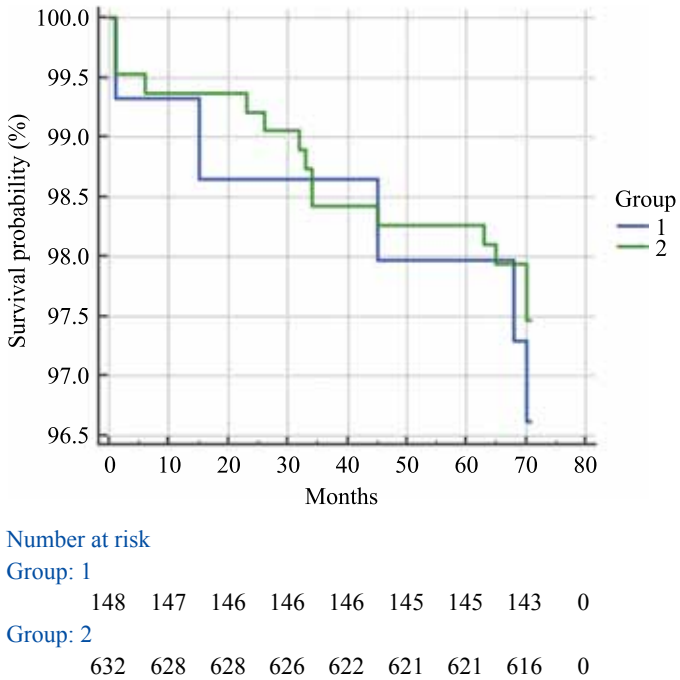
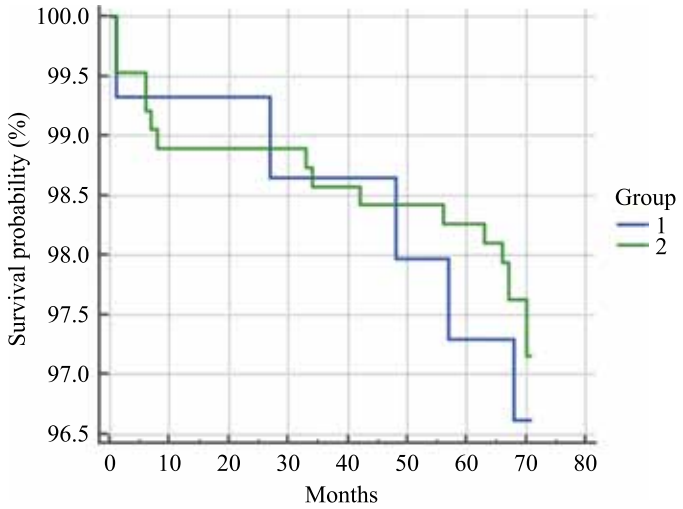


Fig. 3. Death-free survival

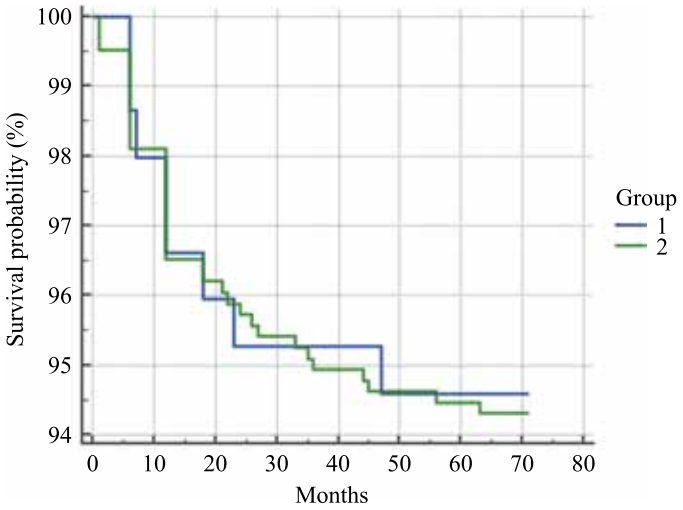
the most likely cause is the distortion of the physical characteristics of blood flow with the formation of stagnation and turbulence zones, which provokes increased neointimal hyperplasia as early as six months after CEA (Fig. 6) [17–19].

Technical errors/errors of primary intervention should also be considered. Bleeding from the anastomotic zone after blood flow had been initiated often requires additional single sutures, which can narrow the arterial lumen [17, 18]. However, according to other authors, another cause of restenosis is implantation of a patch that is too wide, which will lead to increased carotid bulb volume [20]. It is known from the laws of physics that in a situation where the size of the afferent vessel is greater than the total size of the efferent vessels, hydrodynamic resistance increases, conditions for blood stagnation and thrombosis are formed [20]. Such circumstances will undoubtedly lead to restenosis and even occlusion [20]. The use of computer simulation methods can be the way out of the situation [17–19]. So, in the future, in order to



Number at risk
Group: 1
148 147 147 146 146 145 144 143 0
Group: 2
632 625 625 625 623 622 621 614 0

Fig. 4. Myocardial infarction-free survival



Number at risk
Group: 1
148 145 142 141 141 140 140 140 0
Group: 2
632 620 608 603 600 598 597 596 0

Fig. 5. Ischemic stroke-free survival

select a personalized reconstruction technique associated with low risks of arterial lumen loss, a computer model of the carotid bifurcation will be constructed preoperatively for each individual patient [17–19]. Further, thanks to the projection of various CEA methods, as well as virtual implantation of patches of different widths, it will be possible to determine the type of reconstruction that would result in minimal changes in the physical parameters of hemodynamics and would be most preferable for that patient [17–19]. At present, the surgery method is chosen based on the surgeon's preferences, which does not correspond to the principles of personalized medicine [1]. Such actions can be characterized as "at random" because the surgeon does not know the physical changes that would follow after applying this type of reconstruction. Externally, the angioplasty will look perfect, while at the hydrodynamic level, there may be dramatic shifts in flow homeostasis in favor of turbulent nature [17, 18]. Therefore, the surgeon's conservative "restraint" on the most common types of CEA should be eradicated in

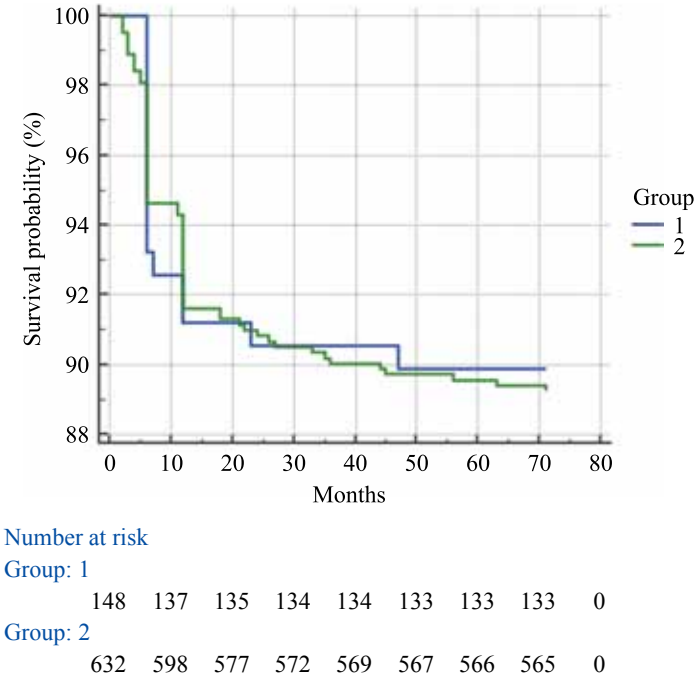


Fig. 6. ICA restenosis/occlusion-free survival

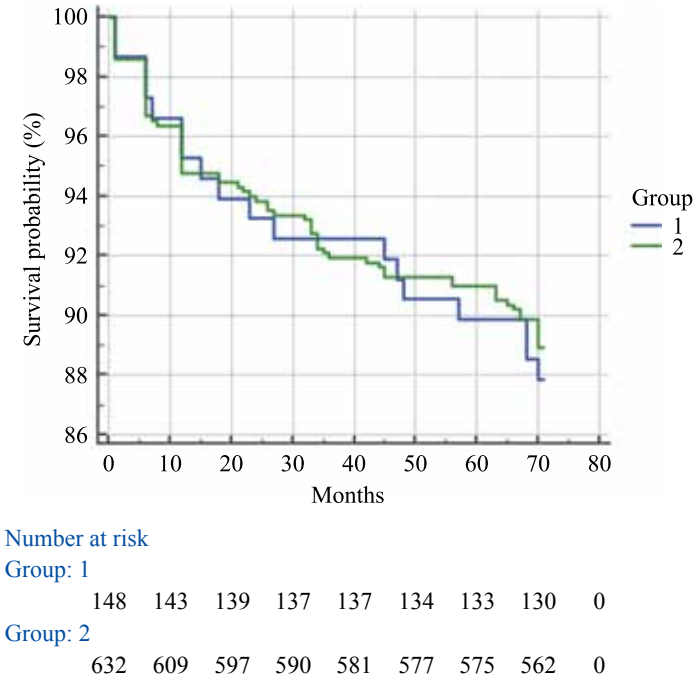


Fig. 7. Combined endpoint-free survival

modern medicine. A vascular surgeon must have a full range of available interventions for precision selection of the most optimal one. In the context of computer-assisted options, such an approach would reduce the incidence of developing vascular lumen loss with an improvement in the quality of life of each individual patient [17, 18]. In addition, it is known that there has always been a human factor and external subjective processes, ranging from troubles in the family to hormonal background, which can affect the psyche of even the most experienced expert, because a surgeon is not a robot. Thus, in the present technical environment, until computer modelling becomes a routine method used in vascular surgery in a personalized manner, the choice of a revascularization strategy should not be based on the preferences of the operating surgeon, as described in the guidelines, but only by a multidisciplinary team meeting.

Geneticists, in turn, add a hereditary component of susceptibility to restenosis [21, 22]. After all, hemodynamics in the reconstruction area changes in everyone, and lumen loss is diagnosed in fact only in one out of every ten persons (Table 4). According to scientists, the presence of some genes and their precision shutdown in the future will allow avoiding activation of conditions for lumen loss, which will lead to optimization of long-term outcomes of classical CEA [21, 22]. However, such tools of influence do not yet exist today.

In summary, it should be emphasized once again that the length of the lesion does not affect the incidence of in-hospital and long-term complications. However, extended plasty objectively requires more time to clamp the arteries. Prolonged cerebral ischemia can lead to intraoperative stroke, which should diminish interest in implantation of a long patch. Current guidelines do not provide maximum safe ICA clamping times. There has been no study on this indicator. The way out of the situation may be the use of cerebral oximetry throughout the CEA. But not all medical institutions have the necessary equipment, limiting themselves, as we do, to retrograde pressure measurement or to Matas test [23–26]. In addition, some authors claim that both retrograde pressure measurement in ICA and cerebral oximetry do not allow to, with high accuracy, determine the cerebral tolerance to ischemia. It has been demonstrated in some studies that intraoperative stroke was still recorded in the presence of optimal parameters under these methods [27–31]. Considering these data, in our practice, if ICA clamping lasted for more than 50 minutes, we interrupted the anastomosis to place a temporary shunt. In our study, this step was not accompanied by ischemic stroke, which emphasized the effectiveness and safety of this procedure. However, if one has the skills to implement other reconstruction types that require less ICA clamping time (ICA prosthetics, formation of a new bifurcation, autoarterial reconstruction, ICA autotransplantation), then one should abandon the classical CEA [6, 7, 10]. In fact, installation of a temporary shunt itself is accompanied by high probability of arterial dissection, distal embolism, ischemic stroke and silent strokes [31–35]. Thus, it is more justified to avoid these risks by considering other possible similar interventions, accompanied by a lower likelihood of adverse cardiovascular events.

CONCLUSION

Implantation of a long patch (≥5 cm) is not characterized by increased incidence of restenosis and all adverse cardiovascular events at in-hospital and long-term follow-up stages. A temporary shunt should be used to prevent development of intraoperative stroke resulting from prolonged ICA clamping. If the surgeon has the skills to perform other types of CEA that are associated with lower risks of complications and restenosis in the long-term follow-up, then the classical CEA should be abandoned, in case of extended atherosclerotic lesion.

The authors declare no conflict of interest.

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THE CONTAMINATION OF HOSPITAL WATER SUPPLY SYSTEMS BY *LEGIONELLA PNEUMOPHILA*

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The risk of severe infectious complications associated with provision of medical care continues to be a pressing issue in modern surgery. *Legionella pneumophila*, characterized by its wide distribution in water supply systems and is highly active in film formation, represents a dangerous/important cause of hospital-acquired pneumonia. Patients requiring immunosuppression, including organ transplant recipients, are in the special risk group. Prevention of hospital-acquired legionellosis in patients at risk is essential due to severe clinical manifestations and high mortality. **Objective:** to summarize the practical experience in detecting contamination of water supply systems by *Legionella pneumophila* strains in multidisciplinary hospitals in Moscow. **Materials and methods.** Isolation of *Legionella pneumophila* strains from water and biofilms of water supply systems in multidisciplinary hospitals in Moscow and serotyping of this pathogen using bacteriological, molecular genetic and enzyme immunoassay methods. **Results.** *Legionella pneumophila* content in water reached high levels. The peculiarities of *Legionella pneumophila* contamination of hot water supply systems included formation of stable biofilms, in which other hospital-acquired pathogens were also identified. The share of *Legionella pneumophila* "SG 1", which causes up to 80% legionellosis cases in the world, was 13% in the water of the hospitals surveyed. The most effective measures for prevention of legionellosis are actions aimed at ensuring water biosecurity. **Conclusion.** There are potential risks of disease in the surgical wards of hospitals providing medical care, including in immunocompromised patients. Due to potential risks, prevention of hospital-acquired legionellosis is a necessary component of ensuring the safety of treatment for immunosuppressed patients.

Keywords: Legionella pneumophila, legionellosis, biofilm, water biosecurity.

Prevention of health care-associated infection (HCAI) is considered an integral component of patient safety during hospital stay. However, if the ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacte*r species) pathogens, as the most aggressive causative agents of severe infectious complications, are well known, it is not sufficient for clinicians to just have a knowledge about *Legionella pneumophila* and legionellosis caused by this pathogen as a hospital-acquired infection.

Currently, the incidence of legionellosis is 1.1 cases per 100,000 of the population in the European Union and 1.62 cases per 100,000 of the population in the United States [1]. The risk group includes patients over 25 years old in hospitals where immunosuppressive therapy is actively used (departments of transplantology, oncology, intensive care, burns, surgical, etc.); patients with diabetes, cardiovascular diseases, respiratory failure; patients whose treatment is accompanied by intubation and lung ventilation; mortality in this group of patients may reach 40–60% [2, 3].

Legionella's high adaptive capacity allows it to colonize artificial water systems; water supply systems and medical equipment serve as reservoirs for the accumulation of the pathogen in cases of hospital-acquired legionella. Unlike planktonic forms, microorganisms that make up biofilms are more resistant to antibiotics and disinfectants [4].

Legionella infection is characterized by the development of multi-organ pathology in patients, high mortality, and absence of specific clinical symptoms, which makes it possible to be differentiated from severe pneumonia caused by other etiological agents [5].

The likelihood of legionellosis cases depends on the level of water contamination with the pathogen, efficiency of aerosols containing Legionella, the rate of spread of the pathogen, as well as the virulence of strains and patient's individual susceptibility. At the same time, the infectious dose of the bacteria required to infect a person cannot be named, since it also depends on the ratio and

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interaction of the listed factors and can vary significantly under specific conditions [6].

All water supply systems are characterized by biofouling processes, or biofilm formation on the inner surfaces of pipes, which leads to secondary microbial contamination of water. In closed water systems, biofilms form on the inner surface of pipes, in shower heads, faucets, and various water filters. The most intensive legionella colonization is shown for rubber and plastic surfaces. However, metal corrosion, which is very common in hot water supply systems with metal pipes, also promotes reproduction of microorganisms to concentrations unusual for natural ecological niches and dangerous for humans [7].

Objective: to summarize the practical experience of identifying contamination of water supply systems by *Legionella pneumophila* strains in multidisciplinary hospitals in Moscow.

MATERIALS AND METHODS

The study was based on the results of bacteriological examination of hot water supply systems in 7 major multidisciplinary hospitals in Moscow; samples were taken in operating rooms, organ transplantation wards, hematology, burns and intensive care units (hereinafter referred to as risk wards).

The applied algorithm for epidemiological assessment of the state of water supply systems consisted of 3 stages:

1) Conducting a preliminary assessment of the epidemiological hazard of the facility. (water screening test using real-time polymerase chain reaction).
2) Assessment of the presence of contamination using a bacteriological method, which determines the exact concentration of the pathogen.
3) Epidemiological hazard assessment based on serotyping of isolated Legionella pneumophila strains.

During sampling, preliminary disinfection of the tap and water drainage were not carried out, which does not contradict the requirements by the National Standard of the Russian Federation "Drinking water. Sampling at water treatment plants and pipeline distribution systems." GOST R 56237-2014.

Samples of water, biofilms, washings from hot water supply systems were investigated in accordance with methodological guidelines MUK 4.2.2217-07 "Detection of *Legionella pneumophila* bacteria in environmental objects" by bacteriological method on BCYE medium using latex agglutination kits SLIDEX (Biomerieux, France), as well as RT-PCR using AMPLI-LEG-RV (CJSC Syntol), a test system for quantitative detection of *Legionella pneumophila*.

Serotype characteristics of the isolated strains were studied using an international panel of monoclonal antibodies by enzyme immunoassay. Monoclonal antibody panel was provided by Dr. J. Helbig and Dr. K. Luck (German Reference Center for Legionellosis, Institute of Medical Microbiology and Hygiene, Technical University, Dresden, Germany) [8].

The effectiveness of the final filtration method and the dynamics of biofilm formation involving *Legionella pneumophila* were evaluated using antimicrobial water filters (PallMedical, UK).

RESULTS AND DISCUSSION

The study examined the peculiarities of *Legionella pneumophila* contamination of hot water systems and identified the factors contributing to it.

Legionella pneumophila culture was isolated in the water distribution systems of all surveyed hospitals, in 14 out 18 surveyed buildings. The percentage of samples in which Legionella was isolated was 41%. The level of Legionella pneumophila contamination in the water supply system varied from 1.2×10^2 to 6.4×10^5 CFU/L. *Legionella pneumophila* concentrations exceeded the risk levels in half of the wards surveyed. Systemic colonization of water distribution networks by Legionella was detected in 4 hospitals (isolated in two or more sections of the water supply system).

In addition, in 9% of the water samples studied in association with *Legionella pneumophila*, microorganisms that are also pathogens of infections associated with health care were isolated: *Acinetobacter spp., Pseudomonas aeruginosa, Brevibacterium vesicularis, Micrococcus luteus*. Species diversity of the isolated microflora indicates that preventive measures taken to ensure water safety are not effective enough.

To determine the epidemiological hazard of potentially dangerous water distribution systems, the serotypic characteristics of the isolated strains of *Legionella pneumophila* were studied. The study confirmed the serotypic diversity of Legionella circulating in hot water systems. The isolated strains belonged to 12 out of 15 *Legionella pneumophila* serotypes. It was found that in 87% of cases, contamination with SG 6 (44%, $p < 0.01$) and SG 5 (26%) serotype strains was detected. The proportion of strains of the first serotype is significantly lower – 13% ($p < 0.01$).

Due to significant contamination of water distribution networks, the epidemiological risk for patients remains significant, because the disease can be caused not only by the Legionella pneumophila serotype SG 1, but also by others. Legionella, which is part of the biofilm, can cause nosocomial *Legionella pneumonia* due to water aspiration by the patient, because the dose of the pathogen capable of causing disease in people with reduced immunity is much lower than for healthy people.

To evaluate the final filtration method, sections of water supply system were selected. A sufficiently high level of contamination with *Legionella pneumophila* serotype SG 1 (more than 10^3 CFU/L of water) was detected.

In the present study, the following pathogen content values in water were taken as reference:

– For non-risk hospital wards, *Legionella pneumophila* level in water was 10^3 CFU\L,
– For risk wards, no *Legionella pneumophila* in water.

For hot water supply systems, a moderate degree of risk occurs when the quantitative level of Legionella in water is 10^3 CFU/L, a high degree of risk occurs at 10^3 CFU/L levels and above. A high degree of risk occurs even in the presence of the pathogen of not only SG 1 *Legionella pneumophila* in water, but also other serotypes and species of Legionella.

The study showed the possibility of completely eliminating *Legionella pneumophila* by means of additional water treatment with 9.9×10^3 CFU/L pathogen levels. There was no additional water protection at the intake points, and the original level of contamination remained. After changing the filter, biofilm samples were examined. Separate microcolonies formed on the outer surface of the filter already on the second day. After a week, biofilm formed on the filter surface. In the biofilm structure, various aquatic microorganisms were isolated, including HCAI pathogens: *Legionella pneumophila, Pseudomonas aeruginosa, Pseudomonas spp., Acinetobacter spp.* and etc.

Analysis of the incidence of legionellosis indicates that the proportion of healthcare-associated legionellosis in different countries ranges from 5 to 20% among all cases of legionella infection. In the United States, health care-associated legionellosis accounts for 23% of all reported legionella infections, with deaths ranging from 9% to 100%. In Italy, pneumonia resulting from legionella is 7.1% of the total number of registered hospital-acquired pneumonia (33.3% mortality in 2008). In the Netherlands, over a ten-year observation period, it was found that 6% of legionellosis cases were associated with transmission in medical institutions [9, 10].

Measures to ensure water safety and prevent legionellosis have become an obligatory component of the prevention of hospital-acquired infections in the United States, Europe, Japan, etc. Prevention is regulated by relevant documents at the national and regional levels aimed at constant monitoring of Legionella levels in water. The effectiveness of these measures is evidenced by the absence of major outbreaks associated with this pathogen. However, foci are periodically recorded in 10–30 cases [6].

Despite species diversity of the *Legionellaceae* family, numbering 50 species, over 90% of cases are caused by the *Legionella pneumophila* species. SG 1 strains cause up to 80% of infections in immunocompromised individuals [11].

External risk factors for the colonization of water systems are: water quality that does not meet the established requirements for both microbiological and chemical indicators; problems in the water distribution system (stagnation and slow flow); piping and tank materials that promote bacterial growth and biofilm formation; insufficient or ineffective water disinfection; water temperature within 25–50 °C; presence of biofilm; aerosol formation; inadequate training of personnel in the maintenance of water distribution systems and prevention of legionellosis [12].

Contamination of medical equipment and instruments associated with intubation and ventilation procedures, surgical intervention, and parenteral nutrition of the patient poses a danger [13]. Inhalation of Legionella-contaminated aqueous aerosol by a patient can occur if breathing devices, ventilation tubing, and nebulizer compartments are rinsed or filled with tap water. Cases of patients being infected during dental procedures and by aspiration during feeding through a nasogastric tube in which enteral feeding mixtures, diluted with water infected with Legionella (SG 6 serotype), were introduced, have been described [14].

In immunocompromised patients, delaying initiation of therapy significantly worsens the prognosis. On average, it takes 8 days from the start of treatment to the response to legionellosis therapy in cancer patients [15]. So, a delay in antibiotic therapy even for a day reduces the effectiveness of treatment; timely diagnosis of legionellosis provides adequate therapy and reduces mortality rates.

Methods of laboratory diagnosis of the infection are standardized on the basis of 3 main methodological approaches: isolation of the pathogen from the detachable lower part of the respiratory tract; identification of a 4-fold or more increase in antibody titers in the serum of patients; determination of soluble legionella antigen in the urine of patients in the acute stage of the disease. Introduction of diagnostic standards in many countries has significantly improved the quality of laboratory diagnosis of infection, which has contributed to increased number of detected epidemic outbreaks and sporadic cases of legionellosis [16].

According to G.M. Galstyan et al., if urine tests are negative for legionellosis in patients with severe pneumonia, then bronchoalveolar lavage examination is necessary to obtain a sufficient number of alveolar macrophages in which legionella reproduce [17, 18].

Disinfection of potentially contaminated water systems and reducing Legionella in them to safe levels is the only real way to prevent legionellosis. The choice of disinfection method is based on analysis of the sensitivity of legionella to various chemical and physical factors. Sodium hypochlorite, monochloramine, chlorine compounds, etc. are usually used for water disinfection [19].

In addition, there is special focus on disinfection and control of medical equipment and instruments in the risk wards, where intensive immunosuppressive therapy is actively used. Operation of medical devices such as a nebulizer is monitored. If the legionellosis pathogen is

isolated from water, additional methods of disinfection of water supply system are used or antibacterial filters are installed at the end points of the water intake in the ward. Restrictions are imposed on patients visiting the shower against the background of severe immunosuppressive therapy; patients who have undergone stem cell or solid organ transplantation are encouraged to use sterile water for brushing teeth, drinking and for rinsing nasogastric tubes; tap water is prohibited in the wards of patients at risk to avoid legionella-contaminated aerosol [20].

Taking into account the need to ensure the safety of water, the Russian Government approved Decree No. 10 dated January 6, 2015 "On the procedure for implementation of production control of the quality and safety of drinking water, hot water". Based on this law, organizations operating water supply systems are required to carry out departmental control of indicators approved by regulatory documents.

Depending on the epidemic situation at the facility, there are three periods of operation of artificial water systems:

– Safe period of operation;
– Risk period when test results indicating epidemically significant levels of Legionella in water are reported;
– Dangerous period when cases of legionellosis associated with colonization of water systems are reported.

In the safe period (in the absence of laboratory-confirmed legionellosis cases), it is necessary to:

– conduct staff training: instructing physicians to be more vigilant about probable cases of healthcare-associated legionellosis, as well as to use reliable diagnostic methods; instructing nursing staff, technical (engineering) staff on measures to prevent hospital-acquired legionellosis.
– control: cases of suspected healthcare-associated legionellosis; laboratory examination (analysis for the detection of antigen in urine, bacteriological analysis of bronchoalveolar lavage) of patients at high risk of infection with suspected legionellosis;
– control over the equipment used for clinical diagnostic laboratory with the necessary diagnostic systems;
– control of operation and disinfection of various medical devices; after cleaning and disinfection of nasal inhalers or other devices, including mechanical ventilation systems, use sterile water to rinse the device; in the absence of sterile water, use filtered water; Use only sterile water to fill the inhaler tank.

In the risk period, it is necessary to take measures provided for by the algorithm of actions during the safe period: to install special water filters in risk wards; to test water for legionella in risk wards within a year (monthly), or within 3 months in other wards.

In a danger period, it is necessary: to register cases of legionellosis or suspicions of legionellosis according to established procedure; exclude the use of potentially colonized water systems by patients from risk wards and risk groups; discuss the results of the investigation of the hotbed of the disease at the commission for the prevention of nosocomial infections; conduct a retrospective epidemiological investigation by analyzing the microbiological, serological and autopsy results to identify possible previous cases.

Thus, general hygienic and disinfection measures can significantly reduce the colonization of water supply systems, while the assessment of the initial level of contamination contributes to determining the most effective strategy for preventive measures. The most effective measures from the standpoint of preventing legionella infection are those aimed at ensuring the biological safety of water.

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POSTOPERATIVE PERICARDIAL EFFUSION: PECULIARITIES OF THE DEVELOPMENT AND COURSE

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Heart transplantation continues to be the gold standard treatment for end-stage chronic heart failure. As with any cardiac surgery, heart transplantation is associated with postoperative complications. One of the most common complications is postoperative pericardial effusion. Heart recipients have a greater risk of developing pericardial effusion than patients after cardiac surgery on their own heart, due to surgical and immunological features. Severe pericardial effusions negatively affect the postoperative period and may be the cause of life-threatening conditions. Identification of risk factors, prevention, early diagnosis and treatment of this disease can significantly reduce the risks of adverse events in this group of patients. The purpose of this literature review is to analyze the development and course of pericardial effusion in heart recipients in world practice.

Keywords: heart transplant, pericardial effusion.

INTRODUCTION

Heart transplantation (HT) remains the only definitive treatment for end-stage chronic heart failure. The International Society for Heart and Lung Transplantation estimates that about 5000 heart transplants are performed annually in the world, and the number of these operations is steadily growing [1]. Russia has also witnessed a significant increase in the number of HT surgeries due to an emerging new donor and recipient selection approach, and improvements in patient management techniques [2]. In recent years, about 300 heart transplants are performed in Russia every year. The Shumakov National Medical Research Centre of Transplantology and Artificial Organs occupies a leading position among transplant centres in the world in terms of number of surgeries performed. Along with increased number of interventions, the number of perioperative complications is also growing. The most severe of them are graft rejection, graft coronary artery disease, heart rhythm disturbances, renal dysfunction, malignant tumors and infectious complications [3]. The attention of clinicians is primarily focused on these problems because they lead to significant deterioration in prognosis after surgery and in the long-term period. However, apart from the main group of complications, there are conditions that also have a high incidence, can lead to life-threatening consequences, and worsen long-term prognosis. One of such complications is pericardial effusion. This complication is typical both for patients after cardiac surgery on their own heart and for HT recipients. In the latter, the incidence of effusion is significantly higher due to different immunological and surgical components [4]. Unfortunately, to date it is impossible to unequivocally identify the causes and mechanisms of the development of this condition due to the multifactorial etiology of the process. Further study of risk factors, identification of possibilities of prevention, early diagnosis and treatment options are all necessary for prevention of adverse events in this cohort of patients.

PERICARDIAL EFFUSION AFTER CARDIAC SURGERY

Pericardial effusion is a common early postoperative complication in patients after heart surgery [4–8]. This complication is the buildup of significant amount of fluid in the space around the heart, which can affect the patient's hemodynamic indicators. The effusion can be idiopathic or result from local or systemic inflammatory reactions [6]. This complication typically manifests itself in the early postoperative period and regresses after 7–10 days. In some cases, it can persist, leading to tamponade [9]. According to most sources, the incidence of clinically significant effusion varies from 1.5 to 25%, depending on the study design and focus [4–8]. The most common causes of this condition are postcardiotomy syndrome, increased bleeding amidst anticoagulant and/or antiplatelet therapy, and lysis of pre-formed clots. Risk factors include prolonged cardiopulmonary bypass time, hypertension, renal failure, increased body surface area, young age, immunosuppression, surgery type and urgency

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[4, 8]. Pericardial effusion is usually classified according to the rate of increase in size, distribution, influence on hemodynamics and composition. The main characteristic of postoperative effusion remains its volume and distance between the parietal and visceral layers during diastole. There are mild (<10 mm), moderate (10–20 mm) and large (>20 mm) pericardial effusions [10]. The classic clinical manifestations of large effusion include the Beck's triad, described as early as 1935 [11]. It involves hypotension, increased pressure in the jugular veins, and muffled heart sounds. However, the manifestation of this triad is typical for acute, "surgical" tamponade, associated with a sharp increase in pressure in the pericardial cavity and cardiac chamber compression, which is often due to surgical complications. Tamponade appearing in the first hours after surgery is usually associated with pericardial hemorrhage, which requires repeated surgical intervention [9]. Acute symptoms also include tachycardia, severe general weakness, shortness of breath on exertion, and chest pain. Local compression symptoms such as nausea, dysphagia, hoarseness, and hiccups may appear. Nonspecific symptoms include cough, lack of appetite, and palpitations [12–14]. Fever is a nonspecific symptom that may be associated with local or systemic inflammation [15]. Pericardial friction murmur is mainly found in patients with concomitant pericarditis [16]. In most cases, the symptoms of pericardial effusion are nonspecific and there are not always classical clinical manifestations in the early stages of the process due to no cardiac chamber compression and compensation of pericardial pressure resulting from pericardial distension. For example, according to E.A. Ashikhmina et al. [4], only 42% of postoperative pericardial effusions are accompanied by hemodynamic changes, and according to P. Meurin et al. [17], in 22%, this complication is asymptomatic within 2 weeks after intervention. In this regard, early instrumental diagnostics is extremely important. Echocardiography is used as the primary diagnostic imaging, volume assessment, and hemodynamic impact. Semi-quantitative assessment of pericardial effusion consists of measuring the rim of fluid between the parietal and visceral pericardial layers by 2D echocardiography [18]. More often, effusion is defined as an echo-negative space, less often there can be adhesions, fibrin threads, or echo-positive clots, which are a sign of active or completed bleeding [19]. An important assessment criterion is to determine the localization of the effusion, including for selecting further surgical tactics. When hemodynamically significant effusion develops, EchoCG may show symptoms such as collapse of various parts of the heart, due to increased pericardial pressure, and inferior vena cava dilation, due to increased venous pressure. Collapse usually occurs at the end of diastole, primarily affecting the right heart, and is a highly sensitive and specific sign of tamponade. Inferior vena cava plethora is manifested by a <50% decrease in its diameter during full inspiration and is a highly sensitive, but nonspecific sign of tamponade [20, 21]. Although EchoCG remains the primary diagnostic tool for detecting or confirming pericardial effusion, CT and MRI should be used when echocardiographic findings are difficult to interpret or there is suspicion of localized or hemorrhagic effusion in the pericardium or thickening. CT and MRI are also used to qualitatively characterize pericardial masses detected by echocardiography [22].

One of the main objectives of modern research is to identify risk factors and predictors of pericardial effusion. According to M. Pepi et al. [5], pericardial effusion is a common complication after cardiac surgery, its frequency and nature depend on the type of intervention; oral anticoagulants are an additional risk factor for cardiac tamponade. The study was conducted on 803 patients. Most of them underwent coronary artery bypass grafting (CABG) surgery or valve replacement. Pericardial effusion was detected in 498 (64%) patients. Moderate or large effusion was found in 30 patients (3.84%). Effusion led to cardiac tamponade in 15 of them (12 took oral anticoagulants). Effusion was more often associated with CABG (75%) than with valve replacement (52%).

Unlike M. Pepi et al., researchers from the Mayo Clinic, Rochester, Minnesota [4] in their retrospective study found that all surgical interventions had a greater risk of pericardial effusion than CABG, and that heart transplantation was considered a separate risk factor for effusions. It has also been reliably proven that previous cardiac operations were associated with lower risk of effusion. The study included 21,416 patients who underwent cardiac surgery. Of these patients, 327 (1.5%) showed signs of moderate or large pericardial effusion. Classic clinical manifestations were detected only in 136 of them, and 280 had nonspecific symptoms. Independent risk factors for effusion were larger body surface area, pulmonary thromboembolism, hypertension, immunosuppression, renal failure, urgency of operation, and prolonged cardiopulmonary bypass.

M. Khassawneh et al. [6] in their study detected postoperative pericardial effusion in 235 (85%) of 335 patients. It was classified as moderate in 70 patients, and large in 15. The researchers also found that small pericardial effusions were more frequent after CABG, while moderate and large effusions were typical for patients after valve replacement. L.B. Weitzman et al. [7] studied 122 consecutive patients after cardiac surgery. One hundred and three (84%) patients had pericardial effusions after surgery. Both studies have similar conclusions that pericardial effusion is a common complication of cardiac surgery; however, most of them regress and do not cause associated complications. The researchers are convinced that patients with effusion do not require prolonged in-hospital follow-up. However,

all patients with previously identified signs of pericardial effusion require outpatient follow-up.

N.K. Khan et al. [8] analyzed the data of 1308 patients within 6 months after surgery for the presence of clinically significant pericardial effusion. The study found that 81 (6.2%) patients had clinically significant pericardial effusion, which required surgical intervention in the late postoperative period (8–87 postoperative days). Haemodynamic instability was present in 34.6% and signs of cardiac chamber compression in 54.3%. The independent risk factors in multivariable analysis were correction of valvular defects, young age and high hemoglobin levels were independent risk factors. Age 60–69 years was associated with lower risk of complications. Results from the above studies confirm the urgency of the problem of pericardial effusion after cardiac surgery and the effect of this complication in the postoperative period.

POSTPERICARDIOTOMY SYNDROME AFTER CARDIAC SURGERY

Touching upon such a topic as postoperative pericardial effusion, one cannot but mention such complication as postpericardiotomy syndrome (PPS). This complication is one of the most common in cardiac surgery [23]. PPS is the development of a systemic inflammatory response, manifested by increased body temperature, chest pain, pleural and pericardial effusions, pericardial thickening, increased C-reactive protein (CRP), pleural and pericardial friction rub. The most dangerous complications of this syndrome are tamponade and constrictive pericarditis [25]. M. Imazio et al. [26] conducted a study involving 360 patients after cardiac surgery. It was found that PPS occurs in 15% of patients during the first 3 months after the operation, 89% had pericardial effusion during the syndrome. Younger patients are more likely to develop the syndrome. J. Lehto et al. [27] found that PPS occurs more often after valve replacement than in CABG. Patients with PPS have a higher mortality rate within the first year of surgery. The primary cause of PPS is thought to be an autoimmune inflammatory response to pericardiotomy and intraoperative mechanical exposure. Unfortunately, there are yet no studies comparing the incidence of the syndrome in patients after interventions on their own heart and in heart transplant recipients. However, it is believed that heart recipients are less susceptible to this syndrome due to suppression of autoimmune factors [28]. U. Sevuk et al. [29] found that intraoperative use of methylprednisolone at a 1 mg/kg dose leads to a lower number of PPS and pericardial effusions, but the severity of effusions was greater in the group receiving methylprednisolone. The study included 200 patients after CABG, 100 of whom received methylprednisolone. A.K. Cabalka et al. [28] who studied 15 patients after heart transplantation at the age of 1 to 17 years, found that PPS was a frequent complication in this group of patients, despite ongoing immunosuppressive therapy. This complication was partially associated with cell-mediated mechanisms, as evidenced by changes in the expression of lymphocyte activation markers. Therefore, the issue of PPS development and incidence in heart transplant recipients requires further study.

PERICARDIAL EFFUSION IN HEART TRANSPLANT RECIPIENTS

The first mention of post HT-transplant pericardial effusion was described back in 1968 by Christian Barnard [30]. Unfortunately, there is still no clear understanding of the pathogenetic mechanisms of this condition due to the multifactorial nature of the process. Most pericardial effusions are known to develop in the first 3 months after HT [31–33], and their incidence in this group of patients is significantly higher than in patients after cardiac surgery on their own heart [4]. According to most sources, incidence of clinically significant effusion in patients after HT varies from 6 to 35% [31–37]. Hemodynamically significant effusions are typically characterized by moderate to large volume and are exudative contents. A peculiarity in the development of this condition in transplant recipients is the influence of several additional factors that are not encountered in patients who undergo interventions on their own heart. Various reports have shown that the occurrence and course of pericardial effusion is influenced by immunosuppressive therapy, anthropometric data of donor-recipient pair, previous cardiac surgery, use of aminocaproic acid during surgery, graft ischemia time, and graft rejection. However, data on these factors vary and there is currently no consensus regarding the main causes of pericardial effusion in patients after HT [31–37].

H.A. Valantine et al. [32] were among the first to conduct a retrospective study with a large sample, addressing the issue of pericardial effusion in patients after H.T. During 1 year, 12 of their transplant population (total, 189) developed moderate or large pericardial effusions. These effusions occurred within 1 month of transplantation in 10 patients and at 3 months and 4.5 years in the other two. Pericardiocentesis was performed because of clinical evidence of increasing effusions in 8 patients. One of the main objectives of the study was to identify the correlation between the occurrence of acute cellular rejection and development of pericardial effusion. Endomyocardial biopsy revealed moderate or severe cellular rejection in 11 out of the 12 patients as the pericardial effusion progressed. Moreover, before the manifestation of pericardial effusion, only 2 out of 12 patients had episodes of moderate rejection. These studies suggest a relationship between the development of moderate to large pericardial effusion and cardiac

transplant rejection. The clinical course and autopsy results in heart transplant recipients indicate a difference in the etiology and prognosis of pericardial effusions in this group of patients relative to patients after cardiac surgery on their own heart. G.R. Ciliberto et al. [35] also found a significant correlation between the severity of acute rejection episodes and pericardial effusion. Pericardial effusions were significantly more frequent in the group of patients with the highest frequency, duration and severity of acute rejection episodes. The study included data from 150 post-HT patients with a 1-year follow-up.

B.F. Vandenberg et al. [31] could not find a correlation between pericardial effusions after transplantation and rejection. In their study, which included 38 patients, the presence of pericardial effusion in patients after their transplantation did not demonstrate independent correlation with chest tube output after operation, cyclosporine therapy, level of blood urea nitrogen, infection, or preoperative diagnosis of idiopathic dilated cardiomyopathy. However, a combination of three factors, namely, cyclosporine therapy, acute rejection, and a preoperative diagnosis of idiopathic dilated cardiomyopathy, yielded an 86% probability of having pericardial effusion. Pericardial effusion was documented in 15 of 38 patients. Moreover, effusion volume was moderate or large in 8 patients. In 60% of patients, there was no evidence of effusion. As described by the authors, the reason for the differing data on correlation between pericardial effusion and acute rejection may be down to different research methodology.

An important factor in the study of pathology is to identify the predictors influencing further development or progression of complications. J.A. Quin et al. [33] studied the influence of 90 different perioperative factors on the development of pericardial effusions. The study included 241 HT recipients. Forty-two patients had moderate or large pericardial effusion develop, and 19 of these patients required drainage. When drainage was required, it was achieved by placement of a subxiphoid pericardiostomy tube. Pericardial effusions were significantly less likely to occur in recipients with a history of previous cardiac surgery. Patients with idiopathic dilated cardiomyopathy, younger patients with lower BMI and high central venous pressure, had a greater risk of complications. The use of hearts from female donors was associated with significant effusion in the postoperative period. Intraoperative administration of aminocaproic acid increased the likelihood of effusion approximately 6-fold. No correlation was found between acute rejection and development of pericardial effusion. Pericardial effusion was detected in 11 (26%) of 42 patients with rejection and 34 (21%) of 161 patients without graft rejection. No graft rejection was detected within 5 years after surgery in 73 ± 7% and 77 ± 3% patients with and without pericardial effusions, respectively.

P.J. Hauptman et al. [34] studied the experience of 203 heart transplants for the presence of pericardial effusion. According to the study, 18 (8.9%) of the 203 transplant recipients developed moderate to large pericardial effusions. Eight patients required pericardiocentesis, and 5 of them subsequently required pericardiectomy in connection with recurrent effusion. None of the 18 patients with significant effusions had a history of previous cardiac surgery. No postoperative pericardial effusion was revealed in 67 patients with previous intervention. In addition to the above factors, the ratio of the recipient's weight to the donor's weight was considered. It was found that in the group of patients with developed effusions, the recipient's weight on average exceeded the donor's weight by 11.9 ± 4.1 kg, while in the group of patients without this complication, the recipient's average weight exceeded the donor's weight by 2.2 ± 1.1 kg. The combination of a significantly greater recipient weight and the absence of previous cardiac surgery predisposed to the development of effusions in 83% of cases. There was no significant difference in the incidence of rejection in patients with and without pericardial effusion. Signs of rejection were found in 6 out of 18 patients with effusion. Factors such as graft ischemic time, cardiopulmonary bypass time, recipient heart size, preoperative use of mechanical circulatory support, postoperative use of anticoagulants, age, sex, and status (according to the United Network for Organ Sharing classification) of patients were not statistically significant factors in the development of pericardial effusion.

One of the most recent retrospective studies on pericardial effusions in heart transplant patients is a work by A.S. Al-Dadah et al. [37]. The study included 91 consecutive patients who underwent orthotopic heart transplantation. A total of 31 (35%) patients developed moderate to large effusions. Only 3 patients with large effusions required drainage; in all other cases the process regressed within 3 months. The only significant factor correlating with effusion was the longer graft ischemic time, which was 180 ± 59 min in the group of patients with significant effusion. According to the authors, a possible mechanism that would implicate the cause of effusion would most likely involve ischemia-reperfusion injury of the graft that would ultimately affect or involve the recipient pericardium. The authors also believe that whatever the etiology of these effusions, they tend to regress within 3 months of surgery. Between 2008 and 2012, Z. Yu et al. [36] evaluated 292 patients within the first 6 months post HT for the development of effusion. In this study, 33 (11.3%) patients developed moderate pericardial effusion. The average time to detection of pericardial effusion was 22.4 ± 18.4 postoperative days. In follow up, 78.8% had resolution of the pericardial effusion, 9.1% had no change in terms of volume and nature of the effusion at 1 year follow up, and 12.1%

had worsening of pericardial effusion requiring surgical intervention. All patients were given a trial of diuretics to reduce the effect of the pericardial effusion prior to intervention and were initiated with tacrolimus and mycophenolate as immunosuppression.

S.F. Stämpfli et al. [38] conducted a study of pericardial effusions in the long-term period after surgery. Hemodynamically irrelevant pericardial effusion unrelated to surgery was found to be a predictor of adverse outcome. Effusions detected during the first year were not included in the study; median follow-up period was 11.9 years. Of 152 patients, 25 developed pericardial effusion. The risk of death and re-hospitalization was 2.5 times higher in the group of patients with effusion than in the group without it.

Prevention of complications is certainly an important factor. With regard to pericardial effusions after transplantation, one way could be the use of prolonged drainage of the postoperative wound using a soft drain. Yun Seok Kim et al. [40] enrolled 250 patients who underwent heart transplantation between July 1999 and April 2012. They received two conventional tubes (n = 96) or two tubes with a soft drain (n = 154). At 1 month after transplantation, 69 patients (27.6%) developed significant pericardial effusion. Among these, 13 patients required surgical intervention. On postoperative day 77, only one patient with the use of a soft drain had pericardial effusion, which required pericardial drainage. According to multivariate analysis, history of previous cardiac surgery and placement of a soft drain were significant factors that prevented pericardial effusion in the postoperative period. However, the average time of prolonged drainage of the postoperative wound with the help of soft drain was 15.6 ± 6.2 days, which may affect the patient's stay in the hospital and development of postoperative wound infection, although there was no increase in these factors in this study.

Some researchers have identified an increased risk of pericardial effusion in the presence of cyclosporin A in an immunosuppressive regimen [39]. To date, this risk factor has no prognostic value since the vast majority of cases no longer use cyclosporine as a basic immunosuppressant. At present, there are no large studies examining the separate effect of modern immunosuppressive drugs on the development of pericardial effusion after heart transplantation. However, some authors point out that the use of immunosuppressants is a risk factor for postoperative effusion [4].

KEY ASPECTS OF THE TREATMENT OF POSTOPERATIVE PERICARDIAL EFFUSION

Pericardial effusions after cardiac surgery often do not manifest clinically; they are detected only on control EchoCG. Therefore, early diagnosis is extremely important and can be of key importance in the further course of this complication. In patients at risk of this complication, such as cardiac transplant recipients, a protocol for routine instrumental examination methods should be established for early and subsequent diagnosis. The treatment strategy is based on the clinical course and EchoCG picture. Moderate effusion is not an indication for surgical intervention; it requires further careful monitoring [36]. Anticoagulant and antiplatelet therapy should be adjusted, diuretic, anti-inflammatory therapy should be prescribed, if there are signs of an inflammatory process. According to the POPE study [41], non-steroidal anti-inflammatory drugs are ineffective in the treatment of moderate to severe pericardial effusions, and should not be prescribed if there are no signs of active inflammatory process due to possible side effects. Colchicine has long been included in the treatment regimen for pericardial effusion, but the POPE 2 [42] demonstrated no effect from the drug. In some cases, the clinical picture develops with the manifestation of classic signs, such as cervical vein distension, tachycardia, weakened heart tones on auscultation, and increased central venous pressure. Manifestation of these signs indicates a fulminant course of the complication and requires immediate action. For hemodynamically significant effusions leading to tamponade, surgical intervention remains the only possible solution. Ultrasound-guided pericardiocentesis is the preferred method, but it cannot always be performed due to inaccessible anatomical location of the fluid (posterior, lateral surface of the heart) or if there is insufficient distance between the pericardial layers due to increased risk of myocardial injury. The undeniable advantage of pericardiocentesis is the minimal invasiveness of the method. In the early postoperative period, the simplest method is to drain the pericardial cavity from the subxiphoid access by separating the previously applied sutures. This manipulation is easy to perform and allows evacuating pericardial effusion of any location in most cases, although it is a more traumatic procedure than pericardiocentesis [43]. To date, there are no clear criteria for choosing a particular surgical tactics for fluid evacuation in pericardial effusions. However, in most of the studies cited, the minimally invasive approach was used more often.

CONCLUSION

Pericardial effusion is one of the most common early postoperative complications in patients after cardiac surgery. The incidence of this complication is significantly higher in heart transplant recipients, although its etiology remains unclear. In the early stages of effusion development, there may be no obvious clinical signs of complication, so timely diagnosis is important. Large pericardial effusions can lead to tamponade and the only treatment for such conditions is emergency surgery. According to various sources, the risk factors

for pericardial effusion in heart transplant recipients are immunosuppressive therapy, initial diagnosis of dilated cardiomyopathy, large anthropometric parameters of the recipient, no previous cardiac surgery, acute heart transplant rejection, and longer graft ischemia time. Most authors agree that patients with identified pericardial effusion require close monitoring. Even hemodynamically insignificant effusion can be a predictor of adverse outcome. It is obvious that identification of risk factors, prevention, early diagnosis, and treatment of this condition can significantly improve the postoperative period and reduce the risks of adverse events in this patient cohort. Further study of pericardial effusions in heart recipients and the development of a clinical diagnostic protocol is a crucial task, which, if addressed, would improve outcomes in modern cardiac transplantation.

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BRONCHIAL COMPLICATIONS AFTER LUNG TRANSPLANTATION

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Bronchial complications are among the main causes of impairing postoperative period and thansplant failure. Severe bronchial complications are very rare but have a high mortality rate. Light forms decrease transplant function and while progressing can leads to life-threatening conditions without required treatment. Nowadays there is a huge necessity in classification of diagnostic and bronchial complications treatment on different terms after lung transplantation. Methods of observation bronchoscopy and interventional bronchology are allowing us to realize prevention, diagnostic and treatment bronchial complications.

Keywords: lung transplantation, bronchial arteries, anastomotic dehiscence, bronchial stenosis, bronchomalacia, bronchial stenting, interventional bronchology.

The development and evolution history of approaches to the treating bronchial complications in donor lung recipients has been quite dramatic. The first lung graft was made by J.D. Hardy et al., in 1963 in a 58-year-old patient with central left lung cancer. The patient's condition was complicated by obstructive pneumonia resulted from tumor occlusion of the left main bronchus, emphysema of the lungs and chronic glomerulonephritis. Considering the respiratory failure events against the background of expressed emphysematous changes, the only possible method of treatment was an attempt to transplant the left lung.

On June 11, 1963, a one-lung graft from a cadaver donor was performed. The immunosuppression protocol included azothioprine, prednisone and mediastinal irradiation. The course of the postoperative period was complicated by the failure of the bronchial anastomosis, which required continuous active aspiration from the left pleural cavity. The patient died on the 18th day after transplantation of infectious complications and renal failure. Autopsy of the left pleural cavity revealed limited empyema and bronchial anastomotic dehiscence in the area of the membranous wall of up to 5 millimeters long [1].

In 1971, C. Bernard et al. performed the third in history transplantation of the cardiopulmonary complex. The patient died on the 23rd day of sepsis against the background of pleural empyema developed as a result of tracheal anastomosis leak [2].

In 1978, J.D. Cooper et al. performed the first of the transplant under extracorporeal membrane oxygenation. The patient died on the 18th day. Autopsy revealed circular necrosis of the graft bronchus of up to 2 cm long [3].

The period from 1963 to 1978 was featured by sporadic reports of transplants performed. Of 38 observations, only 9 patients survived for over 2 weeks after surgery, and only one patient was discharged from the clinic. In the overwhelming majority of cases, adverse outcomes were caused by the failure of the bronchial or tracheal anastomoses [4].

A number of scientific studies and experiments revealed the negative effect of azathioprine and high doses of glucocorticoids on the repair processes in the area of bronchial anastomosis [5]. The introduction of Cyclosporin A (Sandoz Pharmaceutical Company) into clinical practice has significantly improved the immediate outcomes of LT, such as by reducing the rate of bronchial anastomoses leakage [6]. Epiplopexy of bronchial anastomosis and the use of other plastic materials (intercostal muscles, pericardium) to cover it and enhance neoangiogenesis were also proposed as a possible solution to the problem [7]. Revascularization of the bronchial arteries of a pulmonary graft has significantly reduced the incidence of early bronchial complications, but to date this method has not become widespread due to its technical complexity [8].

A lower incidence of bronchial complications during transplantation of the cardiopulmonary complex was established when compared to isolated lung transplantation. That can be explained not only by the preservation of blood supply through the bronchial arteries, but also by the reduction in the time of pulmonary ischemia in the composition of the cardiopulmonary transplant. On the basis of these regularities, the domino transplant was taken as a solution to the problem of bronchial anastomotic dehiscence. The technique consisted in transplanting the cardiopulmonary complex to a patient in need of isolated

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LT, while the heart of the first recipient, under safety conditions, was transplanted to patient No. 2 [9, 10].

Bilateral en-block lung transplantation with the tracheal anastomosis formation was accompanied by an unacceptably high leakage rate, which was the reason for the technique to be widely rejected[11].

Progress in surgical technique, preservation of donor organs, approaches to drug immunosuppression significantly improved both immediate and long-term outcomes of lung transplantation, which made it possible to reduce the rate of bronchial complications from 60–80% in the first year after surgery to 2–18%, while lowering mortality to 2–4% [12–14]. Nevertheless, bronchial complications in donor recipients remain one of the main reasons for the development of adverse outcomes at different times after lung transplantation.

ETIOLOGY OF BRONCHIAL COMPLICATIONS

The bronchial tree is known to receive blood supply through the bronchial arteries starting from the aorta or intercostal arteries and, to a lesser extent, collateral blood supply at the level of the submucosa from the bed of the pulmonary artery. One of the main reasons for the development of bronchial complications (BCs) both in the early postoperative period and in the long term after transplantation is the bronchial arteries transection at the graft removal. The current, generally accepted surgical technique does not imply their routine reconstruction, as a result of which the blood supply to the tissues of the respiratory tract continues to be carried out only by venous blood from the bed of the pulmonary artery through the collaterals of the submucosal layer, which leads to the development of ischemia and dystrophic processes, both in the area of the anastomosis and throughout the bronchial tree. Revascularization of the bronchial arteries of a pulmonary graft can be considered as a method of BC prevention in the early postoperative period [15], but the role of this technique in the prevention of late BC has not been reliably confirmed [16].

The maximum shortening of the stump of the main bronchus of the graft to 1–2 cartilaginous semi-rings from the upper lobe bronchus spur was found to reduce the rate of bronchial anastomosis leakage from 11.1% to 2.6%. In addition, the importance of smooth dissection of the graft root area and minimal skeletonization of the bronchial stump to preserve lymphoid, pericardial tissue and tissue used as plastic material for covering the suture line, preserving microcirculation, and collateral blood supply to tissues in the area of bronchial anastomosis has been proven [17].

Effective collateral blood supply to the bronchial tree of the graft is restored within 2–4 weeks [8]. A number of factors have been established that affect these processes, and therefore are capable of aggravating the phenomena of ischemia and the risks of developing bronchial complications. These factors include:

- Donor lung conservation technique.
- Ischemic-reperfusion injury of a lung graft.
- Primary lung graft dysfunction.
- Graft rejection.
- Infectious and inflammatory changes.
- Prolonged mechanical ventilation with high PEEP values.

It has been proven that the donor lungs preservation in dextran solutions with low Ca concentration (at the rate of 60 ml/kg) with the addition of prostaglandin E to the perfusate, during ante- and retrograde perfusion, allows not only to prolong the length of safe storage (conservation) of donor organs to 12–14 hours and according to some reports and in the experiment, up to 22–25 hours, but also to reduce the BC rate [18].

On the other hand, there are conflicting opinions on the absence of significant differences in the BC rates depending on the duration of ischemia of a pulmonary graft [19], as well as about the greater likelihood of developing BC from the side of the graft implanted in the second place in two-lung transplantation [20].

Ischemic attacks, inflammatory changes and acute rejection events lead to edema of the mucous and submucous layer of the respiratory tract, entailing an increase in vascular resistance at the microcirculatory level. Ischemic attacks can increase, resulting from the reduction in pulmonary blood flow against a background of hypotension, a decrease in cardiac output and the use of vasopressors [21].

Primary dysfunction of the lung graft expressed as an interstitial edema, damage to the alveolar-capillary barrier, shunting and reduction of pulmonary blood flow, aggravates ischemia of bronchial tissues. Primary dysfunction necessitates prolonged mechanical ventilation, often severe (high PEEP), which creates additional risks of BC development [22].

Acute rejection events lead to damage to the alveolar epithelium, vascular endothelium, which subsequently increases the risk of developing bronchial stenosis during the first year after transplantation [23].

At the early stages of the lung transplantation development, there was a generally accepted belief in the negative effect of high doses of corticosteroids on the repair processes in the area of the bronchial anastomosis, and the use of corticosteroids before transplantation was considered as contraindications to surgery [24]. To date, the opinion of the majority of researchers is represented by diametrically opposite views, noting positive effects in the form of a decrease in the rate and intensity of the formation of endobronchial granulation tissue and decreased risk of developing rejection [12, 25].

The effects of proliferative signal inhibitors (Everolimus) expressed in endothelial, smooth muscle cells

and fibroblasts have been proven to reduce the frequency and rate of progression of chronic rejection in the form of bronchiolitis obliterans syndrome. The same effects underlie the principles of combined treatment of bronchial stenosis by a combination of endoscopic bronchoplasty methods and long Everolimus administration. On the other hand, antiproliferative effects can lead to such disastrous consequences as the development of bronchial anastomotic dehiscence at the early stages after transplantation. This makes it possible to administer a four-component immunosuppression no earlier than 3 months after transplantation [26].

Along with ischemia, respiratory tract infection plays an important role in the development of bronchial complications. Necrotic changes in the mucous membrane of the bronchial tree both in the area of the anastomosis and throughout, drug immunosuppression, direct contact of the transplanted organ with the environment, lack of effective ciliary clearance, cough reflex weakening – all these create favorable conditions for infection persistence in the respiratory tract. In this context, of particular importance is a fungal infection that develops with a rate of 15–35% and is represented by *Aspergillus* and *Candida* fungi in 80% of cases [27]. The most aggressive type of fungal infection, *Aspergillus* fungi, is expressed in the form of pseudomembranous or necrotic *Aspergillus*-associated tracheobronchitis and invasive pulmonary aspergillosis. Mortality in the development of generalized fungal infection, especially in the case of invasive forms, reaches 100% [28].

Some studies proved that chronic infection with highly virulent multi-resistant gram-negative microflora (*P. aeruginosa, B. cepacia*) increases the rate of bronchial complications by 29% [29].

Drug-induced immunosuppression is a risk factor for opportunistic infections (*P. carinii; Aspergillosis*, CMV). The likelihood of developing pneumocystis pneumonia in patients not receiving appropriate antimicrobial therapy is more than 80% [30]. The devastating consequences of infection and the development of opportunistic infections are considered in the standard regimen of antimicrobial prophylaxis in lung recipients.

It was found that long mechanical ventilation (50–70 h or longer) and high PEEP values result in damage to the bronchial mucosa and bronchial wall in the anastomotic area and are also associated with high risks of infectious complications [17, 29].

It is argued that performing a "telescopic" bronchial anastomosis in 48% of cases is complicated by development of bronchial stenoses, while the end-to-end anastomosis provides a lower rate of such complications and is thus more preferable [16].

Suture technique is equally important. The generally accepted technique is a combined bronchial anastomosis with the formation of a continuous twisted suture of the membranous part of the bronchus and a single interrupted suture of the cartilaginous part of the bronchus. For effective comparison of cartilaginous half-rings, some authors recommend performing 8-shaped sutures, which allows minimizing the number of probable bronchial anastomosis complications from 18.1 to 2.3% [13].

Despite the lengthy discussions on the benefits of a particular surgical technique and the option of bronchial anastomosis, a consensus has not yet been reached, and the choice is determined by the immediate operation conditions and the surgeon's own experience and preferences. In some cases, performing a bronchial telescopic anastomosis is necessary to compensate for the difference in the diameter of the bronchus of the donor and recipient.

CLASSIFICATION OF BRONCHIAL COMPLICATIONS

Bronchial anastomotic dehiscence

Ischemic disorders in the bronchial tissues of the graft lead to desquamation of the bronchial epithelium and the development of anastomosis of varying severity. The most severe form is necrotic anastomosis's, a condition that potentially threatens the development of bronchial anastomosis failure.

The bronchial anastomotic dehiscence (BAD) is a violation of the integrity and tightness of the suture of the bronchial anastomosis as a result of necrotic changes extending to the entire thickness of the wall of the bronchial stump of the graft, leading to the formation of both length-limited defects and complete anastomosis separation. It develops with a rate of 1 to 10% within 1 to 4 weeks after transplantation and is features with a high mortality rate [12]. The clinical picture differs depending on the degree and extent of the dehiscence.

In some cases, dehiscence is latent, without clear clinical manifestations and is diagnosed during routine bronchoscopy. CT of the chest organs is a highly sensitive method for diagnosing BAD, as it allows both to directly visualize a defect in the bronchial wall and to identify it by indirect signs in the form of a limited peribronchial air accumulation.

The development of a necrotic anastomosis is not an absolute predictor of BAD development; however, it makes it mandatory to conduct regular observational and sanitary bronchoscopy to monitor changes.

In itself, the fact of revealing the bronchial anastomosis dehiscence is not an indication for emergency surgery. The tactics are determined by the length of the defect, the clinical picture and the effectiveness of conservative therapy. With defects of less than 25% of the anastomosis circumference, in the absence of clinical manifestations, waiting is preferred; if a defect is larger than 25% of the circumference, or if symptoms are present, interventional

bronchoscopic or surgical reconstructive interventions are performed [31].

In case the dehiscence of a limited length is developed, self-expanding metal stent implantation is recommended. The choice of the stent option is a subject for discussion; however, most authors are inclined to the implantation of uncoated nitinol stents that do not interfere with neoepithelialization for 6 to 8 weeks [12, 32]. Coated stents prevent effective ciliary clearance of the airway mucosa, creating favorable conditions for infectious colonization of the anastomotic region [33, 34]. The limitation of the time of implantation is due to the risk of granulation tissue growing on an uncoated stent up to its complete occlusion, which significantly complicates its subsequent removal, potentially threatening the formation of a secondary defect that is even larger than the initial one [32, 33].

Some observations on effective therapy of short-length bronchial anastomosis leakage by instillation of surgical glue based on fibrin or cyanoacryalate on the defect area have been published [35, 36]. In case of ineffectiveness of conservative and minimally invasive methods of treatment, attempts are made to close the defect with additional covering of the suture line with the recipient's own tissues with preserved blood circulation (intercostal muscles, pericardium, strand of the greater omentum on the feeding pedicle), and reconstructive bronchoplastic operations are performed. If reconstruction is impossible or in case of development of repeated failure, if the patient's condition allows, transplantatectomy is performed.

Bronchial fistulas

Bronchial fistula is a pathological fistulous communication of the bronchus lumen with anatomically similar structures or cavities, depending on which they are subdivided into bronchopleural, bronchomediastinal and bronchovascular.

Bronchopleural fistulas develop in the early postoperative period and are typically associated with 12% of cases of bronchial anastomoses dehiscence [36]. The clinical picture is featured with the development of pneumothorax, which persists for a long time despite constant active aspiration from the pleural cavity, and subcutaneous emphysema. The presence of a direct communication of the pleural cavity with the bronchus lumen leads to development of pleural empyema, resulting in sepsis. Diagnostic and therapeutic tactics are identical to the corresponding approaches in the case of bronchial anastomosis dehiscence, and the same for bronchomediastinal fistulas [36, 37].

Bronchovascular fistula is a rare complication described in isolated cases [38]. First, it is associated with BA dehiscence against the background of persistent bronchial, most often fungal infection (*Aspergillus, Candida*). In the published observations, the development of bronchovascular fistulas involved the aorta, pulmonary arteries, azygos vein, and left atrium. The formation of a bronchovascular fistula leads to the development of arrosive, often fatal, pulmonary hemorrhage. The development of air embolism has been described in [39] A few publications show cases of rescuing a patient with transplantatectomy or resection of a lung graft of various sizes [40].

Bronchial anastomosis dehiscence and bronchial fistulas in terms of the time of their occurrence are early bronchial complications that develop in up to 3 months after lung transplantation. For periods of more than three months, the following bronchial complications are characteristic:

– Bronchial stenosis.
– Endobronchial hypergranulation.
– Bronchomalacia.

Bronchial stenosis

Bronchial stenosis (BS) is a fixed, breathing act-independent, persistent narrowing of the diameter of the bronchi lumen developing mainly as a result of cicatricial changes with a rate of 1.6–32%. BS can develop at any time after LT but is most often during the first 2 to 9 months after transplantation. Depending on the localization relative to the bronchial anastomosis, they are classified into central and peripheral. The central stenoses are localized directly in the area of the anastomosis or near, but no further than 2 cm. According to various estimates, they are observed in 12–40% of cases. Distal bronchial stenoses are localized more than 2 cm from the bronchial anastomosis and are recorded in 2.5–3% of recipients [41]. The most severe form of peripheral bronchial stenosis is the syndrome of the disappearing intermediate bronchus, which develops with a frequency of about 2%. It has been established that the median survival rate of recipients of donor lungs after the diagnosis of disappearing intermediate bronchus syndrome is about 25 months [42].

The clinical picture is shortness of breath, cough, rales, and the development of obstructive pneumonia in the extreme form. Respiratory function examination shows obstructive breathing patterns. Depending on the degree of stenosis, the condition may be asymptomatic. In some cases, up to 50% of the obstruction of the bronchus lumen can be found accidentally at diagnostic bronchoscopy. Diagnosis is based on characteristic endoscopic and CT images.

Treatment is consistent. The first-line therapy includes methods of interventional bronchology with increasing degree of invasiveness: balloon dilatation, electro- and argon-plasma coagulation, cryotherapy, and implantation of bronchial stents. These techniques are used both independently and in combination.

Balloon dilation (balloon bronchoplasty) is especially preferred if obstructive pneumonia develops as a result of cicatricial stenosis. In 26% of cases, thanks to balloon bronchoplasty, it is possible to achieve stable outcomes without the need for stent implantation in the future. In other cases, at least 2 attempts to dilate bronchial stenosis are recommended before deciding on the bronchial stent implantation [37].

If restenosis develops after repeated balloon bronchoplasty attempts, bronchial stent implantation is indicated [43].

The choice of the type of stent remains a subject for discussion and a specific clinical situation. The main advantage of silicone stents (SS) (Dumont type) is their easy removal even after long periods of implantation. The disadvantage is that a rigid bronchoscopy is needed for its installation.

A metal stent, despite the convenience of implantation, in particular the possibility of implantation through the working channel of a video bronchoscope, has limited indications for use due to the risk of germination by granulation tissue, which limits the time of safe use to 3–4 weeks, otherwise leading to mechanical damage when trying to remove it.

Combined (hybrid) stents of nitinol (an alloy of titanium and nickel), coated with a polymer shell, having all the benefits of metal (ease of implantation) and silicone stents (ease and atraumatic removal), are currently an option of choice. A promising trend in bronchial stenosis treatment is the use of biodegradable and drug-eluting stents [44]. Despite the long existence of bronchial stenting as a therapeutic option for lung transplantation, to date, the advantage of certain stents within the framework of the tasks considered has not been established in randomized trials.

Incorrect stent selection and long implantation periods are accompanied by the development of complications in 50% of cases. Most often, stent migration, parietal and borderline proliferation of tissue granulations, and in the case of uncoated stents and their germination up to complete occlusion, obturation of the lumen with thick bronchial discharge occur [45].

The recommended duration of implantation of a silicone or combined stent is 6–8 months on average; then it is possible to completely resolve cicatricial stenosis as a result of bronchial remodeling. Separate observations show the period of safe implantation of bronchial stents of up to 7 years [46]. It has been established that the median survival rate of patients after bronchial stenting is 82 months versus 22 in patients after isolated balloon bronchoplasty [47].

Endobronchial hypergranulation

The development of endobronchial hypergranulation (EH) is accompanied with impaired ventilation risk due to the risk of airway obstruction. Hyperplastic granulation growths are typically formed in the area of bronchial anastomosis within 3–4 months after lung transplantation, which, according to various estimates, in 7–24% of cases leads to the development of clinically significant obstruction. The growth of granulation tissue is also provoked by trauma to the mucous membrane of the respiratory tract during endoscopic manipulations, laser coagulation, electrosurgical manipulations, and placement of bronchial stents. Infection with fungal flora in the area of bronchial anastomosis is a recognized risk factor for excessive development of granulation tissue [21, 48].

The approaches to endobronchial hypergranulation treatment are controversial. The decisive factor to choose the particular tactics is the degree of obstruction. Waiting tactics are recommended when areas of granulation are detected that cover up to 25% of the bronchial lumen and in the absence of a clinical picture. The question naturally arises about the expediency of waiting since lack of treatment will inevitably lead to continued growth of granulation tissue. In the case of obstruction of the lumen of the bronchus by more than 25% or in the presence of productive symptoms, their removal is indicated [31].

Endobronchial hypergranulations are treated by interventional bronchology techniques. Mechanical removal of granulation tissue with biopsy forceps during flexible endoscopy has advantages in comparison with electrosurgical methods of treatment, when using which excessive trauma to the bronchial wall can lead to inflammatory changes and provoke further growth of granulation tissue. A promising direction in the therapy of hypergranulations is cryotherapy in combination with mechanical removal of frozen tissues, which is accompanied by a lower frequency of relapses. Despite the variety of treatment methods, the repeated development of endobronchial hypergranulations, according to various sources, takes place in 10–50% of cases [12, 14, 37].

Given the high recurrence rate of cicatricial stenosis and endobronchial granulations, new methods and therapeutic approaches are proposed. Brachytherapy [49] and photodynamic therapy [50] are suggested as possible treatment options. A promising direction is the local use of antitumor, antiproliferative agents. Mitomycin C inhibits fibroblast proliferation and is used as a short-term application of a tampon with a solution (concentration 0.1–1 mg/ml, 0.4 mg/ml on average) for 2 to 5 minutes to the bronchial wall area after removal of granulation tissue or to the area of cicatricial stenosis [51].

There is evidence of the effectiveness of a combination of interventional bronchology methods with the appointment of proliferative signal inhibitors (m-TOR inhibitors), but this issue needs further study [52].

In the absence of the effect of the considered conservative, minimally invasive techniques and their combinations in the treatment of bronchial stenosis, recons-

tructive and bronchoplastic surgical interventions, to resection of a lung transplant in various volumes are forced [53, 54].

Bronchomalacia

Bronchomalacia (BM), or expiratory collapse of the airways, is a condition in which exhalation is accompanied by a decrease in the diameter of the bronchial lumen by more than 50% as a result of the loss of the supporting function by the cartilaginous framework of the bronchi, hypotension of myoelastic elements. It is localized mainly in the area of bronchial anastomosis and distal airways [12, 14, 37], which, depending on the severity, can lead to violation of the ventilation conditions.

The development of bronchomalacia is observed in 1–4% of cases within 4 months after LT. The etiology is poorly understood, it is assumed that BM is associated with ischemic damage, persistent infection, and immunosuppression regimens. It is classified depending on the localization into peribronchial (within 1 cm from the anastomotic line) and distal BM [12, 37].

The clinical picture is shortness of breath, more in the supine position, the participation of auxiliary muscles in the act of breathing, difficult expectoration, recurrent infectious attacks, and a chronic barking cough.

Instrumental diagnostics includes the assessment of dynamic expiratory changes in the diameter of the bronchi at chest CT or bronchoscopy [37].

Modern approaches to the therapy of bronchomalacia aimed at reducing the severity of symptoms of the disease and improving the quality of life include conservative approaches, minimally invasive endoscopic methods and methods of surgical correction. In the absence of symptoms, when bronchomalacia is an accidental diagnostic finding, therapy is not indicated. As a conservative method of treatment, non-invasive ventilation of the lungs is used, which prevents the development of collapse of the airways due to the positive end-expiratory pressure. This method is the initial stage in the treatment of bronchomalacia, aimed at reducing shortness of breath, paroxysmal cough, improving sputum discharge, which makes it possible to compensate for the severity of symptoms and is carried out mainly at night or on demand. In case of severity of symptoms, frequent infectious exacerbations, constant dependence on noninvasive ventilatory support, stent implantation is performed. The choice in this case is the Dumont or combined nitinol stent. The stent creates a rigid framework that prevents the expiratory collapse of the airway, reducing the manifestation of symptoms, and improving the patient's quality of life. When the malacia area is localized at the level of the main bronchi, it becomes necessary to use Y- or J-shaped stents, the effective fixation of which in the airway lumen is achieved due to the tracheal segment of the stent. The placement of the stent is carried out with the expectation of remodeling of the bronchus, which is assessed after its removal within 6–8 months. According to some authors, the optimal duration of silicone stent implantation is 9–12 months [47]. In the absence of remodeling of the bronchomalacia region, they are forced to resort to repeated stenting for a long time or to surgical correction.

CONCLUSION

Higher number of patients undergoing lung transplantation, an increase in their life expectancy naturally lead to an increase in the number of bronchial complications diagnosed at different times after the operation. Observational bronchoscopy and interventional bronchology are an important part of a multidisciplinary approach to monitoring donor lung recipients. Timely diagnosis and operative, minimally invasive correction of bronchial complications allows avoiding the development of chronic dysfunction, reducing the quality and shortening the life expectancy of recipients after lung transplantation.

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CURRENT TRENDS IN THE CREATION OF CELL-FREE ALLO- AND XENOTISSUES FOR RECONSTRUCTION OF HEART STRUCTURES

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Tissue engineering has significant potential for solving the problems of durability of biological tissues when used in cardiac and vascular reconstructive surgery. A decellularization technology has been proposed for obtaining a biomaterial, morphologically and functionally similar to the damaged human heart tissue. This review discusses various aspects and models of biological tissue decellularization, including the modern technology of using supercritical carbon dioxide as the most eco-friendly and promising method.

Keywords: heart valve, tissue engineering, decellularization, supercritical carbon dioxide.

Biological prostheses have been used in cardiovascular surgery since the early 1960s, when D. Ross and B. Barratt-Boyes transplanted a cadaveric aortic valve into an orthotopic position in 1962. In 1968, A. Carpentier began using glutaraldehyde for chemical treatment of biological tissue, performing aortic valve replacement with a stented bioprosthesis. Today, porcine aortic and bovine pericardial prostheses are widely used all over the world, even without a stent. About 275,000 artificial heart valves are implanted worldwide every year, of which about half are mechanical and half are biological. This indicates the increasing popularity of bioprostheses in recent decades. However, mechanical and biological prostheses come with some limitations, such as infection, risk of thromboembolism, need for lifelong anticoagulation (mechanical), or limited durability (biological). Allograft is an alternative to mechanical or biological prostheses and has several advantages over existing valves. Homovital (taken from a living heart) and cryopreserved allografts consist of viable tissue relatively resistant to infection and has excellent hemodynamic properties. On the other hand, viability of foreign cells induces immune response, possibly leading to a later, but still, degeneration of the valve. At the same time, antibiotic-sterilized human allograft valves have limited durability due to the lack of living cells inside the matrix [9].

Glutaraldehyde is most commonly used to reduce immune response and xenograft rejection [4, 5, 6, 15, 25]. Although tissue processing reduces its immunogenicity, cytotoxicity and calcification remain the main undesirable components. Calcification plays a major role in degenerative dysfunction of bioprosthetic heart valves, which in turn is initiated mainly by residual dead cells due to glutaraldehyde treatment. The mechanism involves reaction of calcium-containing extracellular fluid with membrane-bound phosphorus, resulting in the formation of mineral deposits of calcium phosphate. In addition, calcification is accelerated by known factors such as young age of the recipient and increased mechanical stress of the bioprosthetic valve cusps. The valve leaflets undergo repeated opening and closing cycles about a billion times during their lifetime. Thus, the structural changes in the native valve occurring with age in the form of sagging of the leaflets, leading to insufficiency or calcification of the leaflets, which causes stenosis, are explainable.

Biological tissues composed of extracellular matrix are used in reconstructive surgery and increasingly find application in regenerative medicine for organ and tissue replacement. Bioengineered valves obtained from acellular xenotissues or decellularized native valve tissue can become the best alternative to mechanical and classical bioprostheses – in experiments they provide repopulation by the recipient's own cells with the possibility of tissue growth and repair. In addition, repopulation valves are considered less prone to calcification and provide ideal hemodynamic parameters. In cardiac surgery, the sources of such materials are allogeneic or xenogeneic tissues for heart valve replacement, creation of "patches" and conduits. However, to date, complete autologous recellularization of implanted acellular heart valves has not been achieved [25]. Valve resellularization is limited only by formation of endothelial recellularization on the leaflet surface. This scenario is much better than cryopreserved valves, which sometimes undergo degeneration and leukocyte infiltration of the entire valve. The fact that repopulation of acellular valves is limited only to the leaflet surface reveals a problem, since it is the leaflet

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tissue that is the main site of degeneration process of the cryopreserved prosthesis [25]. It is expected that without a viable cell population capable of replicating within the valve leaflet, acellular valves will suffer the same fate as cryopreserved valves.

Long-term studies of allo- and xenotissues in cardiac surgery have shown that cellular components of grafts can promote calcification or immune reactions [15, 19, 20, 24, 25]. In order to obtain a biomaterial that is structurally and functionally close to the damaged structure of the human heart and at the same time immunologically safe, decellularization of tissues and organs from humans or animals has been proposed. The goal of all existing protocols is to remove all viable cells while maintaining the integrity of the extracellular matrix. Decellularization methods thus include osmotic, chemical, enzymatic and mechanical. The main problems of decellularization remain to a greater or lesser extent severe disorders in the extracellular matrix structure, increased immunogenicity and thrombogenicity of decellularized biotissue.

The decellularization process is primarily aimed at ensuring immunological inertness and preserving the main structural and functional components of biological tissue, such as proteins, collagen and glycosaminoglycans [5, 6, 10, 25].

The development of decellularization of xenogeneic tissues began in the 1980s. Several enzymes and detergents have been tested to eliminate interstitial cells, but most of these treatments have proved ineffective. The first tissue-engineered porcine heart valve Synergraft® (Cryolife Inc., USA) was developed as an alternative to conventional biological valves. Porcine aortic valve, composite aortic grafts (model 500) or whole pulmonary valve roots (model 700) were made cell-free using the patented Synergraft® technology. However, the SynerGraft technology, using a combination of DNase and RNase enzymes, decellularization, cryopreservation and radiation, has proved ineffective. Already the first results showed that they cannot be used clinically. P. Simon et al. [9] in 2001 reported the results of implantation of Synergraft® prostheses in the right ventricular outflow tract in four male children (age 2.5–11 years). Two patients underwent the Ross procedure and two had homograft replacement. Three children died shortly thereafter. Among the three dead children, one died on day 7 after surgery due to a sudden valve rupture, the second and third died on 6 weeks and 1 year after implantation. The fourth prosthesis was explanted at day 2 after implantation prophylactically. The used method of xenograft decellularization, apparently, did not ensure antigen elimination. Implantation of decellularized allografts gives mixed results. Conventional (cellular) cryopreserved valve allografts cause increased levels of reactive human leukocyte antigens class I and II [15]. Sayk et al. [16] reported macrophage infiltration of a decellularized SynerGraft pulmonary valve allograft as early as week 5 after implantation. Increased levels of donor-specific antibodies against leukocyte antigens class I and II was also found in adult patients who were implanted with an allograft that was decellularized using an ionic detergent sodium dodecyl sulfate [17].

Freezing is one of the physical methods used in tissue decellularization, which includes direct pressure, sonication, and agitation. Rapid freezing of tissue produces intracellular ice crystals that destroy cell membranes and cause cell lysis. The rate of temperature change must be carefully controlled so that ice formation does not disrupt the cell scaffold itself. Although freezing can be an effective method of cell lysis, it can only be used in combination with other methods of removing cellular material from tissue [5].

To remove cellular components from bovine xenopericardium, D.W. Courtman et al. [4] in 1994 described a stepwise process of using detergent and enzymatic extraction to create a cell-free matrix, which represented a promising approach to biomaterials for the reconstruction of cardiovascular structures. Tissue treatment included the use of hypotonic and hypertonic solutions, detergents (octylphenoxy polyethoxyethanol and sodium dodecyl sulfate), as well as DNase and RNAse, which, by inhibiting autolysis, removed all cells from tissues along with lipids [4]. The process resulted in a material composed mainly of elastin, insoluble collagen, and closely bound glycosaminoglycans. Light and electron microscopy confirmed that almost all cellular components were removed without ultrastructural signs of damage to the fibrous components. Biochemical analysis revealed preservation of collagen and elastin and some differential extraction of glycosaminoglycans. Tests for elastic-strength properties have shown that the mechanical properties of the tissues were practically unchanged.

Hypo- and hypertonic solutions effectively remove intact cellular elements. However, numerous studies on antibodies to the major histocompatibility complex (MHC) have found a positive reaction correlating with the intensity of matrix infiltration by T cells in vivo. Obviously, aqueous hypo- and hypertonic solutions are unable to eliminate membrane-bound MHC antigens after osmotic cell lysis. However, such a relatively mild decellularization technique is characterized by a more complete preservation of acellular matrix structures [2].

In order to reduce the risk of tissue matrix damage, Akatov et al. [1] developed a method that was based on the use of EDTA and digitonin. This method induced rapid death of donor cells in grafts, but did not remove dead cells from the matrix. Digitonin is a non-polar detergent, and, by binding to the plasma membrane cholesterol, it disrupts its integrity. EDTA is a chelator of calcium, magnesium and a number of other metal ions and was used by the authors to inhibit accumulation of

calcium and phosphate in mitochondria during cell death. However, this method of processing aortic xenografts did not eliminate immune response, which led to matrix reorganization and damage [1].

Additional tissue treatment with trypsin or nucleases has been suggested. Short-term enzymatic action on the basement membrane cleavage by trypsin has proven to be an effective method for removing the cellular barrier to cell invasion [12]. S. Cebotari et al. washed aortic and pulmonary artery allografts twice with phosphate-buffered saline and incubated them with constant shaking in trypsin/EDTA (0.5% trypsin and 0.2% EDTA) at 37 °C for 48 hours [9]. The decellularized valves were then washed to remove residues and stored in fresh phosphate-buffered saline at 4 °C. Further studies by I. Tudorache et al. comparing the treatment of the pulmonary artery trunk with 1% sodium deoxycholate, 1% sodium dodecyl sulfate or 0.05% trypsin/0.02% EDTA, showed that all methods resulted in complete decellularization of the valve tissue, but only sodium dodecyl sulfate and deoxycholate allowed complete removal of all cells from the pulmonary artery wall and valve [8]. The morphological integrity and safety of the scaffold proteins were significantly higher in the groups treated with the detergent. Enzymatic treatment, on the other hand, led to the destruction of the basement membrane and deterioration of the wall longitudinal extension parameters (stiffness, elasticity, ultimate force, stress and deformation) in the trypsin/EDTA group ($p < 0.05$). All these methods reduce inflammatory and immunological response after implantation and at the same time provide a matrix that is structurally similar to the native valve. However, further studies have shown that sodium dodecyl sulfate can also lead to structural changes in the matrix, changing mechanical properties such as elasticity and extensibility [11].

Qi Xing et al. [13] compared three biological tissue decellularization methods: high concentration (0.5 wt%) of sodium dodecyl sulfate, low concentration (0.05 wt%), and freeze-thaw method. Preservation of the extracellular matrix, mechanical properties, ability to respond in vitro and ability to repopulate cells were assessed. The results showed that treatment with high concentration of sodium dodecyl sulfate removed up to 90% of the DNA, but significantly reduced the mechanical strength of the cell-free matrix. The modulus of elasticity and viscosity decreased by about 80% and 62%, respectively. The freeze-thaw method maintained the structure and mechanical strength of the cell-free matrix, but retained a large amount of cellular components in the scaffold (about 88% DNA). With all three methods, in vitro tests did not induce a significant immune response and were able to maintain cell repopulation in vitro.

The use of decellularization techniques to reduce the immunological potential of xenogeneic tissues and organs is based on the assumption that the cellular component of a xenograft is the only factor contributing to its antigenicity. Approaches for evaluating acellularity of the scaffold after decellularization have mainly involved histological evaluation of residual nuclei [18, 19], although this information does not provide knowledge about the removal of known xenogeneic antigens such as galactose-alpha-1,3-galactose (alpha-gal) and major histocompatibility complex class 1 (MHC I) – in the form of transmembrane glycoproteins contained on the surface of all nucleated cells. Gonçalves A. et al. studied the degree of decellularization of biological matrices from bovine pericardium [18]. The pericardium was subjected to standard decellularization, consisting of hypotonic lysis and DNase/RNase treatment. Additionally, the tissue was treated for 24 hours with solutions: 0.5% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, alpha-galactosidase (5 IU/mL) or phospholipase A2 (150 IU/mL). The tissues were then washed for 96 hours with gentle agitation at 27 °C, and then evaluated using light microscopy. It turned out that standard processing resulted in only partial removal of histological cellularity and persistence of alpha-gal, MHC I and alpha-actin. The addition of deoxycholate treatment resulted in clear acellularity, but preserved xenogeneic antigens. Sodium dodecyl sulfate provided complete acellularity and removal of xenogeneic antigens, with alpha-galactosidase treatment selectively removing alpha-gal from bovine pericardium.

Sodium dodecyl sulfate and sodium deoxycholate are ionic detergents and are effective in dissolving both cytoplasmic and nuclear cell membranes, but tend to denature proteins by disrupting protein-protein interactions. Triton X-100 is the most widely studied non-ionic detergent for decellularization protocols and can be an effective method of decellularization, although its effectiveness largely depends on other methods with which it is combined in a particular protocol [5].

Further studies to more finely assess matrix acellularity included DNA quantification [20], analysis of residual DNA fragment length [21, 22], and assessment of residual cellular structural proteins (without proven antigenicity) [15, 23]. However, there is currently no standard for decellularization success criteria. Thus, the reliability of a cell-free scaffold as a measure for assessing residual antigenicity also requires careful study [15].

The most effective agents for decellularizing each tissue and organ depend on many factors, including the cellular composition of tissue (e.g. liver versus tendon), density (skin or adipose tissue), fat content (brain or bladder), and thickness (skin or pericardium). Importantly, each agent and method used to remove cells will still alter the composition of the extracellular matrix and cause some degree of destruction to its ultrastructure. Minimizing these undesirable effects, rather than preven-

ting them completely, is the goal of any decellularization method [5].

One relatively understudied method worthy of attention at present is the use of supercritical carbon dioxide ($scCO_2$) and ethanol as a medium for cell extraction. A number of researchers have reported that the high permeability and high transfer rate of the supercritical fluid makes this method very effective.

J. Won Lee et al. [27] analyzed lipid extraction with supercritical CO_2 and concluded that supercritical carbon dioxide ($scCO_2$) is an eco-friendly supercritical fluid that is chemically inert, non-toxic, non-flammable and non-polluting. As a green material, $scCO_2$ has desirable properties such as high density, low viscosity and high diffusivity that make it suitable for use as a solvent in decellularization. The growing concern surrounding environmental pollution has triggered the development of green analysis methods based on the use of $scCO_2$ in various laboratories and industries. $ScCO_2$ is becoming an effective alternative to conventional organic solvents.

In addition, $scCO_2$ biotissue treatment cycle can be short, lasting a few hours instead of days required when using other detergents. Eliminating the use of detergents such as sodium dodecyl sulfate will also help reduce damage to the extracellular matrix and decrease cytotoxicity due to detergent residues [3].

In 2008, Sawada et al. [3] presented a study on supercritical decellularization with carbon monoxide. The authors reported adequate removal of DNA and cells, but at the same time found intense tissue dehydration, which caused the tissue to harden, while making it more brittle, potentially threatening the use of the material and creating a major obstacle to progress in cellular technology.

Tissue dehydration is an important parameter determining the suitability of tissue as an implant, and it has been suggested that low water content impairs the mechanical properties of biological tissue, although the minimum degree of hydration to maintain its functional properties remains unknown [9].

Supercritical carbon dioxide containing a small amount of entrainer was an adequate medium for extraction of cell nuclei and cell membranes from biological tissue [5]. Under mild extraction conditions (15 MPa, 37 °C), cell nuclei were completely eliminated within one hour. However, the efficiency of phospholipid removal largely depended on the rate of carbon dioxide transfer into the tissue. In this case, mechanical strength did not decrease even with prolonged treatment. Thus, the authors believe that decellularized tissue can be prepared quickly enough and obtained in a completely dry state, which is beneficial in terms of long-term storage without rotting or contamination.

Taking into account the experience of previous researchers, D.M. Casalia et al. [26] presented a new decellularization technique that preserves the hydration state of the matrix and its mechanical properties. A porcine aortic wall was used for the study, from which all adipose tissues were carefully removed. It was then cut into thin rectangles (approximately 3 cm × 2 cm) and stored in phosphate-buffered saline at 4 °C for at least 48 hours prior to use. Each tissue sample was dried for 15 minutes in a light vacuum using filter paper and a Büchner funnel. Intensive drying in a vacuum oven (37 °C, vacuum 38.1 cmHg) was used as a control. Changes in tissue weight were recorded after 1, 2, 3, 6 and 24 hours. To prevent water extraction from the aortic tissue and to avoid critical dehydration, full thermodynamic equilibrium (i.e. full saturation) between $scCO_2$ and water was first achieved. This equilibrium between $scCO_2$ and water was achieved at a liquid CO_2 flow rate of 5 mL/min or lower. With an increase in the flow rate, the equilibrium could not be maintained. The hydrated $scCO_2$ was then used to treat the matrix. The treatment factor (i.e. total mass of CO_2 per unit mass of hydrated material) and other conditions used (including temperature, pressure and depressurization rate) were set to be similar to those used by Sawada et al. for comparison.

Until the tissue was examined for decellularization, it was stored at –20 °C, prewashed in phosphate-buffered saline and cut into circular slices about 1 cm wide.

Despite high water content (more than 97%) in the aortic wall, complete decellularization was not achieved with $scCO_2$ alone. The researchers included four different additional components in the pre-saturation chamber to determine if they improved decellularization: water, water + Dehypon Ls-54 surfactant (BASF America, Florham Park, NJ), pure ethanol, and a mixture of water and ethanol. After treatment, the tissues were fixed in 10% formalin for at least 24 hours and immersed in paraffin. After slicing and deparaffinization, they were stained with hematoxylin and eosin, or Masson's trichrome stain was used. DNA quantification was performed using DNyzol reagent (Invitrogen, Carlsbad, Calif.). DNA concentration was calculated based on absorbance and initial tissue mass measurements.

The authors concluded that the presented new hybrid method of using $scCO_2$ combines a short processing period and complete decellularization, which was confirmed by histology and DNA quantification (<0.04 μg DNA/mg tissue), while maintaining tissue structure and mechanical properties.

R.S. Hennessy et al. [28] analysed the use of supercritical carbon dioxide for sterilization of decellularized valves. They found that this method is superior to others (gamma irradiation, hydrogen peroxide, ethanol) and may be promising, despite the residual content of peracetic acid in the tissue after treatment, which is one of the ingredients in the sterilization method and helps to maintain tissue sterility for a long time. In the study, in vivo implantation in animals showed no side effects due to the

presence of acid in the heart valve tissue. But research is ongoing to produce completely sterile decellularized heart valves without the presence of peracetic acid.

In conclusion, it must be said that by optimizing decellularization processes, it is possible to obtain valves and other tissues taken from humans and animals that will minimize the donor and patient specificity that is necessary to ensure compatible grafts. However, in order to truly reduce the differences between donors and patients in need of transplantation, it is necessary to develop new tissue engineering methods, since current methods do not provide absolute durability and functionality of decellularized tissues. In addition, recellularization techniques need to be improved to evenly distribute the desired cell types throughout the tissue and ensure sufficient delivery of nutrients and oxygen for optimal cell viability.

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VALVE-SPARING AORTIC ROOT RECONSTRUCTION

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This paper reviews the current main approaches to valve-sparing aortic root reconstruction. The advantages of valve-sparing surgeries are obvious – low mortality, longer survival, better quality of life of the operated patients, since the techniques save the heart's pumping reserves and free the patient from continuous intake of direct-acting oral anticoagulants and laboratory control of the hemostasis system, as well as other prosthesis-associated specific complications.

Keywords: aortic valve, aortic root, aortic insufficiency, valve-sparing surgery.

In 1956, the first successful valvuloplasty was performed in a patient with severe aortic regurgitation by French surgeon C.W. Lillehei, stitching two cusps, thereby eliminating prolapse and insufficiency (Kwasny L., 1913).

The main causes of aortic insufficiency can be divided into two large etiological groups. These are congenital abnormalities of the development of the aortic root and the ascending aorta, entailing a disruption in valve geometry. Considered are both genetic hereditary mutations and sporadic gene changes, as well as disorders in the embryogenesis of the cardiovascular system under the influence of external factors.

The second group of acquired aortic insufficiency includes various inflammatory diseases, in which infectious agents either directly affect the valve leaflets and the aortic wall, or systemic diseases in which antibodies are produced targeting the cardiovascular system, particularly elastin and elastase located in the connective vascular and valve tissues (rheumatic diseases, syphilis, tuberculosis, systemic lupus erythematosus, systemic scleroderma, etc.), as well as non-inflammatory – atherosclerotic, autoimmune. In this case, the pathogenetic process will be associated with local medionecrosis and thinning of the vessel wall; increased internal pressure of the pulse wave provokes expansion, rupture and stratification. It should be noted that the pathological mechanism is mostly triggered in the areas subjected to the highest hemodynamic stress – the aortic root and the physiological bends of the aorta.

Postoperative and posttraumatic cases of aortic insufficiency should be distinguished separately. The degree and severity of aortic regurgitation will be directly determined by the nature and location of the lesion.

All mechanical prostheses have pressure differences, whose magnitude, in addition to the features of the model and its size, depends on the shock output and heart rate. This dependence is not linear in nature and is accompanied by energy loss and extra work with each cardiac cycle. At rest, the most advanced prostheses are characterized by an average pressure gradient of 10 mmHg in the aortic position, which is an additional constant load for the left ventricular myocardium, which can be particularly fatal in the early postoperative period in decompensated patients with reduced ejection fraction. Thrombus formation, bleeding and septic endocarditis are among the specific complications following a valve replacement surgery (Konstantinov B.A., 1989).

However, despite the fact that valve replacement is the standard procedure in most aortic insufficiency cases, valve plasty or valve-sparing surgery should be considered in patients with elastic uncalcified tricuspid or bicuspid valves, aortic insufficiency type I (aortic root enlargement with normal leaflet mobility) or type II (leaflet prolapse) (Lancellotti P., 2008).

The main methods of aortic root reconstruction include (Molchanov A.N., 2017):

- Resuspension – "suspension" of the aortic valve commissures to the reconstructed sinotubular junction;
- Remodelling – excision of all 3 sinuses, cutting out the corresponding matched tubular prosthesis with creation of neosinuses and suturing it to the aortic ring;
- Reimplantation – the aortic ring and leaflets are placed inside the tubular prosthesis.

Resuspension is performed when the sinotubular junction is dilated after its diameter has been restored. The commissures are tightened with sutures on the spacers. When aneurysmal dilatation spreads to the non-coronary sinus, the Wolfe procedure is performed, including reconstruction of the sinotubular junction and non-coronary sinus; otherwise known as partial remodelling (Wolfe W.G., 1983).

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In classic remodelling using the Tirone David II technique (Fig. 1), all sinuses are excised and button-shaped coronary artery ostia are cut out. It is suggested that a prosthesis 1–2 mm smaller than the aortoventricular junction be used. The prosthesis is cut out from the distal end in a U-shape then sutured to the root. For remodelling, spherical Valsalva prostheses are used to create artificial sinuses, thereby reducing the hydraulic effect on the valve leaflets.

The difference with the Yacoub procedure is that the prosthesis is not cut in a U-shape, but in a V-shape. The Tirone David III procedure, like the Hopkins procedure, consists of additional external aortic valve annuloplasty.

The aortic valve can be reimplanted into the prosthesis using the David I and Florida Sleeve procedures, as well as their various modifications.

The David I procedure is a complex technique that involves all components of the aortic root: aortic annulus, aortic valve leaflets, Valsalva sinuses and sinotubular junction (David T.E., 2019).

The David I procedure (Fig. 2) mobilizes the aortic root just below the aortic annulus. The coronary artery orifices are cut out as "buttons". The sinuses are excised, departing from the commissures by about 5 mm. After measuring the annulus diameter, the prosthesis is selected one size larger than the size of the annulus. The prosthesis is fixed by stitching the fibrous ring with separate U-shaped sutures. The commissures are fixed to the prosthesis with three sutures, tightening as much as possible without stretching the prosthesis using polypropylene sutures. The level of location of the commissures should ensure satisfactory coaptation of the aortic valve cusps of at least 4 mm. Next, the sinuses are fixed with a twisted or mattress suture. Additional leaflet plasty is applied as needed and at the surgeon's discretion (Beckmann E., 2019).

This valve-sparing operation was designed to correct dilated aortic annulus and sinotubular junction, but it removed the aortic sinuses and placed the valve in a rigid cylindrical structure (David T.E., 2019). Several studies have shown that the rate of opening and closing of the aortic valve can be reduced by reconstructing the aortic sinus (De Paulis R., 2001, Aybek T., 2005). To solve this problem, several types of prostheses with extensions mimicking the sinuses of Valsalva have been proposed, but most of them are spherical. The aortic root is a cylinder with three bulges; in spherical prostheses, the horizontal plane will be changed into an oblique linear one, which will further affect the durability of aortic valve cusps; the most anatomical is the Uni-graft prosthesis, with three separate sinuses, which showed good hemodynamic outcomes almost similar to physiological indicators (David T.E., 2019). The prostheses are presented in Fig. 3.

A simpler reimplantation option is the Florida Sleeve procedure (Fig. 4). Under this technique, measurement

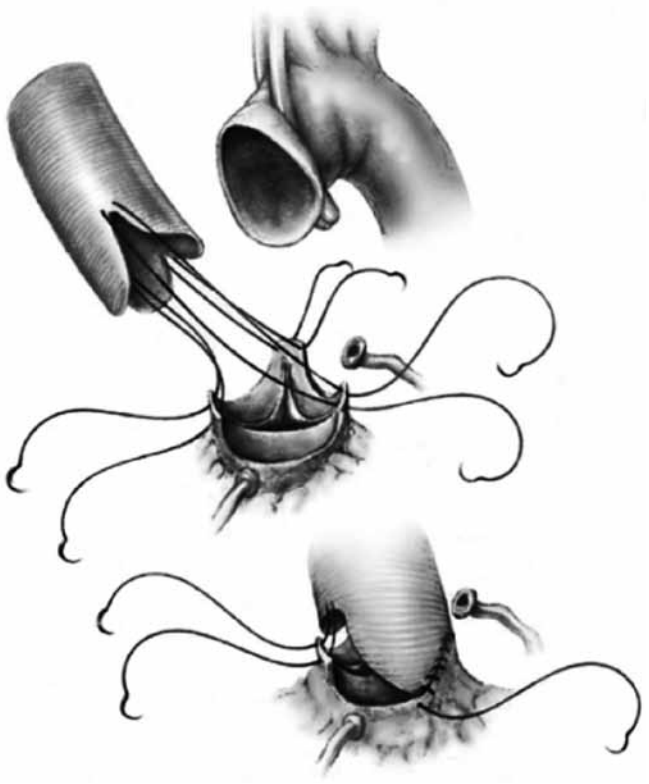

Fig. 1. Tirone David II/Yacoub procedure

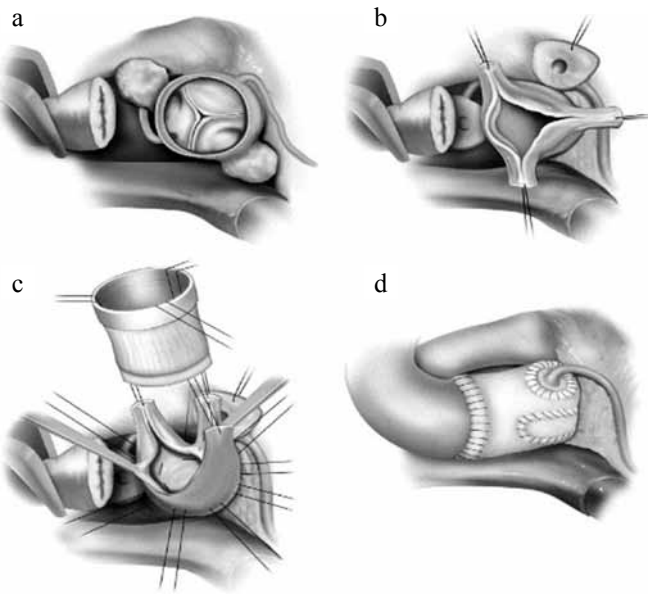

Fig. 2. Aortic valve reimplantation into the prosthesis using the Tirone David I procedure

and selection of a prosthesis are performed only by assessing the diameter of the fibrous ring. The aortic root is placed in the prosthesis by passing the coronary arteries through prepared keyhole-type slots. The sinotubular junction is sutured to the Dacron prosthesis with a curling suture. It is important to position the commissures at the correct height in order to create a satisfactory coaptation of the leaflets. This suture narrows the sinotu-

bular junction to the required diameter. Hegar dilators are used for a more accurate measurement, controlling the final diameter. However, it is not always possible to bring the sinotubular junction to the proper size due to its pronounced dilatation. In such cases, a supracoronary prosthesis with a smaller diameter prosthesis is used for additional narrowing. This technique also allows you to strengthen and fix the aortic root in the required patient anatomical parameters (Hess P.J., 2005).

Uni-graft

Gelweave Valsalva

Fig. 3. Vascular prostheses

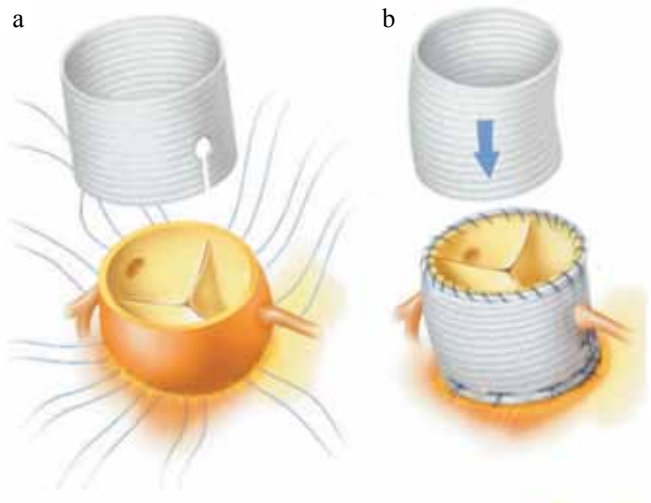

Fig. 4. Aortic valve reimplantation into the prosthesis using the Florida Sleeve procedure

Most patients with aortic root aneurysm have annuloaortic ectasia of varying severity with the development of aortic valve insufficiency. Aortic annuloplasty is an important component of preventing further expansion of the annulus fibrosus and progression of aortic regurgitation. Although a separate annuloplasty can be performed during the remodeling procedure, it is already included in the reimplantation technique (David T.E., 2001, Urbanski P.P., 2013, Michael A., 2018).

The advantages of aortic root reimplantation are confirmed by positive outcomes, lower risk of reintervention and lesser manifestation of aortic insufficiency in the long-term postoperative period (Belov Yu.V., 2006, Liu L., 2011).

According to European studies, dystrophic diseases constitute the main group of patients with aortic insufficiency – about two-thirds of all observations (Iung B., 2003). Among them there is a significant group of patients with elastic uncalcified tricuspid or bicuspid valves, with aortic insufficiency type I (aortic root enlargement with normal leaflet mobility) or type II (leaflet prolapse) (Lancellotti P., 2010; le Polain de Waroux J.B., 2007; Lansac E., 2008).

For this group of patients, many cardiac surgeons recommend valve-sparing techniques for surgical correction of aortic insufficiency. However, an analysis of the database of the Society of Thoracic Surgeons shows that replacement of the aortic valve and ascending aorta are performed in 80% of patients (Detaint D., 2009; Stamou S.C., 2015).

Pressure differences occurring in all mechanical prostheses depend not only on the hemodynamic characteristics of the model, but also on the shock output and heart rate. This dependence is not linear in nature and is accompanied by increased energy consumption of the myocardium at each cardiac cycle. At rest, mechanical prostheses are characterized by an average pressure gradient of 10 mm Hg in the aortic position, which is also an additional constant load for the left ventricular myocardium, which must be borne in mind when managing patients in the early postoperative period, especially in decompensated patients with reduced ejection fraction. Thrombus formation, bleeding and septic endocarditis are among the specific complications after a valve replacement surgery (Konstantinov B.A., 1989).

CONCLUSION

The advantages of valve-sparing surgeries are obvious as they are accompanied by low mortality, longer survival, better quality of life of the operated patients since they save the pumping reserves of the heart and free the patients from constant intake of direct anticoagulants

and laboratory control of the hemostasis system, as well as other prosthesis-associated specific complications.

The authors declare no conflict of interest.

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PRIMARY BILIARY CHOLANGITIS

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Primary biliary cholangitis (PBC), formerly known as primary biliary cirrhosis, is an organ-specific autoimmune disease predominantly affecting middle-aged women. It does not occur in children. PBC prevalence varies depending on the geographic location of the country. Over the past 30 years, there has been an increased incidence of PBC, while significant progress has been made in understanding the pathogenesis of PBC due to the development of innovative technologies in molecular biology, immunology and genetics. The presence of antimitochondrial antibodies and cholestasis on biochemical analysis is sufficient to make a diagnosis, without the need for liver biopsy. Small- and medium-sized bile ducts are the targets of PBC. In the first stage of the disease, granulomatous destruction of the bile ducts occurs; in the second stage, loss of bile ducts, their proliferation, increased size of the portal tracts with chronic inflammation; in the third stage – fibrosis with septal formation, loss of bile ducts and cholestasis; in the fourth stage – liver cirrhosis. Previously, the survival rate of PBC patients ranged from 7.5 to 16 years. However, it has improved significantly with ursodeoxycholic acid and obeticholic acid treatment. If there is no effect from treatment and end-stage liver failure sets in, liver transplantation is performed.

Keywords: primary biliary cholangitis, PBC, pathogenesis, risk factors, ursodeoxycholic acid, obeticholic acid.

INTRODUCTION

Primary biliary cholangitis (PBC), previously known as primary biliary cirrhosis, still remains a recurring issue among hepatologists, transplantologists and physicians of other specialties. Over the past 30 years, significant progress has been made in the study of the epidemiology and pathogenesis of PBC, as well as its diagnosis. Administration of ursodeoxycholic acid (UDCA) in patients with PBC has become a revolutionary milestone in the treatment of this condition, slowing its progression to cirrhosis and end-stage liver failure, as well as reducing the need for liver transplantation. The purpose of this paper is to review the literature on the evolution of ideas about PBC.

BRIEF INFORMATION ON THE EMERGENCE OF THE TERM "PRIMARY BILIARY CHOLANGITIS"

Progressive liver disease with histological signs of cirrhosis, starting from the first description in 1949 [1] and up to 2015, received the stable name "primary biliary cirrhosis", adopted by all hepatologists and gastroenterologists of the world. However, in 2014 and 2015, the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) approved a name change from "primary biliary cirrhosis" to "primary biliary cholangitis" [2]. In 2014, 18 experts in Japan agreed to revise the nomenclature of primary biliary cirrhosis, but there was no unanimous agreement. Seven experts felt that "biliary" and "cholangitis" sounded redundant, and that "cholangitis" does not accurately reflect the pathological changes in the liver of patients with primary biliary cirrhosis. The experts concluded that an alternative nomenclature for primary biliary cholangitis should be created in the future, a name that would more accurately reflect the nature of the disease [3].

The change in the name from primary biliary cirrhosis to primary biliary cholangitis was justified by the following facts: introduction of antimitochondrial antibodies as a tool allowed physicians to diagnose primary biliary cirrhosis at earlier stages before the development of liver cirrhosis; widespread use of UDCA as a first-line drug suppressed progression of the liver disease to a cirrhotic stage among a significant part of patients [4]; in Japan, about 70–80% of patients are asymptomatic [3]. One of the arguments for the name change was that in the English transcription, the abbreviation of both names is the same – PBC. S. Shimoda and A. Tanaka (2016) [5] in accordance with the general agreement called on all members of the Japanese society of hepatologists to use the name "primary biliary cholangitis" for the disease known by the abbreviation PBC.

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EPIDEMIOLOGY OF PRIMARY BILIARY CHOLANGITIS

PBC is an organ-specific autoimmune disease [6] with chronic inflammation and cholestasis [7–11]. The disease progresses to biliary cirrhosis at different rates [12]. Without treatment, the median survival time for patients with PBC is 7.5 years in symptomatic and 16 years in asymptomatic patients [13].

The disease is predominant in women [6, 10–12, 14] over 40 years old, with an incidence of 1 per 1000 [9]. In the United Kingdom, North America and Sweden, the ratio of women to men is approximately 10:1 [15], while in China it is 6.1:1 [16]. According to T. Kogiso et al. (2017) [10], female individuals compose 90% of PBC cases. Unlike other autoimmune liver diseases, PBC does not occur in children [17].

The epidemiology of PBC has been particularly intensively studied over the past 30 years. Most studies have noted a significant increase in the incidence and prevalence of this disease. M.I. Prince and O.F. James (2003) [18] cite numerous possible factors causing the increase in the incidence of PBC. They believe that this may be due to increased exposure to a currently unknown environmental etiological agent, or demographic changes with an increased elderly, at-risk population. Prevalence may have further increased due to increased survival of patients, either due to improved care or earlier diagnosis. In addition, clinicians may have also become more able to recognize PBC based on clinical presentation. The authors conclude that whatever the cause of PBC, the recognized epidemiology of PBC has dramatically changed over the past 30 years. Geographic differences in PBC incidence strongly suggest the presence of as yet unidentified risk factors [18].

In the United States, PBC is relatively rare, up to 39.2 persons per 100,000 population [19]. In the Asia-Pacific region, the overall prevalence of PBC is on average 118.75 (49.96–187.55 range) and the incidence is 8.55 (8.05–9.06 range) persons per million population per year. Prevalence is highest in Japan and China (191.18 per million population), medium in New Zealand (99.16 per million population) and low in South Korea and Australia (39.09 per million population). The 5-year accumulative incidence of decompensation, hepatocellular carcinoma and death/liver transplantation in PBC patients was 6.95% (2.07–11.83%), 1.54% (0.9–2.19%), and 4.02% (2.49–5.54%), respectively [20].

ETIOLOGY AND PATHOGENESIS

The etiology of PBC is poorly understood. Cigarette smoking, nail polish, urinary tract infections and low socioeconomic status have previously been considered as etiological factors, but none of them have been confirmed [21].

The liver is the most important organ controlling immune tolerance. Despite its exceptional ability to induce tolerance, the liver remains a target organ for autoimmune diseases, including PBC [22].

The discovery of mitochondrial autoantigens recognized by antimitochondrial antibodies in 1987 marked the beginning of a new era in PBC research. Since then, significant progress has been achieved in understanding this disease, due in part to the development of innovative technologies in molecular biology, immunology and genetics [23, 24].

PBC is a disease of immune dysregulation, including loss of tolerance to mitochondrial antigens [7]. In 95% of patients, a whole family of antibodies to various mitochondrial antigens is present in the blood serum [25]. The serologic hallmark of PBC is the presence of antibodies to mitochondria, especially to the E2 component of the pyruvate dehydrogenase complex [9].

The mechanisms by which anti-mitochondrial antibodies produce liver tissue injury are unknown. However, the presence of these antibodies has allowed detailed immunological definition of the antigenic epitopes, the nature of reactive autoantibodies and the characterization of T-cell responses. Several mechanisms may now be proposed regarding the immune-mediated bile duct damage in PBC, including the possible role of T-cell-mediated cytotoxicity and intracellular interaction between the IgA class of antimitochondrial antibodies and mitochondrial autoantigens [17]. An imbalance of circulating regulatory and helper T cells may be involved in the pathogenesis of PBC [31].

It is assumed that the pathogenesis of PBC, having an autoimmune mechanism of origin, develops in genetically susceptible subjects. In addition, not only genetic, but also environmental factors are involved in the pathogenesis of PBC [7, 27]. Numerous studies have shown that environmental factors, hereditary genetic predisposition, and loss of tolerance are involved in PBC pathogenesis [28].

Genomic association studies have revealed a strong relationship between certain HLA alleles and PBC [21]. It has been previously shown that only HLA class II loci (HLA-DRB1 *08, *11 and *13) were associated with PBC. Many other loci, including IL12A, IL12RB2, STAT4, IRF5-TNPO3, 17q12.21, MMEL1, SPIB, and CTLA-4, were later found to be associated with the disease. Taken together, this confirms the important role of innate and adaptive immune systems in the development of PBC. Identifying the risk loci associated with the disease may contribute to the development of rational, specific therapies in the future [7].

The mechanism of bile duct damage by antimitochondrial antibodies is associated with an immune attack on aberrantly expressed molecules of the pyruvate dehydrogenase complex-E2 antigens and bile-duct epitheliocytes. Some microbial proteins, through molecular

mimicry, become like pyruvate dehydrogenase complex-E2. Therefore, the immune response can also be directed against certain bacteria in the bile duct wall with damage to their epithelial cells [29].

The multilinear immune response at various stages of PBC development includes the involvement of galectin-3 in the pathogenesis of this disease. Recently, its role in specific binding to NLRP3-inflammasomes and activation of the inflammatory process in PBC models has been described. Galectin-3 is a β-galactoside-binding lectin that plays an important role in a variety of biological processes, including cell proliferation, differentiation, transformation and apoptosis, pre-mRNA splicing, inflammation, fibrosis, and host defence. The NLRP3 inflammasome is a multimeric protein complex that initiates the inflammatory process upon activation [30].

DIAGNOSIS

Anti-mitochondrial M2 antibodies and specific antinuclear antibodies (gp210 and Sp100) are typical and specific for PBC. The presence of these antibodies and cholestasis in biochemical analysis are sufficient to make the diagnosis without a need for liver biopsy [6, 11].

According to Japanese national guidelines, PBC can be diagnosed if there are at least two of the following three signs: elevated cholestatic enzymes, presence of antimitochondrial autoantibodies, and presence of histological signs [6].

The disease is often detected based on abnormal increase in alkaline phosphatase activity, followed by confirmation in the presence of antimitochondrial antibodies [21]. The presence of antimitochondrial antibodies or antinuclear antibodies that are highly specific for PBC in combination with cholestasis is usually sufficient to confidently diagnose PBC [8].

The severity and activity of the disease at baseline and during treatment should be assessed to identify individuals with elevated bilirubin levels, platelet counts below 150, or biochemical disease activity during treatment. Liver ultrasound should be performed to detect overt cirrhosis and splenomegaly; transient elastography to detect increased liver stiffness [8].

The commonly accepted non-invasive measure of the degree of liver fibrosis is the Fib-4 formula, which includes age, aspartate aminotransferase level and platelet count. It has been tested and validated in a variety of liver diseases, including PBC [31]. The aspartate aminotransferase to platelet ratio index (APRI) reflects the presence or absence of progressive fibrosis or cirrhosis in PBC [32].

PATHOMORPHOLOGY

The targets in PBC are small and medium bile ducts [7, 33]. This is because of the fragility of biliary epithelial cells caused by apoptosis, aging, and autophagy [6]. The disease is characterized by chronic progressive destruction of small intrahepatic bile ducts [34, 35] with portal inflammation [11] and eventually fibrosis [17] and cirrhosis [6, 11].

The study of liver biopsies showed that the development of PBC occurs in four stages. At the first stage, there is granulomatous destruction of interlobular and septal bile ducts. At the second stage, there is bile duct loss, their proliferation, increased size of the portal tracts with chronic inflammation (infiltration by mononuclear cells). The third stage is characterized by septal fibrosis, bile duct loss and cholestasis. At the fourth stage features cirrhosis of the liver. This division into stages is conditional, since in different parts of the liver of the same patient, there may be histological changes characteristic of different stages of PBC [36].

According to T. Warnes et al. (2019) [37], liver biopsy is required in the diagnosis of around 20% of patients with PBC. The Ludwig PBC staging system (sinusoidal fibrosis, orcein deposition, bile duct loss, and cholestasis) is of more prognostic value than other staging systems (Ishak and Nakanuma), but the major histological parameter providing independent prognostic value is the presence or absence of sinusoidal fibrosis.

CLINICAL PICTURE

Most patients remain asymptomatic and are diagnosed when cholestasis and elevated alkaline phosphatase levels are detected incidentally [11]. Detection of the disease at a young age (less than 45 years) and male sex are predictors of a more severe course of PBC [8]. The recipient's APRI >2 is negatively associated with patient survival ($P = 0.0018$) [38, 39].

Clinical symptoms include pruritus (itchy skin), dry complexion, fatigue, abdominal discomfort, arthralgia, and bone pain [11]. The most common symptoms in PBC are fatigue and itching, occurring in 85% and 70% of patients, respectively [40, 41]. In patients with PBC, fatigue and itching occur regardless of the severity of the disease [42]. In the work of J.A. Talwalkar et al. (2003) [43], about 55% of patients had itching. Severe pruritus significantly reduces the quality of life of patients [44]. Scratching provides little or no relief, and intense scratching can cause severe skin damage [45]. Nearly three-quarters of patients reported that itching prevented them from sleeping, and 3.6% of patients itched to blood [46]. Cholestyramine is the only FDA-approved drug for the treatment of pruritus in people with PBC. However, it can cause gastrointestinal complications, which limits its clinical use [45].

When examining 97 women with PBC, M.K Prashnova et al. (2018) [47] revealed osteoporosis in 48.9% of patients, and osteopenia in 30.0%. According to the authors, age and duration of menopause were independent predictors of osteoporosis in PBC, and postmenopausal fractures were associated with low dietary protein.

Patients with PBC may have a combination with various other autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis, but most often (in about 60% of patients) with Sjogren's syndrome [48]. There is no consensus on the effect of PBC with Sjogren's syndrome on patient survival. Some authors [49] report that the overall survival of patients with this combination is significantly lower than with PBC alone, while other authors [50] found no such differences.

In the same patient, PBC can be combined with autoimmune hepatitis at the same time. Both diseases have typical clinical manifestations and typical histological features. In PBC, the bile ducts are destroyed and sometimes granulomas form, while autoimmune hepatitis shows severe portal and lobular lymphoplasmacytic inflammation. Nevertheless, a careful analysis of clinical and histological criteria is required to make a diagnosis and prescribe appropriate therapy for both diseases. The first-line therapy for PBC is UDCA, and immunosuppression for autoimmune hepatitis. Both diseases can progress to liver cirrhosis [51]. Familial cross-over between autoimmune hepatitis and primary biliary cholangitis is rare [52]. The authors presented such observations in siblings. If a combination of PBC with autoimmune hepatitis is suspected, liver biopsy is necessary [8].

There are cases of PBC combined with autoimmune hepatitis and generalized sarcoidosis [53]. However, since granulomatous liver damage is observed in both PBC and sarcoidosis, it is necessary to carry out morphological differential diagnosis of these two diseases [54].

EXTRAHEPATIC MANIFESTATIONS OF PBC

Extrahepatic manifestations of PBC include lung damage with involvement of the parenchyma, vessels, pleura, and regional lymph nodes in the pathological process. In the lungs, fibrosis may develop, and the degree of respiratory failure depends on severity of the fibrosis. The most reliable diagnosis method is high-resolution CT scan [55]. The authors believe that due to the possibility of a prolonged asymptomatic course of the pulmonary process with the development of irreversible changes in patients with PBC, it is advisable to conduct screening to be able to timely detect and treat lung lesions.

TREATMENT

The survival rate of patients with PBC previously ranged from 7.5 to 16 years [13]. However, it has considerably improved after treatment with UDCA [56–58], which is the first-line therapy for PBC [59]. Its therapeutic effect is multifaceted: 1) it increases cholesterol saturation of bile, reduces bile viscosity and improves its outflow; 2) it has an anti-inflammatory effect, suppressing the expression of HLA class I antigens on hepatocytes and production of pro-inflammatory cytokines, regulating phagocytosis and peroxidation reactions; 3) activates hepatocyte antiapoptotic mechanisms; 4) influences lipid and glucose metabolism through interaction with nuclear farnesoid X receptors of the small intestine and liver; 5) influences the functional state of the intestine by providing a laxative effect, stimulating intestinal secretion and peristalsis [60].

The British Society of Gastroenterology recommends that oral UDCA at 13–15 mg/kg/day be used as first-line pharmacotherapy in all patients with PBC. If tolerated, treatment should usually be life-long. The use of UDCA in PBC delays histological progression of the disease and prolongs the survival of patients without liver transplantation. It is assumed that progression of the disease slows down due to reduction of cholestatic damage by acting on the target biliary epithelial cells [61]. Although treatment with UDCA shows good clinical results in most patients [62], there remain about 40% of patients with PBC who do not respond adequately to therapy, which is accompanied by a high risk of severe complications [61].

UDCA is a specific treatment with an excellent response in over 60% of patients. When there is no positive effect, treatment can be continued in combination with other drugs such as obeticholic acid (OCA) and fibrates [11]. OCA, a farnesoid X receptor (FXR) agonist, which has been evaluated as a second-line therapy for PBC, has been licensed by the U.S. Food and Drug Administration and the European Medicines Agency for use in patients who show inadequate response to UDCA or are unable to tolerate it [61].

Treatment with OCA in patients with PBC has shown promising results. For instance, initial clinical trials showed that the use of OCA (in addition to UDCA) in patients with PBC with an inadequate response to UDCA significantly reduced serum alkaline phosphatase [21]. A randomized, double-blind trial of the efficacy of OCA in the treatment of PBC showed that approximately 50% of patients also achieved significant reductions in serum alkaline phosphatase, a marker that predicts disease progression, the need for liver transplantation, or patient death [63]. Although there has been a biochemical improvement in treatment with OCA, there is no conclusive evidence that it reduces the severity of clinical outcomes or improves quality of life. In addition, OCA is not suitable for patients with pruritus, as it can worsen it [61]. This drug does not have sufficient therapeutic effect in all patients; approximately 50% of patients may require other therapies [64].

Therefore, there is an urgent need for more effective treatments for this problematic disease. Several other drugs are currently being investigated for therapy in patients with PBC who do not respond to UDCA treatment [21]. Other new drugs currently in clinical development may have fewer side effects. Fibrates have this potential, but there is presently no evidence to support their routine clinical use in PBC [61]. In Japan, bezafibrate is often used for this purpose, but clinical trials have

not been able to clearly demonstrate the effectiveness of this drug [5].

The current focus is on the study of the modulation of nuclear receptor pathways, which specifically and effectively improve bile secretion, reduce inflammation, and attenuate fibrosis. Pharmacological FXR agonists and receptors activated by peroxisome proliferators are effective. Immunotherapy remains challenging as drug targets and pleiotropic immune pathways have not been identified. Symptomatic treatment, particularly pruritus, is a significant goal achieved in the development of rational therapy with apical sodium-dependent bile acid transporter [12]. Cholestatic pruritus is treated with first-line drugs (bile acid sequestrants) or second-line drugs (rifampicin). However, these drugs are poorly tolerated by patients and have side effects [8]. Ademetionine is used to treat increased fatigue/weakness in liver disease, in particular PBC, as one of the most promising drugs, which has significant positive effect on the condition of patients [65]. The patient will require liver transplantation if cirrhosis develops.

CONCLUSION

Primary biliary cholangitis is an autoimmune disease that progresses to biliary cirrhosis at varying rates. Without treatment, the median survival for patients with PBC is 7.5 years in symptomatic patients and 16 years in asymptomatic patients. The disease predominantly affects women over 40 years of age. Currently, there has been a significant increase in PBC incidence and prevalence. Its etiology has not been adequately studied, but it has been established that antibodies to various mitochondrial antigens are formed. The autoimmune mechanism develops in genetically susceptible subjects when exposed to environmental factors. The targets in PBC are small and medium bile ducts with their progressive destruction and the development of cholestasis, leading to portal inflammation and eventually to cirrhosis. Most patients remain asymptomatic. Clinical symptoms include pruritus, dry complexion, fatigue, abdominal discomfort, arthralgia, and bone pain. Treatment is based on the use of UDCA, which is the first-line therapy and is effective in over 60% of patients. When there is no positive effect, treatment is continued in combination with other drugs, such as obeticholic acid and fibrates. Early diagnosis and timely treatment have reduced the number of patients requiring liver transplants. However, if primary biliary cholangitis progresses to an end-stage liver disease, liver transplantation remains the only treatment for such patients.

The authors declare no conflict of interest.

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CULTIVATED AUTOLOGOUS ORAL MUCOSAL EPITHELIAL TRANSPLANTATION

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According to the World Health Organization, corneal blindness is the fourth most common cause of blindness and visual impairment worldwide. In Russia, up to 18% of blindness is caused by corneal damage. Limbal stem cell deficiency (LSCD) is one of the causes of corneal blindness and visual impairment due to anterior epithelial replacement with fibrovascular pannus. Bilateral LSCD may develop in patients with aniridia, Steven–Jones syndrome, and severe corneal burns of both eyes, leading to severe decrease in visual acuity in both eyes and, as a consequence, physical disability associated with blindness. In such cases, cell therapy, based on autologous oral epithelial culture as an alternative to allogeneic limbus transplants, is proposed for reconstruction of the anterior corneal epithelium. This new treatment method promotes corneal reepithelization, better visual acuity, reduced nonspecific ocular complaints and improved quality of life of patients. The effectiveness and significant increase in the frequency of transparent engraftment of donor corneas after cell therapy drives huge interest in this topic all over the world. This review presents literature data on the features of histotopography and methods for obtaining a cultured autologous oral mucosal epithelium, on cell markers that are used to identify epithelial cells, and on methods for creating cell grafts for subsequent transplantation to the corneal surface in LSCD patients.

Keywords: oral mucosal epithelial cells, oral epithelium, stem cell transplantation, cornea, corneal epithelium, limbal stem cell deficiency.

BACKGROUND

The World Health Organization states that corneal blindness is the fourth (5.1%) most common cause of blindness and low vision in the world [1]. Reports have it that as of 2015, there were 23 million unilaterally corneal blind people and 4.9 million bilaterally corneal blind people in the world due to corneal disease [2]. In Russia, up to 18% of blindness is caused by corneal disease [3]. Shortage of donor material, as well as pathogenesis of several corneal diseases resulting in ineffective keratoplasty for one reason or the other have led to exceptionally high demand for new directions of treatment for these conditions to be developed and introduced into clinical practice.

Limbal stem cell deficiency (LSCD) is one of the causes of corneal blindness and visual impairment in corneal pathology. It is known that the anterior corneal epithelium is renewed during life due to local unipotent progenitors located in the limbus called limbal epithelial stem cells (LESC) [4]. In acute extensive alteration or in the case of a chronic process, LESC damage can be irreversible. At the same time, deficiency in function and/or a lack of these cells lead to disruption of the natural epithelial renewal process and is classified as LSCD. In LSCD, the physiological barrier with the conjunctiva is destroyed, which leads to migration of fibrovascular tissue to the surface of the corneal stroma, and causes severe persistent decrease in visual acuity [5].

With progression, LSCD leads to corneal punctuate epitheliopathy and, often, appearance of persistent epithelial defect, significantly increasing the risk of corneal ulceration and perforation [6]. With unilateral lesion, if the paired eye is healthy, the possibilities of social adaptation in such a patient are not considered limited. However, depending on the severity of symptoms, the quality of life can be significantly reduced. In aniridia, Stevens-Johnson syndrome, and corneal burns in both eyes, bilateral LSCD develops, causing a marked decrease in visual acuity in both eyes and, consequently, disability due to blindness. Because of superficial and/or deep corneal neovascularization, keratoplasty in any patient with LSCD is classified as "high risk" due to an unsatisfactory prognosis for transparent engraftment [7]. According to J.S. Friedenwald [8], LSCD is considered as a trigger mechanism for superficial corneal neovascularization. Hence, epithelial reconstruction in this syndrome is a pathophysiologically grounded and justified procedure.

Currently, there is no unified approach to the treatment of LSCD. Many groups of researchers propose various methods and surgical techniques depending on the extent of the process (the extent of limbal corneal zone

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lesion along its circumference), involvement of one or both eyes, and the tear production level [9, 10]. Several clinical studies in bilateral LSCD have investigated the effectiveness of surgical methods of allogeneic corneal limbus transplantation from a deceased donor [11] or from a living relative donor [12]. However, the protocol of pharmacological support for this operation is long-term systemic immunosuppression [13]. To solve this problem, a number of research groups suggest using cell therapy based on cultured oral mucosal epithelial cells [14]. Cell culture for transplantation is obtained under laboratory conditions from a biopsy specimen of the oral mucosa [15]. Early works on this topic suggested the use of cultivated autologous oral mucosal epithelium [16, 17]. The rationality of choosing this type of cells was due to its morphological properties similar to the anterior corneal epithelium [18]. It is non-keratinized, stratified squamous and is in contact with air. According to reports, successful corneal re-epithelialization based on these cells was observed in 72% of cases, with follow-up periods from 1 to 7.5 years [14]. The barrier between the corneal and conjunctival epithelium was restored, chronic inflammation regressed, and visual acuity increased in 68% of patients. Y. Satake et al. showed that engraftment of such an epithelial graft according to the Kaplan–Meier analysis is relatively stable over time, and is 64.8% in the first year, 59.0% in the second and 53.1% in the third. [19]. In the work of A. Baradaran-Rafii et al. [20], after transplantation of cultivated autologous buccal epithelial cells, penetrating keratoplasty was performed for optical purposes. Kaplan–Meier analysis revealed that the corneal graft retained transparency in 92.9% of cases after the first year of observation and in 69.2% at 3.3 years.

Thus, clinical studies show that cultured autologous oral epithelium can be considered as the main method of repairing the corneal epithelium in bilateral LSCD, as well as an alternative treatment option in unilateral LSCD. The prospect of *de novo* epithelialization in severe corneal diseases by transplantation of a cultured autologous oral epithelium is responsible for the increased interest in this topic worldwide.

Objective: to analyze literature data on experimental methods of obtaining a cultured autologous oral mucosal epithelium and to identify the most relevant directions in the development of the technology for transplanting these cells.

FEATURES OF THE STRUCTURE OF THE ORAL EPITHELIUM

By embryogenesis, the epithelial tissues of the oral cavity have heterogeneous origin, which is reflected in their structure and physiological properties [21]. Based on histological studies with staining for specific markers in the oral cavity, it is possible to detect areas of both keratinized and non-keratinized stratified epithelium [22]. Specifically, the epithelium of masticatory surfaces, such as the hard palate and gingiva, is considered to be the keratinized type. The epithelium lining the lower surface of the tongue, soft palate and floor of the mouth, as well as the mucosa of the lips and cheeks (buccal) is classified as non-keratinized [22]. According to literature, buccal epithelium may contain areas of parakeratinization, and may be presented as keratinized along the teeth clamping line [23]. In contrast, the mucosal surface of the lip is lined with a histologically more homogeneous non-keratinized epithelium, which has fewer stratified layers [23]. It is generally known that the corneal epithelium is non-keratinized stratified squamous epithelium [24]; hence, transplantation of cultured cells with keratinization and/or parakeratinization properties for its reconstruction is not an optimal solution.

METHODS FOR OBTAINING AN ORAL EPITHELIAL CULTURE

One of the key issues in the application of cell technologies in clinical practice is the standardization of the culture medium and conditions. It is important to note that for clinical use, it is recommended to use culture media that have no animal components [25], while supplements used to stimulate the growth of a certain cell type (insulin, hydrocortisone, and others) must have a GMP (Good Manufacturing Practice) certificate [26]. It has also been shown that autologous patient serum can be used as a common mitogen in cell transplant production process [27].

Among the culture media used in the clinic as the base for obtaining the buccal epithelium culture, the following were used: DMEM/F12 medium (1:1–1:3) containing 1.05–1.425 mM calcium and medium for keratinocyte growth with a low calcium content: 0.06–0.07 mM [28]. Low calcium content in the medium is a method of culture selection of the epithelium, due to morpho-functional transformation and elimination of fibroblast-like cells [29]. High calcium content, in turn, causes stratification of epithelial cells [30] and can reduce the overall regenerative potential of the future cell preparation.

According to reports, the most common group of culture medium supplements used to stimulate oral epithelial growth includes factors such as insulin, hydrocortisone, human epidermal growth factor (hEGF), triiodothyronine, and cholera toxin [31]. According to our data, the last two factors are supplied as research reagents and are not GMP certified.

Primary oral epithelial cell culture can be obtained by cultivating explants or by treating tissue with enzymes [26]. The first method is relevant if the mucosal biopsy is small (2–4 mm), and enzymatic treatment can lead to

the death of progenitor epithelial cells. The disadvantage of this method is slow growth and potential possibility of culture contamination by fibroblast-like cells from the submucosa of the biopsy specimen. For larger tissue samples, the enzyme treatment technique, which is in two stages, is applicable [26]. The first uses a dispase solution in DMEM medium (1.8 mM calcium) to split the basement membrane. For this, the mucosal tissue is placed in a solution with a 2.4 U/mL dispase concentration for 18 hours at +4 °C (cold version) or at +37 °C for 2 hours (accelerated version). At the second stage, the split-off epithelium is treated with trypsin-versene (0.25–0.02%) to obtain a cell suspension for seeding. According to some authors, the concentration of 4–5 × 10^5 cells per cm^2 is the most optimal for seeding buccal epithelial cells [32]. The primary culture and its passaging are carried out in standard conditions under phase-contrast light microscopy with a change of medium after 1 day. The buccal epithelial cell culture is distinguished by its high proliferative potential and ability to maintain the population during subculturing [31].

IDENTIFICATION OF ORAL EPITHELIAL CELLS IN CULTURE AND TISSUE

The most common technique for identifying oral epithelial cells in culture is immunofluorescent staining of cultured cells. Proliferation markers are among the most important ones, as they make it possible to identify progenitor cells both by the general marker of dividing cells Ki67 [33] and by the more specific ones for oral epithelium p75 [33] and p63 [34]. The cell phenotype is confirmed by staining for epithelium-specific integrin β1 (basement epithelium) [35], vimentin (intermediate filaments) [36], ZO-1 (Zonula occludens-1) (dense intercellular contact protein type 1) [33], connexin-43 (gap junction protein) [36]. Staining for cytokeratin markers detects keratinized (CK 1 and 10) and non-keratinized (CK 4 and 13) epithelium [37, 36]. Cytokeratin 8 and 18 staining can be used to detect cells expressing markers characteristic of leukoplakia and squamous cell carcinoma in situ [38]. Additional staining for CD 44 and 73 markers allows identification of fibroblast-like cells in culture [28]. Oral mucosal epithelium in biopsy specimen can be routinely identified on cross sections using hematoxylin-eosin paraffin staining. For a more detailed characterization of the epithelium in the tissue, immunohistochemical staining for the above markers on cryosections is used.

METHODS FOR CREATING CELL GRAFTS BASED ON CULTURED ORAL EPITHELIAL CELLS

Cell therapy in the context of corneal surface reconstruction cannot be accomplished by simply instilling a suspension of cultured cells. Therefore, carriers or matrices are needed to anchor the cultured cells and create a tight contact between the graft and the cornea [39]. Based on the properties of the cornea in general and its epithelium in particular, it should be understood that they should be transparent, easy to manipulate both during cultivation and in the process of transplantation. It is necessary that the matrix maintains the proliferation of cultured cells and maintains their high viability [31]. In experimental clinical studies, the amniotic membrane (amnion), fibrin gel, and cell layer creation technology were used for transplantation of autologous cultured buccal epithelium [31].

The amnion is a flat membrane that mimics the basement membrane, upon cultivation on which buccal epithelial cells spread horizontally, forming a planar structure [40]. When used as a substrate for the growth and transfer of cultured cells, their quantity and quality (immunophenotype) before transplantation is extremely difficult to assess.

Fibrin glue makes it possible to encapsulate cells in a bulk tissue-engineered construct by sequentially mixing a suspension of cultured cells and glue components [41]. Fibrin glue, which has a registration certificate in Russia (Ivisel®, Johnson & Johnson), has never been studied before as a carrier of oral epithelial cells. Unlike its counterpart (Tisseel®, Baxter), this glue has a shorter biodegradation period due to the absence of antiproteoletic enzyme aprotinin in its formulation. Both adhesives are not autologous products, although they have a high safety profile. Their widespread use is limited due to the complexity of their delivery and storage, which are carried out at temperatures below –20 °C.

The technology of obtaining cell layers was proposed, among others, to create a buccal epithelial cell graft [16]. For this, special laboratory glassware was used, with a heat-sensitive polymer applied to the culture surface [42]. When transferred from an incubator (+37 °C) to room temperature (+20…24 °C), the polymer changes its properties to hydrophobic and allows the separation of the cultured cell layer as a thin film without using enzymes. The resulting cell sheet, however, is a fragile object and also requires fixation to the cornea during transplantation.

Thus, today there are a variety of methods for cultivating the oral mucosal epithelium and methods for obtaining a graft. Almost every stage is variable, from choosing a biopsy site to determining a substrate for cultivation. For the reader's convenience, a clarifying characteristic of the methods is presented (Table).

CONCLUSION

Due to the disabling nature of the diseases causing LSCD, reconstruction of partially or completely lost corneal epithelial cover has been a challenging issue in oph-

thalmology for many decades. Published reports on cell therapy based on cultivated autologous oral mucosal epithelium in patients with bilateral LSCD indicate that it is highly efficient. The application of the new method contributes to corneal re-epithelialization, improved visual acuity, reduced basic nonspecific complaints and better quality of life in patients, most of whom are disabled due to corneal blindness. However, in the literature there are various and often contradictory data on the methods of isolation and cultivation of oral epithelial cells, as well as on the methods of cell graft construction. This may be the reason for obtaining heterogeneous cell populations, and, consequently, incomparable results. The question also remains open as to what determines the best corneal re-epithelialization outcome – cell transplantation, depending on the type of tissue-engineered construct, or the quality of the resulting cells in terms of the ratio of markers. Finally, due to the heterogeneity of the oral mucosa, the properties of an autologous epithelial cell transplant may differ.

Thus, a critical analysis of scientific publications on the problem of therapy with cultured oral epithelial cells in limbal stem cell deficiency allowed us to conclude that today there are some general rules and guidelines underlying this experimental approach. But, at the same time, the cardinal difference in points of view on several key issues requires further research in this direction.

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Table

Methodological approaches used to obtain oral mucosal epithelial cell culture and graft

Methodology	Types	Brief description
Source of nonkeratinized epithelium in the oral cavity [22, 23]	Lips and cheek mucosa, Inferior surface of the tongue, Soft palate, Floor of mouth	The most accessible for biopsy are lips and cheek mucosal surfaces
Method for obtaining primary epithelial culture [26] Cell substrate [40, 41, 42]	Enzymes (dispase, collagenase, trypsin)	Enzymes promote rapid production of cells with reduced viability and are usually used in combination with a feeder layer
	Explants cultivation	Slower cell yield, the niche of local stem cells and the surrounding matrix are preserved
Feeder layer [15, 32] Culture medium [28, 29, 30] Serum [27, 28]	Amniotic membrane	Used in many protocols as a substrate for epithelial cell culturing; it is a transparent membrane, composed mainly of type 4 collagen
	Fibrin gel	Transparent hydrogel obtained from commercial fibrin gel (Tisseel®, Baxter; Evicel®, Jonson)
	No substrate	Cells are cultured on the surface of the culture dish
	Thermo-responsive polymers	When the temperature drops to +20…24 °C, it becomes hydrophobic and separates cells from the culture surface
Source of nonkeratinized epithelium in the oral cavity [22, 23] Method for obtaining primary epithelial culture [26]	3T3 mouse fibroblasts	A confluent fibroblast monolayer inactivated by cytostatic agents or irradiation; enriches the culture medium with growth factors
	No feeder layer	Cultivation without this layer requires the addition of epithelial cell mitogenic stimulants
Cell substrate [40, 41, 42] Feeder layer [15, 32]	“High calcium” (≥1.0 mM)	Activates epithelial cell maturation, promotes active migration and attachment of fibroblasts
	“Low calcium” (≤0.1 mM)	Retains the immature state of the epithelial cell population; prevents migration and attachment of fibroblasts; the basis for selective culture media
Culture medium [28, 29, 30] Serum [27, 28]	Xenogenic	There is a risk of transmission of known and unknown pathogens; batch-to-batch variability
	Autogenous	The disadvantages of xenogeneic serum are eliminated; a cryobanking stock can be created
Specific epithelial growth factors [31]	GMP: insulin, hydrocortisone, human epidermal growth factor (hEGF)	These three growth factors are produced as GMP certified products
	Non GMP: triiodothyronine and cholera toxin	Not released with GMP certification, additional regulatory approval required

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