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https://orcid.org/0000-0003-4983-5896

VILNIUS UNIVERSITY

Kaliyev Rymbay

Novel Method for Normothermic Ex Vivo Preservation of Donor Heart Using Blood Cardioplegia and Conditioning

DOCTORAL DISSERTATION

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Academic consultant – Prof. Dr. Robertas Samalavicius (Vilnius University, Medicine and Health Sciences, Medicine, M 001).
Academic consultant – Prof. Dr. Pranas Serpytis (Vilnius University, Medicine and Health Sciences, Medicine, M 001).

This doctoral dissertation will be defended at a public meeting of the Dissertation Defence Panel: **Chairman – Prof. Dr. Germanas Marinskis** (Vilnius University, Medicine and Health Sciences, Medicine, M 001).

Members:
Prof. Dr. Jutta Arens (Twente University, Netherlands, Technology Sciences, Mechanical Engineering, T 009),
Prof. Dr. Gintaras Kalinauskas (Lithuanian University of Health Sciences, Medicine and Health Sciences, Medicine, M 001),
Prof. Dr. Kestutis Rucinskas (Vilnius University, Medicine and Health Sciences, Medicine, M 001),
Prof. Dr. Eva Strike (Rigas Stradins University, Medicine and Health Sciences, Medicine, M 001).

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VILNIAUS UNIVERSITETAS

Kaliyev Rymbay

Naujas donoro širdies normoterminio *ex-vivo* konservavimo metodas, naudojant kardioplegiją ir kondicionavimą

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Mokslinis konsultantas – prof. dr. Robertas Samalavičius (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina, M 001).
Mokslinis konsultantas – prof. dr Dr. Pranas Šerpytis (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina, M 001).

Gynimo taryba:
Pirmininkas – prof. dr. Germanas Marinskis (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina, M 001).

Nariai:
prof. dr. Jutta Arens (Twente universitetas, Nyderlandai, technologijos mokslai, mechanikos inžinerija T 009).
prof. dr. Gintaras Kalinauskas (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina, M 001).
prof. dr. Kęstutis Ručinskas (Vilniaus universitetas, medicinos ir sveikatos mokslai, medicina, M 001).
prof. dr. Eva Strike (Rigos Stradins universitetas, Latvija, medicinos ir sveikatos mokslai, medicina, M 001).

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CONTENTS

ABBREVIATIONS

ABB	acid-base balance
ALV	artificial lung ventilation
ARICD	Anesthesiology, resuscitation and intensive care department
Cardioplegia	artificially induced temporary stopping of heart or a sharp decrease in heart rate induced by stopping coronary blood flow.
CARMAT	wholly artificial heart CARMAT
CCI	craniocerebral injury
Chronic cardiac failure (CCF)	pathological condition in which the cardiovascular system does not provide the body's oxygen requirements first during physical activity, and then at rest. CCF is characterized by periodic acute conditions (decompensation), manifested by a sudden or, more often, gradual increase in symptoms and signs of CCF (shortness of breath, edema in the lower extremities, general weakness, pulmonary rale, displacement of the apex beat and elevated blood pressure in the jugular veins caused by a structural failure or dysfunction of the heart)
CVM	cardiovascular morbidity
DCS	diseases of the circulatory system
ECMO	extracorporeal membrane oxygenation
ESHP	ex-situ heart perfusion
IHD	ischemic heart disease
Interleukins	cytokines responsible for cell-cell interaction between leukocytes

INTERMACS	(Interagency Registry for Mechanically Assisted Circulatory Support) International register of mechanical circulatory support
ISHLT	The International Society For Heart And Lung Transplantation
LVAD	left ventricular assist device
LVEF - left ventricular ejection fraction (EF)	an indicator that represents the volume of blood pushed by the left ventricle (LV) at the time of its contraction (systole) into the aortic lumen
NRCSC	National research cardiac surgery center
OCS heart– Organ Care System	reproduces the living body conditions for organs. The device sends the donated blood, which is oxygenated and saturated with nutrients through the beating heart. The device allows increasing significantly the time of functioning of the organ before transplantation (up to 8-10 hours). In addition, it helps to examine and even restore the functions of an organ outside the body.
Orthotopic cardiac transplantation (OCT)	one of the main methods of surgical treatment of patients with end-stage cardiac failure, tolerant to drug treatment
TDI (Tissue Doppler Imaging)	color tissue doppler imaging used in conjunction with a pulse doppler in echocardiography to assess the myocardial contractility.
UNOS	United Network Organ Sharing

1. INTRODUCTION

1.1. The research problem and the relevance of the work

Despite improvements of mechanical circulatory support in recent years, heart transplantation remains the approach most likely to improve survival and quality of life in patients with end-stage heart failure (1). Success in heart transplant depends on the quality of the donor heart, procurement, preservation and storage of the graft, the complexity of the operation and duration of graft ischemia (2). Some determinants of successful transplant outcomes are difficult or even impossible to modify, such as the recipient co-morbidities or the quality of the donor heart. On the other hand, it might be possible to improve clinical outcomes by modifying determinants related to procurement and preservation of the graft.

The key factors for success of heart transplantation are duration of preservation of the donor organ and reliable protection from ischemic damage. Representation of the number of patients requiring organ transplantation: the United States is a leader in organ transplantation: 384 patients are registered on the waiting list per 1 million people, of which 91 patients receive transplantation, i.e. 24%. In our country, the figures are much higher, 39 people are registered, and six patients receive transplantation, i.e. 15%. Organ transplantation covers for only about 25 % of the Waiting list across the world. This determines the urgency of the problem and the feasibility of implementing a set of state measures for development of organ donation and transplantation on a global scale. Almost all transplantations were performed using a hypothermal technique involving cooling the organ and introducing a preservative solution with a temperature of 4-6°C, which protects the heart for about 180 minutes (3).

In developed countries, a trend has emerged over the past decade, showing a constant increase in the gap between the growth in the number of patients and the supply of donor hearts. In the UK, 10% of patients on the waiting list die waiting for organ transplantation every year. On the other hand, in the United States, 7 out of 10 hearts are unsuitable for transplantation due to their inadequate preservation: in hypothermia, donor heart with left ventricular hypertrophy cannot be preserved for a long time, it can be well preserved for only a few hours, and the consequences of brain death can lead to poor protection of the donor organ (4). In the UK, only 25% of donor hearts are suitable for transplantation due to the age of the donor and the duration of ischemia. Statistics for the last 20 years in the UK have shown that reducing

ischemia by 1 hour reduces the risk of death in the first year after transplantation by 25% (5). In the United States, a 1-hour reduction in ischemia increases survival by 2.2 years (6). According to the International society for heart and lung transplantation (ISHLT), the risk of rejection of the transplanted organ increases significantly after 3 hours of ischemia (7). Mortality in the first month after heart transplantation is 8% and is caused by primary rejection of the graft, the main causes of which are donor-recipient incompatibility, donor age and duration of ischemia. In the United States, since 2003, the rate of growth in the number of donor hearts is 10% per annum. However, more than half of them cannot be used due to the hypothermic method of protection (8). The increasing demand and limited supply of high-quality donor material has led to serious back-log of needs, which is 3/4 of the number of patients who need transplantation. Moreover, doctor's strive to expand the donor base by relaxing the requirements for the donor organ contributes to the maintenance of high post–transplant mortality-only half of patients with transplanted hearts survive the ten-year milestone (9). The figures above show that avoiding hypothermia will significantly increase the donor base and reduce the growing gap between the need and supply of donor hearts. All these data lead to a change in the paradigm of organ donation, which consists in transition from cooling and preservation of donor organs to their constant normothermic perfusion with oxygen-rich autologous blood. This will allow conducting effective diagnostics, "treatment" of the organ, and its longer preservation, which in general will significantly expand the number of organs available for transplantation (10, 11). With the success of heart transplantation, the eligibility criteria for donor organs are constantly expanding. However, transplantations are limited by the lack of suitable donor hearts. The age of the donor and the severity of ischemia are the main limiting factors. Donors who have significant cardiac dysfunction due to brain death are not currently used due to the lack of a reliable way to predict transplant recovery after transplantation. In order to overcome these limitations, a system of technical support of donor organs has been developed, which minimizes/eliminates the time of cold ischemia and allows restoring the heart function in order to evaluate the parameters of the heart function in natural conditions. Experience shows that the increased use of new perfusion technologies will lead to an expansion of the criteria for organ eligibility and thereby increase their number (12). Modern technologies in transplantology are aimed at the transition from hypothermic to normothermic methods of preserving and transporting donor organs using mobile autonomous

equipment under conditions of their perfusion with a solution enriched with oxygen and nutrients.

Until now, scientific data on conditioning have not been published, and there is very small data on the use of the heart care system, since this is a new method of preserving the organ. There are multicenter randomized studies conducted by Ardehali and coll., which compared methods of cold preservation of a donor heart and OCS system. In addition, the use of this device for elderly donors, donors with reduced cardiac output, was studied. Moreover, all studies used the standard method of myocardial protection Custodiol. Methods of myocardial protection and conditioning during the recovery and long-term transportation of a donor heart using the organ care system have not been studied (13-15).

1.2. Working hypothesis

The hypothesis of this research is to analyze the novel method for preserving a donor heart using blood cardioplegia and conditioning in an organ care system, and to conduct a prospective collected comparative evaluation of two main methods of myocardial protection of patients with ventricular assist devices.

Ex situ heart perfusion (ESHP) allows for a long preservation time and provides the opportunity to assess cardiac function and viability, and metabolism. The current clinically available system relies solely on lactate levels to determine if the heart is suitable for transplant, limiting the ability to predict post-transplant functional recovery. The goal of our study was to evaluate the impact of the treatment of beating heart in normothermic ESHP based on ECMO technology (homemade) on post-transplant graft function Figure 3.

Figure 1. Mobile device Transmedics Organ Care System (OCS). (A) Wireless monitor/controller. (B) Heart perfusion module: (1) aortic flow probe; (2) aortic perfusion line. (C) Instrumented heart: (1) aortic connector; (2) pulmonary artery cannula; (3) left ventricular venting tube.

Figure 2. Map for using the donor organ (heart) care device.

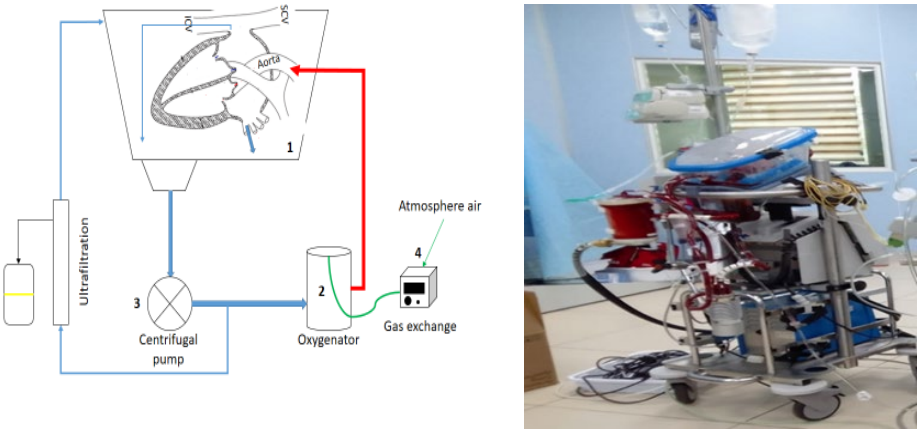


Figure 3. The scheme and picture of portable ex situ perfusion system (our development) 1-integrated box reservoir for a donor heart placement, 2-oxygenator, 3-centrifugal pump, 4- gas exchange using of atmosphere airflow and lines connecting all components.

1.3. Defensive statements

The quality of donor hearts is one of the key determinants of transplantation success (1). The current standard method for organ preservation is cold storage, which exposes the heart to an ischemia period. Ischemia - reperfusion injury inevitably occurs due to ex-vivo transport period, cold storage limits the safe preservation time, increase the risk of post-transplant adverse events, and might increase the risk of primary graft dysfunction (PGD). This technique of organ preservation ensures the acceptable level of protection up to 6 hours. Geographical limitations exist for donor-recipient matching, leading to donor heart underutilization (2, 3). Analyses of large datasets shown that the main predictors of success in heart transplant are the quality of the donor heart, procurement, preservation and storage of the graft, the complexity of the operation, ischemic time and the prognostic factors of the recipient (4). Some determinants of successful transplant outcomes are impossible or difficult to modify, such as the recipient co-morbidities or the quality of the donor heart. However, it is possible to improve clinical outcomes by modifying determinants related to procurement and preservation of the graft.

Despite the development of new technologies in the field of artificial implantation of left ventricle mechanical support and wholly artificial heart, heart transplantation remains the gold standard for treatment of patients with

end-stage cardiac failure. According to the International Society for Heart and Lung Transplantation (ISHLT), approximately 6 million people in the United States are living with heart failure, and some of them die while waiting for advanced surgical treatment or a heart transplant due to limited availability of donor organs. As in the rest of the world, the main factor limiting the number of heart transplants in Kazakhstan is the lack of donor organs and the need for long-term transportation. Therefore, improving the effective protection of the donor organ will ensure timely heart transplantation to patients, including those who are on mechanical circulatory support or have artificial left ventricle, regardless of the geographical location of the donor on the territory of the Republic of Kazakhstan.

1.4. The aim of the investigation

The aim of this study is to evaluate the efficiency of preserving a donor heart using blood cardioplegia and conditioning in an ex situ donor heart perfusion period, and to compare normothermic ex-situ preservation using an organ care system with cold storage of donor hearts in patients who undergo heart transplantation with left ventricle assist devices.

The aim of study was to evaluate the impact of the treatment of beating heart in normothermic ex situ heart perfusion based on ECMO technology (homemade) on post-transplant graft function.

1.5. Research tasks

1. to compare myocardial protection and conditioning during the ex-situ perfusion and long-term transportation of a donor heart in organ care system;
   - Compare lactate trents, notrope doses and duration
   - compare meant ending concentration of interleukin-6 ant Interleukin-8
   - compare postopetative ECMO duration
2. to compare impact of normothermic ex-vivo preservation using organ care system compared with cold storage. in patients with ventricular assist devices;
   - to assess the survival rate of compared groups after transplantation.
3. To evaluate post-transplant graft function after normothermic beating heart ex-situ heart perfusion based on ECMO (our development) in a porcine model:
   - To evaluate venous lactate trend and interleukins
   - To evaluate heart edema (compare heart weight)

- To evaluate myocardial oxygen consumption, echocardiography
- To evaluate histological and MRI picture
- To evaluate coronary blood flow, mean arterial pressure, and heart rate

1.6. Novelty of the work

However, in recent decades, there has been a growing interest in using blood as the basis for CPS during open-heart surgery. The advantages of blood cardioplegia are associated with high oxygen and buffer capacity of red blood cells; energy and plastic substrates for myocardial metabolism; adequate colloid osmotic pressure that prevents the development of vacuolar degeneration; natural antioxidants that reduce the risk of reperfusion injury; reduction in total hemodilution during surgery, which is especially important in case of long-term myocardial ischemia and its reduced functional capabilities. According to many authors, this method has undoubted advantages over other methods for myocardial protection.

Until now, the standard method for recovery and transporting a donor organ was the traditional method using dry ice in saline solution. Today, a portable device capable of crossing all obstacles during transplantation in order to resolve the problems of cold storage method, since, it minimizes cold ischemic injuries through supply of warm blood and oxygen to the heart; optimizes condition of the organ by filling the deficit of the oxygen, nutrients and hormones and provides continuous monitoring and conditioning the condition of the organ just before transplantation.

When analyzing the literature data, the advantages of using the organ care system were revealed, where the duration of transportation is several times higher and the mortality rate was 5%, compared to the cold method, where this indicator was 20% [14].

Myocardial protection is key point in open heart surgery and heart transplantation. Inadequate myocardial protection leads to severe edema, development of ischemic disorders, electrical instability, "stunning" of cells, which is accompanied by development of postoperative complications. According to international publications, there is currently no consensus on the optimal method of protecting the myocardium during heart surgery.

Modern authors believe that the importance of various predictors of early mortality increases sharply when preserving the heart using Custodiol solution in cold conditions for 4 hours or more [16].

However, in recent years, there have been reports of successful donor organ transplantations with a preservation duration of 8-10 hours using the

Organ Care System (OCS) mobile device in figure 1. The system contains new technologies for providing cardiac activity that simulates the conditions of function of organ outside the body and allow it to function close to its physiological state [17-20].

1.7. Practical meaning of the work

The practical significance of the study for the Republic of Kazakhstan is the fact that according to data for 2020, 203 people in Kazakhstan need a heart transplantation per year. In order to increase the number of suitable donor organs and improve transplantation outcomes, we need to develop an effective method for protection of donor organ. The concept "myocardial protection" includes a whole range of methods, including anaesthetic support, surgical approach, hypothermia, and cardioplegia directly.

Cardioplegia is artificially induced temporary cardiac arrest or a sharp decrease in heart rate induced by stopping coronary blood flow.

Currently, there are more than 50 formulas of various cardioplegic solutions (CPR), most of which are prepared empirically and have not been experimentally tested. This figure and continued development of new formulations indicate that researchers and clinicians are dissatisfied with such formulations and justify the search for ways to optimize methods of protecting the myocardium from ischemia.

In recent decades, there has been a growing interest in using blood as the basis for CPS. The advantages of blood cardioplegia are associated with high oxygen and buffer capacity of red blood cells; energy and plastic substrates for myocardial metabolism; adequate colloid osmotic pressure that prevents the development of vacuolar degeneration; natural antioxidants that reduce the risk of reperfusion injury; reduction in total hemodilution during surgery, which is especially important in case of long-term myocardial ischemia and its reduced functional capabilities. According to many authors, this method has undoubted advantages over other methods for myocardial protection [11-15].

The practical application of this method in the Republic of Kazakhstan will allow further development of the heart transplantation program, making it possible to transport the donor organ for long distances and reduce the mortality rate from chronic cardiac failure.

Standard heart preservation

Heart transplantation is the most effective surgical treatment method used to end-stage heart failure. Despite improvement in the management of HTx recipients, the rate of primary graft dysfunction (PGD) is still high, being a severe complications that still represents the leading cause of 30-day mortality after HTx (21-23). The quality of donor hearts is one of the key determinant of transplantation success (24). Donor organ quality, in turn, is determined by a variety of factors including donor age and preexisting disease, the mechanism of brain death, donor management prior to organ procurement, the duration of hypothermic storage, and the circumstances of reperfusion. As demand for solid organ transplantation has increased, so has the use of marginal donors (e.g., those obtained from older donors or from donors with evidence of chronic organ disease or dysfunction prior to brain death) (25). Thus, many cadaveric organs offered for transplantation have preexisting disease or dysfunction prior to the onset of brain death, and although results obtained with marginal doors are generally regarded as acceptable (at least in relation to the waiting list mortality), it is clear that both short- and long-term posttransplant outcomes are not as good when compared with organs obtained from conventional donors (26.27). Furthermore, although the use of marginal donors has led to an increase in the potential donor pool, it has also led to an increased discard rate of cadaveric organs offered for transplantation.

Another factor that adversely affects donor organ quality is brain death. It has been recognized for some time that both the short- and long-term outcomes after cadaveric organ transplantation are significantly inferior to those obtained when the transplanted organ is obtained from a living donor whether the donor is related or unrelated to the recipient (28). Brain death results in a series of hemodynamic, neurohormonal, and pro-inflammatory perturbations, all of which are thought to contribute to donor organ dysfunction.

Hemodynamic Changes

Brain death is accompanied by a series of complex hemodynamic, neurohormonal, and immunological changes. The time course and severity of these changes may vary according to the tempo and nature of the neurological insult leading to brain death. The most severe changes are usually seen in the setting of acute onset of brain death (such as occurs with severe intracranial hemorrhage), which is associated typically with an acute and intense

autonomic discharge, characterized by initial bradycardia (parasympathetic discharge) followed by extreme tachycardia and hypertension (sympathetic discharge). Potential donor organs suffer an ischemic insult during this phase—the heart as a result of a massive increase in workload (29) and the peripheral organs caused by intense peripheral vasoconstriction (30). This autonomic storm has its onset within the first few minutes and usually passes within 15 min. The autonomic storm is also characterized by a sudden increase in cytosolic calcium, which in turn activates enzymes such as lipase, protease, endonuclease, nitric oxide (NO) synthase, and xanthine oxidase (30). These enzymatic changes disrupt normal adenosyl triphosphate (ATP) utilization and generate oxygen-free radicals, which contribute to organ failure. Thereafter, there is a loss of sympathetic tone associated with persistent tachycardia and hypotension. The loss of autonomic tone also results in impaired vascular autoregulation with diminished blood supply and oxygen delivery to organs and tissues. Both initial and late circulatory changes can lead to severe ischemic damage in donor organs before their removal, causing deterioration of the quality of the transplanted graft.

Neurohormonal Changes

Although most investigators accept a link between brain death and disruption of the hypothalamic–pituitary axis, there are conflicting data regarding the hormonal changes that occur during and after central nervous system injury and their influence on hemodynamic parameters and organ quality (31–31). In animal models the hormonal changes fall into two categories: those associated with the autonomic storm represent a transient and massive increase in circulating catecholamines, and those associated with hypothalamic–pituitary failure lead to neurogenic diabetes insipidus and a marked decrease in levels of thyroid hormones and cortisol, at least in animal models (34,35). Metabolic abnormalities associated with these hormonal perturbations include impaired aerobic metabolism despite normal O2 delivery. This has been demonstrated 334 Hicks et al.both globally (36) and in specific organs including the heart (34) and kidney (37). The consequent reliance on anaerobic metabolism results in lactic acidosis (34,36,37) and rapid depletion of high-energy substrates such as ATP (34). Progressive depletion of high-energy stores has been reversed successfully by a combination of T3, cortisol, and insulin administration, suggesting that hormonal changes are the major cause of mitochondrial dysfunction with impaired energy production at the cellular level (38). Some investigators, however, have demonstrated only minor hormonal changes in humans after the onset of brain death (39, 40). An extensive survey of studies on brain-dead

human donors indicates that a reduction in the level of free triiodothyronine (T3) has almost always been documented, but changes in other hormone levels (such as thyroid-stimulating hormone, thyroxine [T4], and cortisol) are variable (39–43). Levels of reverse T3 have been found to be normal or increased after brain death, consistent with a "sick euthyroid" state. Differences between experimental and some clinical findings may be explained by the fact that the former are determined with a uniform mechanism of brain death in highly controlled systems in contrast to the latter group, in which patients suffer brain death by a variety of mechanisms.

Immunological/Inflammatory Changes

Studies investigating the relation between brain death and immunological activation of peripheral organs have demonstrated that the explosive increase in intracranial pressure followed by systemic hypotension upregulates various lymphocyte-and macrophage-derived cytokines on solid organs in rats (44). The hypothesis that brain death increases the immunogenicity of solid organs is further supported by findings that kidneys and hearts transplanted from brain-dead donor animals experience accelerated acute rejection compared to those from living donors (45). Early adhesion molecules (selectins) not present on the vascular cell surface under resting conditions but upregulated rapidly after injury seem to trigger subsequent events. Adherent leukocyte populations express other classes of adhesion molecules (intercellular adhesion molecule; vascular cell adhesion molecule; lymphocyte-function associated antigen-1) and release proinflammatory lymphokines (tumor necrosis factor-α, interferon [IFN]-γ). Expression of major histocompatibility complex (MHC) class I and II molecules is increased. The upregulation of MHC on graft cells is mediated primarily by IFN-γ, itself increased by the brain-death–I/R insult. The mediators of immunological activation of donor organs after brain death have not been determined. The deleterious changes in endothelial surfaces and the increasing immunogenicity of solid organs begin promptly after massive central injury, and it has been suggested that these changes can be partly explained by excessive catecholamine release (30). This hypothesis is further supported by the experimental observation that even short-term administration of catecholamines in brain dead donors is followed by reduced survival and poor initial function after renal allotransplantation in pigs (46).

Finally, the process of transplantation exposes the donor organ to an obligatory period of ischemia and reperfusion. Traditionally, hypothermic storage of the donor organ has been used to protect it from ischemic injury, but donor organs differ markedly in their capacity to withstand hypothermic

ischemia. Data from the Registry of the International Society for Heart and Lung Transplantation indicate that the risk of primary graft failure and death rises dramatically for both the heart and lung as ischemic time increases (46, 47). Based on these data, the maximum recommended ischemic times for the donor heart and lung are 6 and 8 h, respectively.

Currently, static cold storage (SCS) of hearts from donations after brainstem death (DBD) remains the standard practice. SCS combines cardioplegia and hypothermia, which can significantly reduce the energy demand of the donor heart (47). However, despite decades of effort, the cold ischemia time has been limited to 4–6 h. Prolonged cold ischemia and ischemia-reperfusion injury (IRI) have been recognized as significant causes of post-transplant graft failure. According to the International

Society for Heart and Lung Transplantation registration, the survival rate decreases as the ischemic time increases (48). The continuous shortage of donor hearts has always been a major limiting factor for heart transplantation (49). After venting the venous circulation, the ascending aorta is cross-clamped and cold preservation solution is rapidly infused into the aortic root to produce rapid cooling and electromechanical arrest of the heart. Usually the donor heart is then excised and placed in a plastic bag containing approx 1 L of preservation solution. The plastic bag is then sealed and placed in an insulated container packed with ice (between 0 and 4°C), in which it is stored until implantation. Ischemia - reperfusion injury inevitably occurs due to ex-vivo transport period, cold storage limits the safe preservation time, increase the risk of post-transplant adverse events, and might increase the risk of primary graft dysfunction (PGD). This technique of organ preservation ensures the acceptable level of protection up to 6 hours. Geographical limitations exist for donor-recipient matching, leading to donor heart underutilization (2, 3). Analyses of large datasets shown that the main predictors of success in heart transplant are the quality of the donor heart, procurement, preservation and storage of the graft, the complexity of the operation, ischemic time and the prognostic factors of the recipient (4). Some determinants of successful transplant outcomes are impossible or difficult to modify, such as the recipient co-morbidities or the quality of the donor heart. However, it is possible to improve clinical outcomes by modifying determinants related to procurement and preservation of the graft.

A common feature of all methods of donor heart preservation described to date has been the use of hypothermia, which markedly reduces myocardial energy consumption and slows the loss of high-energy substrates. According to the van't Hoff equation, the activity of enzymatic reactions is reduced by approximately 50% for every 10°C reduction in temperature (50). For static

ischemic storage, profound hypothermia (1–4°C in a standard ice chest) has been found to produce satisfactory myocardial protection for up to 6 h in clinical heart transplantation (51). Equivalent levels of myocardial preservation have been reported after 4 h of storage of the canine heart at 4 and 12°C (52). With continuous ex vivo perfusion, excellent myocardial protection may be achieved with lesser degrees of hypothermia (53).

The benefits of hypothermia, however, come at a cost. A major hazard of hypothermia is cell swelling. Normally, the cationic composition of intracellular (high K+, low Na+) and extracellular fluid (high Na+, low K+) is maintained by the membrane Na,K-ATPase pump, which uses energy (ATP) derived from oxidative phosphorylation in mitochondria. The total intracellular colloid osmotic pressure derived from the intracellular proteins and impermeable anions is approx 110–140 mOsm/kg (50). Anaerobic–hypothermic preservation suppresses the Na,K-ATPase pump. Sodium and chloride diffuse into the cell down their ionic concentration gradients, and the water that follows leads to cell swelling.

Hence, in order to prevent cell swelling, impermeable substances must be added to the preservation solution to generate the same amount of osmotic pressure present in the intracellular compartment. Examples include the intravascular impermeants mentioned above. Other impermeants that can be used for this purpose are saccharides such as lactobionate, raffinose, glucose, and mannitol or anions such as citrate, phosphate, sulfate, and gluconate. Another consequence of hypothermic ischemic storage is intracellular Ca2+ accumulation. Under normothermic conditions, myocyte handling of Ca2+ is an energy-dependent process, in which Ca2+ is removed from the cytoplasm (directly and indirectly) by the action of ATPases. Inactivation of these ATPases together with activation of the Na+-H+ exchanger (see below) during hypothermic storage allows Ca2+ to accumulate within the cytoplasm, resulting in Ca2+ overload during storage.

Hypothermia markedly slows myocardial energy consumption but does not arrest it completely. Under hypothermic ischemic storage conditions, the energy dependent processes required to maintain cell viability can only be sustained through anaerobic glycolysis. This results in rapid depletion of high-energy substrates, lactic acid production, and intracellular acidosis. High levels of intracellular lactic acid not only injure cellular organelles, but also can activate macrophages. This, in turn, can lead to cytokine production and the initiation of an inflammatory response (50).

The accumulation of intracellular H+ ion during hypothermic ischemic storage activates a membrane-bound Na-H ion exchanger or antiporter (54) (Fig. 4). This ion exchanger, while quiescent under normal conditions, is

activated by a decrease in intracellular pH and is driven by the transmembrane ionic gradients for Na+ and H+ in an energy-independent process. The Na-H antiporter exchanges intracellular H+ for extracellular Na+. With inactivation of the Na,K-ATPase pump by hypothermia, the resultant accumulation of intracellular Na+ reverses the direction of a second membrane ion exchanger (the Na-Ca antiporter), which exchanges intracellular Na+ for extracellular Ca2+. Hence, the net effect of intracellular acidosis during ischemia is an accumulation of intracellular Ca2+ (54).

One method to prevent acidosis during hypothermia is the addition of hydrogen ion buffers to the preservation solution. Hydrogen ion buffers used for cardiac preservation include potassium phosphate, sodium bicarbonate, magnesium sulfate, and histidine. One of the distinguishing characteristics of Bretschneider (HTK) solution, for example, is its extremely high concentration of histidine in comparison with other organ-preservation solutions (Table 2). An alternative (and possibly more effective) approach to preventing the harmful effects of acidosis is via pharmacological inhibition of the Na-H exchanger (55).

Reperfusion Injury

Although restoration of oxygenated blood flow is essential to the survival of ischemic tissue, the process of reperfusion can paradoxically lead to further tissue injury (56). The severity of this reperfusion injury is directly related to the severity and duration of the ischemic insult that preceded it. Reperfusion injury results in myocyte damage through myocardial stunning, microvascular and endothelial injury, and irreversible cell damage or necrosis (lethal reperfusion injury).

The major chemical mediators are thought to be oxygen-derived free radicals and Ca2+ (56). In addition, there is evidence that white blood cells directly contribute to reperfusion injury after periods of prolonged ischemia (57).

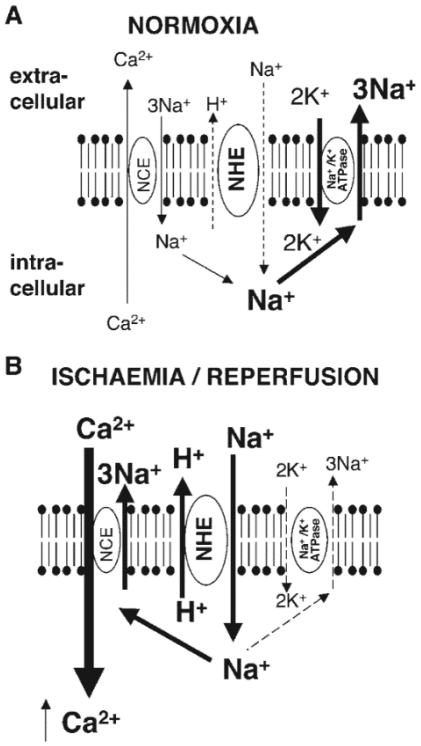


Figure 4. Reperfusion Injury

Activity of the sodium hydrogen exchanger under normal conditions and during ischaemia reperfusion. Under normoxic conditions (Panel A), ATP supply is nonlimiting. Internal sodium is extruded via Na+/K+ ATPase, calcium is extruded via the sodium/calcium exchanger and the sodium/hydrogen exchanger is quiescent. As a consequence of ischaemia (Panel B), ATP is depleted and the Na+/K+ ATPase becomes inactive. Accumulation of H+ as a result of glycolysis, activates the sodium hydrogen exchanger, resulting a large influx of sodium. This sodium is now cleared by the sodium/calcium exchanger resulting in a dangerous intracellular accumulation of calcium which may cause electrical instability, contractile dysfunctions and myocyte death.

Oxygen-derived free radicals

Restoration of oxygen to tissues that have accumulated anaerobic metabolites leads to a burst in the production of oxygen-derived free radicals and oxidants. These include superoxide anion, hydrogen peroxide, hypochlorous acid, hydroxyl radical, and peroxynitrite. Small amounts of

oxygen-derived free radicals are produced as a normal byproduct of a number of essential cellular processes (e.g., mitochondrial energy production and cell-to-cell signaling) but are prevented from causing cell injury by a variety of cellular antioxidant mechanisms. The abrupt increase in cellular levels of oxygen-derived free radicals that occurs during reperfusion after prolonged ischemia is due in part to excess production of free radicals via reaction of xanthine and hypoxanthine with xanthine oxidase. In addition, prolonged ischemia depletes the cell of its antioxidant reserves so that it is less capable of scavenging any excess free radicals generated during reperfusion. Oxygen-derived free radicals contribute to cell injury through a wide variety of chemical reactions including lipid peroxidation, abnormal crosslinking, and cleavage of proteins and DNA disruption.

Potential approaches to the prevention of the burst in oxygen-derived free radical accumulation during organ storage and reperfusion include the addition to the preservation solution of a pharmacological inhibitor of xanthine oxidase, such as allopurinol, and the addition of antioxidant free radical scavengers. Examples include reduced glutathione, mannitol, superoxide dismutase, desferrioxamine, and 21-aminosteroids.

Ca2+ overload during reperfusion

As described previously, there is an accumulation of intracellular Ca2+ during hypothermic ischemic storage due to coupled activation of the Na-H and Na-Ca exchangers. Reperfusion with oxygenated blood initially leads to further activation of the Na-H exchanger resulting in further Ca2+ influx (55). In addition to increased Ca2+ influx, Ca2+ reuptake into the sarcoplasmic reticulum is reduced as a result of depressed activity of the sarcoplasmic reticulum Ca2+ pump (following depletion of intracellular ATP levels). The sustained increase in diastolic Ca2+ has two potentially lethal consequences for the myocyte: sustained contraction (contracture) of actin–myosin proteins and sustained activation of Ca2+-dependent enzymes within mitochondria resulting in mitochondrial failure. Potential approaches to prevention of Ca2+ overload during reperfusion include a reduction in the Ca2+ concentration of the preservation fluid, supplementation of the preservation fluid with Mg2+, which competes with Ca2+ for Ca2+ exchangers and pumps, and the addition of drugs that inhibit Ca2+ influx. These include Ca2+ channel blockers and Na-H exchange inhibitors.

White blood cells

White blood cells are another potential source of oxygen-derived free radicals. Ischemic injury to the vascular endothelium leads to upregulation of

various adhesion molecules, which initiate sticking and activation of circulating white cells and platelets to the vessel lumen (57). In addition to release of free radicals, white cells may physically plug the lumens of microscopic vessels within the reperfused organ, leading to the no-reflow phenomenon (58). The use of white blood cell filters at the time of reperfusion has been shown experimentally and clinically to reduce evidence of reperfusion injury and graft dysfunction (59, 60).

Endothelial injury during ischemia and reperfusion

Under normal physiological conditions, the vascular endothelium synthesizes compounds that induce vascular relaxation and inhibit white cell and platelet adherence to the vessel wall. These compounds include NO, endothelium-dependent hyperpolarization factor, and prostacyclin. Ischemic injury to endothelial cells inhibits production of these compounds upregulating of prothrombotic and pro-inflammatory adhesion molecules as a consequence. Oxygen-derived free radicals generated on reperfusion may further damage the vascular endothelium. For example, superoxide reacts directly with NO, leading to loss of the physiological activity of NO and formation of peroxynitrite, a potent cytotoxic-free radical. Nitric oxide and prostacyclin are potent vasodilators and possess cytoprotective properties that may be beneficial for preservation of allograft function during and after cold ischemic storage. Prostacyclin and related prostanoids have been used to produce maximal vasodilatation within the vascular bed of the donor organ either via prior intravenous administration (61-64) or by addition to the preservation solution (61, 65, and 66). Similarly, NO donors (e.g., glyceryl trinitrate and diazenium diolates—NONOates) have been added to preservation solutions to offset the loss of endogenous NO that occurs during hypothermic storage and reperfusion (67-69). Endothelial injury caused by I/R injury has been implicated as a factor in the development of both acute allograft dysfunction and chronic allograft vasculopathy. Another potential source of endothelial injury is the high K+ concentration of some intracellular preservation solutions such as University of Wisconsin (UW) solution, although this remains controversial. Several clinical studies suggest that the development of coronary allograft vasculopathy may differ according to the type of preservation solution used at the time of transplantation, with two studies reporting higher rates when the heart was stored in UW solution (70, 71).

The key factors for success of heart transplantation are duration of preservation of the donor organ and reliable protection from ischemic damage. Representation of the number of patients requiring organ transplantation: the United States is a leader in organ transplantation: 384 patients are registered

on the waiting list per 1 million people, of which 91 patients receive transplantation, i.e. 24%. In our country, the figures are much higher, 39 people are registered, and six patients receive transplantation, i.e. 15%. Organ transplantation covers for only about 25 % of the Waiting list across the world. This determines the urgency of the problem and the feasibility of implementing a set of state measures for development of organ donation and transplantation on a global scale. Almost all transplantations were performed using a hypothermal technique involving cooling the organ and introducing a preservative solution with a temperature of 4-6°C, which protects the heart for about 180 minutes (3).

In developed countries, a trend has emerged over the past decade, showing a constant increase in the gap between the growth in the number of patients and the supply of donor hearts. In the UK, 10% of patients on the waiting list die waiting for organ transplantation every year. On the other hand, in the United States, 7 out of 10 hearts are unsuitable for transplantation due to their inadequate preservation: in hypothermia, donor heart with left ventricular hypertrophy cannot be preserved for a long time, it can be well preserved for only a few hours, and the consequences of brain death can lead to poor protection of the donor organ (4). In the UK, only 25% of donor hearts are suitable for transplantation due to the age of the donor and the duration of ischemia. Statistics for the last 20 years in the UK have shown that reducing ischemia by 1 hour reduces the risk of death in the first year after transplantation by 25% (5). In the United States, a 1-hour reduction in ischemia increases survival by 2.2 years (6). According to the International society for heart and lung transplantation (ISHLT), the risk of rejection of the transplanted organ increases significantly after 3 hours of ischemia (7). Mortality in the first month after heart transplantation is 8% and is caused by primary rejection of the graft, the main causes of which are donor-recipient incompatibility, donor age and duration of ischemia. In the United States, since 2003, the rate of growth in the number of donor hearts is 10% per annum. However, more than half of them cannot be used due to the hypothermic method of protection (8). The increasing demand and limited supply of high-quality donor material has led to serious back-log of needs, which is 3/4 of the number of patients who need transplantation. Moreover, doctors' strive to expand the donor base by relaxing the requirements for the donor organ contributes to the maintenance of high post–transplant mortality-only half of patients with transplanted hearts survive the ten-year milestone (9).

The continuous enhancement of medical and interventional therapies, as well as the widespread improvement of mechanical circulatory supports,

has made it possible for an aging population with multiplying complications to be considered for heart transplants.

Static cold storage

The use of static cold storage (SCS) for donor graft preservation, aims to stabilize biological tissues by influencing metabolic pathways. Such strategy slows the cellular and extracellular biochemical processes that are responsible for organ degradation during ischemic storage, thus extending a safe storage time up to several hours. The temperature dependence of chemical reaction rates follows the "Arrhenius equation", used to describe the temperature-depending metabolic changes: for every 10°C reduction of temperature below the physiological temperature the metabolic rate for living biological tissues reduces by 50%. Hence, cold storage slows but does not completely arrest cellular metabolism. Consequently, progressive ischemic injury is an inevitable consequence of prolonged SCS and the results of HTx are suboptimal when graft ischemic time is greater than 6 h. Moreover, the negative impact of graft ischemic time is considerably influenced by donor characteristics, as age, left ventricular hypertrophy, mild-to-moderate coronary artery disease and catecholamine support (72).

As reported by the ISHLT Consensus Statement on donor heart and lung procurement (73), the ideal donor graft temperature during storage should probably be kept between 5°C and 10°C. In fact, freezing of any part of the heart is undesirable because freezing and subsequent thawing may cause tissue damage potentially responsible for PGD (74). Indeed, possible freeze injury was detected in 7% of autopsies done on patients deceased for clinically diagnosed PGD (75).

The Paragonix SherpaPak^{TM} cardiac transport system (PSP) is able to guarantee a constant, homogeneous and controlled temperature of the donor heart between 4°C and 8°C, thus minimizing tissue injury due to ice-cold temperature exposure.

The PSP device consists of two canisters, one internal and one external (Figure 5A). The internal canister is filled with cold storage saline solution (4°C–8°C), and the donor heart is submerged into it, after being connected to the canister lid by means of an aortic connector (Figure 5B). The most widely used solutions for heart preservation are the Celsior, the University of Wisconsin (UW) and the Custodiol (histidine-tryptophanketoglutarate HTK). Then, the inner canister is placed into the outer one, creating an insulating air chamber, and outside this system is surrounded by single-use cooling ice packs. A thermometer connected with the internal canister allows continuous monitoring of the temperature (Figure 5C).

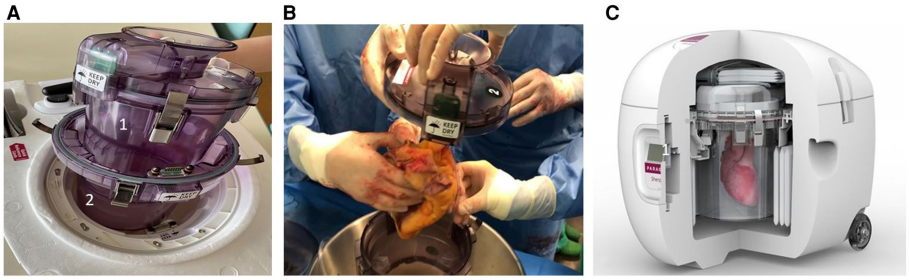


Figure 5. The paragonix sherpa-Pak system. (A) Internal (1) and external (2) canisters of Paragonix Sherpa-Pak. (B) The donor heart connected to the internal canister lid. (C) Overview of the system: (1) display and bluetooth data transmission module.

The GUARDIAN study is a post-market, observational registry of adult and pediatric patients who received a donor heart preserved and transported using either the PSP or standard preservation methods. Using data of 877 patents enrolled in the Guardian heart registry by 16 US centers, two cohorts of 249 patients were propensity matched according to the technique of graft preservation. Although the 1-year survival did not statistically differ between the two cohorts ($p = 0.12$), PSP preservation significantly reduced severe PGD rate, compared to ice-cold storage (4% vs. 10%, $p = 0.01$) (76). The use of PSP has also proved to be cost-beneficial. In a recent study that compared two groups of 87 matched patients (PSP and ice-cold storage), post-HTx costs were significantly lower when donor organs were preserved with PSP. This figure reflected a significant role of PSP in reducing incidences of severe PGD (5.7% vs. 16.1%, $p = 0.03$) and employment of mechanical circulatory support after HTx (21.8% vs. 40.2%, $p = 0.009$), and thus the recipient hospital length of stay (77). Histological analyses of myocardial biopsies taken as soon as the donor hearts were reperfused during HTx, showed that grafts preserved with PSP appeared to have less interstitial edema and myocyte damage compared to those preserved with traditional ice storage (78).

Machine perfusion

Differently from static cold ischemic storage, machine perfusion (MP) is considered an ideal approach for donor organ management to extend the donor pool and/or increase the utilization rate. Perfusion can supply the metabolic need of the myocardium, thus minimizing irreversible ischemic cell injury and death. Two types of heart perfusion systems currently used, which are either hypothermic MP (HMP) or normothermic MP (NMP), have

successfully preserved animal and/or human hearts (79). The longest reported successful human heart preservation time was 16 h with NMP (80). Currently, there is only one commercially available perfusion system for clinical use, the organ care system (OCS), and one recently tested system, the non-ischemic heart preservation system (NIHP) (81, 82). Another approach to extend the donor pool is to utilize organs from donation after circulatory death (DCD) (79, 83). For these donor hearts, MP can provide a platform to resuscitate, preserve, assess and even possibly recondition the cardiac function prior to plan transplantation. Well-designed machine perfusion can theoretically expand the donor pool in different ways. A prolonged safe preservation time allows to utilize remote donor hearts and functional assessment allows to utilize some of the DCD and high-risk donor hearts. Pediatric heart transplantation may have an extra benefit since pediatric donor shortage is even worse, and long transport time occurs more frequently. Despite the growing number of human donor hearts preserved with MP, it remains controversial whether MP is superior to SCS. In this systematic review, we summarize and critically assess all available clinical data on MP of adult donor hearts, highlighting its therapeutic potential as well as the current limitations and shortcomings.

Hypothermic Machine Perfusion

In clinical practice, two types of MP have been used to preserve donor hearts: HMP and NMP. The system temperature was controlled below 10°C during HMP, in contrast to 34°C during NMP.

The rationale of HMP preservation consists in reducing the metabolic requirements of the heart with an optimal and homogenous cooling (below 10^0C), while providing continues metabolic support through perfusion with oxygenated, nutrient enriched medium to limit as much as possible intracellular anaerobic metabolism and acidosis. Experimental study performed on large animal models have suggest that compared to SCS vs HMP could attenuate tissue injures and provide superior myocardial function after heart transplantation (84).

Clinical utilization was limited due to concerns about a reliable functional assessment of this systems. Another major limitation is associated to the risk of edema during HMP preservation and after reperfusion period. Moreover, edema related to HMP employment was reported to be more likely interstitial and reversible, with limited impact on myocardial function after heart transplantation period (85).

We identified three nonrandomized, single-centre studies that used HMP systems (Table 1) (81, 86, 87). Wicomb et al. in 1984 demonstrated the first system for HMP of the human heart used crystalloid cardioplegic solution (86). In this study, 4 patients were perfused with an oxygen- and carbon dioxide-bubbled crystalloid cardioplegic solution at a pressure of 8–10 cm H_2O. All four hearts were transplanted after a total preservation time of 6, 7, 12, or 15 h. Only one patient survived after 16 months with normal heart function (86). Hill et al. in 1997 reported successful heart transplantation with HMP using a colloid cardioplegic solution to perfuse eight hearts with a low flow rate (17 ml per 100 g per hour) for 221 min. For comparison, 13 hearts were preserved with cardiosol (185 min) and 50 hearts with modified St. Thomas solution (187 min). The 7-year survival rate was 70% in the St. Thomas solution group and 100% in the other two groups (87). In the third study, more recently, in 2020 Nilsson et al. preserved six hearts using so called "non-ischemic hypothermic perfusion" (NIHP) with a perfusion pressure of 20 mm Hg at 8°C. A home-made MP perfusate comprised a hyper-oncotic cardioplegic solution supplemented with hormones and erythrocytes. These six NIHP transplantations were compared with 25 SCS transplantations during the same period. The median total preservation time was longer for the NIHP group (223 min; IQR, 202–263) than for the SCS group (194 min; IQR, 164–223). The primary outcome showed a 100% event-free 6-month survival rate for NIHP recipients, compared to 72% for SCS recipients. Furthermore, creatine kinase-muscle/brain, assessed 6 h after ending perfusion, was 76 ng/ml for NIHP compared with 138 ng/ml for the SCS recipients (non-significant), indicating less myocardial damage when using the NIHP method (81). Based on these preliminary promising results, the Xvivo Perfusion AB (Goteborg, Sweden) has patented the NIHP and further developed it to a commercially available device (88).

The Lifecradle Heart Preservation System is a HMP, currently under development, that uses hypothermic, oxygenated, nutrient perfusion at 5ºC, in a controlled and monitored environment. The safety and efficacy of the device will be defined on the basic of clinical evidence, currently pending.

Table 1. Hypothermic machine perfusion

Study	Number of patients	Temperature (°C)	Outcome
Wicomb et al., 1984 (86)	HMP = 4	4–10	Total preservation time 12, 7, 15, and 6 h. One patient survived over 16 months
Hill et al., 1997 (87)	HMP = 8, SCS = 12	Ice-cooling	7-year survival rate 100% in both the HMP and the SCS groups
Nilsson et al., 2020 (81)	HMP = 6, SCS = 25	8	6-month event-free survival rate 100% in the HMP group and 72% in the SCS group

HMP, hypothermic machine perfusion; SCS, static cold storage.

Normothermic Machine perfusion

In order to overcome the disadvantages of static cold storage, research is being carried out on warm preservation storage methods started in parallel. The devices used in both approaches were similar and used warm pulsatile perfusion of the heart with blood to allow ex-situ recovery of a marginally functioning heart or a hearts after cardiac arrest before implantation. Blood has been considered an ideal solution for perfusion because of its excellent oxygen and nutrient delivery properties. Additionally, blood acts as a free radical scavenger and a powerful buffer against acidosis and metabolic toxicity. In addition, blood protects endothelial function and reduces the risk of damage.

Normothermic MP systems perfuse the heart with oxynated blood and enriched solutions, keeping it beating and at a near-physiological condition. Currently the only NMP system commercially available for clinical heart transplantation is the Organ Care System (OCS, TransMedics Inc, Andover, MA). Ex situ perfusion with this device is particularly attractive when "extended criteria" for donor organs procurement have to be further evaluated; this system, bedsides limiting graft ischemic time, allows a real-time monitoring of the donor graft assessing hemodynamic parameters and lactate concentration.

The first step in the establishment of an OCS heart perfusion device is the procurement of the heart. The steps to obtain a heart are very different for brain death donation (DBD) and cardiac death donation (DCD). Patients with cardiac death have neurological injury to have a meaningful life but do not meet the criteria for brain death.

With the OCS, oxygenated donor blood is used to perfuse coronary arteries at a temperature of 34°C with a perfusion pressure of 60–90 mmHg. Lactate concentration is monitored to verify that adequate perfusion is achieved and if it is above 5 mmol/L, the heart is discarded (15). In the PROCEED II trial, five donor hearts were discarded, four because of rising lactate concentrations and one because of technical issues (15).

Twenty-one publications, including eight papers (15, 89, 18, 90-94) and 13 conference abstracts (80, 95-106) presented results from using the OCS at transplantation of DBD hearts with or without a control group (Tables 2, 3). Three of these studies were randomized (Table 2). The only randomized and multicenter study, PROCEED II, which recruited 130 patients from 10 heart transplant centers in the United States and Europe, showed no significant differences in the primary endpoint (30-day patient and graft survival) or secondary endpoints. However, the mean total out-of-body time was significantly longer in the OCS group than in the control group (324 vs. 195 min) (82). The other two randomized studies reported data from single institutional heart transplant candidates, previously enrolled in the PROCEED II study and subsequently followed for an additional one and 2 years (18, 90). There were no significant differences between the OCS and SCS groups regarding changes in intimal thickness for the left main and left anterior descending coronary arteries (90). Chan et al. followed the recipient for 2 years and did not find any significant differences in patient survival, freedom from non-fatal major cardiac events, or cardiac allograft vasculopathy (18).

Thirteen studies (25, 80, 91, 93, 94, 96, 99-103, 105, 106) used the OCS in high-risk cases. High risk was defined as an adverse donor/recipient profile, including an estimated ischemic time longer than 4 h, left ventricular ejection fraction less than 50%, left ventricular hypertrophy, donor cardiac arrest, alcohol/drug abuse, coronary artery disease, recipient mechanical circulatory support, and/or elevated pulmonary vascular resistance. In nine publications, the OCS was compared with SCS (Table 2) (91, 92, 95-101). The results of three of these studies favored OCS perfusion (97, 99, 101), including two studies that used the OCS for high-risk cases (99, 101). The other six studies did not find any significant difference in the primary outcomes (91, 92, 95, 98, 100). The total preservation time was reported in five studies, and it was significantly longer in the OCS groups (91, 95, 96, 99, 100).

Botta et al. compared day-0/day-1 CK-MB levels between an OCS group and an SCS group and did not find any significant difference (96). Falk et al. compared IRI between the OCS and SCS groups by measuring interleukin (IL)-6, IL-8, IL-18, angiopoietin-2, and insulin-like growth factor-binding protein-1 immediately after and 24 h after heart transplant (97). The

results showed that OCS preservation significantly reduced all these proteins. Seven studies compared short- and long-term patient survival rates and found no significant difference between the groups (91, 92, 95, 98-101).

One case report reported two long-distance heart transplantations, with or without the OCS. Although both patients remained well at 6 months with normal cardiac function, the patient who received the SCS-preserved heart had a longer hospital stay (50 vs. 12 days) and a higher cost (AU$234,160 vs. 56,658) compared with the OCS recipient (91). In nine publications, only the OCS was studied (Table 3) (5, 10, 16, 21, 32–35, 40). In general, the OCS preserved heart function well, resulting in a satisfactory postoperative survival rate for the recipients. Two case reports presented successful transplantations after 10 and 16 h preservation time (80, 94). In one study, hearts from both standard criteria donors and marginal donors (outside standard acceptability criteria) were preserved with the OCS, and no significant differences in 1-month, 1-year, and 2-year survival rates were found. However, there was an increased requirement for extracorporeal membrane oxygenation (ECMO) support in the standard criteria donor group (33% vs. 11%) (103).

The OCS was used for DCD hearts in 11 studies (Table 4) (107–117). In clinical practice, DCD hearts are retrieved with either direct procurement and perfusion (DPP) (107–109, 111-115, 117) or thoracoabdominal normothermic regional perfusion (TA-NRP) (110, 113, 115, 117). For DPP, after confirmation of death, a cardioplegic flush is applied. Thereafter, the heart is excised and transported in a beating state using an OCS. For TA-NRP, after confirmation of death, cardiac resuscitation is achieved with the help of an external pump. After weaning from the TA-NRP, cardiac functional assessment is performed using a pulmonary artery flotation catheter and transesophageal echocardiogram. Four studies reported comparable results between the OCS-preserved DCD hearts and the SCS-preserved DBD hearts (111, 113, 115, 117). However, two hearts were discarded after OCS preservation owing to machine failure (111). One study reported a 100% 3-month survival rate in both OCS-preserved DCD hearts and OCS preserved marginal brain donor hearts (114). One study compared post-transplant biopsies for C4d and acute rejection episodes. The results suggested a lower IRI rate and similar patterns of cellular rejection for the OCS-preserved DCD hearts compared with the regular DBD transplantation (116). The other five publications presented successful DCD heart transplantations using OCS (107–110, 112). Messer et al. also compared the DPP plus OCS with TA-NRP plus OCS for DCD hearts and found no significant difference in 30- and 90-day survival rates (113, 115).

Five clinical trials are currently recruiting patients (Table 4) (118-122). Among these trials, three have a randomized design (119, 120, 122) and four are multicenter studies (41, 42, 44, 45). All ongoing clinical trials use patient/graft survival as the primary endpoint and patient/graft survival in a different time frame and/or graft function as secondary endpoints.

Ischemia is the main reason a donor heart can only be preserved within a few hours. The principle of the MP is to avoid ischemia. Both preclinical (123) and clinical (80, 94) studies have shown that successful transplantations after more than 10 h of MP preservation can be achieved. A prolonged preservation time would theoretically benefit the transplantation teams and reduce transplantation costs.

Using MP leads to a longer preservation time (129 min longer in the OCS group and 29 min longer in the NIHP group than in the SCS group) (81, 82). Moreover, MP requires additional surgical and technical support, proprietary equipment, appropriate transport, and additional costs. However, it may reduce the length of stay in the intensive care unit or hospital, postoperative mechanical support, and need for reoperation. Therefore, the total cost and labor demand may be reduced (91).

The figures above show that avoiding hypothermia will significantly increase the donor base and reduce the growing gap between the need and supply of donor hearts. All these data lead to a change in the paradigm of organ donation, which consists in transition from cooling and preservation of donor organs to their constant normothermic perfusion with oxygen-rich autologous blood. This will allow conducting effective diagnostics, "treatment" of the organ, and its longer preservation, which in general will significantly expand the number of organs available for transplantation (89, 77). With the success of heart transplantation, the eligibility criteria for donor organs are constantly expanding. However, transplantations are limited by the lack of suitable donor hearts. The age of the donor and the severity of ischemia are the main limiting factors. Donors who have significant cardiac dysfunction due to brain death are not currently used due to the lack of a reliable way to predict transplant recovery after transplantation. In order to overcome these limitations, a system of technical support of donor organs has been developed, which minimizes/eliminates the time of cold ischemia and allows restoring the heart function in order to evaluate the parameters of the heart function in natural conditions. Experience shows that the increased use of new perfusion technologies will lead to an expansion of the criteria for organ eligibility and thereby increase their number (78). Modern technologies in transplantology are aimed at the transition from hypothermic to normothermic methods of preserving and transporting donor organs using mobile autonomous equipment

under conditions of their perfusion with a solution enriched with oxygen and nutrients.

The organ care system manufactured by Transmedics (USA) provides normothermic perfusion of the organ ex vivo with the blood of the donor itself, excluding prolonged ischemia (Figure 1). From the moment when the organ is retrieved from the donor to the moment when the heart surgeon begins to transplant the organ, the organ remains functioning outside the body, in a state close to physiological. All this time, the organ is stored and transported in the organ care device. The heart beats, and warm and oxygenated blood is perfused through the coronary arteries. The device has been clinically tested in clinics in the USA and Europe. It was used for more than 300 heart transplantations. According to clinical studies, the stated maximum time for autonomous preservation of a donor heart is up to 12 hours, while the actual time is limited to 8 hours. This method was used for the first heart transplantation operations in our country in the OCS (figure 2). The device allows increasing significantly the time of functioning of the organ before transplantation, according to our data, with a maximum duration of 16 hours. In addition, it helps to examine and even restore the functions of an organ outside the body. It is especially important to use this device to assess the condition of an allograft received from a donor after death as a result of circulatory failure. When analyzing the literature data, the advantages of using the organ care system were revealed, where the duration of transportation is several times higher and the mortality rate was 5%, compared to the cold method, where this indicator was 20%.

Until now, scientific data on conditioning have not been published, and there is very small data on the use of the heart care system, since this is a new method of preserving the organ. There are multicenter randomized studies conducted by Ardehali and coll., which compared methods of cold preservation of a donor heart and OCS system. In addition, the use of this device for elderly donors, donors with reduced cardiac output, was studied. Moreover, all studies used the standard method of myocardial protection Custodiol. Methods of myocardial protection and conditioning during the recovery and long-term transportation of a donor heart using the organ care system have not been studied (90-92).

In 2012, we initiated the first heart transplant program in Kazakhstan. Alongside initiatives to increase the donor pool, we sought ways to improve patient outcomes to mitigate the realities of a small donor pool and the long distances over which donor hearts are transported in our country (often >1000 km) (93). In this context, the Organ Care System (Transmedics, OCS) is used at our center and it allows normothermic, beating, perfused ex-vivo donor heart preservation and thus has the potential to reduce the risk related to time-dependent ischemic injury to the donor heart during cold storage (107). The

OCS also allows ex-situ assessment or improvement of non-standard donor hearts, or resuscitation of DCD hearts (18). The results of the PROCEED II study demonstrated a significant reduction in cold ischemic time for the OCS relative to standard cold storage donor preservation. The standard approach for donor heart harvesting is to use of custodiol cardiolplegic solution for arresting the heart, followed by reanimation of the heart in the OCS.

Despite the development of new technologies in the field of artificial implantation of left ventricle mechanical support and wholly artificial heart, heart transplantation remains the gold standard for treatment of patients with end-stage cardiac failure. However, according to the International organization for heart and lung transplantation, world statistics show that in the United States of America alone, about 6 million recipients with cardiac failure die waiting for surgery every year. As in the rest of the world, the main factor limiting the number of heart transplants in Kazakhstan is the lack of donor organs and the need for long-term transportation. Therefore, improving the effective protection of the donor organ will ensure timely heart transplantation to patients, including those who are on mechanical circulatory support or have artificial left ventricle, regardless of the geographical location of the donor on the territory of the Republic of Kazakhstan.

Currently, an intracellular hypothermic crystalloid solution Custodiol (Germany), developed on the basis of Bretschneider's solution, is widely used worldwide for organ care device. A special feature of this solution is its large buffer capacity and long duration of cardioplegic effect.

However, in recent decades, there has been a growing interest in using blood as the basis for CPS during open-heart surgery. The advantages of blood cardioplegia are associated with high oxygen and buffer capacity of red blood cells; energy and plastic substrates for myocardial metabolism; adequate colloid osmotic pressure that prevents the development of vacuolar degeneration; natural antioxidants that reduce the risk of reperfusion injury; reduction in total hemodilution during surgery, which is especially important in case of long-term myocardial ischemia and its reduced functional capabilities. According to many authors, this method has undoubted advantages over other methods for myocardial protection.

Until now, the standard method for recovery and transporting a donor organ was the traditional method using dry ice in saline solution. Today, a portable device capable of crossing all obstacles during transplantation in order to resolve the problems of cold storage method, since, it minimizes cold ischemic injuries through supply of warm blood and oxygen to the heart; optimizes condition of the organ by filling the deficit of the oxygen, nutrients and hormones and provides continuous monitoring and conditioning the condition of the organ just before transplantation.

When analyzing the literature data, the advantages of using the organ care system were revealed, where the duration of transportation is several times higher and the mortality rate was 5%, compared to the cold method, where this indicator was 20% (91).

Myocardial protection is key point in open heart surgery and heart transplantation. Inadequate myocardial protection leads to severe edema, development of ischemic disorders, electrical instability, "stunning" of cells, which is accompanied by development of postoperative complications. According to international publications, there is currently no consensus on the optimal method of protecting the myocardium during heart surgery.

Modern authors believe that the importance of various predictors of early mortality increases sharply when preserving the heart using Custodiol solution in cold conditions for 4 hours or more (93).

However, in recent years, there have been reports of successful donor organ transplantations with a preservation duration of 8-10 hours using the Organ Care System (OCS) mobile device in figure 1. The system contains new technologies for providing cardiac activity that simulates the conditions of function of organ outside the body and allow it to function close to its physiological state (107-110).

Society's self-sufficiency in terms of organ replacement is still far away from being achieved, given the large disparity between transplant demand and donor organ availability. In the attempt to reduce this discrepancy and expand the donor pool, the transplant community has progressively increased the utilisation of extended-criteria donors (ECD) such as older donors, donors with comorbidities and donation after circulatory death (DCD). The main challenge preventing a wider utilisation of ECD and DCD allografts is the higher susceptibility to the ischemia-reperfusion injury (IRI) (1), an unavoidable part of the transplantation process. For this reason, in the last decades, there has been an exponential development on organ reconditioning strategies, in order to enable graft resuscitation and viability assessment prior to implantation (124). Ex-vivo perfusion techniques are usually classified according to the perfusate temperature, hypothermic (<10°C) or normothermic (37°C), with roller or centrifugal pumps used to generate pressure-controlled pulsatile or continuous flow within the organ, via connection to the renal inflow (artery) and outflow (vein) (125). Given the variety of combinations of different parameters and settings (temperature, oxygen, nutrient and/or drug delivery, in situ/ex-situ), machine perfusion (MP) is considered a promising way to expand the criteria of transplantation by optimizing its preservation modalities, potential of organ viability assessment and potentially decreasing the rate and gravity of postoperative complications (126).

Table 2. Normothermic machine perfusion for hearts from donation after brainstem death with static cold storage as the control group.

Study	Number of patients	Total machine perfusion time (min)	Outcomes of interest
Ardehali et al., 2015 (15)	OCS = 67, SCS= 63	OCS = 324, SCS= 195	No difference in 30-day survival rate and SAE between groups
Chan et al., 2017 (18)	OCS = 19, SCS= 19	OCS = 361, SCS= 207	2-year patient survival rate: 72.2% in OCS group, 81.6% in SCS group ($p = 0.38$)
Sato et al., 2019 (90)	OCS = 5, SCS= 13	OCS = 362, SCS= 183	ΔMIT ≥0.5 mm with no significant difference between groups. From baseline to 1 year post-transplant, ΔMIT, maximal intimal area, and percent stenosis were similar between groups
Botta et al., 2017 (96)	OCS = 7, SCS= 95	OCS = 296, SCS= 187	No significant difference in CK-MB post- transplant
Falk et al., 2019 (97)	OCS = 16, SCS = 24	Not reported	OCS perfusion reduces IRI at the cytokine and endothelial level in recipient blood immediately after transplantation
Fujita et al., 2018 (98)	OCS = 29, SCS = 169	Not reported	Survival rate similar between groups
Garcia et al., 2015 (99)	OCS = 15, SCS = 15	OCS = 373, SCS = 204	30-day survival rate: 100% in OCS group and 73.3% in SCS group ($p = 0.03$)
Jain et al., 2017 (91)	OCS = 1, SCS = 1	OCS = 495, SCS = 412	Total cost of OCS transplantation significantly less than SCS transplantation
Koerner et al., 2014 (92)	OCS = 29, SCS = 130	OCS = 313, SCS: not reported	No significant difference in cumulative survival rates at 30 days, 1 year, and 2 years
Kaliyev et al., 2020 (127)	OCS=25, SCS=10	OCS=423, SCS=210	No significant difference in 30-day mortality. Normothermic ex-vivo preservation beneficial for long-time out of body organ preservation in comparison of cold storage especially for recipients on mechanical circulatory support.
Rojas et al., 2020 (100)	OCS = 49, SCS = 48	OCS = 402, SCS = 225	No significant difference in 30-day, 1-year, and 2-year survival rate
Sponga et al., 2019 (101)	OCS = 17, SCS = 70	Not reported	Improved 30-day, 1-year, and 5-year survival rate in the OCS group
Sponga et al., 2020 (95)	OCS = 44, SCS = 21	OCS = 428, SCS = 223	No significant difference in 30-day mortality

IRI, ischemia-reperfusion injury; MIT, maximal intimal thickness; NS, not significant; OCS, organ care system; SAE, serious adverse events; SCS, static cold storage.

Table 3. Normothermic machine perfusion for hearts from donation after brainstem death

Study	**Number of patients**	**Total machine perfusion time (min)**	**Outcomes of interest**
Ayan Mukash et al., 2019 (102)	OCS=47	Not reported	Kaplan-Meier survival estimates 91%, 85%, and 80% at 3 months, 6 months, and 1 year
Garcia et al., 2016 (103)	OCS=60	Not reported	Survival rate similar between regular donor group (n = 24) and extended criteria donor group (n = 36)
Garcia et al., 2014 (93)	OCS=26	371	Survival rate 100% at 1 month and 96% at follow-up of 257 days
Kaliyev et al., 2019 (89)	OCS=43	344	30-day survival 100%
Koerner et al., 2012 (104)	OCS=13	Not reported	1- and 2-year survival rate 89%
Nurmykhametova et al., 2018 (80)	OCS=1	960	Total out-of-body time 16 h, longest out-body time to date
Rojas et al., 2020 (106)	OCS=76	382	Survival rate 92.1% and 82.9% at 30 days and 1 year
Stamp et al., 2015 (94)	OCS=1	611	Total out-of-body time 10 h
Yeter et al., 2014 (105)	OCS=21	388	Freedom from cardiac-related death 95% at 30 days and 6 months, 87% at 1 and 4 years

Table 4. Studies of normothermic machine perfusion for hearts from donation after circulatory death.

Study	Number of patients	Outcomes of interest
Chew et al., 2017 (114)	DCD = 12, MBD = 12	All hearts retrieved with DPP, comparable survival rate between OCS-preserved DCD hearts and OCS-preserved MBD hearts
Chew et al., 2019 (111)	DCD = 23, DBD = 94	All DCD hearts retrieved with DPP, comparable survival rate between OCS-preserved DCD hearts and SCS-preserved DBD hearts
Dhital et al., 2015 (112)	DCD = 3	All hearts retrieved with DPP, survival to date: 77, 91, and 176 days
Garcia et al., 2016 (107)	DCD = 2	Both hearts retrieved with DPP, survival to date: 290 and 291 days
Mehta et al., 2019 (108)	DCD = 7	All hearts retrieved with DPP, 90-day survival rate 86%
Messer et al., 2016 (110)	DCD = 9	8 hearts retrieved with TA-NRP + OCS; all patients survived during follow-up (range, 48–297 days)
Messer et al., 2017 (113)	DCD = 26, DBD = 26	DCD hearts retrieved with DPP or TA-NRP, comparable results of the OCS-preserved DCD hearts and the SCS-preserved DBD hearts
Messer et al., 2019 (115)	DCD = 50, DBD = 50	DCD hearts retrieved with DPP or TA-NRP, comparable results in 30-day survival
Mohite et al., 2019 (109)	DCD = 1	Heart retrieved with DPP, alive to date at 5 months
Page et al., 2017 (116)	DCD = 20, DBD = not reported	Biopsies within first month after transplantation showed significantly lower positive C4d rate in OCS-preserved DCD hearts suggesting a lower IRI rate. During first year, acute cellular rejection (2R) was lower in DCD than DBD group
Page et al., 2018 (117)	DCD = 31, DBD = 31	DCD hearts retrieved with DPP or TA-NRP, comparable results

DBD, donation after brainstem death; DCD, donation after circulatory death; DPP, direct procurement and perfusion; IRI, ischemia reperfusion injury; MBD, marginal brain dead; TA-NRP, normothermic regional perfusion; OCS, organ care system; SCS, static cold storage.

There are several advantages to using OCS system for heart perfusion and preservation during transport from the donor hospital to the recipient hospital.

1) Duration of cold ischemia and donor advanced age are major risk factors for post-transplant morbidity and mortality. As the age of the donor increases, the risk of developing coronary artery disease (CAD) increases. The study showed that when the duration of cold ischemia was shortened, the mortality rate 1 year after transplantation was twice as high. The time increases from 3 hours to 6 hours, and decreases by half if less than 1 hour (128). The OCS system significantly reduces the cold ischemia time of the heart and allows coronary angiography to be performed in online, which is the best method to diagnose CAD (16). Aortic perfusion may also affect the OCS system as a surrogate for diagnosing significant coronary artery disease in donor hearts. In a study of OCS systems, Garcia Saez D et al. reported that increased aortic pressure during cardiac perfusion is a prognostic indicator of coronary artery disease donor heart. Therefore, it is important to constantly monitor aortic root pressure. Although angiography is not available, monitoring of aortic pressure and lactate can help determine the severity of the CAD disease and subsequent decision-making regarding graft survival (93). Non-inferiority study of OCS the PROCEED II study (a prospective multicenter study) demonstrated the outcomes of heart transplantation. Hearts preserved by OCS were comparable to hearts preserved by static cold storage (93). The use of cardiac OCS did not affect daily outcomes or graft survival in heart transplant recipients. Superior preservation compared to standard cold storage methods. There was also no big difference incidence of severe rejection and length of intensive care unit stay in transplant patients is saved using OCS compared to SCS [31]. Interestingly, a single-center retrospective study showed better results. Outcomes of heart transplant patients using OCS and standard cold storage method (127).

Mortality after transplantation in patients with congenital heart disease is high, especially in those who have undergone previous surgery. Additionally, open heart surgery remains a challenge for surgeons due to the complex anatomy and the potential for severe adhesions from previous surgery, and explanting a native heart requires a complex mediastinal incision may be required. Additionally, many cases of pediatric heart transplantation may require complex anatomical reconstruction after cardiectomy and prior to transplantation. Similarly, a patients who previously had have VAD implanted may also benefit from an OCS supported donor hearts due to the anatomical complexity of their previous VAD. To safely perform cardiectomy without

damaging the other structures, transplant preparation may be more challenging and take longer time.

Surgeons should always wait until the donor heart lands near the recipient hospital before performing with the irreversible step of explanting the recipient heart to avoid catastrophe in case of unforeseen event happens. Therefore, organ transport time and the time required for complex mediastinal dissection and reconstruction significantly increase the cold ischemia time of the donor heart, even after arrival in the recipient operating room. In this situation, the OCS system becomes a lifesaver, allowing the surgeon to perform the mediastinal dissection more calmly and consciously (129). Fleck et al. in their study found that pediatric transplant patients had better outcomes when the donor heart was perfused with OCS than those preserved with SCS (130). In a small retrospective study, Ardehali et al. reported that the use of OCS before heart transplantation in patients with VAD appears to improve their 30-day survival compared with SCS (130). However, due to limited sample size, further research will be required to investigate this indication in the future.

The OCS system allows hearts to be transported over long distances, increasing the donor pool. Currently, the heart is procured a radius of a 500-mile to keep the flight time less than two hours. However, in many cases, organs must be discarded because a suitable donor or recipient may be far away. The OCS system allows hearts to be obtained and transported over long distances, potentially increasing the donor pool (131).

OCS can the potentially increase the donor pool by accepting the marginal heart. Currently, hearts with left ventricular hypertrophy, EF (ejection fraction) of 40-50%, recovery time > 20 minutes, and donor age > 55 years are rejected for transplantation due to the risk of primary graft dysfunction. However, many of these hearts can recover completely after short-term improving the coronary perfusion on cardiopulmonary bypass support. The OCS system makes it possible to acquire these marginal hearts and assess their function in real time after establishment of coronary perfusion. If cardiac function improves, these hearts can be successfully transplanted with good results (132). In the EXPAND trial, 75 of these 93 marginal hearts were transplanted (81% utilization rate). The average cross-clamp time and OCS perfusion time were 381 and 279 minutes, respectively. In this study, the 30-day survival rate was 95%, the incidence of severe primary graft dysfunction within 24 hours after transplantation was 11%, and the overall survival and graft survival rates at 24 months were respectively 82% and 95% (133).

3. SUBJECTS, METHODS AND RESULTS OF THE RESEARCH

3.1. Recipients and donors characteristic

A prospective comparative evaluation of myocardial protection and conditioning during the ex-situ perfusion and long-term transportation of a donor heart in organ care system

In this study, we performed randomized, controlled, comparative, prospective analyses data at our center. Between May 2014 and September 2017, 43 patients with heart failure underwent heart transplantation at our institution, and we used the OCS for donor heart preservation in all cases shown in figure 1. Eligible recipients were at least 18 years of age and had to be on the heart-transplant waiting list at our center. Of these, we arrested the donor hearts before explant and before implant using blood cardioplegia and conditioning in 30 cases and in 13 cases, we used standard Custodiol solution for cardioplegia (standard care group). The study received approval through the responsible ethics committee at our institution and all patients provided written informed consent to be part of this study and to allow their data to be used for the analysis. The study group (n=43) which divided in two groups:

1. Myocardial protection during preservation and transportation of a donor heart is carried out using blood cardioplegia and conditioning (Levosimendan and ultrafiltration) (n=30) (BC group)
2. Control group consists of myocardial protection during recovery and transportation of the donor heart is carried out using a crystalloid cold (Custodiol) cardioplegia (n=13) (SC group). Organ transportation, diagnostic and therapy measures were carried out in the OCS system.

The main results of interest were 30-day survival and cardiovascular complications. Moreover, clinical, laboratory, immunological, and ultrasound data were collected from donors and recipients, as well as parameters of the OCS device. Operational and postoperative data, duration of extracorporeal membrane oxygenation (ECMO) (during implantation), and data from tissue dopplerography of the first 7 days were analyzed.

Procedures

All patients underwent orthotopic heart transplantation. After the donor heart was dissected, a double-outlet needle was inserted in the donor's ascending aorta and secured with a 4-0 polypropylene purse-string suture. According to standard procedures for the Transmedics device, 500 ml of priming solution was added to the OCS. After the donor was heparinized (300

IU/kg), the donor blood (1200–1500 mL) was collected prior to antegrade cardioplegia and prior to cross clamping of the aorta. 10,000 IU of heparin was added to the blood collection bag and this was used to prime the perfusion module. In the blood cardioplegia group, a portion of the normothermic blood (500–750 mL) was collected retrogradely for initial dose of cardioplegia. In the standard care group, 1000 ml of standard Custodiol solution (cooled to 4 degrees of Celsius) was used. In both groups, the aorta and pulmonary artery of the donor heart were cannulated and heart connected to the OCS. In the OCS, oxygenated blood was pumped into the aorta, perfusing the coronary arteries. The coronary sinus flow then passes through the tricuspid valve (as both the superior and inferior vena cavae are sutured closed) and is ejected by the right ventricle into a pulmonary artery catheter and returned to the blood reservoir. Then, the heart is reanimated to normal sinus rhythm. The pump flow and solution flow rates of the OCS were adjusted to maintain the mean aortic pressure between 60 mmHg and 90 mmHg and coronary blood flow between 650 mL/min and 850 mL/min. According to standard protocol, samples were taken in the OCS before the donor heart was connected to the OCS. These included donor lactate (CG4+, within 30 minutes of blood collection), baseline OCS lactate and chemistries (CG8+, during priming). Hourly arterial and venous lactates were monitored throughout during OCS time. Periodic arterial chemistry samples were taken during OCS time (approximately every 20-30 minutes). Samples were collected from the arterial and venous sampling port of OCS. The samples were analyzed with a handheld lactate analyzer (i-STAT, Abbott Diagnostics, East Windsor, NJ, USA).At the beginning and end of ex vivo heart perfusion, venous blood samples were taken to assess IL-6 and IL-8 levels (Bio-Rad, Model 680, USA; Model 680 Microplate Reader,USA). Upon arrival at our center, the donor heart were arrested with approximately one liter of normothermic blood cardioplegia in the blood cardioplegia group or Custodiol solution in the standard care group and were disconnected from the OCS for implantation into the recipient. Transplantation and preoperative care proceeded according to the standard procedures of our center in both groups (5). The solution we used in the blood cardioplegia group consisted of blood and crystalloid solution at the ratio of 1:5 and a cardioplegia pressure of 150mmHg.The crystalloid solution in the blood cardioplegia group contained KCl 4% (30 ml), MgSO4 25% (10 ml), NaHCO3 4% (13 ml), Mannitol 15% (6.5 ml), and Lidocaine 2% (2 ml) with blood up to a total volume of 600 ml. In the blood cardioplegia group only, the graft was conditioned with Levosimendan 45µg/kg (using body weight of donor) while in the OCS and hemofiltration with a blood flow of 200–300 ml/h was applied in the OCS in order to protect

and improve donor heart function. Between 100-200 ml/h of plasma was collected and Sterofundin isotonic solution was used to replace the plasma. It was used due to its positive inotropic effect by increasing calcium sensitivity of myocytes by binding to cardiac troponin C in a calcium-dependent manner.

Outcomes of interest were the ex vivo heart perfusion mean change in IL-6 and IL-8 concentration from baseline, ischemic time, perfusion time, hemodynamic measurements and lactate levels. We defined total preservation time as the heart perfusion time while in the OCS. Total ischemic time was defined as time from donor heart explant to recipient implantation minus time in OCS. We also collected electrophysiological data, data on perioperative parameters, including OCS perfusion measures, interleukin 6, 8 and lactate trends. Postoperative recovery and follow-up were defined as inotrope dose, length of stay in intensive care unit, TDI parameters and extracorporeal membrane oxygenation duration (if used).

Statistical analysis

Continuous data are expressed as mean ± standard deviation for continuous data, unless otherwise specified. Categorical data are expressed as counts and proportions. Where possible, a two-sample independent t-Test was used to compare the means. Statistical analyses were performed using SPSS system for statistics.

Results

Recipient and donor population

The donor and recipient characteristics and risk factors are shown in Table 6. In the recipient group, the median age is slightly higher in the standard care group compared to the blood cardioplegia group. Other prognostic risk factors at baseline are similar between the two groups, including gender, body mass index, ILs in donor and proportion of patients who were on a ventricular assist device at the time of transplant.

Comparative analysis of donors and recipients in 2 main groups: the first group - myocardial protection during recovery and transportation of the donor heart is carried out using blood cardioplegia and conditioning using Levosimendan and ultrafiltration (BC group) (n=30); second control group - myocardial protection during recovery and transportation of the donor heart is carried out using standard cold (Custodiol) cardioplegia (SC group) (n=13) are shown in figure 6. The average age in the blood cardioplegia and conditioning group was moderately higher compared to the age of recipients in control group (Custodiol). Other predictive risk factors such as gender, body mass index, and proportion of patients who were on a ventricular assist

device during transplantation were similar between the groups. Most recipients and their donors had the same blood type.

The median (area of distribution) observation time was 255 (30-360) days in the blood cardioplegia and conditioning group and 360 (30-600) days in the control group.

Table 5. Composition of blood cardioplegia

Composition of blood cardioplegia 600 ml:	
KCl 4%	30 ml
$MgSO_4$ 25%	10 ml
Lidocaine 2%	2 ml
$NaHCO_3$4%	13 ml
Mannitol 15%	6,5 ml
Blood	up to 600 ml

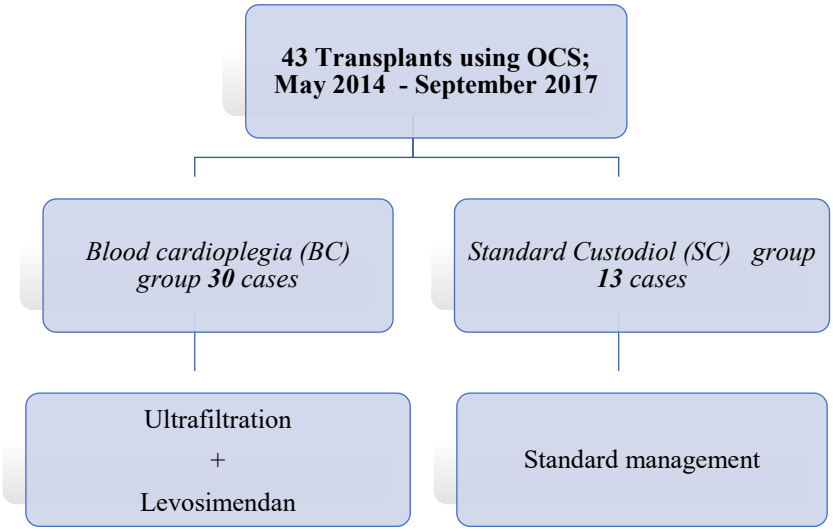


Figure 6. Study protocol

Table 6. The donors and recipients characteristics and risk factors

Donor characteristics	**BC (n=30)**	**SC (n=13)**	**p value**
Age (years)	39 ±11	43 ±15.5	0.2
Male, n (%)	22 (74)	9 (75)	0.95
BMI (kg/m^2)	22.4 ±1.6	22.6 ±2.5	0.08
Cause of death CVA, n (%)	22 (74)	9 (75)	0.95
Other cause of death, n (%)	8 (26)	4 (25)	0.96
Median LVEF (range)	62 (57-65)	63 (59-67)	

Recipient characteristics	BC (n=30)	SC (n=13)	p value
Age (years)	35 ±15	40 ± 12	0.45
Male	89.4% (26 of 30)	75% (9 of 13)	0.51
BMI (kg/m^2)	22.6 ± 2.5	22.8 ± 4	0.1
NICM n (%),	73.6% (22 of 30)	50% (6 of 13)	0.27
Other	26.3% (8 of 30)	50% (6 of 13)	0.25
UNOS 1A+	42.1 (12 of 30)%	33.3% (4 of 13)	0.74
Implanted VAD, n (%)	52.6% (15 of 30)	41.6% (5 of 13)	0.66

Data are expressed as mean ± standard deviation, unless otherwise notedCVA-cerebrovascular accident; LVEF- left ventricular ejection fraction; BMI– body mass index; NICM - non-ischaemic cardiomyopathy; UNOS United Network for Organ Sharing; VAD-ventricular assist device

Electrophysiological findings; OCS data

Ischemic times and perfusion times of donor hearts in the OCS are shown in Figure 6. Mean (±standard deviation) total ischemic time was 75.2 (±22) min in the blood cardioplegia group compared to 82.9 (±8.4) min in the standard care group. Mean ex vivo perfusion time was 282.5 ± 86.7 min in the blood cardioplegia group compared to 247.4 ± 88.4 min in the standard care group (P=0.87). Time of sinus rhythm restoration in OCS and in recipient was significantly lower in blood cardioplegia group Table 7.

All donor hearts had stable perfusion and biochemical characteristics in the OCS and measures were similar between the two groups (Figure 8). Starting concentration of IL-6 and IL-8 were no statistically differences between groups. Ex vivo heart perfusion mean ending concentration of IL-6 and IL-8 was significantly lower in the blood cardioplegia group compared to the standard care group 1493 ng/ml (SD 529.3) vs. 2866 ng/ml (SD 601.2); (p = 0.01), 989ng/ml (SD 453.6) vs. 1274 ng/ml (SD 423.4) (p=0.05) (Figure 9).

Mean venous lactate at the start of perfusion was 2.7 mmol/l (SD 0.7) in the blood cardioplegia group and 3.2 mmol/l (SD 0.8) in the standard care group (P = 0.1). At the end of perfusion, the mean venous lactate was lower in the blood cardioplegia group 4.1 mmol/l (SD 1.9) compared to the standard care group 8.8mmol/l (SD 2.1) (P=0.001) (Figure 10).

The time of ischemia and perfusion of the donor organ in the OCS device is shown in figure 7. The average total time of warm ischemia was 75.2±22 minutes in group 1 compared to 82.9±8.4 minutes in group 2. The average ex vivo perfusion time of the donor organ was 282.5±86.7 minutes compared to 247.4±88.4 minutes in group 1 and 2 (p=0.87), respectively. The average time (standard deviation) for sinus rhythm recovery after switching to organ care devices is 2.6± 1.4 min in group 1 and 8.5 ± 5.8 min in group 2

(p=0.04). The mean time (standard deviation) for sinus rhythm recovery after transplantation to the recipient was significantly lower in the blood cardioplegia group and conditioning compared to the standard Custodiol group 3.2 ± 2.1 vs 7.3 ± 7.1 minutes (p=0.02), respectively, table 7. The average value of troponin I and ABB analyses does not differ statistically significantly.

Perfusion and biochemical parameters in organ care device during the transportation of the donor heart were within the normal range in the comparative groups shown in figure 7. The average change (standard deviation) in Interleukin (IL) 6 and 8 concentrations before transportation before surgery in the organ care device did not differ statistically significantly. The average change (standard deviation) in Interleukin (IL) 6 and 8 concentrations before stopping the organ care device for heart implantation to the recipient is statistically lower in the blood cardioplegia group compared to the Custodiol group - 1493 ng/ml (SD 529.3) vs. 2866 ng/ml (SD 601.2) ;(p = 0.01), 989ng/ml (SD 453.6) vs. 1274 ng/ml (SD 423.4) (p=0.05), respectively, in groups (figure 9).

The mean concentration (standard deviation) of venous lactate at the beginning of perfusion is 2.7 mmol/l (SD 0.7) in the blood cardioplegia group and 3.2 mmol/l (SD 0.8) in the Custodiol group (p=0.1). The mean concentration (standard deviation) of venous lactate at the end of perfusion is 4.1 mmol/l (SD 1.9) in the blood cardioplegia group and 8.8 mmol/l (SD 2.1) (p=0.001) in the Custodiol group (Figure 10).

Postoperative recovery

Median ICU stay was 11 days (range: 4–40 days) in the blood cardioplegia group and 19 days (range: 5–42) in the standard care group. Median time on ECMO who received mechanical support was 29.5 hours (29.5 ± 28.4 hours, n=6) in the blood cardioplegia group compared to 78.4 hours (78.4 ± 89 hours, n = 8) in the standard care group (P = 0.02). Inotrope dose within 72 hours was significantly lower in blood cardioplegia group see Table 7. Cardiotonic support doses (mcg/kg/min) for the first 72 hours were significantly lower in the blood cardioplegia and conditioning group compared to the Custodiol group (Table 8). The average time (standard deviation) spent on artificial lung ventilation is 30 hours (24-73) in the blood group and 78.4 (26-312) hours in the Custodiol group. Median (area of distribution) duration of stay in ARICD is 11 (4-40) days in the blood group and 19 (5-42) days in the Custodiol group. ECMO duration in the blood cardioplegia and conditioning group (29.5 ± 28.4 hours, n=6) compared to (78.4 ± 89 hours, n = 8) in the Custodiol group (p = 0.02). (Table 8).

Indicators of tissue myocardial Doppler imaging on the 7th day after surgery were within the normal range in all groups: indicators of tissue myocardial Doppler imaging on the 7th day after surgery were within the normal range in all groups: S1LV lateral (cm/sec) 7.8(±1.3) in group 1 and 8.5 (±1.4) in group 2), and 8.8 in group 3, S1LV medial (cm/sec) 8.3 (±1.62) in group 1 and 7.68 (±1.23) in group 2, 8.7 in group 3, S1RV (cm/sec) 9.3 (±1.33) in group 1 and 8.35 (±1.39) in group 2, 9.0 in group 3, respectively. LVEF (%) 55.4 (±2.31) in group 1 and 56.5(±7.5) in group 2, and 50 in group 3. The average sinus rhythm recovery time is 5.3 (3.2; 7.3) minutes in group 1 and 7.5 (5.3; 9.7) minutes in group 2, and 7 minutes in group 3.

Inotropic support doses were normal in all groups, but significantly increased on day 3 after transplantation in group 1. The average time (standard deviation) spent on artificial lung ventilation is 163 hours (153;173) in the 1st group and 98 (30;168) hours in group 2, and 75 hours in the group number three.

The average concentration of Interleukin (IL) 6 and 8 before recovery of the donor heart from the device is significantly lower in group 2 compared to group 1, 5.54 vs. 0.29, and 7.0 in the group 3. Preoperative concentrations of IL-6 and 8 do not differ. The average concentration of venous lactate at the beginning of perfusion is 1.7 mmol/l in group 1, 5.3 mmol/l in group 2, and 2.0 mmol/l in group 3. The average concentration of venous lactate at the end of perfusion is 8.7 mmol/l in group 1, 4.1 mmol/l in group 2, and 7.7 mmol/l in group 3, respectively. In all groups, the survival rate was 100% on 30 day after transplantation.

Survival and graft failure

All patients were alive on the 30th days post implant in both groups. Primary graft failure incidence was 3% (n=1) in blood cardioplegia group and 8% (n=1) in the standard care group. One patient developed right ventricular dysfunction 1 month after implant in the standard care group, and one patient in blood cardioplegia group.

Table 7. Time of sinus rhythm restoration (Data are mean – S.D.)

	BC group (n=30)	***SC group (n=13)***	***p value***
Time of sinus rhythm restoration in OCS (min)	2.6 ± 1.4	8.5 ± 5.8	0.04
Time of sinus rhythm restoration in recipient (min)	3.2 ± 2.1	7.3 ± 7.1	0.02

Data are expressed as mean ± *standard deviation*

Table 8. Tissue Myocardial Doppler (at day 7), ICU length of stay, Inotrope dose and ECMO duration.

	Blood cardioplegia group (n=30)	***Custodiol group (n=13)***	***p value***
S^1LV lateral (cm/s) TMD	10 ± 1.6	9.2 ± 1.8	0.73
S^1LV medial (cm/s) TMD	8.93 ± 1.35	8.58 ± 1.6	0.60
S^1RV (cm/s) TMD	10 ± 2.66	8.95 ± 1.96	0.36
LVEF (%) TMD	61.4 ± 2.31	57.5 ± 7.9	0.001
ICU length of stay (days)	11.7 ± 10.3	19.6 ± 13	0.44
Inotrope Dose (mcg/kg/minute IV) **24 hours** *Dobutamine* *Milrinone* **48 hours** *Dobutamine* *Milrinone* **72 hours** *Dobutamine* *Milrinone*	 6.5 ±1.9 1.75 ±1.25 6.0 ± 2.6 *0.2 (n=1)* 3.6 (±0.8) 0.2 (n=1)	 6.5 ± 1.7 1.8 ± 1.3 6.8 ± 1.3 0.3 (n=1) 5.4 (±2.7) 0.2 (n=1)	 0.74 0.90 0.39 0.05
ECMO duration (h)	*29.5 ± 28.4* *n=6*	78.4 ± 89 n=8	0.002

TMD - tissue myocardial Doppler; S^1LV –myocardial velocity associated with isovolumic contraction of left ventricle; S^1RV - myocardial velocity associated with isovolumic contraction of right ventricle; LVEF -left ventricular ejection fraction; ICU – intensive care unit; ECMO –extracorporeal membrane oxygenation.
Data are expressed as mean ± *standard deviation*

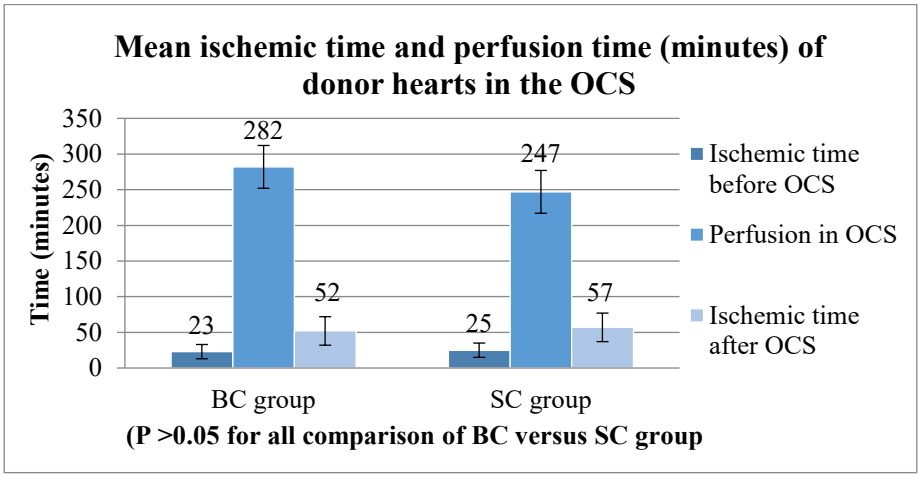


Figure 7. Mean ischemic time and perfusion time (minutes) of donor hearts in the OCS (*P* >0.05 for all comparison of BC versus SC group).

"Error bars represent standard deviation

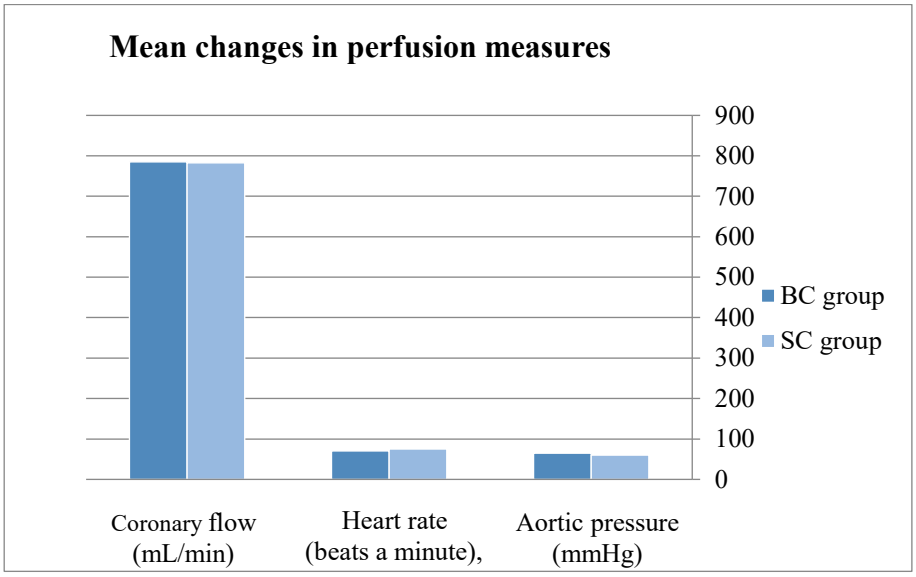


Figure 8. Mean changes in perfusion measures: Coronary flow (mL/min), Heart rate (beats a minute), Aortic pressure (mmHg) in OCS Heart *(P* = NS).

"Error bars represent standard deviation

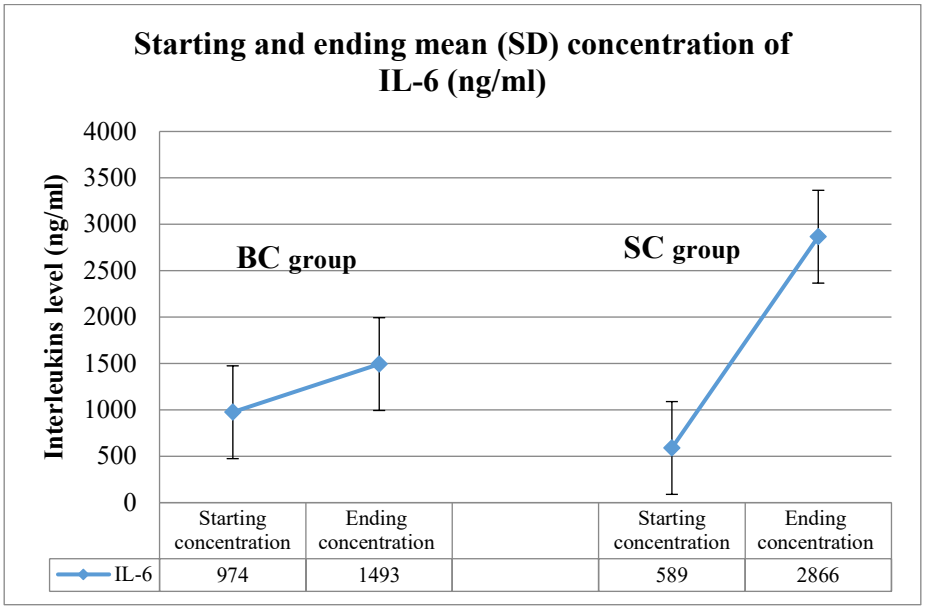
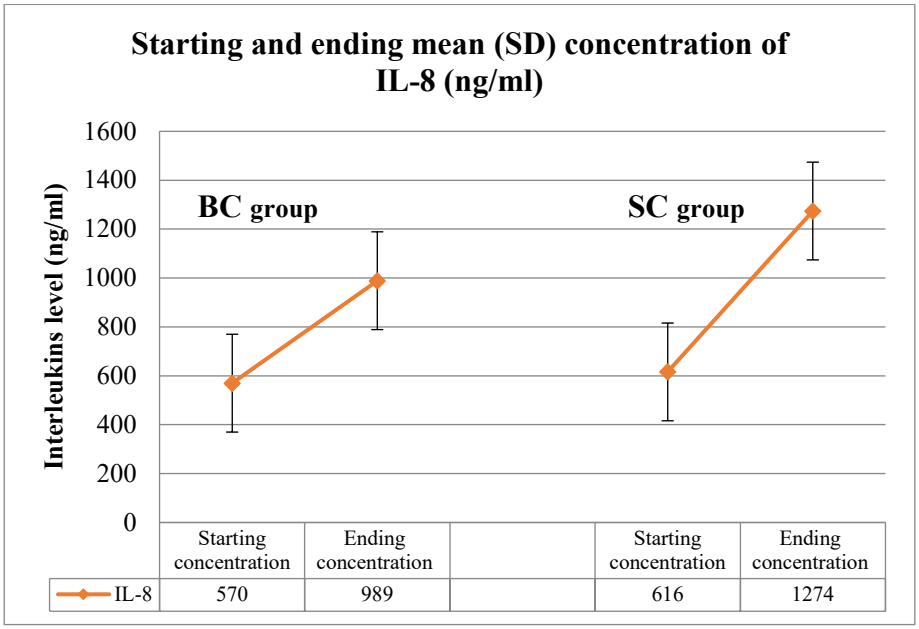


Figure 9. Starting and ending mean (SD) concentration of IL-6, 8 (ng/ml) in the Organ Care System



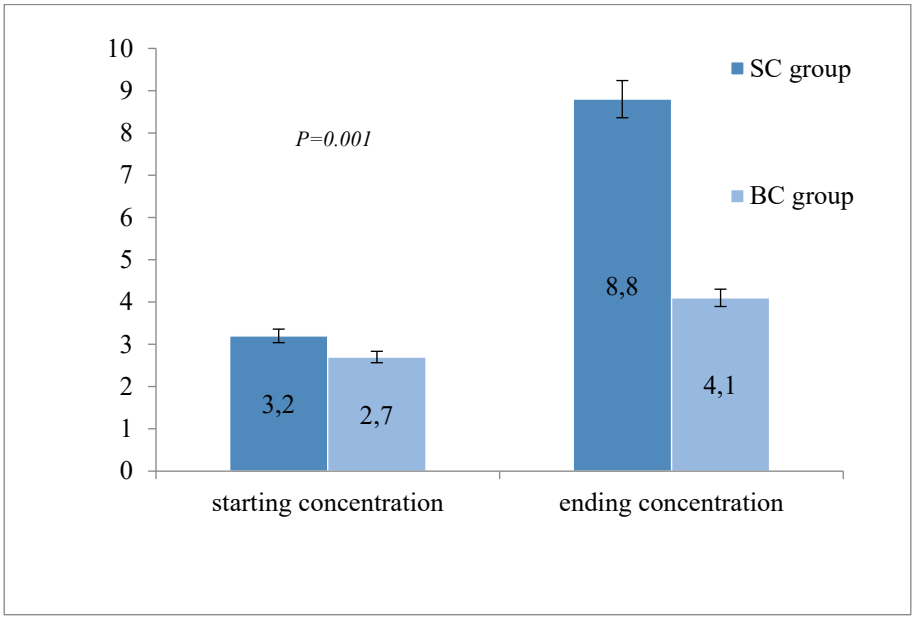


Figure 10. Mean changes in lactate trends (mmol/l). P-value represents the difference of change over time (test)

"Error bars represent standard deviation

3.2. Materials and methods

In this part of study, we performed a single-center experience of impact of normothermic ex-vivo preservation using organ care system compared with Cold storage for prolonged heart preservation especially beneficial for high-risk recipients bridged to transplantation with Mechanical Circulatory Support.

The Heart OCS

The heart OCS (Transmedics Inc, Boston, MA) is composed of an organ perfusion module with disposable and nondisponsible parts and a compact wireless monitor. The monitor displays indicated (online time) organ measurements, such as aortic pressure, coronary flow, blood temperature, and heart rate. The heart is perfused in the resting mode. Warm oxygenated blood is pumped into the aorta, thereby perfusing the coronary arteries, and deoxygenated blood enters the right atrium through the coronary sinus and passes through the tricuspid valve to the right ventricle. The blood is then ejected through the pulmonary artery to the blood oxygenator and is returned to the reservoir.

Procedures

After acceptance of donor heart based on clinical information, our team performed a detailed allograft assessment at the time of donation (transesophageal echocardiography, cardiac output studies using a pulmonary artery catheter, direct evaluation of the coronary arteries, and measurement of left and right atrial pressures). Before aortic cross clamping, the right atrial appendage was annulated using a 34F venous cannula, thereby allowing approximately 1.5 L of donor blood to be collected to prime the OCS module. After the donor was heparinized (300 IU/kg), the donor blood was collected prior to antegrade cardioplegia and prior to cross clamping of the aorta. In blood collection bag was added heparin (10,000 IU) and this was used to prime the perfusion module. Portion of the normothermic blood (500–750 mL) was collected retrogradely for initial dose of blood cardioplegia. The aorta and pulmonary artery of the donor heart were cannulated and heart connected to the OCS with the posterior aspect facing upward and the left atrium and aorta toward the heart chamber. In the OCS, oxygenated blood was pumped into the aorta, perfusing the coronary arteries. The coronary sinus flow then passes through the tricuspid valve (as both the superior and inferior vena cavae are sutured closed) and is ejected by the right ventricle into a pulmonary artery catheter and returned to the blood reservoir. Then, the heart is reanimated to normal sinus rhythm. The pump flow and solution flow rates of the OCS were adjusted to maintain the mean aortic pressure between 60 mmHg and 90 mmHg and coronary blood flow between 650 mL/min and 850 mL/min. According to standard protocol, samples were taken in the OCS before the donor heart was connected to the OCS. These included donor lactate (CG4+, within 30 minutes of blood collection), baseline OCS lactate and chemistries (CG8+, during priming). Periodic arterial chemistry samples were taken during OCS time (approximately every 20-30 minutes). Samples were collected from the arterial and venous sampling port of OCS. The samples were analyzed with a handheld lactate analyzer (i-STAT, Abbott Diagnostics, East Windsor, NJ, USA). Upon arrival at recipient center, the donor heart was arrested with approximately one liter of normothermic blood cardioplegia before transplanting. The graft was conditioned with Levosimendan 45μg/kg (using body weight of donor) while in the OCS and hemofiltration with a blood flow of 200–300 ml/h was applied in the OCS in order to protect and improve donor heart function.

For the standard cold storage group, the donor heart was arrested with the standard heart preservation solution (40C Custodiol). Transplantation and

preoperative care proceeded according to the standard procedures of our center in both groups.

Study Design and Participants

From 2011, when initiated the heart failure program 353 patients were implanted with ventricular assist devices to date and 35 (10 %) of them transplanted (9). Between 2012 and 2018, we performed a retrospective single-center review of prospectively collected data. All patients who underwent heart transplantation with MCS using the OCS Heart (n=25) versus standard cold storage were included in this study. Eligible recipients were at least 18 years of age and had to be on the heart-transplant waiting list. The study received approval through the responsible ethics committee at our institution and all patients provided written informed consent to be part of this study and to allow their data to be used for the analysis. Endpoints included 30-day survival, heart preservation time (ischemic time, OCS perfusion time, out of body time), duration and level of inotropic support, ITU stay-day, Mechanical Circulatory Support after heart transplantation, adverse cardiac events.

Statistical analysis

Results were expressed as mean and standard deviation or median and interquartile range (continuous variables), and counts with percentages (categorical variables). Where possible, a two-sample independent t-Test was used to compare the means. Outcome measures used were 30-day survival. Statistical analyses were performed using STATA version 12 (Stata Corp, Texas, US).

Results

Donor and Recipient population

The donor and recipient characteristics and risk factors are presented in Table 11. There was a trend slightly higher donor age on the OCS group vs CS (41.3±9.3 Vs 38.3±11.5 yo; p= 0.2), with 92% vs 70% male donors. The gender mismatches among the donor/recipients profile in OCS group 3 male donor to 3 female recipient, and in SC group 3 female donor to 3 male recipient. Nineteen donors (76%) vs six (60%) died of spontaneous intracranial hemorrhage, 6 (24%) vs 3 (30%) died of cerebrovascular accidence in OCS vs CS group respectively, and 1(10%) patient died of trauma in CS group.

There was no significant difference in recipient age in OCS and CS group (38.6±11.9 Vs 43.6±12.6 yo; p= 0.2) and 80% (n=20) vs 100% (n=10) were male, respectively. All patients had advanced heart failure (64% vs 70% NICM) in OCS vs CS group. The IMPACT score was a trend towards higher estimated risk of death at 1y in the OCS group (14.2 vs. 10.8% p= 0.083).

In the OCS group 20 recipients was on LVAD support (HeartWare-3, HeartMate II- 10, HeartMate 3- 4, HeartMate 3+ECMO- 1, HeartMate 3 + RVAD-1, RVAD + LVAD (short term biVAD Levitronix)-1) and ECMO-2, total artificial heart (CARMAT)-3 compared in the CS group 10 recipients on LVAD support (HeartWare-2, HeartWare+RVAD-1, HeartMate II-4, HeartMate 3 -2, HeartMate 3 + RVAD (Levitronix)-1). Of the 20 recipients who received LVAD support preoperatively, six versus two patients had an ongoing severe pump pocket infection at the time of transplantation in OCS and CS, respectively. Two patients in OCS group vs one patient in CS group were on inotropic support in addition to MCS preoperatively milrinone 0.1 vs 0.15 mcg/kg/min, dobutamine 7 vs 6 mcg/kg/min, respectively.

OCS assessment

Mean (SD) total ischemic time during preservation was statistically significantly longer in CS group in comparison with OCS group (210 (23) Vs 74.6 (13) min p=0.001). Median ex vivo normothermic heart perfusion time in organ care system was 348.4(132; 955) min. There was significant difference in total out of body time between OCS group 423(67) Vs CS group 210(23) min p=0.002) Table 2.

In the OCS group, allograft had stable perfusion and biochemical characteristics during ex vivo perfusion (Figure 11). Mean venous lactate trend during perfusion is normal level (Figure 2).

Intraoperative and Postoperative course and survival

The mean warm ischemic time for heart implantation was 53.4(12.3) vs 60.2 (11.5) minutes p value 0.8 in OCS and CS group. The allograft total ischemic time was 74.6(13) vs 210(23) minutes p value <0.001. The mean cardiopulmonary bypass time was 279(87) vs 256 (69) minutes p value -0.4. Six (24%) patients in OCS (one patient had RV dysfunction, one patient had sepsis, and other four had biventricular dysfunction) and six (60%) in CS group (two patients had sepsis, and other four had biventricular dysfunction) required ECLS support for weaning from cardiopulmonary bypass (p=0.02). In all the cases, the allograft function improved and ECLS could be weaned median after 4 days, except one patient in OCS group who had developed right

ventricular dysfunction. The median duration on inotropic support was 103(47; 465) vs 236(153; 423) hours p=0.1, mean level of inotropic support 24 hours was dobutamine 7.1(1.6) vs 8.5(1.9) mcg/kg/min p value -0.05, milrinone 0.2(0.3) vs 0.25 (0.4) mcg/kg/min p value – 0.7 in OCS and CS group, respectively. The median ITU stay was 16 days (3; 50) in the OCS group and 20 days (12; 52) in the CS group p=03. Inotropic support duration and level was significantly lower in OCS group Table 2.

All patients were alive on the 30th days post implant in CS groups and 96% in OCS group (p=0.5).

Table 9. The donor and recipient characteristics and risk factors

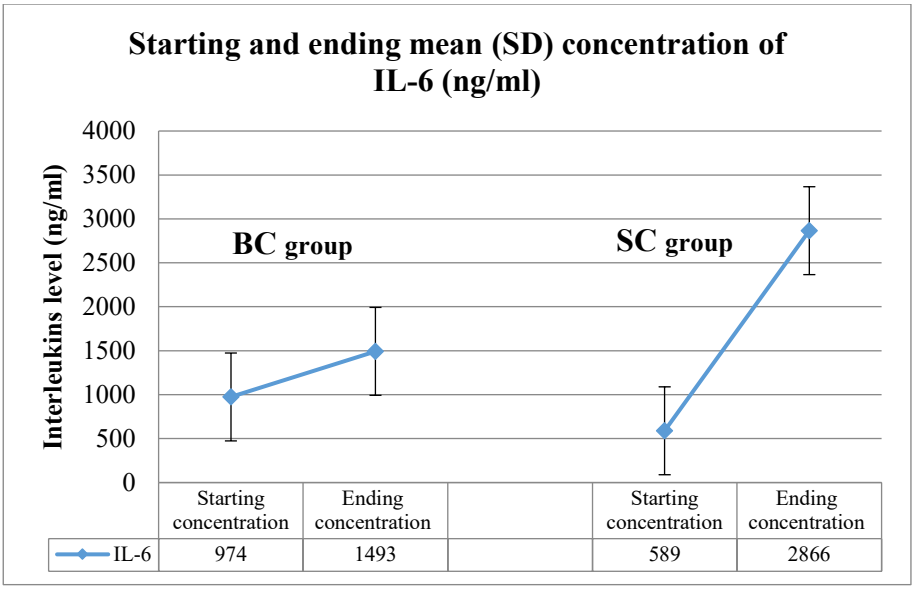
Donor characteristics			
	OCS (n=25)	CS (n=10)	P value
Age (years)	41.3±9.3	38.3±11.5	0.2
Male, n (%)	23(92)	7(70)	0.9
Cause of death, n (%)			
Intracranial hemorrhage	19 (76)	6 (60)	0.9
Cerebrovascular accident	6 (24)	3 (30)	0.9
Trauma		1 (10)	
Median LVEF (range)	58(52-63)	60(54-65)	
Recipient characteristics			
Age (years)	38.6±11.9	43.6±12.6	0.2
Male, n (%)	20(80)	10(100)	0.7
NICM n (%),	16(64)	7(70)	0.9
Median previous sternotomies rate	2(1;5)	1(1;3)	0.1
PVR > 4WU	4(16%)	4(40%)	0.6
Mechanical Circulatory Support			
LVAD, n (%)	20(80)	10(100)	
ECMO, n (%)	2(8)		
CARMAT, n (%)	3(12)		

Data are expressed as mean ± standard deviation, unless otherwise noted.
NICM - non-ischaemic cardiomyopathy; LVEF- left ventricular ejection fraction; LVAD-left ventricular assist device; ECMO- Extracorporeal Membrane Oxygenator; CARMAT- total artificial heart; PVR-pulmonary vascular resistance; WU- Wood unit.

Table 10. Outcomes data

	OCS (n=25)	CS (n=10)	P value
Total ischemic time (minutes)	74.6 ± 13	210 ± 23	<0.001
OCS perfusion time (minutes)	348.4 (132;955)	NA	NA
Mean total out of body time (minutes)	423 ± 67	210 ± 23	0.002
Warm ischemic time (minutes)	53.4±12.3	60.2±11.5	0.8
MCS after Htx (%)	24	60	0.02
CPB time (minutes)	279±87	256±69.2	0.4
Duration Inotropic support (hours)	103 (47; 465)	236 (153;423)	0.1
ITU stay-days	16 (3;50)	20 (12; 52)	0.3
30-day survival (%)	96	100	0.5

CPB- cardiopulmonary bypass



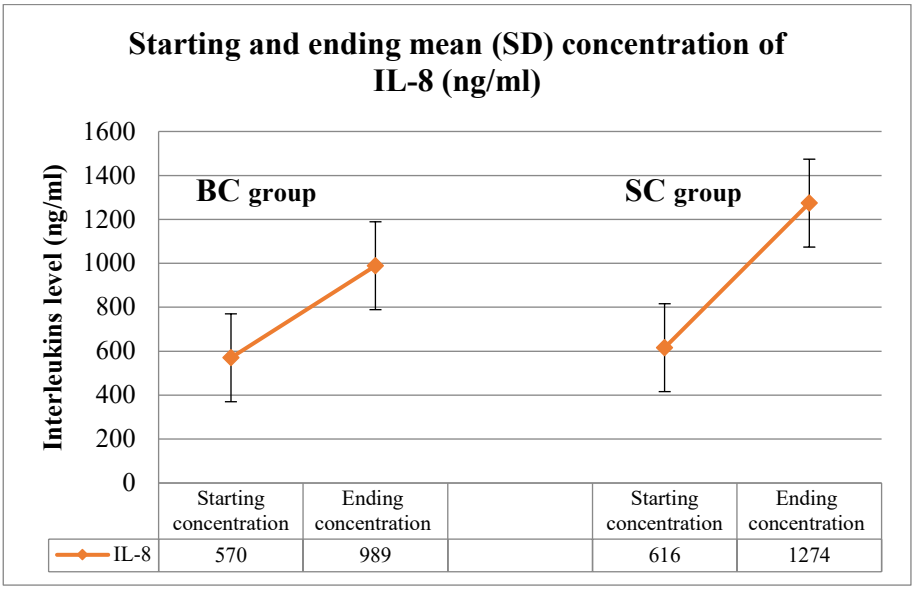


Figure 11. Organ Care System data (Mean SD)

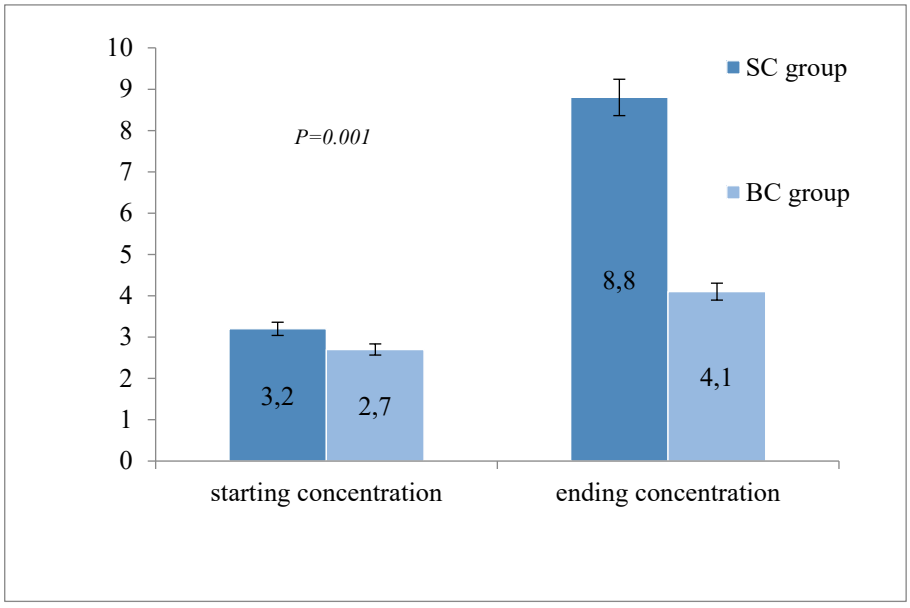


Figure 12. Venous lactate level (mmol/L) before and during OCS support (Mean SD)

"Error bars represent standard deviation

3.3. Materials and methods

To evaluate the impact of the treatment of beating heart in normothermic ESHP based on

ECMO technology (our development) on post-transplant graft function in a porcine model.

A prospective experimental trial was performed to evaluate post-transplant graft function after normothermic beating heart ex-situ heart perfusion based on ECMO. The study was approved by the National Research Cardiac Surgery Center's Animal Care and Use Committee (Protocol No 2022/01-121). A animal care committee approved the experimental protocol, and animals were treated following the Principles of Laboratory Animal Care prepared for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources. Male adult domestic pigs weighing (90-100 kg) were selected to perform 20 normothermic ESHP based on ECMO technology. Five orthotopic heart transplants were made to final graft assessment.

Donor procedure

Anesthesia and monitoring

The food was withheld from each pig for 24 hours and water for 12 hours prior to the surgery. A physical examination was performed to ascertain health status. Intramuscular administration of Atropine sulfate (0.05mg/kg), acepromazine maleate (0.05 mg/kg), and ketamine sulfate hydrochloride (15 mg/kg) was used for the induction of anesthesia. Thiopental sodium (10–15 mg/kg) was administered through an angiocatheter for general anesthesia. Each anesthetized pig was intubated with an endotracheal tube, mechanically ventilated, and given isoflurane (0.5%–4%) to maintain general anesthesia. Three liters of crystalloid solution (Sterofundin) was administered in each animal to compensate initial hypovolemia and to provide upcoming blood drainage. Monitoring during the procedure included invasive arterial blood pressure, central venous pressure, core temperature, urine output, and skin electrocardiography. For transplant cases Swan-Ganz pulmonary artery catheter was used to measure hemodynamics in donor and recipient animals. Epicardial echocardiography was performed in all cases.

Surgical procedure

A median sternotomy was performed, pericardium was opened. The animal was fully anticoagulated with intravenous heparin 300 international

units (IU)/kg to obtain an activated clotting time greater than 480 seconds. A double-outlet needle was inserted in the ascending aorta and secured with a 4-0 polypropylene purse-string suture. Through it 500mL of the normothermic oxygenated blood was collected and used for the blood component of short-acting blood cardioplegia solution. Prior to cardiac arrest, 1000 ml of blood was collected as priming solution of normothermic ESHP system, followed by aorta cross clamping and cardioplegic solution administrating. After complete arrest, heart was dissected and prepared for ESHP.

In heart transplantation cases, the arterial cannulation (22F) in the ascending aorta and bicaval cannulation (26 F and 28F) for venous drainage were performed for cardiopulmonary bypass.

Heart harvesting and Preparation

After the collection of baseline measurements, a single dose of normothermic blood cardioplegia (10-15mL/kg., 36 °C) mixed in ratio of four parts donor blood to one part crystalloid was delivered through a double-outlet needle placed in the ascending aorta, with a cross-clamp placed distally.

The heart was then excised, and stored approximately 15-20 minutes while preparing to be connected to the normothermic ESHP system (Figure 1, 2). In the ESHP system, oxygenated blood is perfused by a centrifugal pump into the aorta (retrograde fashion), perfusing the coronary arteries. The venous return from the coronary sinus was ejected through the pulmonary artery cannula only, and coronary blood flow (CBF) was monitored, as well as blood gases. The heart was oriented in the ESHP system so that aorta was facing upward with the posterior aspect of the heart resting on a pad, which is part of our custom integrated blood reservoir.

Outcomes of interest were mean change in lactate, ischemic time, perfusion time, hemodynamic measurements, TDI parameters and morphology.

Perfusion System Setup

The primary components of the perfusion circuit consisted of ECMO set were: oxygenator, centrifugal pump, 3/8 tubes (Medos, Medizintechnik AG, Germany) which connected to special closed integrated reservoir (our institution made), a gas mixer (our institution made), and flow meter (Medos, Medizintechnik AG, Germany), an ultrafiltration device (Medos, Medizintechnik AG, Germany). The centrifugal pump flow and solution flow rates of the ESHP system were adjusted to maintain the mean aortic pressure between 60 -70 mmHg and blood flow between 500 - 800 mL/min. Root

pressure was measured via a pressure transducer connected to a stopcock on the aortic root cannula.

Pumped coronary sinus blood collected into the integrated reservoir via gravity drainage. Perfusate temperature managed with the heater-cooler of ECMO machine. The hearts were perfused for 6-14 hours, and then arrested with normothermic blood cardioplegia. Five of them were transplanted to recipient animals.

Perfusate Preparation

The ESHP system was primed with blood based perfusate from the donor pig, and diluted to achieve a mean hematocrit of 22% (2% standard error, hereafter SE), making a total volume of ~ 1 L. The oxygenator kept the perfusate at normothermia (The 3T HCU was set to provide an initial perfusate temperature of 36°C) and facilitated gas exchange using 0.1-0.9 liters per minute (LPM) of atmosphere airflow. Approximately 50 mL of crystalloid isotonic solution Sterofundin (B. Braun Melsungen AG, Germany), Levosimendan 2.5 mg (1 mL) were added to the reservoir and recirculated. The system was then de-aired and circulation started at low RPM. One-way valve within 3/8 arterial line was incorporated to maintain aortic root pressure and prevent backflow and risk of air embolism from LV.

Pharmacological support provided via controlled on demand infusion of epinephrine, glucose, insulin, nitroprussid (Goregaon, Mumbai) and sodium bicarbonate.

Active zero balance ultrafiltration (Medos, Medizintechnik AG, Germany) with Sterofundin (B. Braun Melsungen AG, Germany) as a plasma substitution solution was used to maintain the hematocrit and electrolytes in the target normal range Figure 3. The calcium was normalized to 0.80 mmol/L, and the pH was adjusted with NaHCO3 as needed.

Recipient Procedure

We performed orthotopic cardiac transplants in 5 animals. In brief, sedation, anesthesia, and monitoring were performed as in the donor protocol. After median sternotomy, we exposed the heart and great vessels and the superior and inferior vena cava were encircled. Systemic anticoagulation was achieved with an intravenous injection of 30000 U of heparin. Ascending aortic and bicaval cannulations were used to initiate cardiopulmonary bypass (CPB). Normothermia was maintained and flow rates were adjusted to maintain a mean arterial pressure above 50 mmHg. After aortic cross-clamping, the recipient heart was excised and the anastomotic margins

inspected and trimmed. At 6-14 hours of ESHP, the donor heart was flushed with an initial dose of normothermic blood cardioplegia and removed from the perfusion system. For implant, we used a standard bicaval anastomotic technique in sequence: LA, pulmonary artery, and ascending aorta. Cardioplegic protection was consisted of 500mL of a 4:1 mixture of blood/crystalloid and delivered at 36°C every 20 minutes. Before removal of the aortic cross clamp, a 500mg of methylprednisolone was administered. Once the aortic cross-clamp was removed, hearts were reperfused for 60 minutes, and weaned from CPB. Weaning was deemed successful if the animal maintained a mean systolic arterial pressure of 60 mmHg for 4 hours after the discontinuation of CPB. A vasoactive infusion of Dobutamine (5 μg/kg/min) and Norepinephrine (0.1 μg/kg/min) was used to assist in weaning process from CPB. Hemodynamics and biventricular functional assessments were performed at 4 hours postreperfusion, following which, the experiment was terminated.

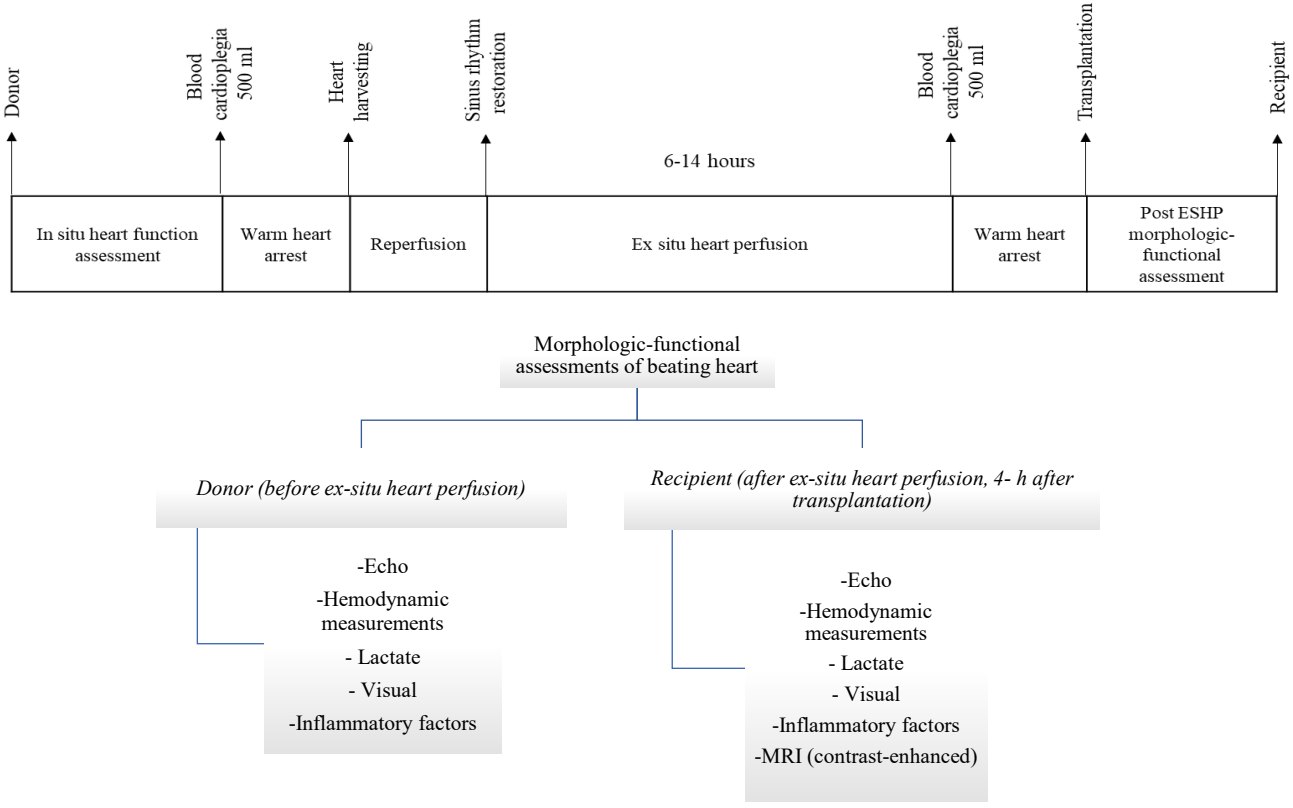


Figure 13. Simplified schema of the experimental design and protocol.

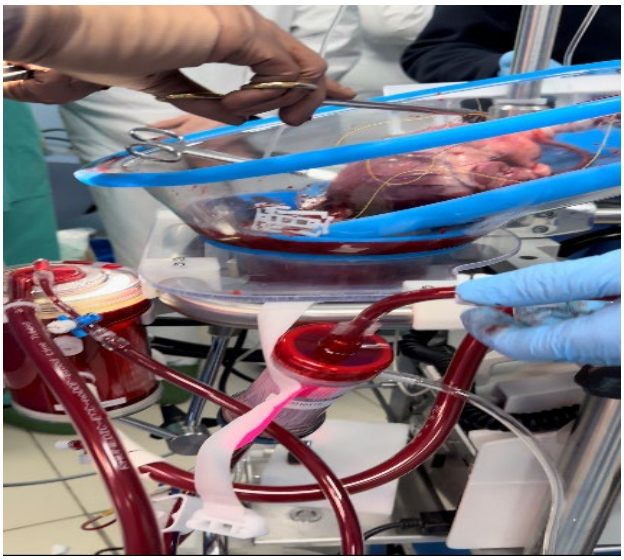


Figure 14. A model of normothermic ex-situ heart perfusion usin continuous perfusate hemofiltration

Experimental measurements

Myocardial function

Measurement of metabolic parameters during normothermic ESHP has been used to assess myocardial function before transplantation. Every 30 minutes samples of arterial and venous blood from the aorta and pulmonary artery were collected for blood gas analysis. Parameters such as pH, lactate level, pO2, pCO2, hemoglobin, hematocrit, oxygen saturation, and electrolytes (ABL 800, Flex, Radiometer, Denmark), IL-1, IL-6, IL-10 were measured and recorded. Myocardial lactate extraction, coronary vascular resistance (CVR), myocardial oxygen consumption (MVO2) were measured.

Coronary vascular resistance (CVR) was calculated as follows:

CVR= (Mean Aortic Pressure−Mean Right Atrial Pressure)/CBF·100g Heart Weight

Indexed myocardial Oxygen consumption (MVO2) was as follows:

MVO2=CBF* (CaO2−CVO2)/100 Heart Weight

Global cardiac function in vivo was evaluated by cardiac output measurement using the pulmonary artery catheter and thermodilution technique (Swan-Ganz catheter, Edwards Lifesciences, Irvine, CA).

Echocardiography

Epicardial echocardiography was performed using a standard transthoracic echocardiographic probe (Philips Epiq, Netherland) during in-vivo and ex-situ periods. The scan protocol includes 3 views: LV short axis,

apical 4, and 2 chamber. In-vivo systolic function was evaluated by ejection fraction (EF) computed using the biplane method of disks (MOD). During ESHP period tissue Doppler parameters were measured.

The primary outcome of this study was the number of hearts successfully transplanted after 6-14 hours of NESHP. Criteria of successful transplantation was defined as: weaning from cardiopulmonary bypass (CPB) without or minimum doses of vasopressor (0.1 g/kg/min Norepinephrine) and/or inotrope (5 g/kg/min Dobutamine) support for the following 4 consecutive hours. Secondary outcomes included CVR during NESHP, lactate concentration, weight gain, hemodynamic parameters prior to heart excision and after separation from CPB, and histologic injury scores.

Statistical analysis

Categorical variables were expressed as group percentages, and continuous variables were expressed as mean +/− standard deviation or median with IQR depending on overall distribution. Normally distributed continuous variables are compared using repeated-measures analysis of variance or paired t-test were appropriate (Minitab 15), and reported as mean ± standard error. A p-value <0.05 was considered statistically significant.

Results of animal model

In vivo measurements

Animal characteristics, myocardial function markers, physiologic parameters, and electrolyte status in vivo summarized in Table 1.

Table 11. Demographic data and measurement of myocardial function, physiologic parameters, and electrolyte status in vivo before perfusion

Demographics	*n=20*
	Mean (SD)
Body weight (kg)	105 (5.6)
BSA (m^2)	7.7 (0.3)
Heart weight (g)	308.4 (84)
Myocardial function indices	
Indexed myocardial Oxygen consumption (MVO2) (ml O2/min/100 g)	5.4 (1.3);
Cardiac output (l/min)	5.8 (0.8)
Cardiac index (l/min/m^2)	3.2 (0.4)
CBF (mL/min)	390 (0.06)
MAP (mmHg)	73(14.6)
Left ventricular ejection fraction	59% (3)

Physiologic parameters	
Arterial lactates (mmol/L)	1.34 (0.59)
Hemoglobin (mg/dL)	10.9 (1.22)
Glucose (mg/dL)	7.7 (2.01)
Temperature (°C)	36.5 (0.2)
pH	7.45 (0.08)
Electrolyte status	
$Ca2^{+}$ (mmoL/L)	1.35 (0.07)
K^{+} (mEq/L)	3.91 (0.7)
Na^{+} (mEq/L)	143.4 (3.96)
$HCO3^{-}$ (mmoL/L)	27.3 (1.79)

CBF (mL/min); MAP (mmHg).

Ex situ analysis

Myocardial function: The mean left ventricular ejection fraction after HTx was 58% (±0.7) and normal TDI parameters trend during ex situ perfusion and after HTx (Figure 4). For over 4 hours post-transplant, there was no evidence of rejection or cardiac dysfunction. Mean cardiac output measured with Swan-Ganz and cardiac index after HTx was 6.0 l/min (±0.7) and 3.1(±0.2) l/min/m^2 respectively. Coronary blood flow, Mean arterial pressure (MAP) and heart rate in normal range shown in Figure 5. All isolated hearts had stable perfusion, biochemical, histological characteristics (semi thin slice of ventricular myocardium x300 has demonstrated myocardium with dystrophic changes in cardiomyocytes of varying severity, serous endo-myocarditis) in the perfusion system and MRI picture (Figure 6). The mean venous lactate trend was within normal levels at the end of perfusion. Proinflammatory parameters, at the beginning and end of ex situ perfusion shown in Figure 7. Hearts in our group developed no clinically significant edema during ESHP, as evidenced by a modest increase in heart weight: 310 [235–500] grams at start compared with 409 [265–610] grams at the end.

Physiologic parameters: Myocardial oxygen consumption (initial 2 hours: 5.2 (1.1); at the end of ESHP 6-14 hours: 4.1 (1.4) (ml O2/min/100 g) and mean venous lactate trend are shown in Figure 7 with normal levels at the beginning 1.4 SD .2 mmoL/L and the end of perfusion: 2.4 SD .2 mmoL/L, respectively. Ischemic times and perfusion times of isolated pig hearts are shown in Figure 5. Mean (SD) ischemic time was 19.2 (±3.3) min. Mean ex vivo perfusion time was 480 (±150) min. Time of sinus rhythm restoration was 2.3 (5.7) minutes.

Perfusate components: Hemoglobin and glucose were maintained steadily throughout 30 minutes perfusion period. Temperature was maintained

at 36 (SD 0 .1) °C during the ex-situ perfusion. Similarly, the perfusate was maintained at a normal range of pH=7.4 (SD 0.1) during the ex situ perfusion period. The concentration of calcium, potassium, sodium or bicarbonate in the electrolyte in the perfusate across the ex-situ period was at a normal range.

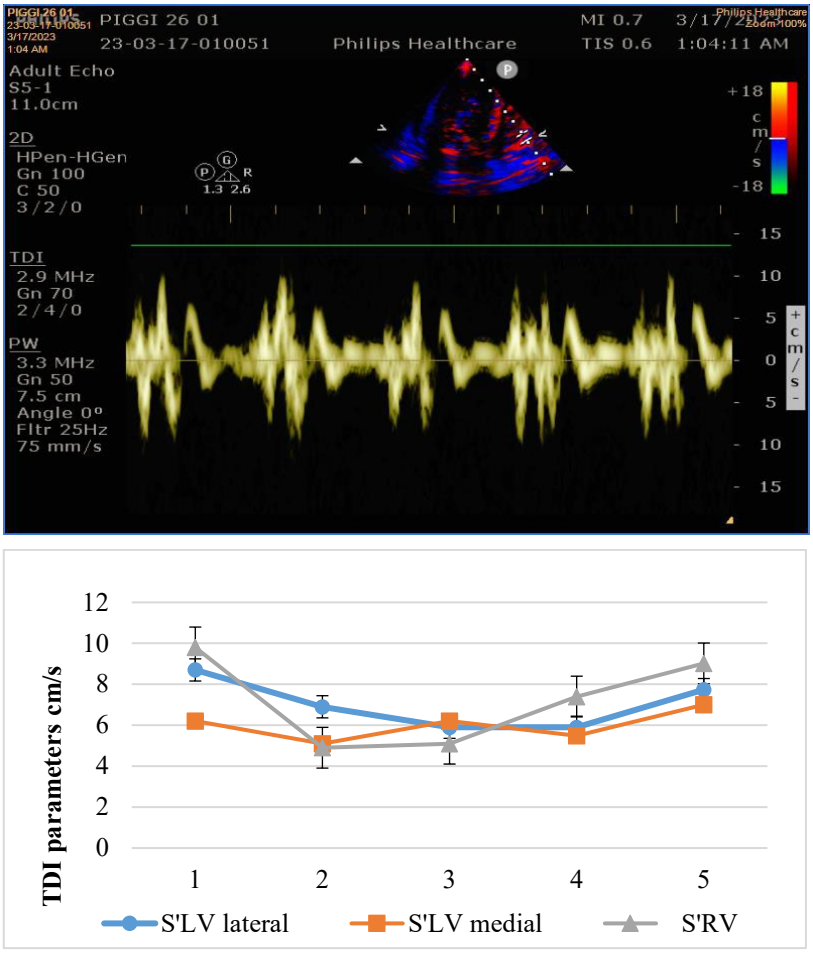
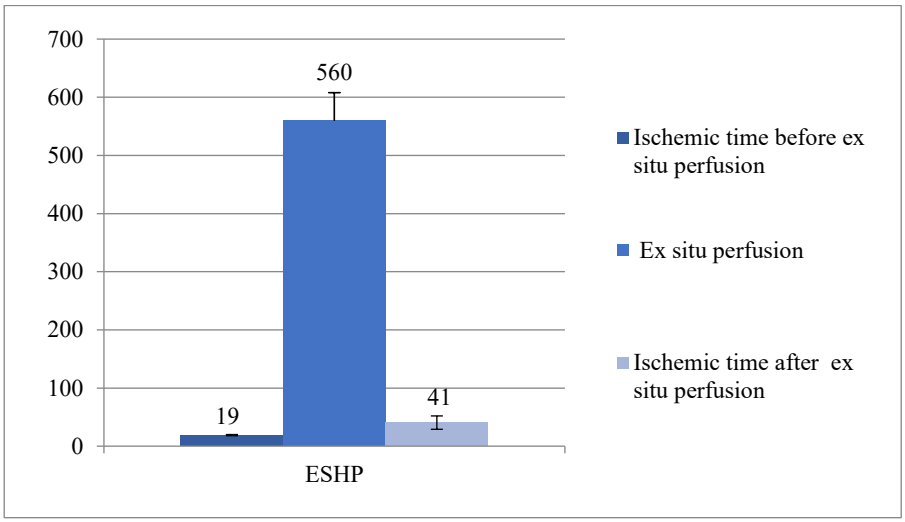


Figure 15. The mean TDI parameters trend during ex situ perfusion (1-donor, 2- 20 min after ex situ perfusion, 3-after four hour ex situ perfusion, 4- before ending of ex situ perfusion, 5- after heart transplantation) and histological characteristics.

The mean tissue doppler imaging parameters trend during ex situ perfusion and after transplantation (1-donor, 2- 20 min after ex situ perfusion, 3-after four hour ex situ perfusion, 4- before ending of ex situ perfusion, 5- after heart transplantation)

$S^{I}LV$ –myocardial velocity associated with isovolumic contraction of left ventricle;
$S^{I}RV$ - myocardial velocity associated with isovolumic contraction of right ventricle.



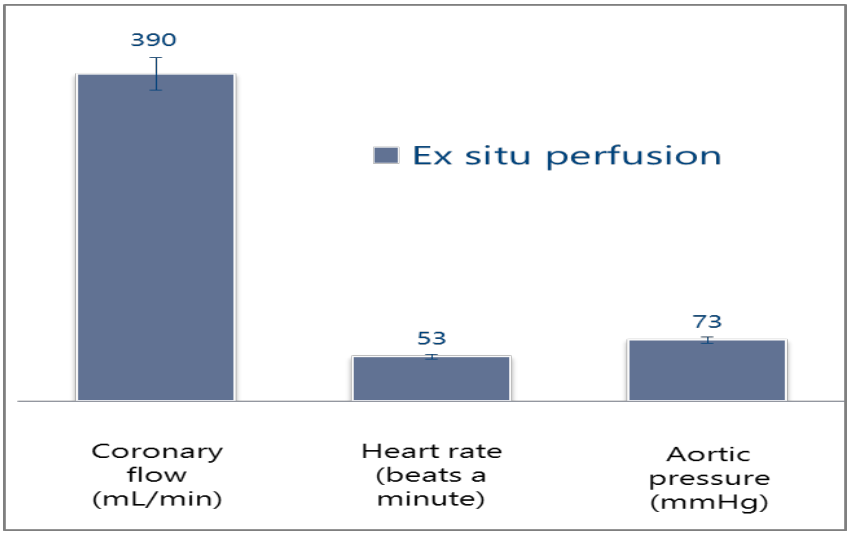


Figure 16. (A) Ex situ heart perfusion data and (B) CF (mL/min), Heart rate (beats a minute), AP (mmHg) of the pig hearts every hour of ex situ perfusion. All data are shown as mean ± SD. The number of animals is n=20.

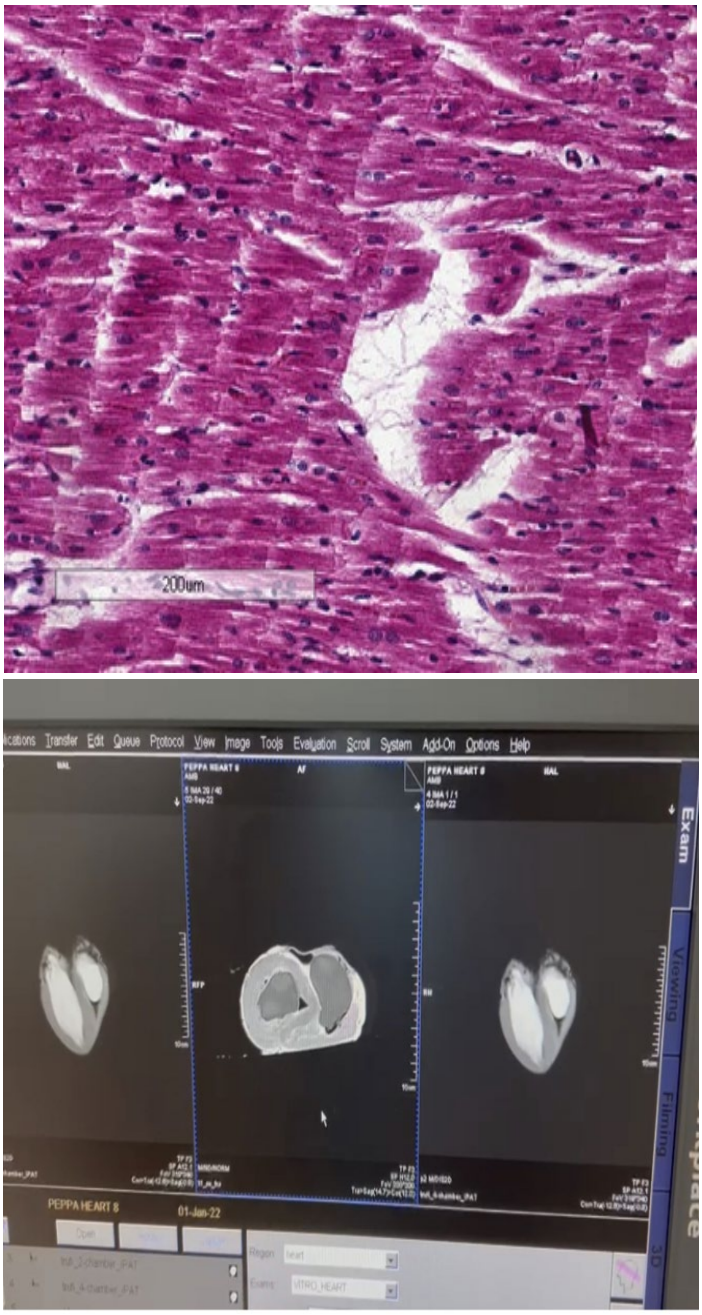


Figure 17. Histological and MRI picture (T1, T2 regimes Siemens Magnetom Avanto 1.5 Tesla).

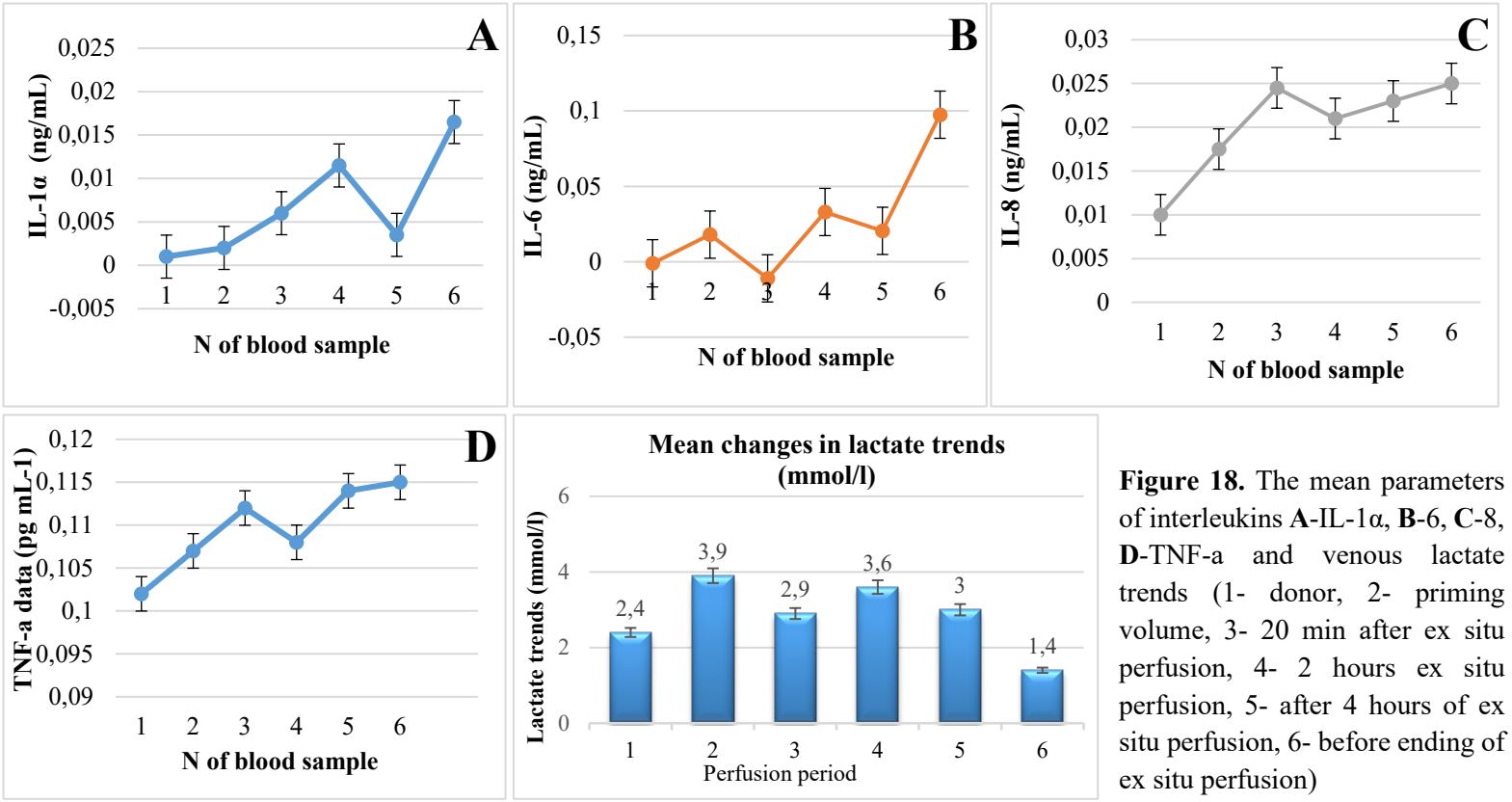


Figure 18. The mean parameters of interleukins **A**-IL-1α, **B**-6, **C**-8, **D**-TNF-a and venous lactate trends (1- donor, 2- priming volume, 3- 20 min after ex situ perfusion, 4- 2 hours ex situ perfusion, 5- after 4 hours of ex situ perfusion, 6- before ending of ex situ perfusion)

4. DISCUSSION

National research cardiac surgery center JSC is the only center in Kazakhstan where a heart transplantation is performed, and the only center in Asia that uses donor organ (heart, lungs) care device Figure 1. During the period from 2012 to 2020, 82 heart transplantation surgeries were performed. Due to the geographical features of the Republic (donor hearts are transported from remote regions >1000 km), remote delivery and preservation of the heart using the organ care system has been developed.

Currently, there are two methods of preserving a donor heart: placing the organ in the cold and using the organ care system (OCS). In case of the first method, biological functions are suspended, and the time of organ ischemia is up to 4 hours. In case of second method, the organ care system reproduces the conditions of living body for organs. The device passes the donated blood which is oxygenated and saturated with nutrients through the beating heart. The device allows increasing significantly the time of functioning of the organ before transplantation (up to 8-10 hours). In addition, it helps to examine and even restore the functions of an organ outside the body. The standard method for preserving an organ in an OCS device is use of cardioplegic Custodiol solution.

One of the disadvantages of Custodiol solution is the low level of potassium. This necessitates the introduction of large volumes of the solution (1000 ml.) and longer exposure to achieve equal ion concentration in the solution and the intracellular fluid of the heart.

In addition, after a single use of Custodiol solution, a long-term cardioplegic effect develops (100 – 120 minutes), which significantly exceeds the time required for placing the heart in the OCS and resuming heart activity. For the latter, on average, it takes 20 minutes.

The advantage of using a blood solution for cardioplegia is the high concentration of potassium ions in the solution, which provides faster cardiac arrest, with a duration of safe myocardial anoxia of 20 minutes. Cardioplegic effect is enhanced by lidocaine contained in the solution, which stabilizes the myocardium, causing a delay in reactivation of fast sodium channels of the cardiomyocyte membrane. Also, the advantages of blood cardioplegia are associated with high oxygen and buffer capacity of red blood cells; energy and plastic substrates for myocardial metabolism; adequate colloid osmotic pressure that prevents the development of vacuolar degeneration; natural antioxidants that reduce the risk of reperfusion injury; reduction in total

hemodilution during surgery, which is especially important in case of long-term myocardial ischemia and its reduced functional capabilities.

To date, the number of patients with heart failure is increasing and, accordingly, there is an increase in the number of patients with mechanical support of the left ventricle (more than 300 implants). Also, 3 surgeries were performed in our center for implantation of wholly artificial heart (CARMAT).

During heart transplantation in patients of this category, there is a need for additional time for safe recovery and preservation of the organ in the device and in these cases, use of organ care device is topical.

During long-term transportation of donor heart (more than 4 hours), the level of lactate increases and edema of organ develops in the device, therefore, the use of ultrafiltration to reduce edema and lactate levels is relevant in our case. In our country, there are problems with regional donor clinics, often the donor heart is in a state of shock with initial tonic support when recovery team arrives. In such cases, we conduct conditioning of the heart in the device using the drug of choice - Levosimendan. The advantage of using a blood solution for cardioplegia is the high concentration of potassium ions in the solution, which provides faster cardiac arrest, with a duration of safe myocardial anoxia of 20 minutes. Cardioplegic effect is enhanced by lidocaine contained in the solution, which stabilizes the myocardium, causing a delay in reactivation of fast sodium channels of the cardiomyocyte membrane. Also, the advantages of blood cardioplegia are associated with high oxygen and buffer capacity of red blood cells; energy and plastic substrates for myocardial metabolism; adequate colloid osmotic pressure that prevents the development of vacuolar degeneration; natural antioxidants that reduce the risk of reperfusion injury; reduction in total hemodilution during surgery, which is especially important in case of long-term myocardial ischemia and its reduced functional capabilities.

The proposed method has obvious advantages over the standard method (Custodiol) for myocardial protection. However, the study in the subgroups of this method showed a high concentration of lactate in blood cardioplegia and levosimendan group (subgroup 1) and blood cardioplegia group (subgroup 3) compared to the blood cardioplegia and ultrafiltration group (subgroup 2). Limited number of donor hearts and unacceptable levels of lactate set limits in our study, which led to insufficient recruitment of patients in subgroups.

According to the Organ Preservation Alliance, almost two-thirds of US donor hearts go un-transplanted, largely due to preservation limits on assessment and matching. The preservation limits also mean that those who

receive grafts often get a suboptimal matching (5). The quality of donor hearts is a key determinant of transplantation success, directly affecting the outcomes (1, 2).

Continuous machine ex-situ heart perfusion at normothermic temperatures (NESHP) is one of the promising approaches to extend organ lifetime. ESHP allows for a long preservation time and provides the opportunity to assess cardiac function, viability, and metabolism. The current clinically available Organ Care System (OCS) relies on metabolic findings, mainly lactate concentration during perfusion to determine if the heart is suitable for transplant, limiting the ability to predict post-transplant functional recovery (6). Reported the "end of perfusion" lactate concentration of less than 5 mmol/L was the most powerful predictor of post- transplantation graft failure (63% sensitivity, 93% specificity) in 30-day outcomes (4). But nowadays increasing donor after cardiac death (DCD) donation process makes it very challenging to rely only on the final lactate concentration, and even on its venous-arterial difference as a parameter of heart metabolism (7, 8). In this context the need for the functional, alongside the metabolic assessment of the hearts perfused with OCS, to determine the eligibility of the heart for safe transplantation is warranted (9).

In our study, we used a group of healthy pigs without neurological determination of death to avoid myocardial dysfunction and damage, and to focus on the impact of the extracorporeal circuit on the heart without a confounder, such as temperature/ischemia-reperfusion injury and hormonal imbalance. In addition, due to the short, acceptable in regular heart surgery ischemic time, in this study we avoided using cold crystalloid cardioplegic solution, to be able to focus on ESHP-related alterations only.

The OCS device runs two times cold crystalloid cardioplegic solution to arrest heart. Therefore, it is still a matter of debate since it is needed just for a short time (20 minutes, appr.) preparation period, resulting in an extra episode of temperature, reperfusion, and ischemia injury increasing the risk of tissue damage, inflammation and edema. Thus, NESHP on ECMO technology with blood-based cardioprotection may offer a safer, more reliable approach for heart preservation.

Johnson et al. reported extended viability of hearts in NESHP to a minimum of 24 hours with the use of hemofiltration, indicating that failure of the perfused heart is caused by factors accumulating in the perfusate which can be removed by hemofiltration (10) [.]. Significant edema leading to primary graft failure during the run may also play a role in the functional decline of the heart. Edema formation can impair diastolic relaxation, and lead

to capillary vasculature collapse causing impairment of myocardial perfusion during ESHP (11-12). Reported edema formation has been significantly lower with blood-based perfusate (13). This concept of ultrafiltration effectiveness was successfully utilized in clinical experience (14-15). Another underlying reason for edema formation during ESHP may be induction of inflammatory response triggered by blood exposure to the extracorporeal circuit. The latter condition may complimentary be cured with hemofiltration or cytosorbtion during ESHP. In our study, we observed trace of inflammatory factors in perfusate for the whole period of ESHP and posttransplant hemodynamic measurements. Subtle level of ILs, TNF-α throughout 6 hours and successful functional posttransplant recovery testifies safety and good functional preservation of our protocol of NESHP. Our study was limited by 6-hour NESHP that is not enough in in the future. It was shown that myocardial functional decline occurs during ESHP exceeding 6 hours via activation of innate immune, oxidative, and endoplasmic reticulum stress (16). The influence of the described method on heart during prolonged NESHP is under investigation by our group, but the cardioprotective effects of ultrafiltration may be sophisticated.

Our study of beating heart in normothermic ESHP based on ECMO technology is non-working model of heart perfusion. Hatami et al. observed significantly better preservation of function in those hearts perfused in semi-physiologic heart chamber pressures working mode (16). The mechanism for such cardioprotective effect has not yet been determined. The functional assessment of the donor heart during ESHP in both modes is restricted to lack of specific tools. Moreover, regardless of the perfusion mode, the ex-situ perfused hearts developed similar significant edema during preservation, which may also hinder organ assessment. Echocardiography is one of well-known non-invasive device for heart function assessment. Its role in ESHP conditions is unknown due to unrevealed of physiological determinants. Correlation of TDI in the setting of ESHP with excellent myocardial performance after transplantation demonstrates promising perspective. The local wall velocities are not the result of the local function, as segments are moved by the action of neighboring myocardial segments. Thus, the velocity differences and velocity gradient are the main measure of regional contraction, and might become the most important employment in the ESHP assessment process. The TDI mechanism for superior functional preservation of hearts perfused in working mode has not yet been determined and is under investigation by our group. Left ventricular TDI gradually decreased during ESHP, with full come back to normal/start level after transplantation. This

parameter remained stable for 4 hours posttransplant before elective termination. Thus, normothermic ESHP based on ECMO technology in non-working model of heart offers a safe and efficient preservation of isolated organ. The mechanisms related to excellent functional preservation of heart are under investigation by our group, but at the moment, cardioprotective effects that may be involved seems to be as follows:

- exclusion of cold crystalloid cardioplegia caused damage;
- short and safe period to restart blood circulation at ESHP device;
- blood based

Heart rate remained stable and did not vary throughout ESHP period.

For the full duration of NESHP all hearts were maintained within the adequate physiologic blood flow and ensuring appropriate oxygen delivery.

No increase in aortic root MAP and coronary resistance were observed.

Chronic cardiac failure (CCF) is an important public health problem. According to statistics, more than 26 million people worldwide suffer from chronic cardiac failure, which indicates the scale of the problem and its relevance. In general population, CCF occurs in 1-2% of people. In Kazakhstan, 4% of the population suffer from CCF (approximately 300 thousand people).

Heart transplantation is still the gold standard in the treatment of patients with end-stage CCF. In this section, the most important factor that determines the prognosis of patients is preservation of the donor organ.

The introduction of the organ care device into clinical practice resulted in taking a new look at the problem of heart transplantation. It is known that the "waiting list" of patients for heart transplantation is constantly increasing, and the number of heart donors remains low.

Thus, the project analyzed data from 48 patients after heart transplantation.

The immediate result of transporting and preserving a donor organ in organ care device was studied. Our final observations show that blood cardioplegia and conditioning is safe and effective method of protecting the myocardium in remote organ recovery and preserving donor hearts in OCS.

Based on the results of the research, patent applications were submitted to the US patent office and the Eurasian patent organization (EAPO), as well as scientific articles were published in international journals, and reports were presented at international conferences (Annex A).

The planned objectives of research work on the project were fulfilled in full and in accordance with the calendar plan.

The OCS has been used to prolong out-of-body time in some cases, expanding possibilities for organ procurement from distant sites (6). This is an important consideration for centers such as which are forced to reckon with long transport distances and increasing rates of mechanical assist devices and fully artificial mechanical support device use in donor recipients.

In this context, we hypothesized that blood cardioplegia could provide near-physiologic conditions (oxygenated environment, normothermic) and could result in favorable patient outcomes. Ischemic time between explant from donor and implant to the OCS is generally between 20–30 minutes, and a single dose of blood cardioplegia has a similar duration of action. In contrast, Custodiol has a longer duration of action and could still be active when the heart is reanimated in the OCS, with unknown effects. In this sense, Custodiol is an intracellular cardioplegic solution which is high in potassium content and can cause arrest related to membrane depolarization. Results of several studies have shown favorable results for the use of blood cardioplegia using measurements such as cardiac enzymes metabolic response (7). The use of induction and reperfusion blood cardioplegia is associated with lower prevalence of post-transplantation right heart insufficiency, arrhythmias, and evidence of ischemia when compared with standard crystalloid cardioplegia (8-11). Adoption of this method of myocardial protection might be indicated to control early morbidity, particularly when poor donor organs are used in high-risk transplant recipients.

In addition, Custodiol must be perfused under hypothermic conditions (4□C), lowering the heart temperature to 15ºC. However, during isolated hypothermia, different ion constellations may lead to cellular edema and impaired electrical activity and to heart fibrillation. Before the onset of cardiac arrest, the energy consumption is increased (12-14). This may cause adverse effects related to the temperature gradient because in the OCS, the donor heart is transported at 34ºC.

It has been demonstrated that the duration of cold ischemia negatively impacts the outcome of transplanted patients and thus can adversely affect organ viability. Peri-transplant injury of endothelium after brain death may initiate immunological processes that accelerate graft arteriopathy (15). The interleukins are a class of cytokines that are produced by leukocytes and have been shown to play important roles in immunological and inflammatory responses. Interleukins 6 and 8 are common cytokines involved in inflammation. IL-6 is an anti-inflammatory cytokine, which plays an important role in inducing acute phase reactions and controlling local and systemic acute inflammatory responses (16, 17). In addition, recent research

suggests that lactate level prior to removal of graft from the OCS is a powerful predictor of graft failure (18). In our Center, we often use donor hearts with high venous lactate (>5 mmol/l) because of the severe shortage of donor organs in our country (19).

There is some evidence suggesting that ultrafiltration (UF) can lead to significant reduction in circulating inflammatory mediators and reduces blood loss and transfusion requirements (20). UF provides its potential advantages, with improvements in hemodynamic, pulmonary, coagulation, and other organ functions. Decrease of blood transfusion requirements as well as reduced total body water and blood loss after the surgery are additional benefits of UF (21, 22). Modified ultrafiltration (MUF) leads to a significant reduction of lipopolysaccharide-binding protein (LBP) and terminal complement complex and was associated with reduced blood loss and postoperative lactate concentrations shortly after surgery (23). MUF can be effective in removing cytokines and adhesion molecules (24). Smaller molecules, such as IL-6, IL-10, tumor necrosis factor (TNF), and endothelin 1 have been shown to be filtered with UF (25).

In our small cohort, the patient outcomes—survival and incidence of serious cardiac-related adverse events at 30 days post implant—were acceptable and demonstrate the feasibility of blood cardioplegia use with the OCS. Lactate trends in the end of ex vivo heart perfusion, inotrope dose at 72 hours and time of sinus rhythm restoration in OCS were statistically significantly higher in the standard care group. Other outcomes, such as OCS perfusion measures and length of ICU stay, were all within the expected range for our center. There was a lower mean ECMO duration in the blood cardioplegia group relative to the standard care group. We commonly use ECMO after heart transplant, during the postoperative recovery period, to reduce the reperfusion time.

Our analysis has several limitations. This is a single center report. Lack of randomization and a small sample size are another limitations, and additional studies, ideally with randomized controlled design, are needed to evaluate the impact of procurement technique and conditioning of the donor heart during transportation might have on outcomes, especially with long ex-vivo times during long distance transportation. Our observations, while preliminary, show mean ex vivo heart perfusion ending concentration of IL-6 and IL-8 were significantly lower in the blood cardioplegia group compared to the standard care group. The use of blood cardioplegia and conditioning could be a safe method for myocardial protection in distant procurement and preservation of donor hearts in the OCS. The independent effects of blood

cardioplegia and Levosimendan are not possible to separate in this study. We can only make comments about the observations we have seen with the combination of blood cardioplegia, Levosimendan and ultrafiltration. For future research, it will be important to separate these interventions and determine their impact individually.

Our study was limited by 6-14hour NESHP, which does not fully investigate the limitations of the system in term of how long it can sustain the heart to remain viable and transplantable that is not enough in future time perspective. We aimed to proof the concept and secure the experiment from the influence of other variables. Another limitation was the determined elective termination of posttransplant period at 4 hours.

5. CONCLUSION

There are several conclusions after evaluation of the two different techniques used for myocardial preservation during the procurement and transportation of the heart using the OCS:

1. The use of blood cardioplegia and conditioning is safe method for myocardial protection in distant procurement and preservation of donor hearts in the ex-situ perfusion conditions.
   a. At the end of ex situ heart perfusion Lactate trends, inotrope dose, and time of sinus rhythm restoration in beating heart condition were statistically significantly higher comparing with the standard static cold storage.
   b. Ex situ heart perfusion mean ending concentration of Interleukin-6 and Interleukin-8 was significantly lower in the blood cardioplegia group compared to the standard care group.
   c. There was a lower mean ECMO duration in the blood cardioplegia group relative to the standard care group.
2. Use of normothermic ex-vivo preservation by organ care system is superior compared with Cold storage for prolonged heart preservation for high-risk recipients bridged to transplantation with Mechanical Circulatory Support.
   a. Mean total ischemic time during preservation was statistically significantly longer in Cold Storage group in comparison with OCS group 210 (23) Vs 74.6 (13) min., respectively.
   b. Significant difference in total out of body time between OCS group 423(67) Vs Cold Storage group 210(23) min ($p = 0.002$), respectively.
   c. In the OCS group, allograft had stable perfusion and biochemical characteristics during ex vivo perfusion.
   d. Normothermic ex- situ preservation of the allograft during transportation with the organ care system is beneficial for long-time out of body organ preservation in comparison of cold storage in recipients on mechanical circulatory support.
   e. The patient outcomes — survival and incidence of serious cardiac-related adverse events at 30 days post implant, were acceptable and demonstrate the feasibility of blood cardioplegia use with the OCS
3. The treatment of beating heart in normothermic ESHP based on ECMO technology in a porcine model demonstrates high safety and efficacy by means of post-transplant graft function:

a. The mean venous lactate and Interleukins trend were within decrease levels at the end of perfusion period.
b. Hearts developed no clinically significant edema during ESHP
c. Myocardial oxygen consumption and echocardiography were normal during perfusion
d. The histological and MRI picture were normal characteristics in the end of perfusion.
e. Coronary blood flow, mean arterial pressure, and heart rate stayed in normal range throughout prolonged organ preservation

6. PRACTICAL RECOMENDATIONS

1. For recipients in unique situations such as previous Total Artificial Heart, or durable Mechanical Circulatory Support, ex situ beating heart perfusion allows long term procurement and safe transplantation.
2. To optimize the allocation logistics and meticulous preparation of the recipients after previous open-heart surgery the ex-situ heart perfusion should be utilized.
3. Continuous perfusate ultrafiltration with a blood flow of 200 to 300 ml/h during ex situ heart perfusion period should be used in order to improve donor heart function by significant reduction of circulating inflammatory mediators.
4. The solution to be used for normothermic heart arrest consists of blood and crystalloid solution at the ratio of 5:1 The crystalloid solution contained KCl 4% (30mL), $MgSO_4$ 25% (10 mL), $NaHCO_3$ 4% (13 ml), Mannitol 15% (6.5 ml), and Lidocaine 2% (2 ml) with whole blood up to a total volume of 600 ml.
5. Machine normothermic perfusion allows the donor heart to be harvested at more distant areas, expanding the list of potential recipients and increasing the chances of gaining a matching donor heart.
6. ECMO technology-based beating heart normothermic ESHP may be used as safe and efficient mean for treatment and graft function assessment throughout prolonged organ preservation.

7. FUNDING

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8. REFERENCE

1. Sunagawa G1, Koprivanac M2, Karimov JH1, et al. Current status of mechanical circulatory support for treatment of advanced end-stage heart failure: successes, shortcomings and needs. Expert Rev Cardiovasc Ther. 2017 15(5): 377-387.
2. Maria Monteagudo Vela, Diana García Sáez, Andre R. Simon. Current approaches in retrieval and heart preservation. Annals of Cardiothoracic Surgery 2018;10.21037/acs.
3. Messer, S. Normothermic donor heart perfusion: current clinical experience and the future / S. Messer, A. Ardehali, S. Tsui // Transp. Int. – 2015. – Vol. 28, N. 6. – P. 634–642.
4. Heart Transplantation Programs Face Significant Challenges [Electronic resource]. Cold storage method is limiting – URL: http://www.transmedics. com/wt/page/heart_tx_challenges_med
5. NHSBT. Organ Donation and Transplantation- Activity figures for the UK as at 7 April 2015 [Electronic resource]. – URL: http://www. organdonation.nhs.uk/statistics
6. Impact of donor cardiac arrest on heart transplantation / K. Southerland, A. Castleberry, J. Williams [et al.] // Surgery. – 2013. – Vol. 154, N. 2. – P. 312–319.
7. HeartWare HVAD for the Treatment of Patients with Advanced Heart Failure Ineligible for Cardiac Transplantation: Results of the ENDURANCE Destination Therapy Trial / F.D. Pagani, C.A. Milano, A.J. Tatooles [et al.] // J. Heart Lung. Transplant. 2015. Vol. 34, Is. 4 (Suppl). S 9.
8. A cardioprotective preservation strategy employing ex vivo heart perfusion facilitates successful transplant of donor hearts after cardio-circulatory death / C.W. White, A. Ali, D. Hasanally [et al.] // J. Heart Lung. Transplant. – 2013. – Vol. 32, N. 7. – P. 734–743.
9. What is the potential increase in the heart graft pool by cardiac donation after circulatory death? / T. Noterdaeme, O. Detry, M. Hans [et al.] // Transpl. Int. – 2013. – Vol. 26, N. 1. – P. 61–66.
10. Organ preservation with the organ care system / R. Yeter, M. Hübler, M. Pasic [et al.] // Applied Cardiopulmonary Pathophysiology. – 2011. – Vol. 15. – P. 207–212.
11. Sabatino M, Vitale G, Manfredini V, et al. Clinical relevance of the International Society for Heart and Lung Transplantation consensus classification of primary graft dysfunction after heart transplantation:

epidemiology, risk factors, and outcomes. J Heart Lung Transplant. 2017; 36:1217-1225.
12. Lund LH, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: thirty-second official adult heart transplantation report—2015; focus theme: early graft failure. J Heart Lung Transplant. 2015;34:1244-1254.
13. Singh SSA, Banner NR, Rushton S, Simon AR, Berry C, Al-Attar N. ISHLT primary graft dysfunction incidence, risk factors, and outcome: a UK national study. Transplantation. 2019;103:336-343
14. Pya Y, Bekbossynova M, Jetybayeva S, et al. Initial 3-year outcomes with left ventricular assist devices in a country with a nascent heart transplantation program. ESC Heart Fail. 2016; 3(1): 26-34
15. Ardehali A, Esmailian F, Deng M, et al. Ex-vivo perfusion of donor hearts for human heart transplantation (PROCEED II): a prospective, open-label, multicentre, randomised non-inferiority trial. Lancet 2015 385(9987): 2577-84.
16. Ghodsizad A, Bordel V, Ungerer M, et al. Ex vivo coronary angiography of a donor heart in the organ care system. Heart Surg Forum. 2012 Jun;15(3):E161-3.
17. Kaliyev R, Bekbossynov S, Nurmykhametova Z. Sixteen-hour ex vivo donor heart perfusion during long-distance transportation for heart transplantation. Artif Organs. 2019; 43:319-320.
18. Chan JL, Kobashigawa JA, Reich HJ, Ramzy D, Thottam MM, Yu Z, et al. Intermediate Outcomes with Ex-Vivo Allograft Perfusion for Heart Transplantation. J Heart Lung Transplant (2017) 36(3):258–63. doi: 10.1016/j.healun.2016.08.015
19. Ravichandran AK, Baran DA, Stelling K, Cowger JA, Salerno CT. Outcomes with the Tandem Protek Duo Duallumen percutaneous right ventricular assist device. ASAIO J. 2018; 64:570-572.
20. Bennett MT, Virani SA, Bowering J, Ignaszewski A, Kaan AM, Cheung A. The use of the Impella RD as a bridge to recovery for right ventricular dysfunction after cardiac transplantation. Innovations (Phila). 2019; 5:369-371.
21. Kobashigawa J, Zuckermann A, Macdonald P, Leprince P, Esmailian F, Luu M, et al. Report from a consensus conference on primary graft dysfunction after cardiac transplantation. J Heart Lung Transplant. (2014) 33:327–40. doi: 10.1016/j. healun.2014.02.027.

22. Singh SSA, Dalzell JR, Berry C, Al-Attar N. Primary graft dysfunction after heart transplantation: a thorn amongst the roses. Heart Fail Rev. (2019) 24:805–20. doi: 10.1007/s10741-019-09794-1.
23. Squiers JJ, Saracino G, Chamogeorgakis T, MacHannaford JC, Rafael AE, Gonzalez-Stawinski GV, et al. Application of the international society for heart and lung transplantation (ISHLT) criteria for primary graft dysfunction after cardiac transplantation: outcomes from a high-volume centre. Eur J Cardiothorac Surg. (2017) 51:263–70. doi: 10.1093/ejcts/ezw271.
24. Kirklin JK, Naftel, Kormos DCRL, et al. Fifth INTERMACS annual report: risk factor analysis from more than 6,000 mechanical circulatory support patients. J Heart Lung Transplant. 2013; 32:141–56.
25. Rosendale, J. D., Chabalewski, F. L., McBride, M. A., et al. (2002) Increased transplanted organs from the use of a standardized donor management protocol.[see comment]. *Am. J. Transplant.* 2(8), 761–768.
26. Taylor, D. O., Edwards, L. B., Mohacsi, P. J., et al. (2003) The registry of the International Society for Heart and Lung Transplantation: twentieth official adult heart transplant report—2003. *J. Heart Lung Transplant.* 22(6), 616–624.
27. Trulock, E. P., Edwards, L. B., Taylor, D. O., et al. (2003) The Registry of the International Society for Heart and Lung Transplantation: Twentieth Official adult lung and heart-lung transplant report—2003. *J. Heart Lung Transplant.*22(6), 625–635.
28. Terasaki, P. I., Cecka, J. M., Gjertson, D. W., and Takemoto, S. (1995) High survival rates of kidney transplants from spousal and living unrelated donors. [see comment]. *N. Engl. J. Med.* 333(6), 333–336.
29. Ryan, J. B., Hicks, M., Cropper, J. R., et al. (2003) Functional evidence of reversible ischemic injury immediately after the sympathetic storm associated with experimental brain death. *J. Heart Lung Transplant.* 22(8), 922–928.
30. Pratschke, J., Wilhelm, M. J., Kusaka, M., et al. (1999) Brain death and its influence on donor organ quality and outcome after transplantation. *Transplantation* 67(3), 343–348.
31. Finkelstein, I., Toledo-Pereyra, L. H., and Castellanos, J. (1987) Physiologic and hormonal changes in experimentally induced brain-dead dogs. *Transplant. Proc.* 19(5), 4156–4158.
32. Macoviak, J. A., McDougall, I. R., Bayer, M. F., Brown, M., Tazelaar, H., and Stinson, E. B. (1987) Significance of thyroid dysfunction in human cardiac allograft procurement. *Transplantation* 43(6), 824–826.

33. Mertes, P. M., el Abassi, K., Jaboin, Y., et al. (1994) Changes in hemodynamic and metabolic parameters following induced brain death in the pig.[see comment]. *Transplantation* 58(4), 414–418.
34. Novitzky, D., Wicomb, W. N., Cooper, D. K., and Tjaalgard, M. A.(1987) Improved cardiac function following hormonal therapy in brain dead pigs: relevance to organ donation. *Cryobiology* 24(1), 1–10.
35. Bittner, H. B., Kendall, S. W., Campbell, K. A., Montine, T. J., and Van Trigt, P. (1995) A valid experimental brain death organ donor model. *J. Heart Lung Transplant.* 14(2), 308–317.
36. Depret, J., Teboul, J. L., Benoit, G., Mercat, A., and Richard, C. (1995) Global energetic failure in brain-dead patients. *Transplantation* 60(9), 966–971.
37. Wicomb, W. N., Cooper, D. K., and Novitzky, D. (1986) Impairment of renal slice function following brain death, with reversibility of injury by hormonal therapy. *Transplantation* 41(1), 29–33.
38. Novitzky, D., Cooper, D. K., and Reichart, B. (1987) Hemodynamic and metabolic responses to hormonal therapy in brain-dead potential organ donors. *Transplantation* 43(6), 852–854.
39. Howlett, T. A., Keogh, A. M., Perry, L., Touzel, R., and Rees, L. H. (1989) Anterior and posterior pituitary function in brain-stem-dead donors. A possible role for hormonal replacement therapy. *Transplantation* 47(5), 828–834.
40. Gramm, H. J., Meinhold, H., Bickel, U., et al. (1992) Acute endocrine failure after brain death? *Transplantation* 54(5), 851–857. 360 Hicks et al.
41. Masson, F., Thicoipe, M., Gin, H., et al. (1993) The endocrine pancreas in braindead donors. A prospective study in 25 patients. *Transplantation* 56(2), 363–367.
42. Harms, J., Isemer, F. E., and Kolenda, H. (1991) Hormonal alteration and pituitary function during course of brain-stem death in potential organ donors. *Transplant.Proc.* 23(5), 2614–2616.
43. Arita, K., Uozumi, T., Oki, S., Kurisu, K., Ohtani, M., and Mikami, T. (1993) The function of the hypothalamo-pituitary axis in brain dead patients. *Acta Neurochir.* 123(1–2), 64–75.
44. Takada, M., Nadeau, K. C., Hancock, W. W., et al. (1998) Effects of explosive brain death on cytokine activation of peripheral organs in the rat. *Transplantation* 65(12), 1533–1542.
45. Pratschke, J., Wilhelm, M. J., Kusaka, M., et al. (2000) Accelerated rejection of renal allografts from brain-dead donors. *Ann. Surg.* 232(2), 263–271.

46. Pienaar, H., Schwartz, I., Roncone, A., Lotz, Z., and Hickman, R. (1990) Function of kidney grafts from brain-dead donor pigs. The influence of dopamine and triiodothyronine. *Transplantation* 50(4), 580–582.
47. Barner HB. Does Myocardial protection Work? Adv Cardiol (1980) 27:223–36. doi:10.1159/000383989.
48. De Beule J, Vandendriessche K, Pengel LHM, Bellini MI, Dark JH, Hessheimer AJ, et al. A Systematic Review and Meta-analyses of Regional Perfusion in Donation after Circulatory Death Solid Organ Transplantation. Transpl Int (2021) 34(11):2046–60. doi:10.1111/tri.14121
49. NHSBT. NHSBT Annual Report on Liver Transplantation (2020-21) (2021). Available from: https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/19867/nhsbt-liver-transplant-report-1920.pdf (Accessed May 16, 2022).
50. Jahania, M. S., Sanchez, J. A., Narayan, P., Lasley, R. D. and Mentzer, R. M., Jr. (1999) Heart preservation for transplantation: principles and strategies. Ann.Thorac. Surg. 68(5), 1983–1987.
51. McCrystal, G., Pepe, S., Esmore, D. and Rosenfeldt, F. (2004) The challenge of improving donor heart prteservation. *Heart Lung Circ.*13, 74–83.Organ Preservation 363
52. Hendry, P. J., Anstadt, M. P., Plunkett, M. D., et al. (1990) Optimal temperature for preservation of donor myocardium. *Circulation* 82(5 Suppl.), IV306–312.
53. Shimada, Y., Yamamoto, F., Yamamoto, H., Oka, T., and Kawashima, Y. (1996) Temperature-dependent cardioprotection of exogenous substrates in long-term heart preservation with continuous perfusion: twenty-four-hour preservation of isolated rat heart with St. Thomas' Hospital solution containing glucose, insulin, and aspartate. J. Heart Lung Transplant. 15(5), 485–495.
54. Lazdunski, M., Frelin, C., and Vigne, P. (1985) The sodium/hydrogen exchange system in cardiac cells: its biochemical and pharmacological properties and its role in regulating internal concentrations of sodium and internal pH. J. Mol. Cell. Cardiol. 17(11), 1029–1042.
55. Karmazyn, M. (1999) The role of the myocardial sodium-hydrogen exchanger in mediating ischemic and reperfusion injury. From amiloride to cariporide. Ann. NY Acad. Sci. 874, 326–334.
56. Verma, S., Fedak, P. W., Weisel, R. D., et al. (2002) Fundamentals of reperfusion injury for the clinical cardiologist. Circulation 105(20), 2332–2336.

57. Jordan, J. E., Zhao, Z. Q., and Vinten-Johansen, J. (1999) The role of neutrophils in myocardial injury. Cardiovasc. Res. 43(4), 860–878.
58. Rezkalla, S. H. and Kloner, R. A. (2002) No-reflow phenomenon.[see comment]. Circulation 105(5), 656–662.
59. Pearl, J. M., Drinkwater, D. C., Jr., Laks, H., Stein, D. G., Capouya, E. R., and Bhuta, S. (1992) Leukocyte-depleted reperfusion of transplanted human hearts prevents ultrastructural evidence of reperfusion injury. J. Sur. Res. 52(4), 298–308.
60. Pearl, J. M., Drinkwater, D. C., Laks, H., Capouya, E. R. and Gates, R. N. (1992) Leukocyte-depleted reperfusion of transplanted human hearts: a randomized, double-blind clinical trial. J. Heart Lung Transplant. 11(6), 1082–1092.
61. Wallwork, J., Jones, K., Cavarocchi, N., Hakim, M., and Higenbottam, T. (1987) Distant procurement of organs for clinical heart-lung transplantation using a single flush technique. Transplantation 44(5), 654–658.
62. Hirt, S. W., Wahlers, T., Jurmann, M., Fieguth, H. G., Dammenhayn, L., and Haverich, A. (1992) Improvement of currently used methods for lung preservation with prostacyclin and University of Wisconsin solution. J. Heart Lung Transplant. 11(4 Pt. 1), 656–664.
63. Nawata, S., Sugi, K., Ueda, K., Nawata, K., Kaneda, Y., and Esato, K. (1996) Prostacyclin analog OP2507 prevents pulmonary arterial and airway constriction during lung preservation and reperfusion. J. Heart Lung Transplant. 15(5), 470–474.
64. Kishida, A., Kurumi, Y., and Kodama, M. (1997) Efficacy of prostaglandin I2 analog on liver grafts subjected to 30 minutes of warm ischemia. Surg, Today 27(11), 1056–1060.
65. Sanchez-Urdazpal, L., Gores, G. J., Ferguson, D. M., and Krom, R. A. (1991) Improved liver preservation with addition of iloprost to Eurocollins and University of Wisconsin storage solutions. Transplantation 52(6), 1105–1107.
66. Changani, K. K., Fuller, B. J., Bell, J. D., Taylor-Robinson, S. D., Moore, D.P., and Davidson, B. R. (1999) Improved preservation solutions for organ storage: a dynamic study of hepatic metabolism. Transplantation 68(3), 345–355.
67. Bhabra, M. S., Hopkinson, D. N., Shaw, T. E., and Hooper, T. L. (1996) Relative importance of prostaglandin/cyclic adenosine monophosphate and nitric oxide/cyclic guanosine monophosphate pathways in lung preservation. Ann. Thorac.Surg. 62(5), 1494–1499.

68. Du, Z. Y., Hicks, M., Jansz, P., Rainer, S., Spratt, P., and Macdonald, P. (1998) The nitric oxide donor, diethylamine NONOate, enhances preservation of the donor rat heart. J. Heart Lung Transplant. 17(11), 1113–1120.
69. Baxter, K., Howden, B. O., and Jablonski, P. (2001) Heart preservation with celsior solution improved by the addition of nitroglycerine. Transplantation 71 (10), 1380–1384.
70. Drinkwater, D. C., Rudis, E., Laks, H., et al. (1995) University of Wisconsin solution versus Stanford cardioplegic solution and the development of cardiac allograft vasculopathy. J. Heart Lung Transplant. 14(5), 891–896.
71. Garlicki, M. (2003) May preservation solution affect the incidence of graft vasculopathy in transplanted heart? Ann. Transplant. 8(1), 19–24.
72. Russo MJ, Chen JM, Sorabella RA, Martens TP, Garrido M, Davies RR, et al. The effect of ischemic time on survival after heart transplantation varies by donor age: an analysis of the united network for organ sharing database. J Thorac Cardiovasc Surg. (2007) 133:554–9. doi: 10.1016/j.jtcvs.2006.09.019
73. Copeland H, Hayanga JWA, Neyrinck A, MacDonald P, Dellgren G, Bertolotti A, et al. Donor heart and lung procurement: a consensus statement. J Heart Lung Transplant. (2020) 39:501–17. doi: 10.1016/j.healun.2020.03.020
74. Hendry PJ, Walley VM, Koshal A, Masters RG, Keon WJ. Are temperatures attained by donor hearts during transport too cold? J Thorac Cardiovasc Surg. (1989) 98:517–22. doi: 10.1016/S0022-5223(19)34352-1
75. Kobashigawa J, Zuckermann A, Macdonald P, Leprince P, Esmailian F, Luu M, et al. Report from a consensus conference on primary graft dysfunction after cardiac transplantation. J Heart Lung Transplant. (2014) 33:327–40. doi: 10.1016/j.healun.2014.02.027
76. D'Alessandro D, Shudo Y, Meyer D, Silvestry S, Leacche M, Sciortino C, et al. Results from over 800 transplant recipients enrolled in the guardian heart registry. J Heart Lung Transplant. (2023) 42:S63–4. doi: 10.1016/j.healun.2023.02.137
77. Voigt JD, Leacche M, Copeland H, Wolfe SB, Pham SM, Shudo Y, et al. Multicenter registry using propensity score analysis to compare a novel transport/preservation system to raditional means on postoperative hospital outcomes and costs for heart transplant patients. ASAIO J. (2023) 69:345–9. doi: 10.1097/MAT. 0000000000001844

78. Lechiancole A, Sponga S, Vendramin I, Ferrara V, Benedetti G, Guzzi G, et al. Graft preservation in heart transplantation: clinical and histological results using three different techniques. J Heart Lung Transplant. (2023) 42:S145. doi: 10.1016/j.healun.2023.02.1608
79. Chew HC, Macdonald PS, Dhital KK. The Donor Heart and Organ Perfusion Technology. J Thorac Dis (2019) 11(Suppl. 6):S938–S945. doi:10.21037/jtd.2019.02.59
80. Nurmykhametova Z, Kaliyev R, Lesbekov T, Bekbossynov S, Bekbossynova M, Kapyshev T, et al. Sixteen Hours Ex-Vivo Perfusion for Heart Transplantation. Eur J Heart Fail (2018) 20:189. doi:10.1002/ejhf.1197
81. Nilsson J, Jernryd V, Qin G, Paskevicius A, Metzsch C, Sjo€berg T, et al. A Nonrandomized Open-Label Phase 2 Trial of Nonischemic Heart Preservation for Human ëHeart Transplantation. Nat Commun (2020) 11(1):2976. doi:10. 1038/s41467-020-16782-9
82. Ardehali A, Esmailian F, Deng M, Soltesz E, Hsich E, Naka Y, et al. Ex-vivo Perfusion of Donor Hearts for Human Heart Transplantation (PROCEED II): a Prospective, Open-Label, Multicentre, Randomised Non-inferiority Trial. Lancet (2015) 385(9987):2577–84. doi:10.1016/S0140-6736(15)60261-6
83. Messer S, Axell R, Colah S, White P, Page A, Parizkova B, et al. Functional Assessment of the Donor Heart Following Circulatory Death and Clinical Transplantation. J Heart Lung Transplant (2016) 35(4):S79–S80. doi:10.1016/j.healun.2016.01.218
84. Michel SG, La Muraglia GM 2nd, Madariaga ML, Titus JS, Selig MK, Farkash EA, et al. Twelve-hour hypothermic machine perfusion for donor heart preservation leads to improved ultrastructural characteristics compared to conventional cold storage. Ann Transplant. (2015) 20:461–8. doi: 10.12659/AOT.893784
85. Wang L, MacGowan GA, Ali S, Dark JH. Ex situ heart perfusion: the past, the present, and the future. J Heart Lung Transplant. (2021) 40:69–86. doi: 10.1016/j. healun.2020.10.004
86. Wicomb WN, Cooper DKC, Novitzky D, Barnard CN. Cardiac Transplantation Following Storage of the Donor Heart by a Portable Hypothermic Perfusion System. Ann Thorac Surg (1984) 37(3):243–8. doi:10.1016/s0003-4975(10)60333-5
87. Hill DJ, Wicomb WN, Avery GJ, Portnoy VF, Collins GM. Evaluation of a Portable Hypothermic Microperfusion System for Storage of the

Donor Heart: Clinical Experience. Transplant Proc (1997) 29(8):3530–1. doi:10.1016/s0041-1345(97)01008-7

88. Perfusion X. Non-ischemic preservation of the donor heart in heart transplantation-a randomized, controlled, multicenter trial (2019). Available from: https://clinicaltrials.gov/ct2/show/NCT03991923 (Accessed June 23, 2022).
89. Kaliyev R, Lesbekov T, Bekbossynov S, Bekbossynova M, Nurmykhametova Z, Novikova S, et al. Comparison of Custodiol vs Warm Blood Cardioplegia and Conditioning of Donor Hearts during Transportation with the Organ Care System. J Card Surg (2019) 34(10):969–75. doi:10.1111/jocs.14162
90. Sato T, Azarbal B, Cheng R, Esmailian F, Patel J, Kittleson M, et al. Does Ex Vivo Perfusion lead to More or Less Intimal Thickening in the First-Year postheart Transplantation? Clin Transpl (2019) 33(8):e13648. doi:10.1111/ctr.13648
91. Jain P, Prichard RA, Connellan MB, Dhital KK, Macdonald PS. Long Distance Heart Transplantation: a Tale of Two Cities. Intern Med J (2017) 47(10):1202–5. doi:10.1111/imj.13568
92. Koerner MM, Ghodsizad A, Schulz U, El Banayosy A, Koerfer R, Tenderich G. Normothermic Ex Vivo Allograft Blood Perfusion in Clinical Heart Transplantation. Heart Surg Forum (2014)
93. García Sáez D, Zych B, Sabashnikov A, Bowles CT, De Robertis F, Mohite PN, et al. Evaluation of the Organ Care System in Heart Transplantation with an Adverse Donor/recipient Profile. Ann Thorac Surg (2014) 98(6):2099–106. doi:10.1016/j.athoracsur.2014.06.098 17(3):E141–5. doi:10.1532/HSF98.2014332
94. Stamp NL, Shah A, Vincent V, Wright B, Wood C, Pavey W, et al. Successful Heart Transplant after Ten Hours Out-Of-Body Time Using the TransMedics Organ Care System. Heart Lung Circ (2015) 24(6):611–3. doi:10.1016/j.hlc.2015.01.005
95. Sponga S, Ius F, Ferrara V, Royas S, Guzzi G, Lechiancole A, et al. Normothermic Ex-Vivo Perfusion for Donor Heart Preservation in Transplantation of Patients Bridged with Ventricular Assist Devices. J Heart Lung Transplant (2020) 39(4):S245. doi:10.1016/j.healun.2020.01.926
96. Botta L, Cannata A, Merlanti B, Campolo J, Gagliardone M, Frigerio M, et al. The OCS Heart System for Ex-Vivo Perfusion of the Donor Heart: A Step toward Future? Artif Organs (2017) 41(9):A23.
97. Falk C, Ius F, Rojas-Hernandez SV, Wiegmann B, Wandrer F, Kaufeld T, et al. Effects of Ex Vivo Perfusion and Il-6 Receptor Blockade on

Ischemia Reperfusion Injury in Cardiac Transplantation. J Heart Lung Transplant (2019) 38(4):S240. doi:10.1016/j.healun.2019.01.591

98. Fujita B, Sievert A, Sunavsky J, Börgermann J, Fuchs U, Gummert J, et al. Heart Transplantation Using the Normothermic Ex-Vivo Perfusion Device Organ Care System for Donor Heart Preservation - Long-Term Outcomes. J Heart Lung Transplant (2018) 37(4):S411. doi:10.1016/j.healun.2018.01.1065
99. García Sáez D, Zych B, Mohite PN, Sabashnikov A, Patil NP, Popov A, et al. Lvad Bridging to Heart Transplantation with Ex Vivo Allograft Preservation Shows Significantly Improved: Outcomes: A New Standard of Care? J Heart Lung Transplant (2015) 34(4): S95. doi:10.1016/j.healun.2015.01.252
100. Rojas SV, Ius F, Kaufeld T, Sommer W, Goecke T, Poyanmehr R, et al. Ex Vivo heart Perfusion for Heart Transplantation: A Single-center Update after 5 Years. Thorac Cardiovasc Surgeon (2020) 68. doi:10.1055/s-0040-1705375
101. Sponga S, Ferrara V, Beltrami AP, Bonetti A, Cantarutti C, Caragnano A, et al. Ex-vivo Perfusion on Marginal Donors in Heart Transplantation: Clinical Resuts and Pathological Findings. J Heart Lung Transplant (2019) 38(4): S42–S43. doi: 10.1016/j.healun.2019.01.089
102. Ayan Mukash A, Kaliyev RB, Nurmykhametova ZHA, Faizov LR, Ashyrov ZHZ, Zhunussov BK, et al. Ex Vivo allograft Perfusion for Heart Transplantation: Single centre Experience. Eur J Heart Fail (2019) 21:468. doi:10.1002/ejhf.1488
103. García Sáez D, Zych B, Mohite P, Sabashnikov A, De Robertis F, Popov AF, et al. Heart Transplantation from Donors outside Standard Acceptability Criteria Using Ex-Vivo Normothermic Preservation. J Heart Lung Transplant (2016) 35(4):S85. doi: 10.1016/j.healun.2016.01.234
104. Koerner M, Tenderich G, El Banayosy A, Schulz U, Brehm C, Koerfer R. Feasibility and Long-Term Results in Recipients of Hearts Procured with Ex Vivo Donor Heart Perfusion. Crit Care Med (2012) 40(12):66–7. doi: 10.1097/01.ccm.0000424503.57952.13
105. Yeter R, Pasic M, Hübler M, Dandel M, Hiemann N, Kemper D, et al. Extended Donor Criteria in Heart Transplantation: 4-year Results of the Experience with the Organ Care System. Thorac Cardiovasc Surgeon (2014) 62, SC44. doi:10.1055/s-0034-1367305

106. Rojas SV, Ius F, Schibilsky D, Kaufeld T, Benk C, Sommer W, et al. Ex Vivo Organ Perfusion for Heart Procurement in High-Risk Transplantations: A Multicenter Study. J Heart Lung Transplant (2020) 39(4):S135. doi:10.1016/j. healun.2020.01.1044
107. García Sáez D, Bowles CT, Mohite PN, Zych B, Maunz O, Popov AF, et al. Heart Transplantation after Donor Circulatory Death in Patients Bridged to Transplant with Implantable Left Ventricular Assist Devices. J Heart Lung Transplant (2016) 35(10):1255–60. doi:10.1016/j.healun.2016.05.025
108. Mehta V, Taylor M, Hasan J, Dimarakis I, Barnard J, Callan P, et al. Establishing a Heart Transplant Programme Using Donation after Circulatory-Determined Death Donors: a United Kingdom Based Singlecentre Experience. Interact Cardiovasc Thorac Surg (2019) 29(3):422–9. doi:10.1093/icvts/ivz121
109. Mohite PN, García Sáez D, Butler AJ, Watson CJE, Simon A. Direct Procurement of Donor Heart with Normothermic Regional Perfusion of Abdominal Organs. Ann Thorac Surg (20 Messer SJ, Axell RG, Colah S, White PA, Ryan M, Page AA, et al. Functional
110. Assessment and Transplantation of the Donor Heart after Circulatory Death. J Heart Lung Transplant (2016) 35(12):1443–52. doi: 10.1016/j.healun.2016. 07.004 19) 108(2):597–600. doi: 10.1016/j.athoracsur.2019.01.087
111. Chew HC, Iyer A, Connellan M, Scheuer S, Villanueva J, Gao L, et al. Outcomes of Donation after Circulatory Death Heart Transplantation in Australia. J Am Coll Cardiol (2019) 73(12):1447–59. doi:10.1016/j.jacc.2018.12.067
112. Dhital KK, Iyer A, Connellan M, Chew HC, Gao L, Doyle A, et al. Adult Heart Transplantation with Distant Procurement and Ex-Vivo Preservation of Donor Hearts after Circulatory Death: a Case Series. The Lancet (2015) 385(9987):2585–91. doi:10.1016/s0140-6736(15)60038-1
113. Messer S, Page A, Axell R, Berman M, Hernández-Sánchez J, Colah S, et al. Outcome after Heart Transplantation from Donation after Circulatory-Determined Death Donors. J Heart Lung Transplant (2017) 36(12):1311–8.doi:10.1016/j.healun.2017.10.021
114. Chew H, Cheong C, Fulton M, Shah M, Doyle A, Gao L, et al. Outcome after Warm Machine Perfusion (WMP) Recovery of Marginal Brain Dead (MBD) and Donation after Circulatory Death (DCD) Heart Transplantation. J Heart Lung Transplant (2017) 36(4):S45–S46. doi:10.1016/j.healun.2017.01.107

115. Messer S, Page A, Berman M, Colah S, Dunning J, Pavlushkov E, et al. First to 50: Early Outcomes Following Heart Transplantation at Royal Papworth Hospital from Donation after Circulatory Determined Death (DCD) Donors. J Heart Lung Transplant (2019) 38(4):S43. doi: 10.1016/j.healun.2019.01.090
116. Page A, Goddard M, Messer S, Dunning J, Parameshwar J, Lewis C, et al. Ischaemic Reperfusion Injury and Allograft Rejection Following DCD Heart Transplantation: Early Results. J Heart Lung Transplant (2017) 36(4): S122. doi: 10.1016/j.healun.2017.01.315
117. Page A, Messer S, Hudson V, Pavlushkov E, Berman M, Dunning J, et al. Early Outcomes from DCD Heart Transplantation: A Single centre Experience. J Heart Lung Transplant (2018) 37(4): S13–S14. doi: 10.1016/j.healun.2018.01.010
118. School HM. Observational Study of the Clinical Use of the Organ Care System (OCS™) in Heart Transplantation (2018). Available from: https://clinicaltrials.gov/ct2/show/NCT03687723 (Accessed July 27, 2020).
119. Perfusion X. Non-ischemic Preservation of the Donor Heart in Heart Transplantation - a Randomized, Controlled, Multicenter Trial (2019). Available from: https://clinicaltrials.gov/ct2/show/NCT03991923 (Accessed July 27, 2020).
120. Nilsson J. Non-ischaemic Heart Preservation versus Standard Cold Storage in Human Heart Transplantation (NIHP2) (2019). Available from: https://clinicaltrials.gov/ct2/show/NCT04066127 (Accessed July 27, 2020).
121. TransMedics. Heart Expand Continued Access Protocol (2019). Available from: https://clinicaltrials.gov/ct2/show/NCT03835754 (Accessed July 27, 2020).
122. TransMedics. Donors. After Circulatory Death Heart Trial (2019). Available from: https://clinicaltrials.gov/ct2/show/NCT03831048 (Accessed July 27, 2020).
123. Steen S, Paskevicius A, Liao Q, Sjöberg T. Safe Orthotopic Transplantation of Hearts Harvested 24 hours after Brain Death and Preserved for 24 hours. Scand Cardiovasc J (2016) 50(3):193–200. doi:10.3109/14017431.2016.1154598
124. Bellini MI, Nozdrin M, Yiu J. Machine Perfusion for Abdominal Organ Preservation: A Systematic Review of Kidney and Liver Human Grafts. J Clin Med (2019) 8:1221. doi:10.3390/jcm8081221

125. Bellini MI, Charalampidis S, Herbert PE, Bonatsos V, Crane J, Muthusamy A, et al. Cold Pulsatile Machine Perfusion versus Static Cold Storage in Kidney Transplantation: A Single Centre Experience. Biomed Res Int (2019) 2019:7435248. doi:10.1155/2019/7435248
126. Husen P, Boffa C, Jochmans I, Krikke C, Davies L, Mazilescu L, et al. Oxygenated End-Hypothermic Machine Perfusion in Expanded Criteria Donor Kidney Transplant: A Randomized Clinical Trial. JAMA Surg (2021) 156:517–25. doi:10.1001/jamasurg.2021.0949
127. Kaliyev R, Lesbekov T, Bekbossynov S, Nurmykhametova Z, Bekbossynova M, Novikova S, Medressova A, Smagulov N, Faizov L, Samalavicius R, Pya Y. Heart transplantation of patients with ventricular assist devices: impact of normothermic ex-vivo preservation using organ care system compared with cold storage. J Cardiothorac Surg. 2020 Oct 27;15(1):323. doi: 10.1186/s13019-020-01367-w. PMID: 33109229; PMCID: PMC7590799.
128. Beuth J, Falter F, Pinto Ribeiro RV, Badiwala M, Meineri M: New strategies to expand and optimize heart donor pool: ex vivo heart perfusion and donation after circulatory death: a review of current research and future trends. Anesth Analg. 2019, 128:406-13. 10.1213/ANE.0000000000003919
129. Rojas SV, Avsar M, Ius F, et al.: Ex-vivo preservation with the organ care system in high-risk heart transplantation. Life (Basel). 2022, 12: 10.3390/life12020247
130. Fleck TP, Ayala R, Kroll J, et al.: Ex vivo allograft perfusion for complex pediatric heart transplant recipients. Ann Thorac Surg. 2021, 112: 1275-80.10.1016/j.athoracsur.2020.12.025
131. Pinnelas R, Kobashigawa JA: Ex vivo normothermic perfusion in heart transplantation: a review of the TransMedics® Organ Care System. Future Cardiol.2022, 18:5-15. 10.2217/fca-2021-0030
132. White CW, Messer SJ, Large SR, et al.: Transplantation of hearts donated after circulatory death. Front Cardiovasc Med. 2018, 5:8. 10.3389/fcvm.2018.00008
133. Schroder JN, D'Alessandro D, Esmailian F, et al.: Successful utilization of extended criteria donor (ECD) hearts for transplantation-results of the OCS™ heart EXPAND trial to evaluate the effectiveness and safety of the OCS heart system to preserve and assess ECD hearts for transplantation. J Heart Lung Transplant. 2019, 38:42. 10.1016/j.healun.2019.01.088

List of publications and published thesis

1. Kaliyev, Rymbay; Lesbekov, Timur; Bekbossynov, Serik, Bekbossynova, Makhabbat; Nurmykhametova, Zhuldyz; Novikova, Svetlana; Smagulov, Nurlan; Medressova, Assel; Faizov, Linar; Ashyrov, Zhanibek; la Fleur, Phillip; Samalavicius, Robertas Stasysa; Pya, Yuriy. Comparison of Custodiol vs warm blood cardioplegia and conditioning of donor hearts during transportation with the organ care system//Journal of Cardiac Surgery 2019. https://doi.org/10.1111/jocs.14162.
2. Kaliyev, Rymbay; Lesbekov, Timur; Bekbossynov, Serik; Nurmykhametova*, Zhuldyz; Bekbossynova, Makhabbat; Novikova, Svetlana; Medressova, Assel; Smagulov, Nurlan; Faizov, Linar; Samalavicius, Robertas; and Pya Yuriy. Heart transplantation of patients with ventricular assist devices: impact of normothermic ex-vivo preservation using organ care system compared with cold storage//Journal of Cardiothoracic Surgery 2020. https://doi.org/10.1186/s13019-020-01367-w.
3. Kaliyev, Rymbay; Bekbossynov, Serik; Nurmykhametova*, Zhuldyz. Sixteen-Hour Ex Vivo Donor Heart Perfusion during Long-Distance Transportation for Heart Transplantation//Artificial organs 2019 – 43(3), c. 319-320.
4. Medressova, Assel; Faizov, Linar; Kuanyshbek, Aidyn; Kaliyev, Rymbai; Myrzakhmetova, Gulzhan; la Fleur, Philip; Pya, Yuriy. Successful heart transplantation after 17 h ex vivo time using the Organ Care System—3 years follow-up. First published: 30 March 2021 https://doi.org/10.1111/jocs.15519.
5. Pya, Yuriy; Kaliyev, Rymbay; Bekbossynov, Serik; et al. 3-year outcomes with ex-vivo allograft perfusion for heart transplantation: Comparison of Custodiol vs warm blood cardioplegia and conditioning. Clinical and Experimental Surgery, 2021, 8(3).
6. Kaliyev, Rymbay; Lesbekov, Timur; Bekbossynova, Makhabbat; et al. Successful Heart Transplantation After 7 h of Cold Storage and Paracorporeal Donor Heart Resuscitation Transplant International, 2022, 35, 10740
7. Lesbekov, Timur; Mussayev, Abdurashid; Alimbayev, Serik; Kalyiev, Rymbay. et al. Prophylactic Awake Peripheral V-A ECMO during TAVR. Journal of Clinical Medicine, 2023, 12(3), 859.

8. Lesbekov, Timur; Nurmykhametova, Zhuldyz; Kaliyev, Rymbay. et al. Hemadsorption in patients requiring V-A ECMO support: Comparison of Cytosorb versus Jafron HA330. Artificial Organs, 2023, 47(4), page 721–730.
9. Nurmykhametova, Zhuldyz; Lesbekov, Timur; Kaliyev, Rymbay. et al. Preliminary report of extracorporeal blood purification therapy in patients receiving LVAD: Cytosorb or Jafron HA330. Journal of Extra-Corporeal Technology, 2024, 56(1), page 10–15.
10. Nurmykhametova, Zhuldyz; Lesbekov, Timur; Kaliyev, Rymbay. et al. Heart preservation with the organ care system for mechanical circulatory support recipients//European Journal of Heart Failure© 2019 European Society of Cardiology, 21(Suppl. S1), 167.
11. Kaliyev, Rymbay; Nurmykhametova, Zhuldyz; Pya, Yuriy. Conditioning of donor hearts during transportation with the organ care system//Progress of ECMO in Lithuania. The 3d Conference. Abstract book 2019 – c. 34-37.
12. Eurasian patent organization B1 041351. (Method of donor heart conditioning) 2022.
13. Patent for utility model of Republic of Kazakhstan (№ 8686) “System for normothermic preservation and long-term transportation of a donor heart” 2023.
14. Kaliyev, Rymbay; Nurmykhametova, Zhuldyz; Pya, Yuriy et al. Successful heart transplantation after 24 hours of ex-situ normothermic perfusion using ECMO technology in an animal model. Under review to JHLT Open journal.
15. Presenting ALEM (Astana Life ex-situ machine) on EXPO2025 April-Ocober 2025 in Osaka, Japan.

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(71)(73) Заявитель и патентовладелец:
АКЦИОНЕРНОЕ ОБЩЕСТВО "НАЦИОНАЛЬНЫЙ НАУЧНЫЙ КАРДИОХИРУРГИЧЕСКИЙ ЦЕНТР" (KZ)

(72) Изобретатель:
Пя Юрий Владимирович, Калиев Рымбай Болатович, Бекбосынов Серик Темирханович, Лесбеков Тимур Достаевич, Нурмыхаметова Жулдыз Аскаровна, Смагулов Нурлан Куандыкович, Новикова Светлана Петровна, Ибраев Талгат Ергалиевич, Аширов Жанибек Зайдинович, Фанзов Линар Ринатович (KZ)

(74) Представитель:
Суюндуков М.Ж. (KZ)

(56) Universitatsspital Zurich: " Levosimendan", 1 января 2012 (2012-01-01), XP055364188, найдено в интернете; URL:http://www.cardiocentro.org/D-Bettex-L evosimendan-in-Perioperative-Cardiac-Failu re-7ab3f300 [найдено 2017-04-12] Other Settings for Levosimendan

Sagiiaschi G. ET AL.: "[Use of uitrafiltration in refractory cardiac decompensation], - PubMed - NCBI", 1 января 1992 (992-01-01), XP055364132, найдено в интернете: URL:https://www.ncbi.nlm.nih.gov/pubmed/14 61535 [найдено 2017-04-12] весь документ

Goti Jp ET AL.: "Cardioplegia for heart transplantation: unmodified UW solution compared with Stanford solution. - PubMed - NCBI", 1 января 1992 (1992-01-01), XP055364162, найдено в интернете: URL:https://www.ncbi.nlm.nih-gov/pabmed/t5 76341 [найдено 2017-04-12] весь документ

(57) Изобретение относится к медицине, а именно к кардиохирургии и перфузиологии. Целью данного изобретения является улучшение результатов трансплантации сердца путем оптимизации кондиционирования сердца описываемым способом. Способ кондиционирования донорского сердца включает в себя комплекс мероприятий: использование кровяного кардиоплегического раствора, ультрафильтрацию и Левосимендан. Для кардиоплегии в организме донора используют нормотермический раствор, который состоит из донорской крови и кристаллоидного раствора в соотношении 5:1 (калия хлорид 4%, магния сульфат 25%, лидокаин 2%, натрий гидрокарбонат, маннитол 15%). Для защиты миокарда и улучшения его кровообращения в перфузат однократно вводится Левосимендан (45 мкг/кг). Для удаления медиаторов воспаления, излишнего количества жидкости, коррекции электролитного состава и гематокрита проводится ультрафильтрация перфузата. Перед пересадкой реципиенту для отлучения донорского сердца от устройства OCS вводится кровяной кардиоплегический раствор.

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ҚАЗАҚСТАН РЕСПУБЛИКАСЫ

РЕСПУБЛИКА КАЗАХСТАН

REPUBLIC OF KAZAKHSTAN

ПАТЕНТ
PATENT

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Система для нормотермического сохранения и длительной транспортировки донорского сердца
System for normothermic preservation and long-term transportation of a donor heart

(73) «Ұлттық ғылыми кардиохирургия орталығы» коммерциялық емес акционерлік қоғамы (KZ)
Некоммерческое акционерное общество «Национальный научный кардиохирургический центр» (KZ)
«National Research Cardiac Surgery Center» Non-profit Joint Stock Company (KZ)

(72)
Пя Юрий Владимирович (KZ) — Pya Yuriy Vladimirovich (KZ)
Калиев Рымбай Болатович (KZ) — Kaliyev Rymbay Bolatovich (KZ)
Лесбеков Тимур Достаевич (KZ) — Lesbekov Timur Dostayevich (KZ)
Файзов Линар Ринатович (KZ) — Faizov Linar Rinatovich (KZ)
Нурмыхаметова Жулдыз Аскаровна (KZ) — Nurmykhametova Zhuldyz Askarovna (KZ)
Медресова Асель Тураровна (KZ) — Medressova Assel Turarovna (KZ)
Жунусов Бауыржан Кажтаевич (KZ) — Zhunussov Bauyrzhan Kazhtaevich (KZ)
Сергиенко Анатолий Викторович (KZ) — Sergiyenko Anatoliy Viktorovich (KZ)
Капиносов Владислав Викторович (KZ) — Kapinossov Vladislav Viktorovich (KZ)
Куанышбек Айдын Саятұлы (KZ) — Kuanyshbek Aidyn Sayatuly (KZ)

ЭЦҚ қол қойылды — Е. Оспанов
Подписано ЭЦП — Е. Оспанов
Signed with EDS — Y. Ospanov

«Ұлттық зияткерлік меншік институты» РМК директоры
Директор РГП «Национальный институт интеллектуальной собственности»
Director of RSE «National institute of intellectual property»

SANTRAUKA

TRUMPINIAI

RBP	rūgščių ir bazių pusiausvyra
DPV	Dirbtinė plaučių ventiliacija
ARITS	Anesteziologijos, reanimacijos ir intensyvios terapijos skyrius
CPS	Kardioplegija - dirbtinai sukeltas laikinas širdies veiklos sustabdymas
CARMAT	dirbtinė širdis CARMAT
KST	kaukolės-smegenų trauma
LŠN	Lėtinis širdies nepakankamumas
ŠKL	sergamumas širdies ir kraujagyslių ligomis
ASL	apytakos sistemos ligos
EKMO	ekstrakorporinė membraninė oksigenacija
ESŠP	ex situ širdies perfuzija
IŠL	išeminė širdies liga
Interleukinai	citokinai, atsakingi už leukocitų ląstelių tarpusavio sąveiką
INTERMACS	Tarptautinis mechaninės apytakos paramos registras (angl. Interagency Registry for Mechanically Assisted Circulatory Support)
ISHLT	Tarptautinė širdies ir plaučių transplantacijos organizacija (angl. The International Society for Heart and Lung Transplantation)
KSPĮ	kairiojo skilvelio pagalbinis įtaisas
KSIF	kairiojo skilvelio išstūmmo frakcija
NAŠCC	Nacionalinis akademinis širdies chirurgijos centras
OCS širdis –	organų apsaugos sistema
OŠT	Ortotopinė širdies transplantacija
SDV	spalvinis doplerio vaizdavimas
UNOS	Jungtinis organų dalijimosi tinklas (angl. United Network Organ Sharing)

1. ĮVADAS

1.1. Problemos aktualumas. Tyrimo svarba ir inovatyvumas

Nors ir naujos technologijos yra kuriamos dirbtinio kairiojo skilvelio ar visos dirbtinės širdies implantavimo srityje, tačiau širdies transplantacija vis dar išlieka pirmuoju pasirinkimu gydant pacientus, kuriems pasireiškia terminalinis širdies nepakankamumas (1). Tačiau, pasak Pasaulinės širdies ir plaučių persodinimo organizacijos, pasaulinė statistika atskleidžia, kad vien tik Jungtinėse Amerikos Valstijose kasmet dėl širdies nepakankamumo miršta 6 milijonai pacientų, laukiančių operacijos. Kaip ir visur kitur pasaulyje, pagrindinis faktorius, ribojantis širdies transplantacijų skaičių Kazachstane yra donorų organų stoka bei ilgas transportavimui reikalingas laikotarpis. Todėl, jei pavyktų užtikrinti efektyvią donoro organo apsaugą, tai užtikrintų savalaikį širdies transplantavimą pacientams, įskaitant tuos pacientus, kuriems taikoma mechaninė pagalbinė kraujotaka, arba kurie jau turi implantuotą dirbtinį kairįjį skilvelį. Tokiu atveju, geografinė donoro širdies padėtis Kazachstano Respublikos teritorijoje taptų nebesvarbi.

Praktinė šio tyrimo reikšmė Kazachstano Respublikai yra ta, kad, remiantis 2020 metų duomenimis, kasmet širdies transplantacijos reikia daugiau nei 200 pacientų. Norint padidinti tinkamų donorinių organų skaičių bei pagerinti transplantacijos išeitis, būtina sukurti efektyvų donorinio organo apsaugos metodą. Sąvoka „miokardo apsauga“ apima didelę metodų įvairovę, įskaitant anesteziją, chirurginę techniką, hipotermiją bei kardioplegiją. Šio tyrimo hipotezė siūlo išanalizuoti naują metodą saugoti donoro širdį, naudojant kraujo kardioplegiją bei kondicionavimą organų apsaugos sistemoje. Šiuo tikslu bus įgyvendintas perspektyvinis surinktų duomenų lyginamasis vertinimas, lyginant du pagrindinius miokardo apsaugos metodus pacientams, kuriems buvo atlikta širdies transplantacija, pasitelkus pagalbinius skilvelių įrenginius.

Ex situ širdies perfuzija (ESŠP) suteikia ilgą apsaugos laiką bei galimybę įvertinti širdies funkciją ir gyvybingumą bei metabolizmą. Šiuo metu kliniškai taikoma sistema taiko tik laktato lygį, kuomet yra nustatoma, ar širdis tinkama transplantacijai. Tai riboja galimybę išprognozuoti funkcinį atsigavimą, įvykdžius transplantaciją. Mūsų tyrimo tikslas buvo įvertinti plakančios širdies palaikymą normoterminiame ESŠP, paremtą EKMO technologija (savadarbe) ir šios sistemos poveikį persodintos širdies transplanto funkcijai.

1.2. Tyrimo tikslas

Šio tyrimo tikslas yra įvertinti donoro širdies apsaugos efektyvumą naudojant kraujo kardioplegiją ir kondicionavimą ex situ donoro širdies perfuzijos laikotarpiu ir palyginti normoterminę ex-situ apsaugą naudojant organų apsaugos sistemą su hipotermine donorinio organo miokardo apsauga pacientams, kuriems atliekama širdies transplantacija, pasitelkus kairiojo skilvelio pagalbinius įrenginius.

1.3. Tyrimo uždaviniai

1. Palyginti miokardo apsaugą bei kondicionavimą atliekant ex situ perfuziją ir ilgalaikį donoro širdies gabenimą organų apsaugos sistemoje;
   - Palyginti laktato kitimo tendencijas, inotropų infuzijos dozes bei trukmę
   - Palyginti interleukino-6 ir Interleukino-8 koncentracijos kitimus
   - Palyginti pooperacinę EKMO trukmę
2. Palyginti normoterminės ex vivo apsaugos poveikį, naudojant organų apsaugos sistemą, su šalta apsauga, taikoma pacientams, turintiems implantuotus pagalbinius skilvelių įrenginius;
   - Palyginti šių grupių išgyvenusiųjų pacientų kiekį po transplantacijos.
3. Įvertinti potransplantacinę transplanto funkciją taikant normoterminį plakančios širdies ex situ širdies perfuzijos metodą, paremtą EKMO (savadarbę sistemą), pasiremiant porcininiu (t.y., kiaulių) modeliu:
   - Įvertinti veninio laktato ir interleukinų kitimo tendencijas
   - Įvertinti širdies edemą (palyginti širdies svorį)
   - Įvertinti miokardo deguonies suvartojimą, echokardiografinius rodmenis
   - Įvertinti histologinius pokyčius
   - Įvertinti vainikinių arterijų kraujo tekėjimą, vidutinį arterinį slėgį ir širdies plakimo dažnį

1.4. Mokslinis naujumas

Pastaraisiais dešimtmečiais buvo vis labiau domimasi galimybe naudoti kraują kaip CPS pagrindą atliekant atviros širdies operacijas. Kraujo kardioplegijos teikiami pranašumai yra siejami su raudonųjų kraujo ląstelių

aukštu deguonies lygiu bei buferine talpa; energija ir substratais miokardiniam metabolizmui; adekvačiu koloidiniu osmoziniu slėgiu, dėl kurio neišsivysto vakuolinė degeneracija; natūraliais antioksidantais, sumažinančiais reperfuzijos traumos pavojų; sumažinama hemodiliucija operacijos metu, kas yra itin svarbu pasireiškiant ilgalaikei miokardo išemijai ir sumažėjusioms funkcinėms galimybėms. Remiantis daugeliu autorių, šis metodas pasižymi neginčijamais pranašumais, lyginant su kitais miokardo apsaugos metodais.

Iki šiol standartinis donoro organo paėmimo ir transplantavimo metodas buvo tradicinis požiūris, kuomet buvo naudojamas sausas ledas ir druskos tirpalas. Šiuo metu jau yra sukurta kilnojamas įrenginys, padedantis įveikti transplantacijos problemas, kadangi buvo išspręsti sunkumai, kildavę dėl hipotermija paremto organo apsaugos metodo taikymo. Tokiu būdu sumažinami šalčio išeminiai pažeidimai, kadangi širdžiai tiekiami šiltas kraujas ir deguonis, pagerinama organo būklė, nes vis papildomas deguonies, mitybinių medžiagų bei hormonų kiekis, bei teikiamas nuolatinis stebėjimas ir organui būtinų sąlygų palaikymas iki pat transplantacijos momento.

Tiriant literatūroje pateikiamus duomenis, buvo nustatyti organų apsaugos sistemos teikiami privalumai. Transportavimo trukmė gali būti kelis kartus ilgesnė, o mirštamumas siekė tik 5 procentus, lyginant su klasikinio organo apsaugos metodo taikymu, kuomet mirštamumo rodiklis buvo 20 procentų.

Miokardo apsauga yra vienas iš pagrindinių faktorių, turinčių didelį poveikį atvirų širdies operacijų bei širdies persodinimo baigčiai. Nepakankama miokardo apsauga veda prie sunkios miokardo edemos, išeminių sutrikimų susidarymo, elektrinio nestabilumo bei ląstelių „sutrikdymo“, po kurių seka pooperacinės komplikacijos. Remiantis tarptautinėmis publikacijomis, šiuo metu nėra sutariama, koks yra geriausias miokardo apsaugos metodas širdies operacijos metu.

Pastarojo meto tyrimai rodo, kad ryškiai padidėja ankstyvasis mirtštamumas, kuomet donorinė širdis transplantacijos metu yra saugoma naudojant Custodiolo tirpalą šaltomis sąlygomis, kuomet šis laikotarpis viršija 4 valandas (3).

Tačiau pastaruoju laikotarpiu pasitaikė pranešimų, kad buvo sėkmingai atliktas donoro organo transplantavimas, saugant jį nuo 8 iki 10 valandų ir naudojant organų apsaugos sistemos (OAS) mobilų įrenginį (Pav. 2). Šioje sistemoje taikomos naujos technologijos, kurios pasirūpina, kad organas funkcionuotų už kūno ribų, ir kurios leidžia organo funkcijai išlikti artimai jo fiziologinei būklei (4-7).

1.5. Praktinė tyrimo reikšmė

Šio tyrimo reikšmė Kazachstano Respublikai yra milžiniška. Remiantis 2020 metų duomenimis, kasmet Kazachstane širdies transplantacijos reikia 203 žmonėms. Nepaisant transplantacijos teikiamos naudos, vis tiek pastebimas širdžių, reikalingų transplantacijai, trūkumas, ir tik ribotas skaičius pacientų, atitinkančių kriterijus tokiai operacijai, šių operacijų sulaukia. Tam, kad būtų padidintas tinkamų donorų organų skaičius bei kad būtų pagerinta transplantacijos baigtis, reikia sukurti efektyvų būdą saugoti donoro organą. „Miokardo apsaugos“ sąvoka apima didelį spektrą metodų, tokių kaip anestezija, chirurginis technika, bei tiesioginė kardioplegija.

Kardioplegija yra kontroliuojamas laikinas širdies veiklos sustabdymas arba žymus širdies veiklos sumažinimas, kuris pasiekiamas, sustabdant kraujo tekėjimą vainikinėmis arterijomis.Šiuo metu yra žinoma daugiau nei 50 įvairių kardiopleginių tirpalų (CPR) formulių, dauguma kurių yra paruošiami empiriškai ir dar net nėra eksperimentais išbandyti. Šis skaičius bei tolesnis naujų formulių kūrimas rodo, kad tiek tyrėjai, tiek ir gydytojai yra esamomis formulėmis nepatenkinti, ir kad nuolat ieškoma būdų, kaip pagerinti miokardo apsaugos nuo išemijos metodus.

Pastaruoju metu vis labiau augo dėmesys idėjai naudoti kraują kaip pagrindą kardioplegijos tirpalams. Kraujo kardioplegijos privalumai yra siejami su raudonųjų kraujo ląstelių aukštu deguonies lygiu bei buferine talpa; energija ir plastiniais substratais iokardiniam metabolizmui; adekvačiu koloidiniu osmoziniu slėgiu, dėl kurio neišsivysto vakuolinė degeneracija; natūraliais antioksidantais, sumažinančiais reperfuzijos traumos pavojų; sumažinama pilnoji hemodiliucija operacijos metu, kas yra itin svarbu pasireiškiant ilgalaikei miokardinei išemijai ir sumažėjusioms funkcinėms galimybėms. Daugelio autorių nuomone, toks metodas turi neabejotinų pranašumų prieš kitus miokardo apsaugos metodus (8-12).

Praktinis šio metodo taikymas Kazachstano Respublikoje suteiktų galimybių toliau plėtoti širdies transplantacijos programą, kadangi atsirastų galimybių gabenti donoro organą dideliais atstumais bei sumažėtų mirštamumo lygis nuo lėtinio širdies nepakankamumo.

2. TYRIMO SUBJEKTAI, METODAI IR REZULTATAI

2.1. Perspektyvinis lyginamasis dviejų pagrindinių miokardinės apsaugos bei kondicionavimo metodų palyginimas ex situ perfuzijos bei ilgai trunkančio donoro organo pergabenimo metu organų apsaugos sistemoje

Šiame tyrime mes atlikome atsitiktinės atrankos būdu atliktą kontroliuojamą lyginamąjį perspektyvinį tyrimą ir apdorojome jo duomenis mūsų centre. Mūsų institucijoje nuo 2014 metų gegužės iki 2017 rugsėjo mes atlikome širdies transplantaciją 43 pacientams, sirgusiems terminaliniu širdies nepakankamumu. Visais atvejais mes naudojome OŠT donoro širdies apsaugojimui. Atrankos kriterijai buvo bent 18 metų amžiaus; be to, pacientas turėjo būti įtrauktas į mūsų centro širdies transplantacijos laukimo sąrašą. Iš šių atvejų, mes registruodavome donoro širdį prieš eksplantavimą ir 30 atvejų mes implantui naudodavome kraujo kardioplegiją ir kondicionavimą, o 13 atvejų mes naudojome standartinį Custodiolo tirpalą kardioplegijai (tai buvo standartinis iki tol taikytas organo apsaugos būdas). Šiam tyrimui už tai atsakingas mūsų institucijos etikos komitetas suteikė pritarimą ir leido panaudoti šio tyrimo duomenis mūsų analizei. Visi pacientai pateikė raštišką informuotą sutikimą dalyvauti tyrime. Tyrimo grupė (n=43) buvo padalinta į dvi dalis:

1. Miokardo apsauga donoro širdies konservavimo ir pergabenimo metu buvo atliekama naudojant kraujo kardioplegiją ir kondicionavimą (Levosimendanas ir ultrafiltravimas) (n=30) (BC grupė)
2. Kontrolinės grupės atveju širdies paėmimo ir transportavimo metu miokardo apsauga buvo atliekama naudojant kristaloidinę šalčio (Custodiolio) kardioplegiją (n=13) (SC grupė). Organo pergabenimas, diagnostika ir terapinės priemonės buvo atliekamos OCS sistemoje.

Pagrindiniai mus dominę rezultatai buvo 30 dienų išgyvenamumas bei širdies ir kraujagyslių sistemos komplikacijos. Be to, buvo surinkti tiek donorų, tiek ir recipientų klinikiniai, laboratoriniai, imunologiniai bei ultragarso duomenys. Taip pat buvo surinkti OCS prietaiso parametrai. Buvo ištirti operaciniai ir pooperaciniai duomenys, ekstrakorporinės membraninės oksigenacijos (EKMO) duomenys (implantavimo metu) bei audinio doplerografijos duomenys per pirmąsias 7 dienas.

Procedūros

Visiems pacientams buvo atlikta ortotopinė širdies transplantacija. Atvėrus donoro širdį, į donoro kylančiąją aortą buvo įsmeigiama dviejų angų

adata, kuri būdavo užtvirtinama su 4-0 polipropileno piniginės siūlės tipo susiuvimu. Remiantis standartinėmis procedūromis, naudojamomis Transmedics įrenginiui, į OCS būdavo įpilama 500 ml paruošiamojo tirpalo. Heparinizavus donorą (300 IU/kg), donoro kraujas (1200–1500 mL) būdavo paimamas prieš užspaudžiant aortą ir atliekant kardioiplegiją. Į kraujo surinkimo maišelį būdavo įdedama 10 000 IU heparino, kad būtų parengtas perfuzijos modulis. Kraujo kardioplegijos grupėje dalis normoterminio kraujo (500–750 mL) būdavo retrogradiškai paimama pradinei kardioplegijos dozei. Standartinės apsaugos grupėje buvo naudojama 1000 ml standartinio Custodiolo tirpalo, atvėsinto iki +4 laipsnių Celsijaus. Abiejose grupėse donoro širdies aorta ir plaučių arterija būdavo kanuliuotos, ir širdis būdavo prijungiama prie OCS. Tuomet deguonies prisotintas kraujas būdavo pumpuojamas į aortą, perfuzuojant vainikines arterijas. Tuomet vainikinio sinuso srautas teka per triburį vožtuvą (kadangi tiek viršutinė, tiek ir apatinė tuščioji venos yra užsiūtos ir uždarytos), ir šis kraujas yra pašalinamas per dešinįjį skilvelį į plaučių arterijos kateterį ir sugrąžinamas į kraujo indą. Tuomet širdis yra atgaivinama iki įprastinio sinusinio ritmo. OCS buvo reguliuojami pumpuojamo srauto bei tekančio tirpalo kiekiai, kad būtų palaikomas vidutinis arterinis kraujospūdis tarp 60 mmHg ir 90 mmHg, o vainikinių arterijų srautas palaikomas tarp 650 mL/min ir 850 mL/min. Pagal standartinį protokolą OCS buvo paimami mėginiai, dar neprijungus donoro širdies prie OCS. Buvo registruojami donoro laktatas (CG4+, ne vėliau nei 30 minučių po kraujo paėmimo), pradinė OCS laktato vertė bei cheminiai rodikliai (CG8+, parengimo metu). OCS metu kas valandą buvo registruojamas arterinis ir veninis laktatas. Reguliariai (maždaug kas 20–30 minučių) buvo atliekami arterinio kraujo cheminiai tyrimai. OCS buvo imami tiek arterinio, tiek ir veninio kraujo mėginiai. Mėginius tyrėme rankiniu laktato tyrimo aparatu (i-STAT, Abbott Diagnostics, East Windsor, Naujasis Džersis, JAV).

Mus domino ex vivo širdies perfuzijos metu IL-6 ir IL-8 koncentracijos pokytis, lyginant su pradine verte, išemijos laikas, perfuzijos laikas, hemodinaminai rodikliai bei laktato lygis. Visą konservavimo laiką mes apibrėžėme kaip širdies perfuzijos laiką OCS naudojimo metu. Visas išemijos laikas buvo apibrėžtas kaip laikas nuo donoro širdies eksplantavimo iki širdies implantavimo recipientui, atmetus OCS laikotarpį. Mes taip pat surinkome elektrofiziologinius duomenis, perioperacinių parametrų duomenis, įskaitant OCS perfuzijos rezultatus bei interleukino 6 ir 8 duomenis ir laktato koncentracijos kitimo tendencijas. Paciento atsigavimo po operacijos ir pooperacinio laikotarpiu stebėjome naudojomų inotropų dozes, laiko,

praleisto intensyviosios terapijos skyriuje, trukmę, SDV parametrus bei ekstrakorporinės membraninės oksigenacijos trukmę (jei ji buvo taikoma).

Statistinė analizė

Nuolatiniai duomenys išreiškiami kaip vidurkis ± standartinis nuokrypis, nebent būtų nurodyta kitaip. Kategoriniai duomenys išreiškiami arba kaip įvykių skaičius, arba kaip proporcija. Kuomet įmanoma, naudojame dviejų mėginių nepriklausomą t-Testą, kad palygintume vidurkius. Statistinius tyrimus atlikome, naudodami SPSS platformą statistikai.

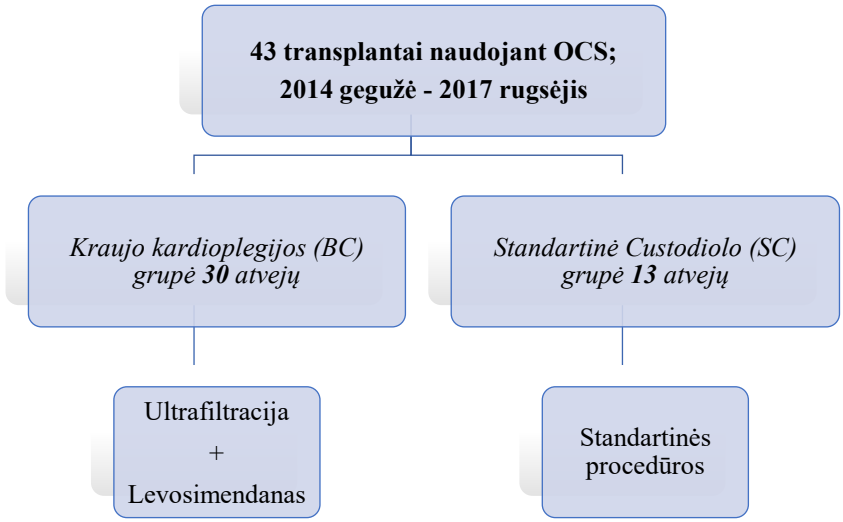
Rezultatai

Recipientų ir donorų populiacijos

Recipientų grupėje amžiaus mediana buvo truputį aukštesnė standartinės apsaugos grupėje, lyginant su kraujo kardioplegijos grupe. Kiti pradinių verčių nuspėjami rizikos faktoriai abiejose grupėse buvo panašūs, įskaitant lytį, kūno masės indeksą, ir IL vertes tiek tarp donorų, tiek ir tarp tos dalies pacientų, kuriems buvo naudojamas pagalbinis skilvelio įrenginys transplantavimo metu.

Buvo atliekamas lyginamasis donorų ir recipientų tyrimas, atsižvelgiant į dvi grupes: pirmoji grupė – miokardo apsauga donoro širdies eksplantavimo ir gabenimo metu atliekant kardioplegiją ir kondicionavimą ir naudojant Levosimendaną ir ultrafiltraciją (BC grupė) (n=30); antroji (kontrolinė) grupė – miokardo apsauga donoro širdies eksplantavimo ir pergabenimo metu vykdoma pasitelkus standartinę šalčio (Custodiolo) kardioplegiją (SC grupė) (n=13). Vidutinis amžius kraujo kardioplegijos ir kondicionavimo grupėje buvo nežymiai aukštesnis nei recipientų amžius kontrolinėje (Custodiolo) grupėje. Kiti prognozuojami rizikos faktoriai, tokie kaip lytis, kūno masės indeksas, ir dalis pacientų kuriems buvo naudojamas pagalbinis skilvelio įrenginys transplantavimo metu, abiejose grupėse buvo panašūs. Dauguma donorų ir jų recipientų priklausė tai pačiai kraujo grupei.

Stebėjimo laikotarpio mediana (pasiskirstymo plotas) buvo 255 dienos (30–360) kraujo kardioplegijos ir kondicionavimo grupėje ir 360 dienų (30–600) kontrolinėje grupėje.



1 paveikslas. Tyrimo protokolas

1 lentelė. Donorų ir gavėjų savybės bei rizikos faktoriai

Donorų charakteristikos	BC (n=30)	SC (n=13)	p vertė
Amžius (metai)	39 ±11	43 ±15.5	0.2
Vyriška lytis, n (%)	22 (74)	9 (75)	0.95
KMI (kg/m^2)	22.4 ±1.6	22.6 ±2.5	0.08
Mirties priežastis CVA, n (%)	22 (74)	9 (75)	0.95
Kitos mirties priežastys, n (%)	8 (26)	4 (25)	0.96
KSIF mediana (diapazonas)	62 (57-65)	63 (59-67)	
Recipientų charakteristikos	**BC (n=30)**	**SC (n=13)**	**p vertė**
Amžius (metai)	35 ±15	40 ± 12	0.45
Vyriška lytis	89.4% (26 iš 30)	75% (9 iš 13)	0.51
KMI (kg/m^2)	22.6 ± 2.5	22.8 ± 4	0.1
NICM n (%),	73.6% (22 iš 30)	50% (6 iš 13)	0.27
Kita	26.3% (8 iš 30)	50% (6 iš 13)	0.25
UNOS 1A+	42.1 (12 iš 30)%	33.3% (4 iš 13)	0.74
Implantuotas VAD, n (%)	52.6% (15 iš 30)	41.6% (5 iš 13)	0.66

Duomenys yra išreikšti kaip vidurkis ± standartinis nuokrypis, nebent būtų kitaip nurodyta; CVA – smegenų kraujagyslių nelaimingas atsitikimas; KSIF – kairiojo stulpelio išstūmimo frakcija; KMI – kūno masės indeksas; NISM – neišeminė kardiomiopatija; UNOS – jungtinis organų dalijimosi tinklas; VAD – skilvelio pagalbinis įrenginys

Elektrofiziologiniai rezultatai; OCS duomenys

Išeiminiai laikai bei donorinių širdžių perfuzijos laikai OCS buvo: vidurkis (± standartinis nuokrypis) visas išeiminis laikas buvo 75.2 (±22) min kraujo kardioplegijos grupėje, lyginant su 82.9 (±8.4) min standartinio gydymo grupėje. Vidutinis ex vivo perfuzijos laikas buvo 282.5 ± 86.7 minutės kraujo kardioplegijos grupėje, lyginant su 247.4 ± 88.4 minutėm standartinio gydymo grupėje (P=0.87). Sinusinio ritmo atstatymo dažnis OCS ir tarp širdies recipientų buvo ženkliai mažesnis kraujo kardioplegijos grupėje.

Visos donorų širdys pasižymėjo stabiliomis perfuzijos bei biocheminėmis savybėmis OCS, ir abiem grupėms buvo taikomos panašios priemonės. Pradinės IL-6 ir IL-8 koncentracijos statistiškai reikšmingai nesiskyrė, lyginant šias grupes. IL-6 ir IL-8 koncentracija besibaigiant ex vivo širdies perfuzijai buvo ryškiai mažesnė kraujo kardioplegijos grupėje, lyginant su standartinio gydymo grupe: 1493 ng/ml (SD 529,3) plg. su 2866 ng/ml (SD 601,2); (p=0,01), 989ng/ml (SD 453,6) plg. su 1274 ng/ml (SD 423,4) (p=0.05).

Vidutinė veninio laktato vertė perfuzijos pradžios metu buvo 2.7 mmol/l (SD 0,7) kraujo kardioplegijos grupėje ir 3.2 mmol/l (SD 0,8) standartinio gydymo grupėje (P=0,1). Besibaigiant perfuzijai, vidutinioji veninio laktato reikšmė buvo žemesnė kraujo kardioplegijos grupėje: 4.1 mmol/l (SD 1,9) lyginant su standartinio gydymo grupe: 8.8mmol/l (SD 2,1) (P=0,001).

Išemijos ir donorinio organo perfuzijos laikas OCS įrenginyje yra vidutinis visas šiltosios išemijos laikas, kuris buvo 75,2±22 minutės 1 grupėje lyginant su 82,9±8,4 minutėmis 2 grupėje. Vidutinis ex vivo donoro organo perfuzijos laikas buvo 282,5±86,7 minutės lyginant su 247,4±88,4 minutėmis 1 ir 2 grupėse (p=0,87), atitinkamai. Vidutinis laikas (standartinis nuokrypis) sinusinio ritmo atsistatymui po perėjimo prie organų apsaugos įrenginio buvo 2,6±1,4 min 1 grupėje ir 8,5±5,8 min 2 grupėje (p=0,04). Vidutinis laikas (standartinis nuokrypis) sinusinio ritmo atsistatymui po transplantacijos organo recipientui buvo reikšmingai mažesnis kraujo kardioplegijos ir kondicionavimo grupėje, lyginant su standartine Custodiolo grupe: 3,2 ± 2,1 lyginant su 7,3 ± 7,1 minutėmis (p=0,02), atitinkamai. Vidutinės troponino I ir RBP tyrimų vertės statistiškai reikšmingai nesiskyrė.

Perfuzijos ir biocheminiai parametrai naudojant organų apsaugos sistemą donoro širdies transportavimo metu lyginamose grupėse visais atvejais buvo normos ribose. Vidutinis Interleukino (IL) 6 ir 8 koncentracijų pokytis (standartinis nuokrypis) prieš išjungiant organų apsaugios įrenginį, kad įgyvendinti širdies implantaciją recipientui, statistiškai reikšmingai

nesiskyrė. Vidutinis Interleukino (IL) 6 ir 8 koncentracijų pokytis (standartinis nuokrypis) prieš išjungiant organų apsaugios įrenginį, kad įgyvendinti širdies implantaciją recipientui, buvo reikšmingai mažesnis kraujo kardioplegijos grupėje, lyginant su Custodiolo grupe: 1493 ng/ml (SD 529,3) lyginant su 2866 ng/ml (SD 601,2); (p = 0,01), 989ng/ml (SD 453,6) lyginant su 1274 ng/ml (SD 423,4) (p=0,05), atitinkamai, skaičiuojant abiejų grupių visumoms.

Vidutinė veninio laktato koncentracija (standartinis nuokrypis) pradedant perfuziją buvo 2,7 mmol/l (SD 0,7) kraujo kardioplegijos grupėje ir 3,2 mmol/l (SD 0,8) Custodiolo grupėje (p=0,1). Vidutinė veninio laktato koncentracija (standartinis nuokrypis) baigiant perfuziją buvo 4,1 mmol/l (SD 1.9) kraujo kardioplegijos grupėje ir 8,8 mmol/l (SD 2,1) (p=0,001) Custodiolo grupėje.

Pooperacinis atsigavimas

Vidutinė laiko, praleisto intensyviosios terapijos skyriuje, medianos vertė buvo 11 dienų (diapazonas: 4–40 dienų) kraujo kardioplegijos grupėje ir 19 dienų (diapazonas: 5–42 dienos) standartinio gydymo grupėje. EKMO trukmės mediana tiems pacientams, kuriems buvo teikiama mechaninis kraujotakos palaikymas, buvo 29,5 valandos (29,5 ± 28,4 valandos, n=6) kraujo kardioplegijos grupėje, lyginant su 78,4 valandomis (78,4 ± 89 valandos, n=8) standartinio gydymo grupėje (P=0,02). Inotropų dozė per pirmąsias 72 valandas buvo reikšmingai mažesnė kraujo kardioplegijos grupėje. Vazoaktyvių vaistų dozės (mcg/kg/min) per pradines 72 valandas buvo reikšmingai mažesnės kraujo kardioplegijos ir kondicionavimo grupėje, lyginant su Custodiolo grupe. Vidutinis laikas (standartinis nuokrypis) praleistas, naudojant dirbtinį plaučių ventiliavimą, buvo 30 valandų (24–73) kraujo kardioplegijos grupėje ir 78,4 valandos (26–312) Custodiolo grupėje. Mediana laiko, praleisto ARITS (pasiskirstymo plotas) buvo 11 dienų (4–40) kraujo kardioplegijos grupėje ir 19 dienų (5–42) Custodiolo grupėje. EKMO trukmė kraujo kardioplegijos ir kondicionavimo grupėje buvo (29,5 ± 28,4 valandos, n=6) lyginant su (78,4 ± 89 valandomis, n=8) Custodiolo grupėje (p=0,02).

Audinio miokardinio doplerio atvaizdavimo duomenys septintąją dieną po operacijos buvo normos ribose visose grupėse: miokardinio doplerio atvaizdavimo rodikliai septintąją dieną po operacijos buvo normos ribose visose grupėse buvo tokie: S1LV šoninis (cm/sec) 7,8 (±1,3) 1 grupėje ir 8,5 (±1,4) 2 grupėje, bei 8,8 grupėje nr. 3, S1LV medialinis (cm/sec) 8,3 (±1,62) 1 grupėje ir 7,68 (±1,23) 2 grupėje, bei 8.7 grupėje nr. 3, S1RV (cm/sec) 9,3 (±1,33) 1 grupėje ir 8,35 (±1,39) 2 grupėje, bei 9,0 grupėje nr. 3, atitinkamai.

KSIF (%) 55,4 (±2,31) 1 grupėje ir 56,5(±7,5) 2 grupėje bei 50 grupėje nr. 3. Vidutinis sinusinio ritmo atstatymo laikas buvo 5,3 (3,2; 7,3) minutės 1 grupėje ir 7,5 (5,3; 9,7) minutės 2 grupėje, bei 7 minutės grupėje nr. 3.

Inotropinių medikamentų palaikomoji dozė buvo normos ribose visose grupėse, tačiau ji reikšmingai išaugdavo 3 dieną po transplantacijos 1 grupėje. Vidutinis laikas (standartinis nuokrypis), praleistas taikant dirbtinį plaučių ventiliavimą buvo 163 valandos (153;173) 1 grupėje ir 98 valandos (30;168) 2 grupėje, bei 75 valandos grupėje nr. 3.

Vidutinė Interleukino (IL) 6 ir 8 koncentracija prieš donoro širdies išėmimą iš įrenginio buvo reikšmingai mažesnė 2 grupėje, lyginant su 1 grupe, 5,54 lyginant su 0,29, ir 7,0 grupėje nr. 3. Priešoperacinės IL-6 ir 8 koncentracijos nesiskyrė. Vidutinė veninio laktato koncentracija pradedant perfuziją buvo 1,7 mmol/l 1 grupėje, 5,3 mmol/l 2 grupėje, bei 2.0 mmol/l grupėje nr. 3. Vidutinė veninio laktato koncentracija baigiant perfuziją buvo 8,7 mmol/l 1 grupėje, 4.1 mmol/l 2 grupėje, bei 7.7 mmol/l grupėje nr. 3, atitinkamai. Visose grupėse išgyvenamumas buvo 100% trisdešimtąją dieną po transplantacijos.

Išgyvenamumas ir transplantuoto organo nepakankamumas

Visi pacientai buvo gyvi 30 dieną po transplantacijos abiejose grupėse. Pradinis transplanto nepakankamumo dažnis buvo 3% (n=1) kraujo kardioplegijos grupėje ir 8% (n=1) standartinio gydymo grupėje. Vienam pacientui iš standartinio gydymo grupės išsivystė dešiniojo skilvelio disfunkcija praėjus 1 mėnesiui po implantavimo; taip atsitiko ir vienam pacientui kraujo kardioplegijos grupėje.

2 lentelė. Audinio miokardinis dopleris (septintąją dieną), trukmė intensyviosios terapijos skyriuje, inotropų dozė ir EKMO taikymo trukmė

	Kraujo kardioplegijos grupė (n=30)	***Custodiolo grupė (n=13)***	***p vertė***
S^{1}LV šoninis (cm/s) TMD	10 ± 1,6	9,2 ± 1,8	0,73
S^{1}LV medialinis (cm/s)TMD	8,93 ± 1,35	8,58 ± 1,6	0,60
S^{1}RV (cm/s)TMD	10 ± 2,66	8,95 ± 1,96	0,36
KSIF (%)TMD	61,4 ± 2,31	57,5 ± 7,9	0,001
Trukmė intensyviosios terapijos skyriuje (dienos)	11,7 ± 10,3	19,6 ± 13	0,44

	Kraujo kardioplegijos grupė (n=30)	***Custodiolo grupė (n=13)***	***p vertė***
Inotropų dozė (mcg/kg/minutė IV)			
24 valandos *Dobutaminas* *Milrinonas*	6,5 ±1,9 1,75 ±1,25	6,5 ± 1,7 1,8 ± 1,3	0,74 0,90
48 valandos *Dobutaminas* *Milrinonas*	6,0 ± 2,6 0,2*(n=1)* 3,6 (±0,8)	6,8 ± 1,3 0,3 (n=1) 5,4 (±2,7)	0,39
72 valandos *Dobutaminas* *Milrinonas*	0,2 (n=1)	0,2 (n=1)	0,05
EKMO trukmė (h)	*29,5 ± 28,4* *n=6*	78,4 ±89 n=8	0,002

TMD – audinio miokrdinis dopleris; S^1LV – miokardo greitis, siejamas su kairiojo skilvelio izovolumininiu susitraukimu; S^1RV – miokardo greitis, siejamas su dešiniojo skilvelio izovolumininiu susitraukimu; KSIF – kairiojo skilvelio išstūmimo frakcija; ECMO – ekstrakorporinė membraninė oksigenacija.
Duomenys yra išreikšti kaip vidurkis ± *standartinis nuokrypis.*

2.2. Vieno tyrimų centro patirtis, vertinant poveikį, kurį padaro normoterminė ex vivo apsauga naudojant organų apsaugos sistemą lyginant su šaltu saugojimu, kuomet širdis yra saugoma ilgą laiką. Nauda pacientams, kuriems, pereinant prie transplantacijos, yra naudojama mechaninė kraujo apytakos parama

Tyrimo struktūra ir dalyviai

Nuo 2011 metų, kuomet buvo pradėta širdies nepakankamumo gydymo programa, 353 pacientams implantuotas dirbtinis kairysis skilvelis. 35 iš jų (10%) buvo atlikta širdies transplantacija (13). Tarp 2012 ir 2018 metų, mes atlikome retrospektyvinį vieno centro perspektyviai surinktų duomenų tyrimą. Visi pacientai, kuriems buvo atlikta širdies transplantacija su MCS naudojant OCS širdį buvo įtraukti į šį tyrimą. Įtraukimo kriterijai buvo bent 18 metų amžius bei tai, kad pacientas buvo įtrauktas į tyrimų institucijos transplantacijos laukimo sąrašą. Tyrimui pritarė mūsų institucijos atsakingas etikos komitetas. Visi pacientai pateikė savo informuotą sutikimą raštyu, kad jie sutinka būti šio tyrimo dalimi ir leidžia, kad jų duomenys būtų naudojami

tyrimui. Tyrimo aprėpties pabaiga buvo išgyvenamumo 30 dienų laikotarpis, širdies išsaugojimo laikas (išemijos laikas, OCS perfuzijos laikas, ir laikas, kurį širdis buvo išimta iš kūno), inotropinių medikamentų infuzijos trukmė ir dozė, gydymo trukmė intensyviosios terapijos skyriuje, mechaninės pagalbinės kraujotakos palaikymo poreikis po širdies transplantavimo, ir nepageidaujamos baigtys, susijusios su širdies veikla.

Statistinė analizė

Rezultatai yra išreikšti kaip vidurkis ir standartinis nuokrypis arba mediana ir tarpkvartilinis diapazonas (nuolatiniams kintamiesiems), bei suskaičiavimui su procentais (kategoriniams kintamiesiems). Kur tik įmanoma, buvo naudojamas dviejų mėginių nepriklausomas t-Testas, siekiant palyginti vidurkius. Naudojamos baigčių vertės buvo 30 dienų intervalas. Statistinė analizė buvo atliekama, panaudojus STATA, versija 12 (Stata Corp, Teksasas, JAV).

Rezultatai

Donorų ir recipientų populiacijos

Pastebėta tendencija, kad donorų amžius buvo truputėlį didesnis OCS grupėje nei CS grupėje (41,3±9,3 lyginant su 38,3±11,5 metai; p=0,2), tarp kurių buvo 92% lyginant su 70% donorų vyrų. Lyčių profilių neatitikimas OCS grupėje buvo 3 vyrai donorai 3 moterims recipientėms, o CS grupėje buvo 3 moterys donorės, kurių širdys buvo transplantuotos trims vyrams recipientams. 19 donorų (76%) lyginant su šešiais (60%) mirė nuo spontaniškos galvos smegenų kraujosruvos, 6 (24%) lyginant su 3 (30%) mirė nuo smegenų kraujotakos sutrikimo OCS lyginant su CS grupe, atitinkamai, o (10%) pacientas CS grupėje mirė nuo traumos.

Nepastebėta reikšmingo skirtumo recipientų amžiaus atžvilgiu, lyginant OCS ir CS grupes (38,6±11,9 lyginant su 43,6±12,6 metais; p=0,2), ir 80% (n=20) lyginant su 100% (n=10) buvo vyrai, atitinkamai. Visi pacientai pasižymėjo pažengusiu širdies nepakankamumu (64% lyginant su 70% NICM) OCS grupėje, lyginant su CS grupe. IMPACT vertės atskleidė tendenciją, kad OCS grupėje pastebėta didesnė tendencija, kad mirtis gali ištikti per vienerių metų laikotarpį (14,2 lyginant su 10,8%; p=0,083).

OCS grupėje 20 recipientų buvo naudojamas pagalbinis KSPĮ prietaisas (HeartWare-3, HeartMate II- 10, HeartMate 3- 4, HeartMate 3+EKMO- 1, HeartMate 3 + RVAD-1, RVAD + LVAD (trumpalaikis biVAD Levitronix)-1) ir EKMO-2, visa dirbtinė širdis (CARMAT)-3, palyginus su CS grupe, kur

10 recipientų buvo naudojamas pagalbinis KSPĮ prietaisas (HeartWare-2, HeartWare+RVAD-1, HeartMate II-4, HeartMate 3 -2, HeartMate 3 + RVAD (Levitronix)-1). Iš tų 20 recipientų OCS grupėje, kuriems buvo naudojamas pagalbinis KSPĮ prietaisas prieš operaciją, šeši OCS pacientai (palyginus su dviem CS pacientais) tuo metu patyrė sunkią „siurblio kišenės“ infekciją transplantacijos metu. Dviem pacientams OCS grupėje ir vienam pacientui CS grupėje buvo teikoma inotropinų medikamentų infuzija bei, MCS atveju, prieš operaciją buvo atitinkamai skiriamas milrinonas (0,1 lyginant su 0,15 mcg/kg/min) ir dobutaminas 7 lyginant su 6 mcg/kg/min.

3 lentelė. Donorų ir recipientų savybės bei rizikos faktoriai

Donorų savybės			
	OCS (n=25)	CS (n=10)	P vertė
Amžius (metai)	41,3±9,3	38,3±11,5	0,2
Vyriška lytis, n (%)	23(92)	7(70)	0,9
Mirties priežastis, n (%)			
Cerebrinė hemoragija	19 (76)	6 (60)	0,9
Ūmus smegenų kraujotakos sutrikimas	6 (24)	3 (30)	0,9
Trauma		1 (10)	
KSIF mediana (diapazonas)	58(52-63)	60(54-65)	
Recipientų savybės			
Amžius (metai)	38,6±11,9	43,6±12,6	0,2
Vyriška lytis, n (%)	20(80)	10(100)	0,7
NICM n (%),	16(64)	7(70)	0,9
Ankstesnių sternotomijų skaičiaus mediana	2(1;5)	1(1;3)	0,1
PVR > 4WU	4(16%)	4(40%)	0,6
Mechaninė parama kraujo apytakai			
KSPĮ, n (%)	20(80)	10(100)	
EKMO, n (%)	2(8)		
CARMAT, n (%)	3(12)		

Duomenys yra išreikšti kaip vidurkis ± *standartinis nuokrypis*

NICM – neišeminė kardiomiopatija; KSIF – kairiojo stulpelio išstūmimo frakcija; KSPĮ – kairiojo stulpelio pagalbinis įtaisas; ECMO – ekstrakorporinė membraninė oksigenacija; CARMAT – visiškai dirbtinė širdis; PVR – plaučių kraujagyslių pasipriešinimas; WU – Woodo vienetas.

4 lentelė. Išeitys

	OCS (n=25)	CS (n=10)	P vertė
Visas išeminis laikas (minutės)	74,6 ± 13	210 ± 23	<0,001
OCS perfuzijos laikas (minutės)	348,4 (132;955)	NA	NA
Vidutinis buvimo ne kūne laikas (minutės)	423 ± 67	210 ± 23	0,002
Šiltosios išemijos laikas (minutės)	53,4±12,3	60,2±11,5	0,8
MCS po Htx (%)	24	60	0,02
DKA laikas (minutės)	279±87	256±69,2	0,4
Inotropinių medikamentų infuzijos trukmė (valandos)	103 (47; 465)	236 (153;423)	0,1
Gydymo intensyviosios terapijos skyriuje laikas (dienos)	16 (3;50)	20 (12; 52)	0,3
30 dienų išgyvenamumas (%)	96	100	0,5

DKA – dirbtinė kraujo apytaka

Intraoperacinė ir pooperacinė eiga bei išgyvenimas

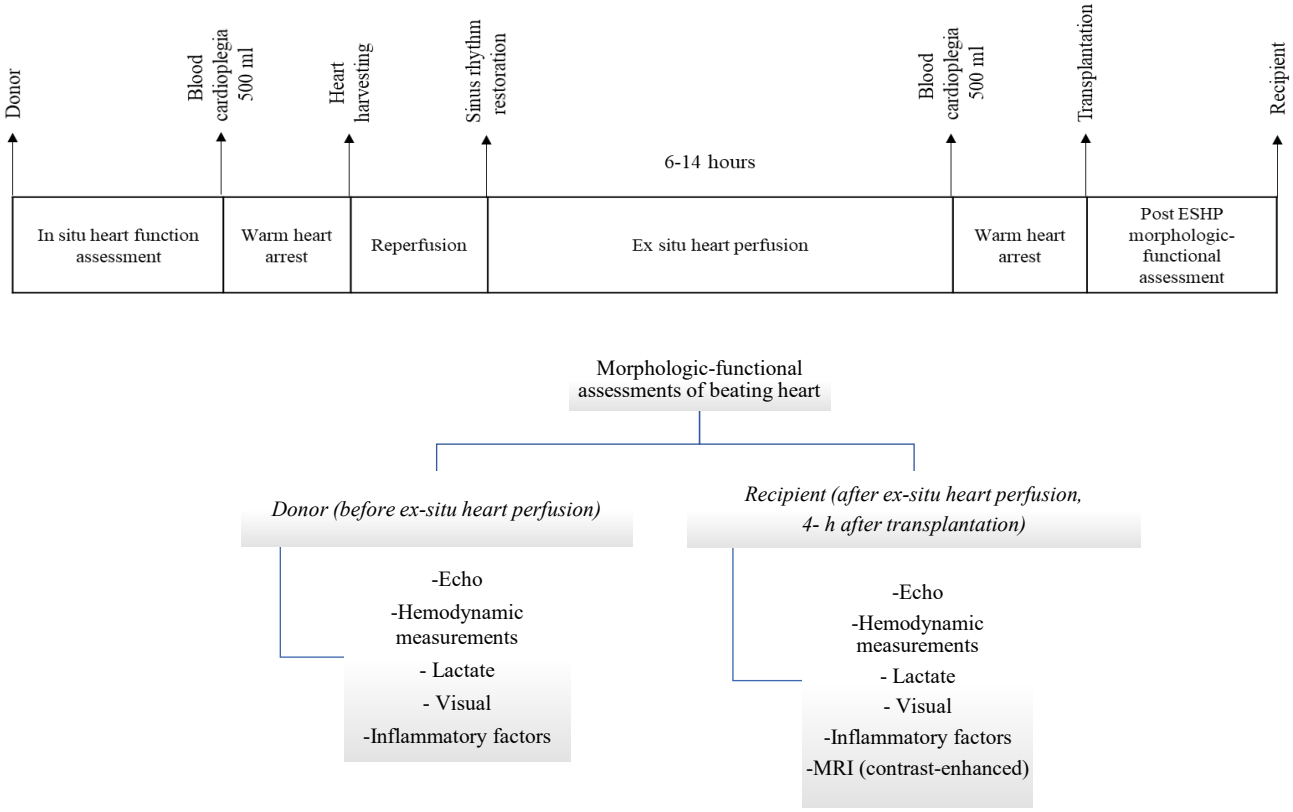
Vidutinis (standartinis nuokrypis) šiltosios išemijos laikas širdies implantacijos metu buvo 53,4(12,3) lyginant su 60,2 (11,5) minutėmis; p vertė buvo 0,8 tiek OCS, tiek ir CS grupėje. Visas alografto išemijos laikas buvo 74,6(13) lyginant su 210(23) minutėmis; p vertė buvo <0,001. Vidutinis dirbtinės kraujo apytakos laikas buvo 279(87) lyginant su 256 (69) minutėmis; p vertė buvo <0,4. Šeši pacientai (24%) OCS grupėje (vienam pacientui pasireiškė RV disfunkcija, vieną pacientą ištiko sepsis, o kitiems keturiems įvyko biventrikulinė disfunkcija) bei šeši pacientai (60%) CS grupėje (du pacientus ištiko sepsis, o kitiems keturiems įvyko biventrikulinė disfunkcija) prireikė ekstrakorporinės gyvybės palaikymo, kadangi atjungimas nuo dirbtinės kraujo apytakos buvo nesėkmingas (p=0,02). Visais atvejais alografto funkcija pagerėjo, ir galimybės atjungti EKMO mediana buvo 4 dienos, išskyrus vieną pacientą OCS grupėje, kuriam išsivystė dešinio skilvelio disfunkcija. Vidutinė inotropinių medikamentų infuzijos trukmė 103(47; 465) vs 236(153; 423) valandos; p=0,1; vidutinės inotropinių medikamentų dozės per pirmąsias 24 valandas buvo dobutaminas 7,1(1,6) lyginant su 8,5(1,9) mcg/kg/min; p vertė <0,05, milrinonas 0,2(0,3) lyginant

su 0,25(0,4) mcg/kg/min; p vertė 0,7 OCS ir CS grupėse, atitinkamai. Vidutinė gydymo trukmė intensyviosios terapijos skyriuose buvo 16 dienų (3; 50) OCS grupėje ir 20 dienų (12; 52) CS grupėje; p=0,3. Inotropinių medikamentų infuzijos trukmė ir dozė buvo reikšmingai mažesnės OCS grupėje.

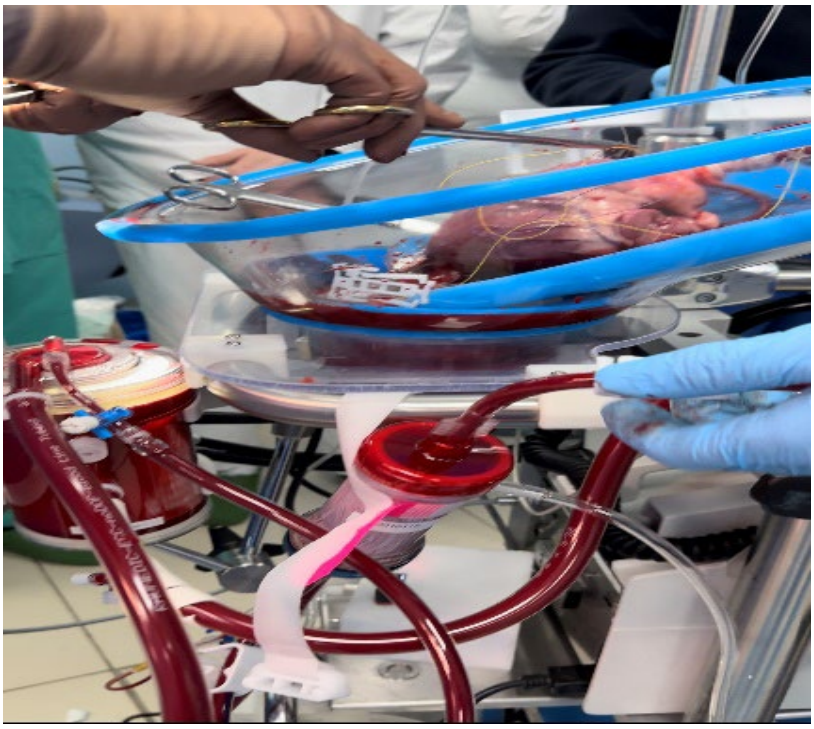
30 dieną po implantacijos visi pacientai buvo gyvi CS grupėje ir buvo 96% gyvų pacientų OCS grupėje (p=0,5).

2.3. Plakančios širdies apsaugos taikant normoterminę ESŠP paremtą ECMO technologija (sukurta mūsų) poveikis transplanto funkcijai po transplantavimo pasiremiant porcininiu modeliu

Buvo atrinktos suaugusios naminės kiaulės (patinai), sveriančios 90–100 kilogramų, kad būtų atlikta 20 normoterminių ESŠP, remiantis EKMO technologija. Kad įvertinti galutinę saugomo organo funkciją, buvo atlikti penki ortotopinės širdies transplantacijos eksperimentai. Mūsų institucijos gyvūnų rūpybos komitetas pritarė eksperimento protokolui, ir su gyvūnais buvo elgiamasi, remiantis Laboratorijos gyvūnų priežiūros principais, pritaikytais medicininiams tyrimams. Taip pat buvo remiamasi Laboratorijos gyvūnų priežiūros ir naudojimo gairėmis, kurias sukūrė Laboratorijos gyvūnų resursų institutas. Šį tyrimą patvirtino Nacionalinis akademinis širdies chirurgijos centras ir jo Gyvūnų priežiūros ir panaudojimo komitetas (Protokolo Nr. 2022/01-121).



2 paveikslas. Supaprastinta eksperimento plano ir protokolo schema



3 paveikslas. Normoterminės ex situ širdies perfuzijos, naudojant nepaliaujamą perfuzato hemofiltraciją, modelis

Donorinės širdies paėmimo procedūra

Anestezija ir monitoringas

Prieš operaciją kiekvienai kiaulei 24 valandas buvo neduodama maisto bei 12 valandų buvo neduodama vandens. Gyvūnai buvo apžiūrimi, kad įvertinti jų sveikatos būklę. Į raumenis buvo suleidžiama Atropino sulfato (0,05mg/kg), acepromazino maleato (0,05 mg/kg), ir ketamino sulfato hidrochlorido (15 mg/kg), kuris buvo panaudojamas sukelti anesteziją. Thiopentalis (10–15 mg/kg) buvo įvedamas per intraveninį kateterį bendrajai anestezijai sukelti. Kiekviena kiaulė, kuriai buvo atlikta anestezija, buvo intubuojama endotrachėjiniu vamzdeliu ir mechaniškai ventiliuojama. Bendrosios anestezijos palaikymui buvo skiriamas isofluranas (0,5%–4%). Kiekvienmam gyvūnui buvo infuzuojama 3 litrai kristaloidinio tirpalo (Sterofundino) kad kompensuoti pradinę hipovolemiją bei pasiruošti artėjančiam kraujo netekimui. Procedūros metu buvo vykdomas monitoringas: invazinis arterinio kraujo spaudimo stebėjimas, centrinio veninio spaudimo stebėjimas, kūno temperatūros registravimas, šlapimo išskyrimo kiekis, bei odos elektrokardiografija. Buvo naudojamas Swan-Ganz

plaučių arterijos kateteris matuoti donorinio ir recipiento gyvūno hemodinamiką. Visais atvejais buvo atliekama echokardiografija.

Chirurginė procedūra

Buvo atliekama vidurinioji sternotomija ir atveriamas perikardas. Gyvūnas būdavo pilnai antikoaguliuojamas su intraveniniu heparinu 300 tarptautinių vienetų (IU)/kg, kad pasiekti aktyvuoto krešėjimo laiką didesnį nei 480 sekundžių. Į kylančiąją aortą buvo įvedama dviejų angų adata ir užtvirtinama 4-0 polypropyleno piniginės tipo siūle. Per šią adatą buvo paimama 500mL normoterminio oksigenuoto kraujo, kuris buvo naudojamas kaip kraujo komponentas trumpo poveikio kraujo kardioplegijos tirpalui. Prieš širdies sustojimą, buvo paimama 1000 ml kraujo kaip parengiamojo tirpalo normoterminei ESŠP sistemai, tuomet aorta būdavo užspaudžiama, ir būdavo įvedamas kardiopleginis tirpalas. Po visiško širdies sustojimo, širdis būdavo paimama ir parengiama ESŠP.

Širdies transplantacijos atvejais dirbtinei kraujo apytakai atlikti būdavo kaniuliuojam aorta (22F) ir bikavalinė kaniuliacija (26 F ir 28F) kad atlikti veninį drenažą.

Širdies išėmimas ir parengimas

Surinkus pradinių matavimų duomenis, buvo įvedama viena dozė normoterminės kraujo kardioplegijos (10–15mL/kg., 36 °C) pateikta keturiomis dalimis donoro kraujo sumaišytomis su viena dalimi kristaloidinio tirpalo, panaudojant dviejų angų adatą, įvestą į kylančiąją aortą, o aortos užspaudimas būdavo atliekama distaliau.

Tuomet širdis būdavo išpjaunama ir saugoma maždaug 15–20 minučių, kol buvo ruošiamasi prijungti ją prie normoterminės ESŠP sistemos. ESŠP sistemoje oksigenuotas kraujas buvo perleidžiamas centrifugine pompa į aortą (atgaline kryptimi), perfuzuojant vainikines arterijas. Kraujas, sugrįžęs nuo vainikinio sinuso, buvo pašalinamas tik per plaučių arterijos kaniulę. Buvo stebima vainikinių arterijų kraujotaka (VAK) bei kraujo dujos. Širdies padėtis ESŠP sistemoje buvo tokia, kad aorta būtų atsukta aukštyn, o galinė širdies pusė remtųsi į padėklą, kuris buvo dalis mūsų kiekvienam atvejui specialiai gaminamo integruoto kraujo rezervuaro.

Mus domino tokios vertės kaip vidutinis laktato pokytis, išemijos laikas, perfuzijos laikas, hemodinaminiai matavimai, SDV parametrai ir širdies morfologija.

Perfuzijos sistemos struktūra

Pirminiai perfuzijos grandinės komponentai EKMO sistemoje buvo oksigenatorius, centrifuginė pompa, 3/8 vamzdeliai (Medos, Medizintechnik AG, Vokietija), kurie būdavo sujungiami su specialiu uždaru integruotu rezervuaru (pagamintu mūsų institucijoje) bei tėkmės matuokliu (Medos, Medizintechnik AG, Vokietija), bei ultrafiltravimo įrenginiu (Medos, Medizintechnik AG, Vokietija). Tėkmės per centrifuginę pompą bei tirpalo tekėjimo greičiai ESŠP sistemoje buvo reguliuojami taip, kad būtų palaikomas vidutinis arterinis slėgis tarp 60 ir 70 mmHg bei kraujo tėkmė nuo 500 iki 800 mL/min. Slėgis aortoje buvo matuojamas per slėgio keitiklį, kuris buvo prijungtas prie čiaupo ant aortos šaknies kaniulės.

Pumpuojamas vainikinio sinuso kraujas būdavo surenkamas į integruotą rezervuarą nupilant naudojantis sunkio jėga. Perfuzato temperatūra buvo reguliuojama, naudojantis EKMO aparato kaitintuvu-aušintuvu. Širdys būdavo perfuzuojamos nuo 24 valandas, o tuomet jų veikla būdavo sustabdoma normotermine kraujo kardioplegija ir persodinamos gyvūnams recipientams.

Perfuzato parengimas

ESŠP sistema buvo paruošiama krauju paremtu prerfuzatu iš kiaulės donorės, kuris būdavo atskiedžiamas, kad pasiekti vidutinį 22% hematokritą (2% standartinė paklaida, toliau žymima SE), ir visas tūris siekė maždaug ~ 1 L. Oksigenatorius palaikė perfuzatą normotermijoje (buvo nustatyta 3T HCU, kad pradinė perfuzato temperatūra būtų 36°C), o dujų apykaita buvo palengvinama, naudojant 0,1–0,9 litro per minutę (LPM) atmosferos oro srauto. Į rezervuarą būdavo įvedama maždaug 50 mL kristaloidinio izotoninio tirpalo Sterofundino (B. Braun Melsungen AG, Vokietija) ir Levosimendano 2.5 mg (1 mL), o tuomet recirkuliuojama. Tuomet iš sistemos būdavo pašalinamas oras, ir cirkuliacija būdavo pradedama žemomis apsukomis. Vienos krypties vožtuvas būdavo įterpiamas ne toliau kaip 3/8 arterinės linijos, kad palaikyti aortos slėgį, neleisti vykti atgaliniam tekėjimui bei išvengti embolizmo pavojaus iš KS.

Farmakologinė parama buvo teikiama per kontroliuojamą (ir pagal poreikį taikomą) epinefrino, gliukozės, insulino, nitroprusido (Goregaon, Mumbajus, Indija) ir kalio bikarbonato infuziją.

Aktyvus nulinės pusiausvyros ultrafiltravimas (Medos, Medizintechnik AG, Vokietija) su Sterofundinu (B. Braun Melsungen AG, Vokietija) kaip plazmos pakaitalo tirpalu buvo taikomas, siekiant išlaikyti hematokritą ir

elektrolitus tikslinės normos ribose. Kalcis buvo pakeliamas iki 0.80 mmol/L, o pH vertė buvo reguliuojama su $NaHCO_3$ pagal poreikį.

Recipiento procedūra

Atlikome ortotopinę širdies transplantaciją 5 gyvūnams. Aprašant glaustai, sedacija, anestezija ir stebėjimas buvo atliekami taip pat kaip ir su gyvūnu donoru. Po viduriniosios sternotomijos, atverdavome širdį ir pagrindinius kraujotakos elementus, ir tuomet apjuosdavome viršutiniąją ir apatiniąją tuščiąsias venas. Sisteminė antikoaguliacija būdavo pasiekiama su intravenine 30000 U heparino injekcija. Kylančiosios aortos bei bikavalinės kaniuliacijos būdavo panaudojamos, kad pradėti dirbtinę kraujo apytaką (DKA). Normotermija būdavo palaikoma, o tėkmės greitis būdavo reguliuojamas, kad vidutinį arterinį slėgį palaikyti virš 50 mmHg. Po skersinio aortos užspaudimo, recipiento širdis būdavo išpjaunama, ir būdavo patikrinami ir palyginami anastomozės kraštai. Po 6–14 valandų ESŠP, donoro širdis būdavo užpilama pradine doze normoterminės kraujo kardioplegijos ir išimama iš perfuzijos sistemos. Širdies implantavimui būdavo naudojama standartinė bikavalinė anastomotinė technika. Buvo taikoma tokia seka: LA, plaučių arterija, ir kylančioji aorta. Kardiopleginė apsauga susidėjo iš 500mL mišinio santykiu 4:1 iš kraujo ir kristaloidinio tirpalo, kuris būdavo įvedamas 36°C temperatūroje kas 20 minučių. Prieš atleidžiant aortos spaustuką, buvo įvedama 500mg metilprednizolono. Kuomet aortos spaustukas jau būdavo pašalintas, širdis būdavo 60 minučių reperfuzuojama ir atjungiama nuo DKA. Atjungimas buvo laikomas sėkmingu, jei gyvūno sistolinis arterinis spaudimas išlikdavo 60 mmHg per 4 valandas po DKA nutraukimo. Vazoaktyvi Dobutamino (5 μg/kg/min) bei Norepinefrino (0.1 μg/kg/min) infuzija buvo naudojama atjungimo nuo CPB procesui palengvinti. Hemodinamikos ir biventrikulinis funkcinis vertinimas būdavo atliekami, praėjus 4 valandoms po perfuzijos. Tuomet eksperimentas būdavo baigiamas.

Miokardo funkcija

Metabolinių parametrų matavimas normoterminės ESŠP metu buvo naudojamas įvertinti miokardinei funkcijai prieš transplantavimą. Kas 30 minučių buvo imami arterinio ir veninio kraujo mėginiai iš aortos bei plaučių arterijos kraujo dujų analizei atlikti. Buvo matuojami ir registruojami tokie parametrai kaip pH, laktato lygis, pO_2, pCO_2, hemoglobinas, hematokritas, deguonies prisotinimas ir elektrolitai (ABL 800, Flex, Radiometer, Danija), IL-1, IL-6, ir IL-10. Buvo matuojama miokardo laktato ekstrakcija, vainikinių kraujagyslių pasipriešinimas (VKP), ir miokardinis deguonies suvartojimas (MVO_2).

Pirminė šio tyrimo baigtis buvo po 6–14 valandų NESHP sėkmingai transplantuotų širdžių skaičius. Sėkmingumo kriterijai buvo apibrėžti kaip: atjungimas nuo širdies ir kraujagyslių šuntavimo (CPB) be vazopresoriaus arba su minimalia jo doze (0,1 g/kg/min Norepinefrino) ir/arba inotropo (5g/kg/min Dobutamino) per 4 iš karto sekančias valandas. Antrinės baigtys buvo VKP per NESHP, laktato koncentracija, įgytas svoris, hemodinaminiai parametrai prieš širdies išpjovimo ir po atskyrimo nuo CPB bei histologinių sužeidimų vertės.

Statistinė analizė

Kategoriniai kintamieji buvo išreikšti kaip grupių procentai, nuolatiniai kintamieji buvo išreikšti kaip vidurkis +/− standartinė paklaida arba mediana su interkvartiliniu diapazonu, priklausančiu nuo bendrojo pasiskirstymo. Normaliai pasiskirstę nuolatiniai kintamieji yra palyginami, naudojant pakartotinių matavimų dispersinę analizę arba porinį t-testą ten, kur tinkama (Minitab 15). Rezultatai pateikiami kaip vidurkis ± standartinė paklaida. p-vertė <0.05 buvo laikoma statistiškai svarbia.

Rezultatai

In vivo matavimai

Gyvūnų savybės, miokardinės funkcijos žymos, fiziologiniai parametrai ir elektrolitų būklė in vivo buvo apibendrinti kaip normalusis skirstinys.

5 lentelė. Demografiniai duomenys ir miokardo funkcijos, fiziologinių parametrų bei elektrolitų statuso in vivo matavimas prieš perfuziją

Demografija	*n=20*
	Vidurkis (SD)
Kūno masė (kg)	105 (5,6)
BSA (m^2)	7,7 (0,3)
Širdies masė (g)	308,4 (84)
Miokardinės funkcijos indeksai	
Indeksuotas miokardo deguonies suvartojimas (MVO_2) (ml O_2/min/100 g)	5,4 (1,3);
Širdies išstūmis (l/min)	5,8 (0,8)
Širdies indeksas (l/min/m^2)	3,2(0,4)
VAK (mL/min)	390 (0,06)
MAP (mmHg)	73(14,6)
Kairiojo skilvelio išstūmimo frakcija	59% (3)

Fiziologiniai parametrai	
Arterinis laktatas (mmol/L)	1,34 (0,59)
Hemoglobinas (mg/dL)	10,9 (1,22)
Gliukozė (mg/dL)	7,7 (2,01)
Temperatūra (°C)	36,5 (0,2)
pH	7,45 (0,08)
Elektrolitų statusas	
$Ca2^{+}$ (mmoL/L)	1,35 (0,07)
K^{+} (mEq/L)	3,91 (0,7)
Na^{+} (mEq/L)	143,4 (3,96)
$HCO3^{-}$ (mmoL/L)	27,3 (1,79)

VAK (mL/min); MAP (mmHg).

Ex situ tyrimas

Miokardinė funkcija: Vidutinė kairiojo skilvelio išstūmimo frakcija po HTx buvo 58% (±0,7) SDV parametrų tendencija ex situ perfuzijos metu ir po Tx buvo normali. Per daugiau nei 4 valandas po transplantacijos, nebuvo jokių atmetimo ar širdies disfunkcijos įrodymų. Vidutinis širdies išstūmis buvo matuojamas Swan-Ganz aparatu, ir širdies indeksas po HTx buvo 6,0 l/min (±0,7) ir 3,1(±0,2) l/min/m^2 atitinkamai. Vainikinė kraujo tėkmė, vidutinis arterinis slėgis (MAP) bei širdies plakimo dažnis buvo normos ribose. Visos atskirtos širdys pasižymėjo stabilia perfuzija, biocheminėmis bei histologinėmis savybėmis (vidutiniškai plona skilvelių miokardo atraiža x300 parodė miokardą su distropiniais pokyčiais kardiomiocituose; sunkumas buvo įvairaus lygio; fiksuotas serozinis endomiokarditas) perfuzijos sistemoje bei magnetinio rezonanso atvaizdavime. Vidutinė veninio laktato tendencija buvo normos ribose baigiant perfuziją. Prouždegiminiai parametrai pastebėti ex situ perfuzijos pradžioje ir pabaigoje. Ištirtoje grupėje širdims neišsivystė jokia kliniškai reikšminga edema ESŠP metu. Tai liudija menkas širdies masės išaugimas: 310 [235–500] gramų pradžioje, lyginant su 409 [265–610] gramais pabaigoje.

Fiziologiniai parametrai: Miokardinis deguonies suvartojimas (pirmosios dvi valandos: 5,2 (1,1); ESŠP pabaigoje, po 6–14 valandų: 4,1 (1,4) (ml O_2/min/100 g); vidutinio veninio laktato tendencija taip pat buvo normos ribose – perfuzijos pradžioje 1,4 SD 0,2 mmoL/L ir pabaigoje: 2,4 SD 0,2 mmoL/L, atitinkamai. Atskirtų kiaulių širdžių išemijos laikai ir perfuzijos laikai. Vidutinis (SD) išemijos laikas buvo 19,2 (±3,3) min. Vidutinis ex vivo perfuzijos laikas buvo 480 (±150) min. Sinusinio ritmo atstatymo laikas buvo 2,3 (5,7) minutės.

Perfuzato komponentai: Hemoglobinas ir gliukozė buvo stabiliai palaikomi per visą perfuzijos 30 minučių laikotarpį. Temperatūra buvo palaikoma 36 (SD 0,1) °C laipsnių lygyje ex situ perfuzijos metu. Perfuzatas taip pat buvo palaikomas normos ribose, laikantis pH=7,4 (SD 0,1) ex situ perfuzijos metu. Kalcio, kalio ir natrio ar bikarbonato koncentracija elektrolite perfuzate visą ex situ laikotarpį buvo normos ribose.

4. APTARIMAS

Per laikotarpį nuo 2012 iki 2020, buvo atliktos 82 širdies transplantacijos operacijos. Dėl geografinių Kazachstano Respublikos sąlygų (donorų širdis dažnai tenka atgabenti iš nuošalių regionų, nutolusių daugiau nei 1000 kilometrų), buvo sukurta organų apsaugos sistema, skirta širdžių atgabenimui ir konservavimui.

Savo tyrime mes naudojome sveikų kiaulių grupę be neurologinio mirties apibrėžimo, kad išvengti miokardo disfunkcijos ir pakenkimo. Vietoj to mes susitelkėme į širdies perfuziją už kūno ribų, neįtraukdami gretutinio faktoriaus, tokio kaip temperatūra ar išemijos-reperfuzijos pažeidimas ir hormonų disbalansas. Be to, dėl trumpo išemijos laiko, kuris yra priimtinas įprastiniu širdies chirurgijos atveju, šiame tyrime mes vengėme naudoti šalto kristaloidinio kardiopleginio tirpalo tam, kad būtų galima susitelkti į ESŠP sukeliamus pokyčius.

OCS įrenginys sustabdydamas širdį du kartus panaudoja šaltą kristaloidinį kardiopleginį tirpalą. Todėl vis dar yra ginčijamasi, kadangi pasiruošimo laikotarpis apima gana mažai laiko (apytiksliai tik 20 minučių), o šioje strategijoje atsiranda papilomas temperatūros ir reperfuzijos bei išemijos pažeidimo epizodas, dėl kurio padidėja pavojus pakenkti audiniams; be to, gali išsivystyti uždegimas arba edema. Tokiu būdu NESHP ECMO technologija, naudojanti krauju paremtą širdies apsaugą, gali pasiūlyti patikimesnį būdą širdžiai išsaugoti.

Mūsų tyrimas, laikant plakančią širdį normoterminiame ESŠP, remiantis EKMO technologija, yra neveikiantis širdies perfuzijos modelis. Hatami et al. pastebėjo, kad kur kas geresnis širdies funkcijos apsaugojimas būdavo pasiekiamas tais atvejais, kuomet širdis būdavo perfuzuojama pusiau fiziologinėje širdies talpykloje, kurioje darbiniu režimu buvo palaikomas spaudimas (14). Šio širdies apsaugos efekto priežastis kol kas dar nėra nustatyta. Funkcinis donoro širdies vertinimas ESŠP metu abiem režimais yra apribotas, kadangi trūksta tam tinkamos įrangos. Be to, kad ir koks perfuzijos

režimas būtų naudojamas, ex situ perfuzuotose širdyse saugojimo metu išsivystydavo žymi edema, o šis faktorius taip pat gali kliudyti organo įvertinimui. Jo vaidmuo įtakojant ESŠP sąlygas yra nežinomas, kadangi kol kas yra nežinoma, kas fiziologiškai apsprendžia šį faktorių. SDV koreliacija ESŠP aplinkoje su puikia miokardo veikla po transplantavimo atskleidžia, kad strategija turi perspektyvų ateityje. Vietinis širdies sienelių susitraukimo greitis nėra vietinės funkcijos rezultatas, kadangi segmentus įveiklina kaimyniniai miokardo segmentai. Tokiu būdu greičio skirtumai ir greičio gradientas yra pagrindinis regioninio susitraukimo matas, ir šis faktorius gali tapti svarbiausiu ESŠP vertinimo procese. SDV mechanizmas siekiant geresnio funkcinio širdžių išsaugojimo, kuomet širdis yra perfuzuota veikiančiu režimu, kol kas dar nėra apibrėžtas, ir mūsų grupė šiuo metu tyrinėja šį mechanizmą. Kairiojo skilvelio SDV ESŠP metu palaipsniui mažėjo, ir po transplantacijos sugrįždavo iki normalaus/pradinio lygio. Šis parametras išlikdavo stabilus 4 valandas po transplantacijos, o tuomet būdavo selektyviai nutraukiamas jo stebėjimas. Tokiu būdu normoterminis ESŠP paremtas EKMO technologija neveikiančiame širdies modelyje suteikia saugų ir efektyvų atskirto organo išsaugojimą.

Įvertinus dvi skirtingas miokardo apsaugos technologijas, atliekant širdies paėmimą ir pergabenimą, naudojant OCS principą, galima pateikti keletą išvadų:

1. Kardioplegijos ir kondicionavimo metodas yra saugus miokardo apsaugos metodas, kuomet donoro širdį reikia paimti geografiškai nutolusioje vietovėje bei išsaugoti ją ex situ perfuzijos sąlygomis.
    a. Širdies ex situ perfuzijos pabaigoje laktato kitimo tendencijos, inotropinių medikamentų dozė bei sinusinio ritmo atstatymas plakančios širdies režimu buvo statistiškai reikšmingai palankesnės, lyginant su standartiniu statišku šaltu širdies saugojimu.
    b. Ex situ širdies perfuzijos Interleukino-6 ir Interleukino-8 koncentracija perfuzijos pabaigoje buvo reikšmingai mažesnė kraujo kardioplegijos grupėje, lyginant su standartinės apsaugos grupe.
    c. Kraujo kardioplegijos grupėje vidutinė EKMO palaikymo trukmė buvo mažesnė, lyginant su standartinės apsaugos grupe.
2. Normoterminės ex vivo apsaugos, naudojant organų apsaugos sistemą, taikymas yra pranašesnis nei šaltos apsaugos taikymas, kuomet širdį reikia išsaugoti ilgą laikotarpį, o recipiento būklė yra itin sudėtinga, pereinant prie transplantacijos naudojant mechaninės cirkuliacijos pagalbą.

a. Vidutinis visas išemijos laikas konservavimo laikotarpiu buvo statistiškai reikšmingai didesnis šalto saugojimo grupėje, lyginant su OCS grupe: 210 (23) lyginant su 74,6 (13) minutėmis, atitinkamai.
b. Buvo nustatytas reikšmingas skirtumas visame širdies buvimo ne kūne laike, lyginant OCS grupę 423(67) su šalto saugojimo grupe 210(23) minučių (p=0,002), atitinkamai.
c. OCS grupėje alografas išlaikė stabilias perfuzijos ir biochemines charakteristikas ex vivo perfuzijos metu.
d. Normoterminis ex situ alografto konservavimas transportavimo metu, naudojant organų apsaugos sistemą, yra naudingas, kuomet organą ilgą laiką reikia saugoti, išėmus iš kūno, lyginant su šaltuoju išsaugojimu, kuomet recipientui teikiama mechaninė kraujo apytakos parama.
e. Pacientų baigtys – išgyvenimas bei sunkūs su širdies veikla susiję sutrikimų atvejai per 30 dienų po implantavimo – buvo priimtinų verčių ribose. Tai atskleidžia, kad kraujo kardioplegija naudojant su OCS yra priimtina ir praktiškai taikytina strategija.

3. Plakančios širdies saugojimas normoterminiame ESŠP, paremtame EKMO technologija, taikant kiaulių modelį, atskleidžia, kad šis modelis yra itin saugus ir veiksmingas, vertinant transplantato funkciją po transplantavimo:
   a. Vidutinės veninio laktato ir interleukinų tendencijos perfuzijos laikotarpio pabaigoje pasižymėjo sumažėjusiu lygmeniu.
   b. Širdyse ESŠP metu neišsivystė klinikiniu atžvilgiu reikšminga edema.
   c. Miokardo deguonies suvartojimo ir echokardiografijos vertės perfuzijos metu buvo normos ribose.
   d. Histologijos tyrimo ir magnetinio rezonanso perfuzijos pabaigoje chararkteristikos buvo normalios.
   e. Vainikinė kraujo tėkmė, vidutinis arterinis slėgis bei širdies plakimo dažnis išliko normos ribose per visą ištęstą organo saugojimo laikotarpį.

5. FINANSAVIMAS

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6. PRAKTINĖS REKOMENDACIJOS

1. Organo unikalios būklės recipientams – tokiais atvejais kai implantuojama visiškai dirbtinė širdis arba kuomet teikiama ilgalaikė mechaninė kraujo apytakos parama, ex situ plakančios širdies perfuzija suteikia galimybę pristatyti organą, kuomet pristatymas užima daug laiko, bei saugiai jį transplantuoti.
2. Siekiant optimizuoti organų alokacijos logistiką bei kruopščiai parengti recipientus po prieš tai vykusios atviros širdies operacijos, turėtų būti naudojama ex situ širdies perfuzija.
3. Nepaliaujama perfuzato ultrafiltracija, palaikant kraujo tėkmę nuo 200 iki 300 ml/h ex situ širdies perfuzijos laikotarpiu turėtų būti naudojama siekiant pagerinti donoro širdies funkciją reikšmingai sumažinant uždegimą sukeliančių mediatorių cirkuliaciją.
4. Tirpalas, kuris turėtų būti naudojamas normoterminiam širdies veiklos sustabdymui, susideda iš kraujo ir kristaloidinio tirpalo, sudaryto 5:1 santykiu. Kristaloidinis tirpalas susidėjo iš KCl 4% (30mL), $MgSO_4$ 25% (10 mL), $NaHCO_3$ 4% (13 ml), Mannitolo 15% (6,5 ml), ir Lidokaino 2% (2 ml); visas kraujo tūris buvo 600 ml.
5. Mašininė normoterminė perfuzija suteikia galimybę, kad donoro širdis būtų paimta labiau nutolusiose vietose. Tuo būdu praplečiamas potencialių recipientų sąrašas, ir išauga tikimybė, kad pacientas gaus atitinkančią donoro širdį art.
6. EKMO technologija paremtas plakančios širdies normoterminis ESŠP gali būti naudojamas kaip saugus ir veiksmingas metodas gerinant ir vertinant transplantato funkciją per ilgalaikį organo konservavimą.

7. DISERTACIJOS TEMA PUBLIKUOTŲ STRAIPSNIAI

1. Kaliyev, Rymbay; Lesbekov, Timur; Bekbossynov, Serik, Bekbossynova, Makhabbat; Nurmykhametova, Zhuldyz; Novikova, Svetlana; Smagulov, Nurlan; Medressova, Assel; Faizov, Linar; Ashyrov, Zhanibek; la Fleur, Phillip; Samalavicius, Robertas Stasysa; Pya, Yuriy. Comparison of Custodiol vs warm blood cardioplegia and conditioning of donor hearts during transportation with the organ care system//Journal of Cardiac Surgery. John Wiley & Sons. eISSN:0886-0440/1540-8191. 2019, 34(10), 969–975. DOI: 10.1111/jocs.14162. [DB: Scopus, Science Citation Index Expanded (Web of Science)]

2. Kaliyev, Rymbay; Lesbekov, Timur; Bekbossynov, Serik; Nurmykhametova*, Zhuldyz; Bekbossynova, Makhabbat; Novikova, Svetlana; Medressova, Assel; Smagulov, Nurlan; Faizov, Linar; Samalavicius, Robertas; and Pya Yuriy. Heart transplantation of patients with ventricular assist devices: impact of normothermic ex-vivo preservation using organ care system compared with cold storage//Journal of Cardiothoracic Surgery. BioMed Central (United Kingdom). eISSN: 1749-8090. 2020, 15(1), 267. DOI: 10.1186/s13019-020-01367-w. [DB: Scopus, Science Citation Index Expanded (Web of Science)]
3. Kaliyev, Rymbay; Bekbossynov, Serik; Nurmykhametova*, Zhuldyz. Sixteen-Hour Ex Vivo Donor Heart Perfusion during Long-Distance Transportation for Heart Transplantation//Artificial organs. Wiley. eISSN: 1525-1594. 2019 – 43(3), p. 319-320. DOI:10.1111/aor.13425. [DB: Scopus, Science Citation Index Expanded (Web of Science)]
4. Medressova, Assel; Faizov, Linar; Kuanyshbek, Aidyn; Kaliyev, Rymbai; Myrzakhmetova, Gulzhan; la Fleur, Philip; Pya, Yuriy. Successful heart transplantation after 17 h ex vivo time using the Organ Care System—3 years follow-up. //Journal of Cardiothoracic Surgery. BioMed Central (United Kingdom). eISSN: 1749-8090. 2021. DOI:10.1111/jocs.15519. [DB: Scopus, Science Citation Index Expanded (Web of Science)]
5. Kaliyev, Rymbay; Lesbekov, Timur; Bekbossynova, Makhabbat; et al. Successful Heart Transplantation After 7 h of Cold Storage and Paracorporeal Donor Heart Resuscitation//Transplant International. Wiley-Blackwell (Denmark). eISSN: 1432-2277. 2022, 35, 10740. DOI: 10.3389/ti.2022.10740. [DB: Scopus, Science Citation Index Expanded (Web of Science)]

CURRICULUM VITAE

Kaliyev Rymbay Bolatovich
Head of operating department with circulatory support laboratory

Experience:
10/2011 – Perfusionist, National Scientific Cardiac Surgery Center, Nur-Sultan

Education:
JSC "Astana Medical University", Astana
2004 – 2010 Specialty "Pediatrics"
2010 – 2011 Internship in pediatric surgery

Additional Information:
Foreign languages: English – fluent

Trainings:
05/2004 – 08/2005 Perfusionist trained, National Research Center for Cardiac Surgery, Astana.
04/06/2005 - 06/06/2005 ECMO trained, National Research Center for Cardiac Surgery, Astana.
07/21/2005 Course Basic Life Support.
02/09/2005 Course Advanced Cardiac Life Support.
09/11/2005 - 27/11/2005 Course Extracorporeal Membrane Oxygenation.
12/09/2009 - 12/01/2010 Cardiac Surgery (Adult), National Research Center for Cardiac Surgery, Astana.
01/08/2010 - 01/12/2011 Transplantologist trained, National Research Center for Oncology and Transplantology, Astana.
03/13/2016 - 03/28/2016 Course Anesthesiology and Intensive Care.

Current Issues.

12/03/2018 - 16/03/2018 Course Perfusion Management during Anesthesia.
01/10/2018 - 05/10/2018 Training Course Extracorporeal Membrane Oxygenation, Kazakhstan, Astana

Professional experience:
08/15/2004 – present Perfusionist, National research cardiac surgery center, Astana

Duties: Carrying out perfusion assessment during cardiac surgery procedures (CABG with EC, CABG off pump, aortic surgery, correction of congenital and acquired valvular heart diseases, LVAD implantation, heart transplantation, TAVI).
Providing special care for patients with various conditions: prolonged respiratory support, ECMO, patients in terminal conditions, organ preservation with Organ Care System.

Personal qualities: Vigorous, hardworking, and communicable.

GYVENIMO APRAŠYMAS

Rimbayus Kaliyevas
Operacinio skyriaus su kraujo apytakos paramos laboratorija vadovas

Patirtis:
2011 spalis – Kraujo perpylimo specialistas, Nacionalinis mokslinis širdies chirurgijos centras, Nur Sultanas, Kazachstano Respublika

Išsilavinimas:
Astanos medicinos universitetas, Astana, Kazachstano Respublika
2004 – 2010: Specialybė: pediatrija
2010 – 2011: Pediatrijos chirurgijos stažuotė

Papildoma informacija:
Užsienio kalbos: Anglų – laisvai bendraujantis (C1-C2)

Stažuotės ir profesinė praktika:
2004 gegužė – 2005 rugpjūtis: Kraujo perpylimo praktika. Nacionalinis mokslinis širdies chirurgijos centras, Astana, Kazachstano Respublika.
2005 birželio 4d. – birželio 6d.: ECMO apmokymai, Nacionalinis mokslinis širdies chirurgijos centras, Astana, Kazachstano Respublika.
2005 liepos 21d.: Gyvybės palaikymo pagrindų kursai.
2005 rugsėjo 02d.: Kardiologinės pakraipos gyvybės palaikymo kursai pažengusiems.
2005 lapkričio 09d. – 27d.: Ekstrakorporinės membraninės oksigenacijos kursai.
2009 rugsėjo 12d. – 2010 sausio 12d.: Širdies chirurgijos apmokymai suaugusiems, Nacionalinis mokslinis širdies chirurgijos centras, Astana, Kazachstano Respublika.

2010 rugpjūčio 01d. – 2011 gruodžio 01d.: Transplantologijos studijos. Nacionalinis onkologijos ir transplantologijos tyrimų centras. Astana, Kazachstano Respublika.

2016 kovo 13d. – 28d.: Anesteziologijos ir intensyvios terapijos kursas.
Šiuolaikinės aktualijos:
2018 kovo 12d. – 16d.: Studijos „Perfuzija anestezijos taikymo metu“.
2018 spalio 01d. – 05d.: Ekstrakorporinės membraninės oksigenacijos kursai. Astana, Kazachstano Respublika.

Profesinė patirtis
Nuo 2004 rugpjūčio 15 iki dabar: Kraujo perpylimo specialistas. Nacionalinis mokslinis širdies chirurgijos centras, Astana/ Nur Sultanas, Kazachstano Respublika.

Pareigos: Atliekamas perfuzijos įvertinimas širdies chirurgijos procedūrų metu (koronarinės arterijos šuntavimas įvairiais metodais, aortos chirurgija, įgimtų ir įgytų širdies vožtuvo ligų korekcija, kairiojo skilvelio pagalbinio įrenginio implantavimas, širdies transplantavimas, transkateterinė aortos vožtuvo implantavimo procedūra).
Teikiama specialioji priežiūra įvairios būklės pacientams: ilgalaikė kvėpavimo parama, Ekstrakorporinė membraninė oksigenacija, terminalinės būklės pacientų priežiūra, organų konservavimas su organų apsaugos sistema.

Asmeninės savybės: energingas, darbštus ir lengvai bendraujantis.

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