

ВЕСТНИК ТРАНСПЛАНТОЛОГИИ И ИСКУССТВЕННЫХ ОРГАНОВ



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ТРАНСПЛАНТАЦИЯ КОНЕЧНОСТЕЙ — НОВАЯ РЕАЛЬНОСТЬ ОТЕЧЕСТВЕННОЙ ТРАНСПЛАНТОЛОГИИ

Глубокоуважаемые коллеги!

Первый в 2026 году номер «Вестника» открывается впервые появившимся в нашем журнале разделом «Новое в клинической трансплантологии». Появление подобной рубрики не только актуально, но и весьма полезно, так как позволяет привлечь внимание к наиболее важным из последних достижений, находящихся на острие разрабатываемых клинических и научных проблем в изучаемой области.

Размещенная в новой рубрике статья С.В. Готье и соавт. посвящена анализу собственного опыта первых операций по трансплантации верхних конечностей. Несмотря на то что все книги, учебники, исторические экскурсы по трансплантологии как в мире, так и в нашей стране обязательно начинаются с красочных иллюстраций и описания чудесного опыта святых Космы и Дамиана, пересадивших пациенту ногу от темнокожего донора, до настоящего времени главным полем деятельности и основным вектором развития отечественной трансплантологии были жизнеспасающие операции по трансплантации солидных органов.

Первая в мире успешная трансплантация верхней конечности была произведена в 1998 году Жан-Мишелем Дюбернаром. С удовлетворением и теплом я вспоминаю наши встречи во время Российско-французского форума по вопросам здравоохранения в 2011 году; «круглый стол» в НМИЦ трансплантологии и искусственных органов имени академика В.И. Шумакова, на котором профессор Жан-Мишель Дюбернар выступил с лекцией «История пересадки композитных тканей: лионский опыт» и состоялось вручение ему медали «Академик В.И. Шумаков».

Публикуемая в настоящем номере статья содержит результаты совместных усилий мультидисциплинарного коллектива по трансплантации верхних конечностей. Без преувеличения, речь идет не только



LIMB TRANSPLANTATION: A NEW MILESTONE IN RUSSIAN TRANSPLANTOLOGY

Dear colleagues,

The first 2026 issue of the Russian Journal of Transplantology and Artificial Organs opens with a new section appearing in our journal for the first time: “New Developments in Clinical Transplantology”. The introduction of this section is both timely and valuable, as it highlights some of the most significant recent advances addressing current clinical and scientific challenges in transplantology.

A paper by Gautier et al., presented in this new section, analyzes the authors’ experience with the first upper limb transplant procedures. Although many books, textbooks, and historical overviews of Transplantology, both international and in Russia, traditionally begin with the well-known 13th-century legend of the miraculous act attributed to the physician-saints Cosmas and Damian, who were said to have transplanted a leg from a dark-skinned donor to a white patient, the primary focus and driving force of transplantology in Russia has long remained life-saving solid organ transplantation.

The world’s first successful human hand transplant was performed in 1998 by pioneering French surgeon, Jean-Michel Dubernard. I recall with particular warmth our meetings during the Russian-French Forum on Healthcare in 2011, including a roundtable discussion held at Shumakov National Medical Research Center of Transplantology and Artificial Organs. On that occasion, Professor Jean-Michel Dubernard delivered a lecture titled “The History of Composite Tissue Transplantation: The Lyon Experience” and was awarded the prestigious Academician Shumakov Medal.

The article presented in this issue reflects the results of the collaborative efforts of a multidisciplinary team specializing in upper limb transplantation. Without exaggeration, this achievement marks not only the emergence of a new direction but also the beginning of a new era in modern Russian transplantology. The expansion of

о новом направлении, но и о новой эре в современной отечественной трансплантологии: расширение клинического опыта всегда ставит и заставляет решать новые научные задачи. Для успешной трансплантации конечности – васкуляризированного композитного (состоящего из различных по составу, строению и функциональным свойствам тканей) трансплантата – решаются задачи создания новых хирургических технологий, совершенствования иммуносупрессии, функциональной реабилитации и др. Наличие высокоспециализированных научно-клинических центров и подготовленных специалистов, современный уровень организации медицинской науки и здравоохранения в нашей стране позволяет быть уверенным в эффективном развитии этого востребованного вида медицинской помощи.

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



clinical experience in this field inevitably brings forward new scientific and practical challenges that require comprehensive and coordinated solutions.

Successful transplantation of a limb, classified as vascularized composite allotransplantation, involves the transfer of complex grafts composed of tissues that differ in structure, composition, and functional properties. Addressing this challenge requires the development of advanced surgical techniques, further refinement of immunosuppressive therapy, and implementation of comprehensive functional rehabilitation programs.

Moreover, the presence of highly specialized research and clinical centers, together with well-trained professionals and the modern organization of medical science and healthcare in our country, provides a solid foundation for successful development of this important and much-needed form of health care.

Sincerely,

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

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THE LAUNCH OF THE UPPER EXTREMITY TRANSPLANTATION PROGRAM: CLINICAL CASES AND KEY ASPECTS OF ORGANIZING WORK IN A NEW AREA OF CLINICAL TRANSPLANTOLOGY

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The loss of an arm (or both arms), not only causes severe physical trauma but also leads to long-term psychological consequences and difficulties in occupational, domestic, and social maladjustment, substantially impairing quality of life. This complex problem is not always addressed effectively with modern high-tech bionic prostheses, which, while enabling the performance of many functional tasks, have inherent limitations. In selected cases, allograft transplantation of an upper limb from a deceased donor may be considered. Until recently, this treatment option was unavailable within the Russian healthcare system. This paper presents the key stages in establishing an upper limb transplant program, including its organizational structure, the roles of its individual components, and the outcomes of practical implementation. The efficacy and safety of the treatment method are illustrated through the first two clinical cases. The experience gained and the outcomes of the first clinical cases are intended to demonstrate the practical feasibility of developing a structured, effective upper limb transplant program that can address the growing demand for this type of high-tech medical care.

Keywords: hand transplantation, distal upper limb allograft, vascularized composite allograft, deceased donation, osteosynthesis, microsurgery, rehabilitation, functional outcomes, immunosuppression.

INTRODUCTION

“War is an epidemic of trauma” – a statement by pioneering 19th-century Russian surgeon Nikolay Pirogov, that vividly captures the devastating consequences of large-scale armed conflicts, which result in a sharp and sustained increase in the number of casualties.

Traumatic amputation of upper limb segments of varying extent, or their irreversible destruction, occurs both as isolated injuries and as part of combined injuries. Although significant advances in exoprosthetics – particularly modern bionic prosthetic technologies – have substantially improved functional rehabilitation, they can only partially restore lost limb function. In carefully selected patients, upper limb transplantation from a deceased donor may therefore be considered as a potential reconstructive option [1].

Upper limb transplantation remains a relatively underexplored area of clinical transplantology within the broader field of vascularized composite allograft (VCA), which also includes face, anterior abdominal wall, and genital transplantation [2]. Globally, the number of VCA procedures performed to date is substantially lower than that of solid organ transplants. This disparity is attributed not only to the technical complexity of the surgical procedures and ethical considerations surrounding the donation of visible body parts such as hands and face, but also to the inherently high immunogenicity of composite allografts. This immunogenicity arises from the presence of multiple heterogeneous tissue types within the graft, including skin, muscle, adipose tissue, and bone marrow components [3, 4].

Despite the use of intensive multicomponent immunosuppressive regimens, rejection rates remain high.

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Moreover, prevention and management of rejection significantly increases the risk of adverse effects associated with immunosuppressive therapy, including life-threatening complications. This highlights a central clinical dilemma in VCA: balancing functional and aesthetic restoration of the upper limb against the substantial risks associated with long-term immunosuppression [5].

Global experience accumulated in leading transplant centers in the field of cross-match–negative transplantation and transplantation in presensitized recipients – i.e., solid organ transplantation associated with a high risk of severe immunological complications – has contributed to the development of more effective and safer immunosuppressive protocols applicable to VCA [3, 4].

In addition, established principles of donor organ procurement, pharmacological cold storage, and transportation – well developed in solid organ transplantation – have been successfully adapted to upper limb VCA procedures. The concentration of such expertise within specialized transplant centers creates the necessary infrastructure and conditions for implementation and expansion of VCA programs.

Furthermore, replantation of amputated native limbs and their segments after traumatic injury, depending on the extent of reconstruction, closely replicates many key stages of transplant surgery. This provides an important clinical platform through which relevant specialists can acquire and refine their experience within routine surgical practice.

These factors, together with a high level of organization and effective functioning of the deceased organ donation system, have created the necessary conditions for the development and subsequent implementation of an upper limb transplant program through collaboration among specialists from leading transplant and trauma centers in Russia.

STAGES OF ORGANIZATION OF THE UPPER LIMB TRANSPLANT PROGRAM

In the first stage, candidate selection criteria were formulated based on international experience [3]. Transplant candidates were considered to be physically healthy male patients aged 18 to 50 years, with average anthropometric characteristics (height ~170 cm, weight ~70 kg), harmonious physique, and isolated amputation of one or both upper limbs not extending above the proximal third of the forearm, and without harmful habits.

Psychological health, strong motivation for upper limb transplantation, strict adherence to medical recommendations, and commitment to long-term treatment were considered essential prerequisites.

Patients who met the selection criteria underwent evaluation at the transplant center to confirm the isolated nature of the traumatic injury and to identify and manage any concomitant conditions. In all cases, the evaluation

as part of the upper limb transplant preparation program concluded with the patient being placed on the waiting list for donor tissue from a deceased donor.

Potential upper limb donors proposed by regional organ donation coordination centers were evaluated according to the following criteria:

- absence of laboratory signs of systemic infection;
- maximum vasopressor therapy with norepinephrine at a dose not exceeding 500 ng/kg/min;
- physical integrity of the donor limb (absence of visible injury, joint deformities, sequelae of previous trauma or surgical interventions, arterial catheterization sites, or signs of thrombophlebitis);
- preserved integrity and functional adequacy of the peripheral vascular bed as confirmed by Doppler ultrasonography;
- absence of osteoarticular pathology based on X-ray findings in two projections;
- absence of tattoos.

Donor–recipient pairs were selected based on anthropometric compatibility (height and weight characteristics, as well as symmetry of the donor's hand in relation to recipient's contralateral hand with respect to shape and size. Compatibility according to blood group and human leukocyte antigen (HLA) profile was also taken into consideration.

Between September 2025 and February 2026, the upper limb transplant working group evaluated two donors who met the established selection criteria. Suitable recipients were identified from the waiting list based on blood group compatibility and anthropometric matching. Distal upper limb transplantations were subsequently performed on September 28, 2025, and February 2, 2026.

Recipient No. 1

Patient A., 48 years old, somatically compensated (healthy), sustained severe combined multiple trauma in 2019 that resulted in the loss of the right upper limb. The stump was located at the level of the proximal third of the forearm (Fig. 1). The patient reported a negative prior experience with prosthetic use. Comprehensive evaluation performed in August 2025 as part of the upper limb transplant preparation program confirmed the technical feasibility of transplantation and revealed no contraindications to the procedure.

Recipient No. 2

Patient B., 40 years old, underwent traumatic amputation of the left upper limb following a mine-blast injury sustained in 2023. The stump of the left upper limb was located at the level of the middle third of the forearm (Figs. 2, 3).

The functional performance of the exoprosthesis used by the patient was assessed as unsatisfactory and inadequate for the demands of an active lifestyle. Evaluation at the transplant center in 2025 revealed no significant

concomitant disease or contraindications to transplantation of the distal segment of the left upper limb.

DONOR STAGE OF DISTAL UPPER LIMB TRANSPLANTATION

In both cases, procurement of the composite upper limb allograft was performed as part of multi-organ retrieval involving the liver, kidneys, and heart. The upper limb procurement team operated simultaneously with the thoracic and abdominal organ retrieval teams.

The donor limb was positioned in abduction on a separate support table. The surgeon performing the explantation was positioned in the axilla. A fishmouth-

shaped skin incision was made along the volar surface, extending from the medial to the lateral epicondyle of the humerus. The skin and subcutaneous tissue were dissected to the level of the brachial fascia, after which the proximal skin flap was mobilized proximally to expose the antecubital fossa. Superficial veins were clipped and divided.

For improved visualization of the neurovascular bundle, the biceps tendon was transected, allowing mobilization of the brachial artery for subsequent cannulation. Following proximal exposure of the brachial artery, dissection continued distally to the bifurcation into the



Fig. 1. 3D reconstruction of CT angiography of the stump of Patient A's right upper limb



Fig. 2. 3D reconstruction of CT angiography of the stump of Patient B's left upper limb

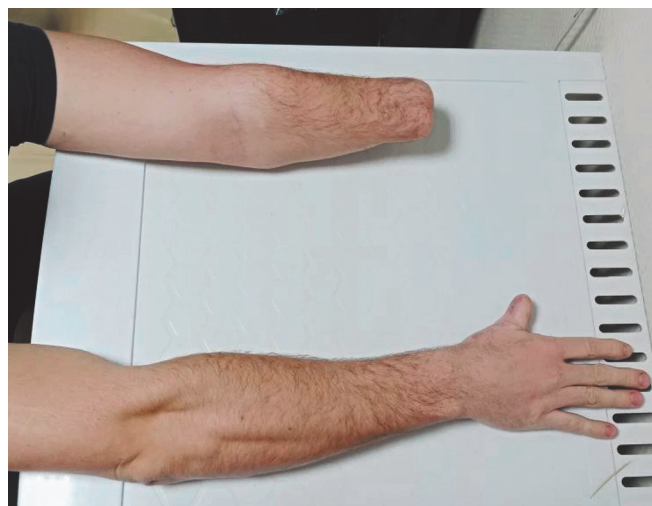


Fig. 3. External view of Patient B's upper limbs

radial and ulnar arteries. The ulnar, median, and radial nerves were subsequently identified and mobilized.

After systemic heparinization, the brachial artery was cannulated using a straight 12-Fr arterial cannula. Custodiol was used as the preservation solution in a volume of 2 L, with a perfusion duration of 10 minutes. From the onset of cold perfusion, superficial and deep veins were transected to ensure passive outflow of the preservation solution.

Upon completion of cold perfusion, decannulation was performed, followed by transection of the brachial artery and accompanying veins. The radial, median, and ulnar nerves were then divided. The flexor muscle group was transected, after which the anterior capsule of the elbow joint was dissected. The limb was flexed at the elbow joint, and the previously formed skin flap was

retracted proximally. After palpating the olecranon, the triceps tendon was transected, exposing the posterior capsule of the elbow joint, which was subsequently divided. Elbow disarticulation completed the donor upper limb procurement procedure.

The graft was wrapped in sterile drapes soaked in preservation solution and packaged in three sterile transport bags. Total procurement time in both procedures did not exceed 60 minutes. The cooled allograft was transported in an isothermal container at +4 °C.

ANESTHESIOLOGICAL MANAGEMENT IN DISTAL UPPER LIMB TRANSPLANTATION

Preoperative preparation of the recipient was carried out at the transplant center simultaneously with graft procurement performed at a separate medical institution.

Intraoperatively, recipients were positioned supine on the operating table. Intravenous premedication included dexamethasone, chlorpyramine, and atropine sulfate, followed by sequential induction of anesthesia using fentanyl (200 µg), 1% propofol (200 mg), and rocuronium bromide (100 mg). Direct laryngoscopy and orotracheal intubation with a 9.0-mm endotracheal tube were then performed.

Mechanical ventilation was maintained using lung-protective settings: peak inspiratory pressure of 10–12 cm H₂O, positive end-expiratory pressure of 4 cm H₂O, respiratory rate of 10 breaths/min, and tidal volume of 5–6 mL/kg. Oxygen concentration in the inspired gas mixture was maintained at 40–45%, while end-tidal carbon dioxide levels were maintained at 4–4.5%. Sedation depth was maintained using isoflurane at 1–2.5 vol%.

Anesthesia and neuromuscular blockade were maintained with continuous infusions of rocuronium bromide at 40–50 mg/h and fentanyl at 50–100 µg/h.

For infusion therapy and hemodynamic monitoring, central venous access was established through the internal jugular vein contralateral to the operative side. A femoral arterial catheter was inserted for invasive hemodynamic monitoring and intermittent arterial blood gas analysis. Additional monitoring included continuous noninvasive heart rate and oxygen saturation assessment. A Foley catheter was inserted for urine output monitoring and optimization of perioperative fluid therapy.

Given the need to avoid vasopressors and/or medications with vasopressor effects, continuous infusion therapy was administered throughout the procedure to maintain stable hemodynamic parameters. Infusion therapy included balanced crystalloid solutions, colloids (albumin and gelofusine), as well as nutritional support via a nasogastric tube using a 10% glucose solution. In addition, 40% glucose solution (800 mg) was administered intravenously, together with prokinetic agents to prevent gastrointestinal paresis. Target hematocrit values were maintained within the range of 25–30%.

At the initiation of vascular reconstruction, sodium heparin was administered intravenously at a dose of 30–35 U/kg to achieve target activated partial thromboplastin time (aPTT) values greater than 200 seconds. Thereafter, prolonged anticoagulant therapy was continued using titrated dosing regimens, maintaining target aPTT values within the range of 50–65 seconds.

Following restoration of blood flow through the principal vessels of the allograft, systemic vasodilators were administered to reduce the risk of reperfusion-associated vasospasm. Systemic glucocorticoids were also given at target doses as part of the combined immunosuppressive protocol.

At the conclusion of the procedure, after the recipient's upper limb had been temporarily immobilized with a plaster splint, a VCA catheter was placed under ultrasound guidance to provide prolonged peripheral analgesia. A 0.5% solution of ropivacaine was administered into the brachial plexus region via a supraclavicular approach to ensure effective postoperative pain control.

Anesthesia was discontinued with the administration of opioid receptor antagonists and sugammadex. The patient was then awakened in the operating room under continued sedation with dexmedetomidine at a dose of 1–1.5 µg/kg/h.

The patients were transferred to the surgical ward for further monitoring and postoperative management.

KEY STAGES OF IMPLANTATION OF A COMPOSITE VASCULARIZED ALLOGRAFT IN THE DISTAL UPPER LIMB

In both cases, an incision was made at the distal end of the forearm stump. The flexor and extensor muscle groups were identified and isolated using a combination of blunt and sharp dissection techniques.

Simultaneously, in a neighboring operating room, the composite allograft was prepared. Bone segments were resected to the required length, and the muscle compartments, together with the associated neurovascular structures, were carefully isolated. After a comparative assessment of the recipient's second forearm length and the stump length, the graft bones were trimmed accordingly.

Osteosynthesis of the ulna was performed using a nine-hole plate with 3.5-mm screws, while fixation of the radius was achieved using a nine-hole plate secured with six screws. After removal of excess muscle from the general flexor group, the superficial muscle group and deep portions of the flexors were isolated.

In both cases, arterial reconstruction was performed by end-to-end anastomosis between the donor and recipient brachial arteries. Under optical magnification, venous outflow was restored through anastomoses of the accompanying veins, including one saphenous vein. In total, the first case involved four venous anastomoses and one

arterial anastomosis, while the second case required five venous anastomoses and one arterial anastomosis.

After blood flow was restored, flexor and extensor tendons were reconstructed. Following mobilization and preparation, epineural sutures were placed on the median, ulnar, and radial nerves. The deep extensor muscle groups on the posterior aspect of the forearm were then sutured.

After completion of reconstruction, the wound was sutured layer by layer. The final appearance of the surgical site is shown in Fig. 4. The limb was immobilized in a position of elbow flexion and approximately 20° of wrist extension. Limb positioning and immobilization were guided by Doppler ultrasound assessment of graft arterial and venous flow. Continuous monitoring using peripheral pulse oximetry was employed to assess microcirculatory perfusion and graft viability.

IMMUNOSUPPRESSIVE THERAPY IN DISTAL UPPER LIMB TRANSPLANTATION

In both cases, preoperative immunosuppressive preparation included initiation of tacrolimus therapy at a dose of 2 mg administered 3 hours before transplantation. In the early postoperative period, the drug was delivered via a nasogastric tube. Subsequent administration followed a standard 12-hour dosing regimen, with dose adjustments performed to achieve target trough concentrations of 12–15 ng/mL.

Induction immunosuppression consisted of two intravenous administrations of basiliximab at a dose of 20 mg, given intraoperatively and on postoperative day 4. Immediately prior to allograft reperfusion, intravenous

methylprednisolone was administered at a dose of 10 mg/kg body weight.

Maintenance immunosuppressive therapy included continued tacrolimus administration with individualized dose adjustment, together with oral methylprednisolone at a dose of 16 mg daily. One week after transplantation, mycophenolate mofetil was initiated at a dose of 2000 mg/day.

EARLY POSTOPERATIVE PERIOD

In both cases, the postoperative course was uneventful. Postoperative management was conducted according to a standardized treatment protocol. The primary goal of treatment was to maintain target hematocrit levels, adequate graft perfusion, appropriate anticoagulation parameters, and prevention of vascular thrombosis, inflammatory complications, and pain syndrome.

During the first 7 postoperative days, infusion therapy was restricted to 1,500–2,000 mL/day. Blood electrolyte levels, acid–base balance, and anticoagulation parameters were monitored every 3 hours.

Continuous monitoring of peripheral pulse oximetry in the transplanted limb was maintained throughout the early postoperative period. Under gastroprotective therapy, cyclooxygenase-1 and cyclooxygenase-2 inhibitors were administered, together with prolonged peripheral regional analgesia. In addition, continuous infusion of prostaglandin E1 at a dose of 60 µg/day and prolonged continuous heparin administration were used to prevent vascular complications.

After 7 days, anticoagulation therapy was transitioned from continuous heparin infusion to intermittent admi-



Fig. 4. Final appearance of the surgical site following completion of the transplant

nistration of low-molecular-weight heparins combined with oral antiplatelet therapy. Antimicrobial prophylaxis included intravenous broad-spectrum antibiotics and antifungal agents, followed by a switch to oral formulations.

Postoperative wounds healed by primary intention in both cases. Delayed bone repair in the osteosynthesis region was observed in both recipients; therefore, osteogenic hydroxyapatite-based compounds were prescribed.



Fig. 5. Demonstration of motor function in Patient A's transplanted hand on postoperative day 120



Fig. 6. X-ray of Patient A's surgical site on postoperative day 120

RESULTS

This article outlines the key stages in the organization and initial implementation of a domestic program for allograft transplantation of the distal upper limb, as undertaken during the preparation and performance of the first two clinical cases.

Recipient A is currently under outpatient follow-up and reports gradual restoration of cutaneous sensation extending from the postoperative scar toward the distal part of the transplanted hand. Thanks to comprehensive rehabilitation measures, the patient demonstrates mobility in all fingers and is regaining motor skills required for daily activities (Fig. 5).

An X-ray of the surgical site shows a well-formed bone callus (Fig. 6). Regular clinical and laboratory follow-up shows no evidence of graft rejection. With tacrolimus concentrations maintained within the target therapeutic range, no adverse effects related to immunosuppressive therapy have been observed. The patient continues rehabilitation and alongside regular outpatient follow-up.

Recipient B, who received a shorter vascularized allograft, demonstrated the ability to partially flex the fingers in the immediate postoperative period, attributed to the preservation of his own forearm muscles in the stump (Fig. 7).

Within 1.5 months after the transplant, an episode of acute rejection was diagnosed based on characteristic clinical findings, including hyperemia, erythematous changes in the graft skin, and graft edema. Laboratory tests indicated increased activity of the T-cell-mediated



Fig. 7. Demonstration of motor function in Patient B's transplanted hand on postoperative day 145



Fig. 8. X-ray of Patient B's surgical site on postoperative day 45

immune response, and treatment with anti-thymocyte globulin was initiated at a dose of 1.5 mg/kg.

Within 24 hours of the first administration, the clinical manifestations described above resolved completely and did not recur. The patient continues comprehensive rehabilitation therapy. A radiograph of the surgical site is shown in Fig. 8.

DISCUSSION AND CONCLUSIONS

Internationally, the number of VCA procedures – particularly upper limb transplantation – remains significantly lower than that of solid organ transplants [1–3]. This is mainly due to limited indications, strict recipient selection criteria, and inherent surgical and immunological challenges. The growing number of patients requiring this type of medical care in recent years underscores the need to develop dedicated upper limb transplant programs.

The combined clinical experience and scientific expertise of leading transplant and trauma centers within the Russian Ministry of Health, including Shumakov National Medical Research Center of Transplantology and Artificial Organs and Priorov National Medical Research Center of Traumatology and Orthopedics, have made it possible to rapidly implement the ambitious task of establishing a new field of clinical transplantology for our country at Shumakov National Medical Research Center of Transplantology and Artificial Organs.

The data presented in this study should be regarded not as a demonstration of a final outcome, but rather as an intermediate stage in addressing key fundamental challenges that previously represented major barriers to the implementation of such procedures in the Russian Federation.

This article did not aim to comprehensively address issues related to referral pathways for transplant candidates or the establishment of a formal waiting list system. Further development of the upper limb transplant program, and of the field more broadly, will require detailed consideration of these aspects, which remain topics for future investigation and publication.

The authors declare no conflict of interest.

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SUCCESSFUL ORTHOTOPIC LIVER TRANSPLANTATION FOR DIFFUSE HEPATIC HEMANGIOMATOSIS IN AN ADULT PATIENT

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Hepatic hemangioma is the most common benign focal lesion of the liver and is usually small and asymptomatic. However, giant hemangiomas may require surgical intervention, while diffuse hepatic hemangiomas is exceedingly rare. We report a rare case of diffuse hepatic hemangiomas in a 59-year-old patient complicated by portal hypertension, inferior vena cava syndrome, liver failure, and Kasabach–Merritt syndrome, who successfully underwent orthotopic liver transplantation (LT) from a deceased donor. This case demonstrates the malignant course of a relatively benign disease that required LT. Although giant liver hemangiomas are a rare indication for LT, in selected cases where conservative and surgical treatments are ineffective or contraindicated, transplantation may be a safe and effective therapeutic option.

Keywords: *hepatic hemangiomas, benign liver tumors, liver transplantation, Kasabach–Merritt syndrome, portal hypertension, inferior vena cava syndrome.*

INTRODUCTION

Hepatic hemangioma is the most common benign liver tumor of mesodermal origin. It consists of blood-filled vascular cavities lined by a single layer of flattened endothelial cells and is primarily supplied by the portal venous system [1]. Giant hepatic hemangiomas represent an atypical form characterized by a diameter greater than 5 cm and often require surgical intervention [2].

Diffuse hepatic hemangiomas (DHH) is a rare condition in which a substantial portion of the liver parenchyma is replaced by multiple vascular lesions. In adults, DHH may be associated with hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu disease), a systemic fibrovascular dysplasia with an autosomal dominant pattern of inheritance. The most common clinical manifestations include recurrent epistaxis and telangiectasias of the skin and mucous membranes, particularly affecting the face, hands, and oral cavity. Involvement of the gastrointestinal tract, lungs, central nervous system, and liver has also been reported [3].

Isolated DHH without extrahepatic manifestations is extremely rare in adults. An even rarer presentation is the coexistence of DHH with a giant hepatic cavernous hemangioma [2, 3]. This combination may be complicated by the development of Kasabach–Merritt syndrome (KMS).

KMS is a rare but potentially life-threatening complication associated with large vascular tumors, including giant hepatic hemangiomas. It is characterized by a form

of consumption coagulopathy resulting from activation of intravascular coagulation, leading to severe thrombocytopenia, hypofibrinogenemia, prolonged prothrombin time, and, in advanced cases, disseminated intravascular coagulation (DIC).

The pathogenesis involves sequestration and destruction of platelets and coagulation factors within the large vascular sinuses of the hemangioma. This process results in rapid clinical deterioration, with a high risk of uncontrolled bleeding, multiple organ failure, and death in the absence of timely intervention.

Given its aggressive and life-threatening course, KMS constitutes a major indication for urgent LT. In cases where conservative and minimally invasive approaches are ineffective, LT as the only definitive treatment in cases where conservative and minimally invasive therapies prove ineffective [4, 5].

The literature to date includes only isolated reports of aggressive disease progression necessitating LT [6].

CLINICAL CASE REPORT

Patient K., a 35-year-old male, first noted abdominal distension in 1999. In 2001, he sought medical evaluation, at which time magnetic resonance imaging (MRI) of the abdomen revealed a cystic mass with irregular contours and septations in the left hepatic lobe, measuring 125×80 mm, with compression of the inferior vena cava and portal vein. Additional lesions measuring 35×25 mm

and 60×60 mm were identified in segments V and VII, respectively. To clarify the nature of these lesions, diagnostic laparoscopy was performed. Intraoperatively, a large tumor measuring approximately 15×15 cm was observed in the left lobe, soft with prominent vascularity. Multiple smaller lesions, ranging from 1.0 to 1.5 cm and visually consistent with hemangiomas, were distributed across both hepatic lobes.

Based on these findings, DHH was diagnosed. The disease remained asymptomatic for several years until 2016. Beginning in 2017, the patient developed ascites and hepatosplenomegaly.

In October 2023, a 59-year-old patient was admitted to the Internal Medicine Department, Shumakov National Medical Research Center of Transplantology and Artificial Organs, presenting with severe weakness, abdominal distension due to tense ascites, abdominal pain, leg edema, and exertional dyspnea. On physical examination, the patient exhibited marked sarcopenia, multiple tophi on the fingers and toes, and tense ascites, with an abdominal circumference of 156 cm.

Laboratory tests revealed signs of liver failure (coagulopathy, hyperbilirubinemia, and hypoalbuminemia), thrombocytopenia, cholestasis, as well as impaired renal function.

Contrast-enhanced computed tomography (CT) of the abdomen demonstrated pronounced hepatomegaly with diffuse parenchymal alterations. The liver had irregular contours and was markedly enlarged, with a craniocaudal dimension of 457 mm in the right lobe and 218 mm in the left lobe. Multiple hypervascular lesions with lacunar contrast enhancement, along with areas of hypoperfusion, were identified in both lobes.

Signs of portal hypertension were present, including dilation of the portal vein (14 mm) and splenic

vein (7.5 mm), esophageal varices, and gastric cardia, splenomegaly, and the presence of a splenorenal shunt. In addition, compression of the inferior vena cava at the hepatic and subhepatic levels was noted (Fig. 1).

The patient underwent diagnostic and therapeutic paracentesis, with a total of 45 liters of light-yellow ascitic fluid evacuated, accompanied by albumin replacement. Medical management included choleretic, hypouricemic, anti-inflammatory, gastroprotective, hypoammonemic, and diuretic therapy. Given the lack of response to conservative treatment and the unfavorable prognosis, orthotopic LT was indicated. The patient was subsequently placed on the waiting list for donor organs at Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow.

Surgical Procedure

In November 2023, the patient underwent orthotopic LT. Owing to the markedly enlarged liver, a bisubcostal laparotomy with an upper midline extension (Mercedes incision) was performed instead of the conventional J-shaped approach (Fig. 2). Considering the high risk of hemangioma rupture and massive intraoperative bleeding during hepatectomy, a cell salvage system (Cell Saver) was employed from the outset of the procedure. Despite technical challenges related to liver mobilization, a cava-preserving (piggyback) hepatectomy technique was utilized to minimize hemodynamic instability and reduce the risk of postoperative acute kidney injury.

Caval reconstruction was performed using an end-to-side cava-caval anastomosis incorporating the hepatic vein orifices, according to the Tzakis technique. Portal and arterial revascularization were achieved sequentially through end-to-end anastomoses using the standard

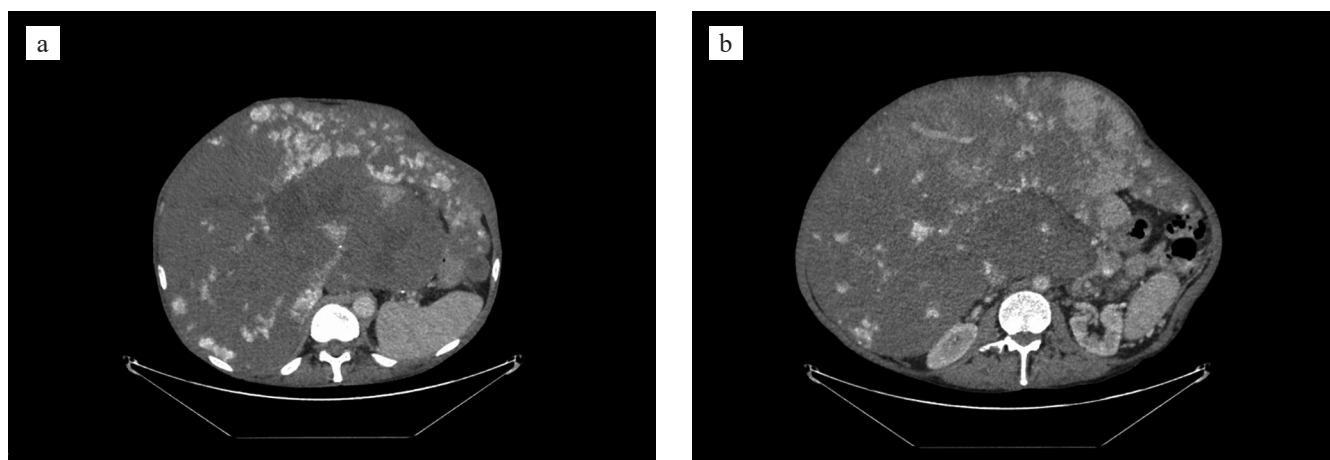


Fig. 1. Contrast-enhanced spiral computed tomography of the abdomen and retroperitoneal space in a patient with diffuse hepatic hemangiomatosis and giant hemangiomas: a, axial section at the liver level during the arterial phase of contrast enhancement: multiple hypodense foci are visible in both lobes of the liver, some of which (>5 cm) have characteristic peripheral nodular enhancement; b, axial section at the liver level during the venous-parenchymatous phase of contrast enhancement: centripetal accumulation of contrast agent in giant hemangiomas is visualized

parachute technique, thereby minimizing the risk of anastomotic kinking or deformation.

Biliary reconstruction was completed via an end-to-end stricture using a 6-0 PDS suture.

The total operative time was 7 hours. Intraoperative blood loss was 1600 mL, with 400 mL reinfused, and

transfusion of 2 units of packed red blood cells was required.

Immunosuppressive Therapy and Postoperative Course

The postoperative period was uneventful, with no complications observed (Fig. 3). The patient was discharged on postoperative day 16 without significant adverse events. The immunosuppressive regimen consisted of extended-release tacrolimus and mycophenolic acid. At one-year follow-up, the patient remains in good clinical condition and continues regular monitoring at Shumakov National Medical Research Center of Transplantology and Artificial Organs. Liver graft function is satisfactory.

DISCUSSION

DHH is a rare disorder characterized by replacement of liver parenchyma by vascular lesions, often associated with hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu), a systemic fibrovascular dysplasia with autosomal dominant inheritance. The most common clinical manifestations include recurrent epistaxis and mucocutaneous telangiectasias involving the face, hands, and mouth. Involvement of the gastrointestinal tract, lungs, central nervous system, and liver has also been reported [3]. Reports of DHH without extrahepatic manifestations in adults are rare.

In the present case, clinical and imaging findings suggest the coexistence of two pathological processes:

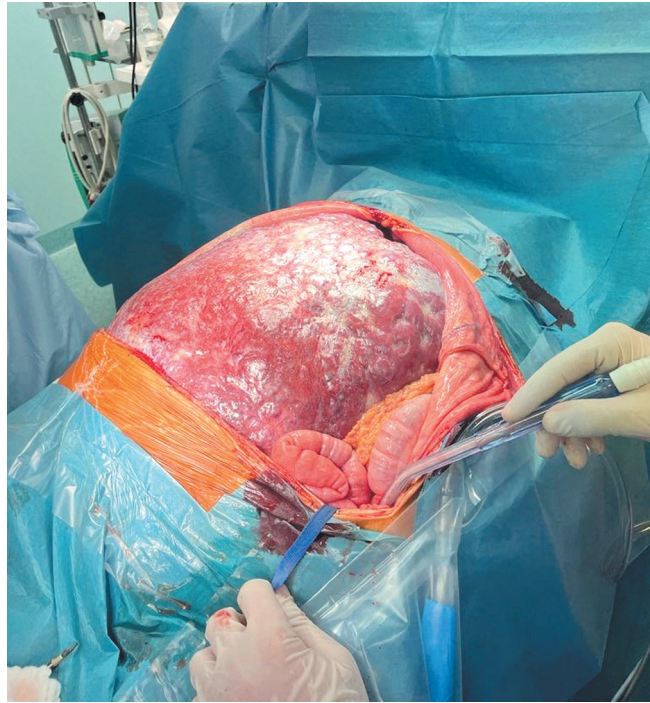


Fig. 2. Subcostal laparotomy supplemented by a midline incision (Mercedes incision)

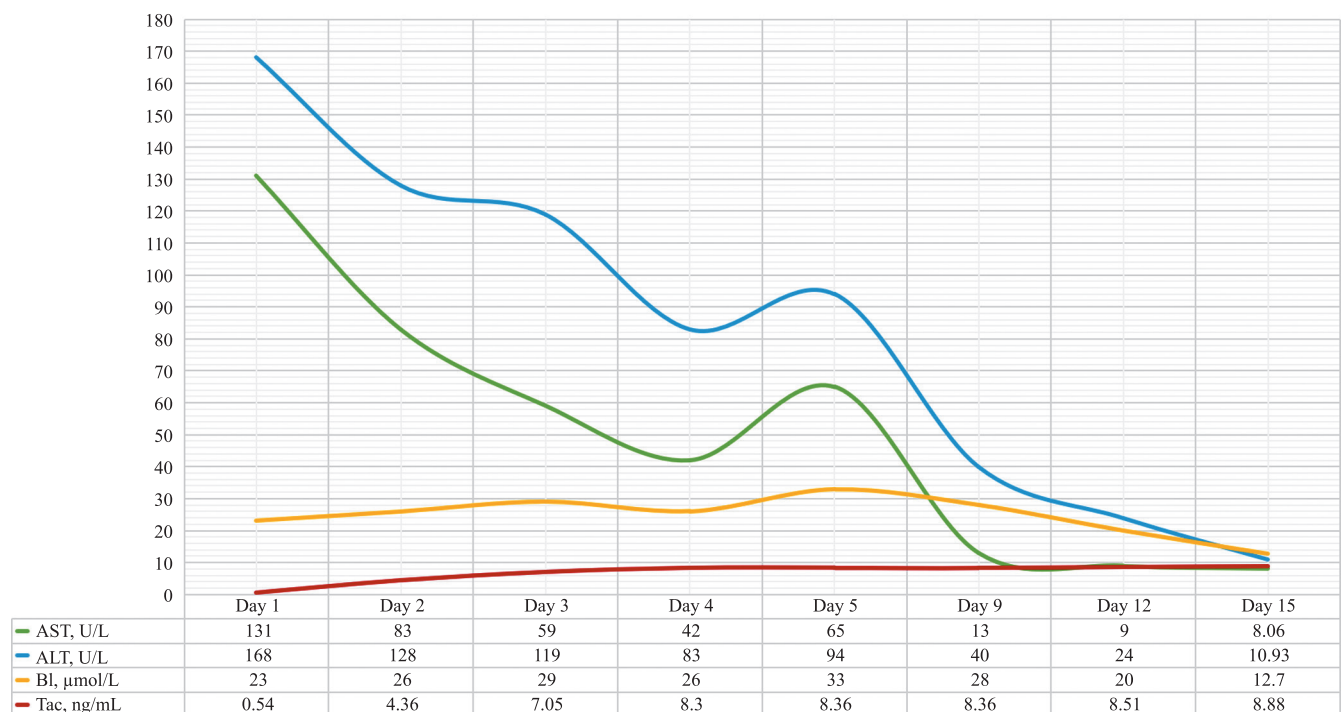


Fig. 3. Dynamics of laboratory parameters in the postoperative period. AST, aspartate aminotransferase; ALT, alanine aminotransferase; Bi, total bilirubin; Tac, tacrolimus level

DHH and multiple cavernous hemangiomas (giant focal lesions). This is supported by abdominal MRI performed in 2001, which showed large cystic-appearing lesions in the left lobe (up to 12.5 cm) and right lobe (segment VII, up to 6 cm), exceeding 5 cm in size and causing compression of major vascular structures – features consistent with giant cavernous hemangiomas.

Additionally, diagnostic laparoscopy at that time revealed numerous small hemangiomas (up to 1.5 cm in diameter), indicating concomitant diffuse hemangiomatosis. In subsequent years, contrast-enhanced CT imaging did not clearly delineate the previously identified large lesions, likely due to the diffuse nature of hemangiomatous involvement, which may obscure lesion boundaries and create the impression of a single diffuse process merging with previously identified large foci [7].

The clinical manifestations of combined giant hepatic hemangiomas and diffuse hemangiomatosis are largely similar and primarily result from vascular ectasia and mass effect. Patients commonly present with abdominal pain, while potential complications include intratumoral hemorrhage, rupture with intra-abdominal bleeding, and high-output cardiac failure due to massive arteriovenous shunting. The latter results from abnormal communication between the hepatic arterial and portal venous systems, allowing high-pressure arterial blood to enter the low-pressure portal circulation [8].

In addition, compression of hepatic venous outflow by hemangiomatous masses may lead to Budd–Chiari syndrome. A pronounced intratumoral consumptive coagulopathy can also develop, resulting in Kasabach–Merritt syndrome, which is characterized by the triad of thrombocytopenia, microangiopathic hemolytic anemia, and disseminated intravascular coagulation (DIC) against the backdrop of a giant vascular liver tumor [4–6].

In our case, the patient exhibited features consistent with classical Kasabach–Merritt syndrome (thrombocytopenia, consumption coagulopathy), along with severe ascites due to vascular compression, reflecting a severe disease course [4, 5].

The optimal treatment strategy for DHH has not been clearly defined and depends on the extent of intact liver parenchyma and the severity of clinical manifestations. In asymptomatic patients, even large hemangiomas may be managed conservatively with regular monitoring. However, the development of symptoms or complications necessitates active intervention.

For localized lesions, standard surgical approaches include hepatic resection or enucleation. Minimally invasive techniques, such as transarterial embolization and radiofrequency ablation, may be used to reduce tumor volume and alleviate symptoms. In selected cases, radiation therapy and anti-vascular endothelial growth factor (anti-VEGF) therapy have also been reported.

However, in diffuse hepatic involvement, these treatment modalities are often ineffective. When extensive tumor burden is accompanied by limited functional liver parenchyma, surgical resection becomes either technically unfeasible or associated with prohibitively high risk. In such cases, liver transplantation remains the only definitive treatment option [7, 9].

In the present case, diffuse vascular liver disease with giant hemangiomas led to life-threatening complications, including portal hypertension, inferior vena cava compression syndrome, and Kasabach–Merritt syndrome, ultimately necessitating orthotopic liver transplantation (OLT). This case highlights that, in carefully selected patients with severe benign vascular liver tumors, transplantation can serve as a viable and potentially curative treatment option.

Following OLT, the patient experienced rapid restoration of liver function. Discharge on postoperative day 16 and sustained clinical stability at one-year follow-up further demonstrate the safety and effectiveness of this approach in such complex clinical scenarios.

Overall, this case underscores the successful role of OLT in the management of DHH with giant hemangiomas.

CONCLUSION

DHH presenting with giant hemangiomas is an extremely rare condition that may result in severe, life-threatening complications, including Budd–Chiari syndrome, portal hypertension, and Kasabach–Merritt syndrome. We report a successful case of OLT in a 59-year-old patient with this condition. This case highlights that even histologically benign liver tumors can follow an aggressive clinical course and may ultimately require transplantation as the only life-saving treatment. OLT should be considered a safe and effective treatment option in rare situations where other treatments are not feasible or have failed.

The authors declare no conflict of interest.

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MAINTENANCE IMMUNOSUPPRESSION AFTER LIVER TRANSPLANTATION: DATA FROM A LOCAL TRANSPLANT REGISTRY

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The steady annual increase in the number of liver transplants (LT) in Russia, together with the expansion of the liver recipient population, underscores the need for large-scale studies on the actual clinical practice of prescribing and managing immunosuppressive therapy. **Objective:** to analyze the structure of maintenance immunosuppressive therapy (MIT) at various time points after liver transplantation and to assess the evolution of approaches to selecting initial immunosuppressive regimens between 2010 and 2024. **Materials and methods.** This single-center, retrospective registry study included data from 568 consecutive LT performed between 2010 and 2024, using grafts from living-related (72%) and deceased (28%) donors. The MIT composition was evaluated at six time points: at hospital discharge and at 1, 3, 5, 7, and 10 years after transplantation. **Results.** At hospital discharge and at 1, 5, and 10 years after LT, calcineurin inhibitors (CNIs) were prescribed in 99%, 96%, 94%, and 90% of patients, respectively. The use of glucocorticoids (St) decreased over time, accounting for 51%, 17%, 10%, and 14%, respectively. Proliferation signal inhibitors (mTOR inhibitors) were prescribed in 13%, 17%, 14%, and 14% of cases, while antimetabolites (A/M) were used in 6%, 7%, 7%, and 14% of patients, respectively. At intervals ranging from one to ten years after transplantation, CNI monotherapy was used in approximately 70% of recipients. Combination regimens included CNI + St in 7%, CNI + A/M ± St in 8%, CNI + mTOR ± St in 8%, and mTOR ± St in 7% of patients. At discharge and at 5 years after transplantation, mTOR-based regimens were more commonly prescribed in patients who underwent surgery for liver tumors (48% and 57%, respectively), A/M-based regimens in patients with immune-mediated liver diseases (22% and 18%), and CNI monotherapy in recipients with viral cirrhosis (52% and 89%). Immunosuppression regimens changed with a frequency ranging from 9% (in the 5–7 year interval) to 49% (in the interval from discharge to 1 year). Over the period from 2010 to 2024, the most notable trends in initial immunosuppression included a shift from immediate-release to prolonged-release tacrolimus, as well as increased use of mycophenolates and mTOR inhibitors, rising from 2% and 4% in 2010–2015 to 9% and 21% in 2019–2024, respectively. **Conclusion.** The underlying etiology of the disease remains the primary determinant in selecting MIT. The high prevalence of CNI monotherapy from 1 to 10 years post-transplant provides a strong rationale for initiating clinical trials to evaluate the safety and efficacy of minimizing or discontinuing immunosuppressive therapy in liver transplant recipients.

Keywords: liver transplantation, immunosuppressive therapy, tacrolimus, cyclosporine, glucocorticoids, mycophenolic acid, azathioprine, everolimus.

INTRODUCTION

Over the past 20 years, the number of LT performed annually in Russia has steadily increased. For instance, the number of procedures rose from 33 in 2004 to 829 in 2023. This growth in transplant activity has been accompanied by a corresponding rise in the population of LT recipients. According to estimates from the Russian Transplant Society, the number of patients living with a transplanted liver increased fourfold between 2013 and 2023 – from 1,150 to 4,644 individuals [1].

In this context, the accessibility and quality of outpatient care for transplant recipients have become increa-

singly important. A central and specific challenge is the long-term management of maintenance IST.

To date, no multicenter studies in Russia have comprehensively evaluated current practices in pharmacological immunosuppression. Most available studies focus either on the early postoperative period or on specific subgroups of recipients. Moreover, studies published more than a decade ago were typically based on small patient cohorts with limited follow-up durations and may no longer accurately reflect actual clinical practice.

This report presents an analysis of the composition and changes in maintenance IST from hospital discharge up to 10 years after LT. In addition, it traces the evolution

of approaches to selecting initial immunosuppressive regimens over the 15-year history of the program at Burnazyan Federal Medical and Biophysical Center, Moscow.

MATERIALS AND METHODS

The data for this analysis were obtained from the Local Scientific Registry of Transplantations at Burnazyan Federal Medical and Biophysical Center. Between May 26, 2010, and December 31, 2024, a total of 568 consecutive LT were performed, including 411 (72%) from living-related donors and 157 (28%) from deceased donors with brain death and preserved circulation at the time of transplantation.

Overall, 549 patients underwent transplantation, comprising 277 (49%) men and 291 (51%) women. Recipient age at the time of transplantation ranged from 18 to 72 years (median [Me], 45 years; interquartile range [IQR], 37–54). The preoperative MELD-Na score ranged from 6 to 42 (median, 16; IQR, 13–21). According to the Child–Pugh classification, 69 patients (12%) were class A, 280 (49%) class B, and 184 (33%) class C; in 35 cases (6%), classification was not applicable.

The most common indications for transplantation were viral-related liver cirrhosis ($n = 202$; 36%), liver tumors ($n = 96$; 17%), including hepatocellular carcinoma ($n = 87$; 15%), cirrhosis due to cholestatic diseases ($n = 86$; 15%), unresectable hepatic alveolar echinococcosis ($n = 49$; 9%), and cirrhosis of unknown etiology ($n = 43$; 8%).

The median duration of static cold ischemia for living-donor transplantation was 0.6 hours (IQR, 0.5–0.7), with a secondary warm ischemia time of 25 minutes (IQR, 21–35). For deceased-donor transplantation, the corresponding values were 8.6 hours (IQR, 7.4–10.0) and 42 minutes (IQR, 34–50), respectively.

At various time points following the primary transplantation, 22 patients underwent one retransplantation, and one patient underwent two retransplantations. In four cases, the primary transplantations had been performed at other institutions. In total, 24 retransplantations were carried out.

Graft loss was defined as either retransplantation or recipient death. All other cases were censored at the date of the last documented follow-up at the Center for Surgery and Transplantology, at which time the recipient was confirmed to be alive with a functioning graft.

As of March 1, 2025, 159 (28%) of the 568 transplanted grafts had been lost, including 8 (1.4%) occurring more than 10 years after transplantation.

Recipient survival at 1, 3, 5, 7, and 10 years was 85% (95% CI, 82–88%), 80% (76–83%), 76% (72–80%), 70% (65–75%), and 64% (57–71%), respectively. Corresponding graft survival rates were 83% (80–86%), 77% (73–81%), 73% (69–77%), 67% (62–72%), and 61% (54–67%).

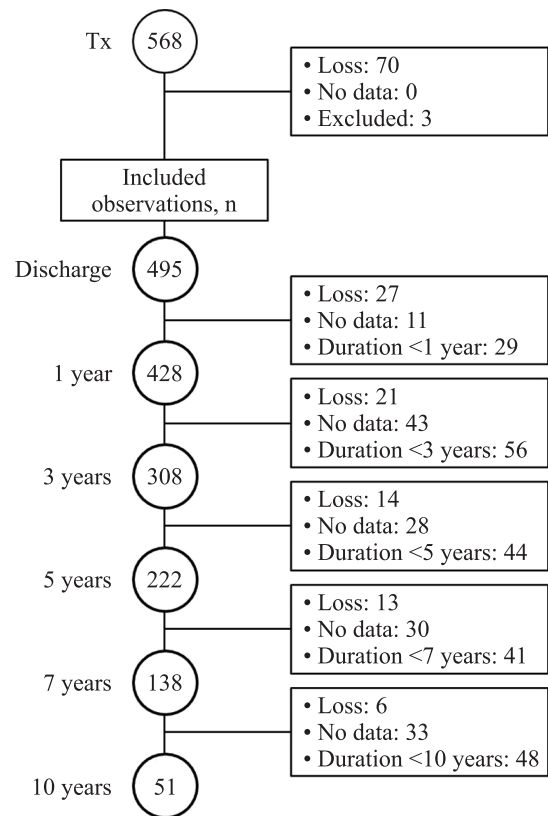


Fig. 1. Study flowchart – distribution of cases by outcomes and time periods after liver transplantation (Tx)

The composition of maintenance IST was evaluated at the time of hospital discharge (approximately 4 weeks after surgery) and at 1, 3, 5, 7, and 10 years after transplantation. At each time point, the number of patients under observation was taken as 100% (Fig. 1). Re-transplants were counted as separate cases. Three cases were excluded from the analysis. In two cases, patients were discharged without IST, as it had been discontinued in the early postoperative period due to sepsis; both patients died within the first year after transplantation. In the third case, the patient independently discontinued tacrolimus monotherapy 3 years after transplantation; the patient remained alive at 5 and 7 years of follow-up but was subsequently lost to follow-up.

IST regimens were categorized according to the classes of agents included in the treatment regimen:

- **Calcineurin inhibitors (CNI):** cyclosporine A (CyA), tacrolimus (Tac), including immediate-release (IR-Tac) and prolonged-release (PR-Tac) formulations;
- **Antimetabolites (A/M):** mycophenolate mofetil (MMF), mycophenolic acid (MPA), azathioprine;
- **mTOR inhibitors:** everolimus, sirolimus;
- **Glucocorticoids (St):** prednisolone, methylprednisolone.

Induction and initial maintenance immunosuppression

In all cases, basiliximab was used for induction therapy at a dose of 20 mg administered intraoperatively and repeated on postoperative day 4. In addition, methylprednisolone was administered prior to graft reperfusion at a dose of 10 mg/kg. From postoperative day 1, methylprednisolone was administered intravenously once daily with stepwise dose reduction: 500 mg → 250 mg → 125 mg → 60 mg when 750–1000 mg had been given intraoperatively, or 250 mg → 125 mg → 60 mg when the intraoperative dose was 500 mg. Beginning on postoperative days 4–5, patients were transitioned to oral methylprednisolone (prednisolone) at an initial dose of 16 (20) mg/day, followed by tapering to 4 (5) mg/day or complete discontinuation by the end of postoperative week 4.

Tac was initiated on postoperative day 3 at a fixed dose of 1 mg, with subsequent dose adjustment to achieve target trough plasma levels of 8–10 ng/mL. When everolimus was included in the initial maintenance immunosuppressive regimen, it was introduced on postoperative days 10–12 at a dose of 3 mg/day, followed by dose titration to maintain target blood levels of 3–8 ng/mL. In such cases, the target Tac concentration was reduced to 3–5 ng/mL.

Antimetabolites (at early stages, exclusively mycophenolates) were added to the CNI + glucocorticoid regimen on postoperative days 10–12 at doses of 720 mg/day for MPA or 1000 mg/day for MMF.

Statistical analysis

Quantitative variables were summarized using the median, interquartile range (IQR), and minimum and maximum values. Qualitative variables were presented as absolute frequencies and percentages. Differences between two independent groups for categorical variables

were assessed using the chi-square test. Differences were considered statistically significant at $p < 0.05$. Statistical analyses were performed using the Jamovi software package (version 2.3.21.0; Jamovi Project, Australia).

RESULTS

Maintenance immunosuppressive therapy at various time points after liver transplantation

The frequency of use of different classes of immunosuppressants varied depending on the time elapsed since LT (Fig. 2). CNIs were the predominant component of maintenance therapy throughout the follow-up period. At the time of hospital discharge, 491 recipients (99%) were receiving CNIs, including cyclosporine A in 24 (5%) patients, IR-Tac in 174 (35%), and PR-Tac in 293 (59%). Between 1 and 7 years post-transplant, the proportion of patients receiving CNIs remained high (94–96%), declining to 90% at 10 years post-transplant.

At the end of the inpatient treatment phase, glucocorticoids (St) in combination with other immunosuppressants were included in maintenance regimens in 253 cases (51%). By the end of the first year after transplantation, the proportion of patients receiving St had decreased to 17%; between 3 and 7 years, it remained at 9–10%, and by 10 years post-transplant, it had increased to 14%. The use of proliferation signal inhibitors showed no significant variation over time, ranging from 13% to 17% across all follow-up periods. mTOR inhibitors were most frequently prescribed during the 1–3-year period (16–17%). Antimetabolites (A/M) were used least frequently, with a prescription rate of 6–7% from discharge up to 5 years post-transplant. However, their use increased at later time points, reaching 10% at 7 years and 14% at 10 years after transplantation.

In total, eight immunosuppressive regimens were used: CNI; CNI + St; CNI + A/M; CNI + A/M + St;

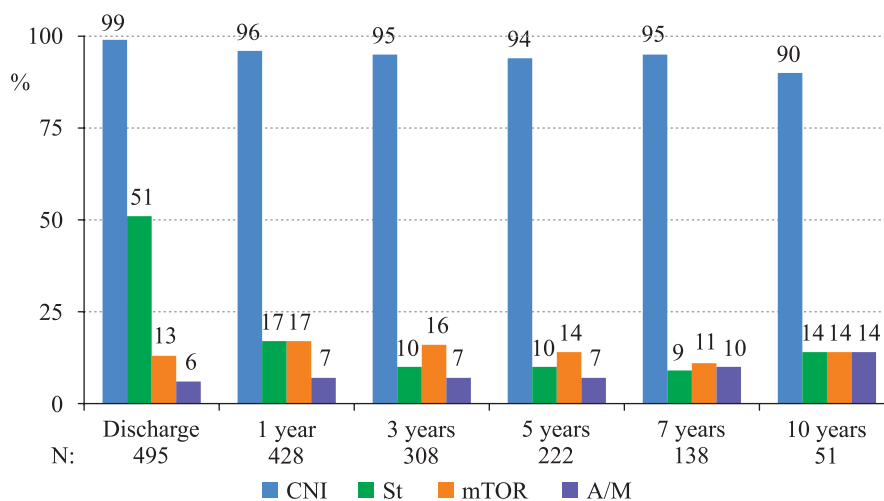


Fig. 2. Immunosuppressants used post-liver transplant. CNI, calcineurin inhibitors; St, glucocorticoids; mTOR, mTOR inhibitors; A/M, antimetabolites

CNI + mTOR; CNI + mTOR + St; mTOR; and mTOR + St. The distribution of maintenance IST according to the number of components in each regimen is presented in Fig. 3a, while Fig. 3b shows the frequency of individual regimens. For clarity, the CNI + A/M and CNI + A/M + St regimens were combined as CNI + A/M $\pm$ St, and CNI + mTOR and CNI + mTOR + St as CNI + mTOR $\pm$ St.

A key trend during the first three years post-transplant was a marked reduction in the use of two- and three-drug regimens: the proportion of patients receiving two-drug therapy decreased from 52% to 19%, while the use of three-drug regimens declined from 9% to 5%. At the

same time, the proportion of patients on monotherapy – primarily CNIs, and less frequently mTOR inhibitors – increased from 39% to 76%.

From discharge through the third year post-transplant, median Tac blood levels gradually decreased from 7.6 to 6.1 ng/mL. From the fifth year onward, levels stabilized at 5.6–5.8 ng/mL. The addition of other agents – A/M, mTOR, and/or St – to maintenance immunosuppressive regimens did not have a significant impact on Tac levels (Fig. 4).

At discharge, Tac levels within the target range of 6–10 ng/mL were observed in 92 patients (50%) receiving monotherapy and in 78 patients (45%) receiving

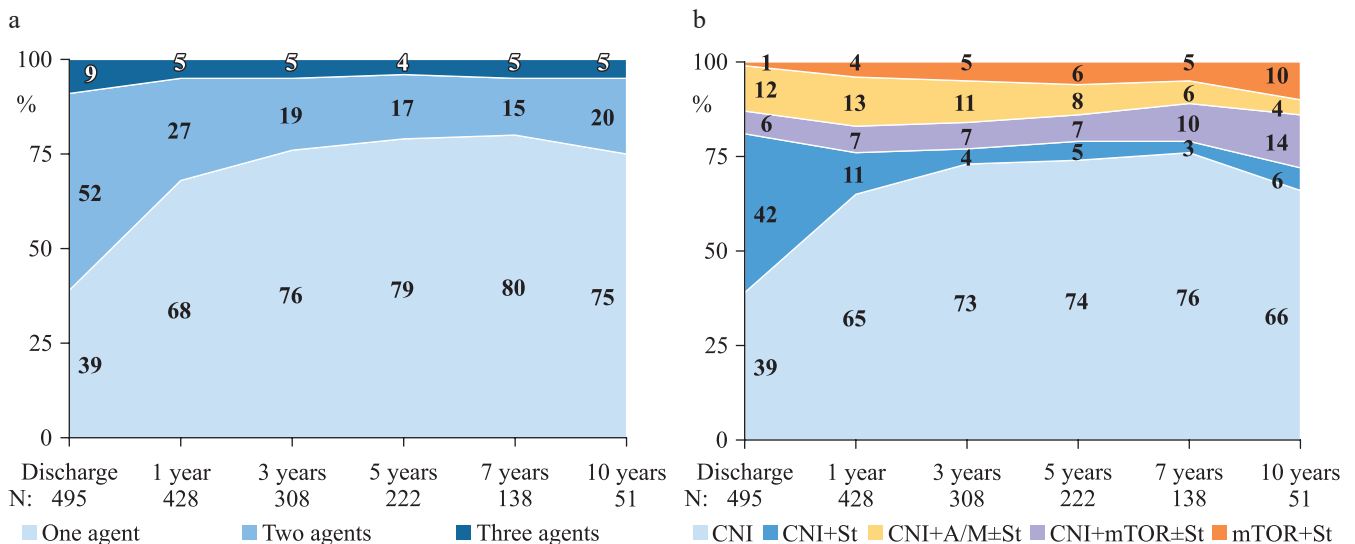


Fig. 3. Structure of maintenance immunosuppression after liver transplantation: (a) number of agents; (b) treatment regimens. CNI, calcineurin inhibitors, St, glucocorticoids; mTOR, mTOR inhibitors; A/M, antimetabolites

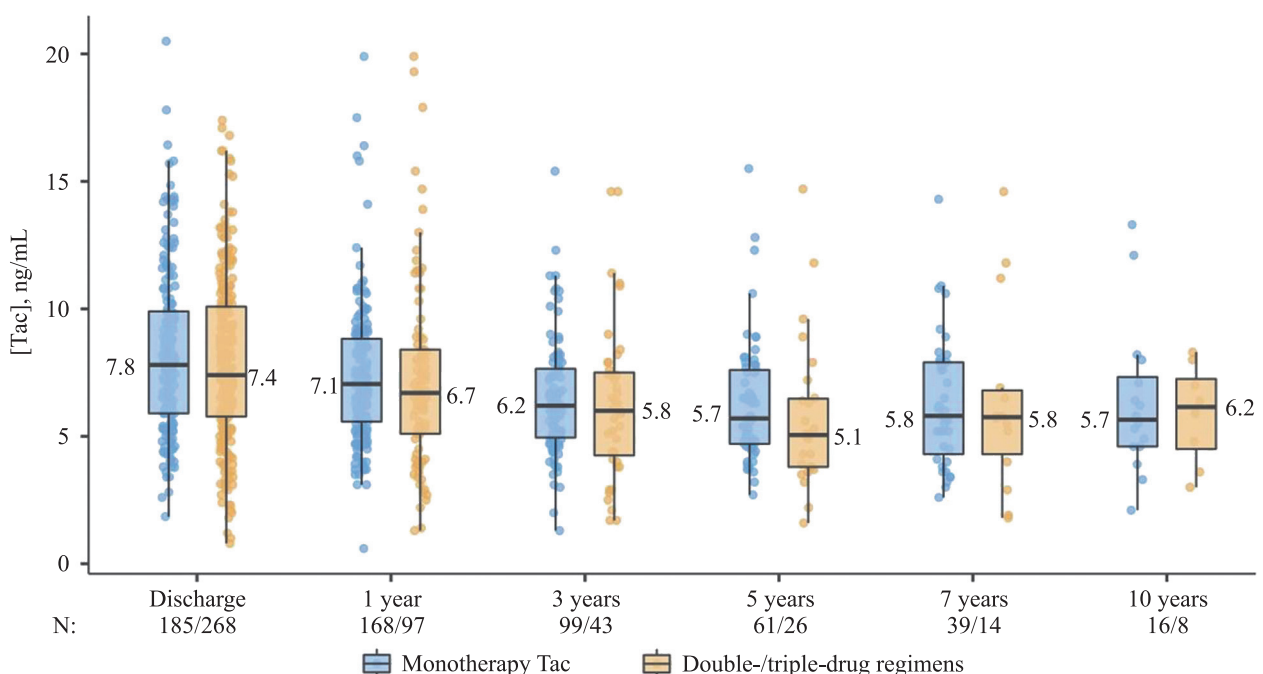


Fig. 4. Tacrolimus trough levels on monotherapy (blue) and double-/triple-drug (yellow) immunosuppression

two- or three-drug regimens. Higher concentrations were recorded in 44 (24%) and 69 (26%) patients, respectively, while lower concentrations were observed in 49 (26%) and 78 (29%).

Over the follow-up period from 1 to 10 years, Tac levels exceeding 6 ng/mL were observed, on average, in 23% of patients receiving monotherapy (Fig. 5a) and in 20% of those on combination IST (Fig. 5b).

At various time points after discharge, modifications of maintenance IST regimens occurred with a frequency ranging from 9% to 49% (Table). In total, 288 discontinuations and 130 additions of immunosuppressive agents were performed. Glucocorticoids were discontinued

most frequently ($n = 201$; 48%), whereas the mTOR inhibitor everolimus was the most commonly added agent ($n = 54$; 13%).

The individual trajectories of maintenance immunosuppression for all patients included in the analysis are presented in Fig. 6. The etiology of the underlying liver disease was a key determinant of maintenance IST strategy. Tac monotherapy was the preferred regimen in patients with viral-related cirrhosis (Fig. 7a): more than half of recipients were receiving it at discharge, and by one year after transplantation, approximately 80% were maintained on this regimen.

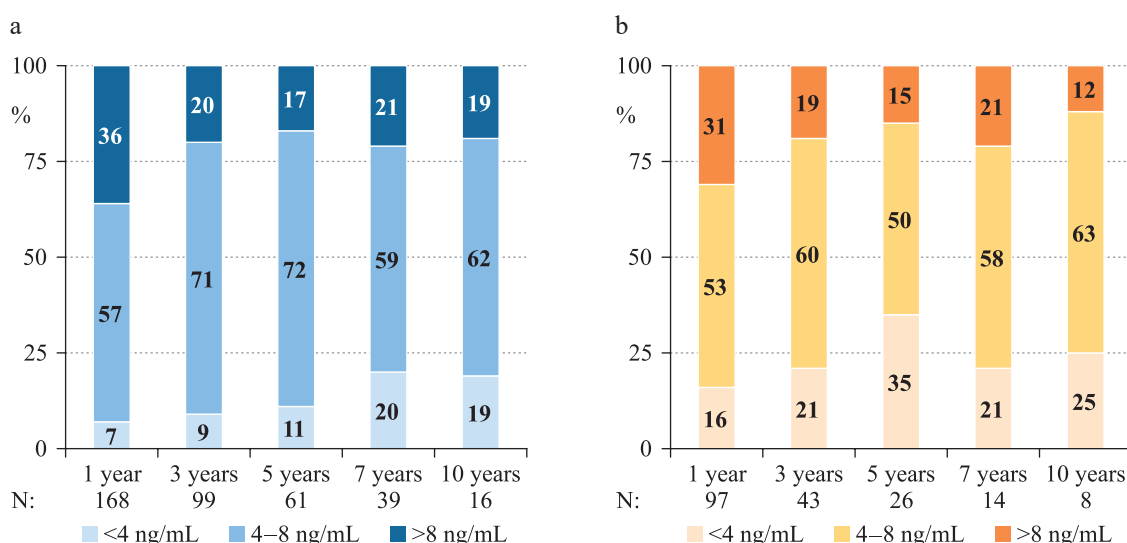


Fig. 5. Liver recipients with tacrolimus trough levels within, above and below the target range (4–8 ng/mL): (a) monotherapy, (b) double- and triple-drug regimens

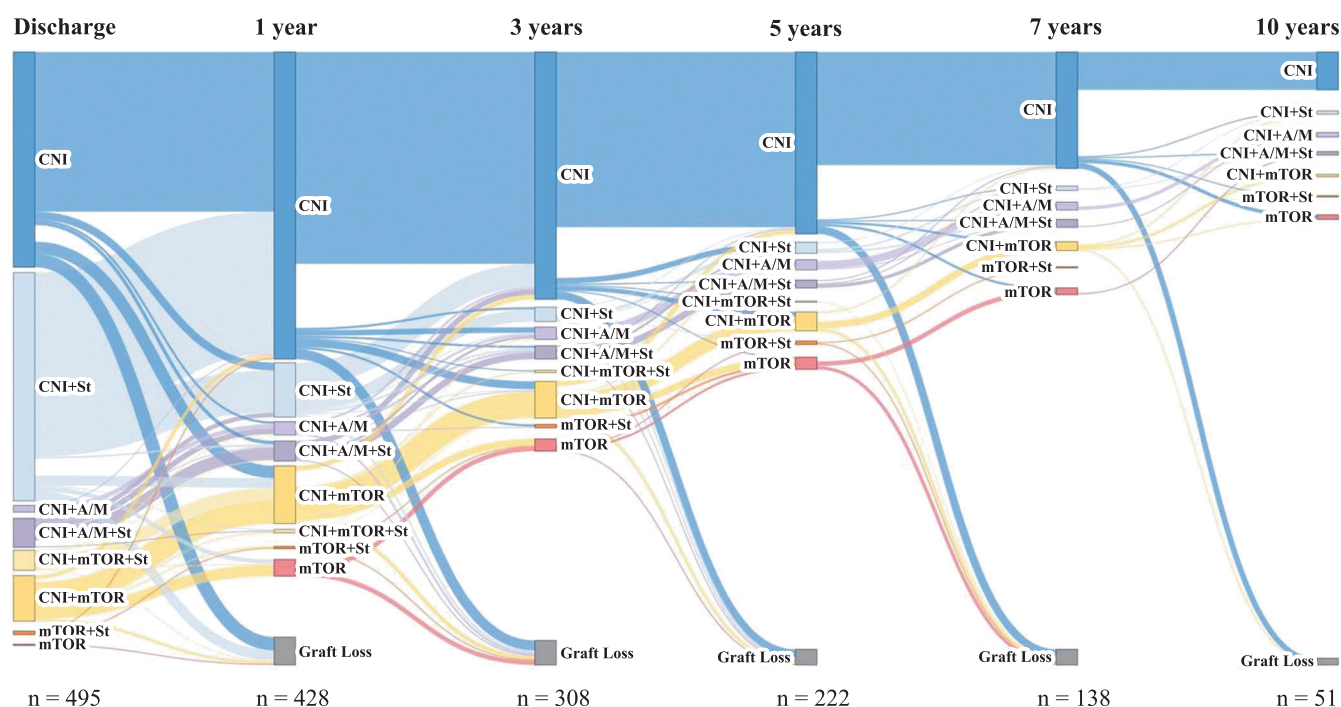


Fig. 6. Individual trajectories of maintenance immunosuppression after liver transplantation

Table

Changes in maintenance immunosuppressive therapy after liver transplantation

Parameter		Discharge – 1 year	1–3 years	3–5 years	5–7 years	7–10 years
Number of immunosuppressive regimens available for modification, n		428	308	222	138	51
Number of modified immunosuppressive regimens, n (%)		209 (49)	70 (23)	33 (15)	13 (9)	11 (22)
Number of additions and cancellations, n (%)		237	90	53	17	21
CNI	addition	1 (<1)	–	–	–	1 (5)
	cancellation	16 (7)	8 (9)	13 (25)	2 (12)	5 (24)
A/M	addition	9 (4)	11 (12)	4 (8)	3 (18)	3 (14)
	cancellation	6 (2)	9 (10)	5 (9)	2 (12)	–
mTOR	addition	26 (11)	13 (14)	7 (13)	4 (23)	4 (19)
	cancellation	6 (2)	5 (6)	7 (13)	1 (5)	2 (9)
St	addition	13 (6)	11 (12)	12 (23)	3 (18)	5 (24)
	cancellation	160 (68)	33 (37)	5 (9)	2 (12)	1 (5)

Note. CNI, calcineurin inhibitors; St, glucocorticoids; mTOR, mTOR inhibitors; A/M, antimetabolites.

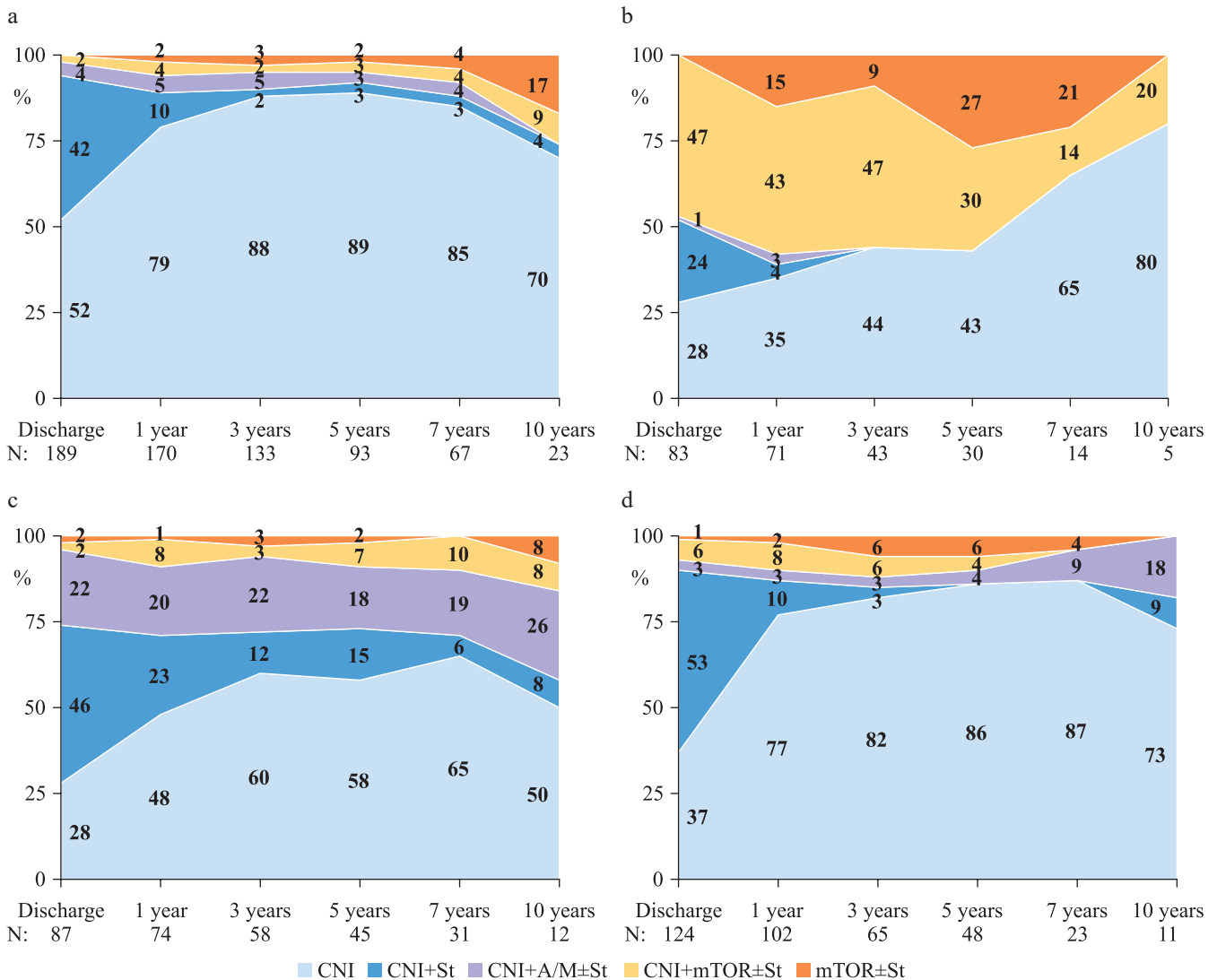


Fig. 7. Maintenance immunosuppressive therapy after liver transplantation for (a) viral cirrhosis, (b) tumors, (c) immune-mediated cirrhosis (AIG, PBC, PSC), (d) parasitic liver disease, cryptogenic and alcoholic cirrhosis, Wilson's disease, and other etiologies

A similar approach was applied in patients undergoing transplantation for unresectable alveolar echinococcosis, cirrhosis of unknown etiology, alcoholic cirrhosis, Wilson's disease, and other indications (Fig. 7d).

At all post-transplant time points, regimens including everolimus were most frequently prescribed in patients transplanted for hepatocellular carcinoma (HCC) and other liver tumors. This group also had the highest proportion of patients not receiving CNIs (Fig. 7b).

Among patients with immune-mediated liver diseases – autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), and primary sclerosing cholangitis (PSC) – antimetabolites were included in the IST regimen in approximately 20% of cases at each time point. In this group, the use of CNI monotherapy increased during the first three years after transplantation, from 28% to 60%, and subsequently stabilized at approximately 55% (Fig. 7c).

The Evolution of Approaches to Selecting Initial Maintenance Immunosuppressive Therapy

Over the 15-year period of the program, the structure of indications for LT has undergone notable changes

(Fig. 8). Beginning in 2019, a clear and persistent trend emerged toward a reduction in the proportion of patients with viral-related cirrhosis without HCC, accompanied by a concurrent increase in the number of transplantations performed for liver tumors. In addition, recent years have seen an increase in the number of transplantations in patients with alcoholic cirrhosis, while the proportion of procedures performed for cholestatic liver diseases has declined.

When comparing two periods of transplant activity – similar in both duration and number of procedures – a statistically significant, approximately twofold increase was observed over time in the proportion of recipients aged over 60 years with diabetes mellitus and arterial hypertension (Fig. 9).

There was also a slight increase in the proportion of recipients with obesity (body mass index >30 kg/m²) and impaired renal function (glomerular filtration rate <60 mL/min/1.73 m²).

These trends, together with accumulated institutional experience, as well as evidence from large international studies and updated clinical guidelines, influenced the approach to selecting maintenance immunosuppressive therapy. During the first five years of the program,

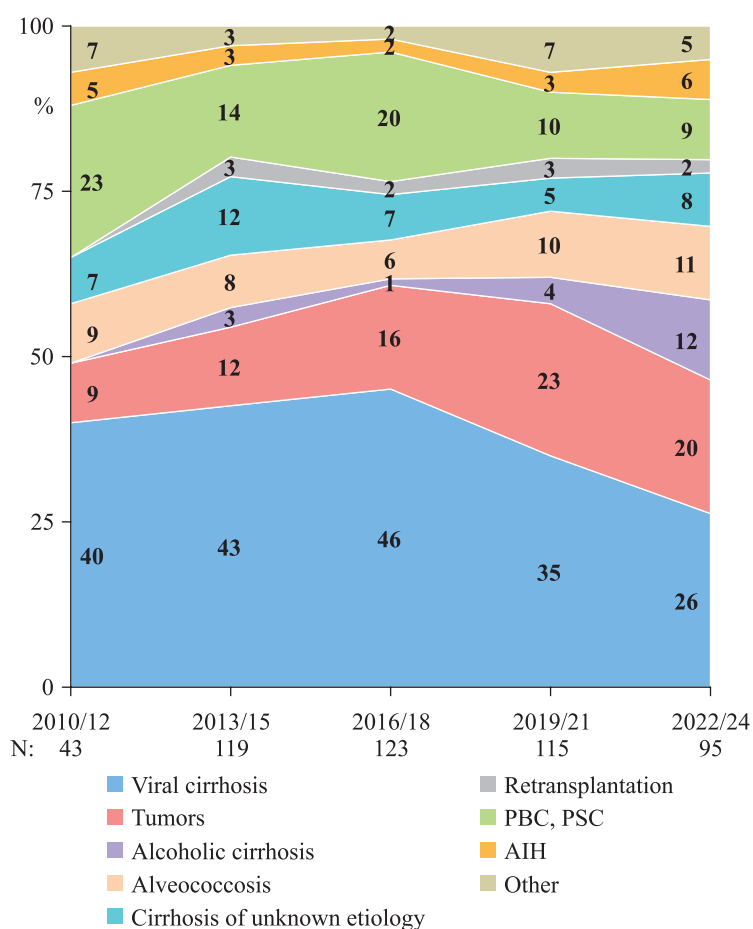


Fig. 8. Changes in the structure of indications for liver transplantation between 2010 and 2024 (discharged patients, N = 495). PBC, primary biliary cholangitis; PSC, primary sclerosing cholangitis; AIH, autoimmune hepatitis

two-drug regimens (CNI + St) predominated; slightly more than one-quarter of patients received CNI monotherapy, while three-drug combinations were rarely used (Fig. 10).

In 2016–2018, compared with the preceding three-year period, the proportion of patients receiving monotherapy doubled, reaching a peak of 55% over the entire observation period. Thereafter, its use declined to approximately 40%, while the increase in two- and three-drug regimens was largely driven by the more frequent incorporation of everolimus-based regimens (CNI + mTOR and CNI + mTOR + St), particularly in patients undergoing transplantation for HCC.

The most notable change in the selection of immunosuppressants for initial immunosuppressive regimens has

been the transition from immediate-release Tac (IR-Tac) to its prolonged-release formulation (PR-Tac). CyA has assumed the role of a “reserve” calcineurin inhibitor, with conversion to CyA in recent years occurring exclusively in cases of serious adverse events (primarily neurological) developed while taking Tac.

The frequency of glucocorticoid use at discharge declined from approximately three-quarters of patients in the early years of the program to one-third in 2016–2018, and has since stabilized at around 50% (Fig. 11).

At the same time, the strategy of early (within 3 months) glucocorticoid withdrawal in patients without immune-mediated liver disease remained unchanged. The timing of steroid discontinuation – whether before or

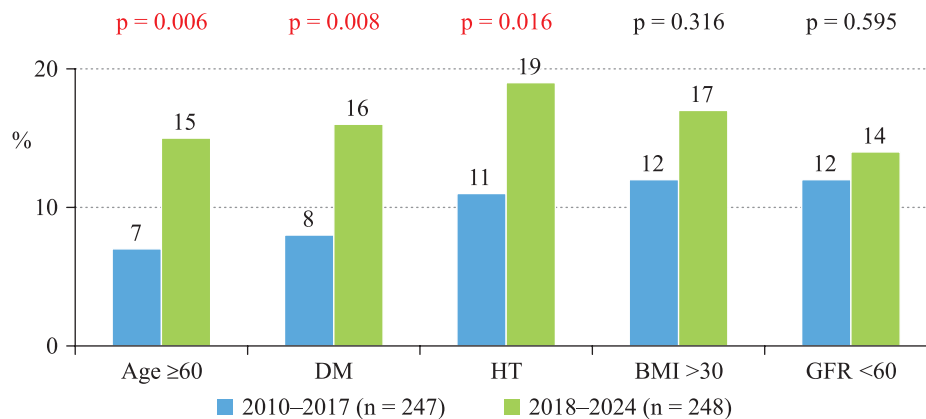


Fig. 9. Proportion of liver recipients at discharge in 2010–2017 (blue) and 2018–2024 (green) with the following characteristics: over 60 years of age, diabetes mellitus (DM), hypertension (HT), obesity (body mass index (BMI) >30 kg/m²), and impaired renal function (glomerular filtration rate (GFR) <60 ml/min/m²)

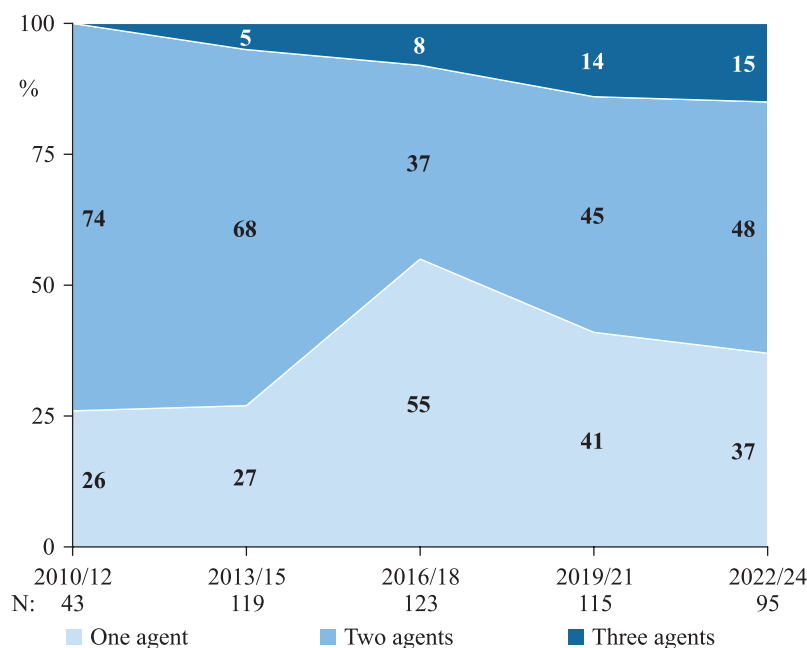


Fig. 10. Single-, dual-, and triple-agent maintenance immunosuppressive regimens at hospital discharge following liver transplantation in 2010–2024

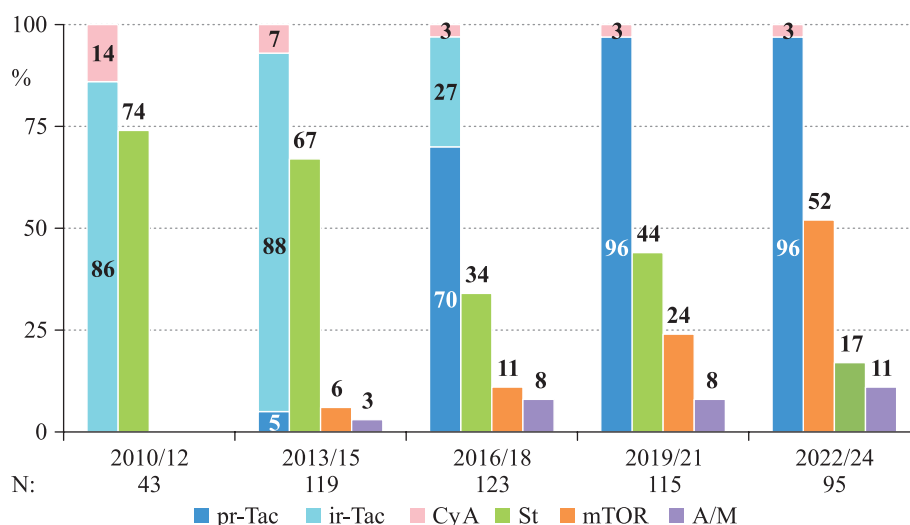


Fig. 11. Frequency of individual immunosuppressants and calcineurin inhibitors at discharge after liver transplantation between 2010 and 2024. pr-Tac, prolonged-release tacrolimus; ir-Tac, immediate-release tacrolimus; CyA, cyclosporine A; St, glucocorticoids; mTOR, mammalian target of rapamycin (mTOR) inhibitors; A/M, antimetabolites

after hospital discharge – was determined by the length of hospitalization and the stability of Tac blood levels.

The practice of prescribing mTOR inhibitors within the first month after transplantation began in 2013. Since 2019, all patients undergoing transplantation for liver tumors, in the absence of contraindications to everolimus, have been discharged on regimens including CNI + mTOR or CNI + mTOR + St. Similar strategies have been employed in recipients with a glomerular filtration rate below <45 mL/min/1.73 m². Until 2016, mycophenolates, primarily MPA, were prescribed mainly to patients with cirrhosis secondary to AIH, and later also to those with PSC.

DISCUSSION

It is well established that, compared with other solid organs, the lower risk of liver graft loss due to rejection permits the safe reduction or discontinuation of intensive IST in many cases. Nevertheless, sustained control of the alloimmune response remains essential for maintaining long-term graft function.

The comparable efficacy of different immunosuppressive regimens provides substantial scope for individualized therapy, taking into account recipient comorbidities, risk factors, and treatment-related complications. However, this flexibility often results in considerable variability in treatment regimens [2], not only between transplant centers but also within a single program and even over the course of treatment in an individual patient.

Clinical trials sponsored by major pharmaceutical companies remain a key driver in the evolution of IST. Their high methodological rigor, advanced technical support, and overall reliability are well recognized. However, when translated into routine clinical practice, the

reported safety and efficacy of novel regimens often do not fully meet expectations.

This discrepancy is typically attributable to several factors [3–5]: highly selective patient recruitment resulting in study populations that are not representative of real-world cohorts; the use of soft (surrogate) endpoints; relatively short follow-up periods that limit assessment of long-term outcomes; and the practical challenges of adhering to strict study protocols in everyday clinical settings. In addition, the potential for bias at the stages of study design, data interpretation, and publication cannot be entirely excluded. In this regard, data from independent research groups and large-scale registry studies are becoming increasingly important from a practical and clinical perspective.

Over the past decade, the principles guiding the initiation and management of IST after LT have remained largely unchanged [6–9]. The introduction of regimens incorporating the mTOR inhibitor everolimus generated considerable expectations; however, their widespread adoption has been limited.

Nevertheless, everolimus-based therapy is strongly recommended for patients with recurrent or *de novo* non-melanoma skin cancer, as well as in combination with reduced (minimal) exposure to CNIs in individuals at high risk of liver tumor recurrence. Furthermore, to mitigate the nephrotoxic effects of CNIs, everolimus – along with mycophenolates and azathioprine – may be used in combination regimens to allow for lower target concentrations of Tac or CyA.

In addition to recipients with oncological risk or impaired renal function, patients undergoing transplantation for immune-mediated liver diseases with a high risk of recurrence require tailored immunosuppressive strate-

gies. In most other cases, Tac monotherapy remains the preferred option for maintenance immunosuppression.

Tac monotherapy – particularly with extended-release formulations – represents an attractive immunosuppressive strategy, as it simplifies the treatment regimen, enhances patient adherence, and avoids adverse effects associated with additional immunosuppressive agents.

With this approach, target Tac levels are typically maintained within the range of 4–8 ng/mL [8]. However, such levels do not always prevent drug-related toxicity and, at the same time, may be insufficient to ensure adequate suppression of the alloimmune response. This may result in prolonged subclinical rejection and an increased risk of graft loss.

Our findings demonstrated no significant differences in Tac levels between monotherapy and two- or three-drug regimens. Over follow-up periods ranging from 1 to 10 years, Tac levels exceeding 6 ng/mL were observed in 23% of patients receiving monotherapy and in 20% of those on combination regimens, suggesting that this pattern is common in routine clinical practice.

In the present study, no comparative analysis of the safety and efficacy of different immunosuppressive regimens was performed. Episodes of acute rejection occurred predominantly within the first months after transplantation, with approximately half arising before hospital discharge. These episodes were rarely associated with severe graft dysfunction, responded to intensification of CNI therapy and/or pulse glucocorticoid treatment, and had a cumulative incidence of no more than 15% over the entire follow-up period. As previously reported [10], rejection led to graft loss in 5 of 500 cases.

Overall, all maintenance IST regimens used – including Tac monotherapy – showed adequate efficacy in preventing acute rejection in both the early and late post-transplant periods.

When interpreting these results, an important feature of the program should be taken into account: the extensive use of living donors. Although current evidence suggests no significant association between the number of A-, B-, and DR-HLA mismatches and the risk of liver allograft rejection [11, 12], the relatively higher degree of histocompatibility in related donor–recipient pairs may represent a key factor enabling the use of less intensive immunosuppressive strategies.

An important, albeit indirect, factor influencing the selection and management of IST is the substitution of original drugs with generic formulations, a practice that began in the 2010s and continues to the present day. There is no objective evidence to suggest that generic formulations of Tac, CyA, everolimus, MPA, or mycophenolate mofetil available in the Russian Federation differ in safety or efficacy from their original counterparts. International data likewise support the feasibility

of using generic immunosuppressants in transplant recipients and indicate no adverse impact on graft outcomes [13–15].

However, the current practice of frequent switching between different brand-name formulations of immunosuppressants provided to recipients under preferential programs raises concerns. Such substitutions may be associated with variability in drug blood levels, necessitating additional laboratory and clinical monitoring, which is not always consistently performed. Moreover, in some cases, a patient's daily dose may be composed of formulations from different manufacturers. In such cases, maintaining immunosuppressant levels at the lower end of the therapeutic range, particularly in the setting of monotherapy, appears risky.

A promising direction in the field of post-liver transplantation immunosuppression is the identification of predictors, biomarkers, and inducers of operational immunological tolerance [16, 17]. Despite the growing body of literature on this topic, withdrawal of immunosuppressive therapy in routine clinical practice is still largely based on a trial-and-error approach, with serum biochemical parameters and histological findings remaining the key guidelines [18, 19].

The decision to discontinue immunosuppression or to reduce it to subtherapeutic levels must therefore be preceded by a careful assessment of potential risks and benefits, the balance of which is highly individualized and not always straightforward [20]. To date, published experience on long-term outcomes in LT recipients maintained on minimal immunosuppression or completely free of IST remains very limited.

CONCLUSION

The analysis showed that the etiology of liver disease necessitating transplantation remains a key determinant in selecting maintenance immunosuppressive strategies. In the final years of the 15-year period under review, there has been an increase in the number of transplants performed for HCC, alongside a rise in recipient age. Consequently, there has been a growing prevalence of comorbidities, including hypertension, diabetes mellitus, obesity, and impaired renal function. These trends are likely to persist and will require substantial adjustments not only in immunosuppressive regimens but also in concomitant therapy.

The basic principles of post-LT immunosuppression are defined in national and international professional society guidelines, which are broadly consistent in their recommendations. In routine clinical practice, adherence to these guidelines ensures, in the vast majority of cases, acceptable transplant outcomes by contemporary standards. However, the concept that immunosuppression remains an “art” is still valid.

This “art” lies not merely in the selection of specific drugs or combinations, but in achieving a balance between rational conservatism – avoiding therapeutic inertia – and timely, and when necessary, decisive yet measured modification of therapy in response to evolving risks or complications.

In the current context, where the emergence of fundamentally new immunosuppressants in the near future appears unlikely, the most realistic strategy for improving long-term transplant outcomes lies in systematic, comprehensive, and regular evaluation of recipients, as well as enhanced adherence to prescribed therapy.

Particular importance should be placed on studies aimed at identifying and validating novel approaches for monitoring the adequacy of immunosuppressive therapy and the strength of transplant immunity. These should include expanded panels of biomarkers, histological and instrumental methods, as well as large-scale multicenter retrospective and prospective studies supported by modern medical data analytics.

This study was conducted as part of the research project “Identification and Management of Factors Determining Long-Term Outcomes of Liver Transplantation in Adult Patients” (EGISU NIOKTR No. 124032000128-9), carried out under a state assignment from the Federal Medical-Biological Agency.

The authors declare no conflict of interest.

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FIRST DIAGNOSIS OF MULTIPLE MYELOMA IN A KIDNEY TRANSPLANT RECIPIENT (A CASE REPORT)

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Background. Kidney graft failure due to recurrence of previously undiagnosed multiple myeloma (MM) is a rare event. This report presents a clinical case of kidney transplantation complicated by graft dysfunction one year after surgery caused by recurrence of undiagnosed MM. **Clinical observation.** A 75-year-old man with a history of arterial hypertension and diabetes mellitus was admitted to the dialysis unit with symptoms of uremia. He had not previously been followed by a nephrologist. Renal ultrasonography revealed diffuse parenchymal changes. Considering the two diseases, chronic kidney disease (CKD C5) secondary to hypertensive and diabetic nephropathy was diagnosed, and maintenance hemodialysis was initiated. After 11 months, the patient underwent deceased-donor kidney transplantation. One year post-transplantation, graft dysfunction developed, prompting a transplant kidney biopsy. Histopathological examination revealed changes characteristic of MM-associated kidney injury, including light-chain cast nephropathy (LCCN, κ type) combined with light-chain proximal tubulopathy (LCPT, κ type), focal segmental glomerulosclerosis, tubulointerstitial nephritis, and acute tubular necrosis. Further evaluation confirmed MM with Bence–Jones proteinuria, stage III B, and myeloma nephropathy of the renal allograft. The patient was transferred to the hematology department for chemotherapy, resulting in partial hematologic remission. However, renal graft function was not restored, and the patient remained on maintenance hemodialysis. **Conclusion.** MM-associated kidney injury is a rare clinical event. In routine clinical practice, thorough pre-transplant evaluation and accurate determination of the etiology of CKD are essential.

Keywords: kidney transplantation, multiple myeloma, cast nephropathy, light-chain proximal tubulopathy.

INTRODUCTION

Patients who have undergone kidney transplantation (KT) are at an increased risk of developing malignant tumors, which impair quality of life and reduce life expectancy. Multiple myeloma (MM) is a B-cell malignancy characterized by the clonal proliferation of plasma cells producing monoclonal immunoglobulin. Although MM is relatively rare in the general population, it is quite common among all hematopoietic and lymphoid tissues, with a frequency of up to 10–15% [1–3].

The course of MM is frequently complicated by renal involvement, leading to the development of acute kidney injury (AKI) as well as acute and chronic kidney disease (CKD), which exacerbate disease severity and prognosis [3, 4]. Kidney damage in MM is heterogeneous and may predominantly affect either the tubulointerstitial compartment or the glomerular apparatus. The most common renal lesion associated with MM is light-chain cast nephropathy (LCCN), which results from excessive production and urinary excretion of monoclonal free immunoglobulin light chains. These light chains exert a direct toxic effect on proximal tubular epithelial cells and contribute to the formation of casts in the distal tu-

bules. In addition to LCCN, other forms of MM-related kidney injury include amyloid light-chain (AL) amyloidosis, characterized by the deposition of monoclonal immunoglobulins with light and/or heavy chains in the glomerular and tubular basement membranes, and, less commonly, light-chain proximal tubulopathy (LCPT). The latter is defined by the accumulation of monoclonal light chains, most commonly kappa, within proximal tubular cells, often accompanied by crystal formation [5–8]. The coexistence of LCCN and LCPT is rare and has been reported only in a limited number of clinical case descriptions [9–11].

There have been isolated clinical reports on cases of primary MM diagnosed in kidney transplant (KT) recipients, resulting in renal allograft dysfunction. Post-KT MM may occur through several mechanisms: (1) recurrence of pre-existing MM, (2) *de novo* MM, or (3) MM associated with the donor kidney [12–16]. In clinical practice, the most frequently encountered scenario is relapse of previously undiagnosed MM in transplant candidates, whereas *de novo* and donor-associated MM are considerably less common.

Histological examination of renal allograft biopsy specimens most often reveals LCCN, while other pat-

terms of graft injury are reported less frequently; in rare cases, LCPT has been reported [13, 14, 17, 18]. A review of the available literature identified only a single reported clinical case documenting the coexistence of LCCN and LCPT in a transplanted kidney in a recipient with subsequently confirmed MM [14].

Here, we present our own rare clinical case of a KT recipient in whom graft dysfunction, developing one year after transplantation, was the first manifestation of previously undiagnosed MM, leading to combined allograft injury in the form of LCCN and LCPT.

CLINICAL CASE

Patient S., a 75-year-old man, was admitted to the emergency department of a medical facility due to a worsening condition. His complaints included increasing weakness, nausea, loss of appetite, and weight loss of 8–10 kg. A few days prior to admission, the patient had undergone self-initiated laboratory testing at a medical center, which revealed markedly elevated serum creatinine (778 $\mu\text{mol/L}$), hyperkalemia (potassium 5.5 mmol/L), and increased blood urea (37.2 mmol/L). Ultrasound examination of the abdominal organs, kidneys, and prostate demonstrated hepatomegaly and diffuse parenchymal changes in the liver, pancreas, kidneys, and prostate. No additional medical records were available at the time of admission.

According to the patient and his relative, he had a long-standing history of arterial hypertension, with maximal blood pressure values reaching 170/100 mmHg, which was well controlled with valsartan (160 mg/day) and indapamide (2.5 mg/day). Four years earlier, he had been diagnosed with type 2 diabetes mellitus and treated with metformin (1000 mg/day), achieving satisfactory blood glucose control.

A week prior to the current deterioration in his condition, the patient experienced an episode of syncope. Subsequent evaluation revealed anemia, with a hemoglobin level of 111 g/L. Upper gastrointestinal endoscopy and colonoscopy were performed; however, no source of bleeding was identified. At that time, serum creatinine was already significantly elevated (590 $\mu\text{mol/L}$), and the patient was advised to see a nephrologist, which was not done.

Upon admission, the patient's condition was moderate. Body temperature was 36.6 °C. Height was 174 cm, body weight 78 kg, and body mass index 25.8 kg/m². He had a normal body habitus with a normosthenic constitution. Skin was jaundiced; no peripheral edema. No abnormalities were detected on neurological or mental status examination, nor were there pathological findings involving the musculoskeletal or endocrine systems. Respiratory rate was 17 breaths per minute. Lung auscultation revealed vesicular breath sounds evenly distributed over all lung fields, without wheezes. Cardiac auscultation demonstrated muffled heart sounds with

a regular rhythm. Heart rate was 93 beats per minute. Blood pressure was 160/80 mm Hg in both arms. The abdomen was of normal configuration, soft, non-tender on palpation, and painless in all quadrants, with no signs of peritoneal irritation. The lower edge of the right hepatic lobe protruded 1 cm below the costal margin. The kidneys were not palpable, and percussion over the lumbar region was painless. Urine output was 500 mL/day over several days. The patient reported frequent urination at night in small volumes.

There was no family history of disease and no known allergies. Epidemiological history was unremarkable: the patient denied contact with infectious sources and reported vaccination against SARS-CoV-2 and hepatitis B virus. Serological testing for HBsAg, anti-HCV antibodies, HIV, and syphilis (RW) was negative.

Laboratory test results. A complete blood count revealed mild anemia with hemoglobin of 113 g/L. White blood cell count ($10.8 \times 10^9/\text{L}$), platelet count ($382 \times 10^9/\text{L}$), and leukocyte differential were within normal limits. Urinalysis was unremarkable; proteinuria was 0.5 g/L. Biochemical blood analysis showed total protein 69 g/L, albumin 46 g/L, urea 62 mmol/L, creatinine 1342 $\mu\text{mol/L}$, glucose 6.5 mmol/L, potassium 6.1 mmol/L, bicarbonate 12.9 mmol/L, and arterial pH 7.27. Bilirubin, liver enzymes, and cholesterol levels were within reference ranges. Lactate dehydrogenase (LDH) was mildly elevated at 259 U/L (reference range 110–210 U/L), and C-reactive protein was 3 mg/L. Glycated hemoglobin (HbA1c) was 7.6%. Serological tests for hepatitis B, hepatitis C, and HIV were negative; SARS-CoV-2 RNA was not detected.

Repeat ultrasound examination demonstrated kidneys of normal size: right kidney 105×62 mm and left kidney 105×64 mm. Renal contours were smooth and well defined. Parenchymal thickness measured 18 mm on the right and 21 mm on the left. The parenchyma exhibited diffusely heterogeneous echogenicity bilaterally. On color doppler flow imaging (CDFI), renal vascularization was preserved and traceable to the capsule. Corticomedullary differentiation was clearly maintained on both sides. The renal pelvicalyceal system was not dilated. Renal sinuses were hyperechoic and contained multiple punctate hyperechoic inclusions measuring 2–3 mm with indistinct acoustic shadows, consistent with microlithiasis. No focal masses were identified.

Conclusion: Ultrasound findings are consistent with diffuse changes in the renal parenchyma.

Echocardiography. Mild dilation of the left cardiac chambers, basal and mid portions of the right ventricle, and ascending aorta was noted. Calcification of the aorta, mitral valve, and aortic valve was present. There were mild regurgitation of the aortic, mitral, tricuspid, and pulmonary valves. Concentric left ventricular hypertrophy was identified. Global left ventricular systolic

function was preserved, with grade II diastolic dysfunction. Minimal pericardial effusion was detected.

Based on the patient's medical history, the presence of two comorbid conditions associated with a high risk of CKD, and the results of laboratory and instrumental investigations, the following diagnosis was established. Primary diagnosis. CKD stage C5 (estimated glomerular filtration rate 8 mL/min), secondary to hypertensive and diabetic nephropathy. Concomitant conditions. Arterial hypertension, stage 3, grade 2, with a very high cardiovascular risk (risk category 4); chronic heart failure, functional class III according to the NYHA classification; and type 2 diabetes mellitus.

Given the high level of azotemia in the setting of reduced diuresis, a tunneled central venous dialysis catheter was inserted, and renal replacement therapy with hemodialysis was initiated. Subsequently, an arteriovenous fistula was created. The patient was discharged for continuation of maintenance hemodialysis on an outpatient basis at the place of residence. The hemodialysis regimen was adequate, with a single-session Kt/V of 1.5–1.7 and a urea reduction ratio of 70–72%. The patient's clinical condition remained stable: blood pressure ranged from 130–140/90 mmHg on antihypertensive therapy; fasting plasma glucose was 5.8–7.0 mmol/L; hemoglobin levels ranged from 88 to 98 g/L; and serum iron, ferritin, calcium, and albumin were within reference ranges. Hyperphosphatemia was noted (1.7–2.2 mmol/L). Following additional evaluation, the patient was placed on the kidney transplant waiting list.

Deceased-donor KT was performed 11 months after initiation of maintenance hemodialysis. Induction and maintenance immunosuppressive therapy were administered according to standard protocols. No intraoperative or early postoperative complications were observed, and the graft demonstrated immediate function. Urine output was 1.8–2.3 L/day. Ultrasound examination revealed the renal allograft located in the right iliac fossa, measuring 121×55 mm (measured medially from the suture line). The parenchyma exhibited increased echogenicity with prominent hypoechoic pyramids, while corticomedullary differentiation was preserved. The renal pelvicalyceal system was not dilated. On CDFI, the vascular pattern was intact, and the renal artery and vein were visualized up to their anastomoses with the iliac vessels. The patient was discharged in stable condition for outpatient follow-up (Table).

Two months after kidney transplant, the patient's condition deteriorated, manifested by a reduction in urine output to 500 mL/day, the development of mild peripheral edema of the shins and feet, and hyperazotemia (Table). Glycated hemoglobin was 6.7%. Polymerase chain reaction testing for cytomegalovirus and Epstein–Barr virus DNA in the blood was negative. A renal allograft biopsy was not performed. During inpatient management, infusion therapy was administered and immunosuppressive therapy was adjusted. This resulted in clinical and laboratory improvement, with restoration of satisfactory graft function (serum creatinine 150 µmol/L, urea 15.7 mmol/L, daily proteinuria 0.7 g). Outpatient follow-up continued.

Table

Laboratory test results for Patient S.

Parameter	Before kidney transplant	After kidney transplant					
		3 days	1 month Discharge from hospital	2 months Deterioration	6 months	9 months	12 months
Hemoglobin (g/L)	140	108	111	111	113	120	89
White blood cells (×10 ⁹ /L)	9.1	5.9	6.4	5.9	6.5	7.2	2.3
– Neutrophils (%)	63	72	65	66	71	69	28
– Lymphocytes (%)	23	14	20	26	23	26	46
– Monocytes (%)	12	13	14	7	7	6	20
Platelets (×10 ⁹ /L)	238	233	252	293	221	234	221
Creatinine (µmol/L)	576	237	157	337	132	100	469
Urea (mmol/L)	18	19.1	12.9	29.6	16.7	12.6	29.3
Uric acid (µmol/L)		440	386	403	388	289	626
Total protein (g/L)	67	61	62	62	67	69	59
Albumin (g/L)	44	43	41	44	48	44	44
Total cholesterol (mmol/L)	5	4.4	4.8	5.1	4.8	5.1	2.9
Glucose (mmol/L)	6	8.8	5.5	8.6	8.5	7.8	6.8
Total calcium (mmol/L)		2.5	2.3	2.4	2.4	2.3	2.5
Phosphorus (mmol/L)		1.31	1.22	1.33	1.23	1.71	1.93
Potassium (mmol/L)	4.7	3.7	4.7	4.9	5.2	4.8	4.2
Proteinuria (g/day)	–	1.2	0.9	0.9	1.77	1.6	2.3
Tacrolimus (ng/mL)	–	8.3	7.8	4.3	7.6	6.4	6.8

One year after KT, the patient's condition deteriorated again, characterized by progressive anemia, leukopenia, and worsening renal allograft function (Table). Polymerase chain reaction testing for cytomegalovirus DNA in the blood was positive, while Epstein-Barr virus DNA was not detected. Ultrasound examination of the renal allograft revealed no significant interval changes. The graft measured 110×44×45 mm (measured medially from the suture line). The parenchyma demonstrated moderately increased echogenicity with preserved corticomedullary differentiation. The pelvicalyceal system was not dilated. On CDFI, intrarenal perfusion was preserved, and the renal artery and vein were visualized to their anastomoses with the iliac vessels. Mycophenolate mofetil was discontinued, antiviral therapy with valganciclovir was initiated, and red blood cell transfusion was performed. Renal allograft dysfunction persisted, prompting a diagnostic biopsy to determine the etiology.

Conclusion. The morphological features were most consistent with renal involvement by multiple myeloma,

including LCCN (κ type) in combination with proximal light chain tubulopathy (LCPT, κ type). Additional findings included focal segmental glomerulosclerosis, tubulointerstitial nephritis, and acute tubular necrosis (Figs. 1–3). Electron microscopy was not performed due to technical limitations.

Based on the morphological findings from the renal allograft biopsy, a hematology consultation and further investigations were undertaken. Bone marrow examination: bone marrow aspirate was hypercellular; with plasma cell (myeloma cell) metaplasia (66.4%); the plasma cells were predominantly large, morphologically polymorphic, and anaplastic, with the presence of multinucleated forms. Erythropoiesis was normoblastic. Urinalysis for Bence-Jones protein was positive. Serum immunoglobulin levels were markedly reduced: IgG 1.7 g/L (reference range 7–16 g/L), IgA 0.13 g/L (reference range 0.7–4 g/L), and IgM 0.04 g/L (reference range 0.4–2.3 g/L). Skull X-ray revealed a focal area of

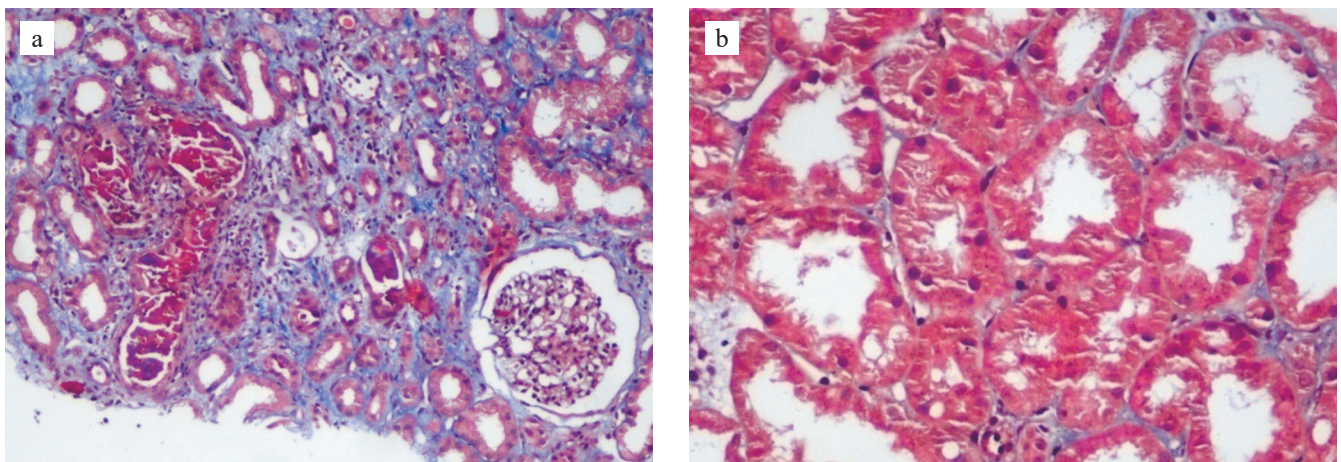


Fig. 1. Biopsy of the kidney transplant: (a) Unchanged glomerulus without mesangial or endocapillary hypercellularity. Interstitial fibrosis with accompanying tubular atrophy. Fragmented xanthophilic casts in the tubular lumen. Masson's trichrome stain, ×100. (b) Xanthophilic granules in tubular epithelial cells. Masson's trichrome stain, ×200

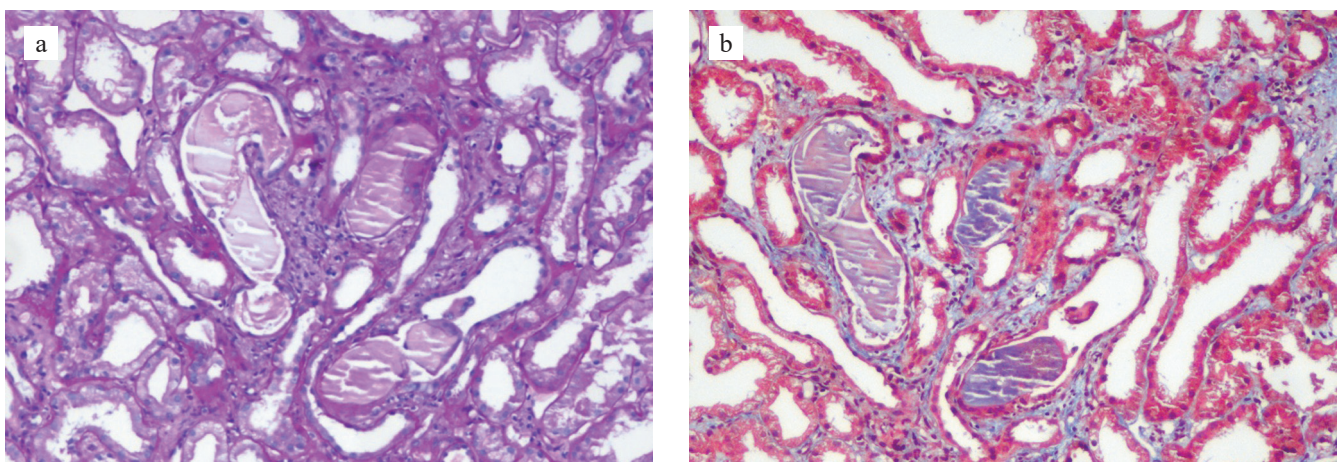


Fig. 2. Fragmented PAS-negative, fuchsinophilic casts, some surrounded by epithelial cells: (a) PAS (Periodic Acid-Schiff) stain, ×200; (b) Masson's trichrome stain, ×200

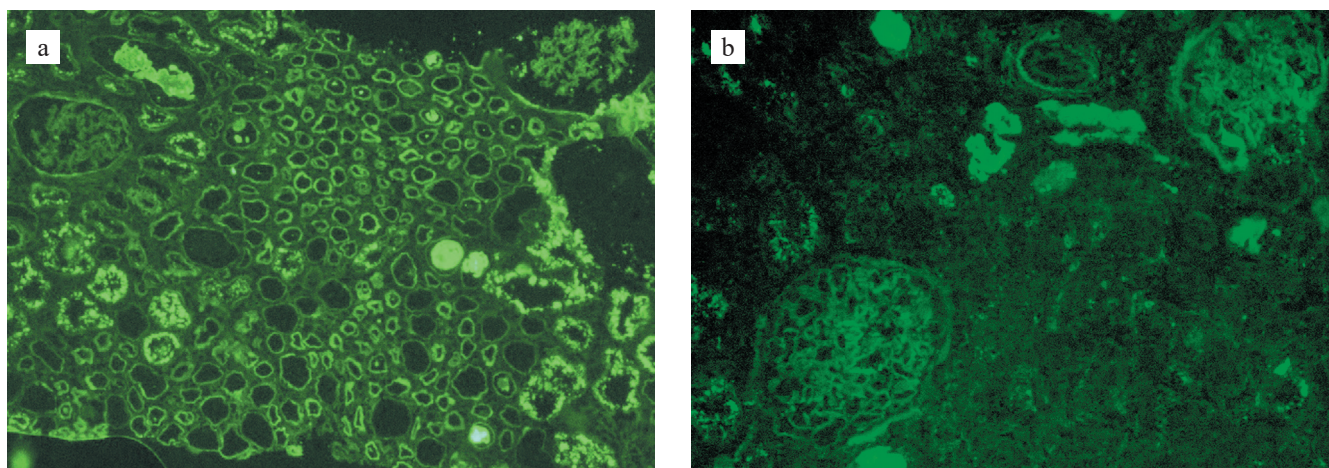


Fig. 3. Immunofluorescence study: (a) Kappa light-chain fluorescence in casts (++) and resorptive granules in the tubular epithelium (++) , $\times 100$; (b) Absence of lambda light-chain fluorescence, $\times 200$

bone tissue brightening is visible at the junction of the right occipital and parietal bones.

Hematological diagnosis: MM with Bence Jones proteinuria, stage III B, complicated by myeloma nephropathy of the renal allograft, characterized by a combination of LCCN and LCPT. The diagnosis was established on the basis of bone marrow cytology (66.4% plasma cells), complete blood count findings (anemia), histological and immunohistochemical analysis of the renal allograft biopsy (LCCN and LCPT), detection of Bence Jones proteinuria, and skull X-ray results (areas of brightening).

The patient was transferred to the hematology ward for chemotherapy. Repeated courses of polychemotherapy resulted in partial hematological remission; however, recovery of renal allograft function was not achieved, and the patient continues maintenance treatment with scheduled hemodialysis.

DISCUSSION

MM in patients with CKD who have undergone KT is rare, with only isolated case reports and small case series reported [12–15]. Since the kidneys are the organs most commonly affected by MM, renal allograft dysfunction is often the first – and sometimes the only – clinical manifestation of the disease. In transplant recipients, differential diagnosis of graft dysfunction includes acute rejection, calcineurin inhibitor toxicity, viral infections, and other causes, including MM; therefore, renal allograft biopsy is a key diagnostic procedure.

Cast nephropathy, the most common renal lesion in MM, is characterized by distinctive morphological changes in renal tissue. Immunofluorescence studies demonstrating monoclonal light chain expression, together with additional hematological investigations, are essential for confirming the diagnosis. This diagnostic pathway was followed in the present clinical observation. One year after KT, the patient developed progressive allograft dysfunction, prompting a biopsy that revealed morpho-

logical features consistent with MM-related kidney injury, subsequently confirmed by further tests.

The biopsy results suggested combined renal involvement, including LCCN and LCPT; however, the latter was not confirmed by electron microscopy. Nevertheless, the presence of one or both patterns of renal injury did not influence the treatment strategy, as the patient was diagnosed with MM for the first time in the post-transplant period. Notably, the coexistence of LCCN and LCPT in a renal allograft represents an exceptionally rare manifestation of MM-related kidney injury and, to our knowledge, has been previously reported only by Singh et al. [14].

Post-transplant MM may develop either as a recurrence of previously diagnosed or undiagnosed disease present before KT, or as *de novo* post-transplant MM [12–15]. A single case of donor-derived MM has also been reported, occurring 7 years after KT; in that case, the kidney donor was the recipient's mother, who was diagnosed with MM 1 year after the transplant procedure [16].

Differentiating between recurrent MM present before transplantation and *de novo* post-transplant MM remains particularly challenging. This diagnostic difficulty is attributable to several factors that complicate the detection of MM in KT candidates. First, older patients, who are at higher risk for MM, often have comorbid conditions closely associated with CKD, which may mask underlying plasma cell disorders. Second, MM may exist in a subclinical or latent form, including non-secretory MM.

In both scenarios, patients typically do not undergo targeted diagnostic evaluations for MM prior to transplantation. Additionally, kidney biopsy is often avoided, as advanced nephrosclerosis is expected to yield limited diagnostic information, while the procedure itself carries a risk of complications [19, 20].

In the present case, we assume that MM was already present prior to KT, and that myeloma nephropathy was

the underlying cause of end-stage renal disease (ESRD) necessitating renal replacement therapy. The patient did not undergo targeted diagnostic testing for MM or kidney biopsy to determine the etiology of CKD, as he had a long-standing history of hypertension and diabetes mellitus (DM), both well-recognized causes of CKD.

In patients with DM, CKD is most commonly attributed to diabetic nephropathy, and kidney biopsy is therefore rarely performed in routine clinical practice. However, accumulating morphological evidence indicates that, even in the presence of DM, a substantial proportion of patients have non-diabetic kidney disease. Such cases are often characterized by atypical clinical features, including the absence of diabetic retinopathy, a short duration of DM (<5 years), hematuria, massive proteinuria, rapid decline in renal function, and extrarenal symptoms of systemic disease [21].

Retrospective analysis of the disease course in our patient before KT revealed several atypical features not characteristic of diabetic kidney disease, most notably severe anemia disproportionate to the degree of CKD and a relatively rapid progression to ESRD, both of which are characteristic of myeloma nephropathy. The diagnosis of ESRD was further supported by the lack of renal function recovery during maintenance hemodialysis, although it is recognized that certain extracorporeal techniques may facilitate the removal of free light chains and lead to restoration of AKI in patients with MM [22, 23].

Also noteworthy was the consistently high normal serum albumin level with low normal (before KT) and reduced (after KT) serum total protein content.

Establishing the correct etiology of CKD is a crucial component of pre-transplant evaluation, as it may significantly influence transplant outcomes due to the risk of disease recurrence in the patient's own kidneys. In this context, the feasibility of performing a native kidney biopsy should be carefully considered, particularly in older patients, including those with DM and hypertension, as well as in cases characterized by an atypical clinical course or rapid progression of renal failure.

Serum and urine protein electrophoresis, commonly used as a screening test for MM, have limited diagnostic sensitivity, underscoring the value of kidney biopsy for definitive etiological diagnosis. When kidney biopsy cannot be performed and the cause of CKD remains unknown, it is recommended to assess monoclonal immunoglobulins in serum and daily urine using immunofixation, with quantification of the M-protein concentration, to rule out MM [24].

The development of *de novo* post-transplant MM in our recipient appears less likely. The patient lacked known risk factors associated with post-transplant MM, including Epstein-Barr virus infection or reactivation, concomitant cytomegalovirus infection, hepatitis C virus infection, or intensification of immunosuppressive therapy during the first episode of graft dysfunction.

Moreover, renal allograft dysfunction was detected relatively soon after transplantation.

In this case, the diagnosis of MM was late – established only after KT, following the onset of graft dysfunction that prompted allograft biopsy, which revealed typical morphological features of severe myeloma nephropathy. Similar clinical scenarios have been reported by other authors [12–15]. It is plausible that performing an earlier transplant biopsy, during the first post-transplant hospitalization (approximately 2 months after surgery), might have enabled earlier diagnosis and initiation of therapy for MM, potentially preserving renal allograft function.

According to reports, several recipients with post-transplant MM experienced restoration of kidney transplant function during chemotherapy, but most patients ultimately required dialysis therapy [14, 25, 26].

The attitude of clinicians toward KT in patients with MM remains ambiguous. The limited number of such procedures can be attributed to the historically incurable nature of MM, the high risk of disease recurrence, and the increased susceptibility to infectious complications following transplantation. However, the introduction of novel chemotherapeutic agents and the widespread use of autologous hematopoietic stem cell transplantation (auto-HSCT) have markedly improved outcomes in patients with MM and ESRD, resulting in survival rates comparable to those of the general MM population.

As life expectancy in MM continues to increase, KT has emerged as a realistic therapeutic option for selected patients. Notably, patients with MM receiving dialysis therapy who have achieved a very good partial response or complete remission should not be excluded from the list of candidates for KT [27–29].

To date, only isolated cases and small series of successful KT in patients with MM have been reported, demonstrating satisfactory allograft function and sustained MM remission lasting several years [30–35]. In particular, Shah et al. [33] reported outcomes in five patients with MM who underwent auto-HSCT followed by KT after a median interval of 27 months. Disease recurrence occurred in two patients at 6 and 16 months post-transplant, while the remaining patients continue to be followed. Death-censored graft survival and overall patient survival were both 80% at four years.

Dykes et al. [34] compared graft and patient survival in KT recipients with pre-existing MM to those in the general KT population and demonstrated comparable graft and overall survival rates between MM patients with ESRD and age-matched controls. Additionally, isolated reports have described successful treatment of MM in KT recipients using modern chemotherapy regimens in combination with auto-HSCT [25, 26].

Importantly, patients who have undergone dual transplantation, autologous stem cell transplantation and KT, represent a particularly challenging group to manage.

These patients require combined immunosuppressive therapy, necessitating careful dose adjustment to minimize cytopenia, which increases the risk of opportunistic infections and other complications [35].

CONCLUSION

The clinical case presented represents a rare case of MM diagnosed in a KT recipient. Post-transplant MM, whether due to recurrence of previously undiagnosed disease or *de novo* MM, is associated with rapid decline and loss of kidney transplant function. In the present case, we have attempted to provide compelling arguments supporting recurrence of MM that remained undiagnosed during the pre-dialysis and dialysis stages of CKD. A thorough pre-transplant evaluation, including accurate determination of the etiology of CKD, is the key to timely diagnosis of MM in KT candidates. Furthermore, the development of renal allograft dysfunction mandates kidney transplant biopsy to establish the underlying cause.

Written informed consent was obtained from the patient for the publication of this clinical case and the results of the renal allograft biopsy.

The authors declare no conflict of interest.

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DE NOVO MALIGNANCIES AFTER LIVER TRANSPLANTATION: EPIDEMIOLOGY, RISK FACTORS, AND MANAGEMENT STRATEGIES

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Background. Liver transplantation (LT) significantly improves survival in patients with end-stage liver disease; however, it is associated with an increased risk of developing *de novo* malignancies. Long-term immunosuppression, viral infections, and unhealthy lifestyle choices increase the risk of post-transplant oncological complications. **Objective:** to summarize current evidence on the prevalence, risk factors, diagnostic, prevention, and treatment of *de novo* malignancies in liver transplant recipients. **Materials and methods.** This paper presents a literature review, including retrospective and prospective studies, meta-analyses, and clinical guidelines published over the past two decades. **Results.** The most common post-transplant malignancies include non-melanoma skin cancer, lymphoproliferative disorders, and solid organ tumors. Major risk factors are prolonged immunosuppression, viral infections, smoking, alcohol use, advanced recipient age, and the underlying liver disease. Current management strategies involve immunosuppression reduction, surgical resection, chemotherapy, and targeted therapy. In particular, mammalian target of rapamycin (mTOR) inhibitors have demonstrated antitumor efficacy in selected patients, particularly those with Kaposi's sarcoma and lymphoproliferative disorders. **Conclusion.** Given the high oncological risk, stratified screening programs and individualized patient management are necessary after LT. Immunosuppression reduction, lifestyle modification, and early detection of malignancies are key factors in improving long-term outcomes.

Keywords: liver transplantation, *de novo* malignancies, immunosuppression, lymphoproliferative disorders, Kaposi's sarcoma, skin cancer, mTOR inhibitors, cancer screening.

INTRODUCTION

LT is a definitive treatment for patients with decompensated end-stage liver disease, acute liver failure, and hepatocellular carcinoma. Advances in surgical techniques, perioperative management, and modern immunosuppressive therapies have significantly increased the number of LT procedures performed and improved long-term patient survival [1].

However, the requirement for lifelong immunosuppression is associated with an elevated risk of serious complications, including infections, cardiovascular events, renal dysfunction, and malignancies. Notably, retrospective studies indicate that up to 20–25% of deaths among patients who survive the early post-transplant period are attributable to the development of *de novo* malignancies [2, 3]. Compared with the general population, LT recipients exhibit approximately a twofold increased incidence of solid tumors and a markedly higher – up to 30-fold – risk of lymphoproliferative disorders [4–8].

Risk factors for the development of *de novo* malignancies following LT include chronic immunosuppression, prior alcohol and tobacco use, oncogenic viral infections, and the underlying liver disease. This review aims to summarize current evidence on the incidence, risk

factors, screening strategies, and treatment approaches for *de novo* malignancies in LT recipients.

Non-melanoma skin cancers (NMSC) are the most common malignancies among Caucasian recipients of solid organ transplants. In LT recipients, NMSC incidence is more than 15-fold higher than in the general population [9, 10]. Among this population, squamous cell carcinoma (SCC) predominates, in contrast to the general population, where basal cell carcinoma (BCC) is more common. Additionally, cases of Merkel cell carcinoma following LT have been reported [11, 12].

NMSC typically develops as a late post-transplant complication, with a median interval of approximately 50 months from surgery to diagnosis [13, 14]. Although these malignancies generally have a limited impact on overall mortality, they can significantly impair quality of life and necessitate ongoing dermatological surveillance.

PREVALENCE AND TYPES OF DE NOVO MALIGNANT TUMORS FOLLOWING LIVER TRANSPLANTATION

Improved long-term survival after LT, coupled with the effects of chronic immunosuppression, has contributed to an increased incidence of tumor-related com-

plications. Evidence from large cohort studies indicates that the risk of developing malignant neoplasms in LT recipients significantly exceeds that of the general population. Moreover, the spectrum of malignancies varies depending on the time elapsed since transplantation, recipient age, the intensity and type of immunosuppressive therapy, and the underlying liver disease [4–8]. The most common malignancies include skin cancers, post-transplant lymphoproliferative disorders (PTLD), and a range of solid tumors affecting different organ systems. Fig. 1 presents the main risk factors for the development of malignant neoplasms after LT.

Table summarizes data from retrospective and prospective studies on the incidence and spectrum of *de novo* malignancies in patients following LT. The table presents key parameters, including the number of patients, duration of follow-up, time to tumor development, overall incidence of malignancies, and the most common tumor types. Standardized incidence ratios (SIRs) are also included, enabling comparison of cancer risk in transplant recipients with that of the general population.

The most reported malignancies include skin cancers, PTLT, lung cancer, gastrointestinal tumors, and head and neck cancers. Reported incidence rates vary widely – from approximately 2% to over 30% – depending on follow-up duration, baseline pathology, and population characteristics.

The following section provides a structured overview of the current literature on the incidence and patterns of *de novo* malignant neoplasms in this patient population.

Skin tumors

A retrospective study reported that the cumulative incidence of skin neoplasms was 14% at 5 years and 24% at 10 years post-transplantation, compared with 11% and 22%, respectively, for non-skin tumors [15]. In another study involving 151 LT recipients, the overall incidence of malignant skin tumors was 22.5%, with squamous cell carcinoma identified as the predominant subtype [16]. Similarly, a prospective cohort study of 161 adult patients demonstrated a progressive increase in the incidence of precancerous and malignant skin lesions over time: 5% at 2–3 years, 12% at 3–5 years, and 28% after more than 5 years of follow-up [17]. The incidence of malignant lesions specifically was 0% at 2–3 years, rising to 9% at 3–5 years and 12% beyond 5 years post-transplant. The main risk factors associated with the development of *de novo* skin tumors are summarized in Fig. 1.

Kaposi's Sarcoma

Kaposi's sarcoma (KS) is a multifocal neoplastic disease affecting the skin and mucous membranes, strongly associated with human herpesvirus 8 (HHV-8). It accounts for approximately 4% of all malignancies in post-transplant patients [18, 19]. The disease primarily develops in individuals with severe immunodeficiency [20]. A higher prevalence of KS is observed among populations from regions with elevated HHV-8 seroprevalence, including Central and Southern Africa, the Mediterranean, the Caribbean, and the Middle East [21].

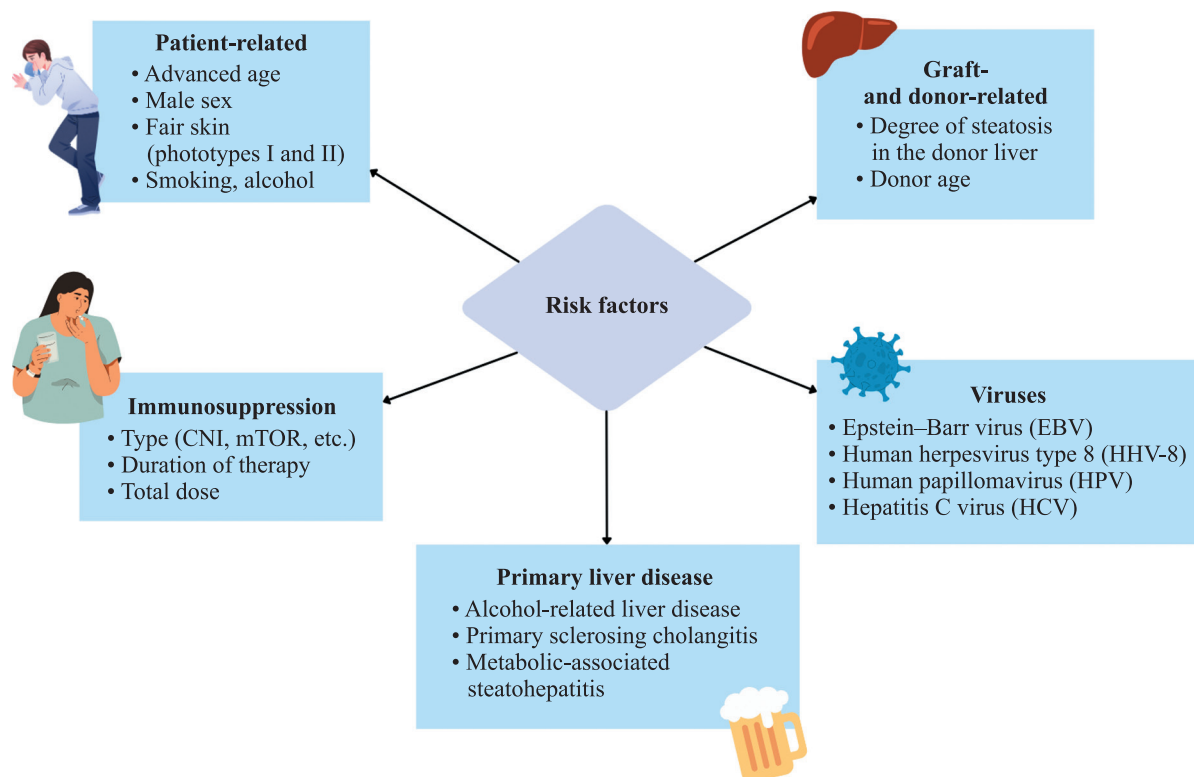


Fig. 1. Major risk factors for *de novo* malignancies after liver transplantation

Table

Prevalence and types of *de novo* malignancies after liver transplantation

Author	Country	Study period	Number of liver transplant recipients	Follow-up (years)	Time to development of <i>de novo</i> malignancies (years)	Overall incidence of <i>de novo</i> malignancies (n, %)	Most common tumors (%)	Overall standardized incidence ratio (SIR)
1	2	3	4	5	6	7	8	9
Frezza et al. [115]	USA	Until 1992	1657	–	–	64 (3.8%)	Basal cell carcinoma – 25%; squamous cell carcinoma (SCC) – 20.3%; melanoma – 6.2%.	–
Krynitz et al. [116]	Sweden	1970–2008	1221	20	–	27 (2.2%)	All malignancies – 3.4 (2.9–4.0); excluding SCC – 2.3 (1.9–2.8); all skin cancers – 16 (12–20); SCC – 32 (24–42)	–
Haagsma et al. [117]	Netherlands	1979–1996	174	5.9	5.1	23 (12%)	Skin and lip cancer – 52%	4.3
Oo et al. [118]	UK	1982–2004	1778	6.5	–	141 (7.9%)	Skin cancer (excluding melanoma) – 36.1%; lymphoma – 12.7%; colorectal cancer – 12.7%; lung cancer – 10%	2.1
Aberg et al. [5]	Finland	1982–2005	540	6.3	5.1	39 (7.2%)	Non-melanoma skin cancer – 25.6%; non-Hodgkin lymphoma – 20.5%	2.59
Finkenstedt et al. [51]	Austria	1982–2007	779	4.1	4.4	105 (12.3%)	Skin cancer – 17%; lung cancer – 16%; oropharyngeal carcinoma – 11%	1.9
Jiang et al. [119]	Canada	1983–1998	2034	3.5 ± 2.8	3.5 ± 2.8	113 (5.5%)	Non-Hodgkin lymphoma – 35.3%; colorectal cancer – 12.3%; lung cancer – 8.8%	2.5
Schrem et al. [120]	Germany	1983–2010	2000	7.3	6.8	120 (6%)	Post-transplant lymphoproliferative disorder (PTLD) – 19.1%; lung cancer – 11.6%; colorectal cancer – 10.8%; gynecological cancers – 8.0%; breast cancer – 6.6%; gastric/esophageal cancer – 5.8%	2
Galve et al. [121]	Spain	1984–1996	1827	–	2.5 ± 1.8	70 (3.83%)	–	–
Mouchli et al. [122]	USA	1984–2012	293	11.5	–	73 (25%)	PTLD – 30.1%; malignant tumors in solid organ transplantation (SOT) – 63%	–
Sanchez et al. [123]	USA	1985–1999	1421	5.5 ± 3.7	–	123 (8.8%)	Skin cancer – 32.8%; lymphoma – 25%; lung cancer – 8%; colon cancer – 5%	–
Wimmer et al. [124]	Germany	1985–2007	609	4.8	5.7 ± 3.7	87 (14.2%)	SCC – 15%; basal cell carcinoma (BCC) – 27.6%	–
Taborelli et al. [125]	Italy	1985–2014	2818	Not applicable	Not applicable	244 (8.6%)	–	–
Shalaby et al. [126]	Italy	1985–2014	2653	3.6 with HCC, 6.6 without HCC	2.7 with HCC, 4.5 without HCC	62 (6.6%) with HCC, 127 (7.4%) without HCC	–	–

Continuation Table

1	2	3	4	5	6	7	8	9
Jiménez et al. [127]	Spain	1986–2000	505	–	–	62 (12.3%)	Skin cancer and Kaposi's sarcoma – 25.8%; PTLD – 21.6%; head and neck cancer – 16%	–
Tjon et al. [128]	Denmark	1986–2007	385	–	–	66 (17.1%)	Non-melanoma skin cancer – 55%; PTLD – 21%	2.2
Engels et al. [7]	USA	1987–2008	37888	–	–	1563 (4.1%)	PTLD – 23.3%	–
Jonas et al. [129]	Germany	1988–1994	458	4.2	3.6	33 (7.2%)	Skin cancer – 24%; PTLD – 21%; gynecological cancer – 21%	–
Kelly et al. [130]	UK	1988–1996	888	–	2 ± 1.5	31 (3.4%)	Skin cancer and Kaposi's sarcoma – 25.8%; lung cancer – 3%	–
Saigal et al. [14]	UK	1988–1999	1140	–	3.8 ± 2.8	30 (2.6%)	Skin cancer – 43%; oropharyngeal cancer – 6%; bladder cancer – 6%; acute leukemia – 6%	–
Yao et al. [131]	USA	1988–2000	1043	6.7	–	53 (5%)	Skin cancer – 32%; gastrointestinal cancer – 21%; hematological tumors – 17%	–
Rademacher et al. [69]	Germany	1988–2006	1616	14	–	322 (19.9%)	Skin cancer – 25.7%; hematological – 15.2%; solid tumors – 60%	–
Watt et al. [37]	USA	1990–1994	798	10	–	271 (34%)	Skin cancer – 54.2%; hematological – 10.7%; solid tumors – 35%	–
Herrero et al. [15]	Spain	1990–2001	187	5.5	–	63 (33%)	Skin neoplasms – 55.5%, non-skin neoplasms – 44.4%	–
Ettorre et al. [132]	Italy	1990–2008	1675	5.2	3.2	98 (5.9%)	PTLD – 18.4%; head and neck cancer – 19.3%; lung cancer – 13.2%; colon cancer – 11.2%; Kaposi's sarcoma – 6.2%	–
Tajima et al. [133]	Japan	1990–2020	1781	~12	–	153 (8.6%)	PTLD – 53%; colon cancer – 9.1%; lung cancer – 7.8%; gastric cancer – 7.8%	1993–1995: 8.12; 1996–1998: 3.11; 2005–2007: 1.31; 2008–2010: 1.34; 2014–2016: 2.27; 2017–2019: 2.07
Benlloch et al. [71]	Spain	1991–2001	772	4.3	–	41 (5.3%)	Solid tumors – 75.6%; hematological – 24.3%	–
Baccarani et al. [8]	Italy	1991–2005	417	6.7	4.2	43 (10.3%)	Non-Hodgkin's lymphoma – 21%; head and neck cancer – 18.6%; Kaposi's sarcoma – 14%	–
Sanaei et al. [134]	Iran	1992–2012	1700	–	5.5	38 (2.2%)	PTLD – 63%; gastrointestinal cancer – 10.5%	–
Sérée et al. [101]	France	1993–2012	11226	–	–	1200 (10.7%)	Lung cancer – 15.6%; esophagus, stomach, colon – 11.8%; larynx cancer 6.3%; oral cavity/pharynx – 6%	All solid tumors – 2.2

End of Table

1	2	3	4	5	6	7	8	9
Jain et al. [87]	USA	1996–2006	1000	6.5 ± 1	3	57 (5.7%)	Skin cancer (including melanoma) – 38.6%; lung cancer – 14%; oropharynx – 12.2%	–
Chatrath et al. [1]	USA	1997–2004	534	5.7 ± 3.2	4 ± 2.2	80 (15%)	Solid tumors – 50%; skin cancer – 30%; hematological – 20%	3.1
Yeh et al. [135]	Taiwan	1997–2011	2127	4.2	–	111 (5.2%)	–	1.5
Park et al. [136]	South Korea	1998–2008	1952	3.5 ± 2.8	3.4 ± 2.4	44 (2.3%)	Stomach – 25%; colon – 20.4%; breast – 9%	Men – 7.7; women – 7.3
Egeli et al. [137]	Turkey	1998–2016	429	8.6	5.3	9 (2%)	Lung cancer – 44.4%	–
Kobayashi et al. [138]	Japan	1999–2022	70	12.1	–	8 (11.4%)	Lung cancer – 50%; PTLT – 37.5%; skin cancer – 12.5%	–
Lucidi et al. [139]	Italy	2000–2015	789	6.75	4	67 (5.5%)	Lung cancer – 16.4%, head and neck – 16.4%; PTLT – 13.4%, breast – 7.4%	–
Mangus et al. [140]	USA	2001–2011	1275	–	–	180 (14%)	Skin cancer – 59.4%, gastrointestinal tumors – 10.5%	–
Yu et al. [141]	China	2005–2011	569	3.5 ± 2.2	–	18 (3.2%)	PTLT – 16.7%	–
Antinucci et al. [142]	Argentina	2006–2014	159	1.1	1.3	12 (7.5%)	Skin cancer – 33.3%	–
Tiwari et al. [143]	India	2006–2017	2100	3.5	–	21 (1%)	Oropharyngeal cancer – 33.3%; lung cancer – 19%; SCC – 9.5%	–

The risk of developing KS in recipients of solid organ transplants is estimated to be up to 500 times greater than in the general population [19].

Although the vast majority of reported cases present with cutaneous lesions, visceral involvement has also been documented and is typically associated with a poorer prognosis [22].

Hematologic Malignancies

PTLT typically develops within the first year following transplantation; however, onset may occur as early as 20 days post-transplant or, conversely, several decades later.

High levels of immunosuppression are considered a major risk factor for early PTLT development. Early-onset PTLT is characterized by the proliferation of B-lymphocytes infected with Epstein-Barr virus (EBV) and is associated with profound suppression of T-cell-mediated immunity, which is intended to prevent graft rejection [25].

In contrast, late-onset PTLT is generally associated with a more aggressive clinical course, monoclonal lymphoid proliferation, and the absence of EBV genetic material in tumor cells [26–28].

Solid Organ Tumors

Solid organ tumors are diagnosed in approximately 1% of LT recipients annually. The cumulative incidence of these malignancies ranges from 3% to 15% at various time points following transplantation [3, 29]. In LT (recipients, the risk of developing solid tumors is estimated to be 2–5 times higher than in the general population [30]. Unlike PTLT, solid organ tumors typically develop more than one year after transplantation, are exceedingly rare in pediatric patients, and occur more frequently in older recipients [3]. An increased risk has been observed in individuals with a history of alcoholic liver disease (ALD) and primary sclerosing cholangitis (PSC) [31]. Recent evidence indicates that solid tumors have become the most common type of malignancy in post-LT patients, surpassing both skin cancers and PTLT in incidence [32].

SKIN CANCER

Risk Factors

Several risk factors contribute to the development of skin cancer in LT recipients. These include advanced age, prolonged and intensive immunosuppressive therapy, infection with human papillomavirus (HPV), and signi-

ficant exposure to ultraviolet (UV) radiation. Individuals with a tendency to sunburn – particularly those with Fitzpatrick skin phototypes II and III – are at increased risk, as are patients with a history of actinic keratosis or prior malignancies, including hepatocellular carcinoma (HCC) and other cancers [15–17, 33, 34].

Additional risk factors include CD4⁺ lymphocytopenia, male sex, blue eye color, red hair, and fair (Caucasian) skin phenotype, as well as the use of induction therapy with monoclonal antibodies [3, 16, 35]. Furthermore, an elevated risk of skin cancer has been reported in patients who undergo LT for PSC or ALD, compared with those transplanted for other underlying liver conditions [14, 16, 34, 36, 37].

Cyclosporine A is considered one of the most significant predictors of cutaneous malignancies. Patients receiving cyclosporine tend to develop skin cancer earlier than those treated with tacrolimus [16]. Moreover, cyclosporine A has been identified as an independent and specific risk factor for post-transplant malignancies [34, 38]. Azathioprine has also been associated with an increased risk of cutaneous squamous cell carcinoma in organ transplant recipients, including those who have undergone heart transplantation [39–41]. Nevertheless, the overall degree of immunosuppression is likely the principal determinant of cancer risk, rather than any single immunosuppressive agent [20, 42].

The primary etiological factor in Kaposi's sarcoma (KS) is infection with HHV-8, although co-infections such as hepatitis B virus, cytomegalovirus, and Epstein-Barr virus may also contribute to disease development [43, 44]. Severe immunosuppression is strongly associated with the onset of KS [18]. Accordingly, individualized adjustment of immunosuppressive therapy represents a key strategy for KS prevention [34].

Although clinical trials investigating novel therapeutic approaches for KS are ongoing, current evidence suggests that the most effective strategy is the substitution of calcineurin inhibitors (e.g., cyclosporine and tacrolimus) with mammalian target of rapamycin (mTOR) inhibitors, such as sirolimus and everolimus [19, 34, 45, 46].

Screening and Prevention

Currently, evidence regarding the effectiveness of surveillance strategies for skin malignancies following LT remains limited. Nevertheless, sustained oncological vigilance is essential, and individualized screening programs should be implemented for patients with identified risk factors [47]. Individuals with a history of risk factors, such as smoking and alcohol use, require more frequent and targeted monitoring. At a minimum, surveillance should be consistent with established oncological screening protocols for the general population [47].

All LT recipients should undergo a comprehensive dermatological examination at least once annually [29,

47, 49, 50]. For patients with additional risk factors, more frequent evaluations – every 3–6 months – are recommended, particularly in those with a prior history of skin cancer or as determined by the treating physician [17, 32, 47–49]. Clinicians should maintain a high index of suspicion when assessing suspicious skin lesions and promptly perform biopsy or excision when malignancy is suspected, even at an early stage [3, 48].

Furthermore, patients must be informed about their elevated risk of skin cancer and educated on preventive measures. These include consistent use of sunscreen, wearing protective clothing and hats, and the use of sunglasses to minimize ultraviolet exposure [13, 29, 47].

In a prospective cohort study involving 779 patients who had undergone total prostatectomy, 105 malignant tumors were detected in 96 (12.3%) of them. The most common were skin cancer (17%), lung cancer (16%), oropharyngeal tumors (11%), and prostate cancer (11%). Patients with early tumor detection had favorable clinical outcomes. The implementation of an intensive follow-up protocol increased the rate of early diagnosis of *de novo* tumors from 4.9% to 13% and improved treatment outcomes [51].

Screening for KS is a complex challenge, as HHV-8 viral load levels in patients are often low and detectable in fewer than half of cases. Furthermore, there are no standardized serological tests [41, 52–54].

Treatment

In general, the treatment of malignant neoplasms in LT recipients involves standard methods – surgery, chemotherapy, and radiation therapy – just as in immunocompetent patients. However, a key difference lies in the need to adjust immunosuppression, particularly for tumors sensitive to the level of immunosuppression, such as KS and PTLC [18].

- **SCC (squamous cell carcinoma):** Superficial lesions may be managed with cryotherapy, electrocoagulation, or curettage, whereas invasive forms require wide surgical excision. These tumors are characterized by an aggressive clinical course, with a high risk of recurrence and metastasis [55, 56].
- **BCC (basal cell carcinoma):** Surgical excision remains the standard of care. In patients with recurrent disease, reduction of immunosuppressive therapy may decrease the risk of new lesions. Radiation therapy can be considered in elderly patients or those unsuitable for surgery. For low-risk lesions, electrodesiccation and curettage are appropriate options. Additional effective treatments include photodynamic therapy, imiquimod, and 5-fluorouracil [56].
- **Malignant melanoma:** Management involves surgical excision, with or without sentinel lymph node biopsy, along with consideration of reducing immunosuppressive therapy [55, 56].

- **Merkel cell carcinoma:** Localized disease is treated similarly to that in immunocompetent patients. In metastatic cases, temporary discontinuation of cyclosporine A may provide clinical benefit; however, the overall prognosis in immunocompromised patients remains poor [55, 56].
- **Kaposi's sarcoma:** There are no standardized treatment guidelines. Therapeutic approaches include surgical resection, radiation therapy, intralesional chemotherapy, reduction or discontinuation of immunosuppressive therapy, and systemic chemotherapy [57]. In some cases, complete remission has been achieved following reduction of immunosuppression or conversion to sirolimus [19, 31, 58, 59]. The efficacy of mTOR inhibitors is attributed to their anti-angiogenic effects, including suppression of vascular endothelial growth factor (VEGF) production and inhibition of endothelial cell proliferation [60, 61]. Additionally, they may inhibit human herpesvirus 8 (HHV-8) replication by modulating viral protein expression [62].

If modification of immunosuppressive therapy proves ineffective, systemic chemotherapy is indicated. The first-line agent is pegylated liposomal doxorubicin, which achieves complete or partial remission in approximately 70% of patients [56, 63]. Other effective agents include vinblastine, bleomycin, taxanes, etoposide, and gemcitabine. Additionally, some studies suggest that prophylactic administration of ganciclovir or other antiviral agents in high-risk patients may offer clinical benefit [56, 64].

HEMATOLOGICAL (LYMPHOPROLIFERATIVE) NEOPLASMS

Risk Factors

Advances in immunosuppressive regimens and supportive care have significantly improved overall survival among LT recipients. However, this increased survival has been accompanied by a higher incidence of *de novo* malignancies. Intensive immunosuppression contributes to profound immunodeficiency and is a major risk factor for the development of post-transplant lymphoproliferative disorders (PTLDs), with a cumulative incidence of approximately 1–2% within the first five years following LT [65].

The risk of PTLD is highest during the first year after transplantation. The type of immunosuppressive agent also plays a critical role: the use of tacrolimus has been associated with a higher risk of PTLD compared to cyclosporine, another calcineurin inhibitor [66]. In particular, drugs that suppress T-cell function are more strongly implicated in the pathogenesis of PTLD.

Most post-transplant lymphoproliferative disorders (PTLDs) are of B-cell origin and are strongly associated with the Epstein–Barr virus (EBV). In EBV-seronega-

tive recipients receiving organs from EBV-seropositive donors, the risk of PTLD development is significantly increased due to the absence of pre-existing immunity [67]. Serological mismatch involving Cytomegalovirus (CMV) may also contribute to an elevated risk. EBV-negative PTLDs are less common and exhibit distinct biological and clinical characteristics. In some cases, these may be linked to previously acquired but undetected EBV infection (Fig. 2) [68].

A single-center study conducted in Germany reported a higher incidence of hematological malignancies in patients who underwent transplantation for Hepatitis C [69]. This association was also supported by findings from a systematic review, which suggested that chronic B-cell stimulation during HCV infection may contribute to lymphomagenesis [70, 71]. However, a large retrospective study from France did not demonstrate a statistically significant association between HCV infection and an increased incidence of hematological malignancies following HCV-related LT [72].

Immunosuppressive therapy, which is essential for preventing graft rejection, disrupts the body's natural antitumor defense mechanisms. In particular, suppression of the T-cell-mediated immune response impairs immune surveillance of atypical cells. Persistent viral infections in the setting of immunosuppression (e.g., EBV, HHV-8), further promote the proliferation of infected cells and their transformation into tumor cells. At the same time, the reduced capacity to eliminate cells with DNA mutations facilitates the accumulation of genetic abnormalities. Together, these processes create a tumor-promoting microenvironment that supports oncogenesis and progression of malignancy.

Clinical Presentation

PTLD typically presents with general symptoms, including fever, night sweats, weight loss, and lymphadenopathy. Extranodal involvement occurs in approximately 50% of cases and may affect organs such as the liver, lungs, and central nervous system (CNS). Laboratory abnormalities may include unexplained cytopenias, elevated lactate dehydrogenase (LDH), hyperuricemia, hypercalcemia, and the presence of monoclonal protein.

According to current classification systems, PTLDs are divided into four main types: non-destructive (early lesions), polymorphic, monomorphic, and classical Hodgkin lymphoma [73]. Early (non-destructive) PTLD is characterized by benign, polyclonal lymphoid proliferation without disruption of normal tissue architecture. Polymorphic PTLD comprises a heterogeneous population of lymphoid cells that do not meet the diagnostic criteria for defined B-cell or T/NK-cell lymphomas observed in immunocompetent individuals. In contrast, monomorphic PTLD fulfills the criteria for established lymphoid malignancies, including diffuse large B-cell lymphoma, Burkitt lymphoma, plasma cell myeloma,

and T-cell lymphomas. Classical Hodgkin lymphoma is the rarest subtype of PTLD.

Poor prognostic factors include a high International Prognostic Index, bulky disease, Epstein–Barr virus negativity, and involvement of the central nervous system or bone marrow, as well as early disease onset following transplantation [74].

Screening and Prevention

There are currently no standardized guidelines for PTLD screening following solid organ transplantation, and surveillance protocols vary across institutions. Early tapering or discontinuation of immunosuppressive therapy, in combination with antiviral prophylaxis, may reduce the risk of PTLD development. Maintaining the lowest effective concentration of immunosuppressive agents – sufficient to prevent graft rejection – also contributes to lowering PTLD incidence.

Whenever possible, transplantation from EBV-positive donors to EBV-negative recipients should be avoided due to the significantly increased risk of disease. Regular monitoring of EBV DNA levels in peripheral blood, with timely adjustment of immunosuppressive therapy in response to rising viral load, may help prevent the onset of PTLD [75].

In selected cases of viral reactivation, administration of the anti-CD20 monoclonal antibody rituximab may be indicated. As the majority of PTLD cases occur within the first year after transplantation, intensified monitoring

is particularly important during this period, with the possibility of gradual de-escalation over time.

Treatment

In patients who have undergone T-cell-depleting therapy and present with systemic symptoms in the absence of infectious or rejection-related causes, PTLD should be strongly suspected and ruled out. Diagnostic evaluation includes measurement of lactate dehydrogenase (LDH) levels, positron emission tomography–computed tomography (PET-CT), and assessment of EBV viral load. In cases where central nervous system (CNS) involvement is suspected, brain magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) analysis are recommended.

The primary goal of treatment is to achieve complete remission while minimizing side effects. Antiviral therapy has not demonstrated efficacy in EBV-positive PTLD. In many cases, initial management with reduction of immunosuppression is effective, typically involving discontinuation of antimetabolites (e.g., azathioprine, mycophenolate mofetil) and a 25–50% reduction in calcineurin inhibitor dosage. Close multidisciplinary collaboration with the transplant team is essential.

Treatment strategies are guided by PTLD subtype. Early (non-destructive) lesions may regress completely within 3–5 weeks following reduction of immunosuppression alone [76]. Polymorphic PTLD generally requires treatment with rituximab, with escalation to chemotherapy in refractory cases. Monomorphic PTLD is

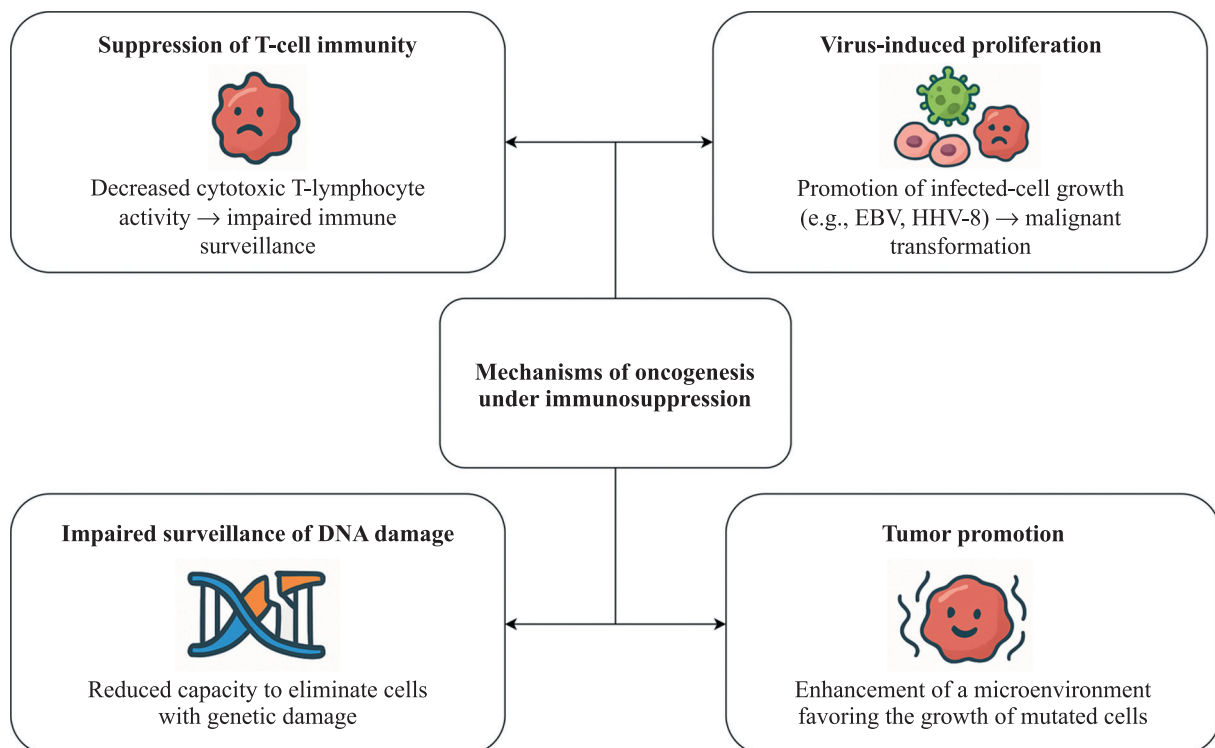


Fig. 2. Key mechanisms of oncogenesis during immunosuppression

managed with first-line chemoimmunotherapy regimens. In some centers, following completion of chemotherapy, patients are transitioned to mTOR inhibitors combined with low-dose prednisolone, with complete withdrawal of calcineurin inhibitors and antimetabolites.

Combination therapy with rituximab and reduction of immunosuppression has been shown to significantly improve overall survival, reaching 73% compared with 33% for reduction of immunosuppression alone [77]. In the PTLT-1 study, a sequential treatment approach consisting of four cycles of rituximab followed by CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine, and prednisolone) achieved complete remission in 57% of patients [78].

Patients who achieved remission after initial rituximab therapy were continued on maintenance treatment, whereas non-responders were escalated to R-CHOP. Notably, approximately 25% of patients were able to avoid chemotherapy altogether, and the median overall survival reached 6.6 years [79]. For highly aggressive disease, particularly in cases with BCL2 and MYC rearrangements, the R-EPOCH regimen may be considered [80].

Alternative combinations, such as rituximab with ibrutinib or with brentuximab vedotin, have not demonstrated superior efficacy and are associated with increased toxicity, especially in the case of brentuximab vedotin [81, 82]. Surgical intervention is rarely indicated and is generally reserved for selected cases, particularly with gastrointestinal involvement complicated by risk of obstruction or perforation.

PTLD relapse following chemotherapy is a major clinical challenge. In a retrospective study of patients with relapsed EBV-positive PTLT, the median overall survival was only 4.1 months [83]. The phase III ALLELE trial evaluated tacelecleucel, an allogeneic, HLA-matched EBV-specific T-cell therapy, which achieved complete remission in 12 of 43 patients and subsequently led to its approval by the European Medicines Agency in 2022 [84]. Brentuximab vedotin may also be considered in the management of relapsed disease.

Epigenetic therapy, including the combination of the histone deacetylase inhibitor nanatinostat with valganciclovir, has been explored in selected cases and demonstrated a partial response in 1 of 4 patients [85]. Additional salvage options include platinum-based chemotherapy regimens, chimeric antigen receptor T-cell (CAR-T) therapy, and autologous stem cell transplantation, with selection guided by disease biology and patient clinical status.

SOLID MALIGNANT TUMORS

Risk Factors

Head and neck tumors (upper respiratory and digestive tracts)

These tumors are associated with smoking and alcohol abuse. They develop from the tissues of the upper respiratory and digestive tracts, including the lips, oral cavity, tongue, nasal cavity, pharynx, larynx (vocal cords), esophagus, and trachea. Patients with alcoholic cirrhosis who undergo LT exhibit a significantly increased risk of developing oropharyngeal cancer compared with those transplanted for other indications [20, 29]. Evidence from a single-center study demonstrated that no cases of oropharyngeal cancer were observed in patients without a history of smoking or alcoholism [86]. Smoking is strongly associated with malignancies of the tongue and larynx, and its carcinogenic effects – well established in the general population – are equally relevant in LT recipients [87].

Disentangling the individual contributions of smoking and alcohol as independent risk factors is difficult, as alcohol enhances the carcinogenic effects of tobacco, and concurrent use of both substances is common [18].

Lung cancer

The main risk factors for lung cancer (LC) in transplant recipients include prolonged post-transplant survival, alcoholic cirrhosis as the indication for LT, and a long history of smoking [44, 88, 89].

Among patients transplanted for alcoholic cirrhosis, the risk of developing LC is approximately 2–4 times higher than in those transplanted for other indications [1, 29]. Data from the U.S. National Institute of Diabetes and Digestive and Kidney Diseases database indicate that these patients exhibit the highest cumulative incidence of LC, reaching 2.0% and 4.8% at 5 and 10 years post-transplantation, respectively, compared with 0.15% and 1.3% in patients with non-alcohol-related indications for LT [37].

Consistent with findings in the general population, smoking remains a major independent risk factor for LC in LT recipients [1, 18, 44, 87]. Notably, the incidence of LC is significantly higher among patients transplanted for alcoholic cirrhosis than among those transplanted for other underlying conditions [18, 90].

Tumors of the gastrointestinal tract

Major risk factors include prolonged follow-up duration, alcoholism, primary sclerosing cholangitis (PSC) with or without inflammatory bowel disease (IBD), obesity, diabetes mellitus, and metabolic dysfunction-associated steatohepatitis (MASH).

In patients with PSC, the risk of colorectal cancer after transplantation significantly exceeds that of the

general population, reaching comparable 10-year population risk levels as early as 5.75 years in men and 3.25 years in women. The presence of concomitant IBD further doubles this risk (OR = 2.06; 95% CI: 1.03–4.15; $P = 0.042$) [91].

Both PSC and MASH are also associated with an elevated risk of pancreatic cancer. The increased incidence of post-transplant gastric cancer may be attributable to the combined effects of chronic immunosuppression and underlying cirrhosis [92].

A retrospective study involving 9,724 patients identified the following as significant predictors of recurrent hepatocellular carcinoma (HCC): donor age >60 years, donor diabetes, body mass index (BMI) ≥ 35 kg/m², severe graft steatosis, and organ procurement following cardiac death with prolonged ischemia time [93].

Active hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, as well as continued exposure to environmental carcinogens, remain significant risk factors.

Urogenital System

Evidence indicates that kidney transplant recipients have an increased risk of both kidney and bladder cancers compared with the general population [6, 7, 94]. Individuals with a prior history of renal cell carcinoma, von Hippel–Lindau syndrome, chronic kidney disease, or polycystic kidney disease are at particularly high risk.

Bladder cancer in transplant recipients is often characterized by a more aggressive clinical course and poorer prognosis than in non-transplanted individuals [95]. In contrast, although prostate cancer may occur in this population, transplant recipients do not appear to have an increased disease-specific mortality compared with the general population [96].

Gynecological Tumors

Female liver transplant recipients are at increased risk of developing cervical and vulvar cancer. This elevated risk is strongly associated with a prior history of cervical dysplasia and persistent HPV infection. Immunosuppression contributes to the reactivation of oncogenic HPV strains and impairs viral clearance, thereby increasing the risk of neoplasia [97].

Screening and Prevention

All LT recipients should be counseled on the importance of complete abstinence from smoking and alcohol use, given their significant contribution to post-transplant malignancy risk. For lung cancer screening, adherence to standard population-based protocols is recommended, as intensified screening strategies have not shown a survival benefit in this population [98, 99]. Annual oral cavity examinations are advised for all recipients, while high-risk individuals (smokers, those with HPV infection) should

undergo a comprehensive annual otorhinolaryngological (ENT) evaluation [49, 100].

Patients with IBD should undergo annual colonoscopy. In contrast, individuals with PSC without concomitant IBD are advised to undergo colonoscopy every 3–5 years [101, 102]. For patients with MASH or a history of HCC after the age of 50 years, colonoscopic screening every 5 years is recommended [49, 91].

According to the European Association of Urology guidelines, annual renal ultrasonography is recommended for high-risk individuals, including those with a prior history of renal cell carcinoma, von Hippel–Lindau syndrome, or autosomal dominant polycystic kidney disease [103].

Screening for cervical and vulvar cancer should follow protocols for immunocompromised women [104].

Treatment

The initial step in the management of *de novo* solid tumors in LT recipients is to reduce immunosuppressive therapy to the lowest effective dose. Although randomized controlled trials are lacking, retrospective studies suggest that conversion from calcineurin inhibitors to mTOR inhibitors is associated with a 55–75% reduction in mortality [105, 106].

Surgical management generally follows the same oncological principles as in the general population. However, transplant recipients are at increased risk of postoperative complications, including infections and higher 90-day mortality rates [107].

If detected early, lung cancer can be radically resected surgically. In patients with significant comorbidities, sublobar resection combined with radiotherapy may be considered [108]. For advanced-stage disease, treatment strategies align with standard approaches, including surgical resection for non-small cell lung cancer and chemoradiotherapy for small cell lung cancer [109].

For esophageal and gastric cancer, esophagectomy or gastrectomy are indicated depending on tumor location. In early-stage disease, endoscopic resection may be a viable option [110, 111]. Surgical and endoscopic resection, chemotherapy, and radiation therapy are also used [112, 113].

Pancreatic cancer is primarily managed surgically, sometimes in combination with neoadjuvant therapy; however, outcomes remain poorer compared with sporadic cases.

Tumors of the upper respiratory tract (oral cavity, oropharynx, and larynx) typically require surgical intervention, frequently combined with chemotherapy and/or radiotherapy. Early detection significantly improves survival rates [114].

CONCLUSION

Skin cancers, PTLDs, and solid organ tumors are the most common *de novo* malignancies following LT and are among the leading causes of morbidity and mortality in this population. Their development is primarily driven by chronic immunosuppression and persistent infection with oncogenic viruses. Additional important risk factors include advanced age, male sex, smoking, alcohol abuse, and underlying conditions such as PSC and inflammatory bowel disease.

In recent years, substantial progress has been made in the development of screening guidelines. Nevertheless, further improvements in patient outcomes will depend on reducing immunosuppression or using alternative medications, as well as strict adherence to risk-adapted screening protocols. Screening measures should be individualized, taking into account each patient's specific risk profile.

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STRUCTURE AND PROVISION OF RENAL REPLACEMENT THERAPY AND KIDNEY TRANSPLANT SERVICES IN KYRGYZSTAN

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We present an overview of the current status and development of renal replacement therapy and kidney transplantation in Kyrgyzstan. The paper outlines the key stages in the evolution of transplant care in Kyrgyzstan and references the regulatory and legal frameworks governing the provision of medical services in this field. In addition, it analyzes transplant activity and the demographic characteristics of kidney donors and recipients.

Keywords: renal replacement therapy, kidney transplantation, transplant center, registry.

BACKGROUND

Kidney transplantation (KT) in patients with end-stage kidney disease (ESKD) in Kyrgyzstan, as in many other countries, is recognized as one of the major achievements of modern medicine. The first successful kidney transplant (KT), performed in 1954, demonstrated the feasibility of the procedure as an effective treatment for patients with terminal renal failure [1]. Since then, KT has become established as the optimal treatment modality for ESKD.

The need to develop a national KT program is supported by evidence showing superior survival rates and improved quality of life in transplant recipients compared with patients receiving other forms of renal replacement therapy [2]. Several studies [3, 4] have demonstrated significant improvements in 1-, 3-, and 5-year KT outcomes over the past 15 years [5].

However, transplant outcomes are largely dependent on the quality of patient management in both the early and long-term postoperative periods. Inadequate follow-up of KT recipients significantly increases the risk of post-transplant complications and negatively affects patients' quality of life and social adaptation [6].

It has been established that the more pronounced the decline in glomerular filtration rate (GFR) in KT recipients, the greater the number of factors that require appropriate management to reduce the risk of further graft dysfunction and loss [7]. These risk factors include the underlying etiology of chronic kidney disease (CKD), elevated body mass index, anemia, as well as comorbid conditions such as diabetes mellitus, heart failure, arterial hypertension, and active malignancy. Implementation of structured educational programs, as well as donor support and systematic monitoring before, during, and after transplantation, represents one of the key components of an effective transplant program [8].

RESULTS AND DISCUSSION

Renal replacement therapy using maintenance hemodialysis in Kyrgyzstan is financed by the Mandatory Health Insurance Fund (MHIF), which has contracts with 37 healthcare institutions, 35 of which are private and 2 are state-owned.

Since March 2022, the MHIF has been responsible for maintaining a registry of patients with stage 5 CKD and for managing a unified national database. As of 1 July 2024, the hemodialysis section of the registry included 2,975 patients [9].

Selection of candidates for organ and tissue transplantation in Kyrgyzstan is carried out by a commission under the Ministry of Health (MoH), comprising medical experts with appropriate training and expertise in transplant medicine [10].

As of February 2025, the National Center for Maternal and Child Welfare (NCMCW) registry included 128 patients listed as KT candidates.

The national KT program in Kyrgyzstan was initiated in 2012, with the first KT procedures performed at two medical institutions: NCMCW and the Research Institute of Cardiac Surgery and Organ Transplantation.

At present, KT in the country is performed exclusively at NCMCW in Bishkek and is limited to living-related donor transplantation, in accordance with regulations issued by Kyrgyzstan's Ministry of Health [11].

Between 2018 and 2024, a total of 63 KTs were performed in Kyrgyzstan, including 62 adult recipients and one pediatric recipient. The annual distribution of transplant procedures is presented in Table.

Between 2023 and 2024, a marked increase in the number of KT procedures was observed. Specifically, 8 procedures were performed in 2023 compared with 21 in 2024, representing a 2.6-fold increase ($p < 0.001$). The

average monthly number of surgeries increased from 0.7 in 2023 to 1.8 in 2024 (Fig. 1).

Men predominate annually in the recipient population (Fig. 2).

As shown in Fig. 2, transplant activity in Kyrgyzstan increased steadily between 2018 and 2024, with a consistently higher proportion of male recipients. Among

Table

Trends in Kidney transplants in Kyrgyzstan (2018–2024). Data from the National Center for Maternal and Child Welfare

	2018	2019	2020	2021	2022	2023	2024
Number of kidney transplants by year	3	9	8	1	13	8	21

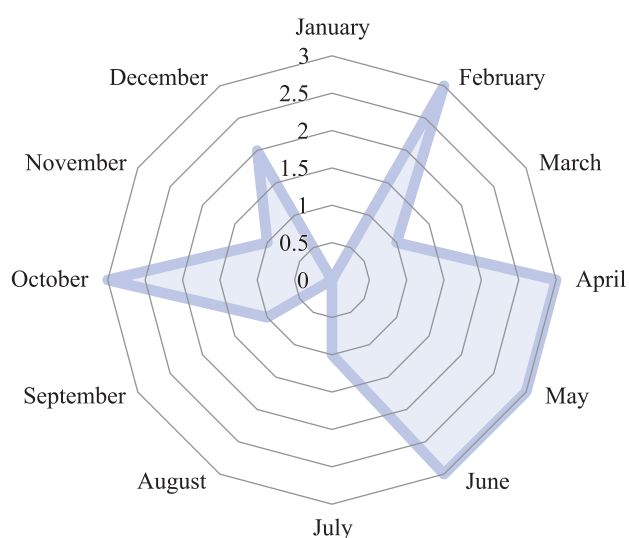


Fig. 1. Monthly distribution of kidney transplant procedures in 2024

donors, males accounted for 55.5% and females for 44.5%.

The significant rise in transplant activity observed in 2024 ($p < 0.001$) can be attributed to the implementation of a new national strategy aimed at developing transplant services and transitioning to a budget-based funding model [12, 13]. Prior to this transition, KT procedures were performed exclusively using living-related donors on a fee-for-service basis [13].

It should be noted that, according to the criteria established by the Patient Selection Commission under Kyrgyzstan's Ministry of Health, only the recipient's biological relatives are eligible to serve as living donors [14].

Fig. 3 presents the age distribution of kidney donors in Kyrgyzstan between 2018 and 2024. Among successful donors, 1 individual was younger than 18 years, 19 were aged 19–30 years, 34 were aged 30–40 years, and 9 were older than 40 years. Over time, with the increasing number of transplant procedures, there has been a trend toward an increase in the number of donors aged ≥ 30 years.

A centralized system for monitoring patients with transplanted organs has now been established in Kyrgyzstan. During the first 3 months following transplantation, all recipients are monitored in Kyrgyzstan. Thereafter, patients – including those who underwent transplantation abroad – are followed up at the outpatient clinic of the National Hospital under Kyrgyzstan's Ministry of Health. This institution is also responsible for providing immunosuppressive therapy to transplant recipients.

In 2024, the number of KT recipients under follow-up in Kyrgyzstan increased by 19.2%, reaching a total of 738 patients.

Previous studies have shown that careful selection of donors and recipients, together with close monitoring of immunosuppressive therapy, are critical for minimizing

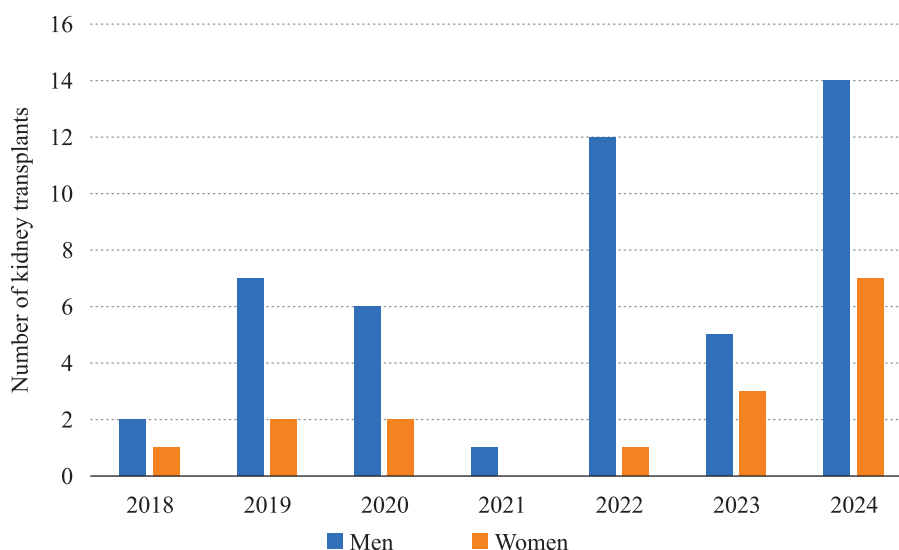


Fig. 2. Kidney transplant activity in Kyrgyzstan (2018–2024)

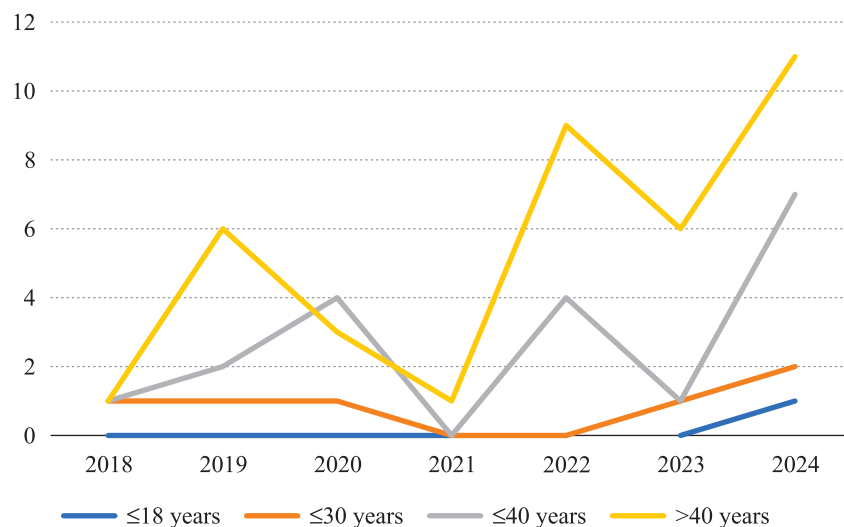


Fig. 3. Age distribution of effective organ donors in Kyrgyzstan (2018–2024)

the risks of infectious complications and graft rejection [15, 16]. Adherence to these principles is reflected in the outcomes of the KT program in Kyrgyzstan: among 63 transplant recipients, only three (4.7%) experienced an episode of acute rejection, and one patient (1.6%) required resumption of maintenance hemodialysis.

CURRENT STATUS AND FUTURE GOALS

As of 2025, transplant medicine is a rapidly developing area of healthcare in Kyrgyzstan. A tissue-typing laboratory has recently been established.

The Cabinet of Ministers of Kyrgyzstan has outlined several strategic priorities for the Ministry of Health. These include further development of the national KT program, expansion of geographic access and number of transplant centers, establishment and continuous updating of a comprehensive registry of patients requiring kidney KT, increasing the number of transplant procedures in line with population needs, ensuring long-term, structured follow-up of transplant recipients, and providing access to immunosuppressive therapy.

CONCLUSION

The successful implementation of a KT program in Kyrgyzstan depends on several key factors, including careful donor–recipient selection, individualized immunosuppressive therapy, monitoring blood levels of immunosuppressive drugs, and timely identification and management of comorbid conditions. Recognition and control of risk factors for graft dysfunction are essential to prevent a return to renal replacement therapy and to improve recipients' quality of life.

FINDINGS

1. Between 2018 and 2024, transplant activity in Kyrgyzstan increased substantially. Male donors predo-

minated (55.5%), with the majority aged 30–40 years ($n = 34$) and 19–30 years ($n = 19$).

2. Living-related donation remains the only source of organs for KT in Kyrgyzstan. A total of 63 kidney transplants were performed between 2018 and 2024, with the highest annual number recorded in 2024 ($n = 21$). Transplant procedures were performed in both adult and pediatric recipients.
3. A centralized system for monitoring transplant recipients and providing immunosuppressive therapy has been established in Kyrgyzstan. As of the end of 2024, 738 patients with transplanted kidneys were under follow-up.

The authors declare no conflict of interest.

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METHODS OF VENOVENOUS BYPASS DURING LIVER TRANSPLANTATION IN HIGH-RISK SURGICAL PATIENTS (A LITERATURE REVIEW)

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Background. The technique of redirecting blood flow from the inferior vena cava (IVC) and portal vein during liver transplantation (LT) offers several advantages, the most important of which is the prevention of intraoperative hypoperfusion-related complications. **Materials and methods.** A literature search was performed in the Scopus, PubMed, and Russian Science Citation Index (RSCI) databases using the following keywords: “liver bypass”, “liver transplantation with venovenous bypass/shunting”, “assisted circulation during liver surgery”, “assisted circulation during liver transplantation”, and “history of transplantology” from the early 1960s through 2025 were considered. In total, 162 articles from Russian and foreign journals were analyzed, of which 44 met the inclusion criteria and were included in the review. **Results.** An analysis of Russian and foreign literature reveals a unified concept regarding the use of venovenous bypass (VVB) systems, as well as the general advantages and limitations of existing techniques. VVB remains a relevant method for maintaining hemodynamic stability and improving postoperative outcomes in liver transplant recipients. Particular attention is given to a novel system and technique for performing VVB that incorporates an oxygenator/heat exchanger and a venous reservoir, allowing the simultaneous use of continuous renal replacement therapy (CRRT). Modern cardiopulmonary bypass techniques are characterized by the creation of optimal conditions for surgical intervention, ensuring a high level of patient safety throughout the procedure.

Keywords: circulatory support, venovenous bypass, liver transplantation.

Venovenous bypass (VVB) is a cardiopulmonary bypass-based technique used in liver transplantation (LT) to divert blood flow from the inferior vena cava (IVC) and the portal venous system. This approach offers several advantages, most notably reducing the need for complete IVC clamping and thereby minimizing the risk of intraoperative hypoperfusion complications. By maintaining venous return and stabilizing systemic hemodynamics, VVB mitigates complications associated with prolonged clamping of major vessels and improves post-LT outcomes.

Furthermore, VVB shortens the duration of the anhepatic phase, which may decrease the risk of ischemia–reperfusion injury to the graft. It also helps attenuate the severity of post-reperfusion syndrome. Although VVB requires an extracorporeal circuit (ECC) and specialized equipment, its integration into routine LT practice has the potential to improve both early and long-term postoperative outcomes by reducing intraoperative complications related to surgical and anesthetic management [1–3].

The objective of this paper is to analyze the evolution of perfusion technologies and describe current VVB techniques used in LT.

A literature search and study selection were conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [4]. The identified studies were systematically analyzed, the findings were interpreted, and conclusions were formulated for inclusion in this review. Searches of the Scopus, PubMed, and Russian Science Citation Index (RSCI) databases initially identified 162 publications. Following a two-stage screening process, 44 relevant studies were selected for the final analysis.

The introduction of VVB in LT has a long history marked by significant milestones. The earliest descriptions of VVB techniques were reported by Francis D. Moore in experimental canine studies conducted in the early 1960s. At that time, passive shunting from the left heart to the superior vena cava (SVC) via the internal jugular vein was employed; however, this approach was associated with a high incidence of pulmonary embolism caused by thrombus formation within the ECC [5].

Subsequently, in 1963, Thomas Starzl published a landmark report describing three attempts at human LT, preceded by seven years of research focused on organ preservation, surgical techniques, and the physiologi-

cal relationships between the liver, pancreas, and other abdominal organs. At that stage, the major limitation remained the inadequacy of immunosuppressive therapy. Modifications introduced in 1962 not only improved the management of rejection episodes within the study but ultimately transformed the field of solid-organ transplantation as a whole.

At that time, VVS was performed using separate active venous drainage systems from both the IVC and portal venous circulation, with blood diverted through the femoral and portal veins into the SVC (Fig. 1) [6, 7].

However, according to the authors of these early studies, significant problems arose due to the high incidence of pulmonary embolism, as the ECC itself proved to be a source of thrombus formation. Initial attempts to address this issue through systemic heparinization and veno-arterial shunting were subsequently abandoned because of severe, life-threatening complications, particularly uncontrolled bleeding [8].

A major breakthrough occurred in the early 1980s, when a research group from University of Pittsburgh introduced an improved VVS technique using specialized equipment and modified perfusion strategies. The adoption of heparin-coated ECC, the use of centrifugal pumps, and the elimination of systemic heparinization enabled more effective venous perfusion while minimizing the severity of coagulopathy [9, 10].

Subsequent advances further improved bypass technology, including the introduction of Biomedicus centrifugal pumps and the Griffith shunt system. The technique was based on combined active venovenous drainage from the IVC and portal venous systems via the femoral and portal veins, with reinfusion into the SVC through the axillary vein. Over time, open cannulation methods were gradually replaced by percutaneous puncture techniques, significantly reducing complications associated with heparinization and massive intraoperative bleeding during LT.

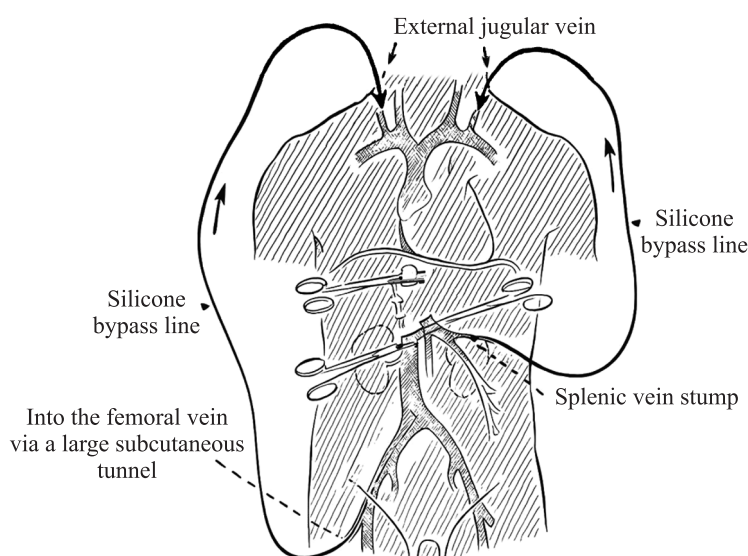


Fig. 1. Schematic representation of blood flow redistribution from the inferior vena cava and portal vein to the superior vena cava system (adapted from Welch C.S. Transplant Bull. 1955 [6])

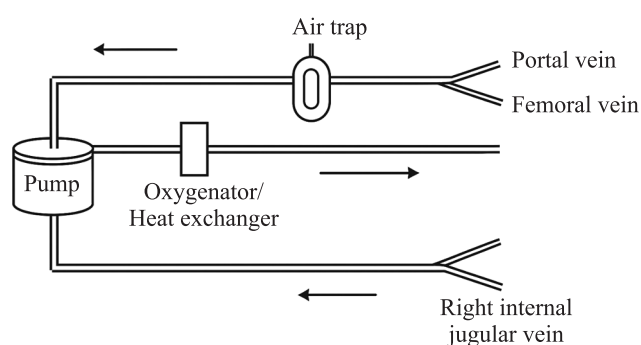


Fig. 2 Schematic representation of veno-venous bypass during liver transplantation (adapted from Butt S.P. et al., The Journal of ExtraCorporal Technology, 2024 [12])

In addition, the development and implementation of rapid autotransfusion systems – first introduced by the Pittsburgh research group – substantially improved intraoperative blood loss management during LT procedures [10, 11].

According to a study by Butt et al., the perfusion system most commonly used for VVS today consists of a circuit system combined with an oxygenator functioning as a heat exchanger (Fig. 2). This key component extends the conventional configuration, which traditionally includes only a centrifugal pump and perfusion tubing for blood drainage and reinfusion, by enabling precise

temperature regulation for controlled patient warming or cooling during surgery.

In addition, the authors supplement the system with bubble sensors on both the inflow and outflow lines, thereby improving procedural safety by reducing the risk of air embolism. Particular emphasis is placed on the use of heparin-coated materials, which decrease the requirement for systemic anticoagulation and enhance circuit biocompatibility.

The authors also note that a hemoconcentrator may be integrated into the VVS circuit when clinically indicated. However, they do not recommend the routine addition of other auxiliary devices because of the increased risk of air embolism. Instead, when required, continuous renal replacement therapy is performed intraoperatively as a separate procedure [12].

The Austin Health Transplant Center in Melbourne, Australia, analyzed 900 liver transplantations performed between 2008 and 2022 and reported that 27 patients (more than 3%) required emergency intraoperative use of VVS. In an additional 16 cases, the use of VVS had been planned from the outset.

Weinberg et al. reported two VVS configurations: in the first approach, active drainage from either the portal vein or femoral vein was directed into the right internal jugular vein; in the second approach, simultaneous shunting from the femoral and portal veins into the axillary vein was performed without the use of an oxygenator or heat exchanger. This latter technique was comparable to the method originally described by Griffith et al. in 1985 [13].

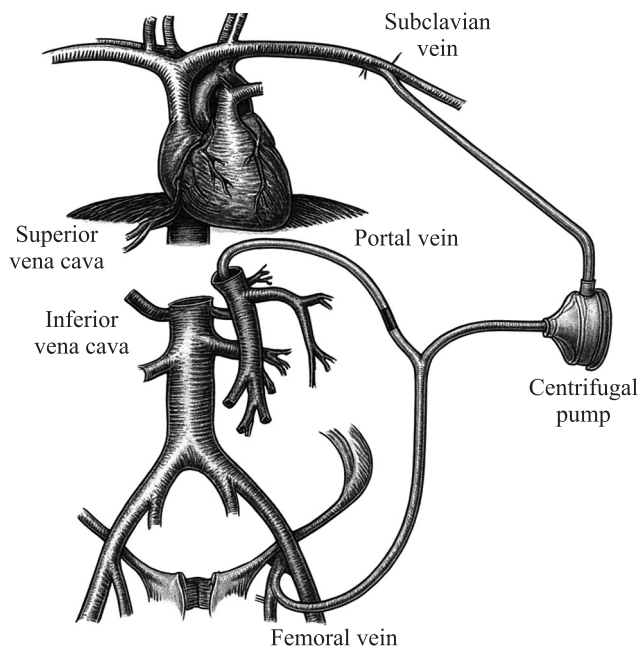


Fig. 3. Veno-venous bypass from the portal and femoral veins to the left axillary vein (adapted from Lokshin L.S., Laptiy A.V., Veno-venous bypass in orthotopic liver transplantation, 1999 [16])

Despite the availability of the technique and its widespread clinical adoption beginning in the 1980s, the routine use of VVS in LT practice has gradually declined worldwide. Several factors contribute to this trend, including the high cost of VVS, the risks associated with insertion of large-bore catheters and the shunting procedure itself, the potential for hypothermia, and the emergence of alternative techniques [3].

Traditional surgical techniques for LT involve complete cross-clamping and resection of the retrohepatic IVC, together with temporary portal vein clamping. These maneuvers may result in pronounced hemodynamic disturbances, including reduced venous return, decreased cardiac output, distal venous congestion, and organ hypoperfusion, all of which can be life-threatening for patients in poor general health [15].

Analysis of the Russian-language literature revealed relatively limited data on the use of VVS between 1990 and 2012, with 2023 considered separately due to increased research interest. In 1999, Lokshin and Laptiy published a paper titled “Veno-venous shunting in orthotopic liver transplantation” in which they reported the use of VVS in 28 patients employing a centrifugal pump with inflow and outflow perfusion lines (Fig. 3). The configuration represented a classical VVS technique. Their study demonstrated the benefits of VVS in preventing ischemic injury to the abdominal organs and kidneys, reducing intraoperative and postoperative complications, and emphasized the necessity of VVS in recipients with certain anatomical features [16, 17].

In 2008, Lutzyk published an experimental study titled “Collateral Venovenous Shunting in Liver Transplantation”, which demonstrated the application of a modified VVS system and technique. The study showed that the method provided stable hemodynamic parameters without significant disturbances in patient homeostasis, despite the use of heparin-coated tubing. The authors also emphasized the advantages of centrifugal pumps over roller pumps, as the latter caused mechanical damage to blood cells and resulted in hemolysis in 58% of cases.

The principal modification compared with the classical VVS configuration was the incorporation of a heat exchanger before reinfusion of blood into the axillary vein, thereby enabling maintenance of a controlled temperature throughout the procedure [18, 19, 21].

Following a prolonged period of limited activity in this field, a significant development occurred in 2023, when the Shumakov National Medical Research Center of Transplantology and Artificial Organs registered a patent entitled “System and Method of Extracorporeal Circulation During Liver Surgery”. In addition, Bondarenko et al. reported the use of a proprietary VVS technique incorporating an oxygenator/heat exchanger and a venous reservoir, allowing immediate reinfusion of shed blood into the SVC system via a double-lumen catheter inserted into the jugular vein (Fig. 4) [20].

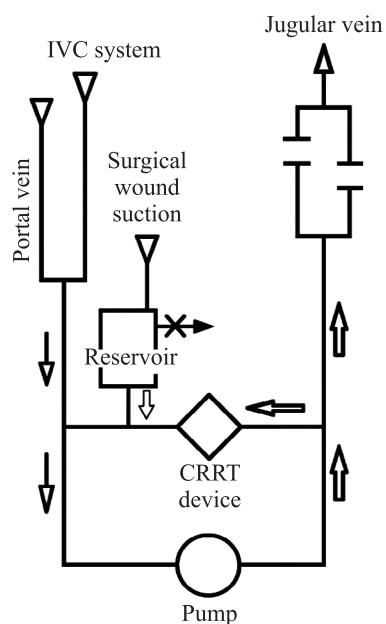


Fig. 4. Schematic representation of veno-venous shunting using an oxygenator/heat exchanger and a venous reservoir [20]. Abbreviations: IVC, inferior vena cava; CRRT, continuous renal replacement therapy

This system and technique combine the advantages of conventional VVS with the ability to control the patient's intravascular volume status, thereby reducing the impact of intraoperative blood loss and decreasing the need for transfusion of blood products during surgery. The percutaneous approach for jugular vein catheterization eliminates many of the disadvantages associated with open surgical access to the axillary vein.

Over the past two decades, VVS techniques have undergone substantial refinement, including the introduction of percutaneous cannulation as a safer and technically simpler alternative to traditional open surgical access. The percutaneous approach has demonstrated advantages in terms of procedural safety and ease of implementation.

Moreover, advances in extracorporeal technologies, including improvements in ECC design, such as integration of heat exchangers to prevent perioperative hypothermia, and the availability of coated (coagulation-inert) circuits for better anticoagulation management, have expanded the clinical applicability and importance of VVS in critically ill patients undergoing LT. Collectively, these innovations have enhanced surgical outcomes [1].

A number of studies evaluating the use of veno-venous/portal (VVP) shunting in LT have reported favorable outcomes. These studies indicate that VVP shunting contributes to intraoperative hemodynamic stability, reduces operative time, and improves postoperative outcomes, particularly in patients with renal dysfunction. Additionally, it has been associated with a lower incidence of complications such as cardiac arrhythmias, pulmonary hypertension, and right ventricular dysfunction. Overall,

these findings support improved recipient safety through a reduction in perioperative surgical risks, thereby reinforcing the role of VVS in minimizing complications and enhancing LT outcomes [2, 3].

In turn, the effectiveness of VVP shunting during LT was further demonstrated by Mossdorf et al., who analyzed 163 consecutive liver transplants performed at a single center following the initiation of its transplant program in 2010. Mean operative time was 269 minutes, while mean warm ischemia time did not exceed 43 minutes. The median requirement for red blood cell and plasma transfusions was 7 and 14 units, respectively. No intraoperative mortality was reported, and the 30-day mortality rate was 3%, with no severe complications attributable to the shunting procedure.

The VVS technique in this series was primarily used to minimize intravascular volume overload, reduce the need for vasopressor therapy, limit myocardial injury, and improve peripheral circulation [14].

In a 2019 comparative study, researchers evaluated the impact of VVS during liver resections involving prolonged vascular isolation and hypothermic organ perfusion. It was found that the use of VVS was associated with significantly reduced intraoperative blood loss ($p = 0.010$) and a lower incidence of postoperative respiratory complications – 15% in the VVS group compared with 64% in patients without VVS ($p = 0.012$).

Although the operative time was longer in the VVS group (460 vs. 375 minutes, $p = 0.023$), no statistically significant differences were observed between the groups in terms of postoperative mortality or incidence of major complications.

These findings highlight the potential benefits of VVS in improving surgical outcomes during complex liver resections involving prolonged vascular isolation and hypothermic liver perfusion. Accordingly, the study supports the recommendation to use this method in such cases [25].

Sakai et al. reported that the introduction of techniques for preserving the retrohepatic segment of the IVC in LT significantly reduced the need for VVS significantly and represented a notable advancement in surgical strategy. Nevertheless, VVS continues to serve as a valuable adjunctive method in LT practice.

Traditionally, venous return cannulation via open access through the axillary vein has been associated with considerable risks, including lymphorrhea, infection, and injury to the brachial plexus. Consequently, in adult LT, percutaneous insertion of the return cannula into the internal jugular vein is now preferred as a safer alternative. This approach not only minimizes procedure-related complications but also improves the overall safety and effectiveness of the surgical intervention [26].

A retrospective study by Sun et al. evaluated the effect of VVS on the development of acute kidney injury (AKI) following LT. Among 1,037 patients included

in the analysis, 247 received intraoperative VVS. The incidence of AKI was significantly lower in patients with pre-transplant renal dysfunction (serum creatinine >1.2 mg/dL) who underwent VVS, and VVS was identified as an independent protective factor against AKI. However, no significant differences were observed in the requirement for renal replacement therapy or 1-year mortality between groups. In patients with normal baseline renal function (creatinine <1.2 mg/dL), the incidence of postoperative hemodynamic disturbances did not differ significantly between VVS and non-VVS groups. Overall, the findings suggest that intraoperative VVS may reduce the risk of post-transplant renal complications in patients with pre-existing renal impairment, although further studies are required to confirm this effect [27].

In another report, Rocco et al. described two cases of complex orthotopic LT in which VVS was employed to manage the porto-mesenteric compartment, with venous grafts anastomosed either to the inferior mesenteric vein (IMV) or the splenic vein (SV). In both cases, a percutaneous femoral-axillary VVS was established prior to abdominal incision to facilitate control of massive collateral circulation within the abdominal wall. In the first case, the IMV was anastomosed to a donor venous graft, while in the second case, severe splenomegaly necessitated splenectomy, after which the SV was similarly anastomosed to the donor venous graft.

In both cases, connecting the distal end of the venous graft to the VVS circuit enabled effective decompression of the porto-mesenteric compartment. This resulted in a reduction in portal hypertension and improved surgical access to the hepatic hilum, which was particularly important in the context of complex dissection following previous extensive surgeries. The described technique is considered both safe and technically straightforward, and it may serve as a valuable alternative in patients requiring VVS who lack standard portal venous access, particularly in cases of severe portal hypertension or repeat LT procedures [12].

An unusual clinical case reported by Salloum et al. described a previously undocumented approach to VVS during LT using a patent paraumbilical vein. In this patient, preoperative computed tomography revealed a significantly dilated paraumbilical vein. Prior to abdominal incision, a percutaneous femoro-axillary VVS was established and subsequently connected to the paraumbilical vein, enabling continuous splanchnic venous decompression throughout the entire surgical procedure.

The successful use of the paraumbilical vein for VVS in this case suggests a promising approach for similar clinical scenarios in the future [28].

In 2024, Butt et al. from Abu Dhabi (United Arab Emirates) reported their experience with VVS in LT in a cohort of 11 patients with varied indications, including high Model for End-Stage Liver Disease (MELD) scores, prior abdominal surgeries with adhesions, recur-

rent spontaneous bacterial peritonitis, portal vein thrombosis, and coronary artery disease with heart failure. The authors reported favorable outcomes, with no vascular complications related to cannulation and no technical issues associated with the VVS circuit. Notably, post-reperfusion syndrome was not observed in any of the cases [12].

The potential benefits of VVS include improved hemodynamic stability during the anhepatic phase due to preservation of venous return, reduced intraoperative blood loss in the setting of portal hypertension, and maintenance of perfusion to vital organs [10, 29]. In addition, patients with metabolic or cholestatic liver diseases – even in the absence of clinically significant portal hypertension – may still be at risk of portal congestion and therefore benefit from VVS or temporary portocaval shunting.

VVS may also facilitate blood flow redirection in cases of complex vascular anatomy or technically demanding surgery, such as massive hepatomegaly, while enabling decompression of the porto-mesenteric circulation [3] and reducing diffuse abdominal bleeding from venous collaterals [14]. Finally, in cases of fulminant liver failure, VVS may help maintain venous return and reduce excessive fluid administration, thereby potentially limiting worsening intracranial hypertension and potential cerebral edema.

However, several authors have reported that in most comparative studies of LT outcomes with and without routine use of VVS, no statistically significant differences were observed in operative time [9–13], blood product transfusion requirements [22, 23, 30], early postoperative mortality [31], incidence of postoperative AKI [24, 32], early postoperative complication rates [24, 33], or length of hospital stay [23, 32, 34]. Based on these findings, some investigators do not recommend routine use of VVS, although they acknowledge that it may still be justified in selected clinical situations where specific indications are present [13].

In line with current clinical trends, the decision to employ VVS should therefore be made on an individual, case-by-case basis, taking into account clearly defined indications and contraindications. Liver surgery in high-risk patients may be associated with substantial acute blood loss, necessitating immediate restoration of circulating blood volume. The use of blood reinfusion systems (CellSaver) does not always provide immediate reinfusion, this process requires a certain amount of time [35, 36].

Thus, while ongoing improvements in VVS systems have enhanced the safety and feasibility of the technique, the resolution of existing challenges has also given rise to new issues that require further investigation. Current practices among Russian scientists, as well as global trends, indicate that LT is increasingly performed without routine use of VVS.

This global transition toward selective, rather than routine, application of VVS – based on specific disease indications, patient condition, and expanded surgical criteria – reflects growing interest in assisted circulation during LT. It also highlights efforts to address current controversies and unresolved issues, with the overarching goal of improving procedural safety.

The authors declare no conflict of interest.

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NONINVASIVE DIAGNOSIS CORONARY COMPUTED TOMOGRAPHIC ANGIOGRAPHY LESIONS OF CORONARY ARTERIES OF THE TRANSPLANTED HEART

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Background. Coronary artery disease (CAD) is one of the leading causes of graft loss after heart transplantation (HT). Owing to cardiac denervation, myocardial ischemia in transplanted hearts is typically clinically silent, necessitating regular screening of recipients to detect transplant vasculopathy. Routine annual invasive coronary angiography (iCAG), however, is associated with potentially life-threatening complications, prompting the search for safe and equally effective non-invasive diagnostic alternatives. Multislice computed tomography coronary angiography (MSCT-CAG) has been widely and successfully used for many years in CAD diagnosis, with a high class and level of evidence, and has long been an alternative to iCAG. This underscores the relevance of evaluating its applicability in heart transplant recipients. **Objective:** to assess the diagnostic effectiveness of MSCT-CAG in detecting cardiac allograft vasculopathy in comparison with iCAG. **Materials and methods.** The study included 46 heart transplant recipients (36 men, 78%) aged 29–68 years (mean age 51.1 ± 10.9 years) who underwent HT between 2012 and 2023. The interval from transplantation to CAG ranged from 201 to 4,285 days (mean 1,097 days). All patients underwent scheduled iCAG and MSCT-CAG. Coronary arteries were evaluated using a 16-segment model. Segments that could not be reliably assessed on MSCT-CAG images were excluded from the analysis. **Results.** Heart rate during MSCT-CAG ranged from 65 to 105 beats per minute (median 90 bpm) and was not adjusted with medication prior to scanning. Invasive CAG allowed assessment of 690 coronary segments, while 683 segments were of diagnostic quality on MSCT-CAG. According to iCAG, coronary lesions were identified in 25 segments. MSCT-CAG detected lesions in 15 segments, yielded false-positive findings in 14 segments, and failed to identify stenoses detected by invasive CAG in 10 segments. X-ray dose was significantly higher during MSCT-CAG (22.6 mSv) compared with iCAG (10 mSv; $p = 0.001$). MSCT-CAG also required a larger volume of contrast medium (90 mL vs. 60 mL; $p = 0.001$). Serum creatinine levels before and after MSCT-CAG were 91.35 ± 18.09 and 95.17 ± 18.53 $\mu\text{mol/L}$, respectively, while glomerular filtration rate (GFR) values were 86.28 ± 17.79 and 82.96 ± 17.72 mL/min/1.73 m². Despite the higher contrast load and compromised renal function due to the nephrotoxicity of immunosuppressive therapy, no cases of contrast-induced nephropathy were observed following MSCT-CAG. Comparative analysis demonstrated that MSCT-CAG had a sensitivity of 60%, specificity of 97%, positive predictive value of 52%, and negative predictive value of 98% relative to iCAG. **Conclusion.** In the diagnosis of cardiac allograft vasculopathy, MSCT-CAG can be used to rule out coronary artery stenosis, demonstrating high specificity (97%) and negative predictive value (98%). The use of MSCT-CAG for the detection of stenosis/restenosis of the coronary vasculature in transplanted hearts requires further study.

Keywords: cardiac allograft vasculopathy, MSCT coronary angiography, heart transplant.

INTRODUCTION

Coronary artery disease is the leading cause of graft functional decline following heart transplantation (HT). Early detection and timely management of cardiac allograft vasculopathy (CAV) are therefore critical for improving long-term outcomes in transplant recipients [1].

According to the 2023 guidelines of the International Society for Heart and Lung Transplantation (ISHLT),

invasive coronary angiography (iCAG) remains the reference standard for diagnosing CAV [2]. At the same time, current clinical guidelines strongly recommend (Class I, Level A) the use of multislice computed tomography coronary angiography (MSCT-CAG) for the detection of coronary artery disease in patients with ischemic heart disease [3]. This has led to increasing interest in the application of this noninvasive modality in heart transplant recipients.

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To date, several studies have demonstrated the high diagnostic accuracy of MSCT-CAG in detecting coronary pathology in this patient population [4–7]. A meta-analysis of 13 prospective studies evaluating MSCT-CAG in patients after HT, which included 615 patients (mean age 52 years, 83% male) and analyzed 9,481 coronary segments, demonstrated that, in HT recipients, MSCT-CAG achieves a sensitivity of 94%, specificity of 92%, negative predictive value (NPV) of 99%, and positive predictive value (PPV) of 67% for the detection of stenoses >50% compared with iCAG. The incorporation of quantitative plaque analysis may further enhance sensitivity for detecting CAV [8].

Overall, current evidence suggests that MSCT-CAG is a sufficiently informative noninvasive modality for visual assessment of the coronary vasculature in HT recipients, with sufficiently high sensitivity (86–89%) and specificity (89–99%). It is important to note that, although iCAG is the gold standard for diagnosing CAV, it requires hospitalization and is associated with procedural risks. The growing number of transplant recipients under long-term follow-up underscores the need for noninvasive screening approaches. In this context, MSCT-CAG may serve as a valuable tool for identifying patients with signs of CAV and assessing disease

progression, thereby helping to define indications and prioritize hospitalization.

Objective: to evaluate the diagnostic effectiveness of MSCT-CAG in detecting CAV in comparison with iCAG.

MATERIALS AND METHODS

The study included 46 heart transplant recipients (36 men, 78%) aged 29–68 years (mean age 51.1 ± 10.9 years) who underwent HT between 2012 and 2023. The interval from transplantation to CAG ranged from 201 to 4,285 days (mean, 1,097 days). The clinical characteristics of the study population are presented in Table 1.

All recipients underwent both elective iCAG and MSCT-CAG. Assessment of the coronary vasculature was performed using the 16-segment model proposed by the American Heart Association [9]. Segments that were not assessable on MSCT-CAG were excluded from the analysis.

Heart rate during the procedure and changes in serum creatinine levels were also recorded. Contrast-induced nephropathy was defined as an increase in serum creatinine of more than 25% from baseline.

Table 1

Clinical characteristics of the patients

Quantitative indicators	M $\pm$ SD / Me	n	min	max
Age, years	51.13 $\pm$ 10.95	46	29.00	68.00
BMI, kg/m ²	27.58 $\pm$ 3.91	46	18.80	35.80
Time from HT, Me (days)	1097.50	46	201.00	4285.00
Serum creatinine level before MSCT-CAG, μ mol/L	91.35 $\pm$ 18.09	46	58.00	140.00
GFR before MSCT-CAG, ml/min/1.73 m ²	86.28 $\pm$ 17.79	46	53.00	119.40
EF, Me (%)	65.00	46	46.00	73.00
EDV, Simpson–L, Me (mL)	78.00	46	59.00	122.00
ESV, Simpson–L, Me (ml)	28.50	46	18.00	59.00

Absolute indicators	Categories	n	%
Recipient's gender	male	36	78.3
	female	10	21.7
Reason for performing the HT procedure	DCM	22	47.8
	ICM	21	45.7
	Other reasons	3	6.5
Diabetes	Present	9	19.6
	Absent	37	80.4
High blood pressure	Present	29	63.0
	Absent	17	37.0
Dyslipidemia	Present	23	50.0
	Absent	23	50.0
Chronic kidney disease	Present	18	39.1
	Absent	28	60.9

Abbreviations: BMI, body mass index; GFR, glomerular filtration rate; MSCT-CAG, multislice computed tomography coronary angiography; EF, ejection fraction; EDV, end-diastolic volume; ESV, end-systolic volume; DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy.

All patients underwent a standard diagnostic workup, including 12-lead electrocardiography (ECG) and transthoracic echocardiography. ECG recordings were obtained using a Megacart system (Siemens, Germany), and echocardiography was performed with a VIVID 9 system (GE Healthcare, USA).

Conventional multiprojection CAG was carried out using the Judkins technique via femoral or radial access on an ALLURA XPER system (Philips, Netherlands). The standard imaging protocol included five projections for the left coronary artery and two for the right coronary artery.

Statistical analysis was performed using standard methods with Microsoft Excel and IBM SPSS Statistics software (version 22).

RESULTS

At the first stage, all patients underwent iCAG. Radial access was used in 63% of cases ($n = 29$), while femoral access was employed in the remaining 37% ($n = 17$). The procedure was technically successful in all cases, with no intra-procedural complications or instances of contrast-induced nephropathy observed.

Based on angiographic findings, a total of 690 coronary segments were evaluated. Of these, 665 segments showed no evidence of lesions, while stenoses were identified in 25 segments. Chronic total occlusions were detected in 2 cases. Previously implanted stents were patent, with no signs of stenosis.

Lesions were localized as follows: in proximal segments – left anterior descending (LAD) artery ($n = 5$), left circumflex (LCx) artery ($n = 1$), and right coronary artery (RCA) ($n = 2$); in middle segments – LAD ($n = 8$), LCx ($n = 1$), and RCA ($n = 2$); and in distal segments – LAD ($n = 5$) and the diagonal branch ($n = 1$). No stenoses were identified in the left coronary artery (LCA), the distal segments of LCx and RCA, or in the second diagonal branch (D2), obtuse marginal artery (OMA), posterolateral artery (PLA) and posterior descending artery (PDA) of the RCA. No cases of coronary circulation involving an intermediate artery were observed.

At the second stage, occurring 1–3 days later, all recipients underwent MSCT-CAG. The procedure was technically successful in 100% of cases. Heart rate during MSCT-CAG ranged from 65 to 105 beats per minute (mean, 90 bpm) and was not pre-adjusted with medication.

According to MSCT-CAG data, 683 coronary segments were of diagnostic quality. Seven segments were excluded from analysis due to inability to reliably assess them: three because of poor image quality, two due to imaging artifacts, and two due to the inability to perform quantitative assessment, with these interpreted as diffuse lesions.

654 segments were free of lesions, while stenoses were identified in 29 segments. Lesions were localized

as follows: proximal segments – LAD artery ($n = 7$), LCx artery ($n = 1$), and RCA ($n = 4$); middle segments – LAD artery ($n = 7$) and LCx artery ($n = 1$); distal segments – LAD artery ($n = 3$) and the diagonal branch ($n = 2$).

No stenoses were detected in the LCA trunk, distal LCx and RCA segments, or in the D2, OMA, PLA, or PDA of the RCA.

The results of iCAG and MSCT-CAG are illustrated in Fig. 1 using the 16-segment coronary artery model proposed by the American Heart Association.

A comparative analysis of MSCT-CAG and iCAG findings showed that, according to iCAG, lesions were identified in 25 segments. MSCT-CAG correctly detected 15 of these lesions, while 14 segments were classified as false-positive. In addition, 10 stenotic segments identified by iCAG were not detected by MSCT-CAG.

Based on these results, the diagnostic performance of MSCT-CAG for detecting CAV was as follows: sensitivity – 60%, specificity – 97%, PPV – 52%, and NPV – 98%.

A comparison of radiation exposure and contrast agent volume between the two methods was also performed (Table 2).

It should be noted that the radiation dose associated with MSCT-CAG was higher than that of conventional CAG (22.6 mSv vs. 10 mSv; $p = 0.001$). In addition, MSCT-CAG required a larger volume of contrast medium (90 mL vs. 60 mL; $p = 0.001$).

An analysis of renal function parameters before and after MSCT-CAG demonstrated minimal changes. Mean serum creatinine increased slightly from 91.35 ± 18.09 to 95.17 ± 18.53 $\mu\text{mol/L}$, while estimated glomerular filtration rate (eGFR) decreased from 86.28 ± 17.79 to 82.96 ± 17.72 mL/min/1.73 m². Further analysis evaluated the dynamics of serum creatinine and eGFR depending on the presence or absence of chronic kidney disease (CKD) (Fig. 2).

In recipients without CKD, serum creatinine levels did not differ significantly before and after MSCT-CAG ($p = 0.896$), measuring 74.36 ± 7.8 and 74.62 ± 8.48 $\mu\text{mol/L}$, respectively. In contrast, patients with CKD showed a statistically significant increase in serum creatinine following MSCT-CAG ($p = 0.005$), from 95.49 ± 17.5 $\mu\text{mol/L}$ before the procedure to 100.17 ± 16.79 $\mu\text{mol/L}$ afterward.

Table 2

Comparison of radiation dose and volume of contrast agent used

Parameters	Groups		p
	Invasive CAG	MSCT-CAG	
Dose, Me [IQR]	10.00 [6.65; 11.30]	22.60 [21.02; 25.00]	0.001
Contrast agent, Me [IQR]	60.00 [50.00; 60.00]	90.00 [90.00; 95.00]	0.001

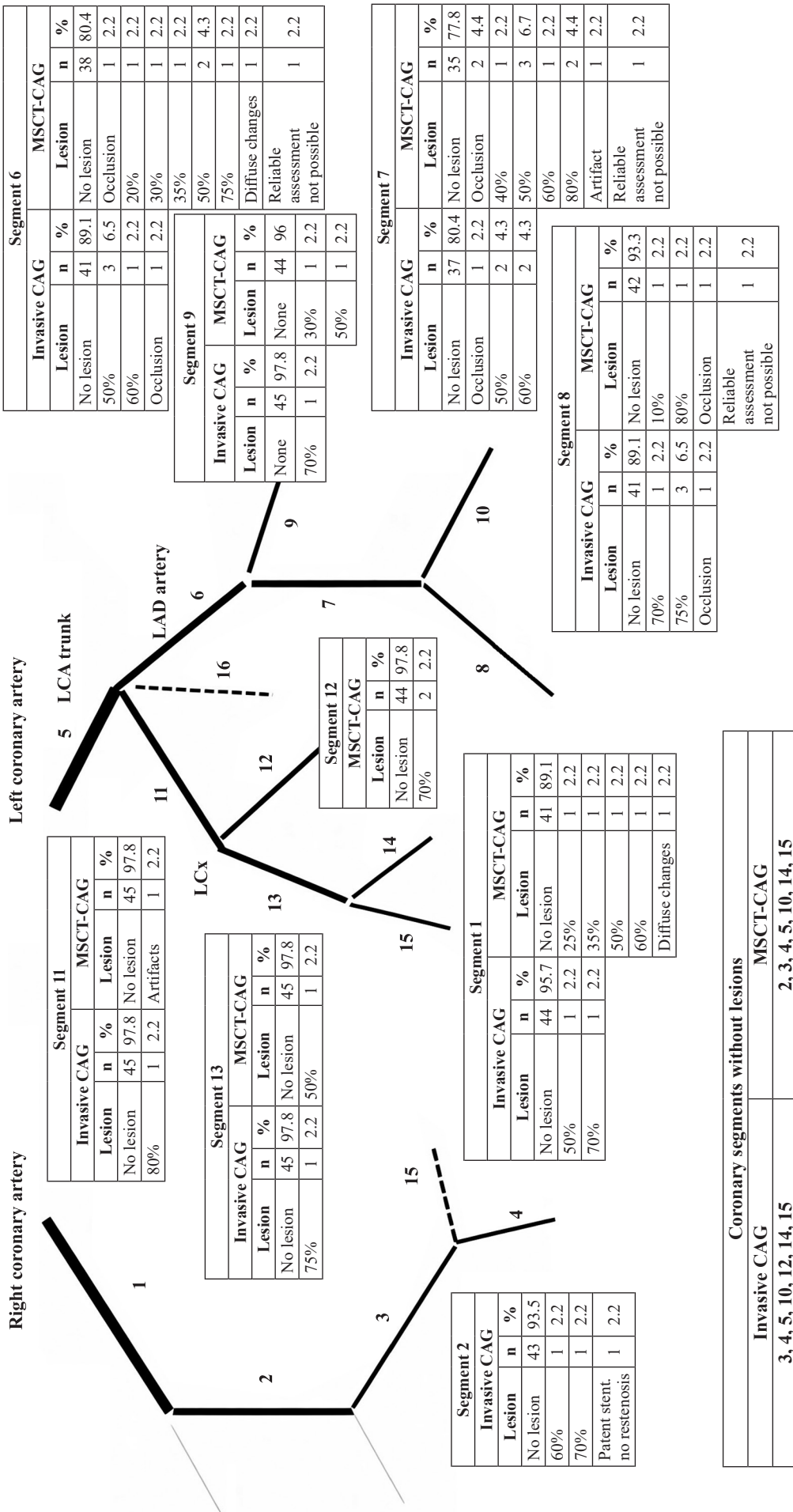


Fig. 1. Results of invasive CAG and MSCT-CAG assessed using the 16-segment coronary artery model according to the American Heart Association classification. Right coronary artery (RCA): segment 1 – proximal RCA; 2 – mid RCA; 3 – distal RCA; 4 – posterior descending artery. Left coronary artery (LCA): segment 5 – left main coronary artery; 6 – proximal left anterior descending artery (LAD); 7 – mid LAD; 8 – distal LAD; 9 – first diagonal branch; 10 – second diagonal branch; 11 – proximal circumflex artery (LCx); 12 – first obtuse marginal branch; 13 – distal LCx; 14 – posterolateral branch; 15 – LCA; 16 – intermediate (ramus intermedius) branch

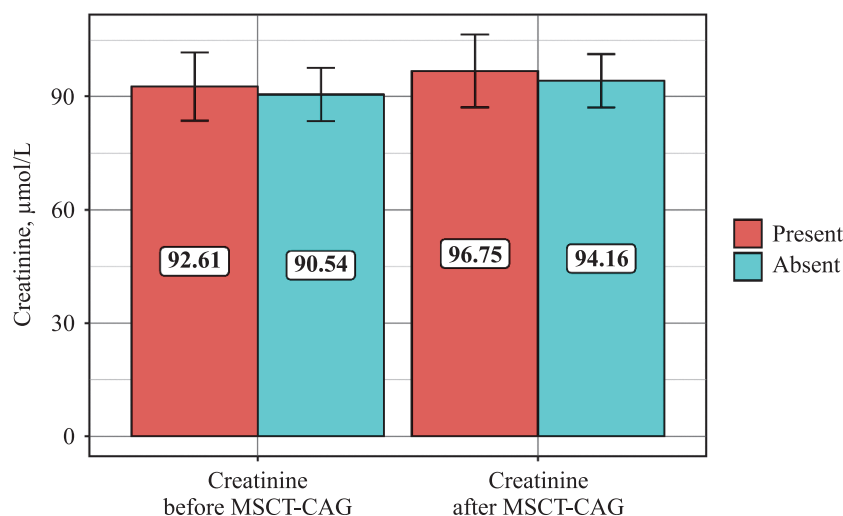


Fig. 2. Analysis of serum creatinine dynamics depending on the presence or absence of CKD

Notably, despite a significant increase in serum creatinine levels in recipients with CKD following MSCT-CAG, this increase did not exceed 5% of the baseline value.

DISCUSSION

Over the past 15 years, the number of HT performed in Russia has increased substantially, reaching 450 procedures in 2024, of which 294 were carried out at Shumakov National Medical Research Center of Transplantation and Artificial Organs in Moscow. As a result, more than 3,000 recipients are currently under follow-up [10].

CAV remains the leading cause of graft loss. According to data from the ISHLT registry, it is detected in nearly 50% of recipients 10 years after transplantation [1, 11]. This underscores the need for continuous surveillance of the coronary vasculature.

Invasive CAG remains the gold standard for diagnosing CAV (Class I, Level of Evidence A, according to the 2023 ISHLT guidelines). However, the procedure is associated with a risk of life-threatening complications (arrhythmias, contrast-induced acute kidney injury, acute cerebrovascular events, coronary artery dissection, and bleeding at arterial access sites). Although the overall incidence of such complications is relatively low (approximately 1.8%), they may be life-threatening, with reported mortality rates of up to 0.1% [12].

In this context, there is a growing need to identify effective, noninvasive alternatives for diagnosing coronary artery disease in transplanted hearts. Recent studies indicate that the diagnostic results from such methods in HT recipients are becoming comparable to those obtained in patients with ischemic heart disease.

In a 2020 study by Foldyna et al., using a second-generation 128-slice MSCT scanner with quantitative coronary segment analysis, 50 HT recipients (84% men) with a mean age of 53.6 ± 11.9 years and a mean post-

transplant follow-up of 6.7 ± 4.7 years were evaluated, including patients both with and without previously confirmed vasculopathy. The interval between iCAG and MSCT-CAG was one day.

Mean radiation dose was 5.8 mSv. Among the study population (42 men and 8 women), the prevalence of CAV was 38% (19/50). At a mean heart rate of 74.1 ± 8.5 bpm, MSCT-CAG provided diagnostic-quality images for 692 coronary segments, of which 632 (91.4%) were comparable with CAG findings, while 56 segments were excluded due to poor image quality. Of the 632 evaluable segments, 190 (30.1%) were proximal and 442 (69.9%) were distal, with distal segments affected more frequently. Quantitative analysis of the coronary wall revealed that fibrous tissue accounted for 44.7%, fibrofatty tissue for 18.6%, non-calcified (soft) plaques for 8.5%, and calcified plaques for 1.0%. Distal segments were affected more frequently than proximal ones.

This study demonstrated that MSCT-CAG is highly effective in detecting clinically significant stenoses ($\geq 50\%$) and shows good agreement with iCAG in HT recipients, with an NPV of 98–100%, sensitivity of 78%, and specificity of 75%. Importantly, MSCT-CAG also enables early detection of CAV through quantitative assessment of plaque volume and composition, thereby facilitating timely adjustment of medical therapy [13].

In a subsequent 2022 study by Ojha et al., the diagnostic accuracy of dual-source MSCT (192-detector, 384-slice) for the CAV detection was evaluated in comparison with iCAG. The study included 38 patients, of whom 27 were men. The mean age of the patients was 33.66 ± 11.45 years, and the time since HT ranged from 10 to 226 months (median: 23.5 months). The prevalence of CAV (stenosis grades 1–5) was 44.7% ($n = 17$) according to MSCT-CAG and 39.5% ($n = 15$) based on iCAG. Clinically significant lesions (CAV grades 3–5)

were identified in 21.1% of patients (n = 8) by MSCT-CAG and in 15.8% (n = 6) by iCAG.

Diagnostic image quality was considered acceptable in 557 of 576 coronary segments (96.7%). Mean radiation dose was 4.24 ± 2.15 mSv for MSCT-CAG and 4.8 ± 1.8 mSv for iCAG, while the average volume of contrast agent used was 42 mL. At the patient level, the detection of signs of vasculopathy of any severity showed a sensitivity of 100%, specificity of 91.3%, PPV of 88.2%, NPV of 100%, and an overall diagnostic accuracy of 94.73%. For the identification of clinically significant stenotic lesions, sensitivity remained 100%, while specificity was 94%, PPV 75%, NPV 100%, and diagnostic accuracy 95%.

Similar results were obtained in the segment-based analysis, with sensitivity, specificity, PPV, and NPV of 96%, 97.6%, 80%, and 99.6%, respectively. Notably, these outcomes were achieved at a relatively low radiation dose (4.24 ± 2.16 mSv), demonstrating high diagnostic accuracy of MSCT-CAG for detecting both early and significant manifestations of CAV when compared with iCAG [9].

When considered alongside the findings of the present study, which demonstrated a high specificity of 97% and an NPV of 98%, these data support the use of MSCT-CAG (including on an outpatient basis) for ruling out coronary lesions in transplanted hearts. This will optimize patient selection for hospitalization.

Another important consideration is the ability of MSCT-CAG to detect clinically significant coronary lesions. Based on both published data and the findings of the present study, the sensitivity of MSCT-CAG in assessing CAV remains insufficient to unequivocally support its use for precise grading of disease severity or for determining indications for revascularization.

It should also be emphasized that the diagnostic performance of MSCT-CAG in detecting coronary lesions is influenced by multiple factors, including the spatial resolution of the CT scanner, the presence of artifacts (e.g., sternal clamps, ECG leads and electrodes), heart rate during image acquisition, and the diameter of the evaluated coronary segments. These limitations highlight the need for further investigation.

CONCLUSION

MSCT-CAG may be considered a useful noninvasive modality for the evaluation of CAV, particularly for ruling out significant stenotic lesions, given its high specificity (97%) and NPV (98%).

Despite the requirement for a higher volume of contrast agent and the presence of baseline renal impairment in many heart transplant recipients due to the nephrotoxic effects of immunosuppressive therapy, no cases of contrast-induced nephropathy were observed following MSCT-CAG in this study.

However, the role of MSCT-CAG in reliably detecting stenoses or restenoses in the coronary vasculature of transplanted hearts requires further study.

The authors declare no conflict of interest.

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TREATMENT STRATEGIES FOR HYPERTROPHIC CARDIOMYOPATHY BASED ON ANATOMICAL VARIANT

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Hypertrophic cardiomyopathy (HCM) is the most common inherited heart disease and is characterized by a variety of manifestations: from morphological features and disease progression to clinical presentation and hemodynamic parameters. Management of HCM should be strictly individualized, considering not only hemodynamic parameters but also anatomical features. Classification based on the presence or absence of left ventricular outflow tract obstruction, as well as the location of interventricular septal hypertrophy (basal, midventricular, and apical), largely determines the optimal management strategy. Drug therapy, surgical myectomy, and alcohol septal ablation are the main treatment options for obstructive HCM, whereas management of the non-obstructive form focuses on symptom control and prevention of complications. Given the risk of sudden cardiac death, timely implantation of an implantable cardioverter-defibrillator in high-risk patients is of paramount importance.

Keywords: hypertrophic cardiomyopathy, left ventricular outflow tract obstruction, surgical myectomy, alcohol septal ablation, heart transplantation.

INTRODUCTION

HCM is the most common inherited heart disease, with an estimated prevalence of approximately 1 in 500 individuals [1].

It is defined by increased left ventricular (LV) wall thickness (≥ 15 mm in at least one myocardial segment in adults) that cannot be explained by abnormal loading conditions such as systemic hypertension [2]. HCM exhibits marked heterogeneity, encompassing a wide spectrum of morphological features, disease progression patterns, clinical manifestations, and hemodynamic profiles [3, 4].

Management strategies for HCM include both conservative and surgical approaches. Surgical options comprise septal myectomy, alcohol septal ablation, and, in advanced cases, heart transplantation (HT). HCM accounts for approximately 1–5% of all heart transplant indications, with post-transplant survival comparable to that observed in other forms of cardiomyopathy [5–7].

The selection of the most appropriate treatment strategy is largely determined by the specific anatomical variant of the disease. This article aims to discuss which treatment is preferable for various forms of this disease.

HCM CLASSIFICATION

Yifan Wang et al. conducted a comprehensive literature review and, drawing on multiple classification systems, systematically outlined the current criteria and distinguishing features of the various HCM subtypes (Table 1) [8].

NON-OBSTRUCTIVE AND OBSTRUCTIVE HCM

A key determinant in the diagnosis and risk stratification of HCM patients is the presence or absence of left ventricular outflow tract (LVOT) obstruction. Understanding the pathophysiological differences between obstructive and non-obstructive forms is critical for selecting the optimal treatment strategy.

Non-obstructive HCM

Historically, HCM was primarily viewed as a condition characterized by dynamic LVOT obstruction. It was this feature that defined its early names: idiopathic hypertrophic subaortic stenosis, hypertrophic obstructive cardiomyopathy, and muscular subaortic stenosis [15, 16].

It is noteworthy that, in recent years, comparatively less attention has been devoted to investigating the natural course of HCM in patients who do not develop LVOT obstruction under any circumstances, including during physical exertion [17–20].

Evidence from a large cohort study ($n = 573$) demonstrated that patients with non-obstructive HCM (unlike those with obstruction) generally exhibit a stable clinical course, often remaining asymptomatic or experiencing only mild manifestations over extended periods. Favorable outcomes in this group were frequently achieved with medical therapy [17].

Medical Management of Patients with Non-obstructive HCM

The management of heart failure in patients with non-obstructive HCM largely aligns with established heart failure treatment guidelines. The primary objective of drug therapy is to reduce LV diastolic pressure and improve ventricular filling. This is typically accomplished through heart rate control using beta blockers, verapamil and diltiazem, alongside the judicious use of loop diuretics. In the absence of LVOT obstruction, cautious administration of nitrates and ranolazine may also be considered to alleviate symptoms in patients complaining of chest pain [3].

Targeted modulation of the renin-angiotensin-aldosterone system (RAAS) using antifibrotic and anti-hypertrophic agents represents a promising adjunctive strategy in the management of HCM. Evidence from a small randomized, double-blind, placebo-controlled trial suggests that losartan may slow the progression of myocardial hypertrophy and fibrosis in patients with non-obstructive HCM [21].

Surgical Treatment of Patients with Non-obstructive HCM

However, there is an important exception to this relatively favorable course of non-obstructive HCM. A small group of patients (estimated at less than 10%) develops

progressive heart failure that is refractory to optimal medical therapy and is associated with severe symptoms (New York Heart Association Class III/IV). This occurs in so-called burned-out HCM, which is characterized by impaired LV contractile function, although in some cases systolic function may initially remain preserved. In such cases, heart transplantation (HT) remains the only definitive treatment option [6, 22, 23].

HT in patients with HCM is associated with favorable outcomes, with survival rates reported to be comparable to – and in some studies exceeding – those observed in other cardiac conditions. Reported post-transplant survival rates for HCM are approximately 85% at 1 year, 75% at 5 years, and 61% at 10 years [6].

Patients with non-obstructive HCM remain at risk for disease-related complications, such as sudden arrhythmic death, thromboembolic stroke, and atrial fibrillation. However, these complications are relatively rare in this patient group. It is emphasized that disease-related mortality in patients with non-obstructive HCM remains low, estimated at approximately 0.5% per year, with excellent long-term survival rates of 99% at 5 years and 97% at 10 years [17, 19, 24–26].

Obstructive HCM

Systolic anterior motion (SAM) of the mitral valve (MV) leaflet is a common echocardiographic finding in HCM, occurring in up to 95% of cases. Its clinical mani-

Table 1

HCM classification (adapted from [8])

Sign	Types
Presence of LVOT obstruction	– Obstructive HCM – Non-obstructive [9]
Types of non-obstructive HCM	– Standard/most common variant – With dilational transformation – Restrictive type – With reduced LV contractile function [10]
Morphological features of the heart and type of LVH	– Basal septal hypertrophy – Septal hypertrophy – Septal hypertrophy with anterior and anteroseptal walls – Apical LVH [11]
	– Septum curved into the LV cavity, forming a crescent-shaped LV cavity – S-shaped septum – Septal hypertrophy with a smooth contour – Apical LVH – Mid-septal hypertrophy [12]
	– Isolated septal hypertrophy – Hypertrophy of the septum and adjacent sections (except the apex) – Hypertrophy of the apex in combination with hypertrophy of other walls – Apical hypertrophy [13]
	– Apical LVH – Mid-septal hypertrophy – Basal septal LVH – Diffuse LVH [14]

Abbreviations: LVOT, left ventricular outflow tract; HCM, hypertrophic cardiomyopathy; LVH, left ventricular hypertrophy; LV, left ventricular.

festations varies widely, ranging from an incidental imaging observation to severe dynamic LVOT obstruction associated with significant hemodynamic compromise. Approximately 60–70% of patients with HCM exhibit either resting or provokable LVOT obstruction [27].

The severity of SAM is graded as follows:

- Mild: transient SAM without contact with the MV;
- Moderate: SAM with leaflet–septal contact lasting less than one-third of systole;
- Severe: SAM with persistent leaflet–septal contact exceeding one-third of systole [28].

Factors predisposing patients to dynamic LVOT obstruction and the SAM effect in HCM have been identified (Table 2) [27].

Interventricular Septum

It has been established that S-shaped deformation of the thickened interventricular septum (IVS) is an independent risk factor for the development of the SAM effect. This effect is attributed to vortex-induced redirection of early, low-velocity systolic blood flow beneath the MV, resulting in anterior displacement of the valve leaflets toward the IVS [27].

Mitral Valve

Normal MV leaflet lengths, as assessed by echocardiography (ECHO), are generally reported as 18–24 mm for the anterior mitral valve leaflet (AMVL) and 11–14 mm for the posterior mitral valve leaflet (PMVL) [29]. In HCM patients, transesophageal ECHO has demonstrated significant elongation of the MV leaflets compared with control subjects. Reported measurements show leaflet lengths of 31 ± 4 mm versus 22 ± 3 mm for the anterior leaflet, and 20 ± 2 mm versus 15 ± 3 mm for the posterior leaflet ($p < 0.00001$ for both cases) [30].

Similarly, a magnetic resonance imaging (MRI) study involving 172 HCM patients and 15 HCM gene carriers

without disease manifestations revealed consistent MV leaflet elongation. The mean anterior leaflet length was 26 ± 5 mm, significantly greater than that observed in controls (19 ± 5 mm, $p < 0.001$). The posterior leaflet was also significantly longer in patients with HCM (14 ± 4 mm versus 10 ± 3 mm, $p < 0.001$) [31].

Excess MV leaflet tissue often extends beyond the zone of coaptation and is referred to as the “residual leaflet”, which contributes to increased leaflet length and surface area. This redundant tissue is typically the first to make contact with the IVS. In HCM, altered intraventricular hemodynamics resulting from abnormal cardiac geometry facilitate the displacement of this residual MV leaflet, thereby promoting LVOT obstruction [32].

Comparative analyses of MV and ventricular dimensions in patients with HCM and the SAM effect with data from the control group showed that in HCM patients, the distance from the MV leaflet closure site to the IVS (C-sept distance) was significantly reduced (12 ± 4 mm vs. 21 ± 3 mm, $p < 0.001$) [33].

Mitral regurgitation in HCM most commonly arises from incomplete closure of MV leaflets. This typically occurs when the PMVL fails to move anteriorly in coordination with the AMVL, either due to reduced length or limited mobility [34]. Accurate differentiation between SAM-related mitral regurgitation and primary MV regurgitation is essential for selecting an appropriate treatment strategy. Assessment of the regurgitant jet direction can aid in this distinction: a posteriorly directed jet is generally indicative of SAM-related regurgitation, whereas a centrally or anteriorly directed jet more often suggests intrinsic MV disease [35, 36].

Earlier theories attributed SAM to the Venturi effect, whereby IVS hypertrophy narrows the LVOT, reducing the cross-sectional increasing and thereby increasing blood flow velocity. The accelerated blood flow displaces

Table 2

Causes of systolic anterior motion (SAM) in HCM (adapted from [27])

Area of pathology	Features
Interventricular septum (IVS)	S-shaped septum
Mitral valve	Elongation of the Mitral valve leaflets (both anterior and posterior)
	Increased distance from the leaflet coaptation point to the apex of the anterior mitral leaflet (the area referred to as the “residual leaflet”)
	Reduced distance between the leaflet coaptation point and the mitral annulus (C–sept distance)
	Venturi effect
	Anterior diastolic motion of the mitral leaflets
Papillary muscles	Papillary hypertrophy
	Increased number of papillary muscles
	Displacement and abnormal attachment of the papillary muscles
	Reduced distance between the papillary muscles
Chordal apparatus	Shortening and chordal fibrosis

the MV leaflets toward the IVS and LVOT, creating a “suction” effect [37].

Currently, a phenomenon known as “diastolic anterior motion” of the MV has been described and considered a precursor to SAM. During late diastole, mitral inflow generates a posteriorly directed vortex that propels the MV leaflets forward even before the onset of systole [38].

Papillary Muscles

Echocardiographic studies have shown that papillary muscle hypertrophy – defined as a short-axis thickness greater than 11 mm at end-diastole – occurs in more than 50% of patients. Cardiac MRI further demonstrates an increased number (2.5 vs. 2.1, $p < 0.001$) and total mass of papillary muscles in individuals with HCM [27, 39].

Displacement of the papillary muscles – whether anterior displacement, basal shift, or apical relocation – can disrupt LV hemodynamics. These alterations may lead to overlap between the LV inflow and outflow tracts [40].

An abnormal attachment of a papillary muscle directly to the AMVL poses a particular risk, as this causes a SAM effect and LVOT obstruction, which worsens the prognosis in HCM [41].

Papillary muscle anomalies have been shown to correlate with higher resting LVOT pressure gradients, independent of IVS thickness or baseline heart rate. Experimental *in vitro* models further suggest that such abnormalities can induce SAM in HCM even in the absence of septal hypertrophy. These variants are estimated to be present in up to 20% of patients with HCM [39, 40, 42].

MORPHOLOGICAL VARIANTS OF INTERVENTRICULAR SEPTAL HYPERTROPHY IN HCM AND TREATMENT STRATEGIES

The clinical course and choice of treatment for HCM are determined not only by the presence or absence of LVOT obstruction but also by underlying morphological features. In particular, the pattern and distribution of IVS hypertrophy is an important factor that must be considered in clinical decision-making. Based on the predominant location of maximal septal thickening, several morphological variants of HCM can be identified, with the basal, mid-ventricular, and apical forms being the most common. These anatomical subtypes carry distinct prognostic implications and play an important role in guiding treatment strategies [43].

HCM with Basal Hypertrophy

Basal IVS hypertrophy is confined to the subaortic region and extends directly to the point where the chords attach to the apex of the AMVL [43].

Focal thickening of the IVS in the subaortic region, which causes the SAM effect and LVOT obstruction, makes this anatomical subtype the most promising can-

didate for various treatment methods aimed at reducing IVS thickness [43].

Pharmacological Treatment of Patients with HCM and Basal Hypertrophy

A meta-analysis of 37 studies involving 1,898 patients showed that, in obstructive HCM, various medications reduce the LVOT pressure gradient to varying degrees [44].

The most pronounced reductions were observed with disopyramide (–43.5 mmHg [95% CI, –51.6 to –35.3]) and cardiac myosin inhibitors (CMIs) (–34.8 mmHg [95% CI, –40.6 to –29.0]). In contrast, beta blockers (–20.7 mmHg [95% CI, –29.4 to –12.0]) and calcium channel blockers (–14.7 mmHg [95% CI, –23.3 to –6.1]) were associated with more modest reductions. An interaction p -value < 0.01 indicates a statistically significant difference between groups.

Within the class of CMIs, mavacamten demonstrated a greater reduction in LVOT gradient compared with aficamten. Among beta blockers, metoprolol was associated with the largest gradient reduction, while verapamil showed the greatest efficacy among calcium channel blockers (p for interaction < 0.01). These findings are consistent with results observed for provokable LVOT gradients [44].

Surgical Treatment of Patients with HCM and Basal Hypertrophy

According to the generally accepted definition, LVOT obstruction in HCM is defined as a peak LVOT pressure gradient of ≥ 30 mmHg, as measured by Doppler echocardiography. However, a higher threshold of ≥ 50 mmHg is generally used to guide consideration of invasive treatment [3].

According to European clinical guidelines [3], invasive treatment is indicated to reduce LVOT obstruction in the presence of the following criteria:

- A resting gradient or maximum induced gradient ≥ 50 mmHg (e.g., during exercise or Valsalva maneuver);
- Moderate to severe mitral regurgitation associated with SAM;
- Presence of symptoms, typically New York Heart Association (NYHA) functional class II–IV;
- Syncope during physical exertion or unexplained recurrent syncope despite optimal medical therapy;
- Atrial fibrillation or moderate to severe left atrial dilatation.

In patients with obstructive HCM who remain resistant to medical therapy, septal myectomy (Morrow procedure) is an effective surgical option. This procedure involves resection of the basal portion of the IVS (transaortic [45] or transmucosal [46] approach), resulting in enlargement of the LVOT lumen and elimination of the

cause of SAM [47]. In cases of severe septal hypertrophy, a more extensive resection may be required, extending the myectomy into the mid-ventricular region beyond the point of mitral leaflet–septal contact (modified Morrow procedure) [48].

For patients at elevated surgical risk or those who are not suitable candidates for surgery, percutaneous alcohol septal ablation may be performed [49, 50]. This procedure involves injecting a small amount of 96% ethanol into the first septal branch of the left anterior descending artery. This induces a controlled localized infarction of the basal IVS, which subsequently leads to LVOT remodeling [51].

In pediatric patients, LVOT obstruction often requires a repeat septal myectomy. This may be due to various reasons, including inadequate myocardial resection during the initial procedure, the presence of a mid-ventricular component of obstruction, papillary muscle anomalies, and postoperative LV remodeling, including regrowth of septal myocardium. Notably, Osiev et al. reported a case of successful alcohol septal ablation in a 6-year-old patient [52].

Table 3 provides a comparative overview of surgical and endovascular approaches for reducing IVS thickness [3, 53].

HCM with Midventricular Hypertrophic Cardiomyopathy

Midventricular hypertrophic cardiomyopathy (MV-HCM) is defined as a pattern of IVS thickening that extends from the MV chordae to the septal region opposite the papillary muscle heads [43].

Isolated MVHCM occurs in less than 10% of patients with obstructive HCM, but it may present with clinical

manifestations similar to those observed in subaortic LVOT obstruction [54–56].

About 25% of these patients have an LV apical aneurysm, and in some of them, a thrombus forms within this aneurysm [3, 57].

Medical Management of Patients with HCM and MVHCM

The main medications used in such cases (beta blockers, calcium channel blockers, and disopyramide) are aimed at reducing LV contractile function and increasing its filling time [58]. These drugs help reduce the pressure gradient in the mid-ventricular region and improve diastolic function in patients with HCM and mid-ventricular obstruction [57, 59].

Two recently published studies, based on analyses of three clinical cases, reported favorable clinical and morphological effects of mavacamten administered at doses of 5–10 mg in patients with HCM and midventricular obstruction. Over a follow-up period of 16–24 weeks, patients demonstrated a reduction in symptom severity, accompanied by a marked decrease in peak mid-ventricular pressure gradient – from 50–77 mmHg to 8–11 mmHg – as well as a 5- to 7-fold reduction in N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels [60, 61]. Additionally, evidence of reverse LV remodeling was observed, including expansion of the LV cavity, reduction in IVS thickness and left atrial size, and improvement in LV diastolic function, while preserving systolic performance [60].

In rare cases, thrombi are found within the aneurysm, which should be treated with long-term oral anticoagulant therapy [57].

Table 3

Comparison of surgical and endovascular treatment methods in terms of reducing septal thickness [3, 53]

Myectomy	Alcohol septal ablation
Perioperative mortality	
Higher	Lower
Surgical complications	
Higher risk of VSD	Higher risk of atrioventricular block (7–20% of patients), requiring implantation of a permanent pacemaker
Higher incidence of postoperative bleeding (OR 0.18; 95% CI 0.11–0.32; $p < 0.0001$)	Higher residual LVOT gradient
Higher risk of the need for intra-aortic balloon counterpulsation	Higher likelihood of repeat intervention (7–20% of patients)
Surgical limitations	
Although the overall risk of VSD after septal myectomy is low, it may be increased in patients with moderate left ventricular hypertrophy (wall thickness ≤ 16 mm)	May be less effective in patients with severe LVH (≥ 30 mm)
Total adjusted treatment cost and hospital length of stay	
Higher	Lower

Abbreviations: VSD, ventricular septal defect; LVOT, left ventricular outflow tract; LVH, left ventricular hypertrophy; LV, left ventricular.

Surgical Treatment of Patients with HCM and MVHCM

In patients with HCM and MVHCM whose symptoms remain refractory to medical therapy, treatment options are more limited than in cases of HCM with basal hypertrophy.

Recent Russian study suggests that extended septal myectomy, combined with interventions on subvalvular structures, can effectively relieve obstruction in both the mid- and basal segments of the left ventricle, while also increasing LV cavity size [62]. When performing myectomy in patients with obstructive HCM in whom subaortic hypertrophy extends into the midventricular region, careful control of the volume of resected IVS tissue is crucial. Transesophageal echocardiography is routinely used to visualize anatomical landmarks within the left ventricle.

In cases with severe basal IVS hypertrophy, septal myectomy via a subaortic (transaortic) approach can be technically demanding. When obstruction involves the midventricular segment, the procedure is typically initiated through a transaortic myectomy, removing the IVS muscle as close as possible to the apex of the heart [43].

In cases of isolated MVHCM, septal myectomy via a transapical approach is generally considered the preferred surgical strategy. Transapical access provides a wide field of view and access to the (IVS and papillary muscles [54].

It is generally accepted that, in the absence of LVOT obstruction, alcohol septal ablation is not indicated for HCM. However, some clinical studies demonstrate that it may be useful for symptom relief and reduction of intraventricular gradients in patients with symptomatic midventricular obstruction [63, 64].

Some studies suggest that cardiac pacing may reduce obstruction and alleviate symptoms in patients with midventricular obstruction [65, 66]. A randomized, placebo-controlled trial involving 17 patients with midventricular obstruction and a mean NYHA functional class III despite optimal medical therapy demonstrated that individualized optimization of pacing parameters can reduce intraventricular obstruction and improve exercise tolerance in patients with severe midventricular obstruction symptoms.

In this study, the mean pressure gradient decreased from 80 ± 29 mmHg prior to pacing to 31 ± 21 mmHg at the optimal pacing configuration, representing an approximate 60% reduction ($p < 0.0001$). Personalized pacemaker programming was associated with an improvement in exercise tolerance, as evidenced by an increase in the distance covered during the 6-minute walk test (328.5 ± 99.9 m vs. 285.8 ± 105.5 m; $p = 0.018$) [58].

Apical HCM

Apical hypertrophy with obliteration of the LV apex is confined to the apex and the distal portion of the IVS [43].

Morphologically, apical HCM can be classified into three subtypes:

- Isolated type: hypertrophy confined to the apical region;
- Mixed type: combined apical and septal hypertrophy, with maximal wall thickness at the apex;
- Relative type: considered an early or less pronounced phenotypic expression of apical HCM [67].

In most cases, apical HCM is asymptomatic [68] and is generally associated with a favorable prognosis [69, 70].

However, extensive hypertrophy in this region can reduce LV end-diastolic volume, contributing to the development of diastolic heart failure [71].

Pharmacological Treatment of Patients with HCM and Apical Hypertrophy

In patients with apical HCM, treatment typically begins with beta blockers. These agents are commonly prescribed in part to reduce the frequency of unstable ventricular arrhythmias. The therapeutic benefits of beta blockers include:

- Reduction of heart rate, particularly during physical exertion;
- Improvement in diastolic heart function;
- Alleviation of symptoms such as chest pain and dyspnea through decreased myocardial oxygen demand;
- Reduction of the mid-ventricular pressure gradient in patients with concomitant mid-ventricular obstruction [68].

The beneficial effects of Verapamil and Diltiazem are attributed to their negative inotropic and chronotropic properties, which lead to a reduction in chest pain, an increase in LV diastolic filling time, and improved myocardial perfusion [72].

Low-dose loop and thiazide diuretics may be used to reduce dyspnea and manage volume overload in patients with apical HCM [68].

RAAS inhibitors are prescribed to slow the progression of myocardial hypertrophy and fibrosis, alleviate symptoms, and reduce mortality [21].

In patients with symptomatic non-obstructive HCM, treatment with Mavacamten has been associated with a significant, dose-dependent reduction in NT-proBNP levels. However, it has not demonstrated a significant effect on symptom relief or improvement in exercise capacity [73].

Surgical Treatment of Patients with HCM and Apical Hypertrophy

Transapical myectomy, aimed at enlarging the LV cavity, is effective in improving diastolic function, reducing symptoms, and potentially delaying or preventing the need for heart transplantation in patients with heart failure secondary to apical HCM [71, 74, 75].

HCM with Hypertrophy of the Entire IVS

Although this variant is rare, patients with obstructive HCM sometimes present with hypertrophy extending to all three zones of the IVS [43]. This phenotype is associated with a less favorable surgical prognosis.

A study published in 2018 evaluated 469 patients with obstructive HCM who underwent surgical myectomy. The cohort included patients with basal hypertrophy ($n = 248$), hypertrophy of the entire IVS ($n = 141$), and diffuse LV hypertrophy ($n = 80$). The mean follow-up period was 2.5 ± 1.4 years. Postoperative assessment showed that patients with basal septal hypertrophy experienced a thinner IVS, lower LV mass, and reduced gadolinium uptake on MRI. This group also demonstrated higher survival rates following myectomy. In contrast, patients with hypertrophy involving the entire IVS and those with diffuse LV hypertrophy exhibited poorer survival outcomes after surgical myectomy [14].

Preclinical HCM

A distinct subgroup among HCM patients consists of genotype-positive but phenotype-negative individuals, commonly referred to as having preclinical HCM [31, 76, 77].

In this group, LV structure and dimensions are typically within normal limits. However, they exhibit structural abnormalities of the MV compared with healthy individuals. These include a longer AMVL (17.1 ± 0.4

vs. 16.0 ± 0.4 mm/m², $p = 0.006$), a thicker PMVL (1.79 ± 0.008 vs. 1.62 ± 0.007 cm, $p = 0.06$), and a reduced distance between the papillary muscles (31.1 ± 0.7 vs. 34.2 ± 0.9 mm, $p = 0.007$).

The prevalence of SAM is also significantly higher in preclinical HCM compared with healthy controls (15.2% vs. 1.6%, $p = 0.006$). These changes are more pronounced in HCM with a distinct phenotype and the presence of genetic markers. Therefore, early diagnosis supported by comprehensive imaging is essential for timely identification, accurate risk stratification, and appropriate selection of management strategies [78].

PREVENTION OF SUDDEN CARDIAC DEATH (SCD) IN PATIENTS WITH HCM

Despite advances in medical and interventional therapies, SCD remains a major clinical concern in patients with HCM. The implantable cardioverter defibrillator (ICD) is an established and effective strategy for both primary and secondary prevention of SCD in this population [3, 4].

Secondary prevention involves ICD implantation in patients who have survived cardiac arrest due to ventricular tachycardia (VT) or ventricular fibrillation, or in those with a documented history of sustained, hemodynamically significant VT [3].

For primary prevention, ICD implantation is recommended in patients with HCM who present with at least one major risk factor for SCD [4] and a calculated 5-year SCD risk of $\geq 4\%$ (Class IIb) to $\geq 6\%$ (Class IIa) according to the HCM Risk-SCD model [3].

Guidelines from both American and European cardiology societies largely overlap in identifying SCD risk factors in HCM, although certain differences remain (Tables 4 and 5) [3, 4].

Table 4

Risk factors for sudden cardiac death (SCD) in patients with hypertrophic cardiomyopathy (HCM) according to the American Heart Association [4]

SCD risk factors	Description
Family history of SCD from HCM	Family history of SCD, judged definitively or likely attributable to HCM in ≥ 1 first-degree or close relatives who are ≤ 50 years of age.
Massive LVH	Maximum wall thickness ≥ 28 –30 mm in any segment within the chamber according to echocardiography or cardiovascular magnetic resonance (CMR) imaging
Unexplained syncope	≥ 1 unexplained episodes involving acute transient loss of consciousness, judged by history unlikely to be of neurocardiogenic (vasovagal) etiology, especially when occurring within the last 6 months
Nonsustained ventricular tachycardia (NSVT)	A significant risk marker is frequent (≥ 3) and prolonged (≥ 10 beats) episodes of NSVT with a high ventricular rate (VR) (≥ 200 beats per minute), as determined by Holter monitoring.
LV systolic dysfunction	Ejection fraction $< 50\%$ by echocardiography or CMR imaging.
LV apical aneurysm	Independent of the size of the aneurysm
Extensive late gadolinium enhancement	Diffuse and extensive late gadolinium enhancement on CMR imaging, representing myocardial fibrosis comprising $\geq 15\%$ of LV mass.

Abbreviations: LVH, left ventricular hypertrophy; LV, left ventricular.

Table 5

Risk factors for sudden cardiac death (SCD) in patients with hypertrophic cardiomyopathy (HCM) according to the European Society of Cardiology [3]

SCD risk factors	Description
Family history of SCD from HCM	– SCD in a first-degree relative <40 years of age, irrespective of a confirmed diagnosis of HCM – SCD in a first-degree relative of any age with a confirmed diagnosis of HCM
Massive LVH	Maximum wall thickness ≥ 30 –35 mm according to echocardiography
Syncope	Unexplained, non- neurocardiogenic syncope, particularly episodes, occurring within 6 months prior to evaluation
Nonsustained ventricular tachycardia (NSVT)	A consecutive series of ≥ 3 ventricular contractions with a frequency of ≥ 120 beats per minute, lasting <30 seconds, regardless of the number and duration of NSVT episodes per day, as well as ventricular rate (VR) during paroxysm. The occurrence of NSVT during or immediately after physical exertion may indicate an increased risk of SCD.
Age	SCD risk is higher in younger patients. In this population, major risk factors, such as NSVT, severe LVH, and unexplained syncope, carry greater adverse prognostic significance.
Left atrial dilation	However, current evidence is insufficient to establish a clear association between SCD and specific parameters such as left atrial area or volume.
LVOT obstruction	However, the prognostic significance of provoked LVOT obstruction and the effectiveness of different treatment strategies (conservative and invasive) in preventing SCD remain poorly understood.

Abbreviations: LVOT, left ventricular outflow tract; LVH, left ventricular hypertrophy; LV, left ventricular.

CONCLUSION

The treatment strategy for HCM should be strictly individualized, taking into account not only hemodynamic status but also detailed anatomical features. HCM classification based on the presence or absence of LVOT obstruction, as well as the localization of IVS hypertrophy, is essential for selecting the optimal management approach.

Medical therapy, surgical septal myectomy, and alcohol septal ablation are the main treatments for obstructive HCM, whereas management of the non-obstructive form primarily focuses on symptom control and prevention of complications.

Given the risk of SCD, timely implantation of an ICD in high-risk patients remain critical components of comprehensive care.

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DONOR HEART CORONARY ARTERY BYPASS GRAFTING

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Objective: to evaluate the initial experience of performing simultaneous heart transplant (HT) with coronary artery bypass grafting (CABG) at Almazov National Medical Research Centre in St. Petersburg. **Materials and methods.** Outcomes of 196 HT performed between January 1, 2016, and January 1, 2025, were analyzed. Patients were divided into two groups: 16 recipients who underwent combined HT + CABG (Group 1) and 180 recipients who underwent standard HT (Group 2). The groups were compared using the following parameters: duration of surgery, graft ischemic time, duration of cardiopulmonary bypass (CPB), duration of inotropic therapy, length of stay in the intensive care unit (ICU), 30-day mortality, and one-year survival. **Results.** The operative time was longer in Group 1, at 312 (286–415); 2020; 1130 min, compared with Group 2, at 268 (225–320); 150; 2150 min ($p = 0.007$). Group 1 also demonstrated a significant increase in graft ischemic time and CPB duration compared to group 2. Median graft ischemic time in Group 1 was 156 (146–180); 120, 240 min, versus 140 (120–160); 40, 240 min in Group 2 ($p = 0.021$). CPB duration was 159 (133–180); 101, 214 min in Group 1 and 128 (101–162); 71–350 min in Group 2 ($p = 0.015$). Despite the more complex nature of the intervention in Group 1, no statistically significant differences were observed between the groups in the duration of inotropic therapy or length of stay in the ICU. Although the 30-day mortality rate was higher in Group 1 than in Group 2 (18.7% vs. 7.8%), this difference did not reach statistical significance ($p = 0.136$). One-year survival rates were comparable between the two groups. **Conclusion.** Simultaneous HT and CABG procedures are associated with longer operative time, prolonged graft ischemia, and longer CPB duration compared with standard HT. However, no differences were observed in the duration of inotropic therapy or early postoperative period. Combined HT and CABG procedures are technically feasible, with 30-day mortality and one-year survival rates comparable to those of standard HT. The use of donor hearts with underlying coronary artery pathology may expand donor selection criteria and increase the number of HT procedures performed.

Keywords: simultaneous heart transplantation, coronary artery bypass grafting, expanded-criteria heart donors, coronary artery stenosis, transplant coronary artery disease, donor-transmitted coronary atherosclerosis.

INTRODUCTION

Despite significant progress over the past decade in the use of mechanical circulatory support (MCS) systems, heart transplantation (HT) remains the only definitive treatment for patients with end-stage chronic heart failure (CHF). The use of these systems as a bridge to HT is associated with high mortality. For example, a study by J.C. Cleveland Jr. et al. reported that approximately 35% of patients receiving MCS died before undergoing HT [1]. Similarly, data from Almazov National Medical Research Centre indicate persistently high mortality rates among patients on extracorporeal membrane oxygenation (ECMO) prior to transplantation, exceeding 40%.

The shortage of donor organs remains a main challenge in transplantation medicine. This issue was further exacerbated during the COVID-19 pandemic. A 2021 international analysis of transplant activity reported a substantial global decline in the number of procedures performed, primarily due to a reduction in deceased donors. In certain countries, the decrease was particularly

pronounced, reaching up to 88% for heart transplantation, 85% for lung transplantation, and 69% for kidney transplantation [2]. COVID-19 outbreaks continue to occur periodically in various countries to this day.

Concurrently, the demographic profile of heart donors has shifted, with the average donor age now approaching 70 years. As a consequence, the prevalence of coronary artery disease (CAD) among donors has increased, being identified in up to 58% of evaluated individuals [3]. The presence of coronary artery stenoses is associated with a heightened risk of early postoperative complications, including acute myocardial infarction following HT.

In response, the European Directorate for the Quality of Medicines & HealthCare recommends routine coronary angiography (CAG) on all heart donors: men >55 years; women >55 years with one or more risk factor; and both men and women aged 45–55 years with two or more risk factors [4]. However, in most transplant centers, despite these recommendations, routine CAG is not consistently performed on potential heart donors.

There are a few isolated reports in the literature addressing HT combined with simultaneous cardiac surgical procedures. Between 2006 and 2008, the Almazov National Medical Research Centre performed the first four cases of cardiac autotransplantation in the Russian Federation, which included simultaneous left atrial reduction and correction of valvular pathology [5].

In 2009, Saito et al. [6] reported a successful case of HT combined with aortic valve replacement. More recently, in 2021, Juneja et al. [7] demonstrated the feasibility of simultaneous liver transplantation and off-pump coronary artery bypass grafting (CABG). Earlier, in 1988, Thomson et al. [8] described the first case of HT combined with CABG performed on a donor heart.

Despite more than three decades since this initial report, there remains a paucity of data – particularly in the Russian literature – regarding the outcomes and feasibility of simultaneous HT and CABG. Expanding the donor pool by including hearts from donors with coronary artery disease may represent a promising strategy to mitigate the ongoing shortage of donor organs.

The aim of this study is to evaluate the initial experience with simultaneous HT and CABG at Almazov National Medical Research Centre.

MATERIALS AND METHODS

This study analyzed the outcomes of 196 heart transplantations performed at Almazov National Medical Research Centre in St. Petersburg, Russia, between January 1, 2016, and January 1, 2025. Among these, 16 procedures involved the use of donor hearts that underwent CABG prior to implantation.

Patients were divided into two groups. Group 1 included 16 recipients (14 men and 2 women) who underwent simultaneous HT with CABG. Group 2 comprised 180 recipients (138 men and 42 women) who underwent standard HT without additional surgical procedures.

The median age of the overall cohort was 52 years (interquartile range [IQR]: 37–58). In Group 1, the me-

dian age was 53 years (IQR: 35–58), whereas in Group 2 it was 51 years (IQR: 37–58). There were no statistically significant differences between the groups in terms of age ($p = 0.916$) or sex distribution.

Prior to HT, all recipients had chronic heart failure classified as functional class III–IV according to the New York Heart Association (NYHA).

In Group 1, the predominant underlying etiology of end-stage heart failure was ischemic heart disease (IHD), accounting for 60% of cases, whereas dilated cardiomyopathy (DCM) was observed in only 10% of patients. In contrast, in Group 2, DCM was the leading cause (45%), followed by IHD (43%).

According to the United Network for Organ Sharing (UNOS) urgency classification, the distribution of patients was as follows: Class IA included 6.25% of patients in Group 1 and 16.67% in Group 2; Class IB comprised 31.25% and 27.22%, respectively; and Class II included 62.50% and 56.11%, respectively ($p = 0.549$) (Fig. 1).

No statistically significant differences were observed between the groups in terms of waiting list duration. The median waiting time in Group 1 was 122 days (IQR: 76–284), compared with 100 days (IQR: 54–177) in Group 2 ($p = 0.547$).

A comparative assessment of pre-transplant functional status showed no significant differences between the groups. According to echocardiography data, left ventricular ejection fraction (LVEF) was comparable: 23% (IQR: 19–28) in Group 1 and 23% (IQR: 18–29) in Group 2 ($p = 0.898$).

The mean pulmonary artery pressure was 44 mmHg (IQR: 35–56) in Group 1 and 47 mmHg (IQR: 38–60) in Group 2 ($p = 0.493$). Pulmonary vascular resistance also did not differ significantly between the groups, measuring 2.93 Wood units (IQR: 2.16–4.75) in Group 1 and 2.74 Wood units (IQR: 2.08–3.88) in Group 2 ($p = 0.585$).

The primary criteria for patient selection for HT included: advanced chronic heart failure (NYHA class III–IV) refractory to optimal medical therapy; reduced

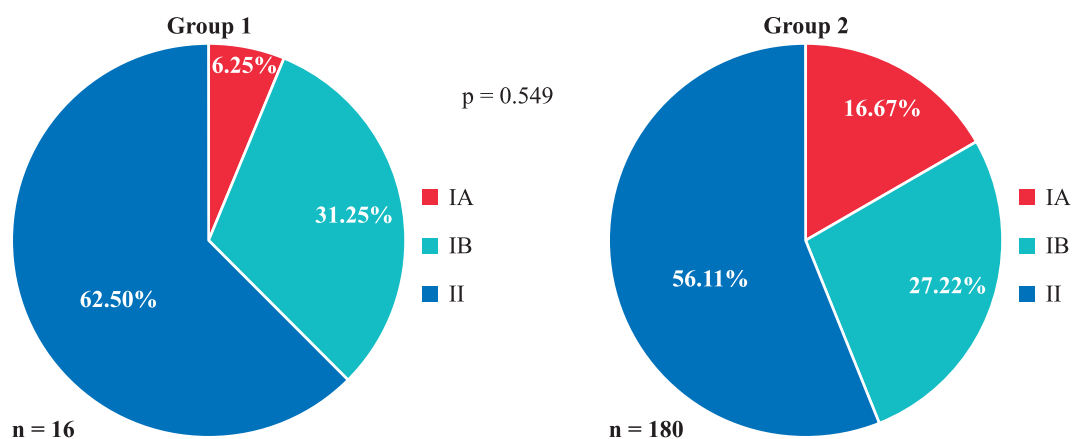


Fig. 1. Urgency of HT according to the UNOS system in patients with simultaneous HT and CABG (Group 1) and standard HT (Group 2)

functional capacity defined by peak oxygen consumption (VO_2) <14 mL/kg/min or $<50\%$ of the predicted value; severe CAD with persistent angina not amenable to revascularization; and malignant ventricular arrhythmias resistant to medical management [9].

A comparative analysis of postmortem heart donors revealed no statistically significant differences between the groups with respect to sex distribution, with male donors predominating in both cohorts – 75% in Group 1 and 67.97% in Group 2 ($p = 0.926$). The mean donor age was 49.40 ± 4.94 years in Group 1 and 45.20 ± 9.86 years in Group 2 ($p = 0.122$). The leading cause of donor death in both groups was stroke, accounting for 75% of cases in Group 1 and 81.25% in Group 2.

All donors received inotropic and vasopressor support prior to organ procurement. The duration of such therapy did not differ significantly between the groups, with a median of 4 days (IQR: 3–4; range 1–10) in Group 1 and 3 days (IQR: 2–4; range 1–9) in Group 2 ($p = 0.213$). Similarly, the length of stay in the intensive care unit

(ICU) was comparable between groups, at 4 days (IQR: 3–4; range 1–10) in Group 1 and 3 days (IQR: 2–4; range 1–9) in Group 2 ($p = 0.213$).

Donor–recipient matching was performed in accordance with established criteria from the specialized literature [10]. Specifically, donor body weight was required not to differ from recipient weight by more than 20%. In cases involving female donors and male recipients, differences in both height and weight were limited to 10%. These criteria were met in all transplant procedures across both groups.

CAG was performed in all donors in Group 1, whereas in Group 2 it was carried out in only 35% of cases. In Group 1, CAG revealed two cases of two-vessel CAD with stenoses of up to 75%, as well as eight cases of single-vessel disease with stenoses also reaching up to 75%. In contrast, no hemodynamically significant coronary stenoses were identified in any of the donors evaluated in Group 2.

An example of single-vessel CAD in a donor heart is presented in Fig. 2, demonstrating a hemodynamically significant stenosis in the proximal anterior interventricular artery (AIV).

The algorithm for deciding whether to perform simultaneous HT and CABG was based on an assessment of donor and recipient parameters (Fig. 3). The primary selection criterion was the UNOS HT urgency classification. Preference was given to patients with more urgent indications for surgery. Another important selection criterion was the patient's prolonged stay on the heart-lung machine compared to other recipients. Since potential heart donors with CAD were in the older age group, efforts were made to select a recipient of a similar age. We also took into account the height and weight of donors and recipients, as well as the number and location of coronary artery stenoses in donors.

The technical feasibility of performing CABG on a donor heart was determined based on the number and anatomical location of coronary artery stenoses. The

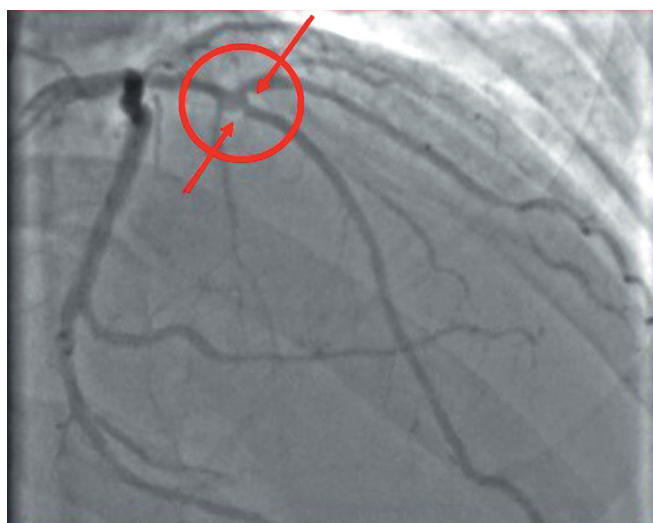


Fig. 2. Donor single-vessel coronary artery disease: stenosis in the proximal segment of the left anterior descending artery

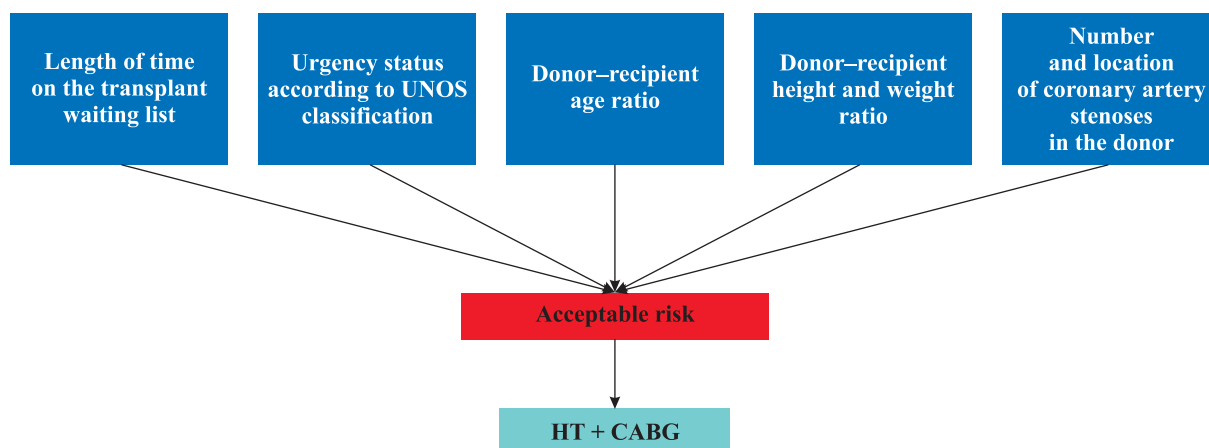


Fig. 3. Algorithm for deciding on the possibility of performing simultaneous HT and CABG

final intraoperative decision regarding the possibility of simultaneous HT and CABG was made by the operating surgeon.

In Group 1, the distribution of procedures was as follows: 14 patients underwent HT with CABG using a single autovenous graft, while 2 patients received HT with a combination of one autovenous graft and one internal mammary artery–coronary graft.

The surgical technique involved a median sternotomy and pericardiotomy, followed by harvesting of the conduit for CABG (saphenous vein). Cardiopulmonary bypass (CPB) was initiated, and the aorta was cross-clamped. After induction of cardiac arrest, the recipient's native heart was excised. The donor heart was then inspected, and a distal anastomosis was created between the autovenous graft and the affected coronary artery of the donor heart (Fig. 4).

Subsequently, the donor heart was implanted using the bicaval technique, and cardiac activity was restored. Coronary perfusion was established either through the internal mammary artery graft or by constructing a proximal off-pump anastomosis between the autovenous graft and the aorta, after which flow was initiated.

In the final stage, hemodynamic stability was achieved through titration of inotropic and vasopressor support, and the CPB system was disconnected.

A comparative assessment of treatment outcomes between the groups was performed using the following criteria: operative time, duration of graft ischemia, CPB time, duration of inotropic support, length of stay in the ICU, 30-day mortality, and one-year survival. Survival analysis was conducted using the Kaplan–Meier method,



Fig. 4. Formation of a distal anastomosis between recipient's autologous vein graft and the affected coronary artery of the donor heart

and differences between groups were assessed using the log-rank test.

Statistical analysis included assessment of normality for continuous variables using the Kolmogorov–Smirnov test. Depending on data distribution, group comparisons were performed using either the Mann–Whitney U test or the independent samples t-test. Continuous variables with non-normal distribution are presented as median (Me) with interquartile range (Q1–Q3) and minimum–maximum values, while normally distributed variables are expressed as mean $\pm$ standard deviation ($M \pm SD$). Categorical variables were compared using Pearson's chi-squared test, and are presented as percentages.

The study was conducted in accordance with the principles of the Declaration of Helsinki, and all patients provided written informed consent for postoperative follow-up by a cardiologist.

RESULTS

A comparison of operative time between the groups (Fig. 5) showed longer procedures in Group 1. The mean operative time in Group 1 was 312 minutes (IQR: 286–415; range 220–1130), compared with 268 minutes (IQR: 225–320; range 150–2150) in Group 2 ($p = 0.007$). This difference is primarily attributable to the additional time required for conduit harvesting and the performance of CABG in Group 1.

A similar pattern was observed for graft ischemia time (Fig. 6) and CPB duration (Fig. 7), both of which were significantly higher in Group 1. Graft ischemia time was 156 minutes (IQR: 146–180; range 120–240) in Group 1 versus 140 minutes (IQR: 120–160; range 40–240) in Group 2 ($p = 0.021$). CPB duration was also prolonged in Group 1, at 159 minutes (IQR: 133–180; range 101–214), compared with 128 minutes (IQR: 101–162; range 71–350) in Group 2 ($p = 0.015$).

No statistically significant difference was found between the groups in the duration of inotropic therapy (Fig. 8). In Group 1, median duration was 7 days (IQR: 5–19; range 1–24), compared with 6 days (IQR: 5–11; range 1–52) in Group 2 ($p = 0.485$).

Despite the greater technical complexity of the surgical procedure in Group 1, no difference was found in the length of stay in the ICU (Fig. 9). ICU stay was 8 days (IQR: 6–19; range 4–31) in Group 1 and 9 days (IQR: 6–13; range 3–119) in Group 2 ($p = 0.974$).

Although 30-day mortality was higher in Group 1 compared with Group 2 (18.7% vs. 7.8%, respectively), this difference did not reach statistical significance ($p = 0.136$) (Fig. 10). The leading cause of death in Group 1 was bilateral polysegmental pneumonia, whereas in Group 2 it was acute right ventricular failure.

One-year survival did not differ significantly between the two study groups (Fig. 11), with survival rates of 81.3% in Group 1 and 87.8% in Group 2 ($p = 0.477$). The maximum follow-up duration was 12 months.

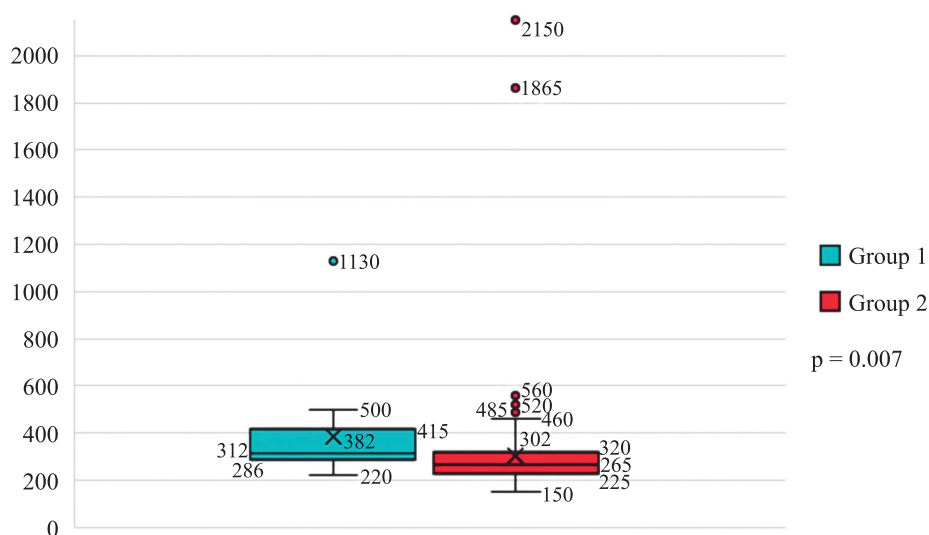


Fig. 5. Duration of the surgical procedure

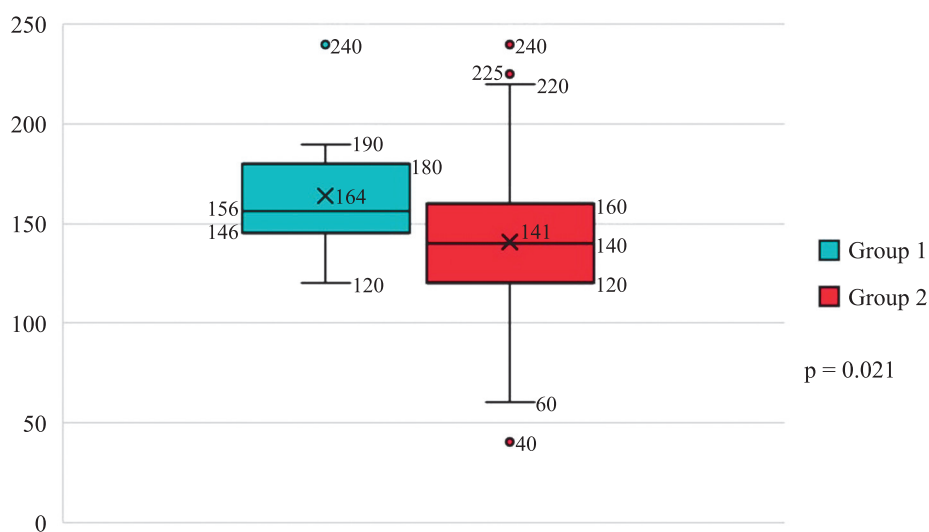


Fig. 6. Graft ischemia time

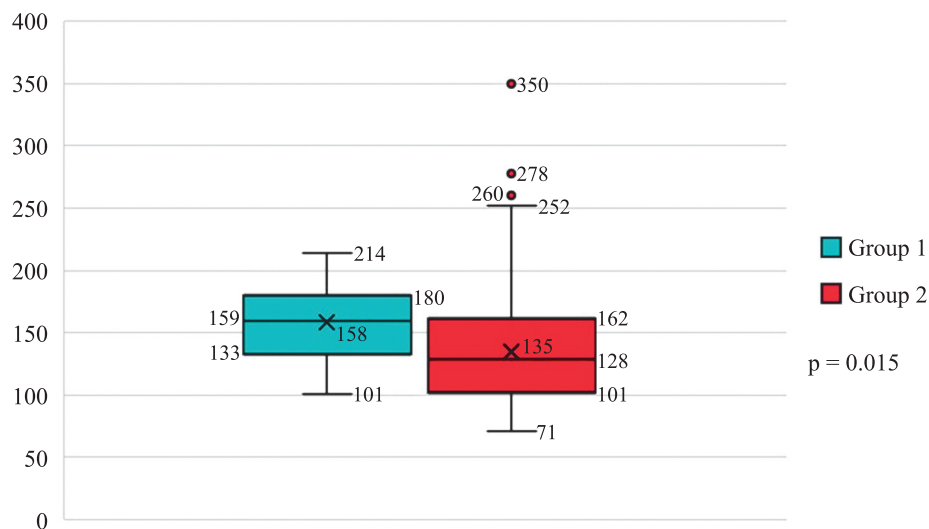


Fig. 7. Duration of CPB

All patients were enrolled in the structured post-transplant follow-up program at Almazov National Medical Research Centre and were monitored throughout the

postoperative period at the center's dedicated transplant cardiology clinic. Patients were not followed up at external institutions or at their place of residence, nor were

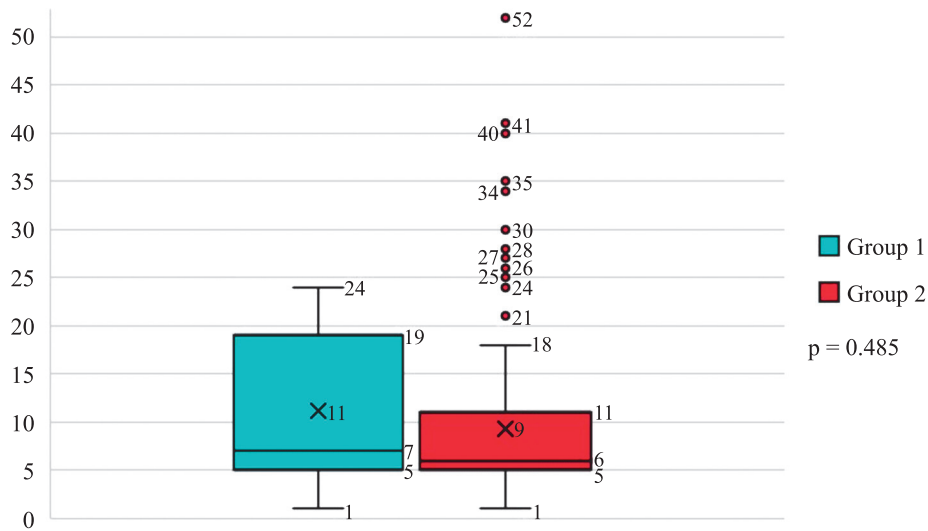


Fig. 8. Duration of inotropic therapy

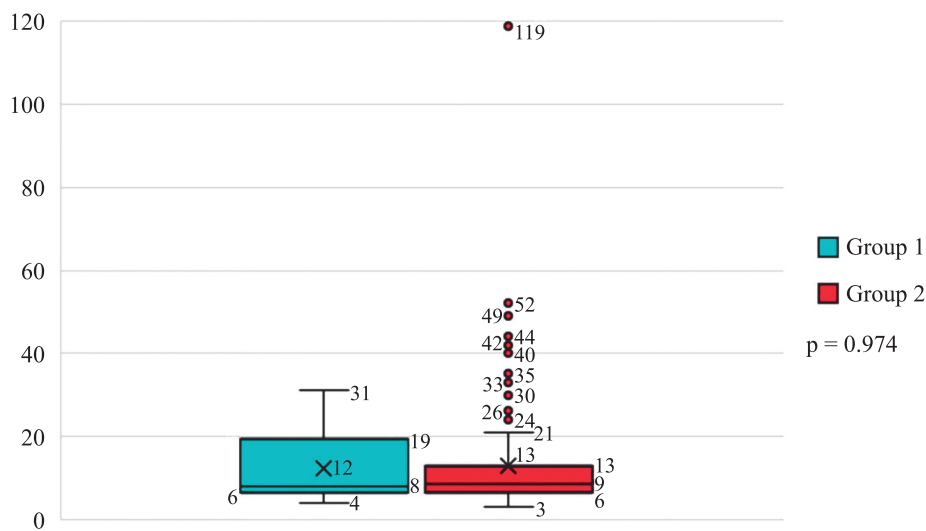


Fig. 9. Length of stay in the ICU

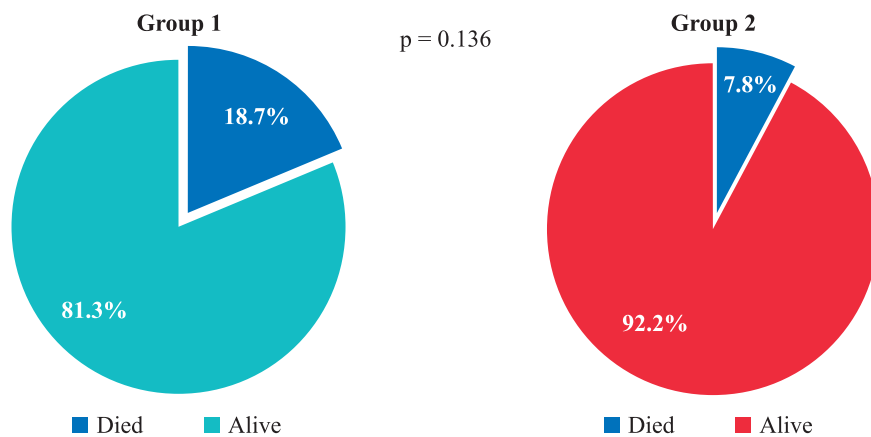


Fig. 10. Post-HT 30-day mortality in the study groups

they transferred to other heart transplant centers within the Russian Federation during the observation period.

Of particular interest in our study is the first clinical case of simultaneous HT and CABG. Patient F., a 64-year-old male with a diagnosis of CAD, coronary atherosclerosis, post-infarction cardiosclerosis with chamber dilation, Lown grade 4A ventricular extrasystoles, paroxysmal atrial tachycardia, severe mitral regurgitation (grade 3), severe tricuspid regurgitation (grade 3), and chronic heart failure (stage IIB, NYHA class II–III), underwent combined HT and CABG.

The donor was a 45-year-old female who died from a ruptured cerebral aneurysm. In the ICU, the donor required 2 days of mechanical ventilation and vasopressor therapy with norepinephrine at 0.45 µg/kg/h. Multiorgan procurement was performed, including the heart and

both kidneys. As noted above, coronary angiography of the donor heart revealed a hemodynamically significant stenosis in the proximal AIV.

Orthotopic HT was performed using the bicaval technique, combined with mammary artery bypass grafting to the AIV and autovenous grafting to the diagonal branch under extracorporeal circulation (ECC) and pharmacologic cold crystalloid cardioplegia (Custodiol). The total operative time was 355 minutes. Donor heart ischemia time was 160 minutes, and ECC duration was 178 minutes.

The recipient was extubated on postoperative day 2. Inotropic therapy was required for 3 days, and on postoperative day 6, the patient was transferred to the cardiology ward for further rehabilitation.

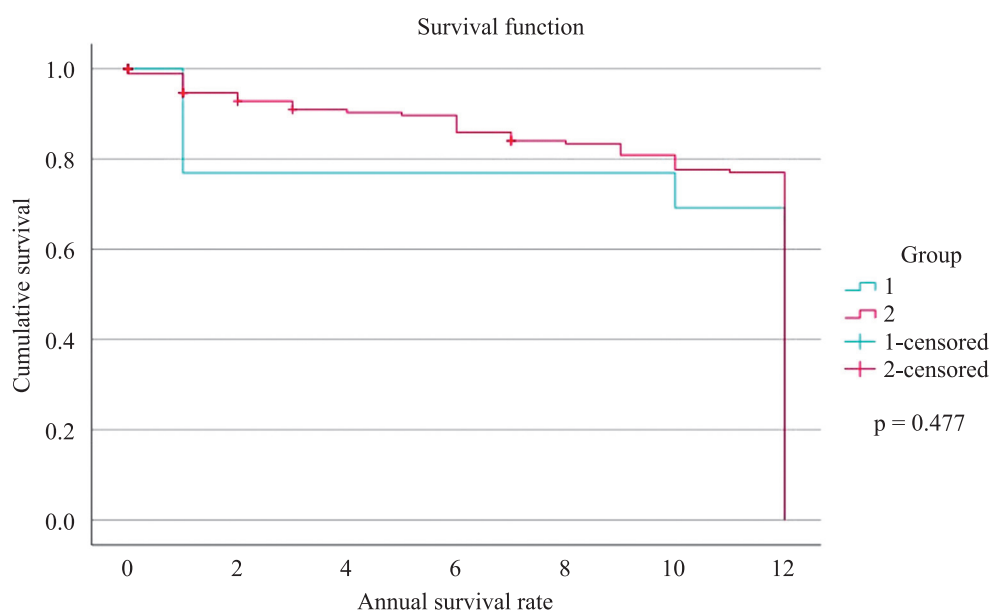


Fig. 11. 12-month survival analysis based on the Kaplan–Meier method

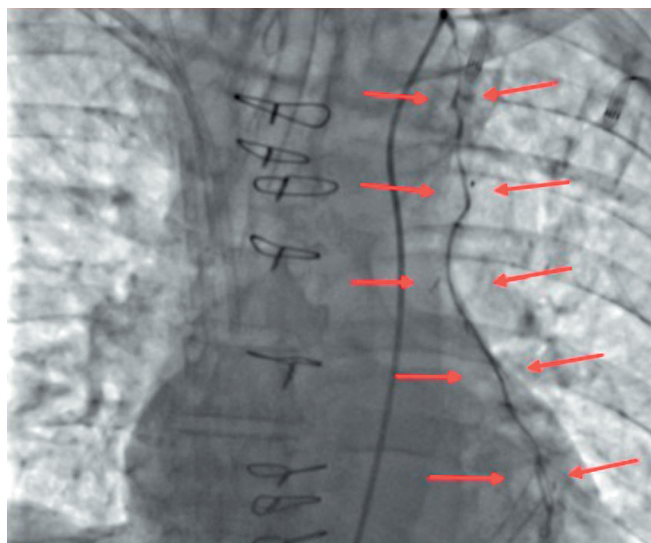


Fig. 12. Recipient's coronary angiography one year after HT

At 1-year follow-up, CAG (Fig. 12) revealed satisfactory graft function. The left main coronary artery showed no hemodynamically significant stenosis. AIV exhibited moderate diffuse proximal changes with 50–60% stenosis distally. The first diagonal branch was free of significant stenosis, while the second diagonal branch had ostial stenosis of 70–80% but was adequately perfused antegradely and via a functioning bypass graft. The circumflex artery, represented by a dominant marginal branch, showed no significant stenosis. The right coronary artery was free of hemodynamically significant lesions. The aortocoronary bypass graft to the second diagonal branch remained patent without significant stenosis.

DISCUSSION

Aortic atherosclerosis is one of the most common conditions in elderly potential heart donors evaluated by

cardiac surgeons. This donor population is of particular clinical interest, as the presence of coronary artery stenosis has traditionally been considered a contraindication to HT.

Although more than 35 years have elapsed since the first report describing simultaneous HT and CABG [8], such procedures remain exceedingly rare.

The largest published series to date was reported by Yang et al. [11] from the Heart Center in Taiwan and included only 11 cases of combined HT and CABG. The early postoperative outcomes were favorable, with no mortality observed within 3 months following surgery. This study thus provided initial evidence that simultaneous HT and CABG is technically feasible and does not appear to increase early postoperative mortality in transplant recipients. Nevertheless, simultaneous HT and CABG procedures continue to be reported only in isolated cases worldwide.

The initial experience of simultaneous HT and CABG at our center demonstrated, on one hand, the technical feasibility of this combined approach and, on the other, its comparable effectiveness relative to standard HT, as shown for the first time in our study.

Despite a more than 100-fold increase in the number of heart transplants performed in our country between 2008 and 2024 as a result of substantial reorganization of donor and transplant services, mortality among patients on the heart transplant waiting list (HTWL) remains high and has not significantly improved, both in the Russian Federation and internationally. The primary limiting factor continues to be the persistent shortage of donor organs, with waiting list mortality rates reported to reach 5–15% [13].

The level of neurological care, particularly for stroke, is improving annually. Therefore, in our view, the continued use of standard donor selection criteria alone is unlikely to significantly increase the number of available donor hearts in the near future.

Over the past decade, the average age of heart donors has increased from 45 to 70 years, while the prevalence of CAD among potential donors now ranges from 42% to 58% [3]. In this regard, it appears reasonable to consider the expansion of donor selection criteria, particularly through inclusion of donors with CAD for HT.

In addition, efforts to address the persistent shortage of donor organs through surgical modification of transplant procedures have been ongoing for decades and continue worldwide. In this context, the experience of four cases of cardiac autotransplantation performed between 2006 and 2008 at Almazov National Medical Research Centre, involving simultaneous reduction of the left atrial cavity and correction of valvular pathology, demonstrated the feasibility of such approaches and, in our opinion, may be considered an early step toward the development of complex simultaneous cardiac transplant procedures [5].

In 2009, Saito et al. [6] successfully performed HT combined with simultaneous aortic valve replacement. In 2021, Juneja et al. [7] reported a series of successful combined liver transplantation and off-pump CABG. In 2017, Patel et al. [13] described HT with simultaneous back-table aortic valve replacement, with the patient discharged in satisfactory condition on postoperative day 11.

However, no publications were identified in the available literature describing similar procedures within the Russian Federation. Accordingly, the results of simultaneous HT and CABG presented in our study may be considered the first reported experience of this approach both at our center and, to our knowledge, in Russian transplant surgery as a whole.

Our study included 196 HT cases, of which 16 patients underwent simultaneous HT and CABG. Comparison of preoperative characteristics between the groups demonstrated that most variables were comparable. The slightly lower baseline LVEF observed in Group 2, despite the absence of statistical significance, may be attributed to the larger sample size in the control group.

The difference in the primary etiology of chronic heart failure – predominantly ischemic heart disease in Group 1 (60%) versus dilated cardiomyopathy in Group 2 (45%) – in our opinion, reflects the significant difference in the patient sample across the groups.

The limited national and global experience with simultaneous HT and CABG raises important questions regarding appropriate patient selection criteria. In the present study, we proposed a decision-making algorithm for assessing the feasibility of combined procedures (Fig. 3). We suggest that potential candidates for simultaneous HT and CABG include patients with prolonged waiting list time, high UNOS urgency status, older recipient age comparable to that of the donor population, and an optimal donor–recipient size match with respect to height and weight parameters.

An important determinant of the technical feasibility of simultaneous HT and CABG is the operating surgeon's intraoperative assessment of the number and anatomical location of coronary stenoses in the donor heart. Integration of these parameters at the final stage of decision-making allows for a balanced evaluation of perioperative risk and, consequently, for determining the appropriateness of performing the combined procedure.

At present, in the Russian Federation, standardized evaluation protocols for potential heart donors older than 40 years do not mandate routine CAG. In our opinion, this issue warrants further discussion. The use of CABG in donor hearts may, on one hand, facilitate more accurate risk stratification and reduce the likelihood of early graft dysfunction. On the other hand, in-depth additional screening of donors could help identify a subset of sub-optimal heart donors whose CAD may still be compatible with successful HT.

In the present study, involvement of more than two coronary arteries was considered significant and was regarded as a contraindication to simultaneous HT and CABG. It can be assumed that simultaneous HT and CABG could also be feasible even with more extensive coronary artery lesions in potential donors.

The first experience in simultaneous HT and CABG at our center demonstrated the key technical characteristics of this combined procedure. Comparative analysis of perioperative parameters revealed a statistically significant difference in total operative time between the groups. The additional time required for conduit harvesting and performance of CABG accounted for this difference, although it remained relatively modest ($p = 0.007$).

Graft ischemia time and CPB duration also differed significantly between the groups. In Group 1, mean ischemia time was 156 minutes (IQR: 146–180; range 120–240), compared with 140 minutes (IQR: 120–160; range 40–240) in Group 2 ($p = 0.021$). CPB duration was 159 minutes (IQR: 133–180; range 101–214) in Group 1 versus 128 minutes (IQR: 101–162; range 71–350) in Group 2 ($p = 0.015$). No statistically significant differences were observed in the duration of postoperative inotropic therapy or length of ICU stay between the groups.

Although one-year survival rates were comparable between the groups, 30-day mortality was higher in the HT + CABG group; however, this difference did not reach statistical significance ($p = 0.136$), likely due to the limited number of cases in Group 1.

CONCLUSION

Thus, the initial experience of our center with simultaneous HT and CABG using donor hearts with CAD allows us to draw the following conclusions:

1. Simultaneous HT and CABG is associated with increased operative duration, longer graft ischemia time, and prolonged CPB time compared with standard HT. However, no differences were observed in the duration of inotropic therapy or the early postoperative course.
2. Simultaneous HT and CABG is technically feasible, with 30-day mortality and one-year survival rates comparable to those of standard HT.
3. The use of donor hearts with pre-existing CAD may contribute to an expansion of donor selection criteria and, consequently, an increase in the number of HT procedures performed.

The authors declare no conflict of interest.

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A COMPREHENSIVE DIAGNOSTIC AND THERAPEUTIC STRATEGY FOR DRIVELINE INFECTIONS IN LONG-TERM MECHANICAL CIRCULATORY SUPPORT

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Objective: to evaluate the clinical efficacy of a comprehensive approach to the diagnosis and surgical treatment of driveline infections (DLIs) in patients with long-term mechanical circulatory support (MCS). **Materials and methods.** A single-center retrospective observational study was conducted at Shumakov National Medical Research Center of Transplantology and Artificial Organs. The analysis included 56 patients with implanted devices for long-term MCS of the left ventricle: AVK-N (2012–2018) – 17; Stream Cardio (2022–2025) – 32; HeartMate 3 (2022–2025) – 7 people. **Results.** DLIs were identified in 18 of 56 patients (32.1%) with long-term left ventricular MCS. In the group managed without the diagnostic and therapeutic DLI algorithm, infections occurred in 8 of 17 patients (47.1%), whereas in the group managed with the algorithm, DLIs were detected in 10 of 39 patients (25.6%). Most DLI episodes developed within the first six months after device implantation. Implementation of the diagnostic and treatment algorithm resulted in a reduction in relapse frequency in patients with extensive forms of DLI (from 2.25 to 1.17 episodes per patient; $p = 0.050$) and a significant decrease in the duration of hospitalization for both local and subcutaneous forms ($p = 0.022$ and $p = 0.014$, respectively). **Conclusion.** The use of the developed algorithm for the diagnosis and treatment of DLIs in patients receiving long-term MCS enabled a systematic approach to patient management, improved the effectiveness of local infection control, reduced hospital length of stay, and decreased the frequency of infectious complications.

Keywords: heart transplantation, long-term mechanical circulatory support, driveline-associated infections.

INTRODUCTION

Long-term MCS has become an essential component in the management of end-stage heart failure over recent decades, serving both as a bridge to transplantation and as destination therapy [1–3]. Advances in device technology, particularly the transition to continuous-flow systems, have significantly improved patient survival and quality of life [1, 2]. However, infectious complications – especially DLIs – remain among the leading causes of rehospitalization, sepsis, and mortality in patients with long-term implantable devices [4–8].

According to international registry data, the incidence of infections at the driveline exit site ranges from 15% to 40%, depending on device type and duration of support [1–3, 8–11]. Despite progress in antimicrobial coatings and advanced wound care strategies, DLIs continue to require a comprehensive management approach that includes early diagnosis, timely surgical debridement, and effective local infection control [1–3, 5, 8, 12]. Particular attention is paid to biofilm mechanisms of pathogen

persistence, primarily *Staphylococcus aureus* and *S. epidermidis*, which complicate eradication and contribute to the recurrence of DLIs [6, 7, 13].

To date, there is no universally accepted protocol or standard of care for managing infectious complications in patients receiving long-term left ventricular assist device support. Therapeutic strategies vary depending on the site of infection, the causative pathogen, device design, and the experience of individual centers. This variability highlights the need for a structured, stepwise, and clinically tailored approach to the diagnosis and management of these complications.

This study presents the results of evaluating the clinical efficacy of a diagnostic and treatment algorithm for DLIs in patients with long-term MCS. The algorithm was developed based on institutional clinical experience and an analysis of treatment outcomes at Shumakov National Medical Research Center of Transplantology and Artificial Organs, and was compared with previously used conventional approaches.

The study evaluated the incidence, clinical and anatomical characteristics, and outcomes of DLIs according to the management strategy employed. In addition, a comparative analysis was conducted of pathogen profiles, timing of infection onset, and duration of hospital stay.

MATERIALS AND METHODS

The study was conducted at Shumakov National Medical Research Center of Transplantology and Artificial Organs. A total of 56 patients were included in the analysis, comprising 53 men (94.6%) and 3 women (5.4%), aged 27–74 years (mean age 56.6 ± 11.5 years), all of whom had implanted long-term MCS devices. The distribution of devices was as follows: AVK-N (Russia) – 17 cases, Stream Cardio (Bio Soft, Russia) – 32 cases, and HeartMate 3 (Abbott, USA) – 7 cases, implanted between 2012 and 2025.

The etiology of end-stage chronic heart failure (CHF) was dilated cardiomyopathy in 26 patients (46.4%) and ischemic cardiomyopathy in 30 patients (53.6%). According to the New York Heart Association (NYHA) functional classification, the majority of patients were in Class III (40 patients, 71%), while Class IV was observed in 16 patients (29%).

The distribution of patients by INTERMACS profiles was as follows: Profile 1 – 5 patients (8.9%), Profile 2 – 14 (25.0%), Profile 3 – 13 (23.2%), Profile 4 – 17 (30.4%), and Profile 5 – 7 (12.5%).

Depending on the management strategy applied for DLIs, patients were divided into two groups. In the group managed without the use of a dedicated DLI diagnostic and treatment algorithm, DLIs were identified in 8 of 17 patients (47.1%). Among patients managed using the algorithm, DLIs were detected in 10 of 39 cases (25.6%).

The first group comprised patients treated using a conventional approach to the prevention and management of DLIs ($n = 8$). This strategy included routine

dressings changes and local antiseptic therapy. Diagnosis was based primarily on clinical signs, such as erythema, edema, and discharge at the exit site. Microbiological cultures and instrumental investigations were performed only when clearly indicated. Empirical systemic antibiotic therapy was initiated and subsequently adjusted according to culture results.

In cases of disease progression, surgical intervention involved incision, extensive drainage, and open wound management, followed by elective closure upon healing. Elective local revision with removal of the felt sheath along the driveline, as well as standardized use of vacuum-assisted closure (VAC) therapy, was not part of this treatment approach.

The second group comprised 10 patients with DLIs who were managed according to a novel diagnostic and treatment algorithm developed at Shumakov National Medical Research Center of Transplantology and Artificial Organs.

In both cohorts, DLIs were classified based on the clinical and anatomical extent of the inflammatory process. Three categories were defined to standardize case description and enable comparative analysis. The stages of progression of driveline infection in long-term MCS are schematically illustrated in Fig. 1.

- **Group A** – localized inflammation confined to the driveline exit site, without extension into subcutaneous adipose tissue or involvement of adjacent structures.
- **Group B** – infection extending into the subcutaneous adipose tissue and tracking along the driveline to its bend at the level of the right hypochondrium (the so-called “critical point”).
- **Group C** – advanced infection involving the pericardial cavity and/or the pump pocket, associated with a high risk of mediastinitis and systemic inflammatory response.

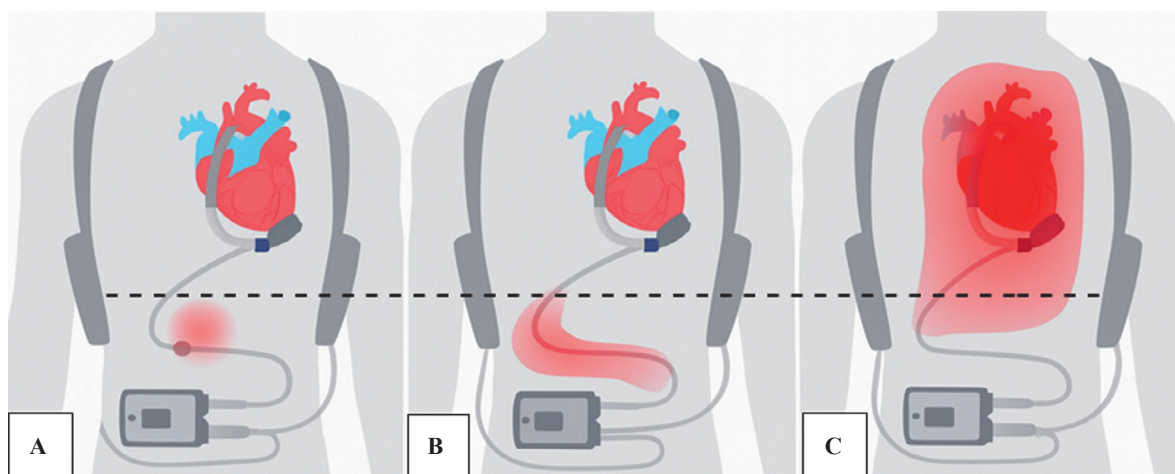


Fig. 1. Stages of progression of driveline infection in long-term mechanical circulatory support. (A) local infection; (B) subcutaneous spread; (C) deep infection with pericardial involvement. The dotted line indicates the “critical point” beyond which further spread of the infectious process occurs

Data were initially processed and tabulated using Microsoft Excel (MS Office Excel 2021), and statistical analysis was performed using StatTech v. 3.1.10 (StatTech LLC, Russia). Nonparametric methods were applied for the comparison of quantitative variables. Results were presented as median and interquartile range (Me [Q1; Q3]) and were analyzed using the Mann–Whitney U test and Pearson’s chi-square test. Categorical variables were expressed as absolute and relative values (n, %) and were compared using Fisher’s exact test. A p-value <0.05 was considered statistically significant.

RESULTS

The Developed Algorithm for the Diagnosis and Treatment of DLIs

Based on clinical experience and analysis of treatment outcomes at the Shumakov National Medical Research Center of Transplantology and Artificial Organs, a structured, step-by-step algorithm for the diagnosis and treatment of DLIs in patients with long-term MCS was developed (Fig. 2).

1. In the presence of pain, erythema, discharge, or fever, a comprehensive clinical evaluation of the driveline exit site is performed. This includes assessment of the extent and intensity of erythema, degree of tissue infiltration, presence of fluctuation, characteristics of the discharge (volume, consistency, and odor), condition of surrounding tissues, as well as the tension and fixation of the driveline. Rapid progression of erythema, the appearance of foul-smelling purulent discharge, fever >38.5 °C, or signs of systemic inflammatory response are considered indications for urgent hospitalization and immediate transition to the next stage of diagnostic evaluation.
2. If infection is suspected, an ultrasound (US) examination of the soft tissues along the driveline tunnel is performed to assess the depth and extent of the inflammatory process and to identify fluid collections or abscess cavities (Fig. 3).
If there is suspicion of infection spreading along the driveline tunnel, a non-contrast CT scan of the chest is performed to evaluate the condition of the pericardium and chest wall (Fig. 4).
The presence of fluid collections or gas inclusions along the course of the driveline or in proximity to the pericardium is regarded as an indication for urgent surgical debridement and consideration of emergency heart transplantation.
3. Microbiological diagnostics. If there are signs of inflammation (erythema, tissue infiltration, seropurulent discharge, tenderness, or bleeding), samples of local discharge are promptly obtained for microbiological culture. Additional swabs are taken from the skin surface, the driveline entry site, and the external sheath of the cable. Prior to initiating antibiotic therapy,

py, blood samples are collected for culture, complete blood count, and biochemical analysis, including measurement of C-reactive protein and procalcitonin levels.

Empirical and then targeted antibiotic therapy.

Pending microbiological results, empirical antibiotic therapy is initiated using broad-spectrum agents targeting the most likely pathogens, primarily staphylococci, including Methicillin-resistant *Staphylococcus aureus*. Following identification of the causative microorganism, antimicrobial therapy is adjusted according to antibiotic susceptibility profiles. The duration and regimen of treatment are individualized based on the severity of the patient’s condition and the extent of the infectious process. Subsequent management is determined by the clinical stage and progression of the infection.

4. Local antiseptic treatment.

At any signs of local inflammation, daily antiseptic care of the driveline exit site is performed using 10% povidone-iodine or 0.05–0.5% chlorhexidine digluconate solutions. In cases of exudation, antiseptic-soaked dressings or local antiseptic baths may be applied. When the patient’s condition stabilizes and discharge is planned, patients are instructed on appropriate self-care techniques. Dressing changes are typically performed at home every 3–4 days, following an individualized care plan established in consultation with the attending physician.

5. Surgical exploration and removal of the outer felt insulation sheath of the driveline (innovative approach).

In cases of persistent inflammation or the formation of abscess cavities or sinus tracts, surgical intervention is indicated, including opening of the infected area, thorough debridement, and necrectomy.

In all long-term MCS systems used in this study, the portion of the driveline located within the subcutaneous tissue is covered with a felt (textile) sheath, whereas the external segment is coated with a silicone layer. A unique technique implemented at our center involves selective removal of the outer felt sheath in the area of active infection.

This approach is applied when infection spreads along the driveline tunnel. During surgical debridement, the felt sheath – which is poorly penetrated by antiseptics – is excised, exposing the underlying silicone surface, which can be more effectively disinfected. Removal of the felt sheath provides several advantages:

1. reduction of the local bacterial load;
2. prevention of capillary spread of infection along the sheath;
3. improved efficacy of antiseptic treatment of the exposed driveline surface.

The technique for removing the felt sheath is illustrated in Fig. 5.

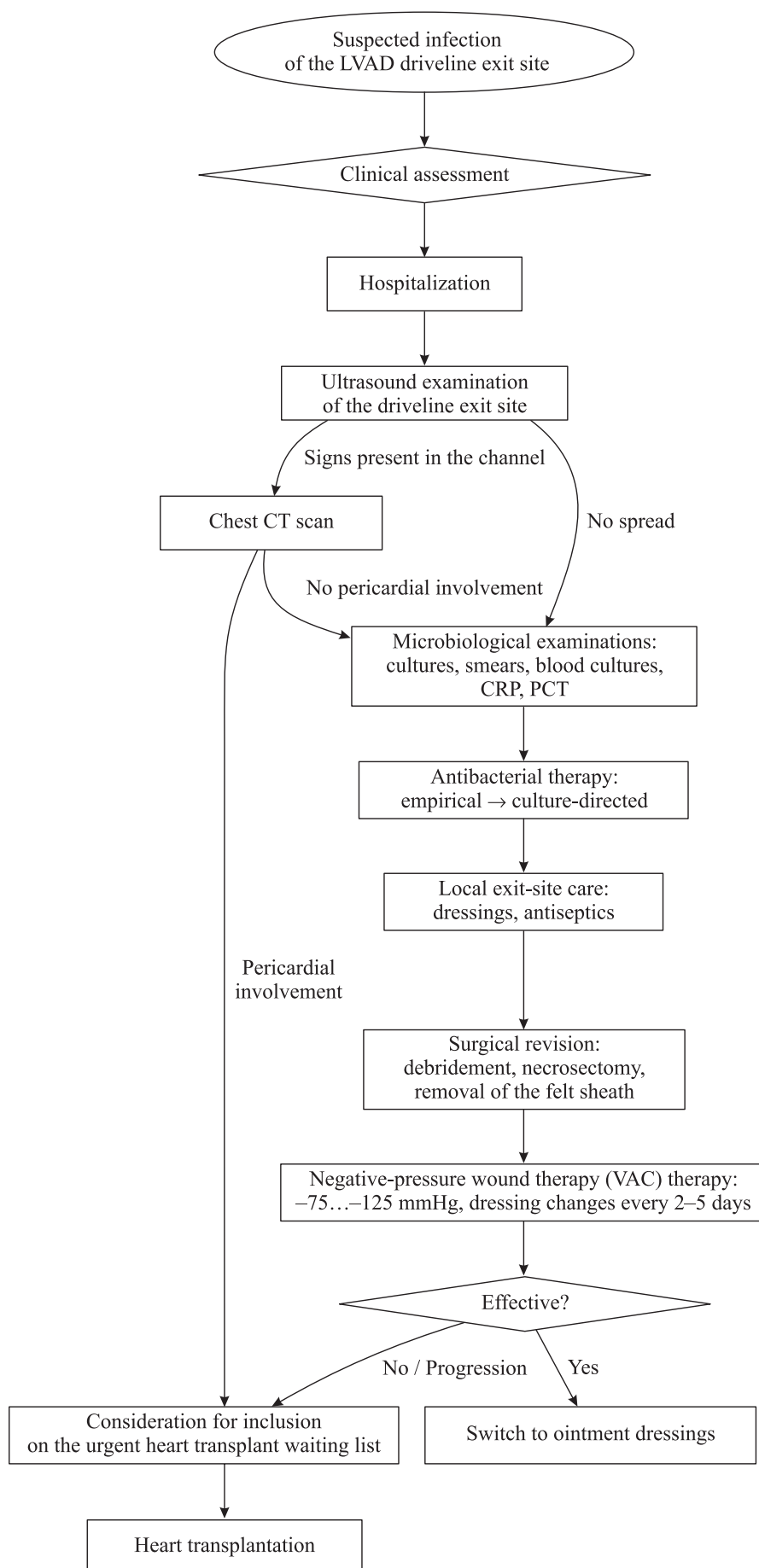


Fig. 2. Algorithm of diagnostic and therapeutic measures for driveline infection in patients with implanted long-term MCS devices

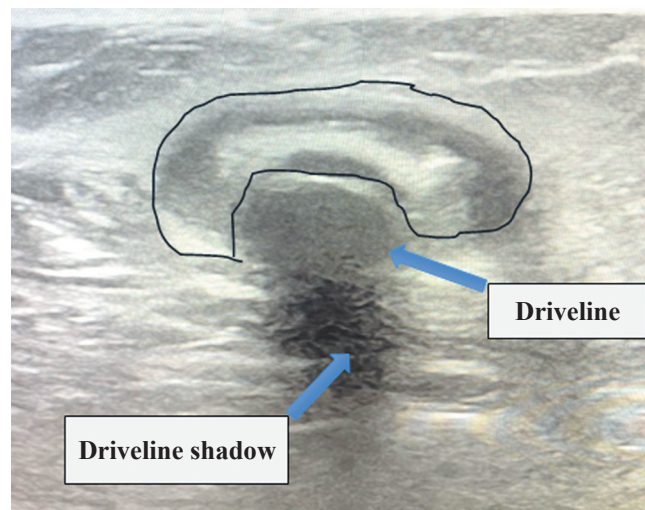


Fig. 3. Ultrasound image demonstrating localized inflammation in the subcutaneous adipose tissue along the driveline of a long-term MCS system. The highlighted area shows a rounded anechoic formation with irregular, indistinct margins and hyperechoic inclusions, with a tissue thickness of up to 4–5 cm, consistent with inflammatory changes and fluid accumulation

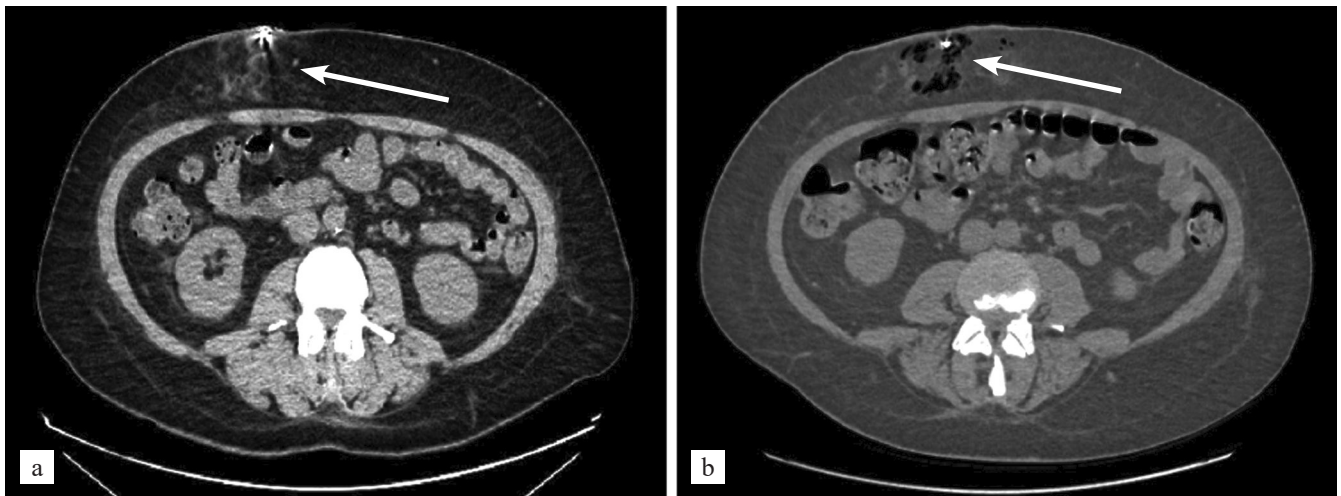


Fig. 4. CT image showing the spread of infection in the subcutaneous adipose tissue along the driveline of a long-term MCS system. (a) The arrow indicates an area of infiltration in the subcutaneous adipose tissue surrounding the driveline. (b) The arrow indicates gas accumulation within the subcutaneous adipose tissue around the driveline

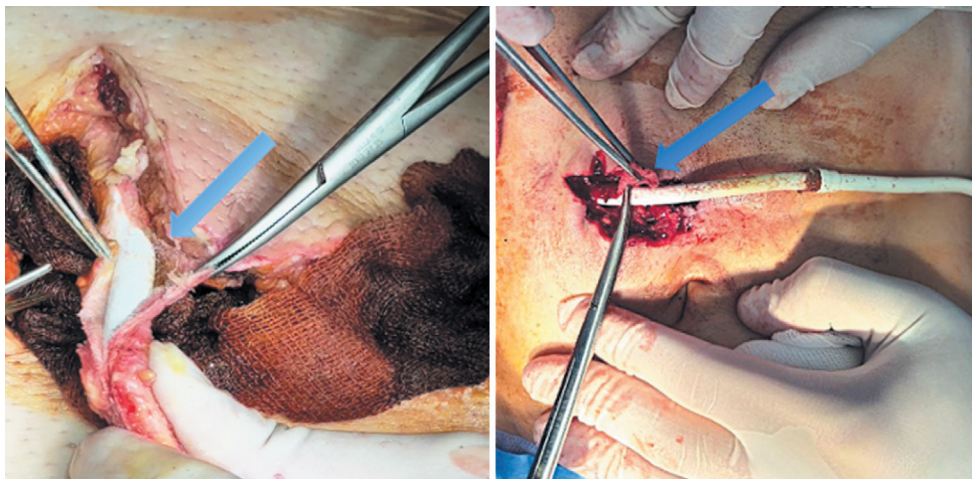


Fig. 5. Surgical removal of the outer felt sheath from the driveline in the area of active infection. Blue arrows indicate the felt covering of the driveline, beneath which lies the silicone layer

This approach enables sustained control of infection without the need for complete device explantation and, in stable cases, allows postponement or avoidance of transplantation. The technique was applied in 6 patients with infection extending into the soft tissues to a depth of at least 5 cm. In these cases, disease progression was effectively limited to the stage of surgical debridement with initial opening of the driveline tunnel to the same depth.

6. After opening the infected tract and performing thorough debridement with removal of necrotic tissue down to viable margins, a VAC system was applied to control infection and promote granulation tissue formation (Fig. 6). In the early postoperative phase, continuous negative pressure of approximately -100 to -125 mmHg was used (reduced to 75 – 100 mmHg in cases of pain or bleeding). Subsequently, an intermittent regimen was applied depending on patient tolerance. Dressings were changed every 48–72 hours in cases of active infection, and every 3–5 days during the stabilization phase. In cases of significant exudate or biofilm formation, intermittent irrigation with 0.9% NaCl or antiseptic solutions (according to institutional protocol) was performed, combined with cyclical aspiration and drainage as required. Efficacy is assessed based on clinical improvement (reduction in erythema/exudation, appearance of mature granulation tissue, and contraction of the wound edges), laboratory dynamics (decrease in C-reactive protein and procalcitonin levels, normalization of white blood cell count, negative or scant repeat cultures, negative blood cultures), and instrumental findings (disappearance of cavities/fluid/gas on ultrasound along the cable). Transition from VAC therapy to ointment-based dressings is indicated in cases of minimal exudate, absence of purulent discharge, fluctuation or odor; granulation tissue covering approximately 80–90% of the wound bed with a residual depth of less than 0.5–1 cm and no residual pockets; and microbiological findings showing negative or minimal growth without resistant strains, accompanied by sustained favorable clinical and laboratory trends.
7. If infection extends along the driveline tunnel toward the chest wall or pericardial region, the patient is placed on the emergency heart transplant waiting list. The region where the driveline bends near the right costal arch is considered a critical anatomical zone; if inflammation reaches this region, it is regarded as a high risk for mediastinal spread and constitutes an indication for transplantation (Fig. 1). This strategy is intended to prevent deep infectious complications, reduce the risk of vascular anastomotic erosion, and improve post-transplant outcomes.

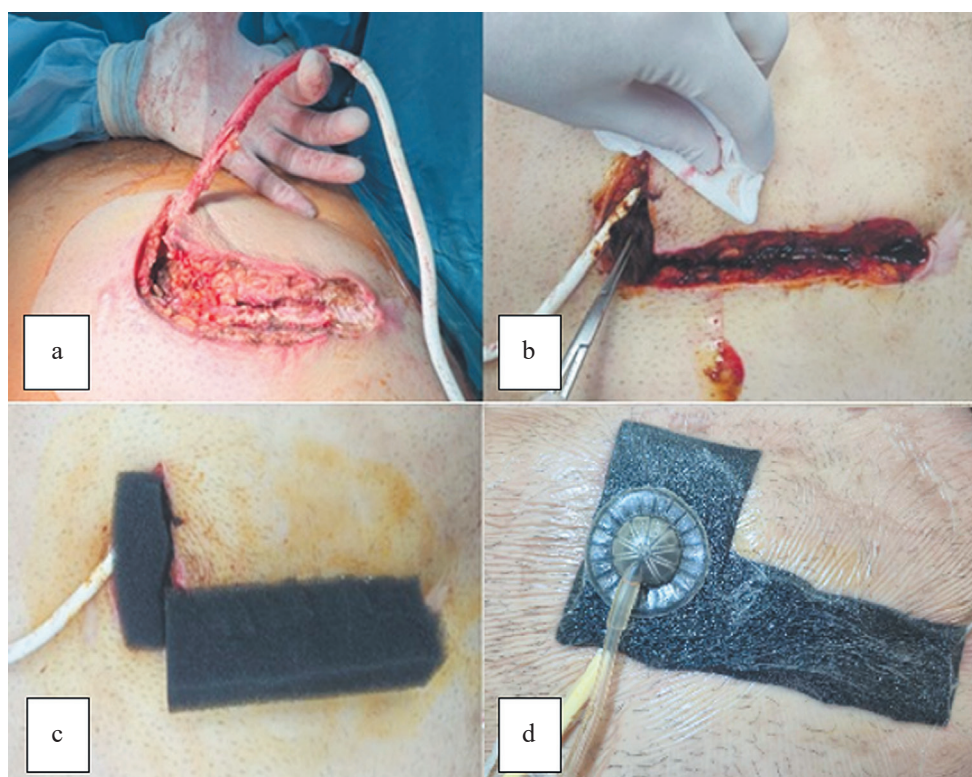


Fig. 6. Stages of surgical treatment of DLI spreading through the subcutaneous adipose tissue along the driveline. (a) Opening of the driveline tunnel with necrosectomy, debridement, and removal of the felt sheath; (b) Antiseptic treatment of the driveline bed; (c) Placement of the negative-pressure wound therapy (VAC) sponge in the driveline bed; (d) View of the functioning VAC system

Comparative evaluation of treatment efficacy in driveline-associated infections in patients on long-term MCS

A retrospective analysis demonstrated that, in the cohort managed without the DLI treatment algorithm (2010–2018), DLIs occurred in 8 patients (47.1% of all implantations). Initially, all cases corresponded to Group A (localized infection). However, in 4 patients, the infection progressed to Group B, and in 1 patient, it advanced to a deep infection involving the pump pocket and pericardial cavity (Group C), necessitating emergency heart transplantation (HT).

It is noteworthy that, in this cohort, the only contraindication to HT in 6 patients was severe pulmonary hypertension, while in the remaining cases the devices were implanted as a bridge to transplantation.

In the group managed using the diagnostic and treatment algorithm (2022–2025), the overall incidence of DLIs was 25.6% (10 of 39 patients). In all cases, the infection initially corresponded to Group A (localized inflammation at the driveline exit site). However, in a subset of patients, progression was observed with spread of infection along the driveline within the subcutaneous tissue to the level of its curvature in the right hypochondrium, corresponding to transition to Group B.

In three patients, the infectious process reached the so-called “critical point”, necessitating urgent inclusion on the HT wait list. In one of these cases, the condition was further complicated by refractory right ventricular failure. In all three patients, severe diabetes mellitus represented an additional adverse factor contributing to the unfavorable clinical course.

The clinical characteristics of the DLI algorithm-treated cohort were influenced by the distribution of baseline contraindications to HT. Among all patients in this group, 25 (64.1%) were deemed ineligible for transplantation due to severe pulmonary hypertension,

while additional contraindications included malignancy in 3 patients, multifocal atherosclerosis in 3 patients, and severe diabetes mellitus in 8 patients.

In the cohort in which the DLI treatment algorithm was applied, 10 cases of Group A infection were initially identified. In 6 patients, progression to Group B was observed, including individuals with diabetes mellitus. Importantly, in none of these cases did the infectious process advance beyond the “critical point”. The combined use of mandatory imaging, targeted antimicrobial therapy, local removal of the felt driveline sheath, and VAC therapy enabled effective resolution of inflammation at stage B, thereby preventing further progression and eliminating the need for emergency transplantation.

Analysis of time to onset and duration of inpatient treatment for driveline-associated infections

The distribution of time to onset of DLIs in both the non-algorithm and algorithm cohorts is presented in Fig. 7. Despite differences between groups, the overall pattern demonstrated that in 77.8% of cases (14 of 18), DLIs developed within the first 180 days post-implantation ($p = 0.62$). In the cohort managed without the algorithm, the onset of DLIs ranged from 18 to 360 days after device implantation. Very early infections (<30 days post-implantation) were observed in 1 patient (12.5%).

DLIs were recorded in 3 patients (37.5%) between 31 and 90 days post-implantation, in 3 patients (37.5%) between 91 and 180 days, and in 1 patient (12.5%) between 181 and 360 days. Overall, in 87.5% of cases, DLIs occurred within the first 6 months after device implantation, indicating a pronounced vulnerability during the early postoperative period.

The occurrence of very early infections, as well as the high proportion of complications within the first 180 days, may be associated with device design charac-

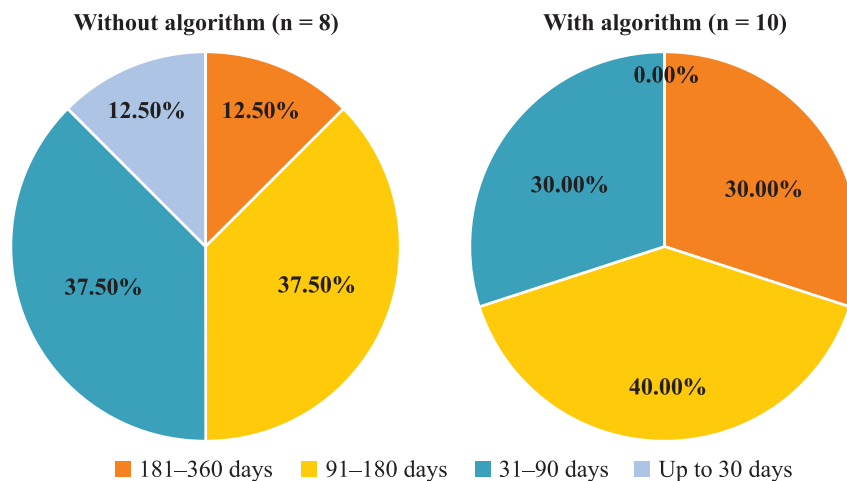


Fig. 7. Distribution of the timing of DLIs in patient cohorts managed with and without the developed diagnostic and treatment algorithm, expressed as a percentage of patients with DLIs

ristics, surgical implantation techniques, and the limited preventive strategies available during the study period.

In the cohort of patients managed using the algorithm, the time to onset of DLIs ranged from 31 to 360 days after implantation. No very early cases (<30 days) were observed. DLIs occurred in 3 patients (30%) within 31–90 days, in 4 patients (40%) within 91–180 days, and in 3 patients (30%) within 181–360 days. Overall, 70% of all episodes developed within the first 6 months after implantation, indicating that the risk of DLIs remains highest during the early postoperative period.

The absence of very early infections and the more uniform distribution of cases throughout the first year of follow-up may reflect improvements in surgical implantation techniques, as well as the implementation of the modified algorithm for prevention and management of DLIs.

A comparative analysis of the duration of hospital stay in patients with DLIs was performed across both cohorts and stratified according to the extent of the inflammatory process. Mean values and standard deviations were calculated for each subgroup. The results of this analysis are presented in Table 1.

In Group A, the mean duration of inpatient treatment in the cohort managed without the algorithm was 26.4 ± 8.1 days ($n = 8$), compared with 20.9 ± 6.5 days ($n = 10$) in the cohort where the algorithm was applied. The reduction in length of hospital stay with the use of the modified algorithm was statistically significant ($p = 0.022$).

At this stage, the infectious process is still limited in nature, and timely diagnosis, adequate antibiotic therapy, and dynamic monitoring play a leading role. Standardizing these stages within the algorithm contributed to a shorter treatment duration and reduced the likelihood of disease progression.

In Group B, a similar trend was observed. The mean duration of hospitalization in the cohort without the algorithm was 59.1 ± 9.6 days ($n = 4$), whereas in the cohort treated using the algorithm, it was significantly lower at 46.5 ± 10.9 days ($n = 6$), with statistically significant differences ($p = 0.014$).

It is important to note that in Group B – where the inflammatory process extends along the driveline within the subcutaneous tissue and approaches the “critical point” – the algorithm calls for early heart transplantation

if this boundary is reached. In the present study, three patients underwent transplantation proactively, before progression beyond the “critical point”. This strategy helps prevent further spread of infection to the pericardium or pump pocket, thereby reducing the need for multi-stage debridement and prolonged hospitalization.

In Group C, within the cohort managed without the algorithm, one patient had a prolonged hospital stay of 72 days due to a severe course of driveline infection. This case required extensive surgical intervention and ultimately led to urgent heart transplantation, prompted by a high risk of mortality associated with potential mediastinal spread of infection and systemic complications. In this clinical context, continuation of mechanical circulatory support was considered unsafe.

Notably, no patients progressed to Group C in the cohort managed with the algorithm. This is attributable to the implementation of an early transplant strategy at the Group B stage upon reaching the “critical point”. Such an approach effectively interrupts disease progression and prevents transition to severe forms of infection.

Across all comparable subgroups, implementation of the modified algorithm was associated with a reduction in the duration of hospitalization; in Groups A and B, these differences reached statistical significance. As expected, progression of the infectious process from Group A to Group B was accompanied by an increase in length of hospital stay. However, patients managed with the algorithm demonstrated significantly shorter hospitalization compared to those treated without it.

The observed effect can be attributed to several key components of the algorithm: standardized early assessment of the extent of infection, timely surgical debridement combined with local removal of the felt driveline sheath and application of VAC therapy, as well as dynamic re-stratification of patients on the HT waiting list upon reaching the “critical point” in cases of unfavorable clinical progression.

Together, these measures not only help prevent progression to deep infection (Group C) but also contribute to a reduction in the overall resource intensity of treatment.

Table 1

Average length of hospital stay during the first year of follow-up

Subgroup	Without algorithm (mean $\pm$ n)	With algorithm (mean $\pm$ n)	Mann–Whitney U test (p)
A (initial)	26.4 ± 8.1 ($n = 8$)	20.9 ± 6.5 ($n = 10$)	0.022
B (transferred from A)	59.1 ± 9.6 ($n = 4$)	46.5 ± 10.9 ($n = 6$)	0.014
C (transferred from B)	72 ($n = 1$)	–	–

Note: Subgroup A includes all patients enrolled in the study at baseline (8 without algorithm, 10 with algorithm). Subgroups B and C reflect the dynamics of the disease and were formed from patients who were initially assigned to subgroup A.

Table 2

Comparative analysis of the proportion of patients experiencing relapses by group and cohort during the first year of follow-up

Group	Cohort	n	1 recurrence n (%)	2 recurrences n (%)	≥3 recurrences n (%)	p, Mann–Whitney U test
A	Without algorithm	8	0 (0.0%)	5 (62.5%)	3 (37.5%)	p = 0.331
	With algorithm	10	3 (30.0%)	4 (40.0%)	3 (30.0%)	
B	Without algorithm	4	0 (0.0%)	3 (75.0%)	1 (25.0%)	p = 0.05
	With algorithm	6	3 (50.0%)	2 (33.3%)	0 (0.0%)	

Analysis of the recurrence rate of driveline-associated infections

Despite the significant reduction in length of hospital stay achieved with the use of the DLI treatment algorithm, the decrease in infection recurrence rates was less pronounced. This finding can be partly explained by the characteristics of the study population. As noted earlier, a substantial proportion of patients in the algorithm-treated cohort had severe diabetes mellitus, which is an independent risk factor for infectious complications.

Consequently, although standardized diagnostic and therapeutic interventions contributed to shorter hospital stays, a tendency toward recurrent infection episodes persisted, particularly in Group A, while in Group B only a partial reduction in recurrence frequency was observed.

These findings prompted a separate, detailed analysis of recurrence rates in the compared cohorts, the results of which are presented in Table 2.

An analysis of the recurrence rate of DLIs during the first year of follow-up showed that, in Group A, the mean number of relapses per patient was 2.38 ± 0.52 in the cohort without the algorithm and 2.00 ± 0.82 in the cohort with the algorithm. The median value in both subgroups was identical (2), and no statistically significant differences were observed ($p = 0.331$). At the same time, both cohorts included patients with multiple recurrences (≥ 3 episodes: 3 of 8 vs. 3 of 10), indicating the presence of a subgroup with a persistent, relapsing course of infection, likely associated with significant comorbidities, including diabetes mellitus.

In Group B, the use of the algorithm was associated with a reduction in recurrence frequency: 2.25 ± 0.50 versus 1.17 ± 0.75 (median 2 vs. 1, respectively), with differences reaching borderline statistical significance ($p = 0.05$). The distribution pattern further supports the clinical relevance of this effect: in the algorithm-treated subgroup, one patient experienced no recurrences, and no cases with ≥ 3 episodes were observed, whereas such patterns were present in the cohort managed using the traditional approach.

In Group C, within the cohort without the algorithm, there was a single patient who did not experience any relapses.

DISCUSSION

The results of this study confirm that DLIs are one of the most common and clinically significant complications in patients receiving long-term MCS. The observed incidence of 32.1% is consistent with findings from both national and international registries, where reported rates range from 15% to 40% [7–9, 11, 15].

The first six months after device implantation emerged as the most vulnerable period for infection development. This likely reflects the phase during which the driveline exit tract is still forming and surrounding soft tissues are adapting to the presence of a foreign surface.

Localized infections were encountered more frequently than disseminated forms. However, it is the disseminated ones that largely determined clinical severity and the need for repeated surgical interventions.

A key determinant of treatment outcomes in this study was the implementation of a structured diagnostic and therapeutic algorithm for DLIs. The use of a comprehensive protocol – incorporating imaging modalities (ultrasound and CT), microbiological confirmation, targeted antibiotic therapy, and standardized surgical debridement – substantially improved control of the infectious process.

A particularly important component of the algorithm was the removal of the driveline's felt sheath when involved in the inflammatory process. This intervention facilitates elimination of biofilm and enables more effective and complete wound debridement.

In addition, the application of VAC therapy played a role in accelerating wound debridement and promoting the transition from the inflammatory to the reparative phase. This was reflected in shorter healing times and reduced hospital stays.

The practical significance of the proposed algorithm lies in reducing treatment duration for localized inflammation confined to the driveline exit site, decreases the frequency of recurrences, and prevents progression to deep, life-threatening forms involving the pericardial cavity and/or pump pocket, including through early heart transplantation, in cases of inflammation spreading to the subcutaneous fatty tissue and progressing along the driveline to its turn in the subcutaneous fatty tissue at the level of the right hypochondrium.

CONCLUSIONS

The developed algorithm for the diagnosis and surgical management of DLIs demonstrates clear clinical benefits. Its implementation is associated with reduced recurrence rates, shorter hospital stays, and a decreased need for repeated surgical debridement.

A comprehensive approach to the diagnosis and treatment of DLIs improves the effectiveness of local infection control and can be considered the optimal management strategy for patients on prolonged MCS.

The authors declare no conflict of interest.

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PREDICTING THE OUTCOME OF HEART TRANSPLANTATION FROM EXPANDED-CRITERIA DONORS USING REGRESSION ANALYSIS

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There is a growing need for tools that enable objective assessment of donor heart quality. One such approach is the use of regression models incorporating donor and recipient risk factors to predict surgical outcomes and potentially expand the donor pool by increasing the number of transplantations. **Objective:** to develop a model for estimating the total risk of one-year mortality in recipients using different categories of expanded-criteria donors (ECDs). **Materials and methods.** The study included 1,500 recipients who underwent orthotopic heart transplantation (OHT) at Shumakov National Medical Research Center of Transplantology and Artificial Organs over an 11-year period, from January 1, 2011, to December 31, 2021. The cohort comprised 1,281 men (85.4%) and 219 women (14.6%), aged 9 to 78 years (median age 49.0 [38.0–56.0] years). The heart transplants performed ($n = 1,500$) were divided into two clinical groups: group 1 (main group) comprised recipients who underwent OHT from ECDs ($n = 1,060$; 70.6%); group 2 (control group) included recipients who underwent OHT from standard-criteria donors ($n = 440$; 29.4%). Donor heart suitability for transplantation was assessed according to the 2023 criteria of the International Society for Heart and Lung Transplantation (ISHLT). **Results.** Donor- and recipient-related indicators were initially evaluated using univariate regression analysis. The final multivariate regression model included five donor-related factors – donor–recipient weight mismatch, donor age, high-dose cardiotoxic therapy, coronary stenosis, and prolonged graft ischemia – and four recipient-related factors – total bilirubin $>40 \mu\text{mol/L}$, creatinine $>110 \mu\text{mol/L}$, international normalized ratio (INR) >1.4 , and pre-transplant peripheral veno-arterial extracorporeal membrane oxygenation (pVA-ECMO). The highest odds ratios were observed for donor age, coronary stenosis, graft ischemia time exceeding 6 hours, and pre-transplant pVA-ECMO support. The predicted one-year mortality rate calculated using regression analysis showed a strong correlation ($R = 0.827$; $p < 0.001$) with the observed one-year mortality rate. Long-term survival was also analyzed across risk groups, with the worst outcomes observed in the high-risk group. **Conclusion.** The proposed statistical model provides a reliable prognostic accuracy for both early and long-term post-transplant survival. Its application at the stages of donor heart evaluation and donor–recipient matching may facilitate the use of a broader donor pool while enabling an objective assessment of recipient prognosis.

Keywords: heart transplantation, mechanical circulatory support, VA-ECMO, regression analysis.

INTRODUCTION

Given the current shortage of donor organs over recent decades, the use of hearts from expanded-criteria donors (ECDs) remains a practical strategy for increasing the availability of heart transplantation (HT), particularly for patients requiring urgent transplantation and/or those with a predicted poorer early or long-term post-transplant survival [1].

In Russian clinical practice, where implantable long-term mechanical circulatory support systems are not yet widely available, the use of suboptimal donor hearts represents a realistic approach to increasing the number of HT procedures performed. In this context, the experience of individual transplant centers is of considerable

importance when determining the suitability of donor organs whose characteristics fall between those of an “ideal” and an “ineligible” donor.

Accordingly, there is a high need for the development of objective tools for assessing donor heart quality. One promising approach involves the creation of regression models incorporating donor- and recipient-related risk factors to predict postoperative outcomes and increase transplant activity [2, 3].

Research objective: To develop a cumulative risk model for estimating the one-year mortality associated with the use of ECDs (including cases of donor–recipient

weight mismatch, donor age >55 years, high-dose cardiotoxic therapy, coronary artery atherosclerosis, graft ischemia time exceeding 5 hours, ejection fraction (EF) <50%, left ventricular myocardial hypertrophy (LVH) >1.5 cm, systemic vascular resistance (SVR), and valvular pathology.

MATERIALS AND METHODS

The study included 1,500 recipients who underwent orthotopic heart transplant (OHT) at Shumakov National Medical Research Center of Transplantology and Artificial Organs over an 11-year period from January 1, 2011, to December 31, 2021. Among the recipients, 1,281 (85.4%) were men and 219 (14.6%) were women. Recipient age ranged from 9 to 78 years, with a median age of 49.0 years (interquartile range: 38.0–56.0 years).

The heart transplants performed (n = 1,500) were divided into two clinical groups:

Group 1 (main group): recipients who underwent OHT from ECDs (n = 1,060; 70.6%); Group 2 (control group): recipients who underwent OHT from standard criteria donors (n = 440; 29.4%).

Assessment of donor heart suitability for transplantation was performed in accordance with the 2023 guidelines of the International Society for Heart and Lung Transplantation [4]. The pre-transplant clinical characteristics of recipients and donors are presented in Table 1.

Statistical analysis was performed using IBM SPSS Statistics 23 (IBM Corp., USA). In the main group, univariate regression analysis was initially conducted to identify factors associated with one-year mortality. Variables demonstrating statistical significance were sub-

Table 1

Pre-transplant clinical characteristics of recipients and donors in the main and control groups (n = 1500)

Indicator	Main group (n = 1060)	Control group (n = 440)	Significance of difference (p)
Heart recipients			
Age, years – Mean ± SD Median [Q1; Q3]	46.3 ± 11.0 48.0 [38; 56]	45.8 ± 13.0 48.0 [37.8; 56]	0.448
Sex (female) – (n/%)	173/16.4	46/10.5	0.002
Weight, kg – Mean ± SD Median [Q1; Q3]	79.5 ± 18.7 79 [66; 92]	79.9 ± 17.0 78 [70; 90]	0.699
BSA, m ² – Mean ± SD Median [Q1; Q3]	1.9 ± 0.2 1.9 [1.7; 2.1]	1.9 ± 0.1 1.9 [1.8; 2.0]	1.000
BMI, kg/m ² – Mean ± SD Median [Q1; Q3]	25.9 ± 5.2 25.5 [22.4; 29.4]	25.7 ± 3.9 25.3 [23.1; 28.4]	0.468
CHF stage IIB (n/%)	603/56.8	313/71.1	0.000
CHF stage III (n/%)	365/34.4	82/18.7	0.000
NYHA functional class Median [Q1; Q3]	3.3 ± 0.5 3 [3; 4]	3.2 ± 0.4 3 [2; 3]	0.000
VA-ECMO – (n/%)	343/32.4	96/21.8	0.000
TPG, mmHg – Mean ± SD Median [Q1; Q3]	10.0 ± 4.2 9 [7; 16]	8.8 ± 4.4 8 [6; 12]	0.000
TPVR, Wood units – Mean ± SD Median [Q1; Q3]	3.7 ± 1.2 2.8 [1.7; 4.0]	2.8 ± 1.5 2 [1.7; 3.6]	0.000
Heart donors			
Age, years – Mean ± SD Median [Q1; Q3]	46.3 ± 11.0 48 [38; 56]	38.5 ± 9.9 39 [31; 46.8]	0.000
Sex (female) – (n/%) Sex (male) – (n/%)	259/24.5 801/75.5	80/18.2 360/81.8	0.01
Female donor–male recipient pair – (n/%)	114/10.8	29/6.6	0.07
Weight, kg – Mean ± SD Median [Q1; Q3]	84.1 ± 17.6 80 [70; 90]	79.3 ± 11.7 80 [70; 87.3]	0.000
Donor-to-recipient weight ratio – Mean ± SD Median [Q1; Q3]	1.0 ± 0.3 1 [0.8; 1.3]	0.9 ± 0.2 1 [0.9; 1.1]	0.000
Stroke (donor) – (n/%)	804/75.8	233/53.0	0.000
MV duration, days – Mean ± SD Median [Q1; Q3]	2.5 ± 1.8 2.0 [1; 3]	2.8 ± 3.8 2.0 [1; 3]	0.038
Eurotransplant Heart Transplant Score, points	26.8 ± 12.4	24.4 ± 10.2	0.000

Abbreviations: SD, standard deviation; BSA, body surface area; CHF, chronic heart failure; NYHA, New York Heart Association; VA-ECMO, venoarterial extracorporeal membrane oxygenation; TPG, transpulmonary gradient; TPVR, total pulmonary vascular resistance; MV, mechanical ventilation.

sequently included in a multivariate logistic regression model.

Independent predictors of one-year mortality ($p < 0.05$) were assigned weighted scores according to their odds ratios (ORs). Predicted one-year mortality scores were then calculated using the derived regression equation. Based on these scores, recipients were stratified into three risk categories: low-risk (≤ 10 points), medium-risk (11–15 points), and high-risk (≥ 16 points).

Predicted mortality rates were compared with observed mortality rates across the risk groups. To assess the accuracy of the model, the correlation between predicted and observed outcomes was evaluated using Pearson's correlation coefficient (r). Long-term survival by risk category was analyzed using the Kaplan–Meier method.

RESULTS

Using univariate regression analysis, 9 expanded-criteria donor variables were evaluated: donor–recipient weight mismatch, donor age >55 years, high-dose cardiotonic therapy, coronary artery stenotic disease,

graft ischemia time >5 hours, EF $<50\%$, LVH >1.5 cm, cardiopulmonary resuscitation (CPR), and valvular pathology. Among these variables, 5 factors demonstrated statistically significant associations with one-year mortality (Table 2).

Considering the potential impact of recipient-related factors on HT outcomes, they were also included in the analysis. Eight recipient parameters were assessed: total bilirubin >40 $\mu\text{mol/L}$, serum creatinine >110 $\mu\text{mol/L}$, international normalized ratio (INR) >1.4 , pre-transplant veno-arterial extracorporeal membrane oxygenation (pVA-ECMO), recipient age >65 years, body mass index (BMI) >30 kg/m^2 , transpulmonary gradient (TPG) >15 mmHg, pulmonary vascular resistance (PVR) >3 Wood units, and type 2 diabetes mellitus. Of these variables, 4 were identified as statistically significant predictors (Table 3).

The final multivariate regression model incorporated 5 donor-related factors and 4 recipient-related factors, demonstrating good predictive performance ($R^2 = 0.64$). The highest ORs were observed for donor age, stenotic

Table 2

Univariate regression analysis of donor-related risk factors for heart transplant from using expanded-criteria donors (n = 1,060)

Indicator	Single-factor (univariate) regression analysis	
	Odds ratio (OR) (95% CI)	p-value
Donor–recipient weight mismatch	1.785 (0.552–1.117)	0.002
Donor age >55 years	2.186 (1.669–2.884)	0.000
High cardiotonic (inotropic) therapy	1.999 (1.003–3.941)	0.005
Coronary stenosis	4.062 (2.070–7.973)	0.000
Graft ischemia time >6 hours	1.608 (1.446–1.867)	0.004
Left ventricular EF $<50\%$	0.698 (0.386–1.262)	0.234
Left ventricular myocardial hypertrophy >1.5 cm	0.880 (0.585–1.326)	0.542
CPR	0.899 (0.305–2.649)	0.848
Valve disease	0.735 (0.153–3.541)	0.701

Abbreviations: EF, ejection fraction; CPR, cardiopulmonary resuscitation.

Table 3

Univariate regression analysis of recipient risk factors in heart transplant from expanded-criteria donors (n = 1060)

Indicator	Univariate analysis	
	OR (95% CI)	p-value
Total bilirubin >40 $\mu\text{mol/L}$	1.531 (0.381–0.740)	0.000
Serum creatinine >110 $\mu\text{mol/L}$	1.528 (0.317–0.879)	0.014
INR >1.4	1.540 (0.391–0.713)	0.000
Pre-transplant pVA-ECMO	2.277 (6.357–14.440)	0.000
Age >65 years	0.290 (0.646–4.317)	0.290
BMI >30 kg/m^2	0.468 (0.515–1.356)	0.468
TPG >15 mmHg, TPVR >3 Wood units	0.680 (0.785–1.450)	0.680
Type 2 diabetes	0.406 (0.646–1.193)	0.878

Abbreviations: OR, odds ratio; CI, confidence interval; INR, international normalized ratio; pVA-ECMO, peripheral veno-arterial extracorporeal membrane oxygenation; BMI, body mass index; TPG, transpulmonary gradient; TPVR, total pulmonary vascular resistance.

coronary artery disease, graft ischemia time >6 hours, and pre-transplant pVA-ECMO (Table 4).

Each statistically significant variable was assigned a weighted point value, and predicted mortality was calculated using a regression-based scoring system derived from the cumulative score (Table 5).

Predicted one-year mortality rates obtained through regression analysis demonstrated a strong positive cor-

relation with observed one-year mortality ($R = 0.827$; $p < 0.001$).

Based on the estimated risk of death within one year after HT, recipients were stratified into three risk categories: low-risk (≤ 10 points), medium-risk (11–15 points), and high-risk (≥ 16 points).

The predicted one-year mortality closely corresponded to the observed mortality across the risk groups,

Table 4

Multivariate regression analysis of donor and recipient risk factors in heart transplantation (n = 1060)

Indicator	Multivariate regression analysis	
	OR (95% CI)	p-value
Donor–recipient weight mismatch	1.961 (0.690–1.324)	0.001
Donor age >55 years	5.852 (0.889–11.184)	0.001
High-dose cardiotoxic therapy	1.560 (0.908–1.974)	0.001
Donor coronary stenosis	8.142 (0.769–15.695)	0.001
Graft ischemia time >6 hours	3.844 (1.497–7.434)	0.001
Total bilirubin >40 $\mu\text{mol/L}$	1.970 (0.662–1.420)	0.001
Serum creatinine >110 $\mu\text{mol/L}$	1.260 (0.339–0.992)	0.047
INR >1.4	1.320 (0.566–1.108)	0.043
Pre-transplant pVA-ECMO	3.910 (0.219–6.538)	0.001

Abbreviations: OR, odds ratio; CI, confidence interval; INR, international normalized ratio; pVA-ECMO, peripheral veno-arterial extracorporeal membrane oxygenation.

Table 5

Risk stratification scale for early postoperative mortality after heart transplantation from suboptimal donors (n = 1060)

Indicator	OR (95% CI)	Points
Donor–recipient weight mismatch (ratio <1.2)	1.961	2
Donor age >55 years	5.852	6
High-dose cardiotoxic therapy	1.560	2
Coronary stenosis	8.142	8
Graft ischemia time >6 hours	3.844	4
Total bilirubin >40 $\mu\text{mol/L}$	1.970	2
Serum creatinine >110 $\mu\text{mol/L}$	1.260	1
INR >1.4	1.302	1
Pre-transplant pVA-ECMO	3.910	4
Maximum total score	30	

Donor–recipient risk scale	Prediction of 1-year mortality
10	11.4%
12	12.4%
14	13.6%
16	15.2%
18	18.5%
20	20.3%
22	22.0%
24	24.4%
26	26.0%
28	28.2%
>29	>30%

Table 6

Distribution of recipients by 1-year mortality risk groups

	Low-risk group	Medium-risk group	High-risk group
Risk scale scores	<10 points	11–15 points	≥ 16 points
Predicted 1-year mortality (%)	≤ 11.2	11.2–14.8	>14.8
Observed 1-year mortality (%)	10.0	12.2	14.6
Number of risk factors (%)			
0–1	100	88.2	29
2–3	–	11.8	68.4
4–5	–	–	2.6

with rates of 10.0%, 12.2%, and 14.6%, respectively (Table 6).

An analysis of long-term survival according to risk category was also performed, with the poorest outcomes observed in the high-risk group (Fig.).

DISCUSSION

Given the persistent shortage of donor hearts, the use of ECD organs remains an important and clinically relevant strategy. It has been suggested that broader utilization of all available donor hearts could substantially alleviate the current organ shortage [5].

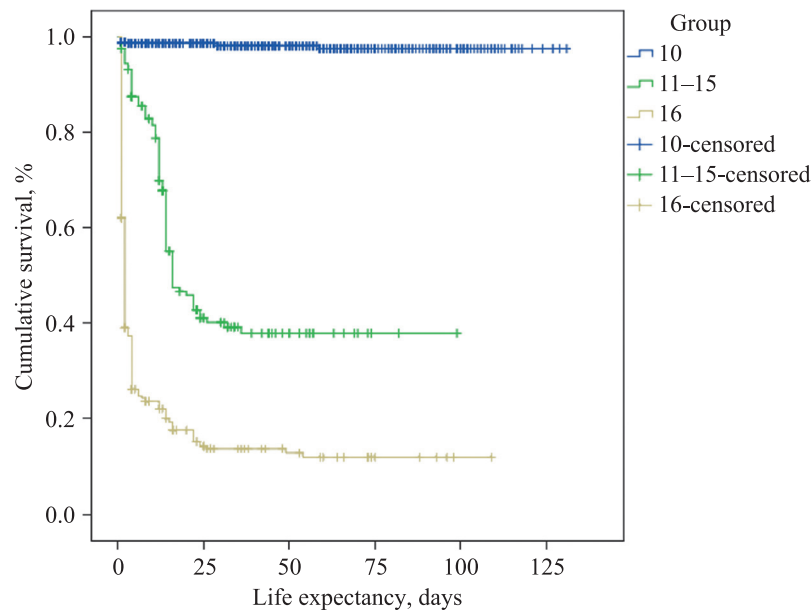


Fig. Long-term survival of heart transplant recipients from expanded-criteria donors stratified by risk group

In the present study, the number of annual HTs performed using ECDs increased from 46% in 2011 to a peak of 72.1% in 2019 during the study period from January 1, 2011, to December 31, 2021. Overall, the majority of the donor pool (70.6%) consisted of ECDs. These criteria included prolonged graft ischemia time, donor age 45–55 years, concomitant but potentially correctable valvular heart disease, coronary artery disease, donor–recipient weight mismatch exceeding 20%, reduced left ventricular systolic function, LVH, high-dose sympathomimetic therapy, prior CPR, methanol poisoning, and combinations of these factors.

HTs from donor organs with one or more risk factors may be justified in recipients requiring urgent transplantation; however, such procedures are associated with an increased likelihood of adverse postoperative outcomes [6]. At present, there are no universally accepted guidelines regarding the use of suboptimal donor hearts, resulting in substantial variability in donor selection strategies among individual transplant centers and regional or interstate organ allocation systems [3, 7].

The increase in the number of HTs performed at many transplant centers has paralleled the expansion of donor eligibility criteria. Outcomes following transplantation using ECDs remain mixed, with studies reporting either comparable or less favorable early- and long-term survival rates relative to standard-criteria donor transplantation. It is evident that the outcomes of HTs from ECDs depend not only on donor-related characteristics but also on recipient pre-transplant factors, including recipient age, presence of chronic pulmonary disease, repeat transplantation, severity of pre-transplant pulmonary hypertension, and multiple organ dysfunction [2, 8].

Given this, it appears justified to develop a statistical prediction model based on extensive institutional expe-

rience and a large series of transplantations performed using ECD hearts. Such a model would enable estimation of the risk associated with HT depending on the presence, number, and combination of expanded donor criteria, as well as their interaction with recipient pre-transplant factors.

Based on univariate analysis, several donor-related risk factors were examined, five of which demonstrated statistical significance: donor–recipient weight mismatch ($p < 0.002$), donor age >55 years ($p < 0.001$), high-dose cardiotoxic therapy ($p < 0.005$), stenotic coronary artery disease ($p < 0.001$), and graft ischemia time >6 hours ($p < 0.004$).

Analysis of recipient-related risk factors identified the following significant predictors: total bilirubin >40 $\mu\text{mol/L}$ ($p < 0.001$), serum creatinine >110 $\mu\text{mol/L}$ ($p < 0.014$), INR >1.4 ($p < 0.001$), and pre-transplant pVA-ECMO ($p < 0.001$).

These variables were subsequently incorporated into a multivariate regression model for predicting the risk associated with HT. The highest ORs were observed for donor age, pre-existing donor stenotic coronary artery disease, graft ischemia time exceeding 6 hours, and recipient pre-transplant pVA-ECMO.

In the present study, predicted 1-year mortality demonstrated a strong positive correlation with observed 1-year mortality ($R = 0.827$; $p < 0.001$). Furthermore, analysis of long-term survival revealed the poorest outcomes in recipients classified within the high-risk group.

CONCLUSION

The developed statistical model provides a reliable prognostic tool for predicting survival in both the early and long-term post-transplant periods. It can be effectively applied during donor heart assessment and

donor–recipient matching, enabling broader utilization of available donor organs while ensuring an accurate assessment of the recipient's prognosis.

The authors declare no conflict of interest.

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RISK FACTORS FOR HUMORAL MYOCARDIAL REJECTION DURING THE FIRST YEAR AFTER HEART TRANSPLANTATION

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Objective: to identify risk factors for antibody-mediated myocardial rejection (AMR) during the first year following heart transplantation (HT). **Materials and methods.** This retrospective study included 162 patients who underwent HT at Almazov National Medical Research Centre between 2010 and 2024. Clinical, laboratory, immunological, and morphological data were analyzed. Sensitization was assessed using Luminex multiplex assays and solid-phase ELISA. AMR was diagnosed based on endomyocardial biopsy with morphological and immunohistochemical evaluation in accordance with the 2013 ISHLT classification. Hemodynamically significant rejection was defined as AMR II–III in combination with graft dysfunction and/or the presence of donor-specific antibodies (DSA). Patients were stratified into three groups: (1) no sign of AMR (control); (2) isolated morphological signs of AMR (AMR II); and (3) hemodynamically significant AMR (AMR III, or AMR II with DSA and/or echocardiographic evidence of graft dysfunction). Discriminant analysis was used to evaluate the factors associated with the development of AMR. **Results.** Morphological signs of AMR were identified in 36.2% of patients, including 9.9% with hemodynamically significant rejection requiring specific therapy. Patients with AMR before HT showed significantly higher sensitization to HLA class I and II antigens (MFI >5000; $p < 0.05$), and following HT, a statistically significant excess of *de novo* antibodies persisted for 6–12 months compared with the control group ($p < 0.05$). In addition, AMR was associated with lower tacrolimus levels during the first two weeks after HT ($p = 0.002$) and reduced antimetabolite doses in the first month post-transplant ($p < 0.001$). Discriminant analysis of 15 variables yielded a model incorporating 9 significant predictors, enabling statistically reliable differentiation of patients with hemodynamically significant rejection from other groups ($F(18,222) = 12.463$; $p < 0.00001$). **Conclusion.** High levels of HLA sensitization before transplantation, suboptimal immunosuppression in the early postoperative period, and predictors identified in the discriminant analysis model are associated with an increased risk of developing hemodynamically significant AMR. The proposed model may be used for risk stratification and to individualize post-transplant follow-up.

Keywords: heart transplantation, antibody-mediated rejection risk factors, donor-specific antibodies.

INTRODUCTION

Cardiovascular diseases are the leading causes of death worldwide [1]. For patients with end-stage heart failure, HT continues to be the only effective treatment option [2]. Over recent decades, substantial progress in transplant outcomes has been achieved, driven by advances in surgical and anesthetic techniques, improvements in monitoring and therapeutic strategies, and the increasing individualization of immunosuppressive regimens [3].

In the context of these developments in modern transplantology, myocardial rejection – provided that optimal donor–recipient matching is achieved and immunosuppressive therapy is appropriately tailored to the recipient's immunological risk – is no longer the leading cause of mortality following HT, even in the early postoperative period [4].

Nevertheless, data from international registries indicate that the incidence of AMR has remained largely unchanged. Importantly, both clinically overt and subclinical forms of AMR have been shown to contribute to graft dysfunction and loss, as well as to the development of cardiac allograft vasculopathy [5].

The problem with AMR lies in the lack of uniform, standardized approaches for its timely diagnosis and, consequently, for the prevention and treatment of this complication. While the need to treat HC-AMR (AMR III) is well established, the optimal management of patients with mild to moderate AMR (AMR I–II), particularly in the absence of clinically significant graft dysfunction or circulating DSA, remains a subject of debate.

As a result, the reported incidence of AMR in the global literature ranges from 10% to 20% and is associated with a significant increase in adverse outcomes.

Histological and immunohistochemical criteria for AMR have been established by the International Society for Heart and Lung Transplantation (ISHLT). These include macrophage and neutrophil infiltration, endothelial swelling, and deposition of immunoglobulins and complement components – particularly C4d – within capillary endothelium. The diagnosis of HC-AMR (HC-AMR), however, relies on a comprehensive assessment that integrates pathological findings from endomyocardial biopsies (histological and immunohistochemical), evidence of graft myocardial dysfunction, and the presence of circulating DSA in the recipient's blood.

Risk factors for the development of AMR are currently the subject of extensive investigation. Proposed predictors include recipient- and donor-related variables such as sex, age, blood group incompatibility, cause of donor death, presence of pre-existing and donor-specific antibodies, patient adherence, the use of induction therapy, and immunosuppressant drug levels [6].

At the same time, advances in desensitization strategies and the increasing individualization of immunosuppressive regimens have significantly modified the prognostic relevance of several of these factors. For instance, recent ISHLT data suggest that the presence of pre-existing antibodies is not invariably associated with an increased risk of AMR, provided that appropriate prophylactic therapy is administered [8].

Particular attention should be directed toward identifying risk factors for HC-AMR, a severe form characterized by rapid progression, biventricular dysfunction, and a high risk of mortality. In this context, identification of predictors specific to this phenotype is essential for improving risk stratification, optimizing post-transplant monitoring, and enabling timely therapeutic intervention [9].

In light of the above, a retrospective analysis of clinical, laboratory, and immunological risk factors associated with the development of hemodynamically significant antibody-mediated myocardial rejection during the first year after heart transplantation appears warranted.

Objective: to identify risk factors associated with AMR episodes within the first year following HT.

MATERIALS AND METHODS

This retrospective study included 162 HT recipients who were followed at Almazov National Medical Research Centre, St. Petersburg, Russia. The inclusion criteria were as follows: patients who underwent HT at the Center between 2010 and 2024, age ≥ 18 years, and provision of informed consent to participate in the study. The exclusion criteria were age < 18 years and refusal to participate in the study.

Clinical and biochemical blood parameters were assessed using an Architect automated biochemical analy-

zer. Blood concentrations of calcineurin inhibitors were measured with a Luminex automated analyzer using an immunofluorescence assay. Drug levels were determined at the trough (C_0), 12 hours after the last administered dose.

Detection of antibodies to human leukocyte antigens (HLA) class I and II, both before and after heart transplantation, was performed using solid-phase enzyme-linked immunosorbent assay (ELISA) and multiplex analysis (Luminex). Recipient sensitization was evaluated based on the percentage of panel-reactive antibodies (PRA), calculated using the Match IT Antibody software (Immucor) for Luminex assays. Patients with anti-HLA antibody levels exceeding 10% PRA and mean fluorescence intensity (MFI) > 5000 were classified as highly sensitized.

Instrumental diagnostic evaluation of AMR included standard transthoracic echocardiography (ECHO) and endomyocardial biopsy (EMB), followed by morphological and immunohistochemical assessment of biopsy specimens.

All patients received standard immunosuppressive therapy consisting of calcineurin inhibitors (tacrolimus), antimetabolites (mycophenolate mofetil or mycophenolic acid), and glucocorticosteroids, along with adjunctive treatments (e.g., antihypertensive and antithrombotic agents) as clinically indicated. Lipid-lowering therapy was routinely prescribed to all recipients, and prophylaxis against cytomegalovirus infection and *Pneumocystis jirovecii* pneumonia was administered during the first six months following transplantation. Patients with a high degree of pre-existing sensitization (PRA $> 10\%$ and MFI > 5000) underwent a desensitization protocol, which included extracorporeal hemocorrection techniques (plasma exchange or cascade plasmapheresis) in combination with intravenous human immunoglobulin administration.

Immediately prior to transplantation, donor–recipient compatibility was assessed using a complement-dependent lymphocytotoxic crossmatch assay.

The diagnosis of AMR was established based on histological and immunohistochemical evaluation of endomyocardial biopsy specimens and classified according to the 2013 ISHLT criteria. Hemodynamically compromising rejection (HC-R) requiring transfusion-based hemocorrection combined with desensitization therapy was defined as either morphologically confirmed severe AMR (AMR III) or moderate AMR (AMR II) accompanied by evidence of graft dysfunction on echocardiography and/or the presence of donor-specific antibodies in the recipient's circulation.

Statistical analysis was performed using IBM SPSS Statistics version 19.0 and STATISTICA version 10.0 (StatSoft Inc., USA). Both parametric and nonparametric

methods were applied to analyze the main patient characteristics. A p -value <0.05 was considered indicative of statistical significance. To identify variables distinguishing the study groups, stepwise discriminant function analysis was employed.

Study Design

Patients were stratified into three groups: Group 1 (recipients without evidence of AMR [control group]); Group 2 (recipients with isolated histological and immunohistochemical features of AMR [AMR II]; Group 3 (recipients with HC-AMR, defined as AMR III or AMR II in combination with DSA and/or echocardiographic evidence of graft dysfunction).

Baseline risk factors for AMR were assessed in patients with and without AMR. Subsequently, discriminant function analysis was performed to identify predictors of HC-R.

RESULTS

A total of 162 patients were included in the study, of whom 71% ($n = 115$) were male. The predominant underlying conditions leading to end-stage chronic heart failure were ischemic cardiomyopathy (50.8%) and dilated cardiomyopathy (40.1%). Other etiologies – including restrictive cardiomyopathy, congenital or acquired heart defects, and storage diseases – accounted for 9.1% of cases.

Prior to HT, 3% of patients with a high degree of pre-existing sensitization underwent a desensitization protocol consisting of transfusion-based hemocorrection (plasma exchange or cascade plasmapheresis) in combination with intravenous human immunoglobulin.

During the first postoperative year, morphological signs of AMR were identified in 36.2% ($n = 59$) of recipients. However, HC-R (Group 3), characterized by graft dysfunction and/or the presence of DSA and requiring intensive therapy, was observed in only 9.9% ($n = 16$) of cases. In 26.5% of recipients ($n = 43$; Group 2), isolated histological findings of AMR (AMR II) were not associated with DSA and did not result in impaired systolic function of the left or right ventricles or clinically significant diastolic dysfunction.

Among the 103 patients in the control group, no AMR episodes were recorded during the first year following transplantation.

No statistically significant differences were observed among Groups 1, 2, and 3 with respect to key clinical, laboratory, and diagnostic parameters, including the severity of chronic heart failure. A trend toward younger age was noted in Group 3 compared with the other groups (median 44 years vs. 52 and 53 years, respectively); however, this difference did not reach statistical significance ($p > 0.05$). The baseline characteristics of the recipients are summarized in Table 1.

Table 1

Recipient's characteristics

Indicator		Group 2 (AMR II according to morphological examination) N = 43	Group 3 (AMR II and III) N = 16	Group 1 (no rejection) N = 103	P
Age, years, Me [LQ; UQ]		52 [41; 59]	44 [30; 54]	53 [41; 57]	NS
Male sex, n (%)		29 (67%)	8 (50%)	78 (76%)	NS
BMI, kg/m ² , Me [LQ; UQ]		24 [22; 27]	22 [19; 24]	24 [21; 27]	NS
Diagnosis, n (%)	IHD	19 (44%)	3 (19%)	43 (42%)	NS
	DCM	11 (26%)	4 (25%)	25 (24%)	NS
	Other	13 (30%)	9 (6%)	35 (34%)	NS
UNOS status 1		8 (18.6%)	6 (37%)	10 (9.7%)	NS
LVEF, Simpson's method (%), Me [LQ; UQ]		23 [18; 25]	23 [14; 27]	22 [17; 27]	NS
LVEDV, mL, Me [LQ; UQ]		251 [193; 330]	200 [149; 325]	242 [207; 313]	NS
LVESV, mL, Me [LQ; UQ]		181 [143; 255]	174 [117; 258]	182 [148; 257]	NS
PVR, Wood units, Me [LQ; UQ]		2.8 [2; 3.7]	3.1 [2.5; 3.6]	2.6 [1.9; 3.4]	NS
NT-proBNP, pg/mL, Me [LQ; UQ]		3964 [2132; 6260]	3142 [1574; 7381]	3792 [2172; 5714]	NS

Abbreviations and group definitions: Group 1, recipients without antibody-mediated rejection (AMR; control group); Group 2, recipients with isolated histological and immunohistochemical evidence of AMR (AMR II); Group 3, recipients with hemodynamically compromising AMR (AMR III, or AMR II with donor-specific antibodies and/or echocardiographic evidence of graft dysfunction). BMI, body mass index; PVR, pulmonary vascular resistance; UNOS, United Network for Organ Sharing urgency status classification; NT-proBNP, N-terminal pro-brain natriuretic peptide; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; ME, median value; LQ/UQ, lower (25th) and upper (75th) percentiles; n, number of observations; NS, no statistically significant difference ($p > 0.05$).

Similarly, when comparing patients with and without histological evidence of rejection, no significant differences were identified in established risk factors, including history of pregnancy, prior blood transfusions, and use of mechanical circulatory support before HT (Table 2).

Analysis of the immunological profiles of patients across the comparison groups revealed a general trend toward higher anti-HLA antibody (AT) levels in recipients with AMR, both overall and across specific HLA classes. Notably, sensitization with AT levels up to 5000 MFI against HLA classes I and II was observed in patients with AMR even prior to transplantation ($p < 0.05$), whereas such sensitization was uncommon in the control group (Fig. 1).

Peak AT levels in the AMR group were detected within the first 6 months post-transplant, with levels exceeding 5000 MFI for HLA class I. In contrast, patients without rejection generally lacked significant sensitization ($p = 0.012$) (Fig. 2).

During the 6–12-month postoperative period, patients with AMR exhibited total AT levels, *de novo* AT, and sensitization to HLA class I that were more than twice those observed in the non-rejection group ($p < 0.05$) (Figs. 3 and 4).

Patients with AMR, like those without rejection, generally received induction therapy. However, rabbit anti-thymocyte immunoglobulin was used as the primary induction agent four times less frequently in patients

Table 2

Risk factors for antibody-mediated myocardial rejection

Indicator	AMR N = 59	No AMR N = 103	P
Pregnancy	18 (31%)	19 (18%)	NS
Blood transfusions prior to HT	20 (34%)	30 (29%)	NS
Mechanical circulatory support prior to HT	11 (19%)	10 (9.7%)	NS
Pre-transplant sensitization	15 (25%)	23 (22%)	NS
Induction immunosuppressive therapy present	46 (81%)	84 (82%)	NS
Rabbit antithymocyte globulin administered after HT	2 (3.4%)	14 (14%)	0.036

Abbreviation: AMR, antibody-mediated myocardial rejection; HT, heart transplantation; NS, no statistically significant difference.

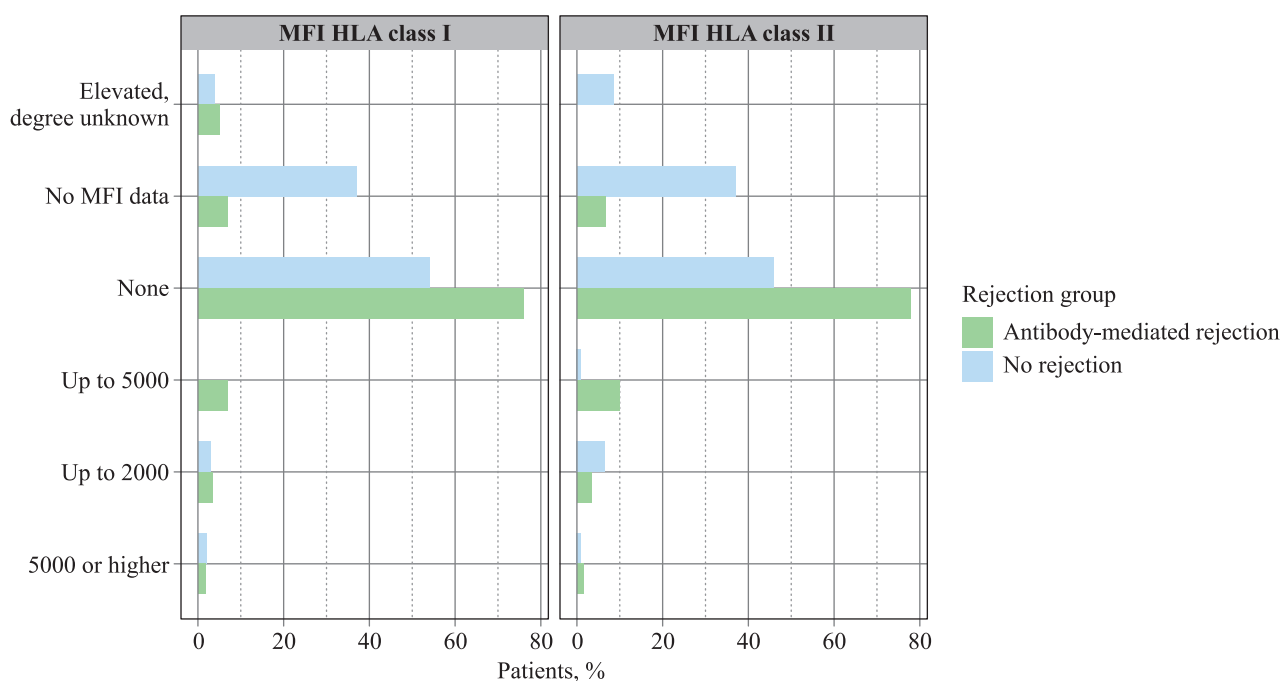


Fig. 1. Mean fluorescence intensity of antibodies to HLA class I and II before heart transplantation in patients with antibody-mediated rejection

with AMR compared to those without rejection. Specifically, only 3.4% of patients in the AMR group received this therapy, versus 14% in the non-rejection group. This difference was statistically significant ($p = 0.036$; $\chi^2 = 4.387$), reflecting a variation in treatment approaches between the groups (Table 2).

Tacrolimus trough levels during the first two weeks after transplantation were lower in patients who deve-

loped AMR compared with those who did not (median 11.4 [8.2–13.6] vs. 12.4 [10.1–15.0] ng/mL, $p = 0.002$). Similarly, immunosuppressant dosing during the first month post-transplant differed significantly between groups ($p < 0.001$), with patients experiencing AMR receiving lower median doses (1000 mg) compared to patients without rejection (1500 mg) (Fig. 5).

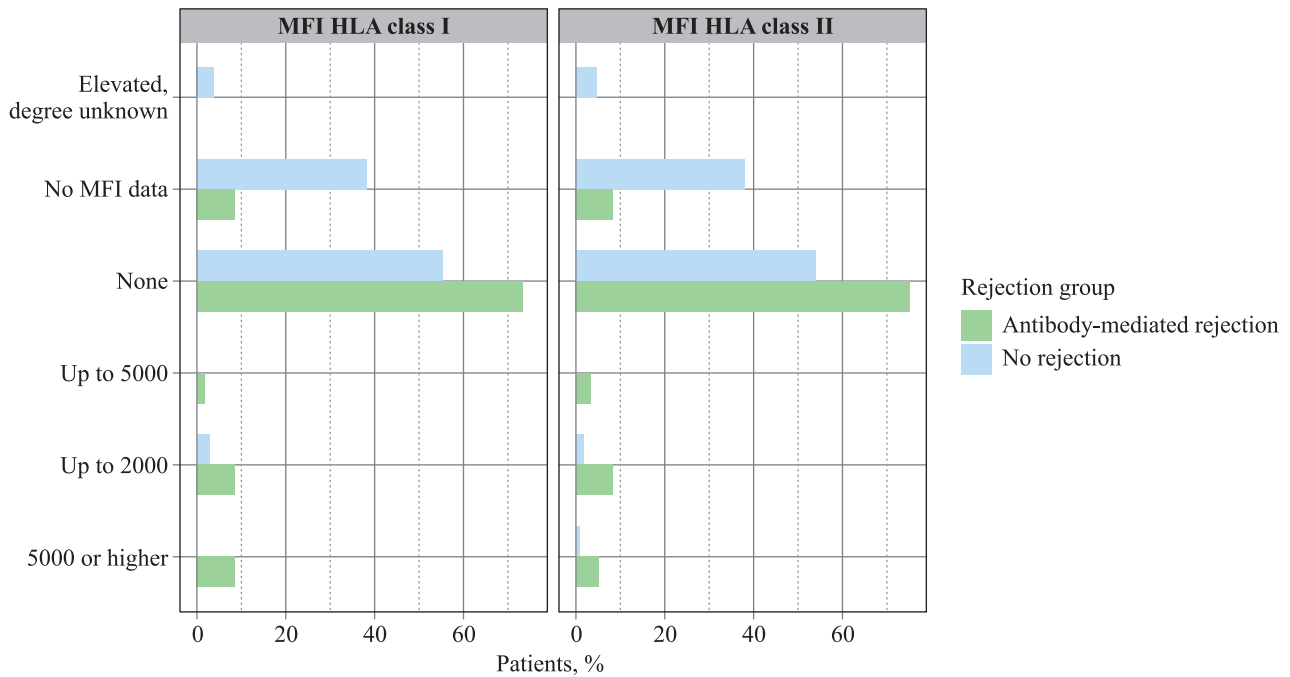


Fig. 2. Mean fluorescence intensity of antibodies to HLA class I and II during the first 6 months after heart transplantation in patients with antibody-mediated rejection

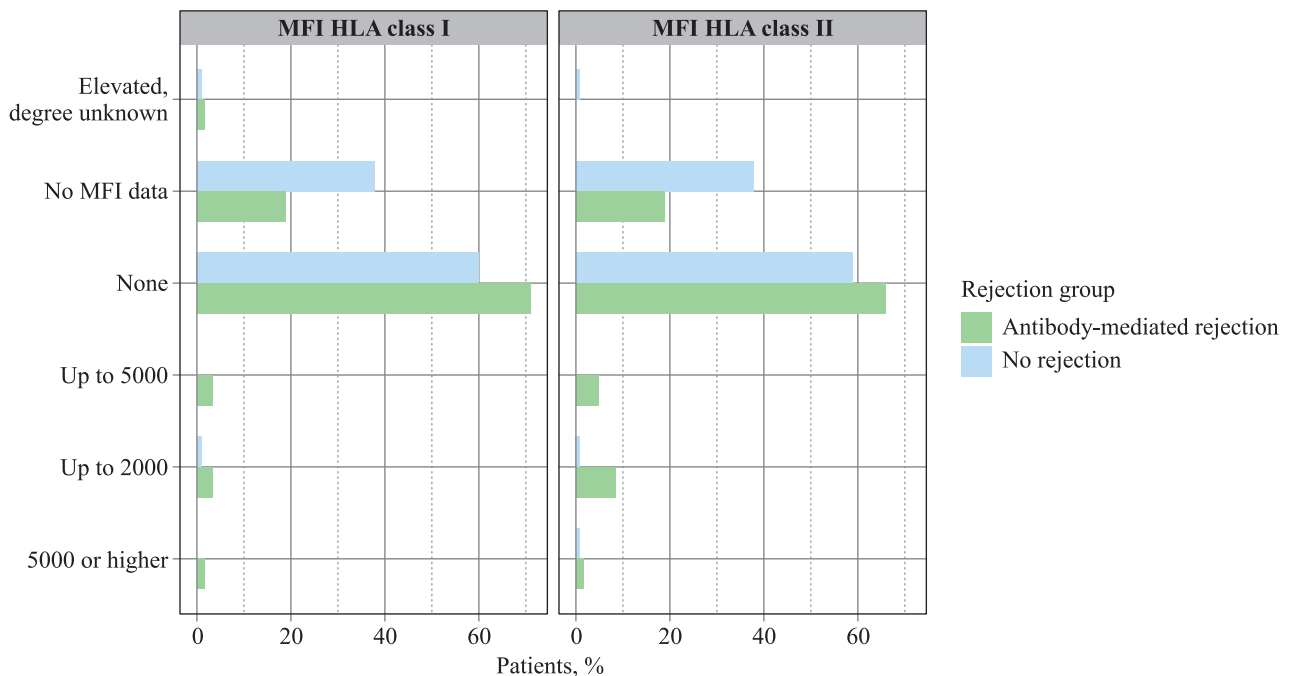


Fig. 3. Mean fluorescence intensity of antibodies to HLA class I and II at 6–12 months after heart transplantation in patients with antibody-mediated rejection

A stepwise discriminant analysis was conducted using a general model incorporating 15 variables. The final model retained 9 features, enabling statistically significant discrimination between patients with HC-R requiring treatment and those without rejection or with subclinical rejection, $F(18, 222) = 12,463$, $p < 0.00001$ (Table 3, Fig. 6).

During the construction of the discriminant function (DF), no misclassifications requiring treatment were observed in the HC-R patient group. Among patients without rejection, 9 misclassifications were identified out of 89 individuals, and in the group with subclinical rejection not requiring treatment, 18 misclassifications occurred among 30 patients. Analysis of these misclassifications revealed no abnormal variables necessitating

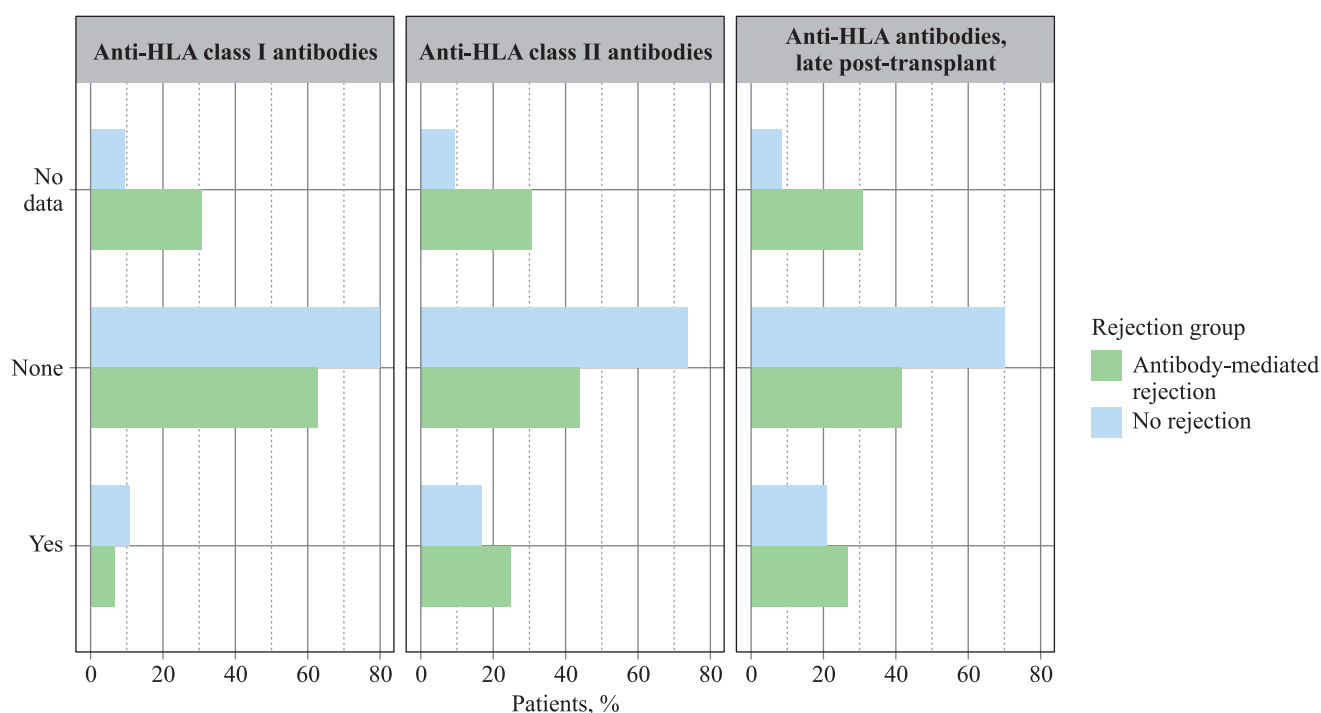


Fig. 4. Levels of pre-existing anti-HLA antibodies and *de novo* anti-HLA antibodies at 6–12 months after heart transplantation in patients with antibody-mediated rejection

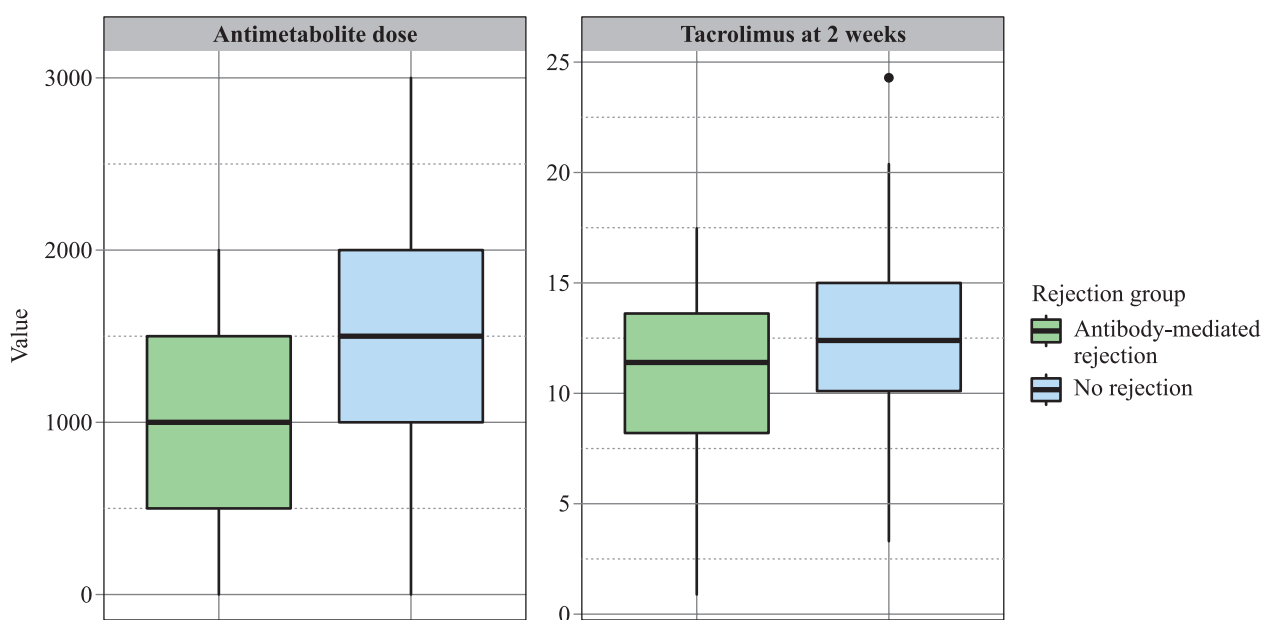


Fig. 5. Antimetabolite dosing and tacrolimus trough level 2 weeks after heart transplantation in rejection and non-rejection groups

Table 3

Discriminant analysis parameters for nine significant indicators
(Wilks' lambda = 0.24739; approx. $F(18, 222) = 12.463$; $p < 0.00001$)

Indicator	Wilks' Lambda	Partial Lambda	F-remove (2,111)	p-value	Toler.	1-Toler. (R-Sqr.)
Post-transplant desensitization therapy	0.630422	0.392419	85.93045	0.000000	0.803934	0.196066
Antimetabolite dose	0.273039	0.906059	5.75427	0.004190	0.760040	0.239960
Tacrolimus trough level at 2 weeks after HT	0.263866	0.937557	3.69638	0.027918	0.936610	0.063390
Patient compliance	0.259201	0.954430	2.64989	0.075127	0.766998	0.233002
Early antibodies after HT	0.256655	0.963899	2.07866	0.129941	0.797085	0.202915
Body mass index	0.262669	0.941829	3.42792	0.035929	0.152929	0.847071
Body weight	0.259829	0.952126	2.79062	0.065695	0.130678	0.869322
Sex	0.257379	0.961188	2.24107	0.111135	0.561731	0.438269
ABO blood group mismatch	0.252805	0.978581	1.21480	0.300684	0.945481	0.054519

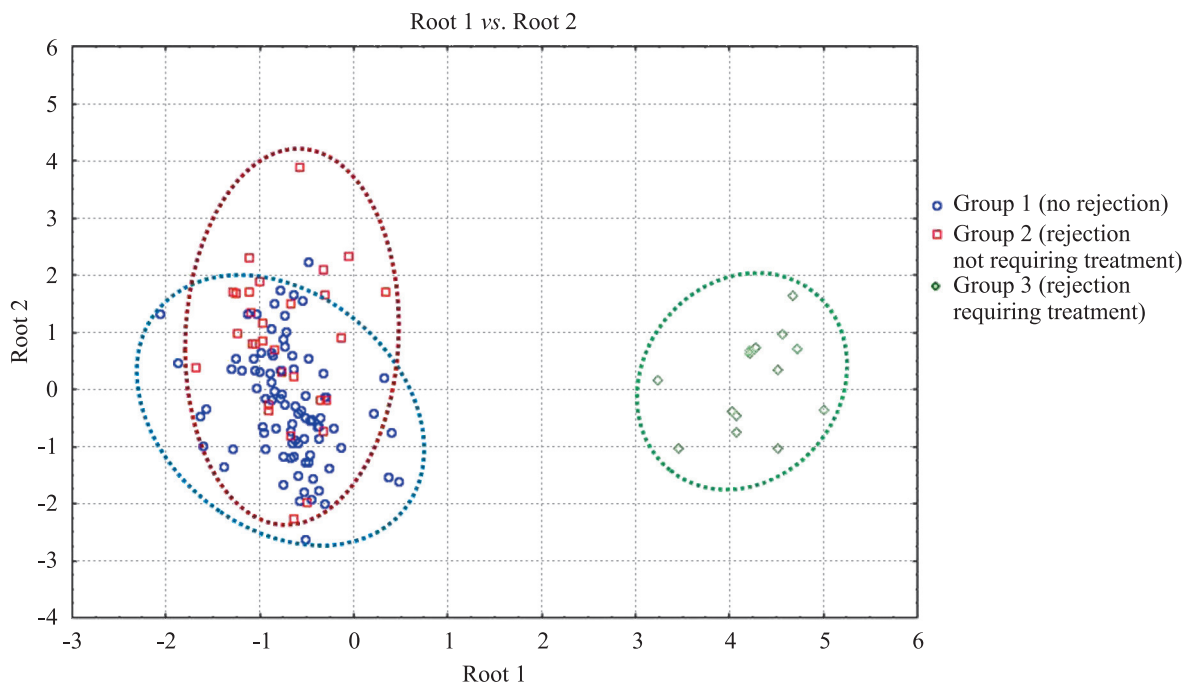


Fig. 6. Graphical representation of the discriminant model

exclusion. Importantly, the DF correctly identified 100% of recipients with rejection requiring treatment.

DISCUSSION

AMR is among the most severe complications following HT, with a substantial impact on both survival and long-term prognosis [10]. It remains a leading cause not only of graft dysfunction but also of cardiac allograft vasculopathy and sudden death [6, 11]. The incidence of AMR) within the first 12 months post-transplant may exceed 30%, a finding corroborated by our data: 36.4% of recipients exhibited AMR according to the 2013 ISHLT criteria, with 9.9% demonstrating HC-R requiring intervention.

Despite advances in noninvasive diagnostic approaches for myocardial transplant rejection, endomyocardial

biopsy continues to be the established “gold standard” for definitive diagnosis [12, 13].

Our results further underscore the clinical heterogeneity of AMR: among the 59 patients with morphological evidence of AMR (AMR II), only 16 required therapeutic intervention. This observation aligns with current understanding that asymptomatic or subclinical AMR is common and necessitates comprehensive evaluation integrating histology, echocardiographic assessment, and serological data [13].

An interesting finding of our study was the difference in the use of induction therapy: only 3.4% of patients who developed AMR received rabbit anti-thymocyte immunoglobulin, compared with 14% in the non-rejection group ($\chi^2 = 4.387$; $p = 0.036$). This observation may suggest a potential protective effect of more intensive in-

duction therapy in sensitized recipients. Previous studies have similarly demonstrated the efficacy of rabbit anti-thymocyte immunoglobulin in preventing AMR among patients at high immunological risk.

In our analysis of risk factors, particular attention was given to the role of DSA. The presence of class I DSA, or both class I and class II DSA, was associated with the highest risk of hemodynamically compromising AMR. These findings are consistent with international guidelines, which highlight the importance of monitoring DSA not only pre-transplant but also throughout the first year following solid organ transplantation.

Our study also revealed that patients who developed AMR were more likely to test positive for anti-mitochondrial antibodies (AMA) prior to HT, with titers ≤ 5000 MFI, whereas sensitization was almost absent in the control group. AMA levels peaked within the first 6 months post-transplant in patients with AMR and remained approximately twice as high as in the control group during the latter half of the year. These findings highlight the importance of prospective immunological screening both before and after HT.

A key outcome of this study was the application of discriminant analysis using 15 clinical and immunological variables to predict HC-R. The final model, which incorporated 9 significant predictors, demonstrated high accuracy in distinguishing patients requiring treatment from those without rejection or with subclinical AMR ($F(18, 222) = 12.463$, $p < 0.00001$). These results underscore the potential utility of discriminant analysis for clinical risk stratification of hemodynamically compromising AMR and for guiding individualized monitoring and therapeutic strategies.

This study identified several key factors associated with AMR, including high levels of pre-existing sensitization, suboptimal induction therapy, inadequate immunosuppressive control, and the presence of *de novo* anti-HLA antibodies. These parameters may serve as the foundation for creating an immunological risk score and developing personalized monitoring strategies.

The future of transplant medicine lies in the integration of molecular diagnostics, predictive analytics, and individualized treatment approaches. Identification of risk factors for HC-AMR and the development of stratification models represent important steps toward improving post-transplant survival and reducing the incidence of complications such as graft dysfunction and cardiac allograft vasculopathy.

CONCLUSION

High levels of HLA sensitization prior to transplantation, suboptimal immunosuppressive control during the early postoperative period, and the development of *de novo* anti-HLA antibodies within the first year af-

ter transplantation are associated with an increased risk of AMR. The discriminant model developed using key clinical and immunological parameters demonstrates potential for risk stratification and personalized patient monitoring. These findings highlight the importance of close immunological surveillance and optimization of immunosuppressive therapy in high-risk recipients. Future studies should aim to validate this model, assess its impact on therapeutic decision-making, and investigate the long-term effects of individualized immunomonitoring on transplant outcomes.

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The authors declare no conflict of interest.

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ASSOCIATION BETWEEN CIRCULATING ANTI-HLA IgG ANTIBODIES AND ADVERSE EVENTS IN HEART TRANSPLANT RECIPIENTS

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Objective: to study the relationship between circulating anti-HLA antibodies and the incidence of adverse events (death and retransplantation) and to evaluate the effectiveness of targeted therapies for graft dysfunction. **Materials and methods.** A retrospective study was conducted among heart transplant recipients hospitalized with signs of circulatory failure (graft dysfunction). All patients underwent coronary angiography, endomyocardial biopsy, and serological testing for anti-HLA IgG antibodies at baseline and, when applicable, after treatment. All heart transplant recipients underwent endomyocardial biopsy with examination of six tissue samples using histological and immunohistochemical techniques, and coronary angiography. Anti-HLA IgG levels in serum were measured using a Luminex device. Follow-up anti-HLA IgG testing was performed only in patients with initially detectable antibodies and after administration of specific therapies for presumed graft rejection. **Results.** The study included 362 heart transplant recipients observed at Shumakov National Medical Research Center of Transplantology and Artificial Organs between January 2018 and November 2024. Participants were aged 18–72 years (mean 48.1 ± 1.3 years) with a mean post-transplant follow-up of 1343.6 ± 125.1 days (95% CI 1218.5–1408.6), comprising 69 females and 293 males. Anti-HLA IgG antibodies were detected in 111 recipients (30.7%). Univariate analysis identified significant associations between adverse events and: repeat heart transplantation ($p = 0.005$), perioperative use of mechanical circulatory support ($p < 0.003$), age under 46 years at hospitalization ($p = 0.023$), and anti-HLA II maxMFI above 5000 ($p = 0.042$). Regression analysis adjusted for anti-HLA II levels showed that only initially elevated anti-HLA II maxMFI levels (>5000) and the persistence of any anti-HLA II levels after etiotropic treatment were associated with the risk of adverse events. **Conclusion.** In heart recipients hospitalized with signs of circulatory failure due to graft dysfunction, the presence of anti-HLA II maxMFI titers above 5000 at baseline, as well as residual anti-HLA II titers after etiotropic treatment for antibody-mediated rejection, are independent predictors of adverse events, including retransplantation and death.

Keywords: heart transplantation, anti-HLA, antibody-mediated rejection.

INTRODUCTION

Heart transplantation (HT) remains the gold standard treatment for end-stage chronic heart failure (CHF) when optimal medical therapy is ineffective and/or radical surgical intervention on the native heart is not feasible. Despite significant advances in diagnostic techniques and therapeutic strategies for heart transplant recipients, graft dysfunction remains a major challenge in modern transplantology. It is a leading cause of recurrent hospitalizations and is associated with an increased risk of adverse clinical outcomes [1].

The human leukocyte antigen (HLA) complex was first identified by French immunologist Jean Dausset in the 1950s as a group of proteins expressed on white blood cells. It was later discovered that antibodies to

HLA (anti-HLA antibodies) play a central role in graft rejection in kidney transplant recipients [2]. In the 1960s, Paul Terasaki and David McClelland developed the complement-dependent lymphocyte cytotoxicity (CDC) assay. This technique enabled the detection of anti-HLA antibodies [3]. Subsequently, various other techniques for detecting anti-HLA have been developed [4].

The HLA system comprises three classes of molecules: class I (loci A, B, and C), class II (loci DR, DQ, and DP), and class III, which includes genes not directly involved in antigen presentation. All are encoded within different regions of chromosome 6. HLA expression on the cell surface enables immune recognition of “self” and “non-self” cells. Class I HLA molecules are expressed on all nucleated cells, whereas the expression of class II HLA molecules is restricted to B cells, activated T cells,

and antigen-presenting cells. Expression of HLA class I and II on allograft vascular endothelium may explain the rejection that occurs in the presence of donor-specific antibodies (DSAs) [5].

Antibody-mediated allograft injury occurs primarily through activation of the complement system. Binding of antibodies to HLA antigens triggers C1q activation and initiates the complement cascade, ultimately leading to tissue damage. In addition, complement-independent mechanisms also contribute to graft injury. DSAs can recruit inflammatory cells via Fc receptor interactions, promoting the release of pro-inflammatory mediators. Antibodies directed against major histocompatibility complex antigens can therefore induce both direct endothelial injury and indirect damage mediated by complement fixation or attraction of inflammatory cells to Fc receptors [6]. As a result, cellular inflammation, thrombosis, hemorrhage, and lysis cause cardiac allograft dysfunction [7].

Despite advances in therapeutic approaches, heart transplant dysfunction and its relationship with circulating anti-HLA antibodies remain an area of active investigation. The aim of this study was to assess the association between serum anti-HLA antibodies and the incidence of adverse outcomes – namely death and retransplantation – and to evaluate the effectiveness of targeted treatment strategies for transplant-related cardiac dysfunction.

MATERIALS AND METHODS

The results obtained in this study were based on the examination of heart recipients who underwent orthotopic heart transplantation (OHT) at Shumakov National Medical Research Center of Transplantology and Artificial Organs. All patients underwent routine endomyocardial biopsy with analysis of six myocardial samples using histological and immunohistochemical methods, coronary angiography, and assessment of serum anti-HLA IgG antibodies using Luminex technology. Repeat testing for anti-HLA IgG antibodies was performed only in patients with initially detected antibodies after completion of targeted anti-rejection therapy.

In the perioperative and early postoperative periods, all recipients received induction therapy with basiliximab followed by triple-drug maintenance immunosuppression consisting of tacrolimus, mycophenolate mofetil/mycophenolic acid, and methylprednisolone. Six months after HT, patients were transitioned to dual-drug maintenance immunosuppression with tacrolimus and mycophenolate mofetil or mycophenolic acid.

Upon the development of heart transplant dysfunction, patients were routinely admitted for inpatient care at Shumakov National Medical Research Center of Transplantology and Artificial Organs. All heart transplant recipients underwent a comprehensive diagnostic evaluation, including endomyocardial biopsy (with analysis of six samples using histological and immunohistochemical

methods), coronary angiography, and assessment of serum anti-HLA IgG levels. Antibody levels were quantified as mean fluorescence intensity (maxMFI) using multiplex analysis on the Luminex® platform.

Repeat measurements of anti-HLA IgG were performed only in patients in whom antibodies were initially detected, following targeted treatment for transplant rejection. Treatment strategies were implemented in accordance with the 2023 clinical guidelines and included pulse corticosteroid (methylprednisolone) therapy, intravenous immunoglobulin, therapeutic plasma exchange, and rituximab infusions [8].

In cases of cardiac allograft vasculopathy (CAV), percutaneous coronary intervention was performed, with or without stent implantation in the affected coronary arteries.

The risk of adverse events was assessed using multiple study factors, such as age, sex, pre-transplant diagnosis, the need for mechanical circulatory support (MCS), and a history of preoperative heart transplant rejection in re-transplant patients. MCS modalities included biventricular assist devices combined with a membrane oxygenator (ECMO) and long-term MCS systems, such as AVK-N and HeartMate 3.

After data collection was completed, all patient data were consolidated into a single electronic database. Statistical analysis was performed using SPSS version 26 (IBM SPSS Inc., USA). Parametric variables are presented as the mean $\pm$ standard deviation ($M \pm SD$), while nonparametric variables are reported as the median (Med) and interquartile range (IQR; Q1–Q3). Comparisons between dependent samples were performed using the Wilcoxon signed-rank test, and comparisons between independent samples were conducted using the Mann–Whitney U test.

The prognostic value of anti-HLA antibody levels for the development of adverse events was evaluated using univariate logistic regression analysis. Accuracy of prediction was assessed using the Hosmer–Lemeshow test, and model performance was quantified using the Nagelkerke coefficient of determination. The statistical significance of the model was assessed using Wald's chi-square test. For all criteria, the critical level of significance was set at 5%, i.e., the null hypothesis was rejected at $p < 0.05$.

RESULTS

This study included blood samples from 362 HT recipients who underwent OHT at Shumakov National Medical Research Center of Transplantology and Artificial Organs between 2010 and 2024. The mean follow-up duration was 1343.6 ± 125.1 days (95% CI: 1218.5–1408.6). The mean age of the recipients was 48.1 ± 1.3 years (range: 18–72 years). Of the total cohort, 69 patients were female (19.1%) and 293 were male (80.9%).

Indications for HT included end-stage CHF due to ischemic cardiomyopathy (ICM) in 116 patients (32.0%), dilated cardiomyopathy (DCM) in 211 patients (58.3%), restrictive cardiomyopathy (RCM) in 5 patients (1.4%), and hypertrophic cardiomyopathy (HCM) in 10 patients (2.8%). In addition, heart retransplantation due to post-transplant graft dysfunction with manifestations of circulatory failure was performed in 15 cases (4.1%). Other indications included peripartum cardiomyopathy (3, 0.8%) and arrhythmogenic right ventricular cardiomyopathy (2, 0.6%). MCS as a bridge to HT was required in 80 patients (22.1%).

Episodes of HT rejection, both cellular and antibody-mediated forms, were evaluated prior to laboratory assessment of anti-HLA antibody levels. A history of rejection episodes was identified in 65 recipients (17.9%), whereas 297 patients (82.1%) had no documented rejection.

Coronary artery vasculopathy of the transplanted heart (CAVTH), as determined by coronary angiography, was identified in 84 heart recipients (23.2%). Histological examination of endomyocardial biopsies revealed acute cellular rejection in 48 patients, with 42 (11.6%) exhibiting R1G-grade, 4 (1.1%) R2G-grade, and 2 (0.6%) R3G-grade rejection. Immunohistochemical examination identified acute antibody-mediated rejection (AMR) in 67 recipients: AMR-1 (I+) in 44 patients (12.2%), AMR-1 (H+) in 15 patients (4.1%), and AMR-2 in 8 patients (2.2%).

The general characteristics of the study population are summarized in Table 1.

The study found that patient sex and a history of rejection episodes prior to OHT in patients with HT dysfunction requiring heart retransplantation were not associated with a worse post-transplant prognosis ($p > 0.05$). In contrast, the use of MCS prior to OHT was associated with a significantly increased risk of adverse postoperative events ($p < 0.003$).

The analysis revealed that recipients with end-stage heart failure (HF) secondary to heart transplant dysfunction had a significantly higher risk of adverse events compared with patients with other preoperative heart pathologies ($p = 0.005$).

Survival analysis revealed that recipients aged 46–72 years had a significantly higher survival rate than younger recipients aged 18–45 years ($p = 0.023$).

Elevated serum levels of anti-HLA IgG antibodies were found to have a statistically significant negative impact on post-transplant prognosis ($p = 0.004$). This part of the study considered anti-HLA antibodies of all classes (both class I and class II) without separately assessing the prevalence of antibodies against specific loci (A, B, C for Class I; DR, DQ, DP for Class II).

A summary of the associations between adverse event risk and the evaluated clinical factors is presented in Fig. 1.

Table 1

Characteristics of the study population (n = 362)

Characteristic		Value
Sex:	Male, n (%)	293 (80.9%)
	Female, n (%)	69 (19.1%)
Age (years) mean $\pm$ SD CI		48.1 $\pm$ 1.3 (CI 95% 46.7–49.4)
Initial diagnosis, n (%):		
ICM		116 (32%)
DCM		211 (58.3%)
RCM		5 (1.4%)
HCM		10 (2.8%)
CTD		15 (4.1%)
Peripartum CM		3 (0.8%)
Arrhythmogenic right ventricular CM		2 (0.6%)
Use of MCS, n (%)		80 (22.1%)
Episodes of transplant rejection prior to study, n (%)		65 (17.9%)
CAV, n (%)		84 (23.2%)
Acute cellular rejection, n (%):		
R0G		314 (86.7%)
R1G		42 (11.6%)
R2G		4 (1.1%)
R3G		2 (0.6%)
Acute antibody-mediated rejection, n (%):		
pAMR-0		295 (81.5%)
pAMR-1 (I+)		44 (12.2%)
pAMR-1 (H+)		15 (4.1%)
pAMR-2		8 (2.2%)
Presence of anti-HLA, n (%)		111 (30.7%)

Abbreviations: SD, standard deviation; CI, confidence interval; ICM, ischemic cardiomyopathy; DCM, dilated cardiomyopathy; RCM, restrictive cardiomyopathy; HCM, hypertrophic cardiomyopathy; CTD, connective tissue dysplasia; CM, cardiomyopathy; MCS, mechanical circulatory support; CAV, cardiac allograft vasculopathy.

According to the study results, transplant CAV did not demonstrate a statistically significant link to the risk of adverse events ($p > 0.05$). In contrast, ACR (specifically R1G-grade rejection) was associated with a poorer prognosis ($p = 0.012$).

Evaluation of AMR did not reveal a statistically significant impact on prognosis for HT recipients at the time of analysis; however, a trend toward significance was observed ($p = 0.073$).

The prognostic effects of different types of ACR and AMR in HT recipients are illustrated in Fig. 2.

After testing serum anti-HLA antibody levels, all HT recipients were stratified into two subgroups: the first consisted of patients with detectable anti-HLA IgG antibodies ($n = 111$, 30.7%), while the second subgroup included recipients without detectable DSAs ($n = 251$, 69.3%).

A comparative analysis between the two subgroups is presented in Table 2.

The two study groups did not differ significantly with respect to demographic characteristics (age and sex) or the incidence of post-transplant complications (acute HT rejection and CAV ($p > 0.05$). However, the use of MCS methods prior to transplantation was significantly more frequent among recipients with detectable serum anti-HLA antibodies, whereas a higher proportion of patients without anti-HLA antibodies had end-stage HF due to dilated cardiomyopathy in the pre-transplant period ($p = 0.02$).

The presence of anti-HLA class I antibodies was not associated with the risk of post-transplant complications, likely reflecting their lower prevalence in the study co-

hort. In addition, circulating anti-HLA class II antibodies did not show a statistically significant association with the development of ACR or CAV ($p > 0.05$). In contrast, elevated anti-HLA class II antibody levels were significantly associated with the occurrence of AMR after HT, with AMR patients exhibiting higher maxMFI values compared to non-AMR patients ($p = 0.004$) (Fig. 3).

To assess treatment effectiveness, serum anti-HLA class II antibody levels were measured before and after targeted therapy for HT rejection. Changes in anti-HLA class II levels before and after treatment are presented in Fig. 4.

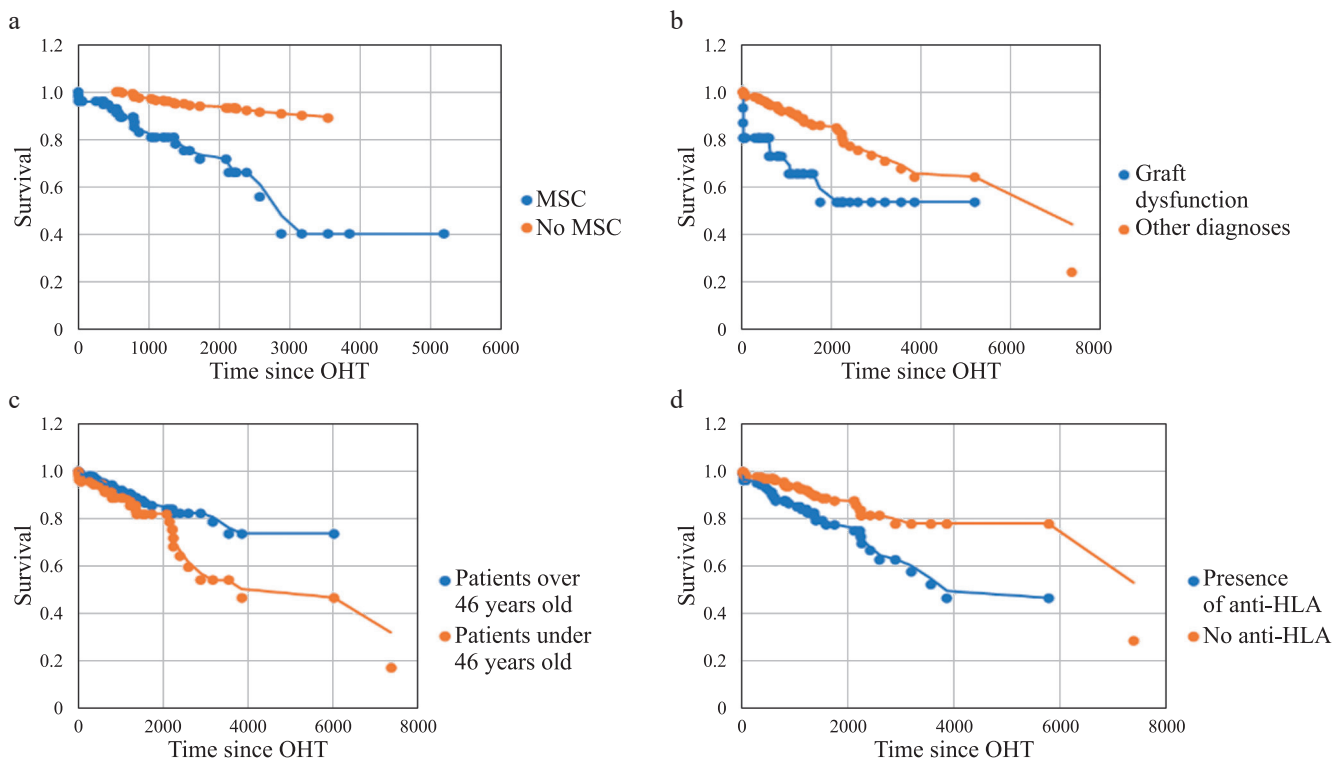


Fig. 1. Comparative analysis of event-free survival depending on MSC (a), graft dysfunction (b), age (c) and presence of anti-HLA antibodies of both classes (d). Note: MCS, perioperative mechanical circulatory support; OHT, orthotopic heart transplantation

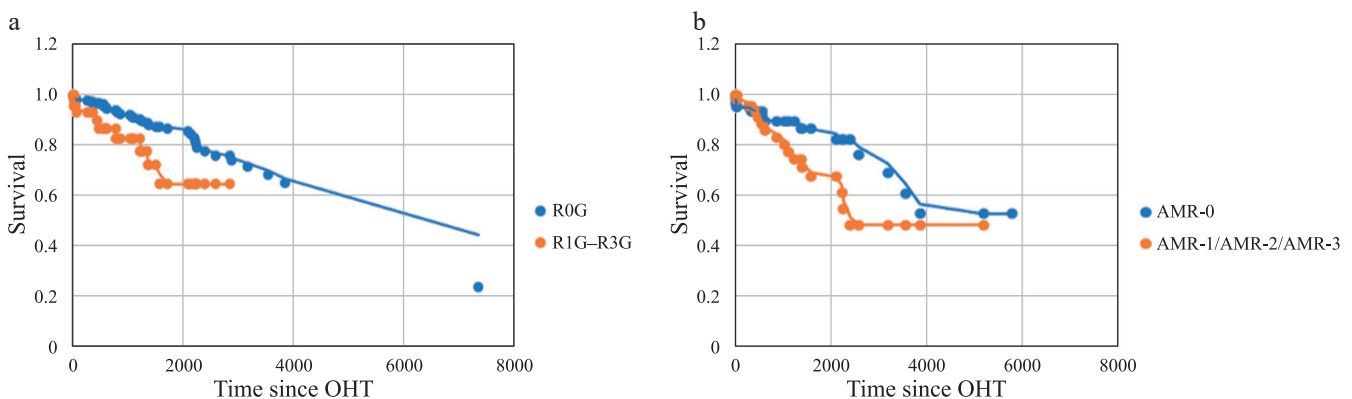


Fig. 2. Comparative analysis of survival without adverse events depending on acute cellular rejection (a) and acute antibody-mediated rejection of heart transplants (b)

Recipients with elevated pre-treatment anti-HLA class II maxMFI levels (>5000) exhibited a significantly poorer prognosis compared with patients whose anti-HLA class II maxMFI <5000 ($p = 0.042$), as illustrated in Fig. 5.

During treatment, patients were categorized into three cohorts: those in whom anti-HLA class II IgG levels be-

came undetectable after treatment (45 patients, 40.5%), those in whom levels decreased but remained detectable (41 patients, 37%), and those in whom the anti-HLA class II IgG levels did not change during treatment (25 patients, 22.5%).

After treatment for HT rejection, serum anti-HLA class II levels were reassessed and correlated with clini-

Table 2

Comparative characteristics of heart recipients with and without anti-HLA antibodies in their blood serum

Characteristic	Anti-HLA positive	Anti-HLA negative	p-value
Number of patients, n	111	251	
Sex (Male), n (%)	78.4%	82.1%	0.25
Age (years) mean $\pm$ SD CI	45.7 $\pm$ 1.3 (CI 95% 47.6–50.6)	49.1 $\pm$ 0.78 (CI 95% 43.1–48.3)	0.12
Cellular rejection, n (%)	27%	8.8%	0.15
Antibody-mediated rejection, n (%)	47.7%	7.7%	0.3
CAV, n (%)	18%	23.1%	0.45
Diagnosis prior to OHT (DCM), n (%)	53.2%	60.3%	0.02
MCS prior to OHT, n (%)	28.8%	19.1%	0.02

Note: SD, standard deviation; CI, confidence interval; CAV, cardiac allograft vasculopathy; OHT, orthotopic heart transplantation; DCM, dilated cardiomyopathy; MCS, mechanical circulatory support.

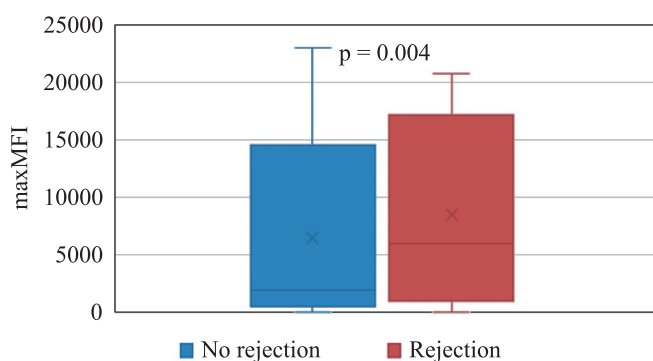


Fig. 3. Comparative analysis of maxMFI of anti-HLA class II in heart recipients with and without antibody-mediated rejection

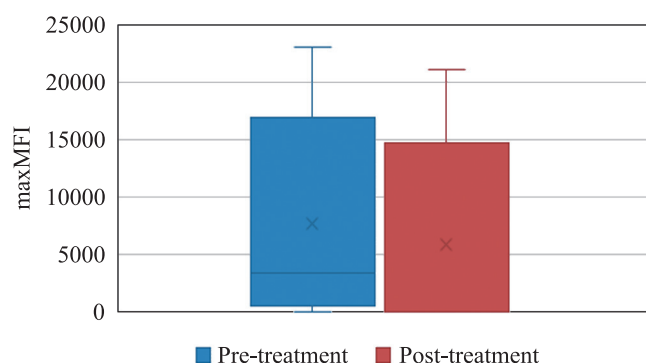


Fig. 4. Dynamics of anti-HLA class II maxMFI levels before and after targeted treatment for acute rejection

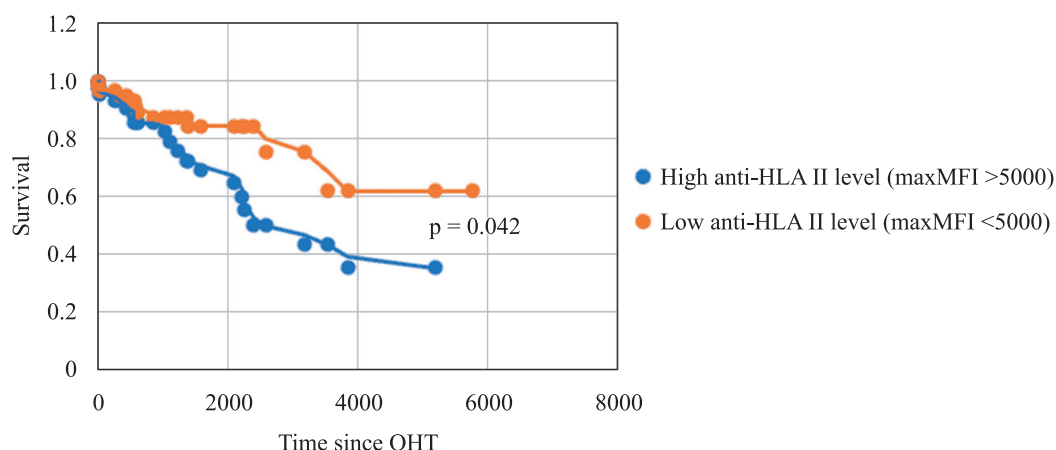


Fig. 5. Risk of adverse events in heart transplant recipients with high and low pre-treatment serum anti-HLA class II IgG levels

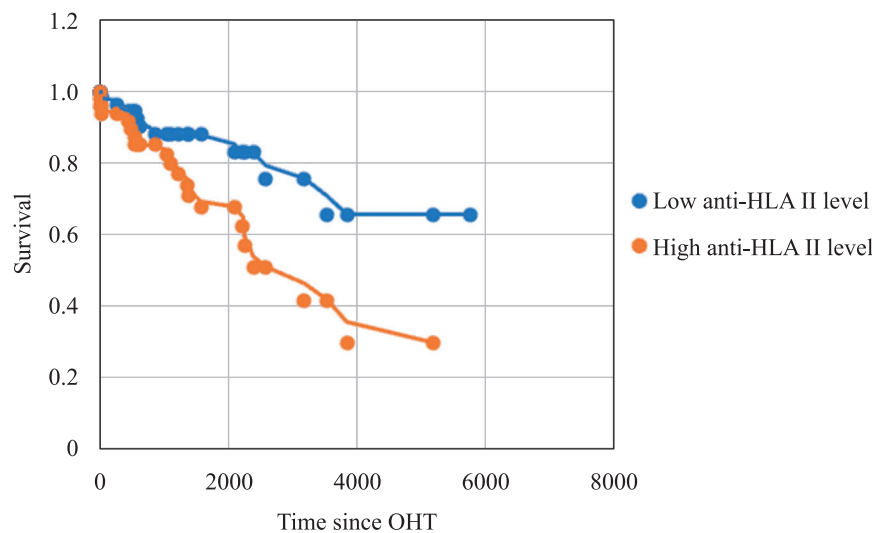


Fig. 6. Comparative analysis of event-free survival depending on post-treatment anti-HLA class II IgG levels

cal outcomes. A favorable dynamic in anti-HLA class II levels was observed, accompanied by a significant reduction in the risk of adverse events ($p = 0.017$), as shown in Fig. 6.

DISCUSSION

The results of this study confirm the significant role of anti-HLA class II antibody levels in the development of AMR and their negative impact on adverse events in heart recipients. Elevated anti-HLA class II levels were strongly associated with AMR ($p = 0.004$), corroborating the findings of Shemakin et al. [9]. Recipients with high pre-treatment anti-HLA class II values ($\text{maxMFI} > 5000$) exhibited a poorer prognosis compared with those with lower levels ($p = 0.042$).

Measurement of anti-HLA antibodies provides a valuable tool for evaluating the effectiveness of therapy for AMR, complementing morphological assessments. In our cohort, treatment for HT rejection resulted in a favorable reduction of anti-HLA class II levels in serum, which was accompanied by a significant decrease in the risk of adverse events ($p = 0.017$).

The study further identified cohorts of patients with complete or partial elimination of anti-HLA class II antibodies following treatment, as well as a cohort in which antibody levels remained unchanged. These findings once again underscore the importance of thorough pre-treatment evaluation of HT recipients and the need for individualized selection of therapy for AMR. Our results are consistent with those of Clerkin et al., who reported that patients with anti-HLA were 151% more likely to experience graft loss – due to death or retransplantation – compared with patients without detectable anti-HLA [9]. Similarly, Smith et al. reported no direct correlation between anti-HLA and CAV in their cohort; however, when examining causes of death, a substantial

proportion of patients with anti-HLA died as a result of CAV or transplant rejection [10].

In our study, patients received targeted therapy for transplant rejection and high immunological risk, consistent with the approaches described by Marco et al. [11], who proposed a diagnostic and treatment algorithm for patients with anti-HLA antibodies, and by Kamath et al. [12]. In addition, we assessed the impact of several factors on long-term outcomes in HT recipients, including the use of MCS, the underlying pre-transplant cardiac pathology leading to end-stage heart failure, and patient age.

Our results showed that prior use of MCS significantly increased the risk of post-transplant adverse events. Moreover, recipients with a pre-transplant diagnosis of “heart transplant dysfunction” as the cause of end-stage HF were more likely to experience postoperative complications. Younger recipients, and those with circulating anti-HLA IgG antibodies have a poorer prognosis compared with patients without such baseline data. The observed effect of MCS on post-transplant outcomes aligns with previously reported data by Chau et al. [13].

Although AMR was not significantly associated with prognosis at the time of analysis, a trend toward significance was noted ($p = 0.073$), highlighting the need to expand the study cohort and extend patient follow-up.

Our findings underscore the importance of measuring anti-HLA antibody levels in patients with HT dysfunction, as these antibodies have a demonstrable impact on the risk of post-transplant complications and negatively affect recipient outcomes. Assessing anti-HLA levels in this patient population allows for individualized selection of optimal therapeutic strategies, which can lead to improved clinical results.

Future studies should incorporate anti-HLA levels into the classification of AMR to guide treatment selection more effectively, in line with recommendations by

Goldberg et al. [15]. Continued research is also needed to refine treatment regimens for transplant dysfunction and to develop measures for its prevention and early detection.

CONCLUSION

The study demonstrates a strong correlation between the presence of circulating anti-HLA antibodies and the development of severe HT dysfunction with an increased risk of adverse events. Early detection of anti-HLA antibodies enables early intervention, helps guide the selection of optimal treatment strategies, and contributes to improved long-term outcomes for heart transplant recipients.

The authors declare no conflict of interest.

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RISK FACTORS FOR ADVERSE OUTCOMES IN PEDIATRIC HEART TRANSPLANTATION

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Heart transplantation (HT) remains the only definitive surgical treatment for end-stage chronic heart failure (CHF). **Objective:** to investigate factors associated with adverse immediate outcomes following HT in children. **Materials and methods.** Between January 1, 2012, and December 31, 2024, 91 HTs were performed in recipients under 18 years of age at Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow. The patients were divided into two groups based on early postoperative outcomes: survivors (n = 79; 86.8%) and non-survivors (n = 12; 13.2%). **Results.** Between 2012 and 2024, a total of 2,190 HTs were performed, including 91 (4.2%) in children. Severe early graft dysfunction occurred in 14 pediatric heart recipients (15.4%), and in-hospital mortality was 13.2%. A high recipient urgency status (UNOS), the use of short-term mechanical circulatory support, and clinical manifestations of multiple organ failure necessitated the expansion of donor heart selection criteria. Receiver operating characteristic (ROC) analysis demonstrated that baseline laboratory parameters influenced transplant outcomes. Serum sodium, lactate, and urea levels, as well as hemoglobin levels, red blood cell and platelet counts, showed statistically significant predictive value, as confirmed by area under the curve (AUC) analysis. Donors in the non-survivor group were significantly older than those in the survivor group. The donor-to-recipient weight ratio was higher among recipients who died in the early post-transplant period. In the non-survivor cohort, significantly higher values were observed for the donor-to-recipient height ratio, donor-to-recipient body surface area ratio, and durations of graft ischemia, anesthesia, surgery, and cardiopulmonary bypass. **Conclusion.** The effectiveness of pediatric HT (hospital survival rate 86.8%) is influenced primarily by recipient urgency status (UNOS). Additional contributing factors include severity of multiple organ dysfunction, donor age, significant donor-recipient anthropometric mismatch, operative time, and intraoperative blood loss.

Keywords: pediatric heart transplantation, heart donor, urgent indications for heart transplantation, risk factors.

INTRODUCTION

Heart transplantation (HT) remains the only definitive surgical treatment for patients with end-stage chronic heart failure (CHF). At present, deceased organ donation from donors younger than 18 years is not practiced in the Russian Federation; therefore, HT using hearts from adult donors is the only available treatment option for pediatric recipients requiring HT.

Numerous single-center and multicenter studies have evaluated the influence of donor- and recipient-related risk factors on HT outcomes. In recipients with a high urgency status, the necessity for urgent transplantation often requires the use of expanded criteria donors, which significantly affects the intraoperative course. Furthermore, transplantation performed in the absence of complete anthropometric compatibility or using organs from older donors may be considered in children requiring

urgent surgery, although such procedures are associated with an increased risk of adverse outcomes [1].

At Shumakov National Medical Research Center of Transplantology and Artificial Organs, experience in pediatric HT accumulated between January 1, 2012, and December 31, 2024, formed the basis of the present study.

Study objective: to investigate factors adversely affecting the immediate outcomes of HT in pediatric recipients receiving donor hearts from individuals older than 18 years.

STUDY POPULATION

Between January 1, 2012, and December 31, 2024, a total of 91 orthotopic HTs were performed in recipients younger than 18 years at Shumakov National Medical Research Center of Transplantology and Artificial Organs. Among the recipients, 36 (39.6%) were girls and

55 (60.4%) were boys, aged 6 to 17 years (mean age: 13.9 ± 2.3 years). The principal causes of end-stage CHF were cardiomyopathy in 81 patients (89.0%) and congenital heart disease (CHD) in 7 patients (7.7%).

The severity of CHF according to the New York Heart Association (NYHA) classification corresponded to functional classes II–IV, with a mean functional class of 3.2 ± 0.5 . In 41 (45.1%) patients, the urgency of HT were classified as United Network for Organ Sharing (UNOS) status 2, 18 patients (19.8%) as status 1B, and 32 patients (35.2%) as status 1A.

Twenty-one (23.1%) HT recipients underwent repeat cardiac surgery. In 36 (39.6%) patients, short-term ($n = 26$ (24.2%)), long-term ($n = 8$ (8.8%)), or a combination (long-term and short-term) ($n = 2$ (2.2%)) of mechanical circulatory support (MCS) was used prior to HT. Pre-HT venoarterial extracorporeal membrane oxygenation (VA-ECMO) lasted for 1–20 ($4.50 [1.75; 6.00]$) days. In 10 (11.0%) recipients, a HeartMate III left ventricular bypass (Abbott Laboratories, USA) was implanted prior to HT, with a duration of 7–1,019 ($609.5 [576.25; 880.0]$) days.

Heart recipients ($n = 91$) were divided into 2 clinical groups:

Group 1 – heart transplants in children from donors older than 18 years with a favorable outcome – surviving recipients ($n = 79$; 86.8%);

Group 2 – heart transplants in children from donors older than 18 years with an unfavorable outcome – deceased recipients ($n = 12$; 13.2%).

Statistical analysis was performed using Statistica 12 and SPSS 26. Quantitative variables are presented as the arithmetic mean and standard deviation, as well as the median and interquartile range. Differences between the two groups were assessed using Student's t-test, as well as the Mann–Whitney U-test in cases of non-normal distribution. A ROC analysis was performed to determine the predictive significance of the quantitative variables. The optimal cutoff value was determined based on the Youden index. A p-value of <0.05 was considered statistically significant.

RESULTS

Between 2012 and 2024, the Shumakov National Medical Research Center of Transplantology and Artificial Organs performed 2,190 HTs, of which 91 (4.2%) were carried out in pediatric recipients. The proportion of pediatric HTs among the total number of HTs performed annually during the study period (2012–2024) ranged from 0.98% in 2013 to 7.2% in 2024 (Fig.).

HT in children ($n = 91$) was performed using donor hearts obtained from individuals with confirmed brain death resulting from either non-traumatic brain injury (BI) in 58 cases (63.7%) or traumatic BI in 33 cases (36.3%). Donor age ranged from 18 to 67 years (mean age: 36.7 ± 11.7 years), while the age difference between donors and recipients ranged from 3 to 58 years (22.6 ± 12.5 years).

The median graft ischemia duration was 176.50 [$137.00; 210.50$] minutes, whereas cold ischemic time was 97.00 [$79.00; 125.50$] minutes. The maximum vasoactive–inotropic score was 11.3 [$7.25; 18.23$]. Median intraoperative blood loss was 800.0 [$500.00; 1400.00$] mL, while postoperative blood loss was 250.0 [$150.00; 375.00$] mL. Postoperative mechanical ventilation lasted for 10.0 [$5.97; 24.25$] hours. Post-transplant MCS was required in 36 recipients (39.7%) for a median duration of 3.0 [$1.48; 4.50$] days.

Severe early graft dysfunction developed in 14 recipients (15.4%). The overall in-hospital mortality rate was 13.2%.

The results of a comparative analysis of pre-transplant recipient characteristics, donor parameters, and intraoperative variables between surviving ($n = 79$; 86.8%) and deceased recipients ($n = 12$; 13.2%) are presented in Table 1.

Deceased patients more often required urgent HT and short-term pre-transplant MCS. In addition, these patients were generally younger and had lower anthropometric characteristics compared with surviving recipients.

Table 2 summarizes the comparative analysis of pre-transplant laboratory parameters in surviving ($n = 79$) and deceased ($n = 12$) recipients under 18 years of age.

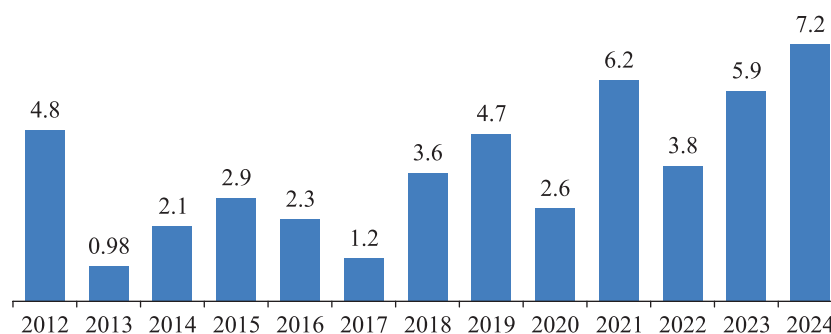


Fig. Percentage of heart transplants (HTs) performed in recipients under 18 years of age relative to the total number of HTs conducted at Shumakov National Medical Research Center of Transplantology and Artificial Organs (October 1, 2012 – December 31, 2024)

Pre-transplant hemoglobin, red blood cell, and platelet levels were significantly lower in the group of deceased recipients. These patients also demonstrated

lower levels of mixed venous oxygen saturation (SvO₂) and serum sodium (Na⁺) ($p < 0.005$). In this same cohort of recipients, significantly higher pre-transplant levels of

Table 1

Pre-transplant clinical characteristics of surviving and non-surviving transplant recipients in the early post-transplant period

Parameters	Heart recipients under 18 years of age		p
	Survivors (n = 79)	Deceased (n = 12)	
Age, years (mean $\pm$ SD)	14.10 $\pm$ 2.13	12.5 $\pm$ 6.9	0.023
Sex, n (%)			
Boys	51/64.6	4/33.3	0.057
Girls	28/35.4	8/66.7	
Weight, kg (Me [Q1; Q3])	50.0 [38.5; 65.0]	31.5 [25.25; 37.75]	<0.0001
Height, cm (Me [Q1; Q3])	163.0 [152.0; 174.0]	145.5 [131.75; 153.0]	<0.0001
BSA, m ² (mean $\pm$ SD)	1.54 $\pm$ 0.31	1.15 $\pm$ 0.27	<0.0001
NYHA FC (Me [Q1; Q3])	3.00 [3.00; 4.00]	4.00 [3.00; 4.00]	0.158
UNOS status (Me [Q1; Q3])			<0.030
1A	23/29.1	9/75.0	
1B	15/19.0	3/25	
2	41/51.9	0/0.0	
Short-term MCS, n/%			<0.003
VA-ECMO	17/21.5	7/66.7	
Other		1/8.3	
Short-term MCS, days (Me [Q1; Q3])	3.60 [1.09; 5.25]	5.00 [3.82; 6.00]	0.239

Note: p – significance level. Abbreviations: SD, standard deviation; Me, median; Q1, first quartile (25th percentile); Q3, third quartile (75th percentile); BSA, body surface area; NYHA FC, New York Heart Association functional classification; MCS, mechanical circulatory support; VA-ECMO, venoarterial extracorporeal membrane oxygenation.

Table 2

Comparative analysis of pre-transplant laboratory tests in surviving (n = 79) and non-surviving (n = 12) heart transplant recipients

Parameters	Heart recipients under 18 years of age		p
	Survivors (n = 79) (Me [Q1; Q3])	Deceased (n = 12) (Me [Q1; Q3])	
Hemoglobin, g/L	125.0 [105.00; 142.00]	96.0 [81.50; 115.0]	0.018
Erythrocytes, $\times 10^9$ /L	4.50 [4.11; 4.98]	3.70 [3.03; 4.23]	0.011
Platelets, $\times 10^9$ /L	210.00 [162.00; 262.00]	122.00 [58.60; 219.00]	0.020
Leukocytes, $\times 10^9$ /L	7.90 [6.65; 8.30]	7.60 [6.00; 7.60]	0.954
Urea, mmol/L	5.50 [4.60; 8.07]	7.9 [6.30; 12.92]	0.014
Creatinine, μ mol/L	63.9 [48.66; 78.25]	48.20 [38.76; 70.75]	0.749
Total bilirubin, μ mol/L	23.20 [12.91; 36.35]	46.00 [10.64; 60.03]	0.042
ALT, U/L	20.90 [16.00; 51.70]	24.50 [11.17; 68.19]	0.373
AST, U/L	29.30 [21.47; 39.70]	32.70 [26.50; 106.34]	0.250
Albumin, g/L	41.40 [24.00; 54.60]	44.40 [41.81; 45.04]	0.310
Total protein, g/L	69.00 [65.50; 74.13]	66.60 [61.15; 75.60]	0.850
NT-proBNP	2530.00 [1514.25; 6393.00]	9016.00 [6207.00; 11825.00]	0.012
pH	7.40 [7.33; 7.40]	7.40 [7.38; 7.45]	0.351
BE, mmol/L	-0.20 [-1.40; 1.98]	0.40 [-3.25; 2.35]	0.821
pO ₂ , mm Hg	38.50 [31.73; 44.63]	31.70 [26.40; 42.45]	0.350
SpO ₂ , %	67.00 [57.10; 75.30]	58.60 [39.10; 71.95]	0.005
Lactate, mmol/L	1.00 [0.90; 1.40]	1.54 [1.35; 2.95]	0.001
Na ⁺ , mmol/L	139.0 [137.0; 140.0]	135.0 [134.0; 138.0]	0.009
K ⁺ , mmol/L	3.50 [3.33; 3.88]	4.0 [3.50; 4.25]	0.042

Abbreviation: ALT, alanine aminotransferase; AST, aspartate aminotransferase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; BE, base excess.

urea, total bilirubin, and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were observed.

Receiver operating characteristic (ROC) analysis further confirmed the prognostic significance of baseline laboratory parameters for HT outcomes (Table 3). Table 4 presents the threshold values of parameters associated with an increased risk of mortality.

The high urgency status (UNOS classification), the requirement for short-term MCS, and clinical manifestations of multiple organ failure in recipients necessitated expansion of the criteria for donor heart utilization.

Table 5 presents the results of the comparative analysis of donor heart characteristics between recipients

who survived and those who died during the early post-transplant period.

Heart donors in the cohort of deceased recipients were significantly older than donors in the cohort of survivors.

Among recipients who died in the early post-transplant period, the donor-to-recipient weight ratio was higher, as were the donor-to-recipient height ratio and the donor-to-recipient body surface area ratio, compared with surviving recipients.

Table 6 presents the comparative analysis of intra-operative characteristics between recipients who survived and those who died during the early post-transplant period.

Table 3

ROC analysis: AUC and 95% CI for pre-transplant laboratory parameters in heart transplant recipients under 18 years of age

Indicator	AUC (95% CI)	p (AUC)
Na ⁺ , mmol/L	0.761 (0.597–0.925)	0.002
Erythrocytes, ×10 ⁹ /L	0.742 (0.567–0.918)	0.007
Hemoglobin, g/L	0.738 (0.567–0.909)	0.006
Lactate, mmol/L	0.737 (0.572–0.903)	0.005
Urea, mmol/L	0.722 (0.562–0.883)	0.007
Platelets, ×10 ⁹ /L	0.692 (0.514–0.869)	0.034
SpO ₂ , %	0.622 (0.406–0.838)	0.27
Total bilirubin, μmol/L	0.577 (0.341–0.813)	0.522
NT-proBNP	0.825 (0.617–1.000)	0.002

Table 4

Optimal threshold values and diagnostic threshold characteristics in heart transplant recipients under 18 years of age, including sensitivity (Se), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV)

Indicator	Threshold (Youden's J statistic)	Se	Sp	PPV	NPV
Na ⁺ , mmol/L	≥139	0.636	0.838	0.368	0.939
Red blood cells, ×10 ⁹ /L	≤3.8	0.636	0.827	0.35	0.939
Hemoglobin, g/L	≤97	0.636	0.813	0.333	0.938
Lactate, mmol/L	≥1.3	0.818	0.703	0.29	0.963
Urea, mmol/L	≥6.6	0.727	0.68	0.25	0.944
Platelets, ×10 ⁹ /L	≤122	0.818	0.68	0.273	0.962
SpO ₂ , %	≤39.8	0.364	0.959	0.571	0.91
Total bilirubin, μmol/L	≥46	0.545	0.827	0.316	0.925
NT-proBNP	≥3,398	1	0.75	0.286	1

Table 5

Characteristics of heart donors for surviving and non-surviving recipients in the early post-transplant period

Parameters	Heart transplant recipients under 18 years of age		p
	Survivors (n = 79) (mean ± SD)	Deceased (n = 12) (mean ± SD)	
Donor age, years	35.6 ± 11.5	43.6 ± 12.9	0.030
Donor-to-recipient weight ratio	1.40 ± 0.57	1.94 ± 1.45	0.020
Donor-to-recipient height ratio	1.05 ± 0.09	1.21 ± 0.14	<0.0001
Donor-to-recipient BSA ratio	1.24 ± 0.27	1.75 ± 0.46	<0.0001

Abbreviation: BSA, body surface area.

Table 6

Intraoperative characteristics of surviving and non-surviving recipients in the early post-transplant period

Parameters	Heart transplant recipients under 18 years of age		p
	Survivors (n = 79) (Me [Q1; Q3])	Deceased (n = 12) (Me [Q1; Q3])	
Duration of anesthesiological assistance, minutes	390.0 [302.50; 451.00]	565.0 [540.00; 765.00]	<0.0001
Duration of surgical intervention, minutes	290.0 [207.50; 348.75]	475.0 [400.00; 690.00]	<0.0001
Heart transplant ischemia time, minutes	170.5 [135.00; 210.00]	187.50 [162.50; 275.25]	0.007
Duration of CPB, minutes	95.0 [78.75; 120.00]	162.0 [99.0; 189.0]	<0.0001

Abbreviation: CPB, cardiopulmonary bypass.

Deceased recipients demonstrated longer durations of graft ischemia, anesthetic management, surgical intervention, and cardiopulmonary bypass.

DISCUSSION

HT is the standard, definitive therapy for children suffering from various irreversible heart diseases associated with end-stage heart failure. Currently, adult donor transplantation is the only viable option for performing HT in recipients under 18 years of age.

Since January 1, 2012, the Shumakov National Medical Research Center of Transplantology and Artificial Organs has been actively developing a program for pediatric HT from deceased adult donors. The number of pediatric HT has increased from 3 in 2012 to 20 per year in 2024.

Given the high demand for urgent HT, selecting a suitable donor organ within standard criteria is extremely challenging. The global medical community has not developed generally accepted criteria for anthropometric compatibility between an adult donor and a pediatric recipient. Body weight has long been used as a traditional indicator for matching donors to recipients [2]. According to international studies, as the prevalence of childhood obesity increases [3, 4], previous assumptions of a linear correlation between heart size and recipient weight may no longer be accurate. A study by Amdani et al. recommends abandoning donor matching based on recipient weight [5].

The donor-to-recipient height ratio also influences HT outcomes. In the study by Amdani et al., both undersized and oversized donors relative to the recipient were associated with an increased risk of graft dysfunction. However, smaller donor hearts appeared to have a more detrimental impact than larger ones. The authors therefore suggest avoiding donor–recipient height differences exceeding 30% [5].

Matching based on body mass index (BMI) and body surface area (BSA), rather than weight alone, has been associated with improved transplant outcomes [6]. In the present study, the donor-to-recipient BSA ratio was identified as a statistically significant predictor of early post-transplant mortality in recipients under 18 years of age.

Currently, pediatric HT increasingly relies on cross-sectional imaging such as computed tomography (CT) and magnetic resonance imaging (MRI) of both donor and recipient to more accurately assess cardiac chamber dimensions and myocardial thickness. However, in clinical practice, this approach may present significant logistical challenges and can prolong the donor–recipient matching process [5].

A method for donor–recipient matching based on chest X-ray imaging combined with anthropometric parameters (donor weight, height, age, sex) and recipient cardiac dimensions obtained from CT scan has also been reported [7]. Mismatch between donor and recipient anatomical dimensions on cross-sectional CT imaging has been shown to independently influence HT outcomes in pediatric recipients [5].

Additional factors associated with adverse post-transplant outcomes in both adult and children include urgent transplantation status, donor age above 45 years, and intraoperative variables such as prolonged operative time, extended graft ischemia time, and increased intraoperative blood loss [8–11]. These findings are consistent with the results of the present study.

CONCLUSION

In-hospital survival among heart recipients younger than 18 years was 86.8%. The outcomes of HT in children are influenced by the urgency status (UNOS classification) and secondary factors related to it, such as severity of recipient multi-organ dysfunction, donor age, significant anthropometric mismatch between donor and recipient, as well as intraoperative factors including prolonged surgical duration and increased intraoperative blood loss.

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CORNEAL XENOTRANSPLANTATION IN OPHTHALMOLOGY: CHALLENGES AND PROSPECTS

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Introduction. This paper evaluates the key structural characteristics of turkey cornea and critically analyzes both the obtained results and existing literature to assess the feasibility of using this xenogeneic material for keratoplasty. Currently, more than 12 million patients worldwide are awaiting keratoplasty. The prolonged waiting times is largely driven by severe shortage of cadaveric human corneas required for the procedure. Recent studies have generated growing optimism regarding the potential of xenogeneic keratoplasty; however, the number of animal donors studied to date remains quite limited. In light of this gap, the objective of this study was formulated. **Objective:** to assess the main structural characteristics of turkey (*Meleagris gallopavo*) cornea and to evaluate its potential use as a xenogeneic material for selective keratoplasty. **Materials and methods.** Corneoscleral buttons (n = 24) were isolated from enucleated turkey eyeballs. In the first stage, the corneal microstructure was examined using scanning electron microscopy and confocal microscopy. In the second stage, the feasibility of cutting out a corneal xenograft suitable for deep anterior lamellar keratoplasty was evaluated. In the third stage, the potential for preservation of the obtained xenografts using xenogeneic turkey corneal material was assessed. **Results.** The turkey cornea was shown to possess all the principal layers characteristic of the human cornea. Its mean thickness was $508 \pm 33.5 \mu\text{m}$, and the presence of Bowman's membrane was confirmed. Preservation of the turkey cornea for up to five days was feasible while maintaining a high endothelial cell density. In addition, the preparation of a xenograft suitable for deep Anterior lamellar keratoplasty from turkey corneal tissue was successfully demonstrated. **Conclusion.** Analysis using confocal microscopy and scanning electron microscopy, together with assessment of xenograft integrity following hypothermic preservation, indicates that turkey cornea represents a promising xenogeneic material for selective keratoplasty. Further studies are warranted to assess its potential application in reconstructive corneal surgery.

Keywords: cornea, turkey, keratoplasty, xenotransplantation, xenogeneic material.

INTRODUCTION

According to recent estimates, there are currently 36 million blind people worldwide [1]. Corneal damage is one of the common causes of vision loss. Currently, restoration of damaged corneal tissue is primarily achieved through keratoplasty – a surgical procedure in which the affected, opacified portion of the recipient's cornea is replaced with a transparent cadaveric donor cornea.

Despite its effectiveness, keratoplasty faces a substantial global demand, with waiting lists exceeding 12 million patients [2, 3]. The primary reason for this shortage is the limited availability of cadaveric donor corneas, which remain the gold standard for transplantation. Consequently, there is growing research interest in the development of alternative graft materials, particularly xenogeneic sources suitable for keratoplasty [4–6].

Historically, the concept of corneal transplantation originated with xenografts. Carl Himly is credited with proposing the idea of xenotransplantation, and his student, Franz Reisinger, performed the first successful cor-

neal transplant in animal experiments in 1818, coining the term “keratoplasty”, which remains in use today [7].

The first clinical corneal xenotransplantation – from an animal to a human – was performed by Richard KISSAM in 1838 using porcine corneas as donor material [8]. Subsequent experimental attempts involving corneas from various animal species produced limited success. Among these, the most favorable outcomes were reported with gibbon corneal grafts, which remained transparent in approximately 50% of cases over a follow-up period exceeding five months [4].

However, the widespread use of primate-derived xenografts has been constrained by several factors. These include their limited reproductive capacity, which restricts the availability of donor material, as well as the potential risk of transmitting zoonotic infections, including viruses closely related to those affecting humans. In addition, ethical concerns regarding the use of species with close behavioral and biological similarities to humans further limit their acceptability.

In contrast, pigs offer several practical and biological advantages over non-human primates, making them a more promising source of xenogeneic material for corneal transplantation [4]. Pigs are widely considered a promising source of donor tissue due to several practical advantages. They are relatively easy and cost-effective to breed, have high reproductive rates with large litter sizes, and can be genetically modified to overcome immunological barriers. In addition, pigs can be raised under controlled, pathogen-free conditions. Ethical concerns are also generally less pronounced, as pigs are commonly used for food in many cultures [9].

However, in regions where Islam or Judaism is practiced, the use of porcine-derived materials may be unacceptable on religious grounds [9]. Moreover, the porcine cornea differs from the human cornea in key morphological characteristics. For instance, its thickness varies considerably depending on the breed, typically ranging from 660 to 990 μm , whereas the normal human corneal thickness is approximately 550–560 μm . Since the cornea is a biological lens, its physical properties are of paramount importance. In addition, porcine cornea differs significantly from human cornea. For instance, in pigs, corneal thickness in the central optical zone is approximately equal to that at the periphery, whereas in humans, thickness gradually increases from the center toward the periphery.

Beyond these morphological differences, the porcine cornea is not suitable for humans because it can elicit a strong immune response in humans, limiting its suitability for transplantation. For example, Song et al. reported that the posterior epithelial layer of the porcine cornea expresses co-stimulatory molecules CD80 and CD86, which activate T-cell-mediated immune responses, leading to graft opacification and rejection [10]. Similarly, Islam et al. demonstrated that native porcine cornea induces the production of multiple pro-inflammatory mediators, including interleukins (IL-1 β , IL-2, IL-6, IL-8, IL-1 α), chemokines (MCP-1, MIP-1 α , MIP-1 β), and other cytokines (TNF, G-CSF, IFN- γ , FGF-basic). The authors suggested that combined C5 blockade with eculizumab and TLR4 inhibition may offer therapeutic potential in porcine-to-human corneal xenotransplantation [11].

Another critical concern is the risk of zoonotic infection. Porcine endogenous retrovirus (PERV) remains the most significant viral agent of concern. PERV is capable of integrating into the porcine genome and has been shown to infect human cells *in vitro*. However, to date, there is no conclusive *in vivo* evidence demonstrating transmission of PERV to human recipients [10].

Due to the marked morphological and immunological incompatibility between porcine and human corneas, recent research has increasingly focused on the use of decellularization techniques to prepare corneal xenografts. This approach enables the complete or partial removal of cellular and immunogenic components while preserv-

ing the native extracellular matrix and its micro- and macrostructural integrity.

Decellularized corneal tissue can function as an *in vivo* scaffold that supports tissue regeneration while maintaining the physiological properties required for corneal replacement. For clinical application, however, an additional sterilization step is necessary to minimize the risk of transplant-associated infections. In this context, gamma irradiation – an established and widely standardized sterilization method – is commonly employed [12].

In recent years, advances in corneal decellularization have made it possible to overcome major immunological barriers to xenotransplantation, enabling the use of animal-derived corneas in the treatment of human corneal diseases. For instance, in 2019, Zheng et al. used similar decellularized porcine xenografts to treat the complications of keratitis and corneal perforations associated with herpes simplex virus infection [13]. Subsequently, in 2020, the same group reported the successful application of similar grafts in the treatment of fungal keratitis, achieving significant improvements in visual acuity [14].

These findings highlight the effectiveness of decellularization techniques and underscore the growing potential of corneal xenotransplantation, driven in part by advances in the processing and preparation of xenogeneic materials [14].

Advances in corneal xenotransplantation have stimulated comparative studies aimed at identifying the most suitable xenograft sources. For example, Liu et al. (2016) reported the results of an *in vivo* experimental study comparing the efficacy of ostrich and porcine corneal xenografts transplanted into rabbits [15]. Their findings demonstrated significantly higher rates of transparent graft engraftment with ostrich corneas compared to porcine corneas. In discussing these results, the authors emphasized the importance of close morphological and histological compatibility between donor xenograft tissue and the recipient cornea. Based on their analysis, they concluded that the human cornea more closely resembles the ostrich cornea than the porcine cornea in key structural parameters.

The high therapeutic potential of interspecies xenotransplantation had already been demonstrated in earlier studies conducted in Russia in the mid-20th century. For example, in 1956, Professor Arkady Pavlovich Nesterov, in his dissertation “Experimental Studies on Heterogeneous Corneal Transplantation”, was the first to demonstrate that avian corneal xenografts – specifically from chickens – yielded the most favorable outcomes in xenokeratoplasty compared with corneas from other animal species [16]. However, due to significant discrepancies in diameter and thickness between the chicken and human corneas, this material is not suitable for contemporary clinical keratoplasty.

In light of these findings, and based on both Nesterov’s work [16] and current scientific literature, Sergey. Borzenok has proposed the turkey cornea as a potentially

promising xenograft source for xenokeratoplasty. At present, there is no global consensus regarding the optimal animal donor for corneal xenotransplantation. Although most studies have focused on porcine corneas, substantial differences in anatomical, topographical, morphological, and refractive properties compared with the human cornea remain key limitations to their clinical application.

Accordingly, the objective of the present study was formulated to address this gap.

Objective: to assess the main structural characteristics and suitability of the turkey (*Meleagris gallopavo*) cornea as a potential xenogeneic material for selective keratoplasty.

MATERIALS AND METHODS

To achieve this objective, 12 turkey heads (*Meleagris gallopavo*) were used, from which 24 corneoscleral complexes were isolated following a standard protocol for harvesting cadaveric donor corneas at an eye tissue bank. In the first stage of the study, intact turkey heads were subjected to confocal microscopy to assess corneal thickness, morphological features, and cellular composition. Confocal imaging was performed using the contact method with an HRT3 confocal microscope (Heidelberg Engineering GmbH, Germany). To further evaluate the microstructural characteristics of the turkey cornea, scanning electron microscopy (SEM) was conducted using a JCM-6000 Plus scanning electron microscope (JEOL, Japan). To this end, corneal samples were fixed in a 4% formaldehyde solution for 24 hours. Dehydration was then carried out using a graded series of acetone solutions as follows: 5% acetone for 20 minutes, 10% acetone for 20 minutes, 30% acetone for 20 minutes, 50% acetone for 20 minutes, 70% acetone for 20 minutes, 90% acetone for 20 minutes, and finally 100% acetone (three changes, 20 minutes each). The dehydrated samples were placed in CPD800A microporous capsules (Quorum Technologies Ltd., UK) and subjected to critical point drying for 30 minutes at 31 °C and 7.39 MPa using a K850 drying unit (Quorum Technologies Ltd., UK), during which the acetone was replaced by liquid CO₂. Subsequently, the samples were mounted on aluminum stubs using carbon tape and conductive adhesive, with the endothelial surface oriented upward. They were then transferred to a Smart Coater vacuum sputtering system (Structure Probe, Inc., USA) and coated with a 5 nm layer of gold (99.9% purity) to provide surface conductivity. Following preparation, the samples were examined using a scanning electron microscope to analyze the microstructural characteristics of the corneal tissue.

In the second stage of the procedure, grafts were prepared from the corneoscleral complexes for deep anterior lamellar keratoplasty (ALK). The excised tissue, consisting of the cornea and a portion of the sclera, was oriented with the corneal endothelium facing upward and secured in this position within the Moria ALK Artificial Anterior Chamber (MORIA Inc., France). Normotony

was established, after which baseline pachymetry of the central corneal thickness was performed using the iVue100 optical coherence tomograph (Optovue, USA).

Subsequently, the Moria Evolution 3E microkeratome (MORIA Inc., France) was used to obtain a lamellar graft of the desired thickness (approximately 350 µm). A final pachymetric measurement of the residual corneal tissue was then carried out to confirm the thickness and adequacy of the prepared graft for ALK.

In the third stage, the prepared grafts for ALK were placed in vials containing Borzenko–Moroz corneal preservation solution and stored under hypothermic conditions (+4 °C). After 24 hours, graft thickness was evaluated using optical coherence tomography (OCT).

The feasibility of long-term preservation of the xenograft was then assessed using xenogeneic turkey corneal tissue. At the end of the 5-day preservation period, final pachymetric measurements were performed with an optical coherence tomograph. Subsequently, the grafts were removed from the experiment and examined using SEM.

RESULTS

Confocal microscopy of the turkey eye revealed that the cornea comprises all the principal layers characteristic of the human cornea. The presence of Bowman's membrane was confirmed at a depth of approximately 50 µm. This layer exhibits hyperreflectivity and an amorphous structure similar to that of the human Bowman's membrane; however, unlike the human cornea, a subepithelial (subbasal) nerve plexus was not observed on its anterior surface.

The stromal layer of the turkey cornea displayed a distinct cross-striated pattern. In the deeper stromal regions, isolated intrastromal nerve fibers were identified. Compared with the human cornea, the turkey cornea showed a considerably lower density and number of both stromal nerve fibers and keratocytes.

It was also observed that corneal thickness increases gradually and uniformly from the central to the peripheral regions. The presence of Descemet's membrane was confirmed. The endothelial layer consists of a monolayer of hexagonal cells with hyperreflective cytoplasm and hyporeflexive intercellular borders. Based on confocal microscopy measurements, the endothelial cell density was determined to be 4373 ± 532 cells/mm².

An example of corneal thickness measurement obtained via confocal microscopy is presented in Fig. 1, while the key morphometric parameters of the native turkey cornea are summarized in Table.

SEM revealed that the stromal layer of the turkey cornea is composed of densely packed collagen fibers, with minimal intercellular spaces. Protein strands were observed interconnecting the collagen fibers in the transverse direction. Electron microscopic analysis of the posterior (endothelial) layer showed a monolayer of hexagonal cells characterized by high cellular density and tight intercellular junctions.

Table
Main morphometric characteristics of native turkey cornea

Corneal thickness, μm	508 ± 33.5
Stromal thickness, μm	440 ± 29.7
Keratocyte density, cells/ mm^2	478 ± 43
Endothelial cell density, cells/ mm^2	4373 ± 532

An example of a scanning electron micrograph of the turkey cornea is presented in Fig. 2.

Corneoscleral complexes were harvested from enucleated turkey eyeballs using a standard protocol for cadaveric donor cornea procurement. The harvesting process is illustrated in Fig. 3.

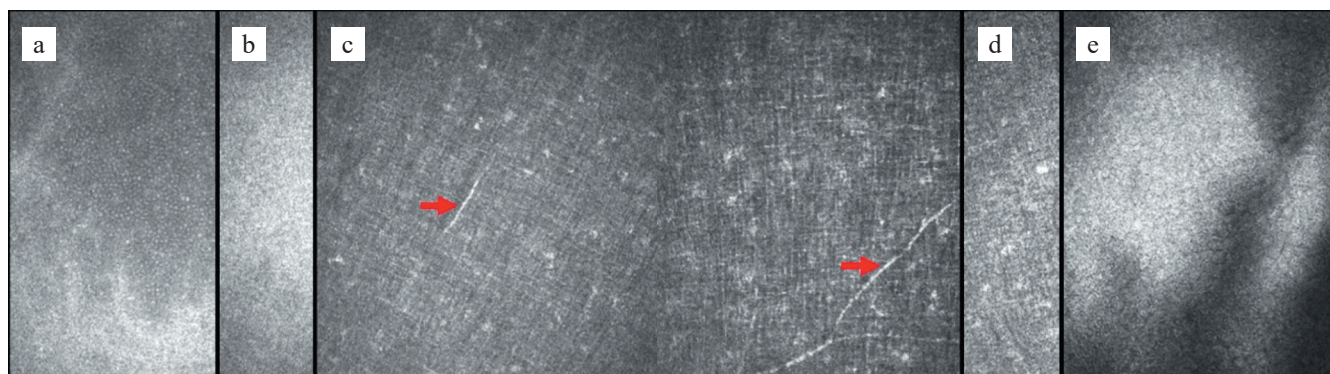


Fig. 1. Confocal microscopy of a turkey cornea: (a) anterior epithelium; (b) Bowman's layer; (c) stroma; (d) Descemet's membrane; (e) posterior epithelium. Arrows indicate stromal nerve fibers

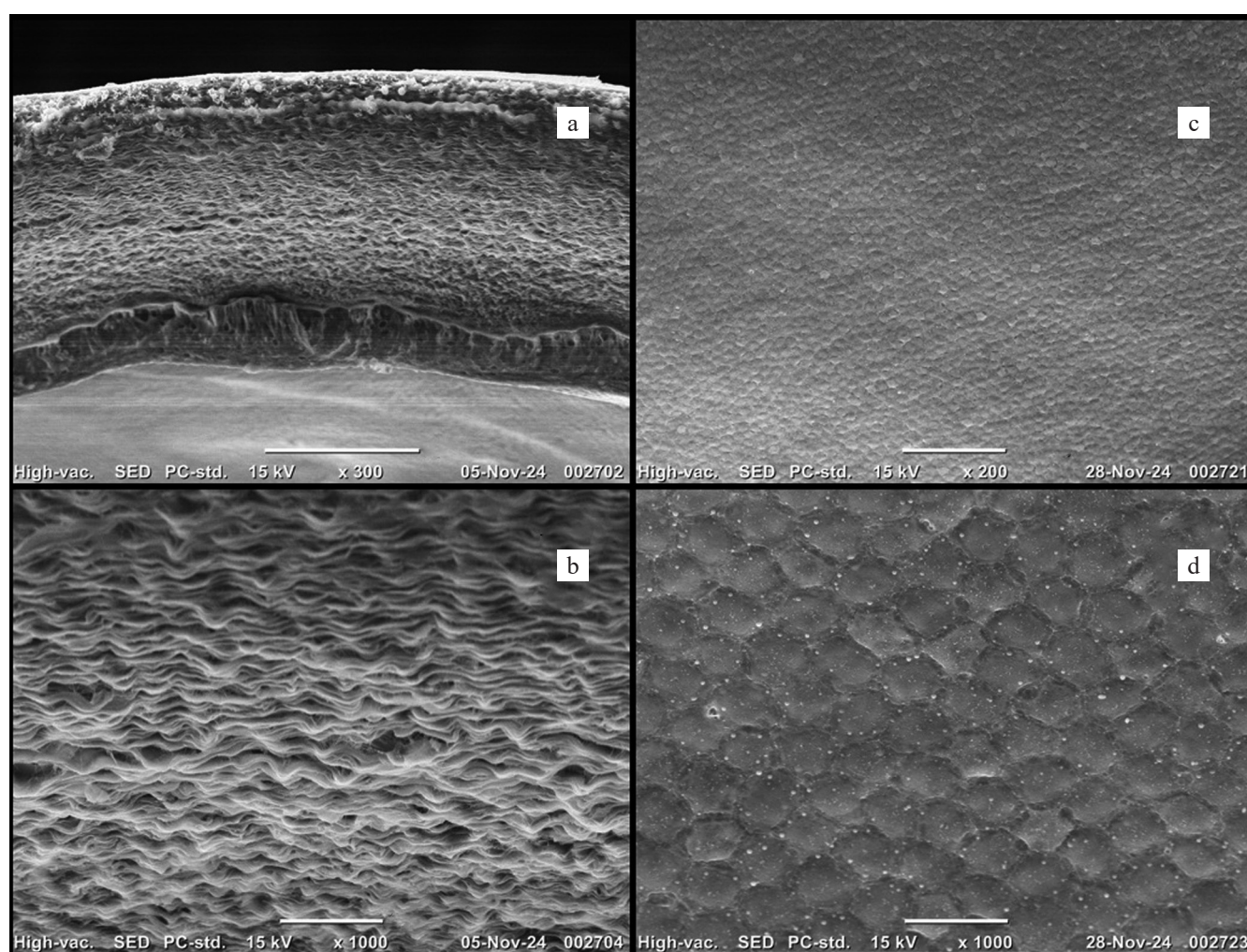


Fig. 2. Scanning electron microscopy of a turkey cornea: (a) overview of the stroma; (b) stromal collagen fibers; (c) overview of the posterior epithelium; (d) hexagonal cells of the posterior epithelium

Grafts intended for ALK were subsequently prepared from the isolated corneoscleral complexes. The mean thickness of the prepared xenografts was $325 \pm 42.9 \mu\text{m}$. Dissection of the xenograft from the endothelial side, combined with the use of a $130\text{-}\mu\text{m}$ blade, enabled more accurate prediction of the final graft thickness. After

24 hours of preservation, graft thickness increased significantly to $385 \pm 53.9 \mu\text{m}$.

An example of graft assessment using OCT after one day of hypothermic storage in a corneal preservation solution, with a measured central corneal thickness of $369 \mu\text{m}$, is shown in Fig. 4.

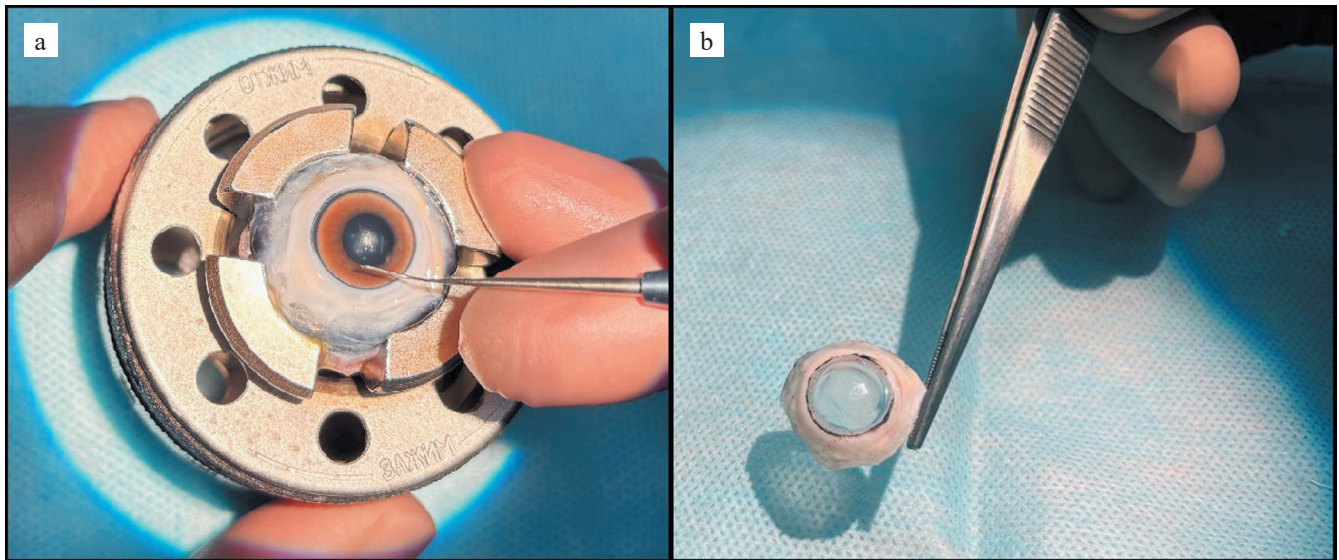


Fig. 3. Procedure for isolating the corneal button (b) from an enucleated turkey eyeball (a)

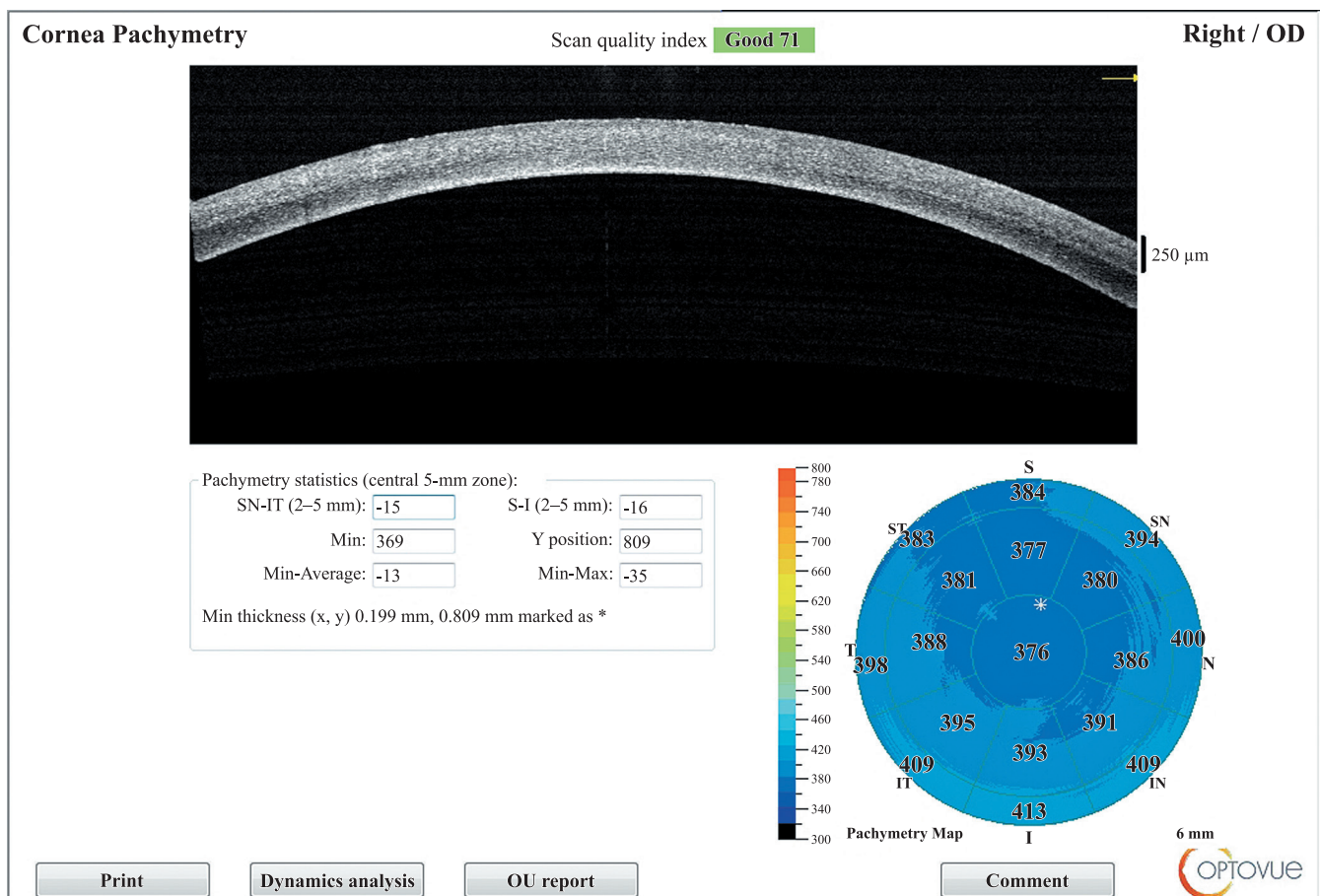


Fig. 4. Optical coherence tomography of the graft for anterior lamellar keratoplasty after 1 day of preservation

The degree of swelling of turkey-derived xenografts used for ALK did not exceed that observed in human corneal grafts at any point during the observation period. As with human donor tissue, the extent of graft edema should be taken into account when preparing xenografts for clinical application.

After 5 days of hypothermic preservation in corneal storage solution, the mean thickness of the xenografts increased to $445 \pm 61.5 \mu\text{m}$. An example of graft evaluation using OCT after 5 days of storage is presented in Fig. 5, showing a central corneal thickness of $433 \mu\text{m}$.

At the end of the 5-day preservation period, samples of the experimental corneal xenografts intended for an-

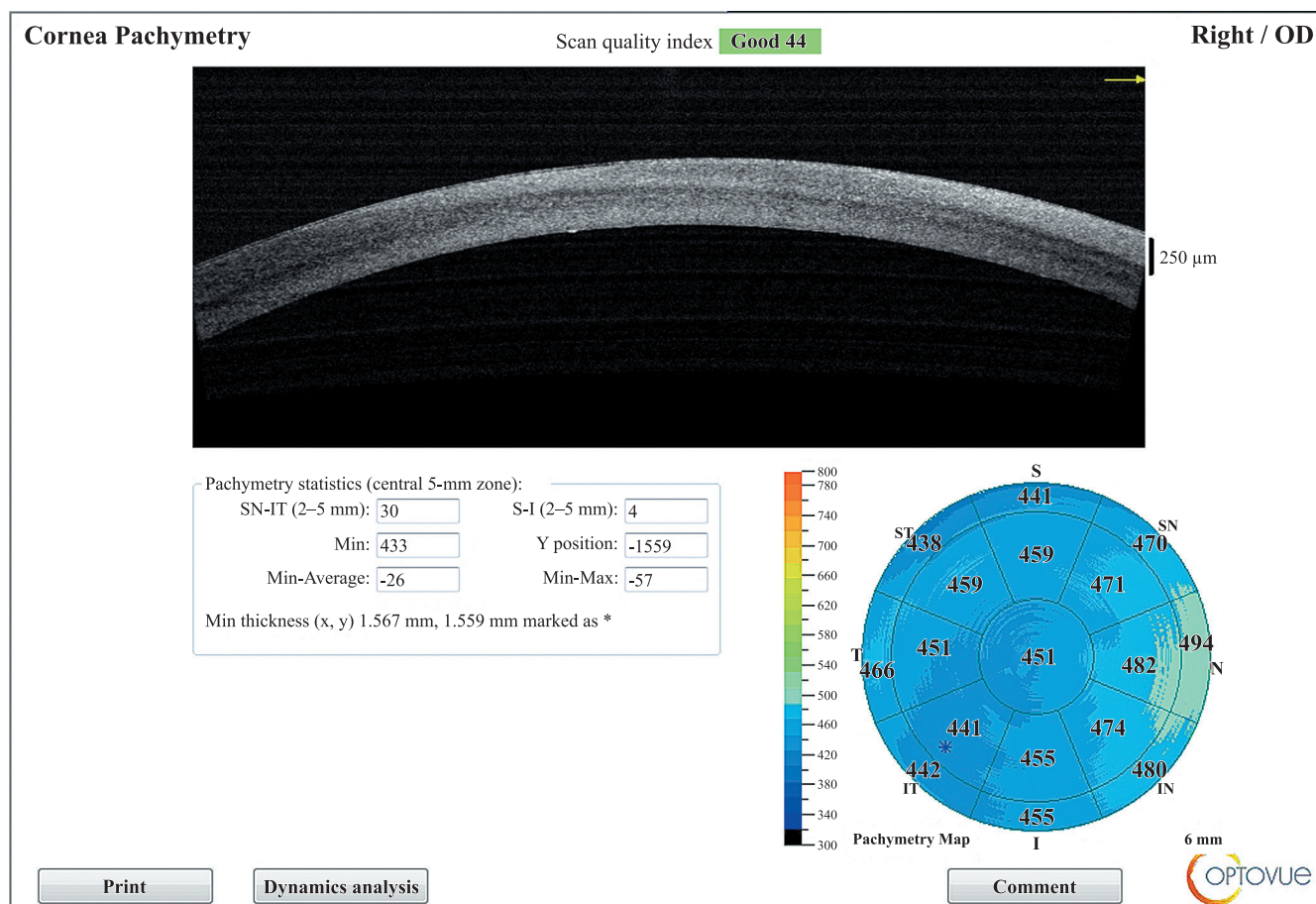


Fig. 5. Optical coherence tomography of the graft for anterior lamellar keratoplasty after 5 days of preservation

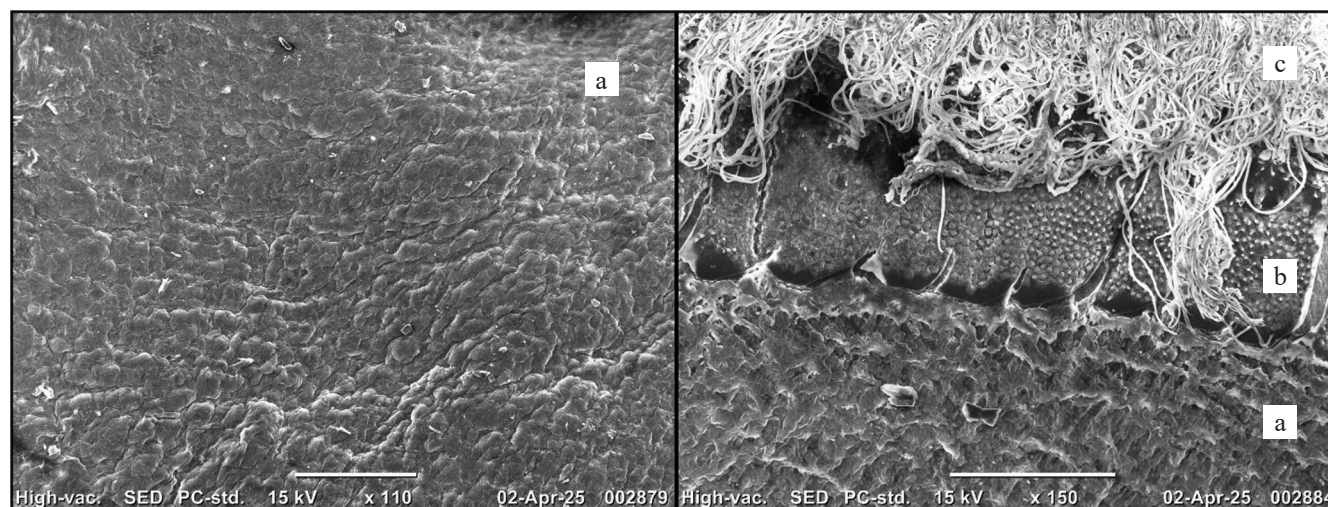


Fig. 6. Scanning electron microscopy of a turkey cornea after 5 days of hypothermic preservation: a, stromal surface after microkeratome treatment; b, endothelium at the periphery of the cornea; c, trabecular meshwork of the eye

terior lamellar xenokeratoplasty were examined using SEM. The analysis showed that the stromal surface, following microkeratome treatment, had a smooth topography, while the collagen fibers retained their characteristic cross-striated organization. Importantly, the endothelial layer composed of hexagonal cells remained preserved, indicating that turkey corneal tissue can withstand hypothermic storage for up to 5 days.

An example of a corneal image after 5 days of preservation, obtained by SEM, is presented in Fig. 6.

DISCUSSION

This study was aimed at the experimental evaluation of the structural characteristics of the turkey cornea and the assessment of its suitability as a xenogeneic material for selective keratoplasty, particularly ALK.

As indicated in the literature, investigations of cadaveric human donor corneas can be readily performed using standard ophthalmic diagnostic tools; however, such approaches may be more challenging when applied to xenogeneic tissues. In this context, the present study demonstrates, for the first time, the feasibility of performing confocal microscopy on native turkey eyes without prior enucleation, thereby enhancing the objectivity and reliability of the obtained data.

All measurements and image analyses were conducted by experienced specialists using calibrated instruments, ensuring consistency and reproducibility across the study.

As a result of this study, several key structural parameters of the turkey cornea were characterized for the first time using confocal microscopy. Mean corneal thickness was $508 \pm 33.5 \mu\text{m}$, with a stromal thickness of $440 \pm 29.7 \mu\text{m}$. Keratocyte density was $478 \pm 43 \text{ cells/mm}^2$, and endothelial cell density was $4373 \pm 532 \text{ cells/mm}^2$.

It was also shown that xenografts of the required thickness for ALK can be reliably prepared from turkey corneas using standard equipment at the Eye Tissue Bank, without the need for additional technical modifications. Furthermore, turkey cornea-derived xenografts were shown to tolerate hypothermic preservation in Borzenko–Moroz corneal storage medium for up to 5 days while maintaining structural integrity. This study presents some of the key parameters of the turkey cornea. At this stage, the findings suggest that the turkey cornea represents a promising alternative for xenokeratoplasty, since widely used porcine corneas differ substantially from human corneas in several critical characteristics, particularly thickness. For instance, the central thickness of the porcine cornea significantly exceeds that of the human cornea, which may necessitate modification or redesign of existing equipment used for preparing grafts for ALK.

In this regard, the turkey cornea shows a notable advantage over the porcine cornea, as its thickness is much

closer to that of the human cornea. Given the critical role of the cornea in the optical system of the eye, such similarity is of particular importance. Unlike the porcine cornea, which exhibits relatively uniform thickness across the central and peripheral regions, both the turkey and human corneas have a thinner central zone with gradual thickening toward the periphery.

These structural differences limit the suitability of the porcine cornea as an optimal xenograft material for keratoplasty. For this reason, there is potential for using the turkey cornea in the development of a xenograft for keratoplasty as a new xenogeneic material.

CONCLUSION

The findings on the fundamental structural characteristics of the turkey cornea – including its overall morphology, corneal thickness, keratocyte density, endothelial cell density, nerve fiber distribution, and stromal collagen organization – support its characterization as a promising xenogeneic donor material for xenokeratoplasty. However, further study is required before it can be used in reconstructive corneal surgery.

The authors declare no conflict of interest.

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EPIGENETICS IN CLINICAL TRANSPLANTOLOGY: DIAGNOSTIC, PREDICTIVE, AND THERAPEUTIC SIGNIFICANCE OF MICRORNA MOLECULES (SYSTEMATIC REVIEW)

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Epigenetics is the study of changes in gene expression that occur without alterations in the primary DNA sequence. These changes are mediated by chemical modifications of DNA, histones, and non-coding RNAs, collectively forming the epigenome, that determines the functional activity of the genome. Epigenetic mechanisms play a fundamental role in cellular differentiation, organismal development, and adaptation to external conditions. In medicine, they have attracted considerable attention due to their involvement in the pathogenesis of oncological, autoimmune, and neurodegenerative diseases. MicroRNAs (miRNAs), as key components of epigenetic mechanisms, play a critical role in controlling immune responses, including those occurring after organ transplantation. This has opened new opportunities for a personalized approach to the management of transplant recipients. Accumulating evidence on the role of miRNAs in solid organ transplantation suggests that integration of omics technologies may expand the existing arsenal of diagnostic criteria, serving as an auxiliary diagnostic tool for monitoring graft function. This systematic review presents a comprehensive analysis of the current literature on the clinical significance of miRNAs in modern transplantology. It highlights the diagnostic and predictive potential of specific miRNAs in relation to the development of complications in recipients of heart, lung, kidney, and liver transplants, and examines current approaches to the use of miRNAs as therapeutic targets.

Keywords: *solid organ transplantation, miRNAs, diagnosis, prognosis, biomarkers, dysfunction, rejection, fibrosis.*

INTRODUCTION

Organ transplantation is a definitive and highly effective treatment for end-stage diseases. However, despite continuous advances in surgical techniques and immunosuppressive therapies, the risk of post-transplant complications that require timely adjustments to treatment, persists [1]. Therefore, the development of new effective approaches aimed at improving long-term graft survival continues to be a key priority in transplant medicine.

For many years, biopsy – despite its inherent risks as an invasive procedure – has remained the gold standard for diagnosing graft pathology. In this context, increasing attention is being directed toward the development of minimally invasive diagnostic approaches. In particular, the identification and monitoring of circulating biomarkers in blood or other biological fluids represent a promising strategy for the early detection of graft dysfunction and the prevention of graft loss [2].

The advent of genomic and post-genomic technologies has represented a major breakthrough in the diagnosis and management of cardiovascular, oncological, and other severe diseases. Current research efforts are increasingly focused on elucidating the molecular mechanisms

underlying the induction of immunological tolerance, as well as on identifying new diagnostic and prognostic markers for risk stratification [3]. In transplant medicine, particular importance is placed on understanding the regulation of innate and adaptive immune responses and exploring the potential for targeted modulation of gene expression.

A rapidly evolving area within this field is epigenetics – a branch of genetics that investigates changes in gene activity that occur without alterations in the primary DNA sequence [4]. The growing number of studies dedicated to the role of epigenetic mechanisms in disease pathogenesis highlights the increasing interest of both researchers and clinicians in this promising area.

A class of small non-coding RNAs (microRNAs or miRNAs) – has attracted considerable attention as key regulators of gene expression and as promising biomarkers and therapeutic targets for a range of socially significant diseases [5]. Since the discovery of miRNA molecules in 1993 by American geneticists Victor Ambros and Gary Ruvkun, numerous research has been conducted to elucidate their biological functions. To date, more than two thousand miRNAs have been identified in the human

genome. The scientists, who revealed a new, fundamental principle of gene regulation, were awarded the 2024 Nobel Prize in Physiology or Medicine.

Given their central role in regulating cellular processes, microRNAs hold substantial potential for application across various fields of medicine, including transplantology, and are likely to remain a major focus of research in the coming years. However, the few systematic reviews on microRNAs in solid organ transplantation have several limitations in their study design. In particular, several systematic reviews exhibit notable constraints:

- the role of miRNAs is often examined within a single type of transplantation [6];
- investigations into the involvement of miRNAs in mechanisms of organ injury are frequently conducted in broader pathological contexts, with transplant recipients representing only a subset of the overall cohort [7];
- the role of miRNA in the development of specific complications is currently being studied [8].

These observations underscore the need for a comprehensive analysis of contemporary literature on the role of miRNAs in transplant medicine, particularly in the context of their involvement in complex immune response cascades and mechanisms of graft injury.

Objective: to systematize and critically analyze published data on miRNAs as key elements of epigenetics, as well as to evaluate their clinical significance in solid organ transplantation.

METHODOLOGY FOR LITERATURE SEARCH

The literature search was conducted using major electronic scientific citation databases – PubMed (www.ncbi.nlm.nih.gov/pubmed) and Russian Science Citation Index (RSCI, <http://www.elibrary.ru>).

To construct search queries in RSCI (last search date: September 20, 2025), the following keyword combinations (English and Russian words) were used: микроРНК* транспл* орган* эпигенетик*; microRNA* transplant* organ* epigenetic*; микроРНК* транспл* орган*; microRNA* transplant* organ*; микроРНК* транспл* печень*; microRNA* transplant* liver*; microRNA* transplant* renal*; микроРНК* транспл* почка*; microRNA* transplant* kidn*; микроРНК* транспл* сердце*; microRNA* transplant* heart*; микроРНК* транспл* легкие*; microRNA* transplant* lung*.

For the PubMed search (last search date: September 25, 2025), the following keyword combinations were used: microRNA transplant organ; epigenetic transplant organ; microRNA transplant liver; microRNA transplant kidney; microRNA transplant renal; microRNA transplant heart; microRNA transplant lung.

The analysis included full-text reviews, meta-analyses, and original research articles published in Russian and English. Selected studies addressed the expression of various miRNAs in solid organ (heart, kidney, liver,

and lung) transplantation, encompassing both clinical studies and experimental animal models. The literature review focused on the following key aspects:

- the relationship between miRNA expression and clinical outcomes of transplantation (development of pathological conditions/complications and patient survival);
- the diagnostic, prognostic, and therapeutic potential of miRNAs;
- methods for quantitative assessment of miRNA expression and interpretation of results across various loci;
- associations between miRNA levels and anthropometric and other recipient parameters;
- analysis of miRNA-mediated signaling pathways and their deleterious and protective effects on the graft;
- the relationship between miRNAs and the regimen and efficacy of immunosuppressive therapy;
- clinical applications.

The exclusion criteria for study selection were as follows:

- studies involving the use of miRNA in bone marrow or stem cell transplantation;
- studies focusing on DNA methylation;
- studies on histone modification;
- publications available only as preprints or conference proceedings;
- duplicate publications.

The overall structure of the literature search and study selection process is presented in Fig. 1.

All publications identified through the search queries across each database were evaluated for relevance to the objectives of the review and consolidated into a unified dataset. The subsequent stage involved the removal of duplicate publications and the exclusion of studies that did not meet the predefined inclusion criteria.

RESEARCH RESULTS

The initial search conducted in the eLIBRARY.RU database yielded 242 publications related to epigenetics and 1,199 publications addressing miRNA in the context of organ transplantation. A parallel search in PubMed identified 676 publications related to epigenetics and 1,541 publications focusing on miRNAs within the broader body of scientific literature.

The earliest publications addressing epigenetics in transplant medicine date back to 1988, with a marked increase in research activity and a peak in the number of publications observed between 2016 and 2020 (Fig. 2a). Studies using the term “microRNA” emerged later, beginning around 2009, with peak publication activity recorded in 2019 (Fig. 2b).

Following the application of inclusion and exclusion criteria, the final dataset comprised 305 publications, which were categorized into three principal groups:

- epigenetics and miRNAs in solid organ transplantation;
- miRNAs in the diagnosis and prognosis of complications;
- miRNA-based drugs, clinical applications.

Published studies clearly demonstrate the growing importance of investigating epigenetic mechanisms –

particularly miRNA – in the development of pathological conditions in solid organ recipients.

However, despite increasing scientific interest, most available studies on the significance of these molecules in organ transplantation remain exploratory in nature. The translation of these findings into routine clinical practice is still limited by several important challenges:

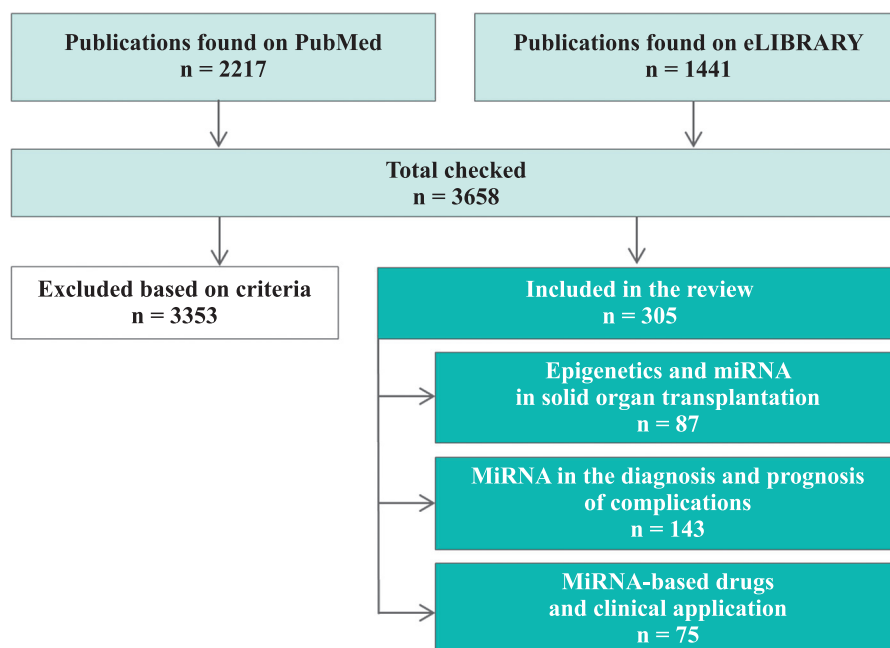


Fig. 1. Flow diagram of the literature selection process for the systematic review

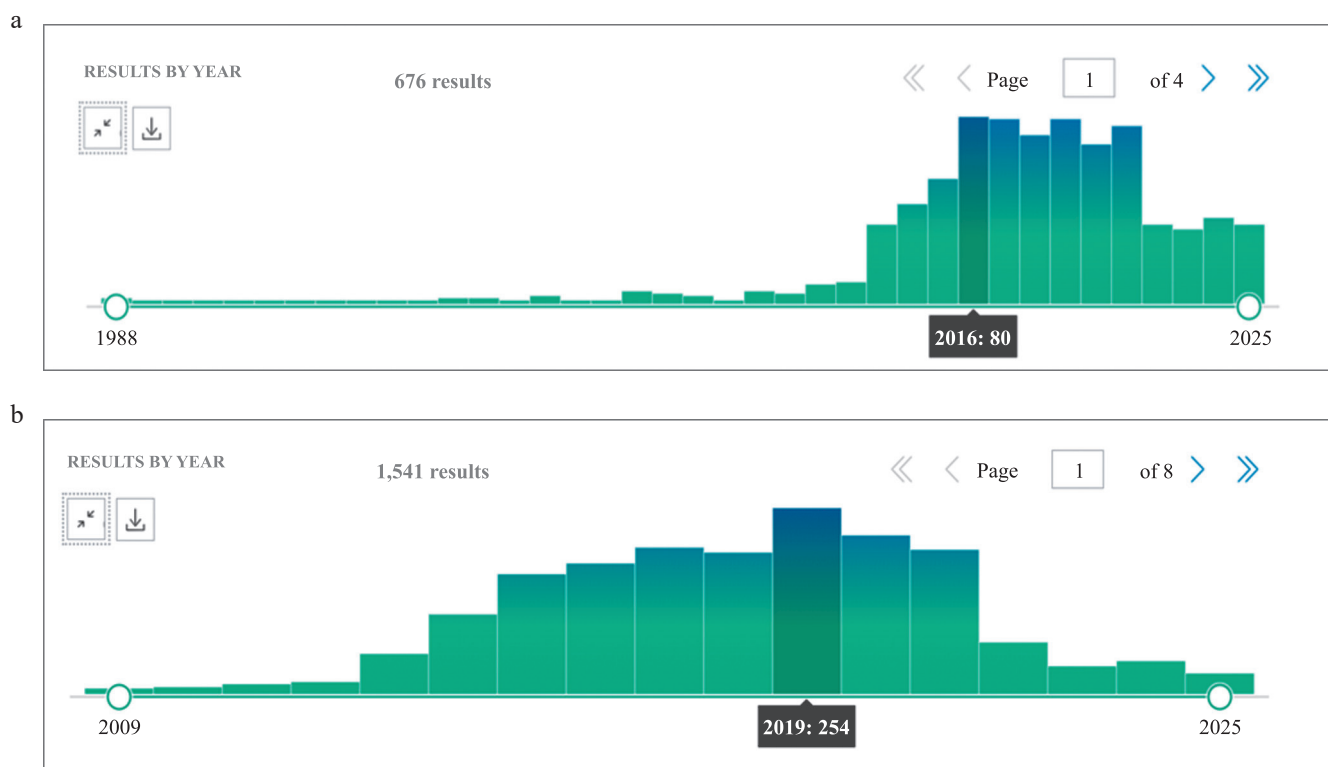


Fig. 2. Distribution by year of publications on PubMed for the following search queries: a) "epigenetic transplant organ", b) "microRNA transplant organ"

the relatively small number of large-scale randomized and prospective clinical trials, lack of standardized and reproducible methods for quantitative assessment of miRNA expression, and inconsistencies in results reported across different studies. The latter is partly due to the large number of miRNA molecules and the pleiotropic nature of their effects in various tissues and organs.

EPIGENETICS AS A NEW FIELD OF SCIENCE

The term “epigenetics” was first introduced in 1942 by English biologist Conrad Waddington to describe the concept of the “epigenetic landscape”, which illustrates the complex and dynamic interactions between environmental factors and the genome during the formation of phenotype [9]. Environmental influences can trigger intricate regulatory cascades that modulate gene activity. Some of these epigenetic modifications can be transmitted across several generations. In this way, the stability of the genetic code ensures species survival, while epigenetic mechanisms provide the flexibility required for adaptation to changing environmental conditions. These processes enable long-term programming of gene activity, including the selective activation or “silencing” of specific genes [10, 11].

Widespread scientific interest in epigenetics emerged at the turn of the 20th and 21st centuries, following experimental evidence demonstrating that organisms can, under environmental influence, inherit patterns of gene activity and subsequently “switch off” these patterns when the external stimulus is removed [12].

The discovery of the unique reversible nature of epigenetic modifications has fundamentally transformed classical views of genetics. Emphasizing the distinction between inherited genetic information and environmentally influenced gene regulation, the distinguished biologist and Nobel laureate Peter Medawar famously stated: “Genetics proposes; epigenetics disposes”. This implies that, beyond the inherited genome, environmental exposures play a decisive role in shaping disease risk over an individual’s lifetime and even across generations.

This concept has been experimentally illustrated in studies involving *Drosophila melanogaster*, where environmental factors such as temperature changes triggered the activation of specific chromosomal elements. As a result, the offspring’s eye color changed from yellow to red.

In 2004, New Zealand researchers Peter Gluckman and Mark Hanson proposed the so-called “mismatch hypothesis”, which suggests that during embryonic development, adaptive programming is initiated in response to environmental cues. This process allows the developing organism to “predict” future environmental conditions and adjust its physiology accordingly. If this prediction is confirmed, the resulting phenotype enhances survival; otherwise, unused adaptation may cause pathology [14].

Empirical support for this concept was provided by studies of individuals born during the Dutch famine of 1944–1945, conducted by epidemiologist Lammert van den Berg and colleagues. These investigations showed that women exposed to severe malnutrition during the third trimester of pregnancy later gave birth to children with reduced anthropometric measures. Despite being raised in relatively favorable postnatal conditions, these individuals exhibited a significantly increased risk of obesity, type 2 diabetes, and hypertension in adulthood compared with those born to well-nourished mothers [15].

This phenomenon was attributed to prenatal metabolic programming – often referred to as fetal “imprinting” – occurring during late gestation, in which intrauterine prediction of famine conditions and the activation of a gene responsible for enhanced nutrient storage in the fetus. Even more remarkable was the fact that the next generation inherited both the anthropometric characteristics of their undernourished parents and the incidence of disease in adulthood.

These fundamental discoveries have provided a new framework for understanding common diseases by distinguishing between purely genetic disorders and those driven by epigenetic alterations. This distinction has important implications for the potential reversibility of pathological processes, making it highly relevant from a national public healthcare perspective. To date, substantial evidence has linked epigenetic dysregulation to the development of oncological, metabolic, and autoimmune diseases.

THE FORMATION OF DOMESTIC EPIGENETICS

The development of epigenetics in Russia has been quite challenging. Nevertheless, our compatriots have made significant contributions to this rapidly evolving field. Among them, Boris Vanyushin (1933–2019) is widely regarded as a foundational figure in early Soviet epigenetic research. His work laid important groundwork for contemporary studies in gerontology, oncology, and biotechnology.

As early as the 1970s, Vanyushin and his team were among the first to demonstrate that methyl groups attached to cytosine residues in DNA play a critical role in gene silencing, and that these DNA methylation patterns are influenced by environmental factors such as stress and nutrition, as well as developmental processes. They also identified DNA hypomethylation as a key mechanism in tumorigenesis, characterized by disruption of normal methylation patterns in cancer cells and subsequent activation of oncogenes [16]. It was this revolutionary discovery that anticipated modern research into the role of the epigenome in oncogenesis. The ideas about the connection between methylation and cancer, put forward at the end of the last century, became the basis for the development of DNA methyltransferase

inhibitors (e.g., azacitidine), used today in the treatment of leukemia. Later, in the 1980s, Vanyushin also described age-associated accumulation of methylation errors, linking epigenetic drift to cellular aging and proposing the concept of age-dependent methylation changes [17].

At the same time, significant advances in the study of epigenetics of stress in plants and animals have been achieved in our country, with the aim of improving agricultural productivity.

Experimental work conducted by Vladislav Khlobovich at the Institute of Cytology and Genetics (Siberian Branch of the Russian Academy of Sciences, Novosibirsk) demonstrated that extreme environmental stressors such as drought and cold can influence the heritable regulation of gene activity in agricultural crops [18].

As a result of a series of experiments, a wheat variety resistant to soil salinization was developed, but more importantly, the possibility of so-called epigenome reprogramming became evident [19].

At the beginning of the 21st century, both in Russia and globally, the advent of whole-genome sequencing technologies marked a major technological breakthrough. These methods, together with advances in molecular profiling and systems biology, significantly expanded the understanding of epigenetic mechanisms and helped define new directions in biomedical and biological research worldwide.

KEY EPIGENETIC MECHANISMS

Epigenetic mechanisms regulate organismal development and cell differentiation, ensuring genomic flexibility and adaptability. They enable cells to respond to changes in environmental conditions through biochemical reactions controlled by specific enzymes [20]. Dysregulation of epigenetic processes is closely associated with a wide range of pathologies.

Three main mechanisms of epigenetic modification are distinguished: genomic mechanisms involving DNA methylation mediated by enzymes; proteomic mechanisms involving post-translational modifications of histones in their unstructured N-terminal domains; and transcriptomic mechanisms involving regulation of gene expression via microRNA molecules.

These processes not only interact to shape the structural and functional organization of chromatin but also complement one another, thereby ensuring the reliability of epigenetic signal transmission.

DNA methylation is the process by which a methyl group (CH_3) is added to cytosine in the presence of guanine, typically within CpG dinucleotides, forming a covalent modification at these sites. The primary biological role of this process is the suppression of transposon transcription (“jumping genes”, which constitute up to 45% of the human genome). Uncontrolled transposon activity may lead to genome instability, chromosomal abnormalities, immunodeficiency, and oncogenic transfor-

mation of cells [20]. As a central epigenetic mechanism, CpG site methylation contributes to genome stability by regulating transposon activity and maintaining long-term silencing of genes essential for normal development and physiological function.

Histone modification is a regulatory mechanism involving chemical alterations of histone proteins, which are responsible for DNA packaging within the nucleus, chromatin organization, and regulation of gene activity. Histones control the accessibility of DNA regions for transcription, while the degree of chromatin compaction and gene expression depends on various post-translational modifications, including methylation, phosphorylation, and ubiquitination [12, 21]. Combinations of these modifications form the so-called “histone codes”, which regulate tissue-specific gene expression.

MicroRNA molecules. Another epigenetic mechanism of gene expression regulation is mediated by RNA interference and involves small non-coding miRNAs, typically 18–25 nucleotides in length. MiRNAs are relatively stable molecules that circulate freely in tissues and biological fluids. According to various estimates, the number of miRNA molecules in the human body may reach approximately 37,000, each of which can regulate multiple target genes simultaneously, forming complex networks of molecular interactions.

IMPORTANCE FOR TRANSPLANT MEDICINE

Epigenetic dysregulation is known to be directly or indirectly associated with the development of various pathological conditions. Within this paradigm, exploring the potential practical application of epigenetic criteria in the field of organ and tissue transplantation appears highly promising. In particular, the identification of novel epigenetic biomarkers of graft rejection and tissue remodeling may provide valuable opportunities for early diagnosis and prediction of post-transplant complications.

The theory of age-dependent methylation [22] has been confirmed by researchers worldwide and served as the basis for the development, in 2013, of an algorithm by American biologist Steve Horvath for assessing the biological age of tissues and organs based on analysis of 353 DNA regions, known as the “epigenetic clock” [23, 24].

It has been shown that the rate of aging of organs and tissues does not always correlate with the chronological age of the organism. Applied to transplantation medicine, this concept suggests that the epigenetically determined “biological age” of a donor organ may serve as a promising independent criterion for donor selection, a predictor of transplant outcomes, and a factor guiding postoperative management strategies in recipients.

The study of the relationship between miRNA and parthanatosis – a form of programmed cell, tissue, and organ death – has also shown significant promise. A correlation has been identified between pre-transplant

miRNA expression levels, cellular aging pathways, and clinical outcomes after transplantation. These findings support the concept of minimally invasive preoperative prediction of outcomes in recipients of heart, kidney, liver, and lung transplants [25].

Patients who have undergone organ transplantation require lifelong immunosuppressive therapy to prevent graft rejection; however, this treatment is associated with an increased long-term risk of malignant tumors and other adverse effects. A key mechanism underlying this risk is epigenetic “silencing” of tumor suppressor genes through promoter hypermethylation [26].

Current research is therefore focused on developing safe epigenetic drugs and biomarkers for early cancer detection, including malignancies associated with prolonged immunosuppressive therapy, which is of critical importance for transplant recipients.

A deeper understanding of the complex interactions between epigenetic alterations, immune responses, and post-transplant pathological processes opens new perspectives for personalized targeted therapy, aimed at long-term, individualized modulation of the recipient's epigenome [27, 28].

MICRORNA IN THE DIAGNOSIS AND PROGNOSIS OF TRANSPLANT PATHOLOGY

The study of potential biomarkers at the pre-translational level represents a qualitatively new step in the early diagnosis of post-transplant complications. In this context, transcriptome analysis enables the identification of miRNA expression profiles that can not only stratify patients according to their immunological risk of rejection or tolerance but also differentiate between types of rejection, thereby providing clinically relevant information for treatment decisions and indications for additional instrumental investigations, including biopsy [29, 30].

A growing number of studies highlight the significance of miRNAs in cardiovascular diseases and in acute rejection of transplanted organs [31, 32]. Because miRNAs regulate key signaling pathways, changes in individual miRNA levels in donor organ tissues or recipient biological fluids correlate with the presence and severity of graft injury. Alterations in miRNA profiles have been detected not only in plasma and serum but also in biopsy samples from transplant recipients with acute cellular rejection. The observation that certain miRNAs originate from immune cells rather than graft tissue suggests that rejection may be predicted even before overt organ damage occurs [33].

Although biomarker levels may be influenced by non-specific factors, consistent changes in circulating miRNA profiles during solid organ rejection, mediated through defined signaling pathways, support their potential utility as both diagnostic and therapeutic targets [34].

Table 1 summarizes the main findings of studies demonstrating the clinical relevance of miRNA expression in complications following heart, lung, kidney, and liver transplantation.

Every year, researchers identify new miRNAs with diagnostic and prognostic relevance for post-transplant complications. Notably, rejection mechanisms in different solid organs are associated either with distinct miRNA expression profiles or, conversely, with the involvement of specific miRNAs in multiple pathological processes across different patient groups, as demonstrated by numerous studies.

An analysis of recent literature shows that most research on miRNAs has focused on heart and kidney transplant recipients, followed by liver transplantation. The smallest number of studies concerns lung transplantation, likely due to both the lower overall number of lung transplants and the challenges associated with assessing graft function and validating research findings.

In heart transplantation specifically, several dozen miRNAs associated with acute rejection have been identified. Their expression levels are most often quantified using PCR-based methods in plasma or serum samples collected from recipients at different time points after transplantation. Variability in the biological material used is partly explained by differences in reagent systems produced by various manufacturers for quantitative miRNA assessment.

The most common of these include miR-10a, miR-31, miR-92a, and miR-155 [35, 44]. Sukma Dewi and other authors have also demonstrated that levels of miR-142-3p, miR-144-3p, miR-101, miR-326, and miR-101-3p may help identify heart transplant recipients with acute cellular rejection [6, 37, 41].

In several studies, Shevchenko et al. reported the diagnostic value of miR-101 and miR-27 in acute rejection of transplanted hearts. In particular, miR-101 levels may allow prediction of rejection even at the preoperative evaluation stage of potential recipients [39, 43]. Additionally, altered miR-27 expression has been associated with myocardial fibrosis, together with changes in miR-339 [40].

Singh et al. described an association between miR-126 and miR-200 expression in recipient blood plasma and the development of cardiac allograft vasculopathy, highlighting their potential role in predicting this complication.

Changes in miR-424 levels in plasma samples from heart and lung transplant recipients have also been observed during Gram-negative bacteremia caused by antibiotic-resistant pathogens, which represent a serious threat in immunocompromised patients receiving lifelong immunosuppressive therapy [42]. In this study, a significant increase in miR-424 levels was detected following confirmation of bloodstream infection.

Table 1

**Key findings on the clinical significance of miRNAs in solid organ transplantation
(heart, lungs, kidney, liver)**

s/n	Authors, reference	Year	Significant miRNAs	MiRNA expression site	Clinical significance
Heart					
1	Duong Van Huyen J.P. et al. [35]	2014	miR-10a miR-31 miR-92a miR-155	Blood serum, transplant tissue	Diagnosis of rejection
2	Singh N. et al. [36]	2015	miR-126 miR-200	Blood plasma	Prognosis of allograft vasculopathy
3	Sukma Dewi I. et al. [37]	2017	miR-142-3p miR-101-3p	Blood serum	Diagnosis of rejection
4	Neumann A. et al. [38]	2017	miR-628-5p	Blood plasma	Diagnosis of vasculopathy
5	Velikiy et al. [39]	2020	miR-101 miR-27	Blood plasma	Diagnosis of acute rejection
6	Shevchenko et al. [40]	2021	miR-27 miR-339	Blood plasma	Diagnosis of myocardial fibrosis
7	Pérez-Carrillo L. et al. [41]	2022	miR-144-3p	Blood serum	Diagnosis and prognosis of acute cellular rejection
8	Shevchenko et al. [42]	2022	miR-424	Blood plasma	Diagnosis of Gram-negative bacteremia
9	Shevchenko et al. [43]	2023	miR-101	Blood plasma	Preoperative prognosis of rejection
10	Bansal S. et al. [44]	2024	miR-155	Blood plasma	Diagnosis of rejection
Lungs					
11	Gharib S.A. et al. [45]	2015	117 miRNA molecules	Epithelial cells	Diagnosis of acute cellular rejection
12	Zhu L. et al. [46]	2018	miR-199b-5p	Blood plasma	Anti-inflammatory effect in rejection
13	Dong M. et al. [47]	2019	miR-27a-3p	transplant tissue	Diagnosis of chronic rejection, obliterative bronchiolitis
14	Palleschi A. et al. [48]	2020	let-7f-5p miR-146b-3p miR-22-5p miR-29c-5p miR-362-5p miR-452-5p	Bronchoalveolar lavage fluid	Diagnosis of acute respiratory distress syndrome and graft dysfunction
15	Shevchenko O. et al. [49]	2021	miR-339	Blood plasma	Diagnosis of bronchial obstruction
16	Dong M. et al. [50]	2023	miR-27a-3p	Blood plasma	Diagnosis and prognosis of obliterative bronchiolitis syndrome
17	Yang J. et al. [51]	2025	miR-124-3p	Blood plasma	Diagnosis of acute graft injury
Kidney					
18	Danger R. et al. [52]	2013	miR-142-5p	Blood plasma	Diagnosis of chronic antibody-mediated rejection
19	Sui W. et al. [53]	2014	miR-181a miR-483-5p miR-557	Blood serum	Prognosis and diagnosis of rejection
20	Vahed S.Z. et al. [54]	2017	miR-150 miR-192 miR-200b miR-423-3p	Blood plasma	Diagnosis of chronic graft dysfunction
21	Cabral A. et al. [55]	2019	miR-27a-5p miR-331-3p miR-885-5p	Blood serum	Prognosis of transplant tolerance
22	de Necochea Campion R. et al. [56]	2025	let 7a-5p miR-29b-3pb miR-99a5p miR-148b-3p miR-148a-3p	Perfusate	Prognosis of graft dysfunction

Continuation Table 1

s/n	Authors, reference	Year	Significant miRNAs	MiRNA expression site	Clinical significance
Liver					
23	Wei L. et al. [57]	2013	miR-21 miR-155	Blood plasma	Diagnosis of rejection
24	Ruiz P. et al. [58]	2020	miR-122 miR-155 miR-181	Blood plasma	Diagnosis of acute cellular rejection
25	Koch P.F. et al. [6]	2024	miR-483-3p miR-885-5p	Blood plasma, Organ tissue	Diagnosis of acute cellular rejection
26	Julian J. et al. [59]	2025	miR-122-5p miR-181a-5p miR-101155-5p	Blood plasma	Diagnosis of rejection
27	Anam M. et al. [60]	2025	miR-452-5p miR-224-5p	Perfusate	Diagnosis and prognosis of rejection
Evaluation of various transplanted organs in a single study					
28	Zhou M. et al. [61]	2016	Liver: miR-22 miR-125b miR-99a miR-192	Organ tissue	Prognosis of graft dysfunction
			Heart: miR-1 miR-133a miR-296 miR-208 miR-499	Organ tissue	Diagnosis and prognosis of cardiovascular events
			Kidney: miR-126 miR-152 miR-182 miR-192 miR-194 miR-204 miR-215 miR-216	Urine	Diagnosis of graft dysfunction and cancer
			Lungs: let-7b miR-16 miR-26a miR-92 miR-125a miR-125b miR-200c	Organ tissue	Diagnosis of tissue fibrosis and cancer
29	Harris A., Krams S.M., Martinez O.M. [62]	2010	Kidney: miR-142-5p miR-155 miR-223	Organ tissue	Cancer prognosis
			Liver: miR-155	Organ tissue	Prognosis of rejection
30	Mas V.R. et al. [63]	2013	Liver: miR-30e miR-296	Perfusate	Diagnosis of graft dysfunction
			Kidney: miR-142-5p miR-155 miR-223	Urine	Prognosis of rejection

End of Table 1

s/n	Authors, reference	Year	Significant miRNAs	MiRNA expression site	Clinical significance
31	Amrouche L., Rabant M., Anglicheau D. [64]	2014	Heart: miR-133a miR-133b miR-208a	Blood plasma	Diagnosis of cardiovascular events
			Liver: miR-30b miR-34a miR-155 miR-222 miR-361 miR-455	Blood plasma	Diagnosis of fibrosis
			Kidney: miR-99a miR-200b miR-200 miR-142-3p	Urine	Prognosis of fibrosis
32	Hamdorf M., Kawakita S., Everly M. [65]	2017	Heart: miR-326 miR-142-3p miR-101	Blood serum	Diagnosis of rejection
			Liver: miR-122 miR-148a miR-194	Blood serum	Diagnosis of rejection
			Lungs: miR-126 miR-146a	Blood serum	Diagnosis of rejection

The diagnosis of lung rejection and fibrosis remains a fundamentally complex issue in transplant medicine, as transbronchial biopsy used to confirm pathological changes and associated graft dysfunction is an invasive procedure with a significant risk of bleeding, bacterial and fungal infections, and other complications. In this context, the development of minimally invasive approaches for monitoring rejection and structural graft changes is of critical importance.

Palleschi et al. demonstrated the involvement of let-7f-5p, miR-146b-3p, miR-22-5p, miR-29c-5p, miR-362-5p, and miR-452-5p in the diagnosis of acute respiratory distress syndrome and graft dysfunction. In this study, miRNA expression was analyzed in bronchoalveolar lavage samples [48].

Attempts to identify biomarkers of acute cellular rejection were also made by a group led by Gharib; however, the 117 miRNAs identified were assessed in epithelial cells, which does not fully meet the criteria for minimally invasive diagnostics [45]. Similarly, Dong et al. identified miR-27a-3p as a marker of chronic rejection and obliterative bronchiolitis, but this was initially performed in transplant tissue samples [47]. Subsequently, the same group evaluated miR-27a-3p levels in blood samples from lung transplant recipients, demonstrating its diagnostic and prognostic value for obliterative bronchiolitis syndrome [50].

Studies by Zhu and Yang demonstrated the involvement of miR-199b-5p and miR-124-3p in the development of acute lung injury and lung transplant rejection based on expression analyses in recipients' blood [46, 51].

The role of miR-339 in tissue fibrosis has also been studied in detail, providing the basis for further evidence of its involvement in bronchial obstruction after lung transplantation. The authors reported a significant increase in miR-339 levels in the plasma of lung recipients during the development of graft structural changes and bronchial stenosis, findings confirmed by invasive video-bronchoscopy [49]. They also noted that the diagnostic performance of miRNA testing improves when combined with measurement of the proteomic biomarker galectin-3.

Studies on the role of miRNAs in kidney transplantation have demonstrated a wide range of biological materials used for analysis, including graft tissue, plasma and serum, graft perfusate, and urine.

In a study by Sui, the analyzed miRNAs identified miR-181a, miR-483-5p, and miR-557 as effective blood markers for the diagnosis and prognosis of kidney transplant rejection [53]. According to Danger et al., chronic rejection can be identified through analysis of miR-142-5p in blood plasma [52].

Amrouche et al. showed a link between miR-142-3p, as well as miR-99a and the miR-200 family (miR-200b and miR-200c), detected in urine, and kidney transplant fibrosis [64]. Studies by Mas and Harris further support the association of miR-142-5p with both graft rejection and cancer development [62, 63].

According to published data, miRNAs in liver transplantation are most frequently studied in recipients' blood plasma or in graft perfusate. Among the most relevant for detecting rejection are miR-122, miR-155, miR-181, miR-224-5p, miR-483-3p, miR-452-5p, and miR-885-5p [6, 57–60]. In addition, miR-30b, miR-34a, miR-155, miR-222, miR-361, and miR-455 have been identified as markers of fibrosis in the transplanted liver [64].

In Russian literature, there is significantly less data on the specific features of miRNA regulation in solid organ recipients compared with international studies, and most research on small non-coding RNAs focuses on their role in oncology.

Only a limited number of institutions in the Russian Federation are engaged in the study of miRNAs in transplant recipients. The leading center in this field is the Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, which has long been involved in the development of minimally invasive technologies for screening and detection of post-transplant complications [66–68].

The data presented reflect the diverse effects of various miRNAs, indicating the need for further studies in large patient cohorts to validate potential biomarkers and develop specific diagnostic panels for each specific type of pathology and transplant.

THE THERAPEUTIC POTENTIAL OF MICRORNA

The discovery of the link between miRNAs and a wide range of diseases has stimulated interest in their use as a novel class of therapeutic agents. This has driven significant interdisciplinary research across biology, chemistry, and medicine aimed at developing miRNA-based therapies. As a result, RNA-based therapy has emerged as a promising approach for treating cancer, while advances in RNA delivery systems have also contributed to the development of modern immunotherapeutic strategies, further accelerating innovation in the pharmaceutical industry.

The development of miRNA-based drugs is particularly promising due to their ability to selectively target specific molecular pathways and simultaneously regulate multiple genes within a single signaling pathway. This capacity enables the modulation of complex biological processes and opens broad prospects for clinical application. To date, miRNAs have been most extensively studied in oncology, with several candidate drugs currently undergoing various stages of clinical trials and regulatory evaluation [69].

To date, among the many miRNA molecules studied to varying extents, two main groups can be distinguished in terms of their medical applications: mimics and antagonists.

MiRNA mimics are synthetic molecules designed to replicate the function of endogenous miRNAs. They consist of short double-stranded RNA sequences which, once introduced into the cell, are incorporated into the RNA-induced silencing complex (RISC), similar to natural miRNAs. These mimics bind to target messenger RNAs and suppress their expression, thereby enabling regulation of specific protein levels. Their potential for correcting pathological conditions associated with miRNA dysregulation is currently under active investigation.

MiRNA antagonists are chemically modified oligonucleotides that inhibit target miRNAs. Commonly referred to as “anti-miRs” or “block-miRs”, they function by binding to target miRNAs to disrupt translation.

In organ transplantation, miRNA-based therapies are being explored to improve recipient survival, reduce the risk of rejection, and mitigate ischemia–reperfusion injury in grafts. Table 2 presents examples of the use of miRNA mimics and antagonists across various fields of medicine, including solid organ transplantation, along with their observed therapeutic effects.

To date, several drugs based on RNA interference have been approved by the U.S. Food and Drug Administration and are in clinical use [82]. The international nonproprietary names of the active ingredients are:

- Patisiran – used for the treatment of hereditary transthyretin amyloidosis; one of the drug's RNA strands binds to messenger RNA transcribed from the TTR gene, thereby inhibiting transthyretin protein synthesis.
- Givosiran – used for the treatment of acute liver failure; a small interfering RNA suppresses the production of δ -aminolevulinic acid synthase (ALAS1) and participates in heme production, acting selectively on liver cells via the carbohydrate marker GalNAc and not entering cells of other organs [83].
- Lumasiran – used for the treatment of primary hyperoxaluria type 1; it targets the liver enzyme hydroxyacid oxidase 1, reducing oxalate levels in urine and plasma.
- Nedosiran – used for the treatment of primary hyperoxaluria type 1; it inhibits hepatic lactate dehydrogenase, an enzyme involved in oxalate production.
- Inclisiran – used for the treatment of primary hypercholesterolemia; it selectively targets the gene responsible for the production of proprotein convertase subtilisin/kexin type 9 (PCSK9) in the liver, enhancing hepatic uptake of circulating low-density lipoproteins and thereby reducing their blood levels [84].
- Vutrisiran – used for the treatment of hereditary and acquired transthyretin amyloid cardiomyopathy and

transthyretin amyloidosis with polyneuropathy; it inhibits messenger RNA regulating transthyretin production, leading to reduced serum TTR levels and decreased amyloid fibril deposition.

In addition, specialists at the Siberian State Medical University have developed a unique-in-its-class microRNA-based drug for the treatment of oncological diseases, aimed at inhibiting the transition of micrometastases into macrometastases [85]. This drug has successfully completed preclinical trials.

The data presented highlight the considerable potential of RNA-based therapies, particularly those leveraging RNA interference, for targeted treatment of various diseases. In the future, these approaches may be adapted to address the challenge of graft rejection in solid organ transplantation.

CHALLENGES AND PROSPECTS

The number of studies investigating the regulation of homeostasis and immune responses via miRNA continues to grow annually. However, a substantial gap remains in understanding the specific contribution of individual miRNAs to the pathogenesis of post-transplant complications. This is largely due to methodological and technological limitations inherent in miRNA research.

Only a limited number of candidate drugs have progressed to clinical trials, and some have been discontinued

due to safety concerns, including toxicity. Further complexity arises from the fact that miRNAs participate in multiple interconnected signaling pathways, making it difficult to identify precise therapeutic targets and increasing the risk of unintended side effects.

In this context, continued research is essential to improve targeted delivery systems and optimize dosing strategies, with the goal of achieving maximal therapeutic efficacy while minimizing adverse effects.

Genomic technologies have enabled the identification of novel and effective biomarkers for transplant pathology. The growing volume of scientific publications has generated extensive datasets, while advances in information technology have created opportunities for their systematic analysis using machine learning algorithms. Improved analytical approaches now allow the integration and interpretation of large-scale medical data, facilitating the identification of complex and previously unrecognized patterns that are critical for understanding biological processes in transplant recipients [86, 87]. Using this genetic profiling approach, several clinically relevant biomarker panels have been developed. Among the most widely recognized are: AlloMap (used to identify patients at increased risk of heart transplant rejection by analyzing the expression levels of 11 genes in peripheral blood), AlloSure (a test based on donor-derived cell-free DNA (dd-cfDNA) for monitoring acute cellular

Table 2

MiRNA-based therapeutics: applications and observed therapeutic effects

Author, reference	Drug	Target	Therapeutic application	Effect
Mimetics				
Ryu Y. et al. [70]	MRX34	miR-34	Cancer therapy (hepatocellular carcinoma, melanoma)	Antitumor activity; clinical trials suspended due to high toxicity
Trang P. et al. [71]	let-7 mimetics	let-7	Lung cancer therapy	Suppression of tumor growth in preclinical studies
Zhao J.L. et al. [72]	miR-125a mimetics	miR-125a	Cancer therapy	Reprogramming of macrophages within the tumor microenvironment
Ramchandani D. et al. [73]	miR708-NP	miR-708	Cancer therapy	Suppression of tumor growth and metastasis
Chioccioli M. et al. [74] Montgomery R.L. et al. [75]	Remlarsen, MRG-229	miR-29	Liver and lung fibrosis therapy	Restoration of miR-29 levels and reduction of fibrosis in preclinical models
Antagonists				
Montgomery R.L. et al. [76]	anti-miR-208a	miR-208a	Heart failure therapy	Prevention of pathological cardiac remodeling and Myh7 activation in response to pressure overload
Thum T. et al. [77]	anti-miR-21	miR-21	Cardiovascular disease and cancer therapy	Inhibition of fibrosis and improvement of cardiac function in preclinical studies
Janssen H.L. et al. [78], Drury R.E. et al. [79], Bonneau E. et al. [80]	Miravirsen (RG-101)	miR-122	Hepatitis C therapy	Reduction of viral load in patients with hepatitis C
Seto A.G. et al. [81]	Cobomarsen (MRG-106)	miR-155	Cutaneous T-cell lymphoma (CTCL) therapy	Antitumor activity with good tolerability

rejection in heart and kidney transplant recipients; it can detect 266 single nucleotide polymorphisms through sequencing to accurately quantify the proportion of donor dd-cfDNA), and Immuknow Cylex (used to assess the level of immunosuppression in kidney transplant recipients [88]).

Despite their clinical promise, these platforms have not yet been widely implemented in routine transplant practice. This limitation is largely due to their effectiveness primarily in detecting clinically manifest rejection, while remaining insufficiently sensitive for early identification of subclinical processes [89].

Moving beyond the concept that a single gene is linked to a single disease has led to the emergence of the modern framework of molecular networks and their integration within the body, encompassing, among other elements, the collective biological functions of microRNAs [90, 91]. It is likely that this approach will, over time, provide answers to some of the most complex questions in transplant medicine.

CONCLUSION

Epigenetics is a rapidly advancing field that has become firmly established in modern biotechnology, medicine, and agriculture. It investigates heritable properties of organisms that are not associated with changes in DNA sequence but are indirectly encoded within the genome. Epigenetic mechanisms – including DNA methylation, histone modifications, and regulation by miRNAs – play a central role in controlling gene expression, cell differentiation, and disease development. This field opens up new opportunities for both diagnosis and treatment.

Based on the results of this systematic review of the literature published in Russian and international electronic databases, focusing on the role of epigenetic mechanisms in solid organ transplantation, a clear and sustained trend toward the comprehensive development of this field of genetics is evident. Although the safety and efficacy of using miRNA as therapeutic targets remain to be fully established, the advantages of circulating miRNAs as diagnostic biomarkers are no longer in doubt.

Owing to their stable levels in the patient's body and their direct involvement in the epigenetic regulation of cellular function, miRNAs open new horizons for understanding the epigenetic determinism of potential organ recipients and donors regarding various transplant outcomes, thereby expanding the potential for effective targeted therapy for recipients.

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CURRENT ISSUES IN THE PREVENTION AND MANAGEMENT OF OXIDATIVE STRESS IN ACUTE RENAL ISCHEMIC-REPERFUSION INJURY

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Renal ischemia–reperfusion injury (IRI), which develops during organ-preserving kidney surgery and particularly during kidney transplantation (KT), remains a major challenge in urology and transplantology, as it can lead to progression of acute kidney injury and chronic graft dysfunction. Conservative strategies aimed at minimizing oxidative stress are especially important in situations where surgical options are limited. In transplantology, IRI is of particular relevance, as KT is the treatment of choice for patients with end-stage renal disease, significantly improving both quality of life and survival compared with renal replacement therapy. A critical stage of the transplantation procedure involves donor organ ischemia (warm and cold), followed by reperfusion after restoration of blood flow in the recipient. The severity of IRI directly influences graft function and is a key risk factor for delayed graft function and acute rejection [1, 2]. Therefore, the search for effective the search for to prevent and correct IRI is critical to improving kidney transplant outcomes. **Objective** to systematize current knowledge on the potential of conservative methods for correcting renal IRI caused by excessive reactive oxygen species (ROS) during organ-preserving kidney surgery and KT under conditions of warm ischemia. **Methods.** A systematic analysis of literature published over the past 10 years was conducted using the PubMed search engine, the Cochrane Library database of evidence-based medicine, and the Scopus unified bibliographic and abstract database of peer-reviewed scientific literature. Particular emphasis was placed on randomized studies evaluating drugs or newly synthesized compounds that suppress ROS formation and restore or enhance the body's antioxidant capacity. **Conclusion.** At the current stage of medical science, considerable attention is focused on substances capable of blocking the molecular mechanisms involved in mitochondrial membrane pore opening, as well as on agents that suppress ROS formation through inhibition of NADPH oxidase and xanthine oxidase. The therapeutic potential of exogenous enzyme preparations (such as superoxide dismutase and catalase), low-molecular-weight catalytic ROS scavengers, and non-enzymatic antioxidants – including supraphysiological doses of ascorbic acid and mitochondria-targeted agents such as mitoquinone and elamipretide – is actively being investigated. In the future, the results of these studies may form the basis for the development of effective antioxidant strategies for the prevention and treatment of renal IRI during organ-preserving kidney surgery and transplantation.

Keywords: renal ischemia–reperfusion injury, reactive oxygen species, oxidative stress, antioxidant protection, kidney transplantation.

1. INTRODUCTION

In urological surgery, acute renal ischemia–reperfusion injury (IRI) is an anticipated consequence of organ-preserving kidney procedures. Such injury may occur during surgical management of benign renal tumors, selected cystic kidney lesions, renal echinococcosis, nephrolithiasis refractory to conservative treatment, nephrotuberculosis, traumatic injury to the renal parenchyma, and, most commonly, localized renal cell carcinoma [3–7].

The issue of renal IRI is equally significant in transplantology. Kidney transplantation (KT) remains the

treatment of choice for patients with end-stage chronic kidney disease, offering substantial improvements in both quality of life and survival compared with long-term renal replacement therapy [8, 9]. A critical phase of transplantation involves ischemia of the donor organ – both warm and cold – followed by reperfusion after restoration of blood flow in the recipient. The severity of renal IRI has a direct impact on graft function and represents a major risk factor for delayed graft function and acute rejection [1, 2, 10].

Therefore, the search for effective strategies for prevention and mitigation of renal IRI is critical to impro-

ving outcomes in both kidney resection and transplantation.

The immediate cause of IRI is the restoration of renal blood flow after a period of suspension. Temporary cessation of perfusion is required during surgery to prevent intraoperative parenchymal bleeding when resecting the affected renal segment or constructing vascular anastomoses. Thus, reperfusion is preceded by warm ischemia induced by clamping the renal artery. Although both ischemia and subsequent reperfusion are essential steps in the surgical procedure – first minimizing blood loss and then restoring organ perfusion – they also initiate a cascade of mechanisms leading to renal tissue injury.

During ischemia, the primary injurious factor is hypoxia (or anoxia), whereas reperfusion introduces normoxic conditions that paradoxically exacerbate tissue injury. The consequences of primary injury and the principal mechanisms of secondary cellular alteration include: 1) Under hypoxic conditions – cellular hypoergia due to impaired ATP production, dysfunction of ATP-dependent ion pumps, and increased generation of reactive oxygen species (ROS), activating free radical-mediated damage to proteins, lipids, carbohydrates, nucleic acids, mitochondria, and cell membranes; 2) Upon reperfusion – an even more pronounced, “avalanche-like” overproduction of ROS.

Subsequent downstream injury mechanisms involve increased permeability of cellular and organelle membranes (including lysosomal membrane destabilization with release of proteolytic enzymes), alterations in redox balance, mitochondrial structural and functional impairment, disturbances in ionic homeostasis (particularly potassium and calcium), metabolic acidosis, and cellular swelling. Edematous cells may compress adjacent microvessels, leading to microcirculatory disorders.

These changes represent a universal, nonspecific cellular response to injury and occur in virtually all cell types, including renal parenchymal cells (nephrocytes), regardless of whether the initiating insult is ischemia or reperfusion [11–13].

2. PATHOGENESIS OF RENAL ISCHEMIC-REPERFUSION INJURY

Ischemia and subsequent reperfusion during organ-preserving renal surgery and KT represents one of the leading causes of acute kidney injury in the early postoperative period [14–16]. The predominant structural damage affects the nephron, particularly the tubular epithelial cells [17]. Over time, this injury may contribute to progression of chronic kidney disease and ultimately chronic renal failure [18–22].

The principal mediators of tissue injury during ischemia and reperfusion are ROS – electrically unstable and highly reactive molecules capable of damaging cellular structures. The primary sources of ROS include the mi-

tochondrial electron transport chain (ETC), as well as enzymatic reactions catalyzed by xanthine oxidase (XO), nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), myeloperoxidase (MPO), and others.

Under conditions of excessive ROS generation – particularly during reperfusion – the imbalance between prooxidant production and antioxidant defense leads to oxidative stress, which plays a central role in cellular and tissue injury. Accordingly, two principal pharmacological strategies are distinguished in the prevention and correction of IRI: 1) Reduction or suppression of ROS production by targeting the mitochondrial ETC and inhibiting ROS-generating oxidases; 2) Neutralization and elimination of ROS by restoring or enhancing the endogenous antioxidant defense system of the cell and extracellular environment [23].

3. MAIN TARGETS FOR PHARMACOLOGICAL CORRECTION OF OXIDATIVE STRESS IN RENAL IRI

3.1. Suppression of the formation of reactive oxygen species

3.1.1. Inhibition of mitochondrial permeability transition pore (mPTP) opening

Under physiological conditions, ETC is responsible for generating up to 90% of all reactive oxygen species (ROS) as metabolic by-products [23, 24]. During ischemia – and particularly upon reperfusion – ROS production increases dramatically. This surge, combined with ischemia–reperfusion–induced disorders in ATP biosynthesis and intracellular calcium homeostasis, promotes the formation of pores at contact sites between the inner and outer mitochondrial membranes. These pores, known as mitochondrial permeability transition pores (mPTP), alter mitochondrial membrane permeability [25–30].

The formation of mPTP leads to further disruption of ionic homeostasis, especially calcium balance, and impairs the energy-synthesizing function of mitochondria. At the same time, it sustains and amplifies ROS generation, thereby perpetuating a vicious cycle of mitochondrial dysfunction. Persistent and uncontrolled mPTP opening can ultimately trigger mitochondrial-dependent apoptotic pathways, contributing to irreversible cell injury.

As defined by Zhou et al. (2023), the opening of mPTP “is a major determinant of mitochondrial dysfunction to induce cellular damage or death” [31]. In light of current evidence, pharmacological inhibition of mPTP opening represents one of the most promising therapeutic strategies for the prevention and mitigation of IRI [32–34].

MPTP is a multiprotein complex that includes the voltage-dependent anion channel (VDAC, also known as porin), the adenine nucleotide translocator (ANT), the peripheral benzodiazepine receptor, and the chaperone protein cyclophilin-D (CypD), one of the 18 known human cyclophilins. Among these components, CypD –

also referred to as peptidyl-prolyl cis-trans isomerase F – plays a central regulatory role in controlling mPTP opening from the inner mitochondrial membrane [35].

Fayaz et al. (2015) metaphorically described cyclophilin-D as “the key to the death door” [36]. Accordingly, pharmacological inhibition of CypD activity is a rational strategy for preventing mPTP opening in pathological conditions associated with mitochondrial dysfunction [37], including renal IRI [38].

One of the earliest agents identified as a CypD inhibitor was cyclosporin A (CsA), a calcineurin inhibitor. CsA is a neutral, lipophilic cyclic undecapeptide composed of 11 amino acids, with a molecular weight of 1202.6 Da, and exhibits high affinity for cyclophilins. Since the early 1980s, it has been widely used as an immunosuppressive and immunomodulatory agent in immunology, transplantation, and oncology [39, 40].

Preclinical studies are currently underway to investigate the potential role of CsA in the treatment of renal IRI. In experimental models of renal IRI in small laboratory animals – typically involving unilateral nephrectomy followed by 30 minutes of occlusion of the renal artery supplying the contralateral kidney and subsequent reperfusion for 60–90 minutes – several protective effects of CsA have been reported. Postconditioning with CsA has been shown to attenuate renal damage caused by IRI [41]. Specifically, CsA administration enhances the calcium retention capacity of renal mitochondria and improves oxidative phosphorylation following reperfusion, although these effects appear to be dose- and time-dependent [42]. In rat models, CsA treatment has also been associated with reduced apoptotic cell counts, decreased severity of microstructural alterations in renal tissue, preservation of tubular epithelial cell viability, and maintenance of overall renal function [43, 44].

However, these findings are not universally supported. For example, Lee et al. (2015) reported evidence of CsA-associated nephrotoxicity, including cytoplasmic vacuolization in glomerular endothelial cells, reduced glomerular size, and a marked decrease in renal blood flow during reperfusion [45]. Similarly, Oliveira et al. (2019), assessing serum creatinine, urea, sodium levels, and histopathological changes in a rat model of 30-minute renal ischemia followed by reperfusion, concluded that “CsA was incapable of preventing the deleterious effects of ischemia-reperfusion injury in rat kidneys” [46].

Briston et al. (2019) attributed the inconsistent and often negative outcomes of preclinical evaluations of CsA to its narrow therapeutic window and the challenges associated with precise dosing, in addition to its inherent nephrotoxic and immunosuppressive properties. This limitation was also highlighted in studies investigating the cardioprotective activity of CsA in myocardial IRI, where the difference between concentrations yielding beneficial effects and those producing adverse outcomes did not exceed 0.2 μM .

Furthermore, it was found that inactivation of CypD does not completely prevent the opening of mPTP but rather delays it until more severe disturbances in intracellular calcium homeostasis develop.

Despite these limitations, inhibition of mPTP opening remains a therapeutically relevant and promising strategy, necessitating the development of alternative approaches [47]. Current research in this area is progressing along two main directions. The first involves the development of novel CypD inhibitors, including derivatives of CsA and sanglifehrin A, as well as small-molecule, non-peptide, low-molecular-weight cyclophilin inhibitors. The second focuses on identification and development of compounds capable of inactivating mPTP through CypD-independent mechanisms [47, 48].

As noted by Briston et al. (2019), advances in these areas could significantly reshape therapeutic strategies not only for IRI but also for a broad spectrum of diseases in which mPTP opening plays a central role in pathogenesis [47].

3.1.2. Inhibition of NADPH oxidases (NOX)

NOX are a family of enzymes that catalyze the formation of the coenzyme NADP^+ from NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate) in the reaction of NADPH with molecular oxygen. The products of the reaction, in addition to NADP^+ , are atomic hydrogen and the superoxide anion radical $\text{O}_2^{\cdot-}$. The generated superoxide in turn, induces the formation of other ROS.

NOX are expressed and localized in all cells capable of phagocytosis (monocytes and macrophages, neutrophils, eosinophils, labrocytes), their biological role is associated with the ability to generate $\text{O}_2^{\cdot-}$ and consists in the implementation of phagocytic defense as such.

In addition, these enzymes may be present in non-phagocytic cells – the so-called non-phagocytic constitutively active NOX, which are always present in the cell, constantly forming $\text{O}_2^{\cdot-}$ and, thereby participating in the regulation of cell proliferation and differentiation, as well as the processes of expression of certain genes at the transcription stage.

NOX exist in the form of several isoforms, among which there are 5 multiprotein NOX complexes (NOX-1–NOX-5) and 2 DUOX dual oxidase complexes (DUOX 1 and 2). NOX consists of four cytosolic and two membrane subunits. Phagocytic NOX are activated through the fusion of membrane and cytosolic components after the latter are translocated to the plasma membrane. The membrane subunits (gp91phox and p22phox) determine the catalytic properties of the activated enzyme, whereas the cytosolic subunits (p47phox, p67phox, p40phox, Rac) perform mainly regulatory functions [49–55].

In the ischemic region, phagocytosis of molecular and cellular structures altered and/or destroyed as a result of oxygen deprivation naturally triggers the activation of

phagocytic NOX, leading to the formation of $O_2^{\cdot-}$ and other ROS. Under conditions of subsequent reperfusion, the generation of ROS increases exponentially, accompanied by intensified free radical damage to nucleic acids, proteins, and lipids, formation of new phlogogens, recruitment of additional phagocytic cells, and further amplification of ROS production. These processes ultimately result in the establishment of a vicious cycle underlying the mechanisms of IRI [56, 57].

Consequently, suppression of ROS formation through NOX inhibition may be a relevant strategy for the prevention and treatment of diseases associated with oxidative stress, including various forms of IRI [58–61].

Apocynin (acetovanillin, 4-hydroxy-3-methoxyacetophenone), an aromatic compound of plant origin, first described in 1883 by German pharmacologist Oswald Schmiedeberg, and diphenylenedione chloride, an aromatic halogen-containing compound, were among the first substances for which NOX inhibitory properties were confirmed. The mechanism of action of apocynin is associated with its ability to inhibit NOX activation by blocking the translocation of the cytosolic subunits p47phox and p67phox to the membrane, whereas diphenylenedione chloride limits the electron transfer function of NOX enzymes.

However, as noted in the review by Wu et al. (2018), both substances possess additional biological activities, including antioxidant effects and can inhibit nitric oxide synthesis, xanthine oxidase, cytochrome P450 reductase, and mitochondrial enzymes. Consequently, it is difficult to unequivocally attribute the protective effects of apocynin and diphenylenedione chloride against IRI solely to inhibition of the NOX enzyme [62].

According to Chokri et al. (2020), over the past decade, several thousand molecules with presumed NOX-inhibitory activity have been screened. From these studies, a number of compounds demonstrating actual inhibitory effects have been identified and classified according to their degree of specificity toward individual NOX isoforms, ranging from low to highly selective [63]. Examples include APX-115 (3-phenyl-1-(pyridin-2-yl)-4-propyl-1-5-hydroxypyrazole HCl), an orally administered compound that has shown nephroprotective activity in experimental models of diabetic kidney injury in mice [64]; NOS31, produced by *Streptomyces* sp., which selectively inhibits NOX-1 activity [65]; and GLX7013114, a selective NOX-4 inhibitor [66], among others. Among the compounds currently investigated for potential clinical use as NOX inhibitors, only one – GKT137831, a direct inhibitor of NOX-1 and NOX-4 – has advanced to clinical trials [67], but the results have not yet been published.

3.1.3. Inhibition of xanthine oxidase

During ischemia and reperfusion, ROS are generated through the xanthine oxidase reaction involved in pu-

rine nucleotide catabolism. Under hypoxic conditions, suppression of ATP biosynthesis occurs alongside an increase in intracellular calcium levels. This rise in Ca^{2+} activates Ca^{2+} -dependent proteases, which induce partial proteolysis of xanthine dehydrogenase – an enzyme that normally catalyzes the formation of uric acid from xanthine under physiological conditions. As a result of this proteolytic modification, xanthine dehydrogenase is irreversibly converted into xanthine oxidase (XO). The XO enzyme subsequently catalyzes the conversion of xanthine to uric acid with simultaneous formation of the superoxide anion radical ($O_2^{\cdot-}$).

At present, the xanthine oxidase reaction is considered one of the principal sources of superoxide generation during oxygen deprivation and subsequent reoxygenation [68]. Consequently, the XO enzyme is a promising pharmacological target for the prevention and mitigation of IRI through the use of XO inhibitors.

Currently available inhibitors of xanthine oxidase include Allopurinol, Oxypurinol (alloxanthine), Febuxostat, and Topiroxostat [69]. These drugs are widely used in clinical practice for the treatment and prevention of hyperuricemia of various origins and gout. However, the feasibility, efficacy, and safety of their use for the prevention and treatment of IRI remain under study.

Allopurinol (a structural isomer of hypoxanthine, a natural endogenous purine) and oxypurinol (a primary metabolite of allopurinol), as pharmacological agents capable of limiting ROS formation through inhibition of xanthine oxidase during renal ischemia–reperfusion, have been investigated in experimental studies by Eremina et al. (2024), Choi et al. (2015), Prieto-Moure et al. (2017), Kang et al. (2023), and Soliman et al. (2023), among others. In these preclinical randomized controlled studies, renal IRI in small laboratory animals (rats or mice) was modeled by subjecting one kidney to approximately 30 minutes of total ischemia while performing nephrectomy of the contralateral kidney. The efficacy of the investigated drugs was evaluated using histological analyses aimed at identifying structural changes in renal tubules and apoptotic cells, together with assessments of renal function, severity of oxidative stress and inflammatory responses, and immunological parameters. The findings of these studies demonstrated the clear therapeutic potential of allopurinol and oxypurinol in reducing renal IRI [70–74].

The nephroprotective properties of febuxostat under conditions of renal ischemia and subsequent reperfusion were investigated by Tsuda et al., with the results published in 2012. The study was conducted on Sprague–Dawley rats and included a control group, a placebo group, and a group treated with febuxostat. According to the findings, animals receiving febuxostat showed significantly lower levels of xanthine oxidase activity and oxidative stress compared with those in the control and placebo groups. Oxidative stress was assessed by

measuring urinary levels of nitrotyrosine, thiobarbituric acid, and 8-isoprostane. In addition, febuxostat-treated animals exhibited improved renal function – evidenced by lower serum creatinine and urea levels – together with reduced tubular damage, a weaker monocyte–macrophage inflammatory response, and fewer apoptotic tubular epithelial cells based on histological examination. As noted by Tsuda et al., these findings collectively indicate the protective potential of febuxostat and support further investigation of this agent as a potential drug for renal protection during IRI [75].

3.2. Neutralization of reactive oxygen species and enhancement of antioxidant protection

Among pharmacological agents capable of neutralizing or removing ROS from the body's internal environment, two major groups are distinguished: enzymatic and non-enzymatic antioxidant drugs [76].

3.2.1. Use of enzymatic antioxidants (SOD, catalase) and their mimetics

Enzymatic antioxidant agents include exogenously administered synthetic analogues of endogenous enzymes that constitute the cellular antioxidant defense system. These enzymes – such as superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferases, thioredoxins, and peroxiredoxins – play a key role in regulating free radical oxidation processes under physiological conditions [77].

Superoxide dismutase (SOD) catalyzes the conversion of the superoxide anion radical into molecular oxygen and hydrogen peroxide according to the reaction: $2\text{H}^+ + 2\text{O}_2^{\cdot -} \rightarrow \text{O}_2 + \text{H}_2\text{O}_2$. Subsequently, catalase (CAT) decomposes hydrogen peroxide into water and molecular oxygen: $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$. Both enzymes are highly specific.

Based on the metal cofactors present in their active sites, SOD isoforms in the human body are classified into copper/zinc-containing SOD (Cu/Zn-SOD) and manganese-containing SOD (Mn-SOD). The enzymatic antioxidant activity of SOD was first described in 1968 by Irwin Fridovich and Joe McCord. Shortly thereafter, research into SOD-based therapeutics began, with their clinical potential being actively explored from the late 1980s.

Initial sources of SOD-based preparations were animal tissues and organs. Subsequently, alternative production methods were developed, including microbiological synthesis [78] using specific strains of yeast, yeast-like fungi, and bacteria, as well as genetic engineering techniques [79, 80]. These methods enabled the isolation of SOD from microbial cells followed by purification using chromatographic techniques [81].

Certain plants also serve as SOD sources, including some melon varieties [82], *Rauvolfia serpentina* [83], and Scots pine seedlings [84], among others. However, native SOD preparations exhibit limited stability and low resistance to proteolysis, with a circulating half-life of no more than 6 minutes. This limitation was recognized soon after methods for isolating SOD from animal and plant tissues were developed. It was precisely this circumstance at that time (the late 1980s and 1990s) that was linked to the highly inconsistent results observed in experimental studies evaluating the therapeutic potential of native (natural) SOD in animal models of IRI affecting various tissues and organs, including the kidneys. Subsequently, more stable and long-acting formulations were developed, including modified forms created by conjugating native SOD with polyethylene glycol (PEG). In addition, low-molecular-weight compounds that mimic the antioxidant activity of SOD – so-called SOD mimetics, which are synthetic metal complexes capable of selectively catalyzing the *in vivo* neutralization of superoxide anion ($\text{O}_2^{\cdot -}$) to form hydrogen peroxide and molecular oxygen – were introduced [85].

As noted by R.G. Goncharov et al. (2023), evidence from specialized medical journals supports the efficacy of exogenous PEG-modified Cu/Zn-SOD in experimental models of IRI affecting skeletal muscle, heart, brain, and liver [86]. Investigations into the effects of native and conjugated SOD preparations in renal IRI began in the late 1980s, with findings reported in studies published between 1985 and 1996 (identified through PubMed database searches). All of these studies were experimental, and most concluded that SOD preparations exert a protective effect, significantly reducing the risk and severity of acute kidney injury associated with renal ischemia–reperfusion.

Despite these encouraging findings from the final decades of the 20th century, this line of research has seen limited advancement since the early 2000s. In this regard, Veronese et al. (2002) observed that both native and PEG-conjugated SOD reduce reperfusion injury in renal and hepatic ischemia. Notably, they highlighted the paradox that, despite a substantial body of positive experimental evidence supporting PEG-SOD, no clinically approved therapeutic applications for humans had been developed at the time [87]. Meanwhile, SOD represents the principal enzymatic defense against the damaging effects of the superoxide anion ($\text{O}_2^{\cdot -}$) in living organisms [88]. As noted in the literature, “...*despite limitations, the SOD enzyme has proved as a powerful tool against diseases, and various forms of conjugates and mimetics have been developed and reported to make it more efficient. Extensive studies are needed in this direction for use of natural SOD-based therapeutics for the prevention and cure of diseases...*” (P. Saxena et al., 2022) [89].

During the 1980s and 1990s, and into the early 2000s, several classes of catalytic ROS scavengers were develo-

ped. These compounds, designed as functional mimetics of SOD and CAT, include manganese-based complexes with selenium, porphyrins, corrole derivatives, and non-aromatic macrocycles. Many of these agents are capable of simultaneously neutralizing both superoxide anion radicals and hydrogen peroxide. While they exhibit broadly comparable SOD-like activity, their catalase-like activity varies depending on structural modifications; for example, the EUK-8 derivative (manganese N,N-bis(salicylidene)ethylenediamine chloride) demonstrates notably enhanced catalase activity [90–96].

The therapeutic potential of several of these compounds in renal IRI has been demonstrated in experimental studies by Gianello et al. (1996), Chatterjee et al. (2004), and others [97, 98]. More recent research has focused on improving existing compounds and developing new manganese-based complexes with novel ligands that function as highly efficient catalytic ROS scavengers and dual SOD/CAT mimetics. Numerous studies have described their biosynthesis, molecular structure, physicochemical properties, and antioxidant activity, supporting their potential for preclinical evaluation as pharmacological agents for the prevention and treatment of IRI in various organs, including the kidneys [99–109].

3.2.2. Use of non-enzymatic antioxidants (ascorbic acid, α -tocopherol, N-acetylcysteine)

As part of ongoing efforts to prevent and treat the consequences of IRI, including in the kidneys, increasing attention is being given to the therapeutic potential of non-enzymatic antioxidant agents. These include ascorbic acid, α -tocopherol, and N-acetylcysteine, as well as mitochondria-targeted compounds such as mitoquinone and elamipretide.

According to Spoelstra-de Man et al. (2018), recent years have seen growing interest in the potential use of ascorbic acid for the pharmacological management of IRI. Its antioxidant efficacy is attributed to its ability to scavenge peroxynitrites (ONOO), hypochlorous acid (HOCl), and superoxide anion radicals. In addition, ascorbic acid inhibits the activity of xanthine oxidase and NADPH oxidases, and facilitates the regeneration of key molecules such as α -tocopherol and tetrahydrobiopterin from dihydrobiopterin. It also supports endothelial nitric oxide synthase function, thereby enhancing nitric oxide bioavailability in the vascular endothelium, among other protective effects [110].

According to studies by Fowler et al. (2014), Zabet et al. (2016), Marik et al. (2016), and others, parenteral administration of high (supraphysiological) doses of vitamin C has been associated with accelerated recovery in both experimental animals and patients in randomized preclinical and clinical studies. Beneficial effects have been reported in conditions such as severe sepsis and septic shock [111–113], post-cardiac arrest states [114], myocardial infarction [115], reperfusion arrhythmias

[116, 117], and ischemia–reperfusion brain injury [118]. Several studies have also reported a significant therapeutic effect of high-dose parenteral ascorbic acid in experimental models of IRI [119–121]. However, despite accumulating evidence supporting its potential efficacy, a number of important issues remain unresolved. These include optimal dosing strategies, timing and duration of administration, and the role of combination therapy with other agents, all of which continue to be subjects of ongoing debate [122].

The antioxidant activity of α -tocopherol has been demonstrated in numerous studies. *In vivo*, it regulates the intensity of lipid peroxidation by scavenging lipid and lipid peroxyl radicals, effectively acting as a “chain-breaking” antioxidant. In addition, α -tocopherol contributes to the regeneration of coenzyme Q and modulates the activity of phospholipase A₂, thereby indirectly influencing arachidonic acid metabolism and the synthesis of prostaglandins and leukotrienes. Under conditions of increased oxidative stress, it helps preserve the biological activity of vitamin A by interrupting peroxidation chains involving polyunsaturated fatty acids. Furthermore, as a synergist of selenium – a cofactor of glutathione peroxidase – it plays an indirect role in the detoxification of lipid hydroperoxides.

Due to its pronounced lipophilicity and hydrophobicity, endogenous α -tocopherol is predominantly localized within the lipid bilayer of biological membranes, contributing to their structural stability. However, these same physicochemical properties limit its clinical use: α -tocopherol cannot be readily incorporated into intravenous perfusion solutions, and when administered parenterally by other routes, its antioxidant effects typically become evident only after 17–24 hours [123].

N-acetylcysteine (NAC) is a cysteine derivative containing a sulfhydryl group and has been used in clinical practice for over 60 years, initially as a mucolytic agent. Subsequently, its antioxidant properties were identified and extensively studied. NAC exerts its antioxidant effects through multiple mechanisms. First, it directly neutralizes ROS via its free sulfhydryl group. Second, it serves as a precursor for glutathione synthesis, contributing cysteine following its deacetylation. Glutathione, synthesized from glutamate, cysteine, and glycine, is a central component of the cellular defense system against oxidative stress [124].

To date, the efficacy of NAC in renal IRI has been primarily investigated in experimental models involving 30–45 minutes of ischemia followed by 1–24 hours of reperfusion. These studies have evaluated parameters such as renal function, mitochondrial activity, mitochondrial homeostasis, and histological changes. Overall, the findings support the protective role of NAC in this context and underscore the need for further investigation in clinical settings [125–128].

3.2.3. Mitochondria-targeted therapy (mitoquinone, elamipretide)

In experimental models of renal IRI in small laboratory animals, Liu et al. (2021), Mao et al. (2022), and others observed pronounced nephroprotective effects following intravenous administration of the mitochondria-targeted antioxidant mitoquinone (MitoQ). This compound consists of ubiquinone (coenzyme Q10) conjugated to a triphenylphosphonium cation, which facilitates its targeted delivery to mitochondria.

Under IRI conditions, MitoQ effectively protected the kidneys from the negative effects of free radicals, maintained adequate renal function, reduced mitochondrial damage and the formation of ROS. In addition, it reduced apoptosis in proximal tubular epithelial cells, and contributed to the restoration of mitochondrial membrane potential and ATP synthesis [129–132].

Elamipretide, also known as peptide SS-31, is a mitochondria-targeted tetrapeptide synthesized in 2000 by Hazel Szeto and Peter Schiller (reflected in the abbreviation “SS”). Structurally, it is D-Arg-dimethylTyr-Lys-Phe-NH₂ and is capable of selectively accumulating within mitochondria, where it exerts antioxidant effects by neutralizing mitochondrial ROS [133].

According to studies by Szeto et al. (2017) and Huang (2024), administration of SS-31 for six weeks following experimentally induced renal ischemia in rats primarily preserves mitochondrial integrity, demonstrating a pronounced mitoprotective effect. This is accompanied by reduced expression of inflammatory markers, preservation or restoration of the structure of tubular epithelial cells, glomerular endothelial cells, and podocytes, decreased rates of apoptosis and necrosis in tubular epithelium, and inhibition of glomerulosclerosis and interstitial fibrosis.

These findings support the concept that mitochondrial protection with SS-31 represents a promising therapeutic strategy. As highlighted by Szeto et al. (2017), targeting mitochondrial dysfunction in IRI may offer a novel approach to preventing the progression to chronic kidney disease following renal IRI [134–136].

4. CURRENT CLINICAL AND EXPERIMENTAL APPROACHES TO MINIMIZING RENAL IRI

In addition to pharmacological strategies aimed at correcting oxidative stress, hypothermia plays a fundamental role in the prevention of renal IRI, particularly in the context of transplantation. Topical cooling of the isolated kidney using saline or preservation solutions supplemented with pharmacological cryoprotectants remains the gold standard for extending the organ's tolerance to ischemia and reducing the severity of reperfusion injury [137, 138]. This approach is designed to decrease tissue metabolic activity, thereby limiting the production of ROS and the development of lactic

acidosis during the ischemic phase. Russian research in cryobiology and organ preservation have also contributed significantly to the development of effective perfusion and storage solutions for transplant organs [139, 140].

Among pharmacological interventions used in clinical practice, N-acetylcysteine (NAC) has attracted considerable attention. Owing to its antioxidant properties and its ability to enhance intracellular glutathione levels, NAC has been evaluated in multiple studies as a potential agent for prevention of contrast-induced nephropathy and for mitigating renal IRI [141, 142]. However, its routine clinical application for prevention of renal IRI in kidney transplantation still requires validation through large-scale, well-designed clinical trials.

Thus, the most promising strategy appears to be a combined approach that integrates mechanical methods of organ protection (such as hypothermia) with adjunct pharmacotherapy targeting key links in oxidative stress pathogenesis.

5. CONCLUSION

During organ-preserving renal surgeries and, in particular, kidney transplantation under conditions of warm ischemia, the risk of renal IRI is present in all patients. Therefore, such procedures should ideally incorporate preventive and corrective strategies aimed at minimizing IRI-induced structural and functional impairments to the kidney.

The first kidney resection in the history of organ-preserving surgery was performed in 1887 by the surgeon Vincenz Czerny (German Bohemia). Since that time, and up to the present day (the end of the first quarter of the 21st century), clinically validated and effective methods for protecting organs and tissues – including the kidney – from IRI have not yet been fully developed. However, the active search for such technologies has never ceased and continues today.

Given the central role of ROS in the free-radical mechanisms underlying IRI, numerous studies have focused on developing pharmacological strategies for organ protection that target ROS generation, neutralization, and clearance. At the current stage of medical science, particular attention is given to agents capable of inhibiting the molecular mechanisms of mitochondrial membrane permeability transition pore opening, as well as suppressing ROS production through inhibition of NADPH oxidase and xanthine oxidase.

The therapeutic potential of exogenous enzymatic preparations (e.g., superoxide dismutase, catalase), low-molecular-weight catalytic ROS scavengers, and non-enzymatic antioxidants such as supraphysiological doses of ascorbic acid, as well as mitochondria-targeted agents like mitoquinone and elamipretide, are being investigated. It is anticipated that the outcomes of these studies will contribute to the development of effective antioxidant-based strategies for the prevention and treat-

ment of renal IRI during organ-preserving kidney surgery and transplantation.

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METHOD FOR THE ISOLATION OF HUMAN PANCREATIC ISLETS FOR CLINICAL TRANSPLANTATION IN TYPE 1 DIABETES MELLITUS

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Islet transplantation is a modern method of treating severe type 1 diabetes mellitus (T1D). However, multiple factors can negatively affect both the efficiency of islet isolation and subsequent transplantation outcomes. Consequently, ongoing efforts to optimize isolation techniques have led to the emergence of new unique protocols. The **objective** of this study is to evaluate the effectiveness of a modified method for isolating pancreatic islets (islets of Langerhans) from deceased donors for clinical transplantation in recipients with T1D. **Materials and methods.** Pancreatic islets were isolated using a modified technique that excluded the use of the Ricordi islet isolator during the enzymatic digestion stage, as well as density gradient centrifugation during the purification stage. Islet identification was performed using dithizone staining. Islet viability was assessed by fluorescent staining with acridine orange and propidium iodide. Functional activity was evaluated by determining the stimulation index using enzyme-linked immunosorbent assay (ELISA). Histological examination of the native pancreas ($n = 3$) included routine staining, as well as immunohistochemical analysis targeting the main types of islet cells. **Results.** Pancreatic islets isolated from the pancreas of deceased donors using the modified technique retained their structural integrity and demonstrated variability in size and shape. The total islet yield was $630,000 \pm 30,000$, with a viability rate of $90 \pm 3\%$ and a stimulation index of 1.41 ± 0.01 . These findings indicate the high quality of the isolated biomaterial and confirm the ability of β -cells to respond to changes in glucose content. Morphological assessment of the donor pancreas demonstrated preserved structural integrity of the islet apparatus, supporting the potential for obtaining viable and functionally active islets. The efficiency of islet isolation during enzymatic pancreatic processing was also confirmed. **Conclusion.** Optimization of the methodological approach enabled the development and validation of a modified technique for processing the pancreas of deceased donors, resulting in the isolation of a substantial number of viable and functional islets of Langerhans. The characteristics of the obtained islet graft support its suitability for clinical transplantation in T1D patients.

Keywords: pancreatic islets, pancreas, transplantation, type 1 diabetes mellitus.

INTRODUCTION

Pancreatic islet (PI) transplantation can be considered an alternative to whole-pancreas transplantation, as it is less invasive, sparing patients from major surgery [1, 2]. It represents a modern treatment for type 1 diabetes (T1D), particularly in patients complicated by a high susceptibility to severe hypoglycemia and glycemic instability [3, 4].

Clinical studies conducted over the past three decades suggest that restoration of endogenous insulin production through islet transplantation – which secretes a full spectrum of bioactive peptides – may achieve metabolic outcomes that are not attainable with conventional insulin therapy alone [5–7].

Evidence also indicates the beneficial effects of islet transplantation in slowing the progression of secondary

complications of T1D, including improved outcomes in diabetic nephropathy and reduced progression of diabetic retinopathy and neuropathy [8, 9]. It has been found that islet transplantation may support sustained graft function and prolong the survival of concomitant kidney allografts in most recipients [10].

PI transplantation in patients with T1D is primarily based on the Edmonton Protocol introduced by Shapiro et al. in 2000 [11]. The key stages of successful PI isolation include: 1) Enzymatic digestion of the pancreas, which leads to dissociation of pancreatic tissue and release of the islets from the surrounding exocrine tissue; 2) Purification of isolated islets from exocrine tissue using techniques such as incubation, filtration, and density-gradient centrifugation [12].

After isolation and purification, the islet cell suspension is evaluated for identity, purity, viability, functionality,

and sterility in order to ensure the safe transplantation of an adequate quantity of insulin-producing cells into the recipient [1, 5].

It is important to note that, despite substantial advances in clinical PI transplantation, several challenges continue to limit its widespread implementation. Achieving insulin independence in a single recipient often requires islets obtained from more than one donor pancreas because of the complexity and unpredictability of islet isolation outcomes. At each stage of islet preparation, numerous factors may adversely affect isolation efficiency and, consequently, transplant outcomes [13].

According to data from the Collaborative Islet Transplant Registry, the success rate of islet graft isolation is approximately 52.5% [14]. As a result, ongoing research is focused on optimizing islet isolation techniques, leading to the development of new and improved protocols [15–17].

Previously, researchers at Shumakov National Medical Research Center of Transplantology and Artificial Organs developed and tested an original method for isolating the islets of Langerhans from rat and rabbit pancreases, as well as from fragments of human pancreatic tissue. This technique reportedly enabled a 2.1-fold increase in islet yield while preserving their morphofunctional properties when compared with previously published protocols [18–22].

This study evaluated a modified protocol for PI isolation from deceased donors. The proposed technique eliminates the need for the costly Ricordi perfusion system during the enzymatic digestion stage, as well as the use of a Ficoll density gradient during the purification process.

The **aim of the study** was to assess the effectiveness of the modified method for isolating PIs from deceased-donor pancreases for subsequent clinical transplantation in recipients with T1D.

MATERIALS AND METHODS

Pancreatic islet isolation

PIs were isolated using a modified technique developed on the basis of traditional isolation protocols [23, 24].

The pancreas specimens were obtained during multi-organ procurement from deceased donors ($n = 3$) with confirmed brain death who met established criteria for optimal pancreatic donation.

The harvested pancreas was removed from a triple-layer sterile bag containing cold preservation solution (+4 °C) (Custodiol; Dr. Franz Köhler Chemie, Germany) and placed in a sterile tray. Surrounding adipose tissue, blood vessels, and adjacent connective tissue were carefully removed. Under sterile conditions, a sample of the preservation solution was collected for bacteriological assessment of pancreatic explant sterility.

The pancreas was subsequently weighed and rinsed with cold Hanks' Solution (PanEco, Russia) supplemented with a 2% solution containing penicillin, streptomycin, and amphotericin B (Thermo Fisher Scientific, USA). The gland was then cut into 6–8 equal parts. A working enzymatic solution consisting of collagenase NB1 (activity: 20 PZ U/g tissue) and neutral protease NP (activity: 1.5 DMC U/g tissue) (SERVA Electrophoresis GmbH, Germany) was added at a volume of 2 mL per gram of pancreatic tissue.

The stretched pancreatic tissue was mechanically minced into approximately $1 \times 1 \times 1$ cm³ fragments, transferred into a sterile container, and incubated at 37.0 °C for 12–15 minutes until free islets became visible in samples of the digested tissue suspension. Enzymatic activity was terminated by adding three times the volume of cold (+4 °C) Hanks' solution.

The islets were purified from exocrine tissue by filtering the enzymatically digested pancreatic tissue through a 600 µm mesh filter (Sigma-Aldrich, USA). An equal volume of complete culture medium based on DMEM supplemented with 1.0 g/L glucose (PanEco, Russia), 10% fetal bovine serum (HyClone, USA), 1% HEPES buffer, 2 mM alanyl-glutamine (PanEco, Russia), and a 1% antibiotic-antimycotic solution containing penicillin, streptomycin, and amphotericin B (Thermo Fisher Scientific, USA) was added to the filtrate, which was subsequently allowed to sediment for 40 minutes. The filtrate was then centrifuged once for 2.5 minutes at 110 g using a Rotina 38R centrifuge (Hettich Zentrifugen, Germany). Freshly isolated islets obtained from the resulting pellet were resuspended and transferred into culture flasks containing complete growth medium.

The islets were incubated at 37 °C in a CO₂ incubator (Series 8000 WJ, Thermo Fisher Scientific, USA) under humidified conditions (100% relative humidity, 5% CO₂) for 12 hours to enhance purification and facilitate removal of dead or apoptotic cells.

Following incubation, the islets were collected for subsequent infusion by centrifugation for 3.5 minutes at 180 g. The resulting islet pellet was washed twice more in Hanks' solution under identical centrifugation conditions to remove residual culture medium. The final islet suspension was then resuspended in the prepared infusion solution for transplantation.

Identification of pancreatic islets

Immediately after isolation, the islets were identified by staining the suspension with dithizone (Lenreactiv, Russia). This staining method not only confirms the presence of insulin-producing beta-cells among the isolated cells but also enables assessment of the purity of the islet suspension by identifying other cellular components that do not stain with dithizone.

The working dithizone solution was prepared immediately prior to staining by dissolving 10 mg of dithizone

in 2 mL of dimethyl sulfoxide (PanEco, Russia) and 8 mL of Hanks' solution, followed by filtration through a sterile syringe filter with a pore size of 0.22 μm (Corning, USA).

A sample of the islet suspension was mixed with the working dithizone solution in a 2:1 volume ratio and incubated at 37 °C for 20 minutes. Evaluation of staining results and counting of the stained islets were performed using a Nikon Eclipse TS100 inverted fluorescence microscope (Nikon, Japan).

Assessment of islet viability

Viability of the isolated islets was assessed after 12 hours of incubation by fluorescent staining with acridine orange and propidium iodide (PanEco, Russia). For staining, a portion of the islet suspension was transferred into a Petri dish and mixed with the working dye solution at a 2:1 volume ratio, followed by incubation in the dark for 20 minutes. Viable islets were subsequently identified and counted using an inverted fluorescence microscope.

Assessment of the functional activity of pancreatic islets

The functional activity of the isolated islets incubated for 12 hours was evaluated using the stimulation index, defined as the ratio of insulin concentration secreted under glucose stimulation to the basal insulin concentration.

To determine basal insulin secretion, the conditioned culture medium was replaced with a fresh medium containing a low glucose concentration (2.8 mmol/L). After a 60-minute incubation under standard conditions, medium samples ($n = 5$) were collected for measurement of basal insulin levels.

Subsequently, the medium was replaced with a fresh medium containing a high glucose concentration (25 mmol/L) to stimulate insulin secretion. After an additional 60-minute incubation under standard conditions, medium samples ($n = 5$) were again collected. Insulin levels in all samples were determined using an enzyme-linked immunosorbent assay (ELISA) reagent kit manufactured by Vector-Best.

Histological examination

Samples of the pancreas ($n = 3$) and pancreatic tissue obtained after enzymatic digestion ($n = 3$), were subjected to morphological examination using standard histological staining methods. The samples were fixed in 10% buffered formalin, dehydrated through a graded alcohol series, cleared with chloroform, and embedded in paraffin. Sections 4–5 μm thick were prepared using an RM2245 microtome (Leica Microsystems, Germany), followed by deparaffinization and rehydration.

The sections were stained with Mayer's hematoxylin and eosin (BioVitrum, Russia), as well as by the Masson staining method using a ready-to-use reagent kit (BioVitrum, Russia). Histological evaluation and pho-

tomicrography were performed using a Nikon Eclipse 50i microscope equipped with a digital camera (Nikon, Japan).

To identify the main types of islet endocrine cells, immunohistochemical staining was performed using antibodies against insulin (Abcam, UK) and glucagon (Merck, Germany). Detection was carried out using a standard horseradish peroxidase method with the Rabbit Specific HRP/DAB (ABC) Detection IHC Kit (Abcam, UK).

Statistical analysis

Statistical analysis was performed using SPSS software (version 26.0). The Student's t-test was applied to assess the statistical significance of differences in mean values between samples when evaluating the functional activity of the isolated PIs. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Characteristics of the islet graft

A schematic representation of the isolation of PIs from a donor pancreas using the modified method is presented in Fig. 1.

The modified technique enabled the isolation of a substantial number of islets of varying sizes from donor pancreases ($n = 3$) while preserving their structural integrity. The isolated islets were predominantly round with smooth surfaces. In some preparations, minor surface irregularities were observed, corresponding to residual fragments of surrounding exocrine tissue (Fig. 2a).

Dithizone staining imparted a characteristic red-orange coloration to the islets (Fig. 2b), facilitating their identification and counting. From a single human pancreas, we were able to isolate $630,000 \pm 30,000$ islets.

Observation showed that the isolated islets retained their original morphological characteristics after 12 hours of incubation. Fluorescent staining with acridine orange and propidium iodide demonstrated predominantly green fluorescence within the islets, confirming their viability (Fig. 2c). Isolated propidium iodide-positive non-viable acinar cells were detected only in the surrounding culture medium. Overall, viability assessment using vital staining indicated that $90 \pm 3\%$ of the isolated islets remained viable.

After 12 hours of incubation, the insulin level in culture medium samples was 4.4 ± 0.8 mU/L, demonstrating the functional ability of beta-cells within the isolated islets to secrete insulin. After glucose stimulation, insulin level increased to 6.2 ± 0.7 mU/L, representing a 41% increase (Fig. 2d). Accordingly, the calculated stimulation index was 1.41 ± 0.01 , indicating the ability of beta-cells to respond to changes in glucose concentration.

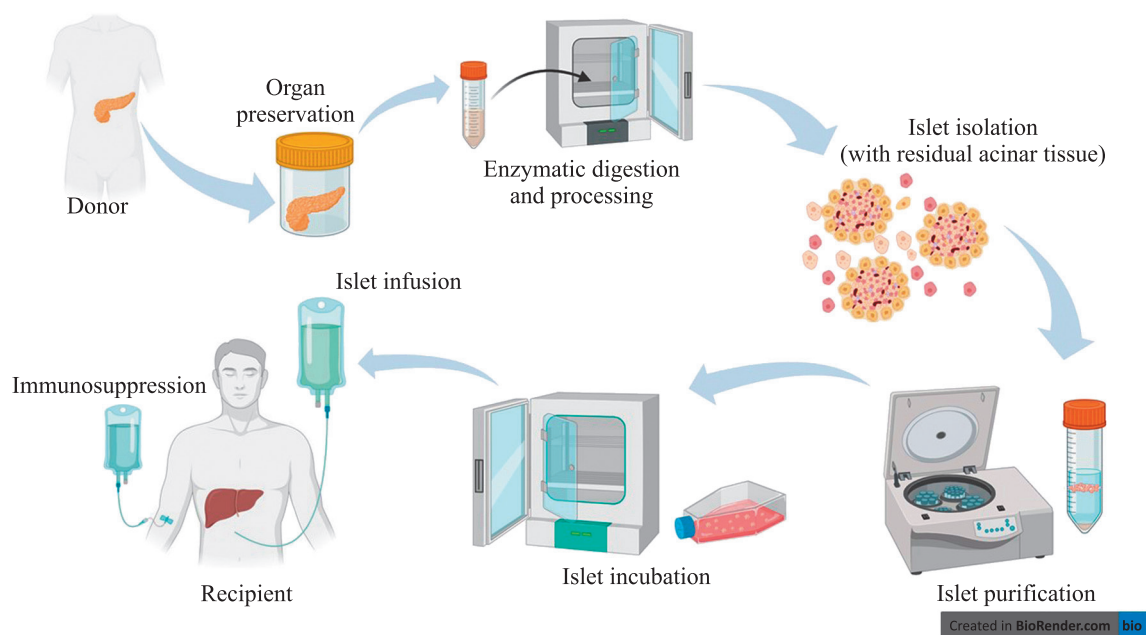


Fig. 1. Schematic representation of the isolation of pancreatic islets from the pancreas of a deceased donor

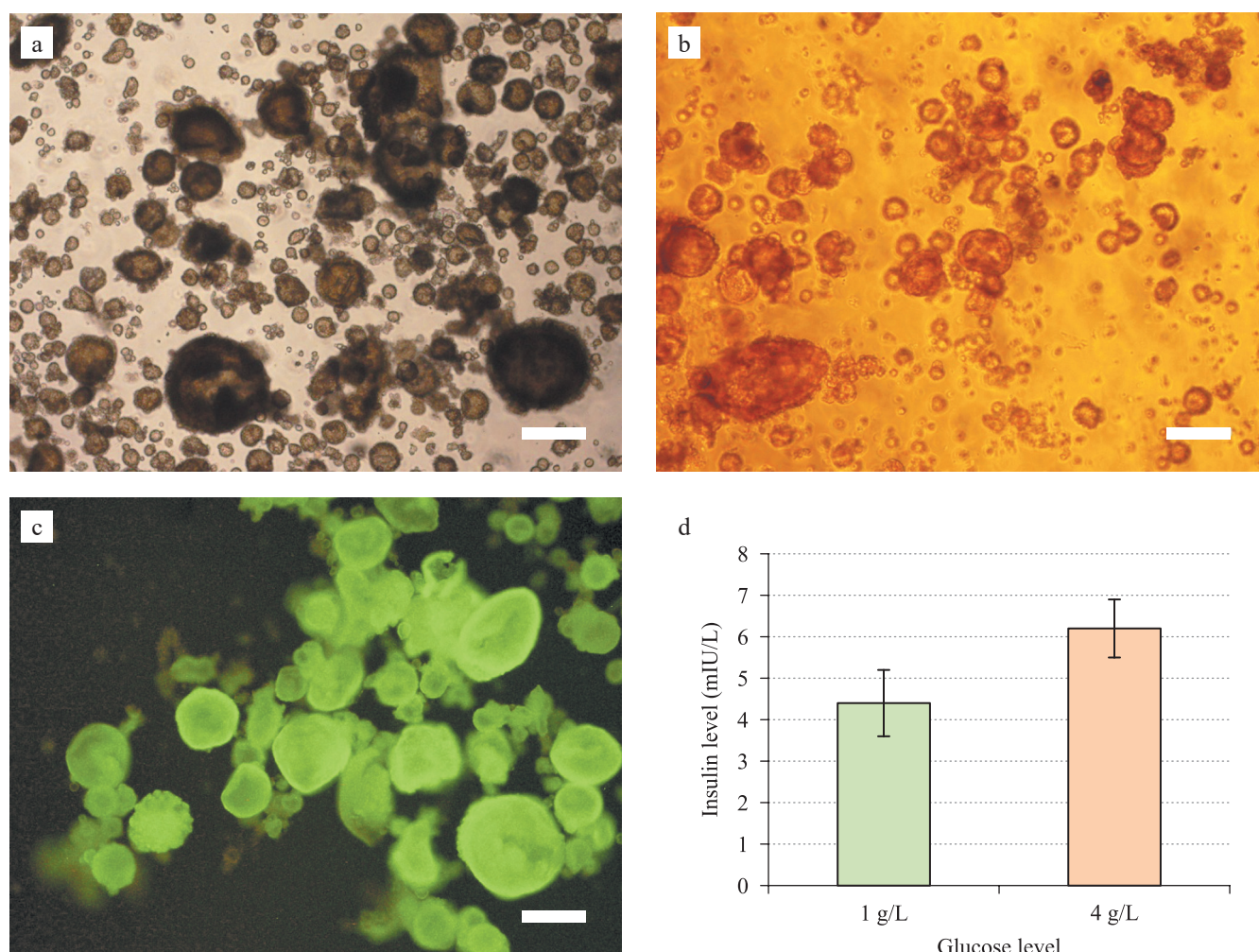


Fig. 2. Freshly isolated human pancreatic islets. (a) phase-contrast microscopy without staining; (b) dithizone staining; (c) islets incubated for 12 hours, assessed by fluorescent staining with acridine orange and propidium iodide; (d) comparative analysis of insulin secretion by islets before and after glucose stimulation. Incubation time: 12 hours. Scale bar: 100 μ m

Morphological study

The initial condition of the pancreatic tissue was assessed by examining donor pancreases with comparable baseline characteristics (donor age: 49–54 years; gland weight: 97–100 g). Histological evaluation revealed no significant pathological alterations in the pancreatic pa-

renchyma, indicating adequate preservation of the organ in the preservation solution.

The glandular parenchyma retained an intact lobular architecture in all examined samples. A mild degree of lipomatosis was observed in some exocrine lobules (Fig. 3a, 3c). No signs of fibrosis was detected; collagen

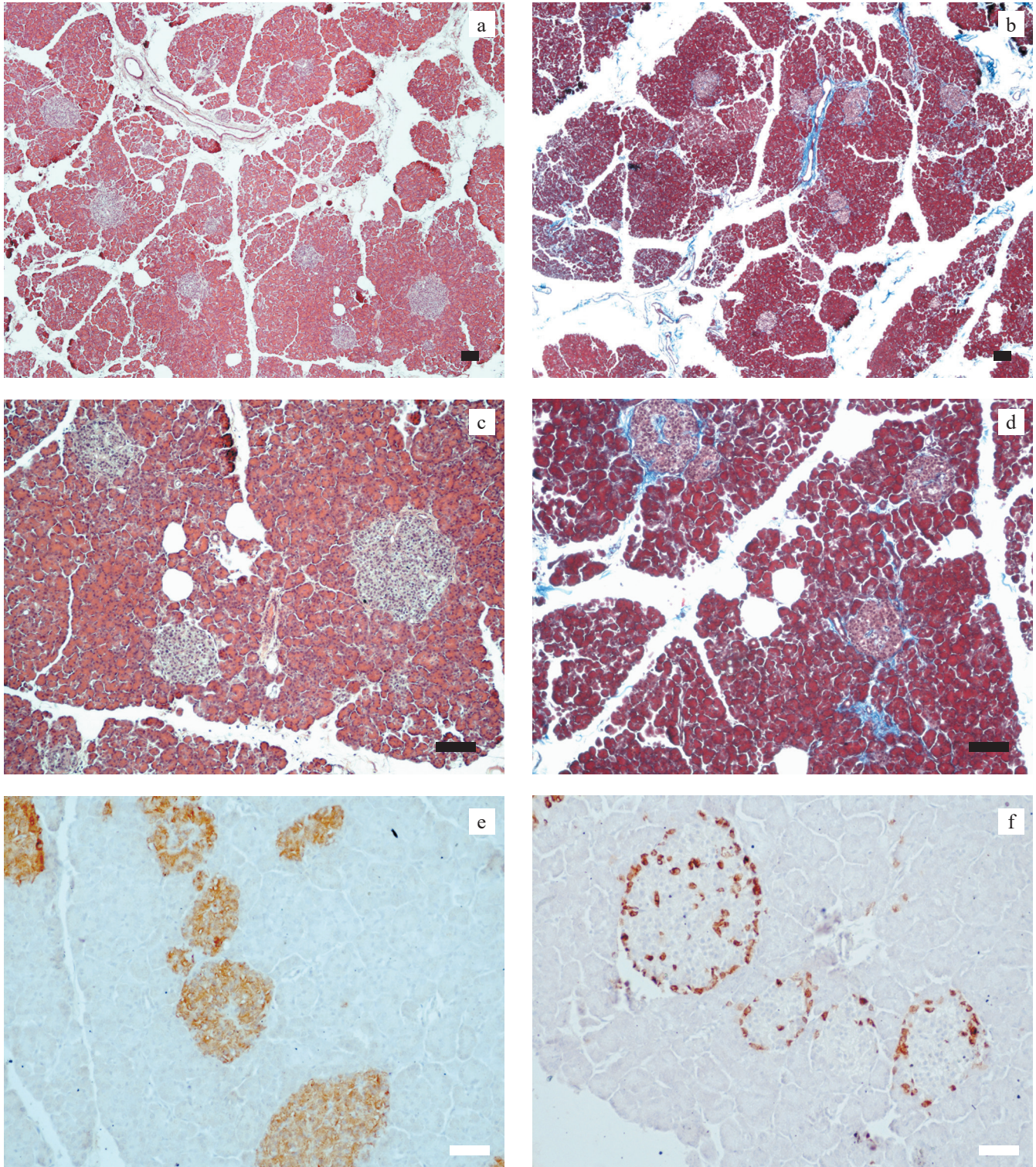


Fig. 3. Donor pancreas. (a, c) H&E staining; (b, d) Masson's trichrome staining; (e) Immunohistochemical staining for insulin; (f) Immunohistochemical staining for glucagon. Scale bar: 100 μ m

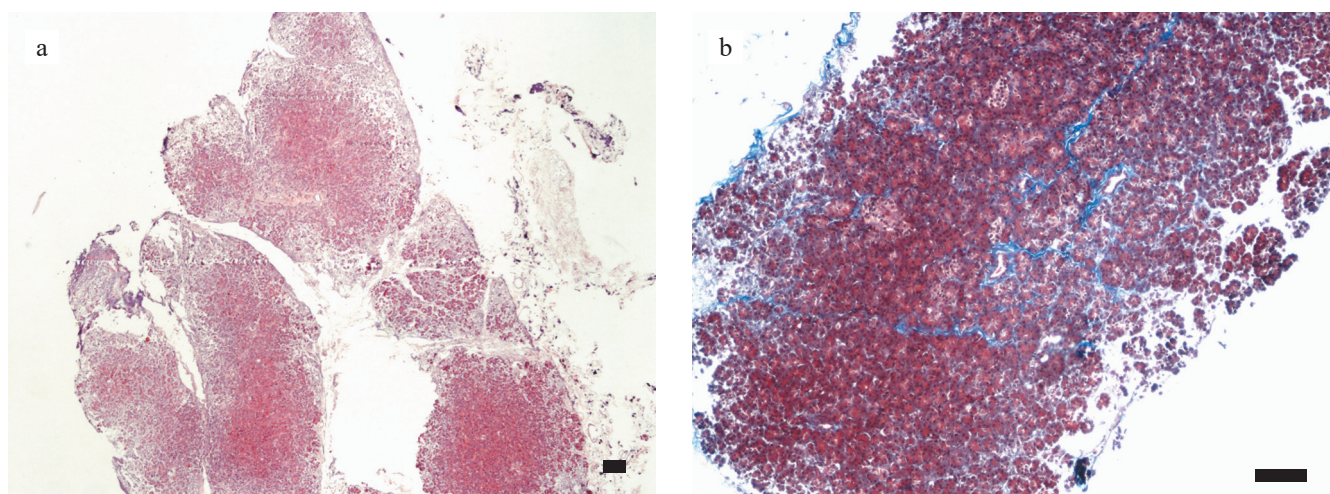


Fig. 4. Pancreatic tissue after enzymatic digestion. (a) H&E staining; (b) Masson's trichrome staining. Scale bar: 100 μ m

fibers (stained blue) were identified predominantly in the periductal regions (Fig. 3b, 3d).

Occasionally, isolated lobules contained small focal areas of pancreatocytes with mild dystrophic changes. Numerous well-defined islets of Langerhans were distributed throughout the parenchyma. These islets exhibited a typical rounded morphology and compact structure without signs of pathological alteration (Fig. 3a–d).

Immunohistochemical staining of the main types of islet cells (beta- and alpha-cells) demonstrated positive immunoreactivity for insulin and glucagon in all examined samples (Fig. 3d, 3e). Brown granules of the precipitate abundantly filled the insulin-positive beta-cells, which constituted the major cellular component of the islets (Fig. 3d). The less abundant glucagon-positive alpha-cells were scattered in a mosaic pattern throughout the islet structure (Fig. 3e).

These findings indicate preservation of the structural integrity of the islet apparatus in the examined pancreases and confirm their potential for yielding viable and functionally competent islets.

An assessment of the efficiency of islet yield from the pancreas during enzymatic processing was further supported by morphological analysis of the digested pancreatic tissue. The results demonstrated tissue dissociation within pancreatic lobules, with either complete absence or only minimal residual islets remaining in the processed lobular structures (Fig. 4a, 4b).

A modification of the traditional protocol was introduced to optimize the methodological steps for isolating PIs, with the aim of increasing yield while preserving their morphofunctional integrity. Thus, particular attention was paid to minimizing the adverse effects associated with individual stages of pancreatic processing [25], thereby reducing structural damage and loss of islet viability during isolation.

In the modified approach, the enzyme solution was administered intraparenchymally rather than intraduc-

tally. This adjustment reduced ischemia duration and significantly accelerated and facilitated pancreatic tissue distension. Specific skills for intraparenchymal injection ensured uniform distribution of the enzymatic solution throughout the tissue.

Given that certain properties of density gradient media – such as hypertonicity, high viscosity, and possible endotoxin contamination – may adversely affect the morphofunctional state of isolated islets [26], the density gradient purification step was abandoned. Instead, purification was performed using complete growth medium and Hanks' saline solution under controlled centrifugation conditions. The selected centrifugation regime proved to be equivalent and productive due to efficient separation of cellular and tissue components.

We believe that eliminating the potentially undesirable effects associated with density gradient media and avoiding additional islet washing may have a positive impact on islet viability.

Our results were comparable with those reported in similar studies on PI isolation using the Ricordi perfusion system and density gradient during the purification process [5, 14].

In global clinical practice, the established quality criteria for an islet product include a yield exceeding 5,000 islet equivalents per kilogram of recipient body weight, viability of at least 70%, and a stimulation index greater than 1 [14]. The islet grafts obtained using the modified method met these internationally accepted requirements, demonstrating an adequate yield, confirmed cellular identity, high viability, and satisfactory functional activity.

CONCLUSION

As a result of optimizing methodological techniques, a modified protocol for processing the pancreas from deceased donors was validated, enabling the isolation of a substantial number of viable and functionally active PIs.

The mean yield of isolated islets was $630,000 \pm 30,000$, with a viability of $90 \pm 3\%$ and a stimulation index of 1.41 ± 0.01 , indicating high-quality preservation of the isolated biomaterial and the ability of beta-cells to respond to changes in glucose levels.

The characteristics of the obtained islet graft show that the resulting cellular material is suitable for transplantation in patients with T1D.

The authors declare no conflict of interest.

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TECHNIQUE FOR DECELLULARIZATION OF CORNEAL LENTICULES FOR SUBSEQUENT KERATOPLASTY

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This article addresses strategies for reducing corneal graft rejection and explores alternatives to full-thickness corneal transplantation. The objective of the study is to develop a technique for removing cellular material from human corneal lenticules obtained during refractive surgery for use as a biomaterial in keratoplasty. A decellularization procedure was developed in which lenticules were immersed in a 1% aqueous solution of sodium lauryl sulfate for 24 hours, followed by detergent removal using a buffered 0.9% NaCl solution for 5 days. Decellularization effectiveness was confirmed by the absence of genetic material using Hoechst staining and hematoxylin & eosin staining. Histological analysis of the biomaterial after preservation for various periods demonstrated no signs of biodegradation of the acellular lenticules, which remained sterile for at least 12 weeks. A comparative experimental study in rabbits demonstrated the efficacy and safety of the proposed technique.

Keywords: *keratoplasty, corneal lenticule, rabbit, experiment, histology.*

INTRODUCTION

The demand for donor corneal tissue remains high, driving ongoing research aimed at reducing graft rejection rates and identifying alternatives to full-thickness corneal transplants [1, 2]. The aim of this study is to develop a technique for decellularizing human corneal lenticules obtained during refractive surgery, with a view to their subsequent use as biomaterials in keratoplasty.

MATERIALS AND METHODS

A procedure was proposed for removing cellular components from surgical specimens. Corneal lenticules obtained during refractive laser surgery were immersed in a 1% aqueous solution of sodium lauryl sulfate (SLS) under aseptic and antiseptic conditions. The stromal fragments were maintained in the solution for 24 hours, after which the residual SLS was removed by sequential rinsing of the lenticules in sterile buffered 0.9% NaCl solution, with five washing cycles of 24 hours each. All procedures were performed in a Class II laminar flow hood. As there are currently no universally accepted standards for assessing decellularization efficiency, evaluation was based on the absence of detectable genetic material, as determined by Hoechst staining and hematoxylin and eosin (H&E) staining [3].

The required concentration of the SLS solution and incubation time for lenticule processing were determined

experimentally. Corneal stromal fragments were treated under the following conditions: 0.1% SLS for 1 day ($n = 10$), 0.1% SLS for 2 days ($n = 10$), 1% SLS for 1 day ($n = 10$), while untreated lenticules served as controls ($n = 10$). Next, the samples were stained with Hoechst dye (Invitrogen, Cat. No. HN-3570, Lot 822389) at a dilution of 1:300 and incubated for 10 minutes. The lenticules were then washed twice in Tris–Tween buffer (pH 7.6) for 5 minutes each and mounted in Fluoromount (Diagnostic Biosystems, REF K024, Lot P 939-B). Fluorescence visualization was performed using a Nikon Eclipse 80i research microscope equipped with a DIH-M epifluorescence attachment.

To rule out possible biodegradation of lenticules following decellularization, histological analysis was performed after storage in sterile tubes containing buffered saline solution for varying durations. The biomaterial was kept in a dark environment at 2–5 °C for 7, 16, 21, 42, 70, and 86 days. Fresh, untreated lenticules served as the control group (No. 7).

Prior to histological examination, the storage media were tested for sterility; no microbial growth was detected. All lenticules were then fixed in 10% neutral formalin, rinsed in running water, dehydrated through graded alcohols, and embedded in paraffin. Histological sections were prepared and stained with H&E.

The specimens were examined under a Leica DM LB2 microscope at magnifications of 50×, 100×, 200×, and 400×. Digital imaging and documentation were performed using a Leica Aperio CS2 slide scanner.

To evaluate the efficacy and safety of the proposed decellularization technique, a comparative experimental study was conducted using an animal model. Acellular lenticules (Group 1, $n = 10$) and untreated lenticules (Group 2, $n = 10$) were transplanted into sexually mature Soviet Chinchilla male rabbits. Under aseptic conditions and intravenous anesthesia, the animals underwent standardized corneal injury in the form of superficial keratectomy (diameter 4.5 mm, depth approximately 150 μm). Lenticule transplantation was performed according to a previously described technique [4].

Postoperatively, the animals received standard ophthalmic therapy, including antimicrobial agents (adjusted for antibiotic sensitivity), anti-inflammatory treatment, and keratoprotective support: moxifloxacin 0.5% or tobramycin 0.3% (three times daily for 14 days), dexame-

thasone 0.1% (for 21 days), and ofloxacin ointment 0.3% (twice daily for 10 days).

The rabbits were examined daily and were euthanized at predefined time points (1, 4, and 8 weeks) via intravenous overdose of an anesthetic combination (zolazepam hydrochloride + tiletamine hydrochloride), following photographic documentation. Eucleated eyeballs were then subjected to histological processing and analysis using the methodology described above.

RESULTS

The following results were obtained from experiments on the decellularization of corneal stromal lenticules. In untreated lenticules (control), numerous keratocyte nuclei were clearly visualized. In contrast, treated samples exhibited only background fluorescence and isolated nuclear elements, indicating the presence of minimal residual nucleic acid fragments (Fig. 1). The median (Me) number of cell nuclei per field of view was 6.5 [6.0; 7.75] in the group incubated in 0.1% SLS for 1 day, 2.5 [2.0; 3.0] in the group incubated in 0.1% SLS

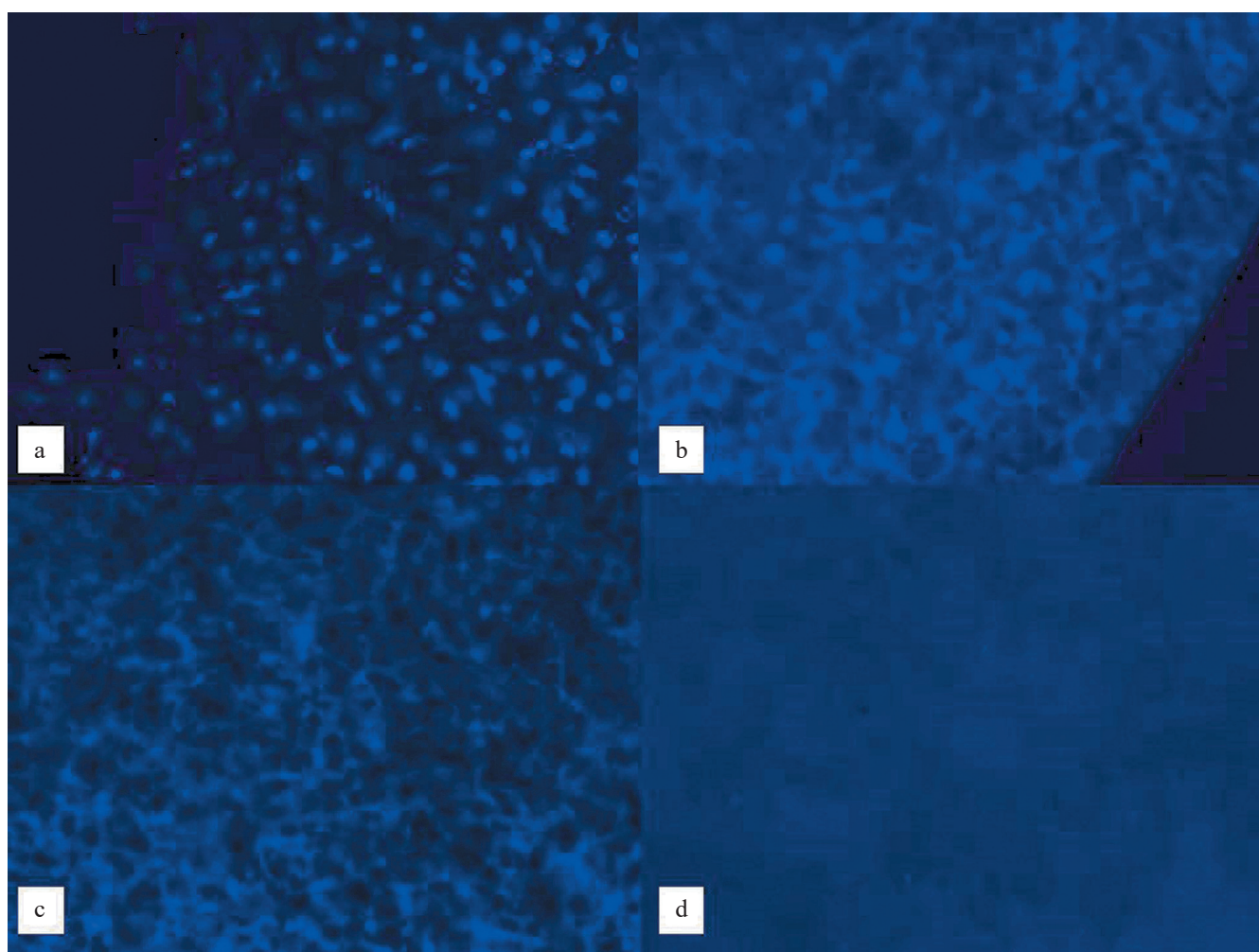


Fig. 1. Fragments of the corneal stroma stained with Hoechst dye ($\times 100$): (a) control; (b) lenticule exposed to a 0.1% SLS solution for 1 day; (c) lenticule exposed to a 0.1% SLS solution for 2 days; (d) lenticule exposed to a 1% SLS solution for 1 day

for 2 days, and 1.0 [0.25; 1.0] in the group treated with 1% SLS for 1 day. These differences were statistically significant (Mann–Whitney U test). Based on these findings, treatment with a 1% SLS solution for 24 hours was identified as the optimal protocol for effective decellularization of corneal lenticules.

Histological examination of lenticules preserved for varying durations and stained with H&E demonstrated that, irrespective of exposure time to the 1% SLS solution, the stromal architecture differed from that of untreated tissue. Specifically, treated samples were characterized by the absence of cellular elements, more uniform hydration, and slight fraying at the lenticule edges.

Specifically, sample No. 7 (control) represented a corneal stromal lenticule obtained by femtosecond (Fs) laser extraction without additional processing. In this sample, the stroma contained a normal population of keratocytes – basophilic, elongated spindle-shaped cells located between stromal fibers. The stromal fibers were densely packed, and the edges appeared sharply delineated. In contrast to experimental samples (Nos. 1–6), the

presence of isolated slit-like spaces suggested uneven stromal edema (Fig. 2).

Additionally, rounded microcavities, occasionally connected by thin bridges, were observed, likely reflecting microcavitation effects induced by femtosecond laser exposure. It can be assumed that immersion in the decellularization solution, followed by preservation in buffered 0.9% NaCl, leads not only to the removal of cellular components but also to uniform stromal hydration (swelling) accompanied by moderate disruption of collagen fiber organization.

In the *in vivo* experiment, the early postoperative course (up to 10–14 days) was similar in both groups and was characterized by a moderate inflammatory response, including episcleral vessel injection, serous conjunctival discharge, and perifocal corneal edema. Corneal sutures remained intact, and the grafts were well adapted to the recipient corneal surface.

However, from days 10–14 onward, animals in Group II (untreated lenticules) exhibited a marked increase in inflammation, including expansion of corneal

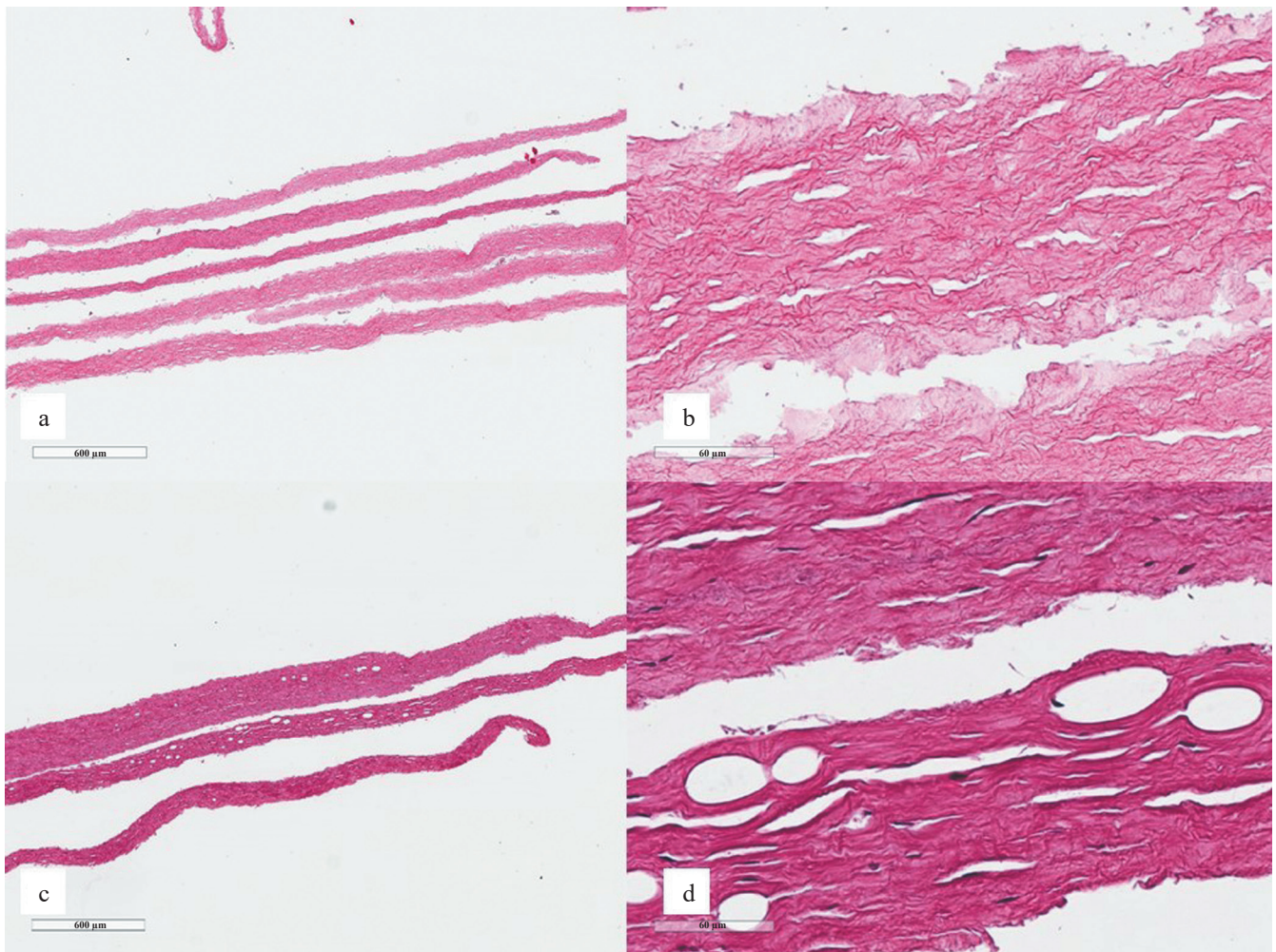


Fig. 2. Microslide of decellularized corneal material and intact lenticules (H&E stain): (a, b) lenticules subjected to decellularization, showing moderate stromal hydration due to exposure to saline solution, with visible slit-like spaces and microcavities; (c, d) intact lenticules containing normal keratocyte density, with compact stroma and microcavities connected by bridges

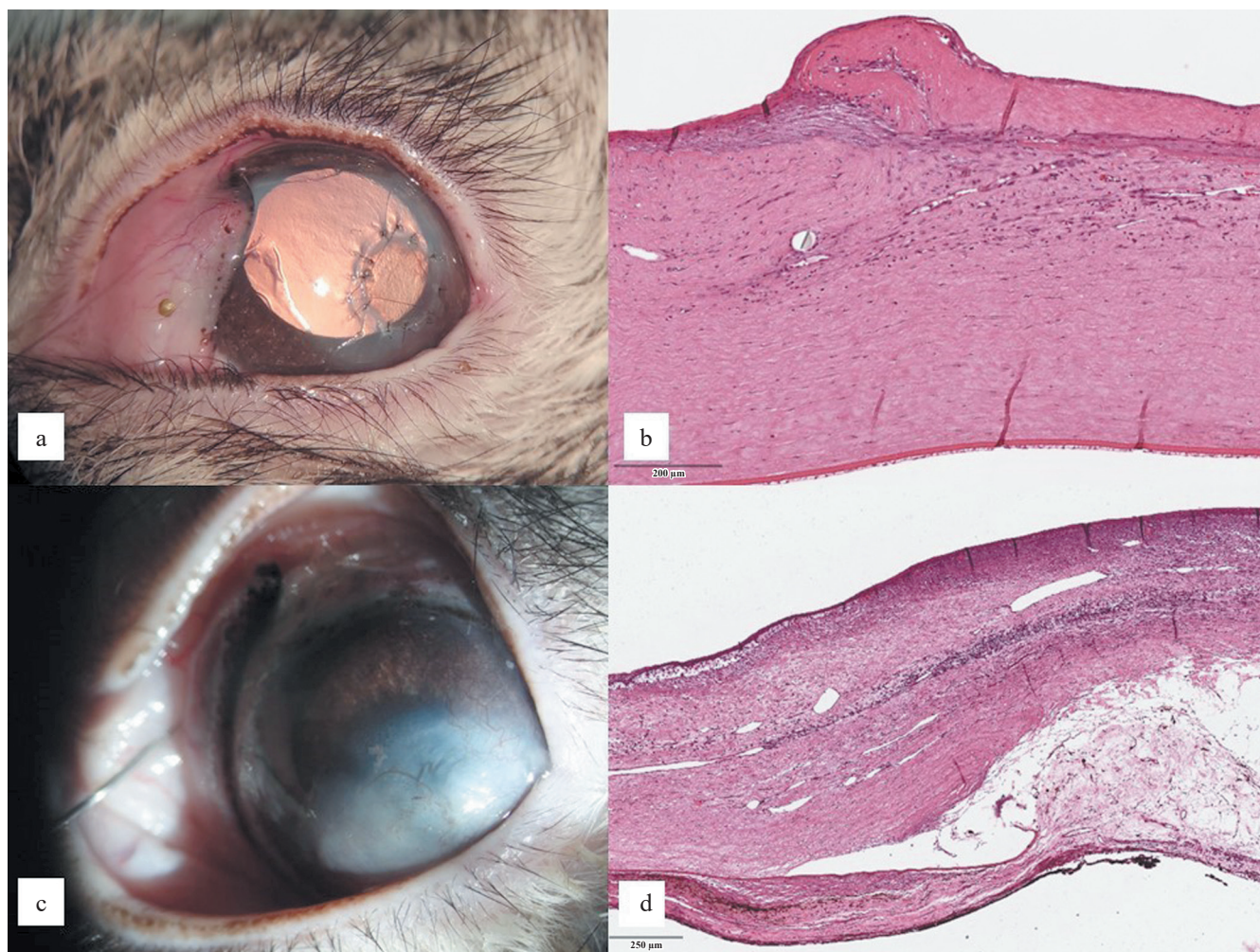


Fig. 3. Clinical and pathological correlation during the postoperative period: (a) photo of the anterior segment of a Group I rabbit, 8-week observation; (b) corneal micropreparation from a Group I rabbit, H&E stain; (c) photo of the anterior segment of a Group II rabbit, 8-week observation; (d) corneal micropreparation from a Group II rabbit, H&E stain

edema, neovascularization, profuse mixed conjunctival discharge, iris vessel injection, and the presence of cellular suspension in the anterior chamber. Anti-inflammatory therapy was intensified with the addition of betadine instillations, while corneal cultures remained sterile. Between 30 and 60 days, this group developed vascularized corneal opacification and extensive anterior synechiae. Light microscopy revealed xenograft degradation and pronounced inflammatory cell infiltration (lymphocytes, eosinophils) at the implantation site, consistent with immune-mediated graft rejection [5–6].

In contrast, the postoperative course in Group I (acellular lenticules) remained favorable throughout the study. The grafts retained transparency, corneal edema resolved by week 8, and the corneal surface became fully re-epithelialized. Histological analysis demonstrated recellularization of the initially acellular stromal matrix by recipient cells (Fig. 3).

CONCLUSION

The proposed technique for decellularizing human corneal lenticules obtained during refractive surgery ensures an effective and sufficient level of cellular removal, as demonstrated by comparative transplantation experiments using decellularized and intact lenticules in a rabbit model.

The procedure is technically simple and can be implemented directly in a clinical setting, enabling rapid preparation of graft material. Furthermore, the decellularized lenticules can be preserved for at least 3 months without evidence of structural degradation, allowing for the establishment of a readily available tissue reserve for emergency tectonic keratoplasty.

The authors declare no conflict of interest.

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EMERGING THERAPEUTIC STRATEGIES FOR TYPE 1 DIABETES

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Modern medical research identifies type 1 diabetes mellitus (T1D) as one of the most pressing global health challenges, with the incidence steadily increasing by up to 500,000 new cases annually. Current treatment approaches do not provide optimal glycemic control, as they are mainly aimed at compensating for endogenous insulin deficiency, which can significantly compromise patients' quality of life. For a long time, researchers have sought a treatment strategy capable of achieving a rapid, safe, and reliable cure for T1D. Yet, due to the insufficient understanding of the mechanisms driving the autoimmune response underlying this condition, modern medicine has not been able to develop an etiologic therapy that could ensure complete recovery. A wide range of therapeutic developments targeting different pathogenetic mechanisms of T1D are currently underway, all aimed at supporting optimal glucose metabolism. Their objectives include preventing or slowing disease progression (delaying the onset of the clinical stage) and facilitating glycemic control in affected patients. Despite substantial progress, none of these innovative strategies has yet reached widespread clinical application. The primary obstacles remain the lengthy timelines required to complete full cycles of clinical trials and the need to address limitations revealed during the research process. This review presents the leading modern approaches to T1DM therapy, with a focus on insulin therapy, immunotherapy, and cell-based strategies. Clinical trial data are analyzed, highlighting both advantages and limitations from practical and economic perspectives. The approaches discussed represent the most promising avenues and are expected to play a central role in future treatment of T1DM.

Keywords: type 1 diabetes mellitus, pancreas, immunotherapy, cell therapy, insulin therapy.

INTRODUCTION

Type 1 diabetes mellitus (T1D) is a polyetiological, multifactorial disease, the central mechanism of which involves an autoimmune response triggered by genetic predisposition in combination with various environmental factors. As a result of this immune-mediated aggression, pancreatic beta cells are destroyed, leading to an absolute insulin deficiency.

T1D is considered a socially significant disease, with its incidence growing steadily worldwide. According to the Russian Federal Diabetes Registry, as of 2023, the number of patients with T1D in Russia reached 277,100, representing 5.58% of the total diabetes population [1].

At present, the standard method of replacement therapy for T1D – insulin therapy – remains widely used in clinical practice. The introduction of modern insulin formulations, pump insulin therapy, continuous glucose monitoring systems, and closed-loop technologies has improved glycemic control in many patients. However, this approach often has low effectiveness due to its high resource intensity, requiring considerable time, concentration, and clinical expertise to demonstrate target therapeutic indicators.

Conventional insulin therapy also imposes significant lifestyle restrictions, particularly regarding diet and physical activity, and the use of repeated injections in conjunction with the complex pharmacokinetics of insulin preparations frequently results in suboptimal glycemic control. This is reflected in the high incidence of severe hypoglycemic episodes and the continued progression of late complications associated with chronic hyperglycemia, such as diabetic neuropathy (41.3%), diabetic retinopathy (28.9%), and diabetic nephropathy (22.8%) [2].

Over the past few decades, numerous efforts have been made to address the existing limitations of insulin therapy through transplantation-based technologies. Approaches such as isolated and combined pancreas–kidney transplantation, xenotransplantation, allotransplantation, and autotransplantation of pancreatic islet cells have been explored and continually refined. However, these methods are characterized by limited efficacy, frequent postoperative complications, and a dependence on immunosuppression, which restrict their application to severe clinical cases.

Current research trends focus on optimizing existing transplantation approaches and developing novel therapeutic strategies aimed at preventing disease onset and

slowing or halting the progression of late complications. The genetic contribution to disease susceptibility and the precise mechanisms underlying the autoimmune response in T1D remain incompletely understood.

Modern technologies, particularly those targeting specific pathogenetic pathways, are now being actively investigated. Several of these experimental strategies have already shown promising results in clinical trials, suggesting the potential not only for significantly improved disease control but also for the achievement of long-term clinical remission in T1D patients.

INSULIN THERAPY

Insulin therapy remains the gold standard for managing T1D. The classic treatment regimen involves daily administration of basal insulin, supplemented by bolus injections of short-acting insulin before meals. This section discusses modern strategies aimed at overcoming the limitations of conventional insulin therapy, forming the foundation for the development of next-generation insulin formulations.

Speed of activation and duration of action

The use of bolus insulin prior to meals requires a certain pause. Modern ultra-short-acting insulin analogues, which exist primarily in hexameric conformation, exhibit a delayed onset of pharmacological effect – usually 5 to 15 minutes after injection. This pharmacokinetic lag restricts their clinical flexibility and can contribute to postprandial hyperglycemia in patients with T1D.

To address these challenges, ultra-rapid short-acting insulin analogues have been developed. These formulations consist of shorter insulin molecules that achieve faster absorption and earlier metabolic activity, thereby mimicking the physiological profile of endogenous insulin secretion more closely. A promising example of this class is AT247 (Arecor Therapeutics plc, UK), an ultra-rapid insulin analogue. In Phase I trials [3], AT247 demonstrated superior glycemic control compared with traditional short-acting analogues such as insulin aspart, due to its accelerated absorption (onset of action within 1–10 minutes) and optimized pharmacokinetic–pharmacodynamic characteristics.

Current trends in the development of basal insulin formulations are focused on creating long-acting analogues that require only once-weekly administration. Their prolonged duration of action is primarily attributed to enhanced binding affinity to serum albumin. Such formulations not only improve treatment convenience but also promote more stable glycemic control, as they lack a pronounced peak of activity.

A notable example is insulin icodec (Novo Nordisk, Denmark), which has been approved for clinical use in Canada. Its extended pharmacokinetic properties result from specific structural modifications, including the re-

placement of three amino acid residues, leading to a half-life of approximately 196 hours [4]. Clinical trials have demonstrated its therapeutic equivalence to insulin degludec, although a higher incidence of hypoglycemic events has been reported, necessitating individualized dose adjustment [5].

Another promising once-weekly basal insulin currently in development is efsitora (Eli Lilly, USA). In clinical studies, efsitora has shown a reduced frequency of nocturnal hypoglycemic episodes and a decreased requirement for short-acting insulin compared with insulin degludec [6].

Glucose-sensitive insulins

Patients with T1D must not only coordinate insulin injections with meals but also adjust doses according to physical activity, which can precipitate hypoglycemia. The development of glucose-sensitive “smart” insulins, which activate only under hyperglycemic conditions and become inactive once physiological glucose levels are restored, offers a potential solution. These formulations more closely mimic endogenous glucose regulation, enabling optimized glycemic control while minimizing the risk of hypoglycemia.

A promising advance in this area is the creation of an oral glucose-sensitive insulin, which eliminates injection-related challenges. This formulation exhibits glucose-dependent activity and consists of insulin complexed with a silver compound and a chitosan–glucose polymer coating. The coating protects the insulin molecule from acidic gastric degradation, maintaining its bioactivity as it transitions into the alkaline environment of the duodenum [7]. After intestinal absorption, insulin accumulates in hepatocytes, forming a metabolic depot. Under hyperglycemic conditions, enzymatic cleavage of the complex triggers a controlled release of insulin into the bloodstream, achieving dose-dependent effect without the risk of hypoglycemia.

An alternative technological approach to glucose-responsive insulin delivery involves the creation of insulin conjugates with amphiphilic polymers, in which surface charge modulation enables glucose-dependent insulin release. Following oral administration, these micellar nanostructures are efficiently absorbed in the gastrointestinal tract and transported via the portal circulation to the liver, where they form a metabolic depot [8].

The rapid activation of insulin when blood glucose rises is most similar to the physiological synthesis of insulin by the pancreas. The drug is in the preparation stage for clinical trials, demonstrating stable 24-hour glycemic control while maintaining a high safety profile in preclinical studies on animal models.

Insulin pump therapy

Patients with T1D are most often children or young adults who lead active lifestyles, which significantly influences blood glucose variability and necessitates frequent monitoring and dose adjustment. Insulin pump therapy has become increasingly prevalent as a means of achieving a more physiological glycemic profile. These devices enable precise, programmable insulin delivery, allowing hourly dose adjustments tailored to individual needs. This flexibility is particularly beneficial for managing phenomena such as the “dawn phenomenon” and for delivering extended boluses during prolonged food intake.

There are now closed loop systems (CLS) that integrate an insulin pump, a continuous glucose monitoring (CGM) device, and an automated control algorithm – embedded either within the pump or as a separate module – to analyze CGM data and regulate insulin delivery with minimal user intervention. In parallel, non-commercial open-source systems, such as DIYAPS (Do-It-Yourself Artificial Pancreas Systems), have emerged. These user-driven platforms operate outside traditional regulatory frameworks and are based on publicly available algorithms that can be customized to individual preferences [9].

The implementation of such technologies aims to enhance patients’ quality of life by reducing the cognitive and emotional burden associated with routine glucose monitoring and carbohydrate counting (CC). Clinical studies have shown that the use of closed-loop and open-source systems reduces both the risk and frequency of hypoglycemia and increases the percentage of time spent within the target glucose range [10].

However, current closed-loop insulin delivery systems remain technologically imperfect, with several key limitations related to the lack of integration of parameters that modulate the glycemic profile, such as heart rate, sweating intensity, physical activity levels, and the rate of gastrointestinal nutrient absorption. This factor necessitates periodic manual recalibration of algorithmic settings, thereby precluding their static use without dynamic monitoring.

A clinical case reported by Ametov et al. [11] highlights the potential risks of improper system configuration. The case involved a patient using an unregulated AndroidAPS (AAPS) platform paired with an Accu-Chek insulin pump (Roche, Switzerland) and a FreeStyle Libre 1 continuous glucose monitoring system (Abbott, USA). After two years of use without algorithmic adjustment, the patient was hospitalized with severe hypoglycemia precipitated by alcohol consumption without corresponding insulin dose modification.

Current closed-loop insulin delivery systems are not yet fully autonomous, and patient participation remains essential for effective glycemic control and the maintenance

of carbohydrate metabolism compensation. Given the continued need for user oversight, the impact of these systems on quality of life remains a subject of debate. While several studies have reported improvements in patient-reported outcomes, findings across the literature are inconsistent.

According to a meta-analysis by X. Jiao et al. [12], when comparing closed-loop systems with conventional methods such as standard insulin pump therapy and multiple daily injections, no statistically significant differences were observed in quality-of-life measures or diabetes-related distress. The authors suggested that the potential benefits of closed-loop systems may be offset by the challenges associated with understanding and operating complex technological interfaces.

A promising direction for future research is the development of fully autonomous bihormonal systems, which combine the delivery of insulin with glucagon (or, in some designs, pramlintide) to replicate physiological pancreatic regulation. However, a 2022 meta-analysis by B. Zeng et al. [13] revealed that, despite a reduction in hypoglycemic events, bihormonal systems achieved no significant improvement in time-in-range compared with single-hormone analogues and were associated with an increased incidence of gastrointestinal side effects.

Alternative routes of insulin administration

A major limitation of conventional subcutaneous insulin therapy is the occurrence of local injection-related complications, including lipodystrophic tissue changes and, in some cases, abscess formation. To mitigate these adverse effects, research has focused on alternative routes of insulin administration, with oral and inhalation formulations emerging as the most promising options.

The oral route offers several advantages, being both safer and more physiologically relevant. It mimics the natural pathway of endogenous insulin, which first passes through the hepatoportal circulation, ensuring primary hepatic exposure similar to insulin secreted by pancreatic beta cells. Numerous oral insulin formulations have been developed to overcome challenges such as acidic degradation in the stomach and enzymatic cleavage, as well as to enhance intestinal absorption. Notable examples include ORMD-0801 (Oramed Pharmaceuticals, Israel), I338 (Novo Nordisk, Denmark), and IN-105 (Biocon Limited, India) [14].

An innovative advancement in this field is the self-orienting microdelivery applicator (SOMA) – a miniature autonomous system for targeted insulin delivery to the gastric mucosa [15]. SOMA consists of a microneedle containing insulin and a compressed spring encapsulated in a sugar-based coating. The device is engineered such that its center of gravity aligns with the needle tip, enabling proper orientation within the stomach. Upon dissolution of the sugar coating by gastric fluid, the spring

mechanism is activated, inserting the insulin-loaded needle directly into the gastric wall.

A promising advancement in the optimization of T1D therapy is the development of inhaled insulin formulations, the most notable of which is Afrezza (MannKind Corp., USA) – currently the only inhaled insulin approved for clinical use. Afrezza is an ultra–short-acting insulin designed for use in combination with long-acting basal analogues to control postprandial hyperglycemia.

This delivery route enhances the efficiency of insulin absorption by providing a large surface area of contact with the drug, which enters the systemic bloodstream and acts within 30 minutes. Clinical studies have demonstrated that Afrezza significantly reduces the frequency of hypoglycemic episodes compared with insulin lispro, although coughing has been reported as a common side effect [16].

A major advantage of inhaled insulin is the ability to provide postprandial glucose correction without the cumulative effects or prolonged activity associated with subcutaneous injections, owing to its short half-life and rapid clearance [17, 18].

Other methods of insulin administration are being developed, but they have not yet been fully studied or have certain disadvantages from a practical and economic point of view (Table).

Hepatic-directed insulin

Another innovative approach in insulin therapy is the hepatic-directed vesicle insulin (HDV) system, designed to target insulin delivery directly to the liver. This technology utilizes vesicular microdisks that facilitate the transport of insulin – regardless of its route of administration – to hepatocyte receptors.

Under normal conditions, pancreatic insulin reaches the liver via the portal venous system, where a portion is inactivated to prevent hypoglycemia, while the remainder promotes hepatic glucose uptake and suppresses hepatic glucose production, effectively preventing postprandial hyperglycemia. Peripheral insulin administration results in a reduced proportion of hepatic glucose utilization and an increased uptake by muscle tissue, leading to glycogen depletion and a greater risk of hypoglycemia [23].

The HDV system aims to restore this physiological distribution, thereby normalizing hepatic and peripheral glucose metabolism. Its use has been shown to reduce nocturnal hypoglycemia, improve glycated hemoglobin levels, and enhance overall glycemic stability. Clinical trials of HDV–insulin lispro (HDV-L) showed both improved glycemic control and a lower incidence of hypoglycemic episodes compared with conventional insulin lispro [24]. Additionally, the insulin delivery system to the liver helps to increase the rate at which it starts working and reduce the required doses.

IMMUNOTHERAPY

One of the key mechanisms in the pathogenesis of T1D is the autoimmune reaction directed against pancreatic β cells and molecules involved in insulin metabolism. T cell–mediated immune aggression, together with the production of autoantibodies, plays a central role in this process. The clinical course of T1D progresses through three distinct stages [25]. In the first stage, the autoimmune process begins, characterized by the appearance of specific antibodies in the blood. Stage 1 diabetes is defined by the presence of at least two of five possible autoantibodies: ICA (against β cells), anti-GAD (against glutamate decarboxylase), anti-IA-2 (against

Table

Alternative insulin delivery routes [14]

Delivery route	Drugs	Characteristics
Intranasal	1. Cell-penetrating peptides 2. Chitosan 3. Ammonium salts	Administering insulin via these routes effectively lowers blood glucose levels. The use of molecules that enhance cell membrane permeability can improve insulin absorption and glycemic control.
Buccal	PharmFilm (MonoSol; Midatech, USA; UK)	A promising minimally invasive method of administering insulin in the form of a rapidly dissolving mucoadhesive film. These films have a rapid onset of action due to the rich vascularization of the oral cavity [19]. Undesirable effects include mucosal irritation and discomfort, which can lead to inadvertent swallowing of the drug.
Transdermal	1. Microneedles for insulin delivery 2. Jet insulin injector 3. U-Strip patch (Dermisonics, Inc., USA)	A potentially promising route of insulin administration due to its minimal invasiveness. Studies have shown its effectiveness, safety, and patient preference over parenteral forms of insulin [20]. Current research is focused on improving insulin transport through the skin and enhancing bioavailability.
Rectal	1. Labrazol 2. HPMC-co-PAM-co-PMAA hydrogel	The advantages of this method of insulin administration are rapid and relatively complete absorption into the systemic circulation [21]. The drug bypasses the harsh gastric environment, although the presence of a pronounced mucosal barrier can reduce efficacy. Various enhancers can be used to improve insulin delivery via these routes [22].

tyrosine phosphatase), IAA (against insulin), and ZnT8 (against zinc transporter 8). At this point, β -cell function and blood glucose levels remain normal. In the second stage, progressive autoimmune destruction of β cells results in impaired glucose tolerance or impaired fasting glycemia. The third stage corresponds to the clinical manifestation of T1D and the need for insulin to maintain normal glucose levels.

T1D has a fairly long preclinical phase characterized by progressive autoimmune destruction of pancreatic β cells. Modern immunomodulatory therapies selectively target key components of the pathological immune response, helping to preserve the residual function of insulin-producing cells and prevent the onset of absolute insulin deficiency. Administering such therapies during stages 1 and 2 can delay the clinical manifestation of T1D. Moreover, studies have shown that some patients retain residual insulin secretion even years after diagnosis [26], and immunotherapy in these cases can significantly improve glycemic control.

Most immunotherapeutic strategies aim to enhance the activity of regulatory T lymphocytes, suppress autoreactive T cells, or reduce autoantibody production by B lymphocytes. However, many existing approaches require prolonged use of non-selective drugs that deplete immune cells and cause immunodeficiency, limiting their widespread use. Therefore, increasing attention is being directed toward new selective immunomodulatory agents that are currently in development or already in clinical use.

Several studies have demonstrated that functional or quantitative deficiency of regulatory T cells (Tregs) plays a critical role in the pathogenesis of T1D [27], leading to uncontrolled activation and proliferation of autoreactive T lymphocytes. Targeting this mechanism helps induce stable immunological tolerance to pancreatic islet antigens, which clinically manifests as stabilization of glycemic control and improved metabolic regulation.

Correction of Treg deficiency can be achieved through two main approaches: transplantation of Treg cell material in cases of absolute deficiency, or activation of the endogenous Treg pool via antigen-specific immunization with islet antigens. A key prerequisite for therapeutic success is the selective suppression of autoimmune responses directed specifically against β -cell antigens, thereby minimizing systemic immunosuppression. The most promising strategies involve avoiding broad, non-specific Treg activation – instead focusing on generation of *ex vivo*-optimized Tregs for reinfusion into patients or direct *in situ* stimulation [28].

Immunotherapy targeting quantitative Treg deficiency

This type of immunotherapy aims to compensate for quantitative Treg deficiency through cell-based approaches.

One strategy involves *ex vivo* autologous transplantation of expanded Tregs. Modification of these cells before reinfusion enhances therapeutic efficacy while avoiding the need for systemic immunosuppression. Clinical studies assessing the safety and efficacy of this approach have reported no clinically significant adverse events [29].

Within two years following therapy, patients demonstrated increased C-peptide levels and reduced requirements for exogenous insulin, with two cases achieving complete insulin independence [30]. Optimization of outcomes using polyclonal Tregs can be achieved by combining this approach with low-dose interleukin-2 (IL-2) therapy [31]. Experimental evidence suggests that this combination expands both transplanted and endogenous pools of functionally active Tregs and enhances their survival; however, it may also stimulate the proliferation of cytotoxic cell populations.

Ex vivo therapy using expanded polyclonal Tregs, PTG-007 (PolTREG, Poland), has demonstrated high therapeutic efficacy. In patients with early-stage T1D, this treatment resulted in insulin independence lasting up to 24 months and reduced insulin requirements for up to 12 years [32]. The combined use of Tregs and Rituximab has proven to be the most effective.

However, a key limitation of *ex vivo*-expanded Treg therapy is the potential phenotypic instability of these cells, which may lead to their transformation into pathogenic autoreactive effector T cells. Therefore, long-term monitoring of T-cell specificity is essential, along with rigorous *in vitro* quality control before reinfusion into patients.

Preclinical studies in experimental animal models indicate that antigen-specific Tregs provide superior therapeutic efficacy compared to polyclonal populations [33]. Nonetheless, the technological challenge lies in isolating these antigen-specific cells, as they are predominantly localized within the target tissue – the pancreatic islets.

Immunotherapy targeting Tregs quality deficiency

There is an alternative therapeutic approach that offers significant advantages in terms of cost-effectiveness and clinical feasibility compared to *ex vivo* cell-engineering techniques. *In situ* antigen exposure stimulates the activation of antigen-specific Tregs directly within target tissues, thereby enhancing immune tolerance.

Recent advances in bioengineering have enabled precise targeting of immune components, site-specific drug delivery, and controlled release mechanisms [34]. For instance, gold nanoparticles can serve as delivery vehicles, carrying proinsulin peptide molecules as autoantigens to induce antigen-specific tolerance [35]. Another promising delivery platform involves genetically modified strains of *Lactococcus lactis* engineered to produce hu-

man proinsulin and interleukin-10, thereby promoting antigen-specific immunological tolerance [36]. Monotherapy with AG019 (Precigen ActoBio, Belgium) has led to reduced HbA1c levels and a 24% increase in C-peptide six months after administration [37].

One of the most studied therapeutic strategies for T1D is antigen-specific immunotherapy using GAD-alum (Diamyd), developed by Diamyd Medical (Sweden). This approach employs recombinant human GAD65 protein, a major islet autoantigen. The mechanism of action involves the induction and expansion of regulatory T cells (Tregs), which helps preserve and enhance the residual functional activity of pancreatic β cells. Clinical efficacy has been demonstrated in patients with newly diagnosed T1D (within 6 months of onset) who are GAD65-seropositive and carry the HLA DR3-DQ2 haplotype. The therapeutic regimen includes three intralymphatic injections into the inguinal lymph nodes, administered at monthly intervals, in combination with vitamin D supplementation [38].

Clinical studies have reported lower mean glucose levels, reduced frequency of hyperglycemia episodes, and longer durations within target glycemic ranges over a 15-month follow-up period. Preservation of endogenous insulin secretion was observed in a substantial proportion of patients for 30–43 months. One observation documented sustained β -cell functional activity for up to 8 years following treatment. Current investigations are assessing the use of booster doses administered 31.5 months post-therapy to extend the duration of clinical benefit [39].

Another promising therapeutic approach for T1D is IMCY-0098 Imotope (Imcyse SA, Belgium), designed to slow disease progression and prevent early onset of T1D. This compound is a linear synthetic peptide incorporating the C20-A1 epitope of proinsulin. Its mechanism of action involves the induction of CD4⁺ regulatory T-cell proliferation, leading to targeted elimination of antigen-presenting cells and autoreactive effector T lymphocytes involved in pancreatic β -cell destruction [40]. Preliminary clinical trial data have demonstrated a favorable safety profile, and ongoing studies are assessing the therapeutic efficacy of this approach [41].

Immunotherapy targeting T-cell immunity in general

One of the most studied agents in this class is Teplizumab (Provention Bio Inc., USA), a humanized monoclonal antibody targeting the CD3 receptor on T lymphocytes, engineered to lack the Fc fragment. Its mechanism of action involves the induction of apoptosis in activated effector T cells, thereby modulating the autoimmune response and reducing the intensity of pancreatic β -cell destruction. At the same time, the drug promotes cytokine-mediated proliferation of regulatory T cells (Tregs), contributing to restoration of immune

homeostasis [25]. A major advantage of Teplizumab is that it does not require long-term administration and does not induce chronic immunosuppression, setting it apart from conventional immunomodulatory therapies. In 2022, the U.S. Food and Drug Administration (FDA) approved Teplizumab for delaying the onset of clinical T1D in individuals aged 8 years and older with stage 2 (preclinical) disease markers. The therapeutic regimen consists of a 14-day course, which has been shown to delay progression to insulin dependence by 2–4 years. In some clinical reports, disease onset was delayed by up to 11 years, although efficacy is closely correlated with the disease stage at initiation and the preserved functional β -cell mass.

This drug can also be used to enhance β -cell viability in individuals with early clinical-stage T1D. Clinical trial data indicate that two 12-day courses of Teplizumab administered to patients with newly diagnosed T1D (within 6 weeks of diagnosis) result in a significant increase in stimulated C-peptide levels by week 78 [42].

Key advantages of Teplizumab include its short treatment duration (12–14 days), in contrast to agents requiring continuous or repeated administration, and the absence of long-term immune suppression. Laboratory studies have shown that Teplizumab induces selective functional depletion of CD8⁺ effector T lymphocytes targeting β -cell antigens, thereby reducing the intensity of autoimmune response [43]. Meanwhile, there is a rapid recovery of immunocompetent cell populations in peripheral blood without an associated increase in infectious complications. Reported adverse effects, such as transient lymphopenia, mild skin reactions, and headache (cephalalgia), are generally self-limiting and do not require additional medical intervention [44].

Cell therapy

Current research in T1D treatment is increasingly focused on islet cell (IC) transplantation strategies aimed at restoring physiological glycemic regulation and endogenous insulin secretion [45]. Despite its significant therapeutic potential, this approach faces major technological challenges, including the identification of an optimal source of insulin-producing cells (IPCs), ensuring their long-term viability, and preventing immune rejection.

In recent years, attention has shifted toward the use of stem cells (SCs), both allogeneic and autologous, due to the limited success of donor allogeneic or xenogeneic islet transplantation. The demonstrated oncological safety of alternative IPC sources has further accelerated the development of stem cell-based protocols.

Numerous experimental systems have been proposed to address key challenges such as maintaining cell survival, enhancing functional activity, and protecting the graft from immune-mediated aggression, which in T1D is intensified by the underlying autoimmune pro-

cess. Modern approaches employ genetic or physical protective mechanisms to eliminate the need for systemic immunosuppression, thereby minimizing complications.

The optimal implantation site remains under investigation, as no anatomical location has yet shown clear superiority. Current research efforts are particularly focused on approaches that demonstrate potential for medium-term clinical applicability.

Transplantation of insulin-producing cells derived from stem cells

A promising direction in cell therapy for T1D is the autotransplantation of insulin-producing islets derived from chemically induced pluripotent stem cells (CiPSCs). While the liver remains the conventional site for islet implantation, alternative implantation sites are being actively explored. A phase I clinical trial demonstrated the efficacy of islet transplantation under the rectus abdominis muscle in a patient with T1D [46]. This site allows improved monitoring of the graft and facilitates safe removal if necessary. Preclinical trials in primates confirmed the oncological safety of the cell line used. The patient received 1.5 million insulin-producing cells, generated using a modified pluripotent stem cell differentiation protocol [47]. Insulin independence was achieved 75 days post-transplantation and maintained throughout a 12-month follow-up. The patient remained in stable normoglycemia, with >98% time in range and glycated hemoglobin (HbA1c) around 5%.

It should be noted, however, that the patient was already on immunosuppressive therapy due to a previous liver transplant, which limits objective assessment of the transplant's immunogenicity. In theory, the use of autologous SCs minimizes rejection risk, though the possibility of recurrence of autoimmune destruction of β -cells cannot be ruled out. To obtain more definitive conclusions and assess potential long-term complications, data from larger patient cohorts with extended follow-up periods (≥ 5 years) are needed.

Other studies have also reported successful transplantation of islets differentiated *in vitro* from autologous SCs [48].

An alternative approach involves the use of donor embryonic stem cells differentiated into islet cells (ICs). Transplantation of such islets into the liver has shown promising efficacy [49]. However, achieving insulin independence still requires immunosuppressive therapy. Consequently, current research focuses on developing technologies that can provide sustained protection of the graft from autoimmune and alloimmune aggression without systemic immunosuppression.

Mechanical protection methods

Various encapsulation strategies have been explored to enhance the efficacy of IPCs transplantation [50]. The-

se methods shield the graft from immune reactions while maintaining adequate metabolic exchange to sustain cell functionality. Promising results have been reported with alginate-based microcapsules coated with PMETAC polymer [51]. Coatings containing 0.4% PMETAC exhibit selective permeability, supporting cell viability and enhancing the functional activity of transplanted IPCs. The demonstrated stability, target selectivity, and low cytotoxicity of this system in both *in vitro* and *in vivo* models highlight its potential for further clinical development.

The Cell Pouch system (Sernova, Canada) has gained considerable recognition as an implantable device designed to provide a favorable microenvironment for the long-term and efficient function of insulin-producing cells (IPC). The system consists of a biocompatible chamber made of non-resorbable polymer materials, shaped into cylindrical compartments. Following subcutaneous implantation, a well-vascularized biological niche forms within approximately 14 days, creating an optimal site for the localization of encapsulated pancreatic ICs.

Clinical results have demonstrated long-term cell survival, minimal adverse effects, normalization of HbA1c levels, and insulin independence lasting up to four years [52]. However, these outcomes were achieved under concurrent immunosuppressive therapy. To ensure reliable protection of IPCs from autoimmune destruction, conformal coating technology, which is currently undergoing clinical evaluation, has been proposed [53]. This approach employs a mechanically stable polymer hydrogel matrix that envelops the transplanted islets, providing selective permeability for biomolecules while preserving physiological hormone secretion kinetics. Preclinical trials have shown that such modified cell transplants, when implanted into pre-vascularized biocompatible chambers, achieve normal glycemic control and sustained insulin independence.

Genetic protection methods

Allogeneic stem cell therapy VX-880 (Vertex Pharmaceuticals, USA) has demonstrated high clinical efficacy. IPCs transplanted into the hepatic portal vein system show strong engraftment and significant functional activity by day 90 post-transplant. This therapy leads to reductions in HbA1c levels, insulin requirements, and episodes of severe hypoglycemia [54]. However, its widespread clinical use remains limited by the need for long-term immunosuppressive therapy. As a result, it is currently reserved for T1D patients who are resistant to standard treatment and experience frequent, uncontrolled hypoglycemia, where preventing life-threatening complications outweighs the risks associated with immunosuppression.

Clinical trials are underway for a new therapy, VX-264 (Vertex Pharmaceuticals, USA), a next-generation

therapy using encapsulated ICs that eliminates the need for systemic immunosuppression. In parallel, Vertex Pharmaceuticals and ViaCyte are advancing a CRISPR/Cas9 genome editing strategy [55], which has demonstrated preclinical safety. This approach involves genetically modifying IPCs to remove specific markers that typically trigger immune rejection, thereby enabling transplantation without immunosuppression.

Further innovations aim to enhance both safety and functional integration of genetically engineered cells by implanting them in optimized microenvironments [56]. A key example is VCTX210 therapy (CRISPR Therapeutics; ViaCyte, USA; Switzerland), based on implantation of differentiated pluripotent SCs into specialized implantation devices designed to promote vascular integration and support physiological metabolic function.

Another approach is being developed by Sana Biotechnology (USA), which employs allogeneic islet transplants derived from induced pluripotent SCs or donor tissue. These cell lines are genetically engineered using HIP (Hypoimmune Platform) technology, enabling immune evasion without the need for immunosuppressive therapy [57]. In a patient with T1D, intramuscular transplantation of donor hypoimmune islets showed strong survival and high functional activity of ICs over a 28-day follow-up period [58].

Controlled cell therapy

In cell-based therapies, evaluating treatment safety and efficacy is essential yet often challenging. Minutia (USA) is developing a stem cell replacement therapy that enables *in vivo* monitoring of transplanted cells. The forearm is proposed as the implantation site, while nanosensor technology is used to monitor cell status in real time [59]. These nanostructured biosensors, integrated into transplanted cells, can detect phenotypic changes and assess immune system activity. Continuous feedback on the performance of IPCs provides critical information for predicting transplant rejection and guiding subsequent treatment tactics.

Pancreatic tissue engineering

A key challenge in islet transplantation is the reduced lifespan and functional activity of ICs due to the loss of their extracellular matrix (ECM). The natural microenvironment of the pancreas not only provides structural support but also facilitates intercellular communication and interaction with cytokines. Reconstructing this microenvironment for ICs using various cell types and biomaterials enhances vascularization and oxygenation, promoting optimal cell adhesion and proliferation at the transplant site.

Recent advances in tissue engineering and 3D bioprinting have opened the possibility of creating pancreatic tissue-engineered constructs (TECs) that closely

mimic the native architecture, composition, and functionality of the human pancreas [60]. Such constructs should integrate both non-cellular components (e.g., hydrogels, polymers) and cellular elements (e.g., IPCs and supporting cells) to ensure physiological insulin secretion and normoglycemia.

Active research focuses on developing decellularized matrices that replicate the spatial and biochemical characteristics of native pancreatic tissue [61, 62]. The major advantage of these matrices lies in their low immunogenicity, achieved through complete removal of cellular material during decellularization. This approach has shown promising results in preclinical trials, with ongoing efforts aimed at identifying optimal materials and transplantation sites.

For instance, intrasplenic implantation of pancreatic TECs composed of floating islet-like cultures and a biodegradable, collagen-containing matrix in rats demonstrated good morphological preservation and sustained functionality. However, its hypoglycemic effect was less pronounced compared to intraperitoneal transplantation of similar TECs [63]. Moreover, intraperitoneal transplantation of a bioartificial pancreas – constructed from a decellularized human pancreas combined with allogeneic islets – in a rat T1D model resulted in more stable and prolonged glycemic control than transplantation of isolated islets of Langerhans [64].

The integration of these tissue-engineered platforms with advanced immunomodulatory strategies holds the potential for achieving a qualitative breakthrough in T1D treatment. Nonetheless, successful clinical translation will require extensive multi-phase preclinical and clinical studies to confirm the long-term safety, biocompatibility, and functional efficacy of these technologies.

COMBINATION OF ISLET CELL TRANSPLANTATION AND IMMUNOTHERAPY

An effective strategy to overcome the limitations of systemic immunosuppression in cell therapy is the use of its selective alternative – immunotherapy. One of the most promising developments in this field is Tegoprubart (Eledon Pharmaceuticals, USA), a therapeutic agent whose mechanism of action is based on selective blockade of the CD40L ligand. This therapeutic strategy induces dysregulation of intercellular signaling interactions by inhibiting costimulatory signals critical for T-lymphocyte activation [65].

Unlike traditional immunosuppressive drugs, Tegoprubart avoids systemic toxicity while effectively preventing both acute and chronic graft rejection. Preliminary clinical data demonstrate its high therapeutic efficacy in the context of islet cell transplantation: treatment with Tegoprubart resulted in a 3–5-fold increase in graft survival compared to standard immunosuppression protocols. Patients achieved normoglycemia without

the use of exogenous insulin within 2–4 weeks after transplantation, accompanied by a 2.7–3% reduction in HbA1c levels [66].

These findings underscore the potential of targeted immunomodulation as a means to minimize the risks associated with long-term immunosuppression.

DISCUSSION

The advances summarized in this review highlight a range of innovative strategies offering promising prospects for the treatment of T1D. Given the complex and heterogeneous pathogenesis of this disease, as well as its variable clinical presentation, it remains impossible to establish a standard universal treatment protocol. Consequently, there is an increasing shift toward personalized therapeutic approaches, in which different modalities are tailored to the specific case to achieve optimal outcomes.

At present, insulin therapy remains the cornerstone and only widely implemented treatment for T1D. Despite its inherent limitations, this approach is likely to remain indispensable in clinical practice for a long time to come. However, continuous scientific progress and accumulated clinical experience are driving its evolution. Modern insulin formulations and delivery systems have greatly enhanced glycemic control and minimized interference with daily life activities.

Modern advances in immunotherapy are already being successfully applied in the treatment of the first patients and are gradually entering broader clinical practice. These approaches can delay the onset of T1D in individuals with preclinical disease and improve glycemic control in patients with preserved residual islet cell function. The scientific community has proposed distinct T1D endotypes, each characterized by specific etiological and pathogenic mechanisms. Selecting an appropriate immunotherapy mechanism based on these endotypes may significantly enhance treatment efficacy.

Meanwhile, progress in cell therapy offers new opportunities for patients with complete β -cell destruction and absolute insulin deficiency. In such cases, technologies ensuring robust protection of transplanted cells from immune attack become crucial. Among emerging strategies, the combination of islet cell transplantation and targeted immunotherapy appears particularly promising, as it enables selective modulation of immune pathways while improving overall therapeutic effectiveness.

CONCLUSION

T1D remains a major challenge for modern medicine. Despite significant progress in existing treatment methods, adequate disease control is still difficult to achieve in most patients. However, emerging modern approaches, developed and refined over many years, are now approaching widespread clinical application. Several of these innovations are in the final stages of clinical

trials, while others have already received approval for clinical use.

The variety of available solutions helps address the specific limitations of individual therapeutic modalities. The high level of modern science, combined with a personalized approach to treatment, offers strong prospects for a substantial improvement in the overall quality of life of patients living with T1D.

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STUDY OF THE HEAD-CAPACITY CURVES OF AN AXIAL PUMP UNDER HIGH BACK-PRESSURE CONDITIONS

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Objective: to study the hydraulic characteristics of an axial pump under conditions where the external back pressure at the outlet exceeds the maximum pressure generated by the pump, and to determine the corresponding head-capacity curves (HCC) in this operating mode. **Materials and methods.** A hydraulic test bench was designed and assembled for the experiments, capable of generating outlet back pressure up to 200 mmHg. Measurements were performed using axial blood pump Stream Cardio (Russia). A series of tests was conducted at rotational speeds ranging from 7,500 to 10,000 rpm. Initial excess pressures of 85, 100, and 120 mmHg were applied. **Results.** The experimental data obtained on the hydraulic test bench made it possible to determine the complete HCC of the axial pump, including the reverse-flow region corresponding to negative flow rates. At a flow rate of 0 L/min, the pressure drop across the pump was 85 mmHg. At a pressure drop of 100 mmHg, the flow rate reached –2.5 L/min, and at 120 mmHg it reached –5 L/min. The HCC in the negative flow range represents a continuous extension of the pump characteristic curve, preserving a similar slope and exhibiting an approximately linear behavior.

Keywords: heart failure, mechanical circulatory support, pump flow rate, pump pressure, axial pump, reverse flow.

INTRODUCTION

Implantable cardiac assist devices are widely used in clinical practice to manage end-stage chronic heart failure. They serve as a critical bridge to heart transplantation or as destination therapy in patients with contraindications to transplantation [1]. Several commercially available systems are currently in routine use worldwide. Among the most common systems are HeartMate III and HeartMate II (Thoratec Corp., USA), as well as HeartWare (Medtronic, USA) [2].

There is a growing demand for devices whose technical specifications enable prolonged mechanical circulatory support (MCS) while maintaining a high quality of life for patients. A key engineering parameter of such systems is the head-capacity curve (HCC), which describes the relationship between the pressure head generated by the device and the resulting flow rate. The shape of the HCC is largely determined by the design of the flow path within the pump. For example, Fig. 1 illustrates the HCC of the axial-flow HeartMate II pump and the centrifugal-flow HeartMate III pump within their typical operating ranges [3]. Axial-flow pumps are characterized by a flatter relationship, often approximated by a hyperbolic function. In contrast, centrifugal pumps, such as the HeartMate III, exhibit a logarithmic relationship ($y = \log_a x$, where $0 < a < 1$). Analysis of these

specifications shows that, at the same flow rate of 3 L/min, the HeartMate II pump generates a pressure head of approximately 70 mmHg, whereas the HeartMate III produces approximately 85 mmHg. Thus, at the same

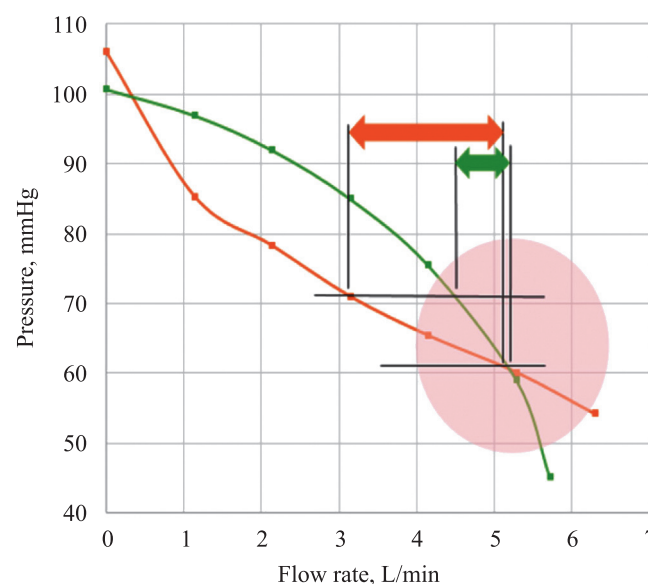


Fig. 1. Head-capacity curves of the HeartMate II (9,000 rpm; axial-flow pump, orange line) and HeartMate III (5,400 rpm; centrifugal-flow pump, green line). The orange circle indicates the typical operating range

flow rate, the two systems operate at different pressure heads and, consequently, different outlet pressures, resulting in distinct hemodynamic effects within this range.

In the region highlighted in orange (Fig. 1), the flow–pressure characteristics of both pumps converge. At a flow rate of approximately 5 L/min, each system generates a pressure gradient of about 60 mmHg, indicating comparable hemodynamic performance. Under these conditions, aortic pressure pulsations occurring during systole, when the centrifugal HeartMate III pump operates in parallel with the native heart, exert a reduced influence on overall cardiac output compared with the axial-flow HeartMate II system.

Fig. 2 presents the HCCs of the left ventricular pump (LVP) integrated into the medical device “Universal System for Mechanical Support of the Pumping Function of the Left and Right Ventricles of the Heart” (Dona-M LLC, Russia). These characteristics are shown as a family of curves describing the relationship between the pressure head generated by the LVP and the flow rate at different rotor speeds.

Within the rotational speed range of 7,500–10,000 rpm, the pump generates a pressure head of approximately 45–85 mmHg at a flow rate of 5 L/min. A reduction in rotor speed below 7,500 rpm is associated with a corresponding decrease in the pressure head produced by the pump.

Most studies describing HCCs focus primarily on the positive flow range. However, under real-world conditions, the opposite scenario may occur during pump operation. In the presence of pulsatile flow generated by the left ventricle, transient increases in aortic pressure may exceed the pressure head produced by the pump. This can result in reverse (retrograde) flow through the device, a phenomenon that requires careful consideration and further investigation.

Such situations have been reported when systemic arterial pressure exceeds the pressure generated by the MCS system for various reasons [4–6]. This imbalance

can typically be corrected by increasing the pump rotor speed, thereby restoring adequate hemodynamics. For example, in the case of the LVP, at a pressure difference of 100 mmHg and a rotor speed of 8500 rpm (Fig. 2), the flow rate is approximately 1 L/min. If the pressure exceeds 100 mmHg at the same rotor speed, the flow rate decreases further and may become negative, indicating the onset of reverse flow.

Several studies have reported that a reduction in pump rotor speed may lead to reverse blood flow through the device [7–10]. Ultrasound evaluation of this phenomenon in the HeartMate II pump demonstrated a bidirectional flow pattern (Fig. 3), characterized by a positive spectrum during systole and a negative spectrum during diastole [11].

Under physiological conditions, at the onset of diastole, aortic pressure remains relatively high, while left ventricular pressure is low, facilitating ventricular filling. The aortic valve prevents retrograde blood flow from the aorta into the left ventricle. However, during MCS, an alternative pathway for blood flow between the left ventricle and the aorta is established. Similar to the function of the left ventricle, the mechanical pumping system creates a direct flow of blood from the left ventricle to the aorta. However, the mechanical support system lacks a valve mechanism, which can lead to blood flowing in the opposite direction from the aorta back into the left ventricle.

Since the pump operates in parallel with the native heart, a physiological pressure difference may develop during diastole in which aortic pressure exceeds left ventricular pressure and may surpass the maximum pressure head generated by the pump. Under MCS conditions, the function of the aortic valve is indirectly maintained through appropriate regulation of pump operating parameters. Specifically, it is essential to ensure that the pressure generated at the pump outlet supports forward (positive) flow at a given flow rate.

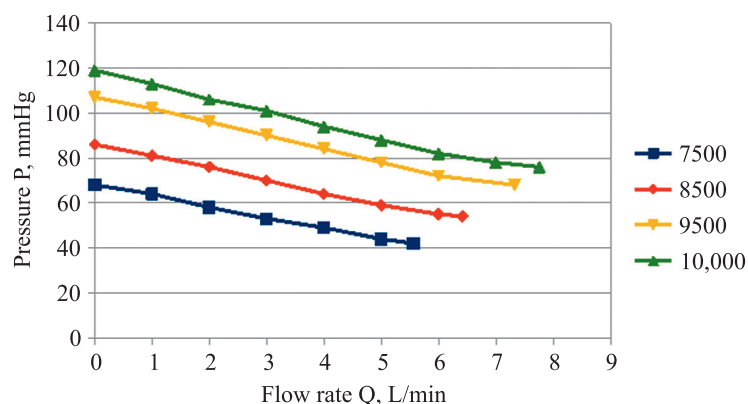


Fig. 2. Head-capacity curves of an axial pump at rotor speeds ranging from 7,500 to 10,000 rpm. A pronounced, nearly linear plateau is observed across all operating modes, indicating high sensitivity of pump blood flow to pressure fluctuations in the aorta

The required parameters are achieved by adjusting the rotor speed. The corresponding pressure and flow characteristics at different speeds can be derived from the pump's HCC or monitored via the device control system. However, reverse flow remains a complex challenge, requiring not only optimization of device settings but also appropriate medical management to stabilize systemic arterial pressure.

The objective of this study is to evaluate the performance of an axial-flow pump under conditions in which systemic pressure exceeds the pressure generated at the pump outlet, to identify the HCCs at which backflow is likely to occur, and to come up with recommendations for optimizing pump rotor speed.

MATERIALS AND METHODS

A dedicated experimental test bench (Fig. 4) was developed to evaluate pump performance under high outlet (back) pressure.

The system consisted of two tanks (tank 1 and tank 2) connected by a 1/2-inch diameter flexible tubing line. The axial-flow blood pump under investigation was installed within the main circuit. The direction of forward (positive) flow during pump operation is indicated by arrow 1 in the figure.

Flow measurements were performed using a dual ultrasonic flow sensing unit (Flowmeter; SONOFLOW CO.55/160, Germany) mounted on the main line. Because each sensor is capable of measuring flow in only one direction, two sensors were arranged in opposing orientations to enable detection of both forward and reverse (negative) flow.

Pressure difference between the pump inlet and outlet was recorded using a differential pressure sensor (Pressure Sensor; Motorola MPX5050, Japan). Tank 1 was equipped with a manual pneumatic pump and a sealed design, allowing controlled generation of elevated hydrostatic pressure within the system. Fluid pressure in

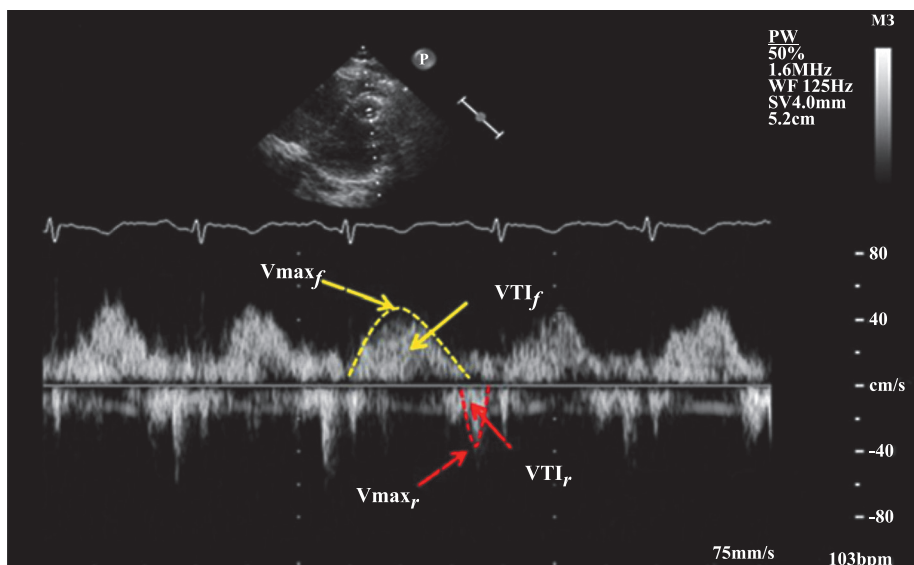


Fig. 3. Signal spectra for positive flow (yellow) and negative flow (red) regimes of the axial pump

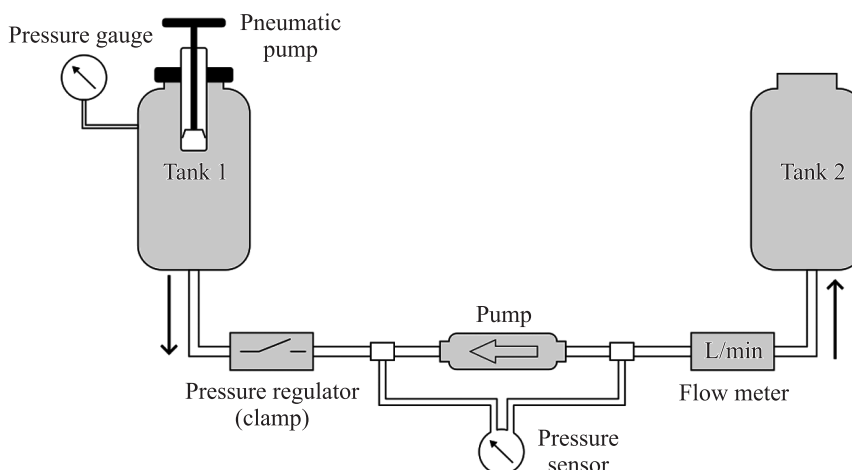


Fig. 4. Schematic diagram of hydraulic test bench

the system was monitored by measuring the air pressure above the liquid surface using a mechanical dial pressure gauge. Signals from the ultrasonic flow sensors and the differential pressure sensor, along with operational parameters of the pump – including average rotor winding current (reflecting the torque generated by the rotor), average rotor speed, and total current consumption of the pumping system – were recorded via an analog-to-digital converter module (E14-140, L-CARD). Data were acquired at a sampling frequency of 20 Hz and stored on a personal computer for subsequent processing and analysis using specialized software.

Tank 1 was filled with 10 L of distilled water to ensure complete filling of the system and removal of air from the circuit. The fluid-filled tubing and the axial pump were initially isolated using a pressure regulator positioned at the pump outlet. An initial overpressure was then generated inside tank 1 using a manual pneumatic pump. Following system preparation, the axial pump was initiated at a predefined rotor speed set by the control unit.

A series of experiments was conducted at rotor speeds ranging from 7,500 to 10,000 rpm. The initial gauge pressure varied between 85 and 200 mmHg.

RESULTS

The temporal profiles of backflow rate (Q , L/min) and backpressure (P , mmHg) at a rotor speed of 8,500 rpm are presented in Fig. 5.

The figure demonstrates four distinct phases of system behavior:

1. Phase 1 (0–25 s). Steady-state equilibrium prior to opening the main line. The circuit is closed, flow rate is zero, and pressure drop across the pump is 85 mmHg, which corresponds to the pump's HCC at a rotor speed of 8,500 rpm (Fig. 2).

2. Phase 2 (25–26 s). Transient response following opening of the main line. A rapid increase in outlet pressure is observed, accompanied by a sharp rise in backflow rate due to high backpressure. The flow rate enters the negative range, indicating reverse flow. This occurs as fluid from tank 1, where a pre-established elevated pressure of 200 mmHg exists, enters the pump inlet. This pressure significantly exceeds the 85-mmHg pressure drop predicted by the HCC at 8,500 rpm. Consequently, fluid is driven through the pump from the high-pressure region (tank 1) toward the low-pressure region (tank 2).
3. Phase 3 (26–35 s). Steady-state reverse flow. This phase is characterized by sustained retrograde flow under approximately constant operating conditions of pressure and flow rate, corresponding to the primary objective of the experiment. During this interval, fluid pressure in tank 1 gradually decreases due to reduction in fluid volume and the associated decrease in air pressure within the sealed chamber.
4. At this point, the pump develops a pressure head of 85 mmHg at a rotor speed of 8,500 rpm, consistent with its HCC. At this point, there is no fluid flow in either the forward or reverse directions (flow rate = 0). The pump thus operates in a state functionally analogous to a closed aortic valve at a systemic pressure of 85 mmHg. In this situation, a further decrease in systemic pressure results in forward flow into the aorta, whereas an increase above the pump-generated pressure head leads to reappearance of retrograde flow.

The data obtained during Phase 3 allow us to plot the pressure–flow $P(Q)$ relationship and determine the HCC for the negative flow region. Fig. 6 presents the pressure drop generated by the pump (P) as a function of backflow

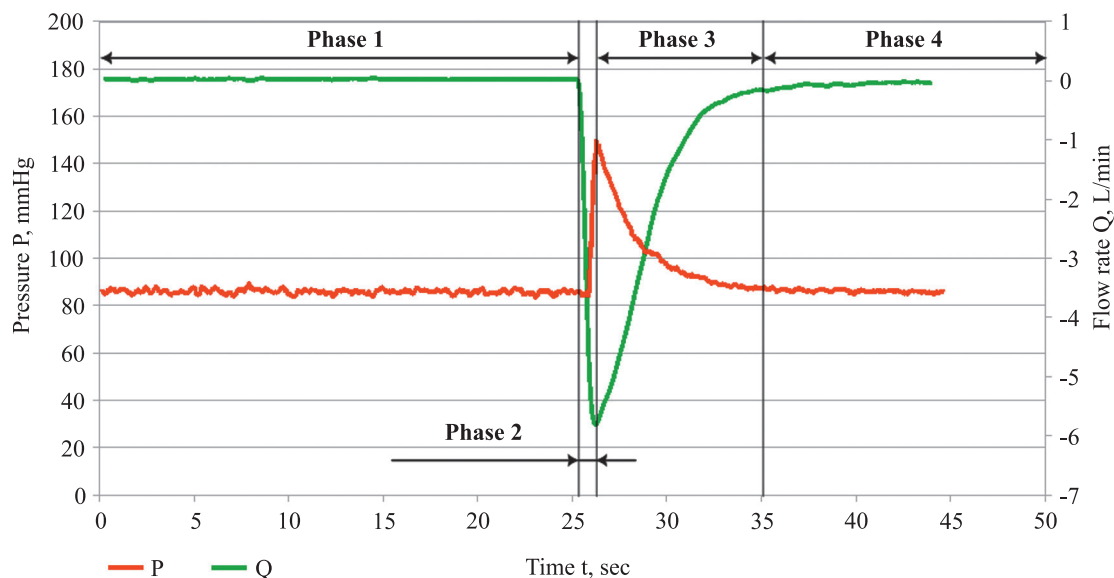


Fig. 5. Reverse flow rate through the axial pump under excess back pressure (rotational speed 8,500 rpm; initial back pressure 200 mmHg) after mathematical processing

rate (Q) at different initial pressures (85–200 mmHg in Tank 1) and a constant rotor speed of 8,500 rpm.

At zero flow ($Q = 0$ L/min), pressure drop across the pump is 85 mmHg. With increasing reverse flow, the system exhibits a progressive increase in pressure drop: at a pressure of 100 mmHg, flow rate is -2.5 L/

min, and at 120 mmHg, it reaches -5 L/min. Further increases in backflow result in an approximately linear rise in pressure drop. As shown in Fig. 6, the initial pressure primarily shifts the range of measurable values, while the HCC values, which are close to linear, remain unchanged.

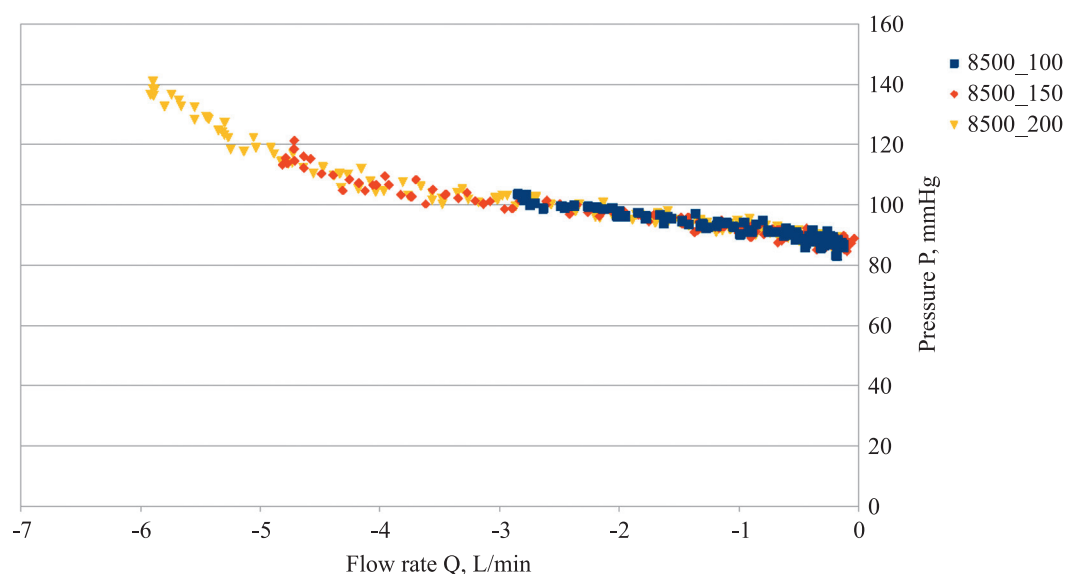


Fig. 6. Head-capacity curves at a rotational speed of 8,500 rpm under different initial pressure conditions

Table

Reverse flow values at different pump rotor speeds

Pump rotor speed (rpm)	Pressure difference P (mm Hg)	Flow rate Q (L/min)
7500	100	-5
7500	90	-4
7500	80	-3
7500	75	-2
7500	70	-1
7500	65	-0.1
8000	110	-5
8000	95	-4
8000	90	-3
8000	85	-2
8000	81	-1
8000	75	-0.1
9000	127	-5
9000	116	-4
9000	112	-3
9000	106	-2
9000	104	-1
9000	97	-0.1
10000	150	-5
10000	140	-4
10000	136	-3
10000	132	-2
10000	124	-1
10000	121	-0.1

The resulting pressure drop and flow rate values obtained at different pump rotor speeds are summarized in Table.

The overall HCCs in the backflow region for all experimental conditions are shown in Fig. 7.

Analysis of the obtained data in comparison with previously established HCCs (Fig. 2) enabled us to determine the total HCC, including the negative flow region, which corresponds to reverse flow conditions.

DISCUSSION

As a result of the conducted experiments, HCCs for an axial-flow pump used in a MCS system were obtained across a range of outlet backpressures and rotor rotational speeds. The shape of the curves in the reverse flow region remains consistent with previously reported HCC behavior. The extent of the negative flow domain was limited by the technical capabilities of the experimental setup.

In the negative flow region, the pressure–flow relationship is preserved, allowing, if necessary, estimation of reverse flow rate (L/min) as a function of rotor speed and system pressure. Thus, the obtained HCC data, including the reverse flow domain, have practical significance and may be used for optimization of operating parameters in MCS systems.

The negative flow rate values obtained in this study represent a continuous extension of the HCC into

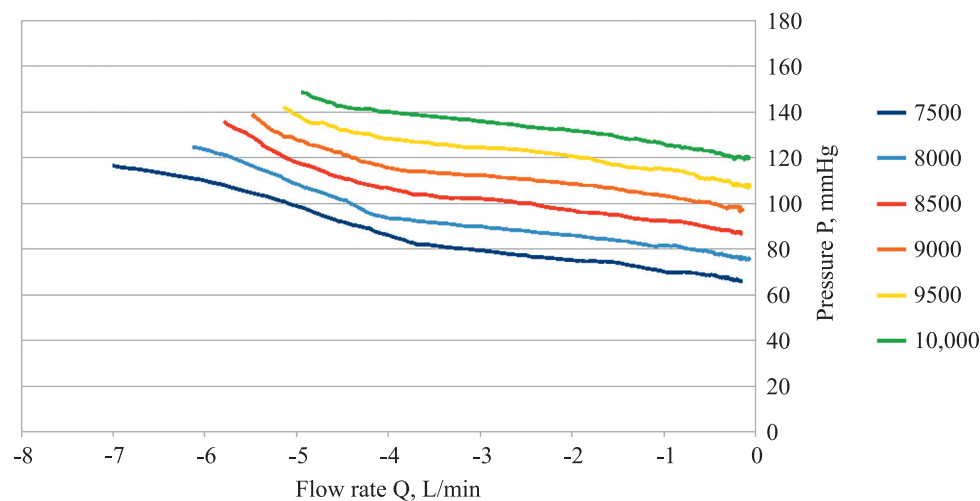


Fig. 7. Head-capacity curves in the negative flow range

the reverse flow region (Fig. 2), while preserving the slope of the characteristic curve. In clinical conditions, particularly during the use of MCS in parallel with a beating heart, the occurrence of retrograde flow from the aorta into the left ventricular cavity during diastole may reduce overall system efficiency. This can result in unpredictable hemodynamic variations and increased risk of complications.

CONCLUSION

The occurrence of backflow in axial-flow pumps used in MCS systems has been identified in the literature as a clinically relevant factor associated with adverse outcomes [4, 7, 10], which is consistent with the findings of the present study.

Despite its clinical significance, reverse flow is not routinely considered when optimizing operating parameters of extracorporeal circulation systems, including widely used clinical devices of various types.

The authors declare no conflict of interest.

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CYLINDRICAL PROSTHETIC VALVES FOR THE TRICUSPID POSITION: PROSPECTS AND LIMITATIONS

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Tricuspid valve (TV) replacement for primary regurgitation is a relatively rare procedure. Bioprosthetic valves are generally preferred because they do not require anticoagulant therapy and tend to degenerate more slowly in the tricuspid position than in the mitral or aortic positions; however, their durability remains limited, particularly in young patients. In contrast, mechanical prosthetic valves require rigorous anticoagulant therapy due to low blood flow in the right heart chambers. Prosthesis selection is especially challenging in cases of infective endocarditis with fibrous annular abscess. The aim of this study is to analyze experimental data on cylinder valves and to review the first clinical experiences reported worldwide. The development of new prosthesis models using inert and more durable materials may address current limitations of TV implants, optimize surgical techniques, and improve patient quality of life in the long-term.

Keywords: tricuspid regurgitation, cylinder valves, bioprosthetic valves, infective endocarditis.

Severe primary tricuspid regurgitation caused by infective endocarditis is most commonly associated with infections following pacemaker lead implantation, prolonged intravenous catheterization during chronic dialysis or cancer treatment, and intravenous drug use. Mechanical prosthetic valves in the tricuspid position are particularly prone to thrombosis due to low blood flow velocity, whereas bioprosthetic valves (BPVs) are susceptible to degeneration and calcification, especially in younger patients. Moreover, no currently available prosthetic valve accurately replicates the native ellipsoidal geometry of the tricuspid annulus or is specifically designed for this anatomical position. Tricuspid valve replacement is often complicated by conduction disturbances due to the proximity of the atrioventricular conduction system, which runs through Koch's triangle near the septal leaflet. In addition, the risk of recurrent infective endocarditis after valve replacement, especially among people who inject drugs, remains high.

The concept of a cylindrical atrioventricular prosthetic valve was first proposed by James Cox and colleagues [1] in the late 1980s, based on the hypothesis that native heart valves function as simple tubular structures whose walls collapse under external pressure. The authors tested a range of materials, including an early experimental scaffold derived from porcine small intestinal submucosa (PSIS), for implantation in both the mitral and tricuspid positions. Their findings demonstrated that the tubular valve design was capable of restoring normal hemodynamics to the prosthetic valve.

EXPERIMENTAL EVALUATION OF CYLINDRICAL PROSTHETIC VALVES

In 2013, Fallon et al. [2] published the results of a small experimental study evaluating cylindrical prostheses fabricated from CorMatrix extracellular matrix (ECM) implanted in the tricuspid position in a sheep model. Echocardiographic examination, together with macroscopic and microscopic histological analyses, showed that the cylindrical valve functioned as a competent tricuspid BPV for more than 12 months without evidence of degeneration or calcification. Histological examination of the explanted constructs further revealed signs of endothelialization and adaptive tissue remodeling.

In 2016, Ropecke et al. [3] conducted a study in a porcine model to implant a cylindrical ECM graft in the tricuspid position and to evaluate its biomechanical and physiological properties in comparison with the native TV. After implantation, the annular area and circumference of the cylindrical prosthesis were smaller than those of the native valve, despite preoperative sizing intended to reproduce the maximum circumference of the natural tricuspid annulus. On average, the circumference of the implanted cylindrical valve was 20% smaller, a discrepancy attributed by the authors to the design of the prosthesis and surgical implantation technique. Importantly, analysis of native tricuspid annular geometry was consistent with prior observations, demonstrating that the annulus assumes a multiplanar shape during systole.

Hsu et al. from Florida International University (USA) subsequently investigated the hemodynamic per-

formance of cylindrical prosthetic valves implanted in the tricuspid and mitral positions. A 26-mm cylindrical valve constructed from PSIS (CorMatrix Cardiovascular) was sutured to a custom 3D-printed valve holder. Hydrodynamic testing was performed using a pulse duplicator system (Vivitro Labs) with 0.9% saline solution. Flow was measured between the right atrium and ventricle, while pressure sensors were positioned in the right atrium, right ventricle, and aorta. Tests utilized a heart rate of 70 beats per minute, with a stroke volume of 50 mL for the tricuspid valve and 71.4 mL for the mitral valve. The tricuspid cylindrical prosthesis demonstrated a transvalvular pressure gradient of 3.97 mmHg and a regurgitation fraction of 7.70% [4].

CLINICAL APPLICATION OF CYLINDRICAL PROSTHESES

In 2016, Murala et al. [5] reported a case involving implantation of a manually constructed cylindrical prosthetic valve in a 15-month-old male infant (10 kg) with pulmonary atresia who had previously undergone two balloon pulmonary valvuloplasty procedures, followed by open TV repair. Despite these interventions, the patient developed severe tricuspid regurgitation and stenosis, along with pulmonary valve regurgitation. An attempted repeat TV repair resulted in further clinical deterioration, prompting the decision to proceed with valve replacement.

The native tricuspid annulus measured 20 mm. The low effectiveness of stented bioprosthetic valves in this age group is well known. Therefore, the surgical team elected to fabricate a cylindrical valve from CorMatrix tissue intraoperatively. This approach was chosen with the intent of preserving the option for future transcatheter valve implantation. The proximal edge of the cylindrical prosthesis was folded inward by approximately 3 mm to create a double-layered suture ring, and the cylinder length was initially set at 24 mm (1.2 times the annular diameter). Distally, the prosthesis was secured to the three papillary muscles using mattress polypropylene sutures, while the suture ring was attached to the tricuspid annulus with a continuous polypropylene suture.

However, significant central regurgitation occurred during ventricular filling. The procedure was repeated using a longer cylindrical prosthesis measuring 35 mm (1.7 times the annular diameter), which resulted in a competent valve with good hemodynamic performance and no residual regurgitation.

Preoperative echocardiography was used to assess the extent of valvular destruction and to determine the native annular diameter and chordal length. The cylindrical prosthesis was made from a CorMatrix sheet, with the diameter matched to the patient's fibrous annulus. Prosthesis length was determined either echocardiographically as the distance from the annulus to the papilla-

ry muscle tips or according to a standardized criterion of 120% of the cylinder diameter. After clamping the aorta, protecting the myocardium, and stopping cardiac activity, all infected areas were excised down to healthy tissue. Following aortic cross-clamping, myocardial protection, and cardioplegic arrest, all infected areas were excised down to healthy tissue. Three ventricular attachment points were then identified for fixation of the cylindrical prosthesis (Fig. 1). Owing to variability in the number and spatial distribution of right ventricular papillary muscles, fixation sites were selected to be approximately equidistant, at 120° intervals, with the anterior papillary muscle typically serving as the reference point when selecting other fixation points. Depending on the condition of the subvalvular apparatus after debridement and surgeon preference, sutures were placed at the tips, bases, or bodies of the selected papillary muscles. Fixation was achieved using mattress suture (usually 4-0 or 5-0 polypropylene or polyester) with or without pledgets. Several noteworthy technical observations were reported. Notably, despite complete excision of the septal leaflet in several cases, no patients developed complete atrioventricular block, suggesting a potential advantage over conventional prosthetic valve replacement. The cylindrical prosthesis was sutured circumferentially to the annulus using a continuous suture technique. An additional advantage of the cylindrical design may be preservation of native annular geometry and the conical configuration of the right ventricle, even in cases requiring extensive excision of infected chordae.

In all but one patient, tricuspid regurgitation was of recent onset, and significant right ventricular remodeling had not yet occurred. There were no in-hospital deaths. Intraoperative papillary muscle rupture occurred in one patient, and delayed rupture developed in another patient seven days after surgery; both complications were successfully corrected. One patient required implantation of a stented xenopericardial prosthesis 22 months later due to cylindrical valve dysfunction. At 6-month follow-up, one patient with a history of intravenous drug use developed fungal endocarditis involving the cylindrical prosthesis and underwent successful reimplantation with a CorMatrix cylinder. In the remaining patients, with follow-up extending up to 18 months, prosthesis function remained satisfactory with no evidence of structural failure.

Based on echocardiographic assessment of the patient's anatomy, a cylindrical prosthesis constructed from CorMatrix extracellular matrix was fabricated intraoperatively during the reoperation. The prosthesis circumference was calculated as the tricuspid annular diameter multiplied by π , and the prosthesis length as 1.3 times the annular diameter. Three attachment points to the papillary muscles were selected at approximately 120 degrees apart and reinforced with CorMatrix tissue

patches. The proximal edge of the cylindrical prosthesis was sutured circumferentially to the tricuspid annulus using a continuous suture technique.

Echocardiography performed on postoperative day 5 showed only minimal regurgitation across the prosthesis. At 11-week follow-up, the cylindrical valve showed excellent functional performance with no evidence of regurgitation. The patient was clinically well, the infection had resolved completely, and she was able to return to work and study. The authors highlighted the minimal use of non-biological material as a key advantage of the cylindrical prosthesis; however, they emphasized that definitive conclusions cannot be drawn without long-term follow-up data [7].

SYNTHETIC AND BIOLOGICAL SCAFFOLDS FOR VALVE SURGERY

In recent years, various synthetic and natural scaffolds have been employed in tissue engineering and regenerative medicine to create tissues and organs seeded with autologous or stem cells, with the aim of overcoming immunological barriers. After implantation of synthetic scaffolds into the human body, the immune system initiates a foreign body response characterized by early neutrophil and macrophage infiltration and release of proinflammatory cytokines at the implantation site. As a result, most synthetic implants become covered by dense fibrous tissue and functionally isolated from the host. This foreign body response may persist until the implant is completely destroyed or surgically removed; otherwise, it can progress to chronic inflammation [8].

Tissue-engineered decellularized ECM scaffolds have great potential to address donor shortages and reduce immunological rejection. ECM scaffolds are biomaterials

derived from various tissues and are intended to function as temporary biological scaffolds that allow the patient's own cells to colonize and repair tissues. These materials have demonstrated the ability to promote constructive tissue remodeling across a wide range of tissues in both preclinical animal models and human clinical trials. However, the host immune response to decellularized tissues is influenced by several factors, including the effectiveness and methodology of the decellularization process, the tissue of origin, the implantation site, and recipient-specific characteristics. Decellularization is a critical step in ECM scaffold production, as it removes immunogenic cellular components and thereby significantly reduces immunogenicity, enhancing the biocompatibility of the resulting scaffold.

However, the use of these bioscaffolds continues to pose significant immunological challenges. Decellularization is defined as the removal of DNA and other cellular components while preserving the native architecture and biochemical composition of the ECM. It is currently the most effective strategy for reducing tissue and organ immunogenicity. However, incomplete removal of cellular remnants during decellularization may trigger a strong immune response after implantation. On the other hand, complete removal of cellular materials can damage the ultrastructural integrity of the scaffold, adversely affecting scaffold function, durability, and regenerative capacity. Thus, the immunogenicity of ECM scaffolds is influenced by multiple interrelated factors. First, decellularization protocols must be optimized to ensure effective removal of cellular components while preserving ECM composition and structural integrity. Second, additional antigen-removal strategies are required to eliminate residual immunogenic epitopes after

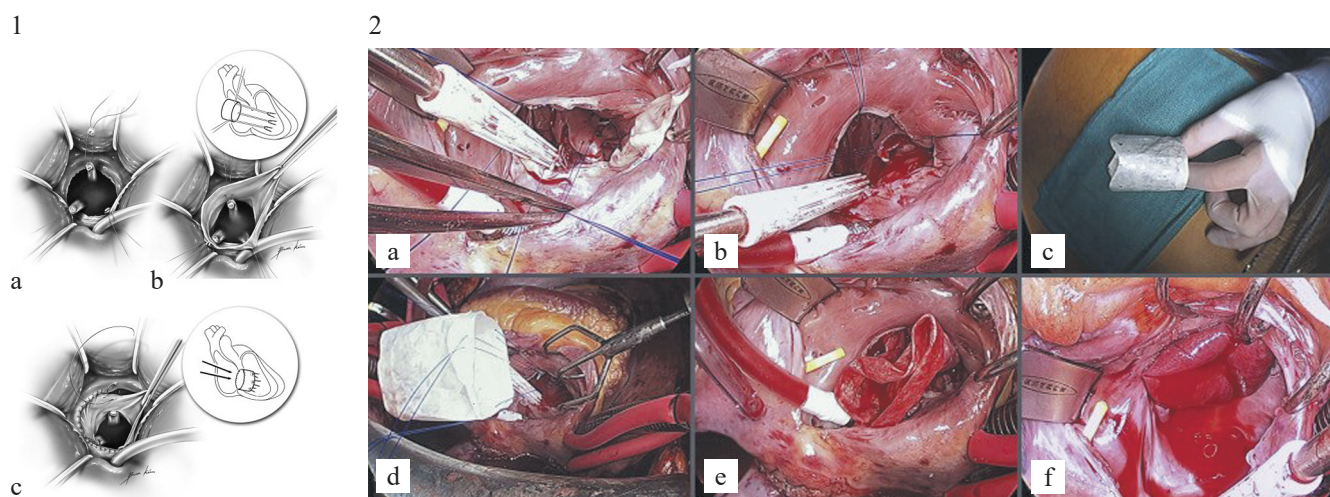


Fig. 1: 1 – schematic representation of the operation (a, suturing the papillary muscles, b, fixing the sutures to the lower edge of the prosthesis, c, fixing the sutures to the rings); 2 – intraoperative view of the stages of the operation (a, b, suturing at the papillary muscle attachment points, c, extracellular matrix valve prior to implantation, d, distal edge of the valve secured to the attachment points, e, proximal edge of the valve circumferentially sutured to the annulus, f, demonstration of valve function during saline insufflation)

decellularization. Third, reliable detection methods and more effective washing protocols must be developed to remove residual cytotoxic agents. Fourth, source tissues should be selected for greater immunocompatibility. Fifth, the degradation rate of the decellularized scaffold must be carefully controlled to prevent either a persistent foreign body reaction with chronic immune activation or excessively rapid degradation leading to acute cytokine release and graft failure. Finally, sterilization methods must be chosen to minimize damage to the decellularized scaffold [9–11].

In a study by Padalino et al. evaluating the long-term outcomes of ECM scaffolds used for reconstructive surgery in congenital heart disease, 5 of 13 patients (38.4%) who underwent valve reconstruction required repeat valve replacement after a mean follow-up of 25.2 months. The authors concluded that the durability of ECM scaffolds appeared inferior to that of autologous pericardium [12].

Nevertheless, the flexibility and reduced thickness of ECM scaffolds may represent an advantage in patients with low body weight, in whom even autologous pericardium can be excessively thick and bulky for small anatomical structures. Moreover, the potential for colonization by autologous cells remains a promising feature of ECM-based materials. In addition, implantation of ECM into viable native valve tissue for augmentation purposes may facilitate tissue regeneration and adaptive remodeling. In contrast, leaflet enlargement using ECM in the setting of a dysplastic valve may result in infiltration by inflammatory cells and neointimal thickening, without any cellular remodeling.

Histological analysis comparing two materials, CorMatrix and autologous pericardium, conducted by Zaidi et al. at the Boston Pediatric Cardiology Center, showed significant differences between the two materials. CorMatrix remained structurally intact with minimal macroscopic change throughout the observation period; however, it elicited a pronounced inflammatory response lasting up to 9 months. This response was characterized by dense infiltration of eosinophils and multinucleated giant cells. No evidence of scaffold resorption, constructive remodeling, or formation of tissue resembling native valve structures was identified. In many specimens, a thick neointimal layer developed over the material, suggesting that CorMatrix was covered with a membrane. In contrast, autologous pericardium did not provoke a significant inflammatory response. Although this difference may partly reflect longer implantation durations for pericardial samples – during which inflammation would be expected to have resolved – even specimens retrieved at earlier time points (e.g., 14 and 163 days) showed no inflammation. By comparison, CorMatrix samples continued to exhibit active inflammatory changes at similar intervals. Notably, focal calcification was

observed in autologous pericardial patches, typically localized around suture lines [13].

CYLINDRICAL PROSTHETIC VALVES MADE FROM AUTOLOGOUS PERICARDIUM

In 2023, Miyamoto et al. [14] developed a template for constructing cylindrical bioprosthetic valves from autologous pericardium for use in atrioventricular valve replacement. For each bioprosthetic valve, the circumference of the native mitral or tricuspid annulus was first measured precisely. Then, the appropriate template, which was originally designed to correspond to the native annular dimensions, was then selected, and the pericardial patch was cut accordingly. The harvested pericardium was treated with 0.6% glutaraldehyde for 2 minutes and subsequently rinsed three times in saline. The proximal edge of the pericardial patch was folded outward to create a loop, and the lateral edges were sutured with several stitches to form a complete cylindrical structure corresponding to the annular circumference. To assess the reproducibility and functional consistency of the cylindrical autopericardial prosthesis across different cardiac anatomies, the authors conducted experiments in 15 animal hearts, including 9 Dorset sheep, 3 goats, 2 dogs, and 1 miniature pig. Prior to valve implantation, the native mitral or tricuspid valve, along with its tendinous cords, was excised.

During implantation of the cylindrical prosthetic valve, the posterior leaflet of the new valve was aligned with the septal leaflet of the tricuspid valve. After implantation, saline was injected into the ventricle to assess valve function. The closed configuration of the implanted valves, leaflet mobility, coaptation length, and the presence or absence of regurgitation were carefully evaluated. Coaptation length was determined using a staining technique. None of the implanted valves showed regurgitation.

The authors emphasized that the *ex vivo* findings demonstrate the structural advantages and potential clinical benefits of the developed cylindrical prosthesis. Nevertheless, further *in vivo* studies, including long-term follow-up, are required to fully assess the potential of this novel BPV.

Researchers from Poland [15] proposed that eliminating artificial materials and implanting exclusively autologous biological tissue could reduce the risk of recurrent infective endocarditis (IE). Their study included seven consecutive patients with active IE who underwent implantation of a cylindrical valve constructed from the patient's own pericardium in the tricuspid position. The decision to use a pericardial cylinder was made due to complete destruction of the native tricuspid leaflets.

Transthoracic echocardiography was performed to evaluate the extent of leaflet destruction, the severity of regurgitation, the size of the fibrous annulus, and leaflet

height (defined as the distance from the annular edge to the heads of the papillary muscles).

All procedures were performed via median sternotomy, which also allowed harvesting of pericardial tissue. A rectangular pericardial patch measuring 10×4 cm was excised and fashioned into a cylinder with a diameter of 30–32 mm. The cylinder length was approximately 120% of its diameter. Three fixation points were marked at 120° intervals, corresponding to the anticipated attachment sites at the right ventricular papillary muscles. In two patients, the cylinder was made from a xenopericardial patch.

The native tricuspid valve, along with all infected tissue, was completely excised. The papillary muscle heads were identified, and the three marked points on the pericardial cylinder were secured to the posterior and septal papillary muscles using 5-0 Gore-Tex sutures. If the anterior papillary muscle was underdeveloped, the corresponding fixation point on the cylinder was attached to the interventricular septum at the level of the other papillary muscle heads. The proximal end of the cylinder was then anastomosed to the tricuspid annulus using a continuous 5-0 Prolene suture, with gradual adjustment to accommodate any diameter mismatch between the native annulus and the prosthetic cylinder. After implantation, a saline (water) test was performed to verify valve competence and ensure adequate sealing (Fig. 2).

Isolated implantation of the pericardial cylinder was performed in only two patients. The remaining five patients required additional surgical procedures, including prosthetic valve implantation in other positions, radiofrequency ablation for atrial fibrillation, closure of a ventricular septal defect (VSD), closure of a patent foramen ovale (PFO), and coronary artery bypass grafting (CABG).

The postoperative follow-up period ranged from 2 to 32 months (mean duration: 17 months). During follow-up, no cases of recurrent IE involving the pericardial

cylinder were observed. However, degeneration of the pericardial cylinder leading to stenosis occurred in three patients. Two of these patients had a history of intravenous drug use. One of them died five months after surgery due to recurrent IE affecting an aortic prosthesis. The second patient developed early cylinder degeneration three months postoperatively and required reoperation. The third patient with cylinder degeneration underwent a successful transcatheter valve-in-valve procedure.

The authors concluded that implantation of a pericardial cylinder in the tricuspid position effectively eradicates tricuspid valve IE and minimizes the risk of recurrence. The use of either autologous or bovine pericardium allows complete restoration of valve function and represents an effective and reproducible surgical technique. Furthermore, degeneration of the cylindrical prosthesis with subsequent stenosis can be managed using balloon valvuloplasty or transcatheter valve-in-valve implantation.

As can be seen, substantial efforts have been made toward the development and clinical implementation of cylindrical BPVs for the tricuspid position. Nevertheless, the choice of material for prosthesis fabrication remains a central challenge. The absence of a supporting frame reduces tissue stress at the attachment sites, which may enhance durability, even when using well-established biomaterials such as fixed xenopericardium or autologous pericardium.

Tissue-engineered constructs represent a promising alternative; however, their clinical application remains uncertain due to limited availability and inconsistent data regarding long-term durability and immunogenicity. In addition, certain technical challenges related to implantation persist and require further refinement.

Overall, the development of a cylindrical prosthetic valve for the tricuspid position is a highly promising direction in cardiac surgery. Its adaptable sizing is particularly advantageous for underweight or small-framed

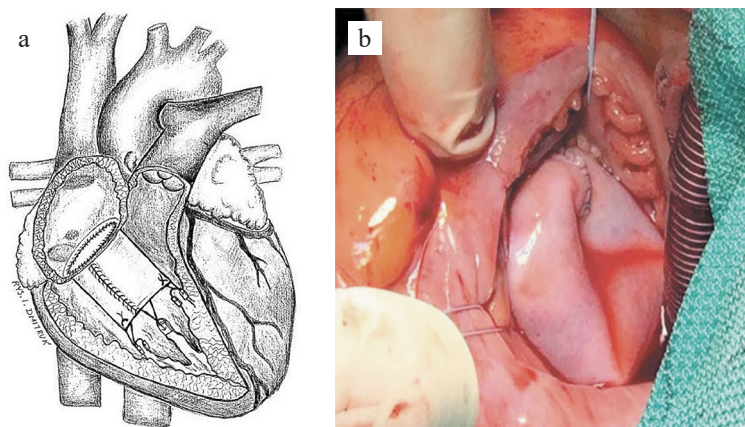


Fig. 2. (a) Schematic representation of an implanted cylindrical prosthesis. (b) Intraoperative view of a cylinder made from pericardium

patients. Continued investigation and accumulation of clinical experience are therefore essential.

The authors declare no conflict of interest.

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INFLUENCE OF LEAFLET CALCIFICATION PATTERNS ON THE BIOMECHANICS OF BIOPROSTHETIC MITRAL VALVES

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Objective: to identify characteristic patterns of calcium distribution in explanted bioprosthetic heart valves and evaluate their influence on the biomechanics of the device. **Materials and methods.** Thirty-three bioprosthetic mitral valve leaflets explanted due to structural valve degeneration were analyzed. Multislice computed tomography (MSCT) images were used to identify pathological calcification within each leaflet. Calcified regions were segmented from top-view projections using a radiographic density threshold of 130 HU. The resulting dataset was clustered according to the number of pixels representing calcified areas, yielding three distinct classes: no calcification, mild calcification, and severe calcification. For each class, a three-dimensional computational model of the bioprosthesis was constructed. Biomechanical behavior was evaluated numerically in a series of computer simulation experiments using the finite element method. Each model included the supporting frame and three valve leaflets, with physiologically relevant boundary conditions simulating pressures in the left atrium and left ventricle. The analysis assessed maximum principal stress, strain, and their spatial distribution across the prosthesis. **Results.** Calcification of one or two valve leaflets resulted in a slight reduction in the average stress and strain values of the intact leaflet – from 0.319 to 0.303 MPa and from 0.134 to 0.130 mm/mm, respectively. Increased calcium content also lowered the peak stress and strain values, from 2.884 to 2.117 MPa and from 0.384 to 0.333 mm/mm. A clear relationship was observed between calcification pattern and local stress concentrations, which exceeded the leaflet's mean stress values by 40–50%. Co-localization of mild or severe calcification clusters on one or two leaflets produced qualitative alterations in the closure mechanism, including “overlap” of mineralized leaflets over adjacent intact ones. **Conclusion.** The findings demonstrate a relationship between the stress–strain behavior of bioprosthetic valve leaflets and the spatial pattern of calcification. While an increase in calcium volume up to 28% does not substantially affect mean stress or strain values, it significantly reduces their peak values.

Keywords: mitral valve, bioprosthetic heart valve, calcification, MSCT segmentation, clustering, finite element method, biomechanics.

INTRODUCTION

In Russia, 2,526 bioprosthetic heart valves (BHVs) were surgically implanted in 2022 [1], with more than 1,600 procedures performed using minimally invasive techniques [2]. Unlike mechanical valves, BHVs do not require long-term anticoagulant therapy and provide hemodynamic performance comparable to that of native heart valves. However, according to reports, more than half of implanted BHVs require replacement within 10–15 years due to structural valve degeneration (SVD) [3]. The main cause of SVD is calcification of the valve apparatus [4, 5]. This process is characterized by progressive calcium deposition within prosthetic tissues, leading to reduced leaflet elasticity and impaired functional mobility.

However, despite extensive research on this issue, there remains no consensus on the root cause of BHV calcification. Several studies highlight the limited understanding of the processes involved [6]. Some authors

attribute calcification to immune-mediated responses, including reactions to residual xenogeneic cells [7] or to fixation and stabilization treatments applied to bioprosthetic materials [8]. Sinusas [9] has emphasized that, in addition to valve design and hemodynamic factors, mechanical stresses play a critical role in the development of SVD.

Advances in analytical tools have expanded the investigation of SVD to include numerical modeling approaches, which provide insight into the stress–strain state of BHVs and their components [10–14]. For instance, Qin et al. (2020) investigated calcification using 3D, patient-specific models of six bicuspid aortic valves [14]. Using finite element method (FEM), the authors evaluated valve biomechanics while accounting for calcium deposits on leaflet surfaces and demonstrated a direct relationship between areas of high mechanical stress and calcification sites.

Similarly, Arzani et al. (2017) demonstrated the influence of leaflet stretching on both the direction and

intensity of mineralization in a native aortic valve model [15]. Their findings suggested that calcification initiates at leaflet attachment sites and progresses centripetally, driven by stiffness mismatches between calcified regions and surrounding tissue.

Overall, the findings reported above do not allow for the formulation of a unified, albeit multifactorial, opinion about the root cause and mechanisms of calcification progression. Therefore, the present study aims to investigate the relationship between mineralization patterns and mechanical stress distribution using our own collection of explanted BHVs in the mitral position, combined with numerical biomechanical analysis.

MATERIALS AND METHODS

The study material consisted of UniLine prosthetic xenopericardial heart valves (NeoCor, Russia) explanted electively due to SVD. All procedures were conducted at the surgical department of the Research Institute for Complex Issues of Cardiovascular Diseases in Kemerovo. Between 2015 and 2024, a total of 11 mitral-position BHVs were included in the study, with no cases excluded. The duration of prosthesis function in the analyzed cohort had a median of 5.33 years (interquartile range: 0.96–9.62), with a minimum follow-up of 0.50 years and a maximum of 13.33 years.

All BHVs in the collection were examined using clinical multislice computed tomography (MSCT), which provides adequate visualization of calcific deposits, on a LightSpeed™ VCT 64 scanner (General Electric, USA). During scanning, the samples were placed on a flat surface with the commissural pillars facing upward. The CT acquisition parameters were as follows: tube voltage 120 kV, tube current 160 mA, gantry rotation time 0.9 s, scan duration 6.8 s, and table speed 39.37 mm/revolution. Image reconstruction was performed with a slice thickness of 0.625 mm using a standard reconstruction kernel. Maximum intensity projection (MIP) images were generated from the MSCT images of the BHVs. After that, the three leaflets were manually extracted from each such image of the prosthesis and oriented in a single position for further clustering (Fig. 1). As a

result, a total of 33 valve leaflets ($N = 33$) were included in the analysis.

Subsequently, the resulting set of slices was analyzed in two consecutive stages: identification of typical calcification patterns through clustering, followed by numerical modeling of the stress–strain state based on the characteristics of the identified clusters.

Calcification clusters

The extracted valve leaflets were clustered according to the number of pixels corresponding to calcified regions. In accordance with published data, areas of pathological mineralization were defined as having an X-ray density of 130 Hounsfield units (HU) [16, 17] for images in 8 bits of gray scale (0–255 gray intensity). After quantifying the number of calcified pixels for all 33 leaflets, the median and quartile values for the sample were calculated. On the basis of these indicators, three distinct image groups were identified.

Numerical modeling

After obtaining the cluster data, biomechanical analysis was performed using FEM in the Abaqus engineering analysis environment (Dassault Systèmes, France) with an explicit solver. The 3D computational model of the bioprosthetic valve comprised three leaflets and a two-component supporting frame, incorporating polypropylene and nickel–titanium elements. The material properties of all prosthetic components were defined based on manufacturer-provided specifications [18] and literature sources [19, 20]. All components were assembled into a single model using a pairing approach, as previously described in our earlier work [21]. Boundary conditions applied to the valve apparatus corresponded to normotensive pressure [22] at a heart rate of 70 beats per minute (Fig. 2).

The resulting 3D model was subsequently modified in accordance with the clustering results obtained in Stage I of the study. To do this, images of the calcium areas of each cluster were superimposed on the prosthesis leaflets, assigning the corresponding properties to the elements of the calculation grid based on data from the

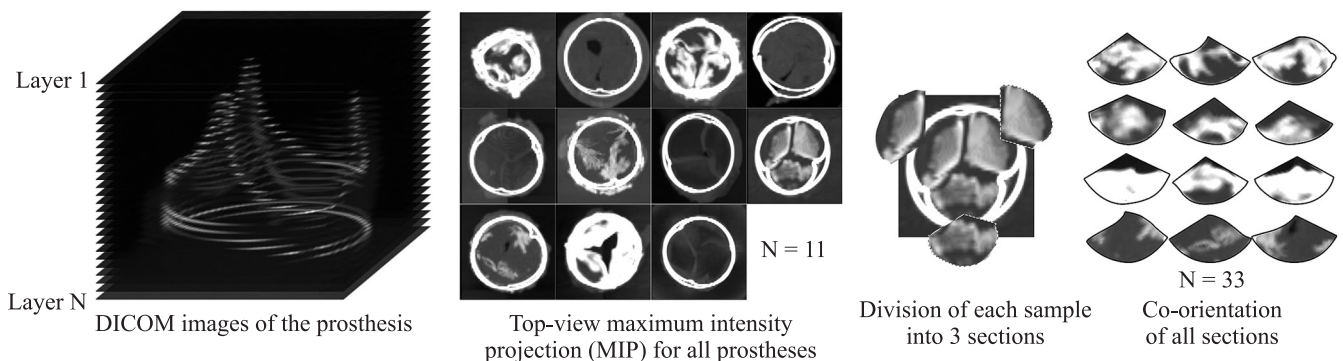


Fig. 1. DICOM image processing design for calcification intensity clustering

literature [19]. The unchanged material (non-calcified) was modeled as bovine xenopericardial tissue stabilized with ethylene glycol diglycidyl ether, as used in the manufacture of prostheses. The mechanical properties of this material and their application for FEM modeling have been described previously in our work based on experimental data [23]. Leaflet–leaflet interactions were defined using a linear soft contact formulation (“linear pressure–overclosure”) with a normal stiffness of 0.2 and Coulomb friction in the tangential direction with a friction coefficient of 0.2. The numerical analysis focused on maximum principal stress (S) and material strain (LE).

Statistical analysis

Descriptive statistical methods were applied. Quantitative data were presented as the mean $\pm$ standard deviation or as the median with the 25th and 75th percentiles, as well as minimum and maximum values, where appropriate. All statistical calculations were performed using built-in functions of the NumPy library (version 1.24.4) in the Python programming environment (version 3.9).

RESULTS

Calcification clusters

During clustering, three clusters were identified (Fig. 3a): no calcification, defined as images with a pixel count below or equal to the median (≤ 6 pixels); mild calcification, characterized by pixel counts between the median and the third quartile (6–473 pixels); and severe calcification, defined as pixel counts equal to or exceeding the third quartile (≥ 473 pixels). The resulting calcium distribution patterns were conditionally labeled

as clusters 0, 1, and 2, respectively. For numerical modeling, valve regions corresponding to mild and severe mineralization (clusters 1 and 2) were classified as calcified, which corresponded to probability values exceeding 50% (Fig. 3b).

To assess clustering quality, the silhouette was calculated [24], and yielded a value of 0.44, indicating moderate yet acceptable separability among the three identified groups (no calcification, mild calcification, and severe calcification).

Numerical modeling

The clusters obtained in the previous stage were subsequently mapped onto the mitral valve prosthesis model. Each such model was labeled using a triplet notation that reflects the cluster assignment for each of the three valve leaflets (Fig. 4a). This approach yielded ten unique calcification patterns: 0-0-0, 0-0-1, 0-0-2, 0-1-1, 0-1-2, 0-2-2, 1-1-1, 1-1-2, 1-2-2, and 2-2-2. In this notation, 0 denotes a leaflet without calcification, 1 corresponds to the mild calcification cluster, and 2 represents the severe calcification cluster (Fig. 4b).

The intact BHV, in which all three leaflets were free of calcification (configuration 0-0-0), was used as the initial state (Fig. 5).

Numerical modeling revealed localized peak values of maximum principal stress ($S = 2.884$ MPa) and material strain ($LE = 0.385$ mm/mm), concentrated in the commissural strut region at the leaflet attachment edge. In contrast, stress and strain within the leaflet dome were substantially lower, not exceeding $S = 0.500$ MPa and $LE = 0.160$ mm/mm. Torsional deformation of the valve apparatus was observed in the central region, demonstrating qualitative agreement with data from full-scale

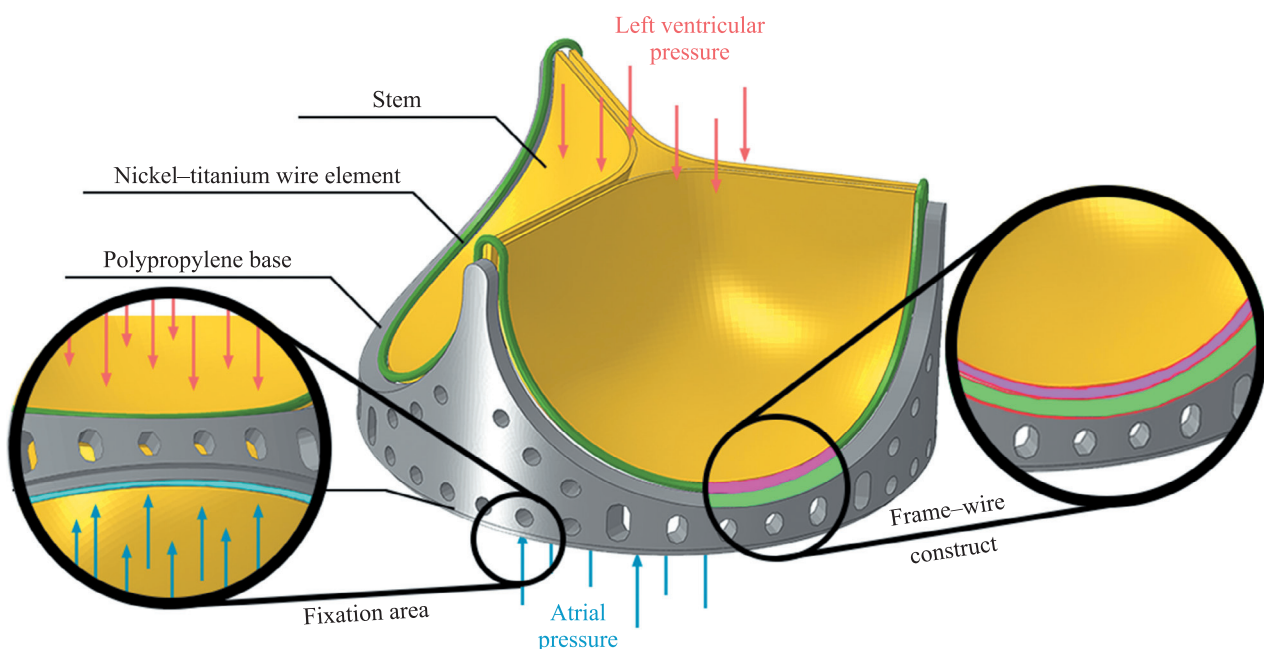


Fig. 2. Visualization of the numerical computation model with indicated boundary condition regions

experimental studies of similar aortic-position prostheses [25]. Subsequent addition of calcification clusters to the model can be interpreted as progressive valve

stenosis, ranging from minor focal inclusions (0-0-1) to extensive leaflet damage (2-2-2). The results are shown in Fig. 6.

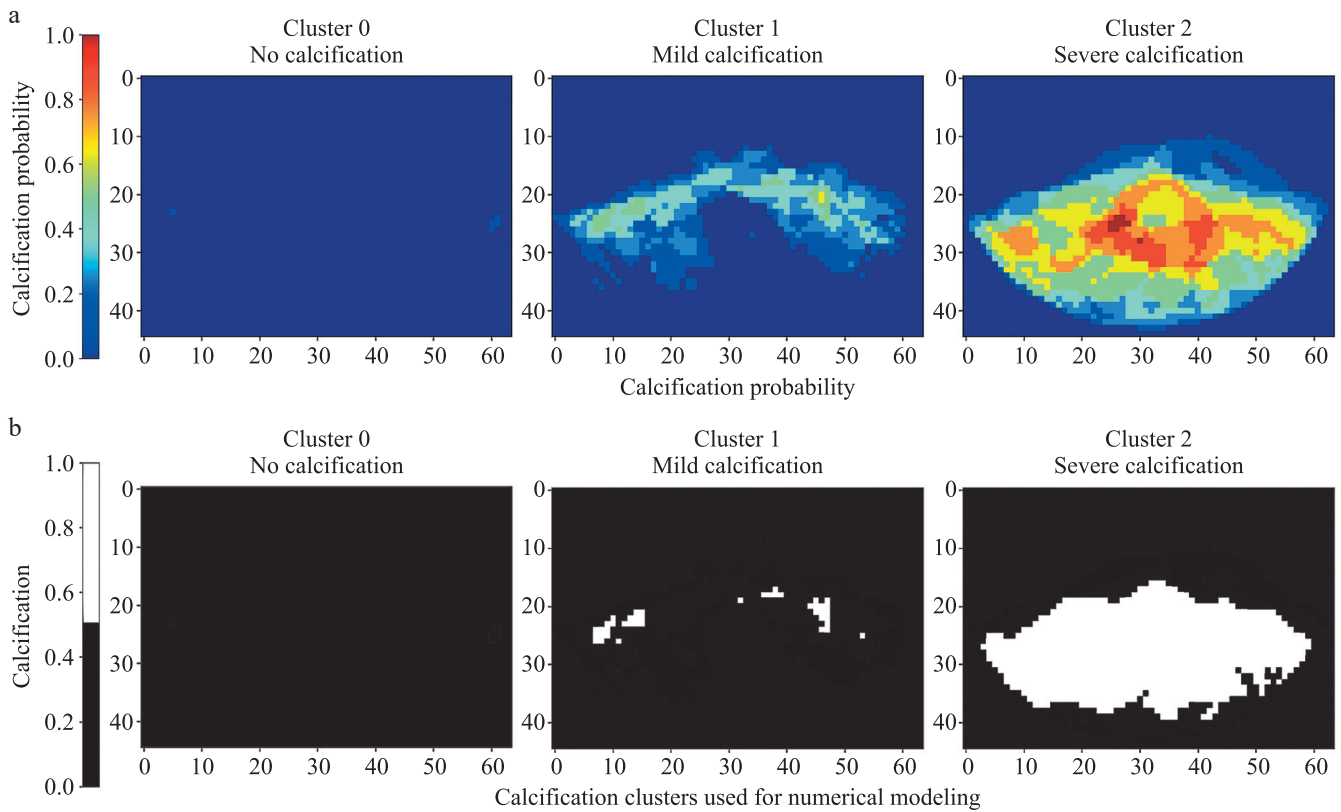


Fig. 3. Results of explanted prosthetic valve processing. a: Calcification clusters obtained; b: Clusters with calcification probability of [0.5–1] (fractions)

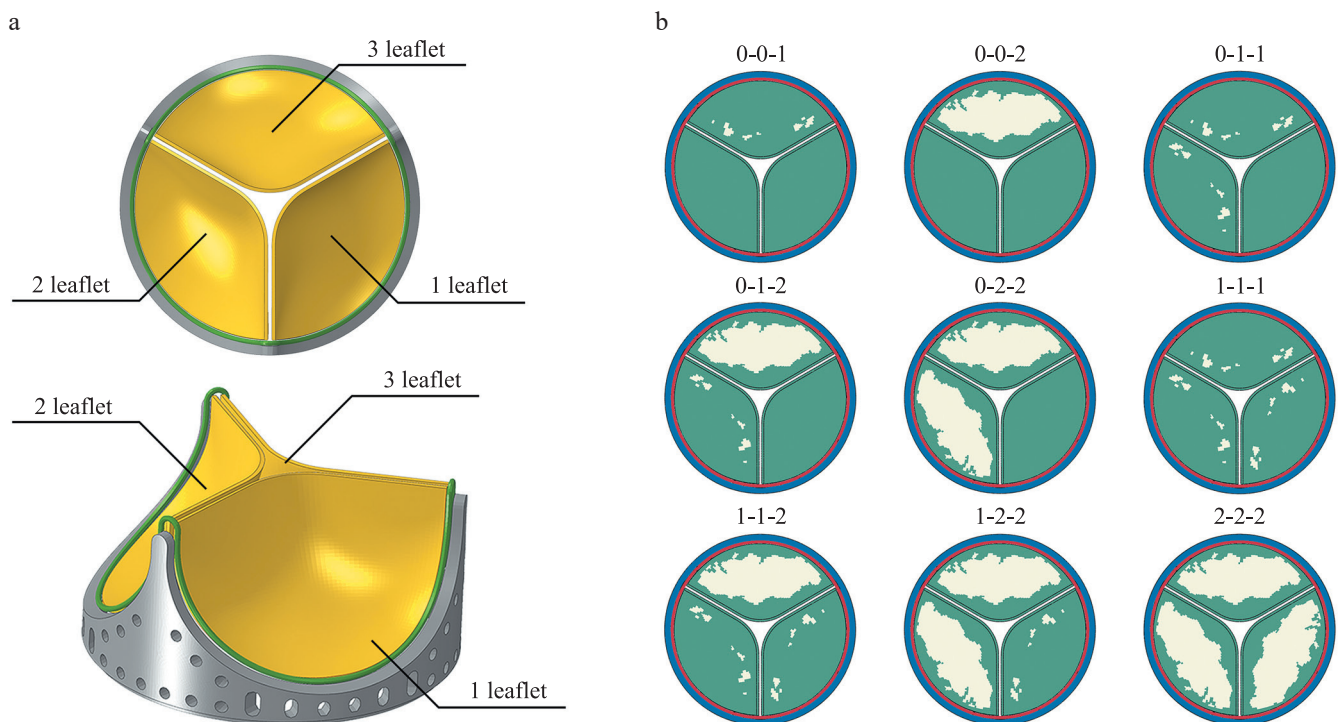


Fig. 4. Principle of forming the triplet number: (a) serial numbering of valve leaflet models; (b) superposition of calcification clusters onto the leaflet model

Numerical analysis of the prosthetic valve models (Fig. 6a–b) showed that inclusion of the second calcification cluster (triplets 0-0-2, 0-1-2, 0-2-2, 1-1-2, 1-2-2, and 2-2-2) led to the emergence of re-

gions with elevated stress and strain in the suture edge zone – features absent in the intact case. In these models, maximum principal stress increased to $S = 2.035\text{--}2.812$ MPa (+283–391%), while ma-

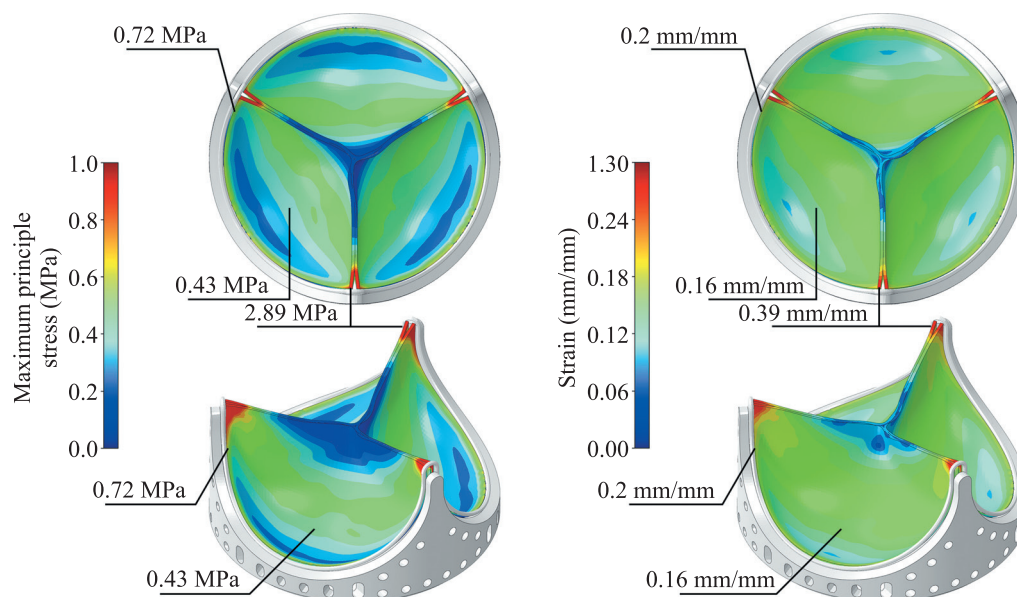


Fig. 5. Results of numerical simulation performed for the intact prosthesis

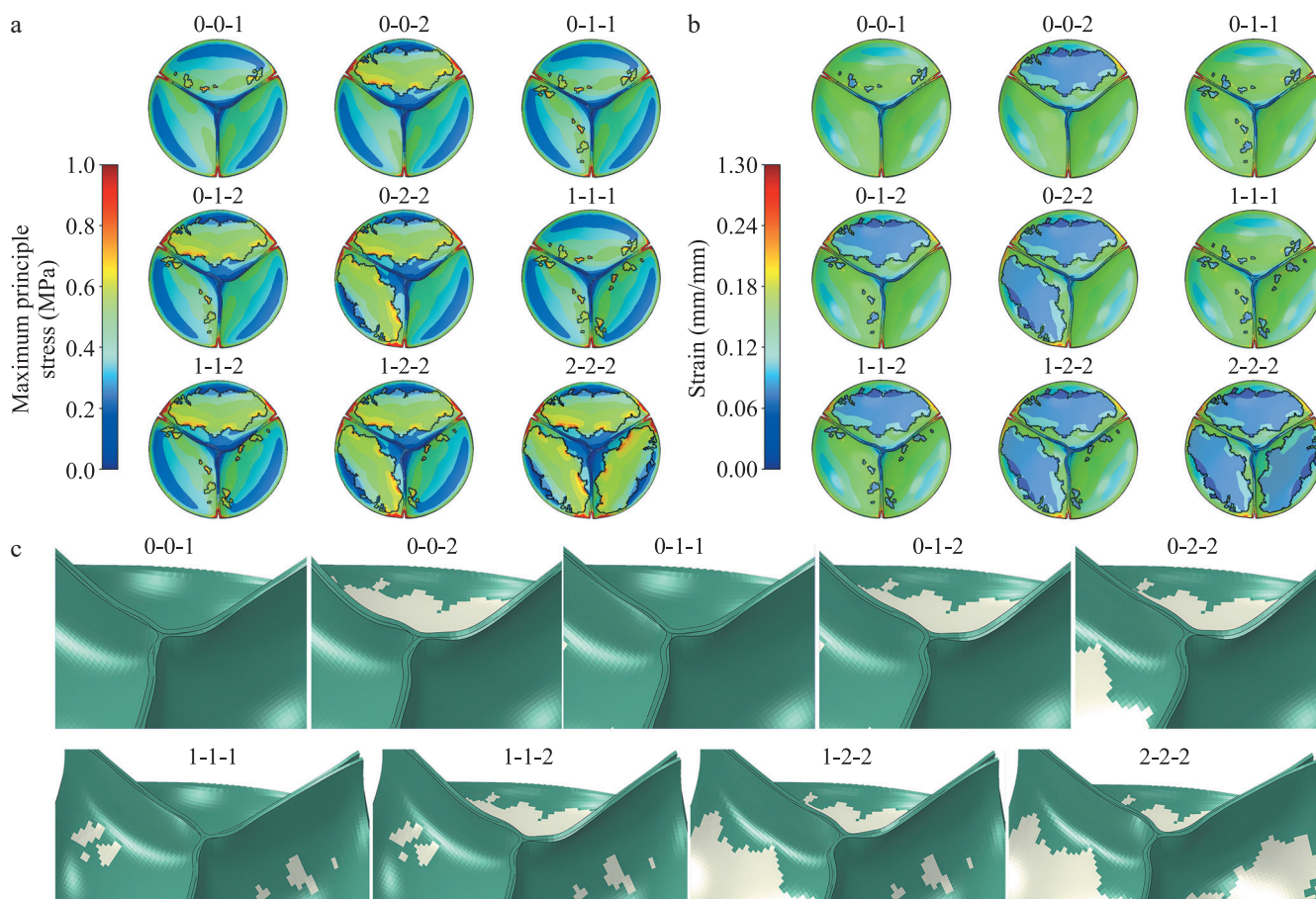


Fig. 6. Results of numerical modeling of calcium deposit clusters. Top-view contour plots show (a) maximum principal stress (MPa) and (b) strain (mm/mm). The contours of calcium clusters are outlined in black. (c) Enlarged isometric views of the valve closure region, where green indicates the bioprosthetic material and white denotes mineralized areas

terial strain rose to $LE = 0.327\text{--}0.384$ mm/mm (+161–189%).

The addition of the first cluster (triplets 0-0-1, 0-1-1, 0-1-2, 1-1-1, 1-1-2, and 1-2-2) did not have such an effect.

There are distinct local increases in peak stresses in the mineralization zones for the first cluster (triplets 0-0-1, 0-1-1, 0-1-2, 1-1-1, 1-1-2, 1-2-2) $S = 1.175$ MPa (+276%) and the second cluster $S = 1.193$ MPa (+280%) compared to the intact model in a similar zone ($S = 0.426$ MPa). In addition, a decrease in maximum strain $LE = 0.108\text{--}0.117$ mm/mm (a drop of 32%–26%) was noted for both clusters.

Qualitative analysis of valve closure revealed changes in the distribution of both maximum principal stress and strain fields. In cases where only the first or second cluster is present in the model on the third leaflet (Fig. 6c: 0-0-1 and 0-0-2; see Table 4), a reduction in the mean strain values ($LE = 0.132 \pm 0.039$ mm/mm and $LE = 0.120 \pm 0.046$ mm/mm, respectively) compared with the intact model ($LE = 0.134 \pm 0.037$ mm/mm) resulted in leaflet “overlap” during coaptation. A similar behavior was observed in configurations with one unaffected leaflet (Fig. 6c: 0-1-1 and 0-2-2). Model 0-1-2 is distinctive in that it includes all three cluster variants. The second and third leaflets exhibited a smooth, untwisted coaptation, whereas the first (intact) leaflet showed the greatest degree of bending. This finding indicates that the presence of calcification on even a single leaflet

(clusters 1 or 2) alters the mechanical behavior of the entire valve apparatus, leading to overlap or excessive bending of non-calcified (cluster 0) leaflets within the coaptation zone. In contrast, when all three leaflets were affected by calcification (Fig. 6c: 1-1-1, 1-1-2, 1-2-2, and 2-2-2), the prosthesis demonstrated a symmetrical closure pattern, with no single leaflet overlapping the others.

The next stage of the study evaluated the effect of progressive calcification – defined as an increase in the total proportion of rigid inclusions up to 42% of the leaflet volume in configuration 2-2-2 – on the peak and mean values of maximum principal stress and strain. The results are shown in Fig. 7.

The image shown (Fig. 7) demonstrates that increasing the degree of mitral valve calcification leads to two interrelated effects. First, the peak values of maximum principal stress showed a clear monotonic decrease (Fig. 7a; Tables 3, 5): from $S = 2.884$ MPa in the intact valve (Fig. 5, configuration 0-0-0) to $S = 2.774$ MPa (–3.8%) in the model with one calcification cluster on each leaflet (Fig. 6a, 1-1-1; Table 5), and further to $S = 2.117$ MPa (–26.6%) in the fully calcified configuration (Fig. 6a, 2-2-2; Table 5). A similar trend was observed for strain (Fig. 7b; Tables 4, 6): peak strain decreased from $LE = 0.384$ mm/mm in the intact model to $LE = 0.378$ mm/mm (–1.6%) for configuration 1-1-1, and to $LE = 0.333$ mm/mm (–13.3%) for configuration 2-2-2. Notably, in all cases, these peak values were loca-

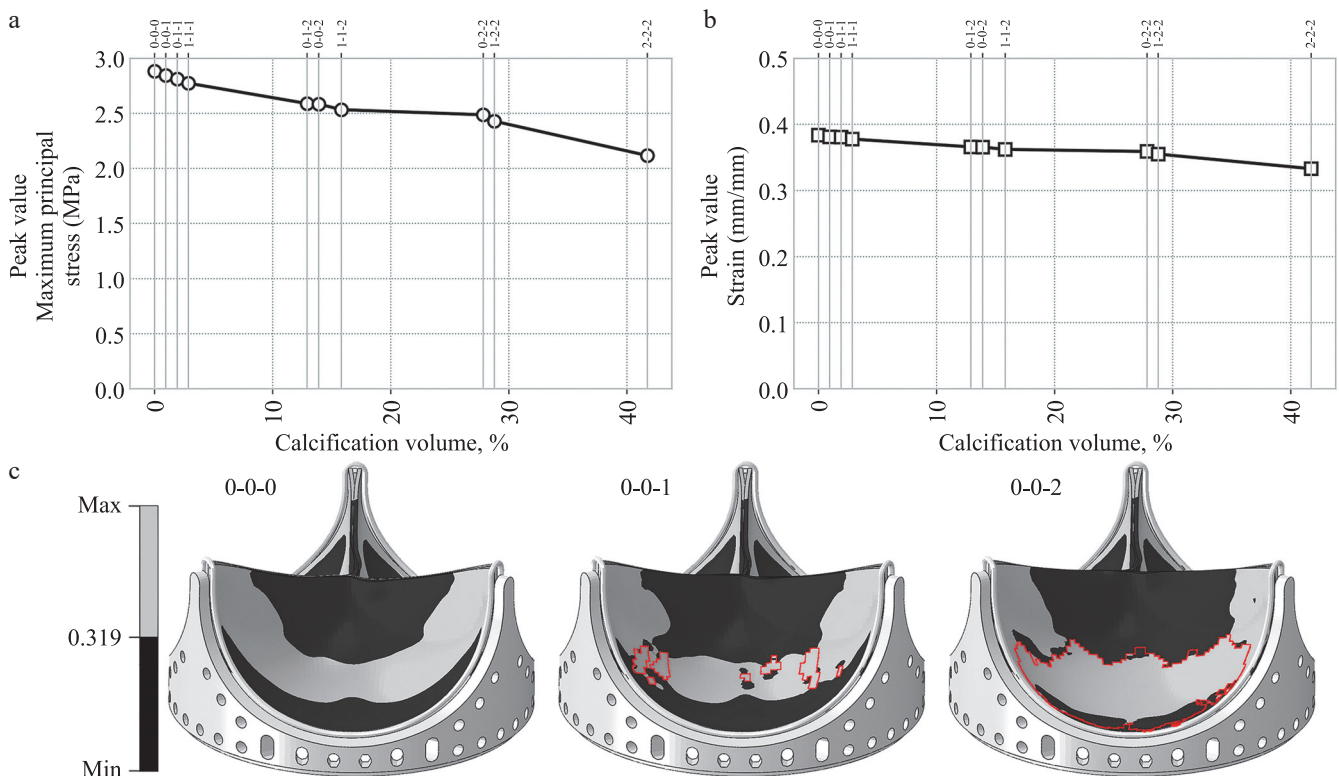


Fig. 7. Assessment of changes in peak values. (a) Maximum principal stress (MPa, S). (b) Strain (mm/mm, LE). (c) Binary map of zones with increased risk of calcification, with calcification cluster boundaries highlighted in red

lized in the region of the commissural pillars along the suture edge (Fig. 6a).

When assessing the differences in the stress-strain state, cluster 1 consistently exhibited higher mean values of both maximum principal stress (Table 5; $S = 0.621$ MPa) and strain (Table 6; $LE = 0.060$ mm/mm) than cluster 2 ($S = 0.514$ MPa and $LE = 0.050$ mm/mm, respectively). Overall, increasing calcification volume was associated with a rise in the mean values and standard deviations of both maximum principal stress (Table 3) and strain (Table 4), with average stress increasing from $S = 0.319$ MPa in the intact prosthesis to $S = 0.360$ MPa in the case of complete leaflet mineralization.

Secondly, spatial localization of calcification influenced the adjacent, non-calcified leaflets (Table 1; triplets 0-0-1, 0-0-2, 0-1-1, 0-1-2, and 0-2-2). Compared with the intact model, these configurations demonstrated a general tendency toward reduced peak maximum prin-

cipal stress values (by up to 22.3% from $S = 2.884$ MPa) as well as a decrease in mean stress across the leaflet (by approximately 5% from $S = 0.319$ MPa). Average stress levels across the entire leaf in the presence of only one cluster (Table 1, triplets 0-0-1 and 0-1-1) increased slightly by 0.6–0.9%, and in the presence of only the second (0-0-2 and 0-2-2) – decreased by 2.2–5%. When both cluster types were co-localized, a comparable decrease in mean stress was observed (–1.9%; Table 1, configuration 0-1-2).

When strain was analyzed, the intact model showed peak and mean strain values of $LE = 0.384$ mm/mm and $LE = 0.134$ mm/mm, respectively. Overall, the presence of calcification was associated with reduced peak leaflet strain (Fig. 7b; Table 2, Table 6). Cluster 1 had a negligible effect on leaflet stiffness, with peak strain decreasing by only 0.8% and 3.9% in triplets 0-0-1 and 0-1-1, respectively.

Table 1

Changes in maximum principal stress (S) in leaflets of Cluster 0 compared with the intact model

Model no.	Peak value, MPa (+/–% change)	Average value, MPa (+/–% change)
0-0-0	2.884	0.319
0-0-1	2.845 (–1.4%)	0.321 (+0.6%)
0-0-2	2.570 (–10.9%)	0.312 (–2.2%)
0-1-1	2.632 (–8.7%)	0.322 (+0.9%)
0-1-2	2.536 (–12.1%)	0.313 (–1.9%)
0-2-2	2.242 (–22.3%)	0.303 (–5.0%)

Table 2

Changes in maximum principal stress (S) in leaflets of Cluster 0 compared with the intact model

Model no.	Peak value (mm/mm) (+/–% change)	Average value (mm/mm) (+/–% change)
0-0-0	0.384	0.134
0-0-1	0.381 (–0.8%)	0.134 (0%)
0-0-2	0.365 (–5.0%)	0.132 (–1.5%)
0-1-1	0.369 (–3.9%)	0.134 (0%)
0-1-2	0.363 (–5.5%)	0.132 (–1.5%)
0-2-2	0.342 (–10.9%)	0.130 (–3.0%)

Table 3

Statistical analysis of the maximum principal stress (MPa, S) for the model as a whole. Negative values indicate compressive stress

Model	Avg.	Standard deviation	Min	Q25%	Q50%	Q75%	Max
0-0-0	0.319	0.178	–0.123	0.193	0.325	0.439	2.884
0-0-1	0.321	0.194	–0.131	0.191	0.318	0.442	2.845
0-0-2	0.335	0.202	–0.136	0.191	0.334	0.455	2.585
0-1-1	0.322	0.197	–0.133	0.189	0.312	0.444	2.812
0-1-2	0.332	0.198	–0.136	0.189	0.325	0.455	2.590
0-2-2	0.348	0.213	–0.138	0.185	0.344	0.482	2.487
1-1-1	0.322	0.200	–0.137	0.187	0.306	0.446	2.774
1-1-2	0.335	0.208	–0.140	0.186	0.320	0.469	2.534
1-2-2	0.348	0.216	–0.142	0.182	0.336	0.490	2.429
2-2-2	0.360	0.223	–0.142	0.176	0.361	0.524	2.117

Table 4

Statistical analysis of strain (mm/mm, LE) for the model as a whole

Model	Avg.	Standard deviation	Min	Q25%	Q50%	Q75%	Max
0-0-0	0.134	0.037	0.004	0.105	0.141	0.162	0.385
0-0-1	0.132	0.039	0.007	0.103	0.138	0.163	0.381
0-0-2	0.120	0.046	0.007	0.086	0.126	0.158	0.366
0-1-1	0.131	0.040	0.003	0.101	0.136	0.162	0.381
0-1-2	0.121	0.045	0.007	0.089	0.126	0.157	0.366
0-2-2	0.106	0.050	0.007	0.059	0.107	0.149	0.359
1-1-1	0.130	0.041	0.005	0.099	0.133	0.162	0.378
1-1-2	0.117	0.047	0.007	0.082	0.121	0.155	0.362
1-2-2	0.105	0.050	0.007	0.059	0.104	0.146	0.355
2-2-2	0.093	0.050	0.008	0.051	0.079	0.134	0.333

Table 5

Statistical analysis of maximum principal stress (MPa, S) stratified by clusters. Negative values indicate compressive stress

Model	Cluster number	Avg.	Standard deviation	Min	Q25%	Q50%	Q75%	Max
0-0-0	0	0.319	0.178	-0.123	0.193	0.325	0.439	2.884
0-0-1	0	0.321	0.190	-0.131	0.193	0.326	0.441	2.845
	1	0.322	0.201	-0.130	0.188	0.305	0.445	2.807
0-0-2	0	0.312	0.182	-0.136	0.190	0.317	0.431	2.570
	2	0.380	0.231	-0.136	0.193	0.381	0.541	2.585
0-1-1	0	0.321	0.190	-0.130	0.192	0.326	0.443	2.632
	1	0.322	0.200	-0.133	0.187	0.306	0.446	2.812
0-1-2	0	0.313	0.173	-0.136	0.190	0.318	0.434	2.536
	1	0.311	0.189	-0.131	0.183	0.295	0.429	2.422
	2	0.380	0.232	-0.136	0.193	0.383	0.543	2.590
0-2-2	0	0.303	0.173	-0.137	0.186	0.308	0.419	2.242
	2	0.370	0.227	-0.138	0.185	0.372	0.534	2.487
1-1-1	1	0.322	0.200	-0.137	0.187	0.306	0.446	2.774
1-1-2	1	0.314	0.192	-0.140	0.183	0.298	0.433	2.486
	2	0.379	0.231	-0.136	0.192	0.381	0.542	2.534
1-2-2	1	0.301	0.180	-0.142	0.180	0.286	0.413	2.166
	2	0.371	0.228	-0.138	0.185	0.372	0.535	2.429
2-2-2	2	0.360	0.223	-0.142	0.176	0.361	0.524	2.117

Table 6

Statistical analysis of strain (mm/mm, LE) stratified by clusters

Model	Cluster number	Avg.	Standard deviation	Min	Q25%	Q50%	Q75%	Max
0-0-0	0	0.134	0.037	0.004	0.105	0.141	0.162	0.384
0-0-1	0	0.134	0.039	0.007	0.104	0.140	0.163	0.381
	1	0.130	0.041	0.014	0.100	0.134	0.161	0.381
0-0-2	0	0.132	0.038	0.014	0.103	0.138	0.161	0.365
	2	0.097	0.052	0.007	0.053	0.084	0.139	0.366
0-1-1	0	0.134	0.039	0.012	0.104	0.141	0.163	0.369
	1	0.130	0.041	0.003	0.099	0.134	0.161	0.381
0-1-2	0	0.132	0.037	0.015	0.104	0.138	0.161	0.363
	1	0.127	0.039	0.025	0.097	0.130	0.158	0.354
	2	0.097	0.052	0.007	0.053	0.084	0.140	0.366
0-2-2	0	0.130	0.037	0.029	0.101	0.136	0.158	0.342
	2	0.095	0.051	0.007	0.052	0.082	0.137	0.359
1-1-1	1	0.130	0.041	0.005	0.099	0.133	0.162	0.378
1-1-2	1	0.128	0.040	0.007	0.098	0.131	0.159	0.360
	2	0.097	0.052	0.007	0.053	0.084	0.139	0.362
1-2-2	1	0.125	0.038	0.025	0.097	0.127	0.156	0.337
	2	0.095	0.051	0.007	0.052	0.081	0.137	0.355

In contrast, cluster 2 produced a moderate stiffening effect: peak strain decreased by 5.0% and 10.9%. The co-localization of both cluster types resulted in a comparable reduction in peak strain (5.5%), similar to that observed for triplets 0-0-2. In terms of average values, cluster 1 did not cause any changes, while cluster 2 (triplets 0-0-2 and 0-2-2) and the combination (triplet 0-1-2) reduced them by 1.5% for all except 0-2-2 (–3.0%).

A qualitative analysis of the stress–strain state maps showed that, in the intact prosthesis, a distinct zone of elevated stresses was present along the leaflet dome, with maximum principal stress values ranging from $S = 0.44$ to 0.48 MPa, exceeding the mean stress level of the entire model ($S = 0.319$ MPa) (Fig. 7b). Notably, this region coincided with cluster 1 location, which in the present study was conditionally interpreted as representing an early stage of calcification. The stress level in this dome region was approximately 40–50% higher than the average stress value across the entire leaflet of the intact model ($S = 0.319$ MPa, see Table 3).

DISCUSSION

Numerical modeling results for the intact variant revealed a stress distribution pattern known for BHVs [26, 27]. The maximum principal stress ($S = 2.885$ MPa) and strain ($LE = 0.385$ mm/mm) were observed in the commissural pillar zone, whereas lower values were detected in the leaflet dome ($S = 0.450$ MPa and $LE = 0.145$ mm/mm, respectively). Similar findings have been reported by Nestola et al. [28] who investigated incompressible fluid flow through an aortic BHV using a fluid–structure interaction (FSI) approach. Their results demonstrated that peak stresses are concentrated near the leaflet attachment zones and commissures, which is consistent with the areas of peak mechanical stress identified in our FEM model. Thus, despite methodological differences between FEM and FSI simulations, both approaches indicate that the commissural pillar region represents a critical zone of stress concentration. The elevated stress levels observed in these areas (up to 2.885 MPa) are primarily associated with tensile loading of the valve material during closure and are in good quantitative agreement with previously published data, reporting peak stress values in the range of 2.087 – 2.500 MPa [29–31].

The sequential inclusion of calcification clusters in the model demonstrated that progressive mineralization, associated with an increase in the volume fraction of mineral inclusions up to 42%, led to a gradual stiffening of the valve leaflets and a corresponding reduction in their strain (strain decreased by approximately 11% when comparing the intact model [0-0-0] with the fully calcified configuration (2-2-2), with LE declining from 0.384 to 0.333 mm/mm (–13.3%). This effect could potentially lead to an increase in afterload on the myocardium and elevate the risk of left ventricular hypertrophy during long-term use of prostheses affected by SVD [32, 33].

Previous studies have linked reduced leaflet mobility and valve stenosis to increases in the mean diastolic pressure gradient and changes in intracardiac hemodynamics. These findings are further supported by published data demonstrating that rigid inclusions within the mitral valve increase local hydrodynamic stresses, thereby promoting regurgitation and flow turbulence [34].

This study revealed differences in the distribution of mechanical stresses within the prosthesis in the presence of calcium deposits of varying severity. These findings are consistent with the results reported by Qin et al. [14], who showed, using an aortic valve model, that localized mechanical stress gradients in BHVs may act as initiators of subsequent calcification, thereby creating a self-reinforcing cycle of material degradation. FEM-based investigations of calcified valve leaflets similarly indicate that mechanical stress is a critical factor in both the initiation and spread of calcification [14, 35]. In particular, the work of Arzani et al. [15], which focused on modeling the function of the native aortic valve, demonstrated that calcium accumulation can originate and progress in areas subjected to increased circumferential strain. This observation closely aligns with our results, in which maximum principal stresses were concentrated at the boundaries of calcium deposits, in the suture margin area.

In addition, the results showed that although calcification reduced peak mechanical loads within the leaflet tissue, it simultaneously changed the valve closure pattern in the central zone, leading to overlapping of calcified leaflets onto relatively intact ones. This phenomenon may be associated with increased risk of accelerated wear at the free edge of the BHV [3, 36]. According to reports [3, 37], such alterations contribute to regurgitation, increased transvalvular pressure gradients, elevated hydraulic resistance, and reduced effective volumetric output of the prosthesis. Experimental studies [20, 30, 31, 38, 39] support these observations and emphasize the importance of accounting for both mechanical and hemodynamic factors when evaluating the durability and functional performance of BHVs.

Identification of regions where maximum principal stresses exceeded the average values in the intact model (Fig. 7b) revealed a clear spatial overlap between stress levels of 0.44 – 0.48 MPa (1.4–1.5 times higher than the mean value of $S = 0.319$ MPa) and areas corresponding to early-stage calcification. Comparable findings were reported by Qin et al. [14], who demonstrated that localized stress concentrations in aortic valve leaflets correlate with both the direction and rate of mineralization growth and are closely related to average stress levels. The model proposed by Arzani et al. [15] showed that rigid inclusions generate zones of reduced strain, which in turn promote further progression of mineralization and establish a self-sustaining cycle of calcification. This effect was attributed to localized stiffness gradients at the boundaries of mineralized regions, leading to stress

redistribution and strain amplification in adjacent tissues. Notably, mitral valve prostheses undergo higher mechanical stress compared with other valve positions, primarily due to hemodynamic characteristics [40].

In summary, the following conclusions can be drawn from the results:

1. Calcification of the mitral BHV is closely related to the stress–strain state of the valve: lower average values of maximum principal stress are associated with a reduced likelihood of mineralization developing in the leaflet dome.
2. Areas where the maximum principal stress exceeds the average by 40–50% correspond to the locations of calcification clusters;

CONCLUSION

The study conducted on 33 explanted BHVs showed that calcification patterns significantly influence the biomechanical behavior of bioprosthetic mitral valves. Three distinct mineralization patterns were identified: no calcification (cluster 0), mild calcification (cluster 1), and severe calcification (cluster 2). FEM analysis revealed a spatial correlation between regions of maximum principal stress exceeding $S > 0.319$ MPa in the intact model and the locations of calcification clusters, with these areas exhibiting a 40–50% higher local stress in the valve leaflet dome. Importantly, the introduction of clusters 1 and 2 into the valve leaflet did not alter the localization of these high-stress regions. FEM results indicated that the presence of at least one cluster 0 or 1 did not significantly influence mean maximum principal stress (decrease $\leq 5\%$) or mean strain (decrease $\leq 3\%$).

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The authors declare no conflict of interest.

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FEATURES OF IgG ANTIBODY DEPOSITION IN EPOXY-TREATED XENOGENEIC BIOPROSTHETIC HEART VALVES

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Objective: to evaluate changes in the immunogenicity of epoxy-treated xenogeneic bioprosthetic heart valves (BHVs) during *in vivo* function by detecting human IgG antibodies in the biomaterial of explanted samples. **Materials and methods.** Fourteen BHVs explanted during repeat valve replacement were examined. Of these, three valves were removed at 1, 20, and 42 days after implantation, while 11 valves had functioned for periods ranging from 3 to 25 years. Histological sections were prepared from the valve leaflets and analyzed by immunohistochemistry using antibodies against human immunoglobulin G (IgG), as well as Russell–Movat pentachrome staining. Selected leaflet fragments were additionally examined by scanning electron microscopy. **Results.** BHVs removed due to early dysfunction after 1, 20, and 42 days of implantation showed no signs of biomaterial degeneration; however, small thrombi were detected on their leaflet surfaces. In contrast, valves that had functioned for 3 to 25 years exhibited clear features of structural degeneration of the biological tissue, including leaflet tears and extensive calcification. These BHVs were also characterized by moderate macrophage infiltration and a slight increase in pannus on the valve surface. Immunohistochemical staining of histological sections for human IgG revealed intense antibody deposition within the biomaterial of all examined BHVs, irrespective of implantation duration. Positive IgG staining was localized along the fibers of the xenogeneic tissue and was absent in recipient tissues, including thrombi and pannus. **Conclusion.** The biological component of BHVs retains its immunogenic properties even after long-term (up to 25 years) function in the recipient's body.

Keywords: *bioprosthetic heart valves, structural valve degeneration, IgG antibodies, immune response, immunohistochemistry.*

INTRODUCTION

The gold standard for treating severe valvular heart disease is replacement of the affected valves with biological or mechanical prosthetic valves [1, 2]. In recent decades, xenogeneic bioprosthetic heart valves (BHVs), derived from chemically stabilized animal tissues such as the porcine aortic complex and porcine, bovine, or equine pericardium, have been increasingly adopted for this purpose [3–5]. Compared with mechanical prostheses, BHVs offer the advantage of low thrombogenicity, allowing patients to avoid lifelong anticoagulation therapy and its associated risk of hemorrhagic complications. However, their long-term durability remains limited: approximately 50% of BHVs require replacement within 15 years due to prosthetic dysfunction associated with structural valve degeneration (SVD) [6–8]. Immune-mediated rejection of the implant is considered one of the principal mechanisms driving the development of SVD [9, 10].

Several research groups have reported dense white blood cell (WBC) infiltration in BHV leaflets explanted from patients with SVD [11–14]. In addition to cellular immune components, deposits of humoral immune

factors – including IgM and IgG antibodies, the complement component C4d, and the membrane attack complex C5b-9 – have been identified within a damaged valve biomaterial [14–16]. Notably, the contribution of antibodies and complement to SVD pathogenesis has been less extensively studied than that of cell-mediated inflammation [17]. Antibody-mediated mechanisms are typically considered in terms of opsonization of xenogeneic BHV tissues, which facilitates subsequent WBC recruitment [17]. By binding via their Fab regions to residual animal antigens within the biomaterial, antibodies can engage Fcγ receptors on monocytes and other immune cells through their Fc domains, thereby promoting cellular activation and adhesion to the BHV surface [18]. Infiltrating WBCs within the biomaterial subsequently release matrix-degrading factors – including proteolytic and oxidative enzymes, as well as calcium-binding proteins – contributing to progressive degenerative changes in the implant flaps [9, 10].

Valve replacement with xenogeneic BHVs is known to induce a marked (2- to 5-fold) increase in recipient antibody titers directed against specific animal antigens, including galactose-α-1,3-galactoside (α-Gal) and N-

glycolylneuraminic acid (N-GNA) [16, 19, 20]. Elevated levels of antibodies to both α -Gal and N-GNA have been detected for up to 2–4 years following BHV implantation [16, 19]. However, these initially heightened antibody titers tend to decline approximately 2 years after valve replacement, indirectly suggesting antigen elimination from the biomaterial [16]. Clinical observations are consistent with our previous immunohistochemical findings, which demonstrated gradual degradation of N-GNA within BHV tissue, with near-complete elimination occurring approximately 2.5 years after implantation [21].

Taken together, these findings suggest a decline in the residual immunogenicity of BHVs over time. This observation appears to contradict histological evidence, which shows that the most pronounced WBC infiltration occurs not at early but at later stages of implant function [11–14].

To address this discrepancy, we evaluated changes in BHV immunogenicity by assessing the presence of human IgG antibodies in tissues from explanted xenografts that had functioned for varying durations, ranging from 1 day to 25 years.

MATERIALS AND METHODS

Study samples

The study included 14 epoxy-treated xenogeneic BHVs removed from recipients during repeat valve replacement in the aortic ($n = 2$) and mitral ($n = 12$) positions. All prosthetic valves were manufactured by NeoCor CJSC (Kemerovo, Russia), including UniLine ($n = 6$) and Tiara ($n = 2$) xenopericardial valves, as well as PeriCor ($n = 2$) and KemCor ($n = 4$) xenoaortic

implants. Notably, only Tiara prostheses were used for aortic valve replacement.

In this series, three xenopericardial valves were explanted after short-term function (1, 20, and 42 days). The removal of the valve after 1 day was unrelated to prosthesis dysfunction and was performed during emergency surgery due to rupture of the posterior wall of the left ventricle. The Tiara valves functioning for 20 and 42 days were explanted due to frame deformation leading to device failure. The remaining 11 BHVs were removed due to SVD after 3 to 25 years of implantation.

Overall, xenopericardial BHVs demonstrated a shorter functional lifespan compared to xenoaortic prostheses – 68 [45.5–76.5; 34–78] months versus 194 [168.8–240.8; 129–300] months, respectively (data presented as median, interquartile range, and minimum–maximum values: Me [25–75%; min–max]). A detailed description of the studied BHVs is provided in Table.

Histological and immunohistochemical staining

The explanted BHVs were placed in sterile 0.9% sodium chloride solution and transported to the laboratory. After macroscopic examination, the valves were processed for histological and immunohistochemical analysis. Fragments of the valve apparatus were embedded in Neg-50™ rapid tissue freezing medium (6502, Thermo Fisher Scientific, USA), and 6- μ m sections were prepared using an HM525 cryostat microtome (Thermo Fisher Scientific, USA). The sections were mounted onto glass microscope slides. Intact BHVs of the UniLine and KemCor models were used as controls.

Table

Key characteristics of the studied bioprosthetic heart valves and their recipients

Case	Patient gender	Patient's age at the time of reoperation, years	BHV model	BHV lifespan
Early dysfunction				
1	Female	71	UniLine	1 day
2	Female	66	TiAra	20 days
3	Male	75	TiAra	42 days
Late dysfunction				
4	Male	72	UniLine	34 months
5	Female	77	UniLine	63 months
6	Male	66	UniLine	68 months
7	Female	37	UniLine	75 months
8	Female	72	UniLine	78 months
9	Female	67	PeriKor	129 months
11	Male	68	PeriKor	182 months
12	Female	70	KemKor	184 months
10	Female	59	KemKor	204 months
13	Male	69	KemKor	221 months
14	Male	60	KemKor	300 months

Note: BHV, bioprosthetic heart valve.

To differentiate the major components of the extracellular matrix within the valve leaflets, Russell–Movat pentachrome staining was performed using a commercial kit (ab245884, Abcam, UK) according to the manufacturer's instructions.

Human IgG was detected in tissue sections using a specific primary antibody (MAA544Hu22, Cloud-Clone Corp., PRC) in combination with the NovoLink Polymer DS immunohistochemical staining kit (RE7150-CE, Leica Biosystems, USA). Sections were first fixed in 4% paraformaldehyde for 10 minutes at room temperature and subsequently washed three times in phosphate-buffered saline (PBS, pH 7.4) for 5 minutes each. Immunohistochemical staining was then performed according to the manufacturer's protocol. The anti-human IgG antibody was diluted 1:16,000 in 1% bovine serum albumin (BSA) in saline. Sections were incubated with the primary antibody in a humidified chamber at 4 °C for 18 hours.

After staining, sections were mounted with VitroGel mounting medium (HM-VI-A250, BioVitrum, Russia) and coverslipped. Negative controls were processed in parallel by omitting the primary antibody.

Light microscopy and image processing

Stained sections were visualized by scanning slides with an MT5300L automated biological microscope (Meiji Techno, USA). Digital image acquisition and processing were performed using QuPath software (v0.6.0) [22].

Scanning electron microscopy

Ultrastructural analysis of explanted BHV leaflets was performed using scanning electron microscopy (SEM). Sample preparation followed a previously established protocol [23]. Briefly, biomaterial fragments were fixed for 24 hours (in two 12-hour cycles) in 10% neutral buffered formalin (B06-003, ErgoProduction, Russia) at 4 °C. Samples were then washed three times in PBS (pH 7.4) for 10 minutes each and sequentially incubated in 1% buffered and 2% aqueous osmium tetroxide solutions (19110, Electron Microscopy Sciences, USA) for 16 and 40 hours, respectively.

Following fixation, tissues were washed four times in PBS (15 minutes each) and dehydrated through a graded ethanol series (50%, 60%, 70%, 80%, and 95%; two changes of 15 minutes per step). Samples were then stained with 2% uranyl acetate in ethanol (22400-2, Electron Microscopy Sciences, USA) for 16 hours. Subsequent dehydration was carried out in 95% ethanol (two 15-minute changes), followed by isopropanol (06-002, ErgoProduction, Russia) and acetone (6-09-20-03-83, EKOS-1, Russia), each for 2 hours. The samples were then impregnated with a 1:1 mixture of acetone and epoxy resin (13900, Electron Microscopy Sciences, USA)

for 16 hours, followed by incubation in pure epoxy resin for 24 hours, and finally immersed in fresh epoxy resin at 60 °C for 24 hours.

After resin hardening, samples were ground and polished using a TegraPol-11 system (Struers, Denmark), contrasted with Reynolds lead citrate (17810, Electron Microscopy Sciences, USA) for 7 minutes, and rinsed in double-distilled water (three times for 5 minutes each).

Finally, the specimens were carbon-coated using a Leica EM ACE200 sputter coater (Leica Microsystems, Germany) and examined with an S-3400N scanning electron microscope (Hitachi, Japan) in high-vacuum mode at an accelerating voltage of 10 kV, using backscattered electron detection.

RESULTS

Macroscopic examination of the explanted BHVs revealed no evidence of biomaterial degeneration in the three samples removed during the early postoperative period (1, 20, and 42 days), whereas all remaining valves exhibited characteristic features of SVD.

UniLine xenopericardial valves showed extensive calcification, predominantly localized in the commissural regions, as well as at the base and dome of the leaflets. In xenoaortic valves (PeriCor and KemCor), large calcific deposits were also observed, accompanied by structural damage, including commissural tears and perforations of the leaflet domes.

Moderate connective tissue overgrowth (pannus) was identified at the base of the leaflets and within the commissural regions on the outflow side of BHVs. Pannus was noted in valves that had been in use for more than 3 years.

Additionally, isolated microthrombi were detected on the surface of all examined leaflets, including those from valves explanted at 1, 20, and 42 days. However, no large thrombotic masses or vegetations were observed.

Histological analysis of Russell–Movat pentachrome-stained sections revealed a comparable histopathological pattern in xenopericardial and xenoaortic BHVs. In both implant types, the leaflets were composed predominantly of collagen, with no evidence of elastic fibers or mucopolysaccharides, although minor fibrin deposits were detected on their surfaces (Fig. 1a). Pannus tissue on the leaflets was readily distinguishable due to its high mucopolysaccharide content (Fig. 1b).

Cellular infiltrates were mainly localized to the superficial layers of the biomaterial and along the valve surface (Fig. 1c). In BHVs explanted within 1–42 days, recipient cells were observed only sporadically, whereas valves removed after longer periods exhibited dense cellular clusters. It should be noted that in xenoaortic – but not xenopericardial – valves, foci of cellular infiltration extended into the tissue parenchyma, particularly around

the edges of perforations and large calcific deposits. Ultrastructural examination indicated that these cellular clusters were predominantly composed of canonical macrophages, with occasional neutrophils present (Fig. 1d).

The immunohistochemical analysis revealed intense positive staining for human IgG antibodies in all examined BHVs, independent of their type or duration of

implantation (Fig. 2). It is important to note that the positive signal was localized specifically to the biomaterial fibers, whereas microthrombi and pannus on the leaflet surfaces did not exhibit IgG staining, indicating that the antibodies were selectively bound to the biomaterial. In contrast, intact control BHV tissues showed no positive staining.

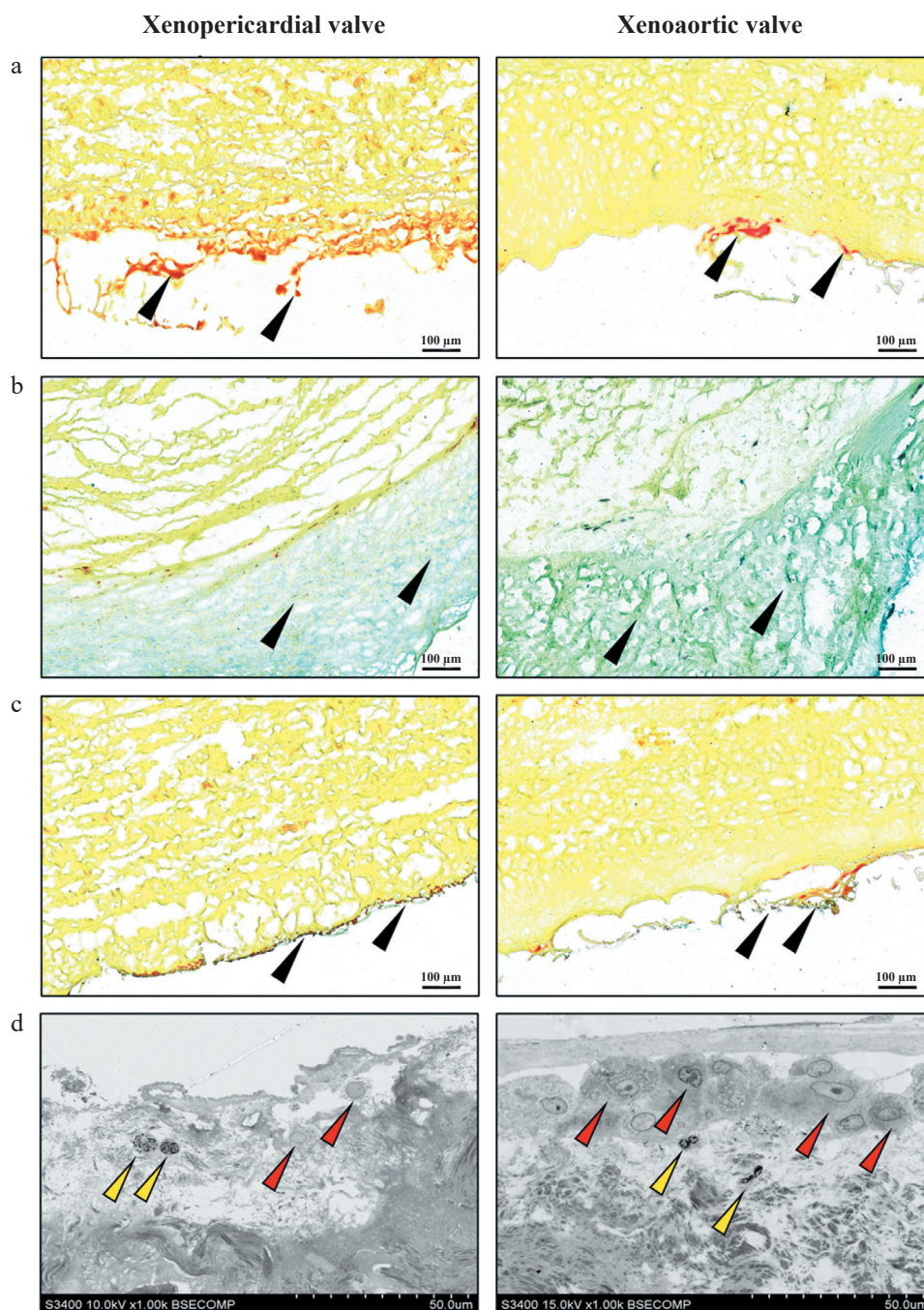


Fig. 1. Movat pentachrome staining of tissues from the studied bioprosthetic heart valves. (a) Leaflets of xenopericardial and xenoaortic valves composed predominantly of collagen fibers (yellow staining), with no evidence of elastin or mucopolysaccharides; but minimal fibrin deposits are present on the surface (red staining; arrows indicate microthrombi). (b) Pannus formed on the surface of the bioprosthetic valve apparatus shows a dense structure composed of collagen fibers rich in mucopolysaccharides (green staining; arrows indicate pannus). (c) As observed in both xenopericardial and xenoaortic valves, recipient cells infiltrate mainly the surface and superficial loose layers of the biomaterial (arrows indicate recipient cells). (d) Electron microscopy demonstrates that the cellular infiltrates within the bioprostheses consist predominantly of macrophages (red arrows) with occasional neutrophils (yellow arrows)

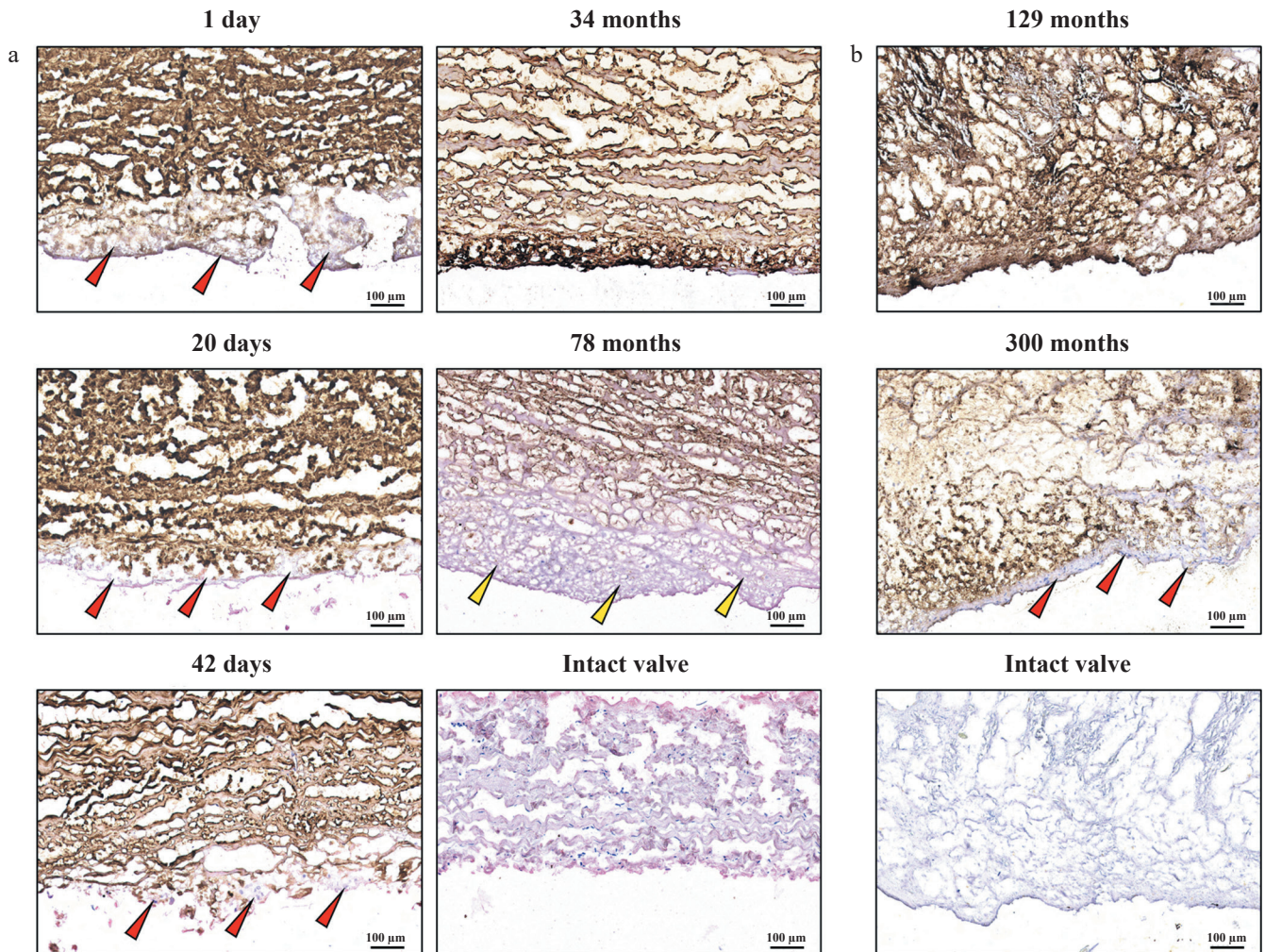


Fig. 2. Immunohistochemical staining of the studied bioprosthetic heart valves. Biomaterial from both xenopericardial (a) and xenoaortic (b) valves explanted from patients was intensely stained with anti-IgG antibodies, regardless of the implantation duration (bioprostheses with the shortest and longest functional periods are shown). In contrast, recipient-derived tissues, including thrombi and pannus, exhibited no positive staining for human IgG (red arrows indicate microthrombi; yellow arrows indicate pannus)

DISCUSSION

In all xenogeneic BHVs examined, the biomaterial contained both human IgG immunoglobulins and clusters of WBCs, primarily macrophages and neutrophils. The pattern of cellular infiltration – particularly the absence of lymphocytes – indicates a nonspecific immune response, consistent with findings from other studies [11–14]. Notably, although recipient antibodies rapidly deposited on the leaflets within the first hours of valve function, cellular invasion occurred much later. Only isolated monocytes and macrophages were observed on BHVs removed in the early postoperative period (1, 20, and 42 days), whereas valves functioning for three years or more exhibited extensive leukocyte infiltrates. These findings suggest that opsonization of chemically fixed xenobiomaterial by antibodies is not the primary trigger for immune cell migration and adhesion to the implant.

An interesting finding of this study is that the deposition of human immunoglobulins in the biological

component of BHVs does not diminish over time: all examined valve leaflets showed intense positive staining for human IgG antibodies, regardless of implantation duration. Although previous studies have reported the gradual elimination of certain animal antigens (e.g., α -Gal and N-Gly) in BHVs, our results indicate that the xenobiomaterial remains highly immunogenic even 25 years post-implantation [16, 21]. This persistent immunogenicity is likely due to porcine and bovine collagen stabilized with di-epoxide compounds, which forms the core of the valve biomaterial and may itself act as an antigen, eliciting an antibody-mediated immune response. Additionally, 19 proteins have recently been identified in glutaraldehyde-fixed bovine pericardium that trigger specific antibody production in BHV recipients [20]. Most of these protein antigens are subcellular and appear to derive from residual donor cells [20]. This underscores the importance of the immunogenic role of the biomaterial's cellular component.

The mechanisms by which antibody deposition in BHV tissues accelerates SVD development remain unclear, but the role of the antibody-mediated immune response in valve degeneration has been demonstrated *in vivo*. For example, pretreatment of glutaraldehyde-stabilized biomaterial fragments with antibodies enhanced calcification in mouse, rat, and rabbit subcutaneous implantation models [16, 24–26].

It should be noted that this study is the first to evaluate changes in the immunogenicity of epoxy-treated xenogeneic BHVs over time. However, it has limitations: most importantly, we have not identified the specific xenobiomaterial components that trigger antibody production and binding to BHV tissues. Future studies to identify these antigens and investigate their stability could substantially advance the development of immunologically inert implants.

CONCLUSION

Intensive deposition of human IgG immunoglobulins occurs in epoxy-treated xenogeneic BHV tissues as early as the first day post-implantation. Notably, the capacity of epoxy-treated xenobiomaterials to bind antibodies remains preserved even after 25 years of valve function in the recipient. This indicates that their immunogenic properties persist despite the gradual degradation of certain carbohydrate xenoantigens previously documented.

COMPLIANCE WITH ETHICAL PRINCIPLES

The study adhered to the principles of Good Clinical Practice and the World Medical Association's Declaration of Helsinki. Approval was obtained from the local ethics committee of the Research Institute for Complex Issues of Cardiovascular Diseases (Protocol No. 19, November 6, 2018). All participants provided written informed consent.

This work was conducted as part of the comprehensive program of fundamental scientific research at the Siberian Branch of the Russian Academy of Sciences, under the Research Institute for Complex Issues of Cardiovascular Diseases (Project No. 0419-2022-0001), titled: "Molecular, cellular, and biomechanical mechanisms of cardiovascular disease pathogenesis in the development of new treatment methods, including personalized pharmacotherapy, introduction of minimally invasive medical devices, biomaterials, and tissue-engineered implants".

The authors declare no conflict of interest.

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HYDROXYAPATITE AS A MATERIAL FOR TRANSPLANTOLOGY: CLINICAL EXPERIENCE AND PROSPECTS OF APPLICATION

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The centrepiece of this analytical review is the metabolism of hydroxyapatite in its natural, bone, and synthetic forms, where the mitochondria-mediated mechanism may serve as the leading mechanism. The possibility that osteoblast mitochondria play an important role in the initial stages of bone mineralisation is discussed. Furthermore, the paper highlights the key role of mitochondria in the metabolism of synthetic hydroxyapatite. Differences between the results of *in vivo* and *in vitro* studies using synthetic hydroxyapatite of different morphologies are also detailed. It is noted that long-term infiltration with immune cells and *in vivo* studies are necessary to adequately evaluate hydroxyapatite as a bone-plastic material. Particular attention is given to the interaction of hydroxyapatite with immune cells and its ability to affect the ribosomes and mitochondria of cells. Due to its mechanical properties, scalability, and potential use for the treatment of extensive bone defects of tumor origin, hydroxyapatite is a promising material. This study also highlights the importance of further development of *in vitro* research methods in the context of their biomimeticity. Overall, this work offers a theoretical direction for future studies of hydroxyapatite as a bone grafting material and emphasises the value of *in vivo* studies.

Keywords: *hydroxyapatite, bone grafting, nanoparticles, immune response, mitochondria.*

INTRODUCTION

The basis of modern medical technologies aimed at bone tissue regeneration is the widespread use of grafts, the main component of which is hydroxyapatite. The diversity of grafts and their constituent materials used is an equal reflection of the extremely large variety of pathological conditions of bone tissue requiring the application of regenerative medicine technologies.

At the same time, the use of any grafts requires preliminary detailed analysis of the immune response in response to the introduction of this or that material. In this context, the unique properties of such materials as hydroxyapatites and their compositions [1, 2], including exceptional phenomena in the context of immune response [3–5], become extremely important. In this regard, there is a need for a more detailed presentation of hydroxyapatites and their compositions as the main components for the fabrication of grafts to restore structural and functional pathologies of bone tissue.

NATIVE FORM OF HYDROXYAPATITE: DESCRIPTION OF STRUCTURE AND FUNCTIONS IN THE BODY

First of all, it is necessary to note the fact that hydroxyapatite is the native form of bone tissue calcium and occupies 70–90% of the bone tissue matrix volume. In bone tissue, hydroxyapatite is represented in the form of crystals of small size and is characterized by the stoichiometric formula $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ [6]. Accord-

ing to the age of a person, three main ranges of their average size are distinguished: up to 6 six years of age, 188–215 nanometers; between 6–19 years of age, 232–252 nanometers; and in adulthood, 252–283 nanometers [7]. Together with collagen I, which occupies up to 90% of the organic phase of bone, it forms the spectrum of the main structural and functional features of bone tissue [8].

The initial onset of mineral component formation in bone tissue is ensured by the energy-dependent transport of Ca^{2+} ions and PO_4^{3-} anions into the mitochondria of osteoblasts (Fig.). Upon reaching the solubility threshold, micropackages of amorphous calcium phosphate or Posner clusters with the proposed formula $\text{Ca}_9(\text{PO}_4)_6$ are precipitated; these are thought to be stabilized by an organic component represented by acidic non-collagenous proteins, including osteocalcin, osteopontin, and bone sialoprotein. Further release of amorphous calcium phosphate may occur via two pathways: direct transport across the mitochondrial membrane or reverse phagocytosis [9].

At the initial stage, the vesicular contents form shapeless accumulations of calcium phosphate in close association with collagen fibers. During subsequent ion diffusion, nascent hydroxyapatite platelets are formed, which, upon contact with one another, lead to a marked increase in the degree of crystallinity [10]. As a result, partially under the influence of non-collagenous proteins [11–13], lamellar structures 2–4 nm in thickness are formed, with their longitudinal axis oriented parallel to the axis of collagen fibrils (Fig.) [6].

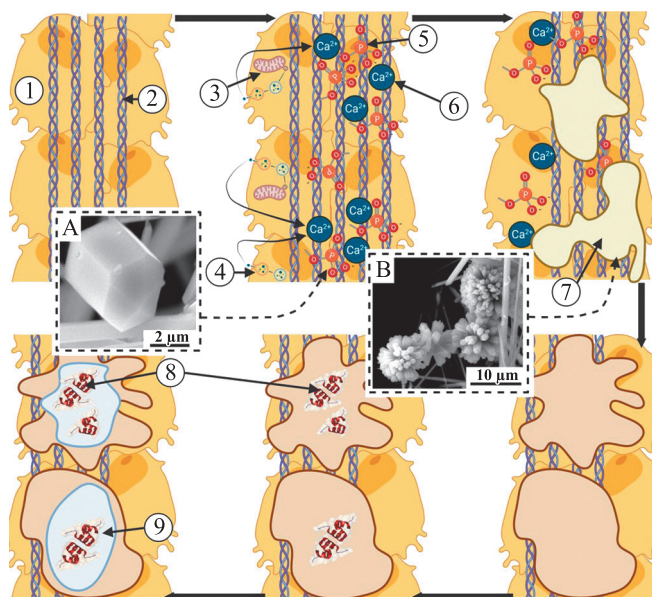


Fig. Scheme of the stages of collagen fibril calcification

Collagen fibrils act as an organizing component in the formation of growth centers of hydroxyapatite crystals [9, 15]. The initiation of hydroxyapatite crystals formation depends on amino acid patterns, in particular, the triplet of amino acids glycine-proline-hydroxyproline, which is the dominant motif of tropocollagen. This process is caused by the formation of ionic bonds between calcium atoms, oxygen atoms of carbonyl groups of the polypeptide backbone, and side chains of proline and hydroxyproline [16]. If we consider a quasi-hexagonal model, lysine at position 108, glutamic acid at positions 110, 116, 582, and 815, and arginine at positions 350, 581, and 816 form a stereochemical pocket for optimal binding of calcium ions and phosphate ions [9]. The functionality of non-collagen proteins is largely due to the effect of osteocalcin. This protein plays a guiding role in the process of intrafibrillar appearance of hydroxyapatite crystals in type I collagen fibrils [17, 18].

As a rule, bone tissue hydroxyapatite is carbonated with carbonate to some extent. The normal range of carbonate substitution is 2–9% [19]. Substitution by carbonate ions occurs at the positions of phosphate ions (PO_4^{3-}) (major B-type substitution) and hydroxyl groups OH^- (minor A-type substitution), thereby provoking a change in crystal structure and molar Ca/P ratio [19, 20]. In the case of hydroxyl ion substitution, which is characteristic of bone apatite, a state of hydroxyl deficiency is formed, which has different degrees of severity depending on the type of bone tissue [21]. Subsequently, it increases the solubility of bone material [22].

As a consequence of this substitution, the mineral component of bone plays the role of the main depot of calcium, phosphorus, magnesium and a number of other mineral elements; this process has a high regulatory potential for the metabolism of these elements in the whole

organism [19, 23]. It is quite obvious that substitution by the mentioned elements affects numerous parameters of the crystal lattice. Iron and strontium have a positive effect on the dimensional characteristics of its individual elements, while zinc and magnesium have the opposite effect. For magnesium, zinc, and iron, a negative effect on the crystallinity of hydroxyapatite was revealed, for strontium – positive [23]. It is also noteworthy that the indicated effect of changing the crystalline microstructure of the mineral component demonstrates a striking heterogeneity in the composition of a single bone [24]. Relatively high strontium content is characteristic of regions with high metabolic activity and newly formed bone structures [25].

The most regular pattern of heterogeneity is the difference of surface and inner layers of hydroxyapatite. The surface layer is in the form of hydrated amorphous calcium phosphate 0.8 [19] or 1–2 nanometers thick [26] and a more crystallized core [19]. Hydroxyapatite is characterized by an extremely flexible microcrystalline structure, suggesting an extremely broad list of elements suitable as substituents. This may account for a wider and more localized range of possible biological properties of native bone hydroxyapatite.

The fundamental reason for the structural strength and high resistance to ionic substituents is the hexagonal organization of hydroxyapatite [19]. However, in some cases, the loss of hexagonal structure and formation of monoclinic organization of the crystal lattice is possible [19], which largely depends on the degree of substitution of chlorine anion Cl^- [27]. Changes in the organization of the crystal lattice of hydroxyapatite are also possible as a result of age-related changes [28]. The observed liability has prompted a search for other constituents of the inorganic phase, such as tricalcium phosphate, dicalcium phosphate dihydrate [19] and octacalcium phosphate [29]. For the latter, no direct evidence has been shown for its presence in bone structure [19], except in pathological conditions [27] under relatively low pH conditions. However, there are studies that confirm the presence of a fixable amount of octacalcium phosphate in bone tissue under normal conditions [10].

As for the higher-level organization of the mineral component of bone tissue, the formation of lamellar structures of hydroxyapatite is generally accepted [19]. Spirally twisted needle-like crystals of apatite, located within and between collagen fibrils [10, 30], merge laterally and are organized in the form of spiral subplates along the loading axis. The defined chiral structure of the latter is the basis for tertiary helical plates. The further formation of the mineral base ordered in time and space with the formation of collagen structures results in a multilevel architecture of the inorganic component of bone [30]. The results of modern studies of the dimen-

sional parameters of each level of organization of the inorganic component of bone are presented in Table.

Having analyzed a number of scientific studies of structural, physicochemical and physiological properties of the mineral component of bone tissue, we aim to emphasize the fundamental role of native hydroxyapatite on the scale of the whole organism, defining it as the most valuable and convenient material in bone grafting. The spectrum of features of native hydroxyapatite presented in this section, beginning with the stoichiometric flexibility of the crystal lattice and ending with the complex process of its formation in the living organism, dictates completely new requirements for synthetic analogs of bone hydroxyapatite as a transplantation material.

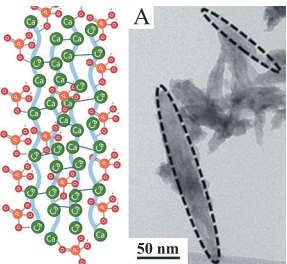
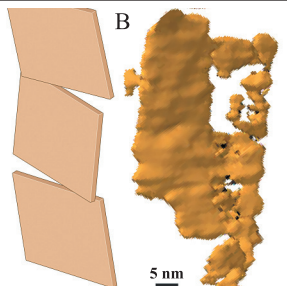
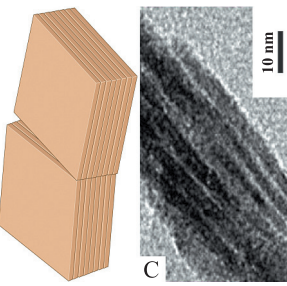
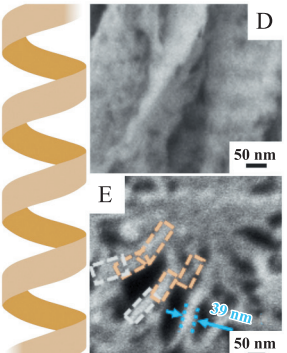
Among them, the multilevel character of the organization, formed in close interaction with the organic component, including the cellular environment, stands out.

HYDROXYAPATITE AS A BONE GRAFTING MATERIAL

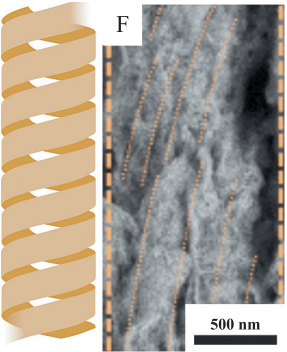
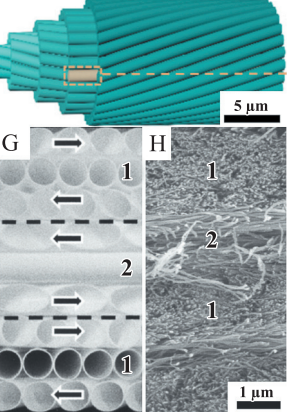
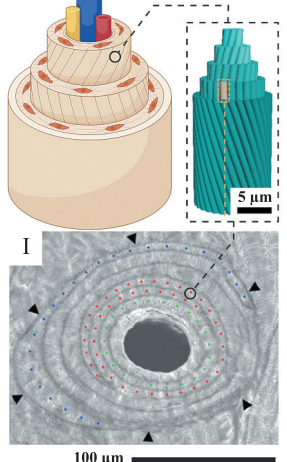
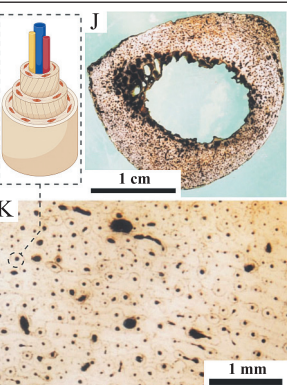
The generally recognized “gold standard” in bone grafting is the use of autologous bone material. Autologous bone grafting has been successfully used in clinical practice for a century [35, 36]. This necessitates a comprehensive analysis of the main advantages of using this method in order to form a kind of reference heuristic model in the development and improvement of alternative sources of material for bone grafting.

Table

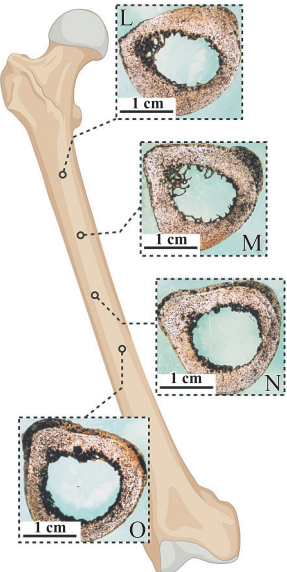
Hierarchy of bone tissue mineral component organization

	Hierarchy	Morphology	Size	Step length and chirality
Needle crystal		Thin, twisted long ribbon	Thickness ~5 nm Width 5 to 10 nm Length 50–100 nm	~1.5 μm right-handed
Subplate		Needle-shaped crystal merging laterally at an angle	Thickness 5 nm Width 20 to 60 nm Length 50–100 nm	~4.7 μm left-handed
Plate		Twisted mineral laminae composed of subplates	Thickness 20–40 nm Width 60–150 nm Length 50–100 nm	~4.6 μm left-handed
Mineral fibrils		Mineral plates spirally merge at an angle	Width 60–150 nm Length: Micron level	~2.9 μm right-hand side

Continuation of Table

	Hierarchy	Morphology	Size	Step length and chirality
Bundles of mineral fibrils		Mineral fibrils spirally merge at an angle	Diameter 1–2 microns Length: Micron level	Coils: 4.2–6.5 μm right-handed
Lamellar units		The fibril bundles are rotationally stacked layer by layer	Diameter 6–12 microns	Coils: 48–653 μm , left/right; Stacking: $\sim 47.6 \mu\text{m}$, right-handed
Haver's canals		Lamellar units are spirally twisted around the blood vessel in the form of coaxial cylinders	Diameter 100–300 microns	Coils: 617–5,167 μm , right/left-handed; Stacking: $\sim 184 \mu\text{m}$, right-handed
Compact bone		The haversack channels are laid in layers in a circular pattern	Thickness $\sim 3 \text{ mm}$	Coils: 13.5–119.4 cm, right/left-handed; Stacking: $\sim 9.7 \text{ mm}$, left-handed

End of Table

	Hierarchy	Morphology	Size	Step length and chirality
Solid bone		Anatomical shape with curve	Diameter ~3 cm	Left-handed

Notes. A, transmission electron micrograph of Mg^{2+} -doped needle microcrystal (adapted from [31]); B, model of a crowded mineral subplate formed by lateral fusion of needle microcrystals; C, transmission electron micrograph of twisted mineral plates, showing the stacking of subplates; D, scanning electron micrograph of spiral mineral fibrils, cross section; E, scanning electron micrograph of spiral mineral fibrils from a lateral perspective; F, scanning electron micrograph of spiral bundles of mineral fibrils from a side view (model and micrographs adapted from [30]); G, model of twisted plywood showing the ratio of transverse and longitudinal lamellae; H, scanning electron micrograph showing the alternation of transverse lamellae (1) and longitudinal lamellae (2) (model image and micrograph adapted from [32]); I, scanning electron micrograph showing the structure of the lamellar system: red dots display a closed ring structure; green dots display a spiral organization and overlap after a 360° turn; blue dots form a sickle moon structure and display an incomplete ring (adapted from [33]). J and K, cross section of the right femur at a distance of 14 centimeters from the proximal end; L, cross section of a horse femur stained with mercury sulfide, showing the arrangement of osteons in the repeat sheets of the capillary network; M, cross section of the right femur at a distance of 19 centimeters from the proximal end; N, cross section of the right femur at a distance of 23 centimeters from the proximal end; O, cross section of the right femur at a distance of 25 centimeters from the proximal end (photographs and microphotographs adapted from [34]).

There is also a clear need to analyze the main problems of using autografts in order to use them in the formation of alternative solutions.

Starting from the reference value of bone autografts, we should define the attributes of an “ideal graft”. First, it should possess the properties of autogenous bone, which include biocompatibility, osteoconductivity, and osteoinductivity. Second, the graft material should be easy to use, safe, and cost-effective [35].

The key conditions for successful bone tissue regeneration formulated in the “diamond concept” are worth mentioning from the generally accepted positions. Its essence is that successful healing of bone defects requires the presence of viable osteogenic cells or their precursors, a suitable connective tissue matrix, adequate vascularization, and a time- and space-specific profile of growth factors [37].

In this context, it is necessary to emphasize the main properties and the distribution spectrum of their values for bone grafts used in clinical practice:

1. The range of values of the structural parameter of the bone implant. Bone grafts have different ability to

withstand mechanical loading. For unmodified grafts based on cortical bone, a relatively high resistance to mechanical loads is shown in comparison with grafts based on cancellous bone. Thus, the mechanical properties of bone grafts without additional modification directly depend on the properties of the donor site [38].

2. Spectrum of osteogenic properties. The ability of the graft to initiate neoosteogenesis [39] due to the preserved pool of graft cells including osteoblast precursors [40] is extremely important. Due to the presence of cellular elements, necessary growth factors and matrix framework, such a graft is able to modulate angiogenesis, adequate perfusion [41, 42] and activity of progenitor cells [43].
3. Osteoinductive properties. Autologous graft is characterized by the presence of an exhaustive set of growth factors necessary for regeneration, providing proliferative and differentiative potential of progenitor cells [44–46].

4. Osteoconductive properties. Ability to provide an optimal environment for normal metabolism, proliferation and differentiation of cell populations [47].

The material for autologous bone graft can be spongy or cortical bone. The advantage of the former is relatively high osteoconductive properties due to a significant concentration of osteogenic cells and growth factors. The main disadvantage of this type of graft is its low ability to provide structural support at the early stages of graft integration [35].

It should also be noted that despite all its undeniable advantages over alternative bone grafting strategies, the methodology of graft isolation continues to improve. A prime example of this is the use of femoral medullary bone chips obtained using the Reamer Irrigator Aspirator System (RIA – Synthes®, Inc. West Chester, USA) [48]. With equivalent rates of graft integration compared to traditional iliac crest harvesting, this method demonstrates a lower risk of chronic pain, infection, and excludes damage to the lateral cutaneous nerve of the femur as a result of compression or contusion [49]. However, the risk of complications such as cortical bone perforation and subsequent bleeding is still high [50].

Against this background, it should be emphasized that the autogenous cortical bone graft is also characterized to a greater extent by osteoconductive properties under the condition of lower biological activity compared to the spongy autologous graft. The next problem is the high duration of revascularization [35, 51]. For this reason, vascularized cortical grafting material that provides osteogenic and osteoconductive properties can be used [35]. In this direction, there is also a continuous development of strategies for the allocation of transplant material that ensure a reduction in the risks of donor site pathologies while maintaining the necessary integrative parameters [52]. However, the development of technologies takes place not only within the framework of improving the techniques of obtaining one or another type of autologous grafts separately. Of particular importance are the works in the direction of optimal combination of the presented types of transplant materials, which allows obtaining positive results, including in their clinical use [53, 54].

Despite the benchmarks of autologous bone grafting material in the context of its osteogenic, osteoconductive, and osteoinductive properties, the number and variety of studies aimed at finding alternative strategies for graft preparation continue to grow. This is due to the fundamental disadvantages of all methods of obtaining autologous graft material: firstly, the limited volume of potential graft material [55, 56]; secondly, the possibility of developing complications of the donor bone site [49, 50]. Moreover, when taking autologous grafting material, the risk of bleeding increases, which still remains one of the main problems, including when using the latest methods of bone implant obtaining [50]. Thus, there are

studies that confirm a significant incidence of complications both after traditional grafting from the anterior and posterior iliac crest (19.37%) and after using the Reamer Irrigator Aspirator system (6%). The general list of complications included the development of infection, hematoma, seroma, chronic pain, fracture, as well as vascular and nerve damage [50]. In a study conducted by A.J. Suda et al. it was shown that the volume of the removed transplant material has a great influence on the risk of serious complications [57]. Thus, we can say that the main limitation of autologous bone transplantation is the small volume of potential grafting material.

In this context, the development of a number of alternative methods for obtaining transplantation material is extremely important. This series includes obtaining allogenic [58–60] and xenogenic transplantation material of natural origin [59, 61], as well as the creation of new biomimetic transplantation materials of artificial and bioengineered origin [59, 62, 63].

NATIVE SUBSTITUTES FOR AUTOLOGOUS BONE GRAFTING MATERIAL: ALLOGENEIC AND XENOGENIC BONE GRAFTS

Functionally, the closest of all the listed alternative methods is allogeneic grafting material. The main advantage of this material is its relatively high availability [64]. Therefore, it has been used in clinical practice for reconstruction of extensive bone injuries for many years. Like autogenous graft, allogeneic graft material has a high degree of similarity to the structure of native bone: it has similar mechanical properties, is biocompatible to a certain extent, and ultimately has osteoinductive and osteoconductive properties [60], which are limited [65], probably due to the need for decellularization of the graft material [60]. As noted by some authors, the key problems of this method are the lack of unified decellularization protocols and the potential risk of infectious disease transmission [65].

In addition, the choice of a specific decellularization and delipidization strategy represents a critical factor determining both the biological and clinical performance of allogeneic bone grafts. The use of chemical reagents required for effective removal of cellular and lipid components may be associated with cytotoxicity, thereby necessitating rigorous control and complete elimination of residual agents from the graft structure [60, 65]. Moreover, aggressive processing procedures inevitably result in partial loss or degradation of biologically active matrix components, which may contribute to the reduced osteoinductive and osteogenic properties of decellularized allogeneic materials observed in clinical practice [65].

Conventionally, allogeneic bone grafts can be divided into two main groups. The first group includes various variations of allogeneic bone graft proper – decellularized bone graft of donor origin of one type. The second

group includes demineralized bone matrices obtained by acid treatment of allogeneic bone graft in order to isolate bone collagen matrix [66]. It is important to note that there is an extremely wide range of methodological solutions to obtain allogeneic [67–69]. This, on the one hand, creates the problem of forming a unified protocol for wide implementation in clinical practice. On the other hand, it gives grounds for a personalized approach, which is actively used in clinical practice.

At the same time, demineralized bone matrix is an allogeneic bone material that was modified in accordance with the general provisions of the Marshall Urist technique [66]. It should be noted that this method, which has already become traditional, has undergone a significant number of modifications since its first use [70]. For example, the preparation of commercial demineralized bone matrix has wide limits of variability among different manufacturers, including the use of different acid solutions, changes in the duration of demineralization, different temperature regimes and methods of decontamination [70, 71].

In comparison with autologous grafts, allogeneic transplant material demonstrates some characteristic features. The common property, which directly depends on the main stage of obtaining all allogeneic grafts – decellularization, is the absence of pronounced osteogenic properties [66, 72]. Nevertheless, according to the “diamond concept” [37], allogeneic bone grafting material has high osteoconductive and osteoinductive properties.

A certain degree of bone allograft effectiveness in clinical practice is largely due to its osteoconductive properties and high value of mechanical support [66] in bone injuries requiring a large volume of grafting material [73, 74]. As in autologous transplantation, the most pronounced osteoconductive properties are characteristic of the allograft of cancellous bone [35]. The variability of the content of osteoconductive agents in the final graft material is largely due to the duration of storage and preparation conditions [38, 75]. Alongside storage duration and preparation conditions, the decellularization process represents an important source of variability in the content of osteoconductive agents. The choice of decellularization methodology directly affects the degree of preservation of extracellular matrix components, including those responsible for osteoconductive properties. Consequently, differences in decellularization protocols may lead to partial loss of biologically active matrix constituents, further contributing to the heterogeneity of functional properties observed in allogeneic bone grafts [60, 65].

Taking into account the facts stated in the conclusion of the previous section, we need to focus our attention on clinical practice. As it has already been mentioned, the main indication for the use of allogeneic grafting material is extensive bone tissue damage requiring a significant volume of grafting material [75–78]. The time

interval of allografting is of particular importance in this context. Since this type of transplant material, despite decellularization [79], has a relatively high frequency of immunogenic rejection [80]. At the same time, in case of extensive bone injuries, including those caused by infectious agents [81], there may be an immune hypersensitivity reaction to an allogeneic graft due to already existing inflammation [66]. Therefore, in clinical practice, the strategy of delayed bone grafting (after 6–8 weeks after the primary surgery) is often used [81, 82].

The current trend in the use of allogeneic bone grafting material is the use of cellular bone matrices. The essence of this method is the implantation of allogeneic mesenchymal stem cells into the structure of the osteoconductive and osteoinductive base. The cellular component is obtained by isolating multipotent stromal cells from cadaveric bone marrow, adipose tissue, and chorion tissue [83]. However, some authors speak about the controversial efficiency of their use [84].

A combination of allogeneic and xenogenic bone grafting materials can also be used, where the former performs the role of an osteoinductive agent and the latter performs a mechanical role [85]. The category of xenogenic bone grafts should be noted separately: bovine bone material is often used for their production [86–88]. There are data confirming high osteoconductive properties of xenogenic bovine bone material [89], while the independent use of xenogenic bone grafting material, despite the positive postoperative outcome in individual cases [90], demonstrates poor quality of clinical outcome [91, 92]. The main negative results of xenotransplantation are fibrous encapsulation of the graft [93] and improper fusion, which is manifested by pain syndrome [94]. Moreover, due to the extremely high duration of xenograft integration (57 weeks) compared to allograft (16 weeks), many researchers have decided that xenografts should not be used independently [91].

Summarizing the data presented in this section, it is worth noting that, on the one hand, the direction of allogeneic transplantation material use is constantly replenished with original methods, including the use of additional categories of transplantation materials. However, on the other hand, in this area there is a noticeable problem with the standardization of the existing methodological base [95]. In clinical practice, this leads to the need to use strategies of personalized medicine, limiting the prevalence of application.

USE OF HYDROXYAPATITE-BASED MATERIALS FOR BONE GRAFTING IN THE RUSSIAN FEDERATION

In the Russian Federation, hydroxyapatite (HA)-based materials represent one of the most widely used classes of synthetic bone graft substitutes in traumatology, orthopedics, maxillofacial surgery, and dentistry.

Their extensive clinical implementation is associated with high biocompatibility, pronounced osteoconductive properties, chemical stability, and the possibility of reproducible large-scale manufacturing, which is especially relevant under conditions of limited availability of autologous bone grafts and the need to reduce donor-site morbidity [96–98].

The development of hydroxyapatite-based biomaterials in Russia has traditionally relied on fundamental studies in inorganic chemistry, crystallography, and materials science. Significant contributions have been made by Russian research groups focused on calcium phosphate ceramics, composite systems, and bioactive coatings for load-bearing implants [96–100]. As a result, a broad range of HA-containing materials – dense and porous ceramics, granules, composite scaffolds, and plasma-sprayed or biomimetic coatings – has been introduced into experimental and clinical practice.

From a functional perspective, hydroxyapatite-based materials used in Russian clinical settings are primarily regarded as osteoconductive matrices providing mechanical support and spatial guidance for bone regeneration [97, 101]. These materials facilitate the adhesion, migration, and differentiation of osteogenic cells but generally lack intrinsic osteogenic potential. Consequently, they are frequently applied in combination with autologous cancellous bone, bone marrow aspirate, platelet-rich plasma, or growth factor-containing formulations, in agreement with the principles of the “diamond concept” of bone regeneration [102, 103].

A key advantage of synthetic hydroxyapatite materials over allogeneic and xenogeneic grafts is their extremely low immunogenicity and absence of risks associated with pathogen transmission. This property is of particular importance in reconstructive procedures performed under conditions of chronic inflammation, post-traumatic bone defects, and infectious complications, where immune hypersensitivity reactions to biological grafts may compromise clinical outcomes [104, 105].

An actively developing research direction in Russia involves the modification of hydroxyapatite through ionic substitution and the creation of composite materials. Substitution of calcium and phosphate ions with magnesium, zinc, strontium, silicon, or carbonate groups is considered an effective approach to regulate crystallinity, solubility, resorption rate, and biological activity of HA-based grafts [96, 106–108]. These strategies aim to reproduce the stoichiometric flexibility and structural heterogeneity characteristic of native bone hydroxyapatite, thereby improving the integration and functional performance of synthetic implants.

Thus, hydroxyapatite – based materials used in the Russian Federation occupy an intermediate but strategically important position between biological grafts and fully inert synthetic implants. Their application reflects a broader trend toward standardized, controllable, and

biologically rational approaches to bone regeneration. Further progress in this field is closely associated with the integration of fundamental knowledge on native bone mineral organization and advanced material engineering solutions.

DEVELOPMENT OF SYNTHETIC HYDROXYAPATITE-BASED MATERIALS FOR BONE REGENERATION

The accumulated knowledge on the multilevel organization, stoichiometric flexibility, and biological functionality of native bone hydroxyapatite has formed the conceptual basis for the development of synthetic hydroxyapatite-based materials intended for bone grafting and tissue regeneration. Unlike biological grafts, synthetic materials offer the advantages of controlled composition, reproducible physicochemical properties, unlimited availability, and reduced risks of immunogenic reactions and disease transmission [24]. These characteristics have positioned synthetic hydroxyapatite as one of the key components in modern strategies for bone defect reconstruction.

The earliest generations of synthetic hydroxyapatite materials were primarily designed to reproduce the chemical composition of bone mineral, focusing on stoichiometric $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ with high crystallinity and structural stability. However, subsequent studies demonstrated that highly crystalline stoichiometric hydroxyapatite differs significantly from native bone apatite in terms of solubility, surface reactivity, and biological performance [109]. This discrepancy prompted a shift toward biomimetic approaches, aiming to approximate not only the chemical composition but also the structural hierarchy and dynamic behavior of natural bone mineral.

One of the central directions in the development of synthetic hydroxyapatite-based materials is the regulation of their micro- and nanostructure. The creation of porous ceramics and scaffolds with controlled pore size, interconnectivity, and surface roughness has been shown to be critical for promoting vascularization, cell migration, and osteointegration [110, 111]. Macroporosity facilitates tissue ingrowth and nutrient transport, while microporosity and nanoscale surface features enhance protein adsorption and osteoblast adhesion, thereby improving osteoconductive performance [112].

Another important strategy involves chemical modification of hydroxyapatite through ionic substitution. Incorporation of biologically relevant ions such as magnesium, zinc, strontium, silicon, and carbonate groups into the crystal lattice allows modulation of crystallinity, dissolution kinetics, and biological activity of synthetic materials [106, 113]. These substitutions are intended to mimic the non-stoichiometric nature of native bone apatite and to impart additional functional properties,

including stimulation of osteogenic differentiation, angiogenesis, and antimicrobial activity [114].

In addition to monophase hydroxyapatite systems, composite materials combining hydroxyapatite with biodegradable polymers, collagen, bioactive glasses, or other calcium phosphate phases have been actively developed. Such composites aim to overcome the intrinsic brittleness of ceramic materials and to provide improved mechanical performance, especially in load-bearing applications [115]. Furthermore, hybrid systems enable a closer integration between inorganic and organic components, reflecting the natural organization of bone tissue and facilitating gradual material resorption synchronized with new bone formation.

From a biological perspective, synthetic hydroxyapatite-based materials are predominantly classified as osteoconductive scaffolds. Their limited intrinsic osteoinductive potential has stimulated the incorporation of biologically active molecules, including growth factors, peptides, and cellular components, into hydroxyapatite matrices [116]. These approaches are consistent with the principles of the “diamond concept” of bone regeneration, emphasizing the synergistic interaction between scaffold architecture, cellular elements, biochemical signals, and vascular supply [101].

Overall, the development of synthetic hydroxyapatite-based materials represents a transition from chemically inert bone substitutes toward multifunctional, biomimetic systems with tunable structural and biological properties. The ongoing integration of insights from bone biology, materials science, and regenerative medicine is expected to further enhance the clinical efficacy of hydroxyapatite-based grafts and to expand their application spectrum in the treatment of complex bone defects.

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