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RĪGAS STRADIŅA UNIVERSITĀTE

Normunds Sikora

MYOCARDIAL PROTECTION AND CARDIOPULMONARY BYPASS IN PEDIATRIC CARDIAC SURGERY. BIOMECHANICAL AND STRUCTURAL PROPERTIES OF NEONATAL CORONARY ARTERIES

Summary of the Promotional Work
Speciality – Cardiac Surgery

Riga, 2013

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The promotional work was carried out in the Clinic for Pediatric Cardiology and Cardiac Surgery as well as in the Biomechanical Laboratory and Institute of Anatomy and Anthropology of Rīga Stradiņš University.

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The open session of the Promotional board of Surgery of Rīga Stradiņš University will be held on the 13th of June, at 15.00 in the Hippocrate auditorium, Rīga Stradiņš University, Dzirciema Street 16, Riga.

The thesis can be acquired in the Library of Rīga Stradiņš University and in the homepage: www.rsu.lv

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Topicality of the work

One of the most important issues in pediatric cardiac surgery is myocardial protection to have good outcomes. As it is crucial to ensure an adequate heart function after aortic occlussion and cardiopulmonary bypass, which is needed for the surgical repair of congenital heart diseases, the care must be taken regarding this matter.

As congenital heart diseases are various and most surgical repairs are complex, it is crucial to maintain an adequate myocardial protection, especially when operating on neonates. Besides it has to be remembered that the immature myocardium has structural and functional characteristics different from those of the adult myocardium. It is widely accepted that the immature heart has a greater tolerance to ischemia than the adult or mature heart, but it is more vulnerable to the increased amount of water, which is an issue for those using the crystalloid cardioplegia [*Jones et al.,2006; Milerova et al.,2010; Vinten-Johansen et al.,2000*].

Cardioplegia is full electromechanical standstill of the heart when cardioplegic solution is injected into coronary arteries with a pump. Tissue preservation is achieved by conserving energy stores through rapid arrest which decreases the ongoing metabolic rate and minimises changes induced by ischemia with specific protective agents. The cardioplegic solution contains high concentration of potassium, which usually goes together with magnesium, procaine and hypothermia to increase the effectiveness. The most important goal of cardioplegia is to protect the heart against ischemic damage of myocardium.

Within long period of time there has been introduced many different methods to prevent myocardium from ischemic lessions in cardiac surgery. However, there are two strategies basically used worldwide nowadays in pediatric cardiac surgery – cold crystalloid and cold blood cardioplegia. The

main diference imbetween is that in second case cardioplegic solution is given into coronary arteries mixed with oxygenated blood beforehand. There are still many discussions regarding the choise of the best method for myocardial protection and in majority of cases it depends mainly on the surgeon and experience of the institution. However, the most popular has become the cold blood cardioplegia with or without „hot shot" in pediatric cardiac surgery, which is more physiologic for the immature heart and improves the early postoperative period [*Amark et al.,2006, Caputo et al.,2002 , Fan et al.,2010, Mauney et al.,1995,Modi et al.,2006, Nomura et al.,2001, Poncelet et al.,2011, Shahzad,2008, Warner et al.,1988*].

When cardioplegic solution is injected into coronary arteries with a pump in order to ensure myocardial protection, it is necessary to determine the correct delivery pressure to avoid damage of the heart. It has to be taken into account that coronary arteries in neonates are immature and much more fragile and easier to damage. There are many structural, physiologic, biomechanical and metabolic diferences comparing to adults, therefore the myocardium of neonates may be more prone the pressure injury in pediatric than in adult cardiac surgery. Too high perfusion pressure would damage coronary arteries and myocardium. On the other hand, prevention of intraoperative myocardial damage depends also on the completeness of delivery of cardioplegic solution, therefore the right infusion pressure should be used.

Ensuring optimal cardioplegia infusion pressure is still an issue, especially in neonatal cardiac surgery. It could be due to the reason that it is very difficult to do researches in this field because of many technical and ethical problems. Recomendations can be found in literature regarding the „safe" delivery pressure [*Irtun et al.,1997, Lindal et al.,1990, Vinten-Johansen et al.,2000*]. However, they are more based on the practical experience of different centres of pediatric cardiac surgery or different animal models. As a result, the aim of our study was to establish the pressure, which is not harmful

for neonates, taking into consideration the biomechanical and structural properties of coronary arteries of neonates.

One of the main goal in modern pediatric bypass equipment is to reduce size of the extracorporeal circuit in order to minimize the prime volume. Even though nowadays there has been developed smaller tubing and oxygenators, which reduces priming volume significantly, it is still an issue in pediatric cardiopulmonary bypass (CPB). High priming volume can produce a low hematocrit on CPB in small infants, which results in decreased tissue oxygenation and reduction of plasma proteins and clotting factors, decrease of the colloid osmotic pressure (interstitial edema), electrolyte imbalance, exaggerated release of stress hormones with activation of complement, white blood cells and platelets.

The use of donor blood itself has several disadvantages, including transmission of viral particles, complement activation, induction of a transfusion reaction, infusion of lactate, glucose and potassium, and citrate-phosphate-dextrose infusion [*Jaggers et al.,2000, Keidan et al.,2004, Ratcliffe et al.1986, Smith et al.,2008, Strauss,2000, Sumpelmann et al,2001*], therefore this should be avoided as much as possible. However, for the reason of hemodilution most institutions use packed red blood cells in their priming solutions.

To improve the quality of myocardial protection and CPB in pediatric cardiac surgery in order to decrease the perioperative mortality of patients we started to employ the method of cold blood cardioplegia for myocardial protection in 2007. Besides we strongly started to measure the delivery pressure when giving cardioplegia. In addition, we started to include in the priming of CPB as fresh PRBC as possible. As we observed clinically the positive influence of these changes on immature myocardium, decission to do a scientific research regarding this topic was made.

Aim of the study

To improve the quality of myocardial protection and CPB in pediatric cardiac surgery and to define the „safe" and effective delivery pressure of cardioplegia in neonates taking into consideration biomechanical and structural properties of neonatal coronary arteries.

Objectives

1. To obtain the clinical and laboratory data from 100 operations of congenital heart defects as well as from patients's early postoperative period.
2. To analyse the data regarding the lactate concentration in the blood samples taken from artery and coronary sinuss before and after aortic occlusion.
3. To analyse the data of echocardioscopy obtained in patients's early postoperative period.
4. To analyse the level of Troponine I in patients's early and late postoperative period.
5. To evaluate the effectiveness of cold crystalloid and cold blood cardioplegia used in pediatrci cardiac surgery, taking into account the results obtained.
6. To study biomechanical and structural properties in samples of coronary arteries of neonates and adults retrieved from autopsies and to compare the results.
7. To define the „safe" and effective delivery pressure of cardioplegia in neonates taking into consideration the results of the research.
8. To obtain and to analyse data regarding the concentration of potassium, natrium, lactate, glucosis and pH in PRBC's used for the priming of CPB in pediatric cardiac surgery.

9. To evaluate the influence of storage time of PRBC on the concentration of potassium, natrium, lactate, glucosis and pH in PRBC's used for the priming of CPB in pediatric cardiac surgery, taking into account the results of the study.

Presented ideas

1. Cold blood cardioplegia provides better myocardial protection than cold crystalloid in pediatric cardiac surgery, especially in younger patients with smaller weight and whenever longer aortic occlusion and CPB time is expected.
2. The delivery pressure of cardioplegic solution should not exceed 100 mmHg in neonates to avoid the damage of immature coronary arteries.
3. The PRBC's used for for the priming of CPB in pediatric cardiac surgery should be as fresh as possible to avoid the unexpected complications.

Scientific and practical novelty of the promotional work

One of the most challenging questions in pediatric cardiac surgery is myocardial protection to have good outcomes. It is most important to ensure an adequate heart function after aortic occlussion and cardiopulmonary bypass needed for the surgical repair of congenital heart diseases, therefore the care must be taken regarding this matter.

As congenital heart diseases are various and most surgical repairs are complex, it is crucial to maintain an adequate myocardial protection, especially when operating on neonates.

There has been introduced many different methods to prevent myocardium from ischemic lessions in cardiac surgery. However, the most popular nowadays in pediatric cardiac surgery are cold crystalloid and cold blood cardioplegia.

Within a long period of time there was used the cold crystalloid cardioplegia with *St.Thomas* solution for myocardial protection in the Pediatric Cardiology and Cardiac Surgery, Children's University Hospital with variable success. As the results of our research indicates, this strategy is very good when operating not very complex congenital heart lessions. However, taking into account specific properties of immature myocardium and long aortic occlusion time needed for correction of complex congenital heart lessions, frequently the results of surgery were not as good as desirable due to the cardiac failure seen after long aortic occlusion time. Therefore for the first time in Latvia there had been introduced the strategy of cold blood cardioplegia for the myocardial protection in pediatric cardiac surgery. Within a long period of time this is proved to provide better myocardial protection in many centres of congenital heart diseases worldwide, especially in younger patients with smaller weight and whenever longer aortic occlusion and CPB time is expected. However, it was never used before in Latvia.

After the introduction of the new method for myocardial protection it was possible to operate successfully also younger patients with smaller weight and more complex congenital heart lessions. Therefore we can speculate that one of the most important reasons why the perioperative mortality has been improved in Clinic for Pediatric Cardiology and Cardiac Surgery is the change of the strategy for myocardial protection in pediatric cardiac surgery. It is scientifically proved also with the results of our research.

Nowadays the cold blood cardiolegia is employed for the myocardial protection on a regular basis in our clinic, especially in younger patients with smaller weight and whenever longer aortic occlusion and CPB time is expected. Besides, taking into account our experience, in 2011 there had been also successfully introduced the strategy of cold blood cardioplegia for myocardial protection in adult cardiac surgery in the Latvian Centre for Aquired Cardiac Surgery.

When cardioplegic solution is injected into coronary arteries with a pump in order to ensure myocardial protection, it is necessary to determine the correct delivery pressure to avoid damage of the heart. Coronary arteries in neonates are immature and much more fragile and easier to damage. There are many structural, physiologic, biomechanical and metabolic diferences comparing to adults, therefore the right infusion pressure should be used. Too high perfusion pressure would damage coronary arteries and the myocardium . On the other hand, prevention of intraoperative myocardial damage depends also on the completeness of delivery of cardioplegic solution.

Frequently cardioplegic solution is delivered into the aortic root without meassuring the infusion pressure leading to the cardioplegia being delivered with higher or lower perfusion pressure than desired. It may result in coronary arteries or myocardium being damaged. Unfortunately, it has to be admitted that the infusion pressure had not been measured until the strategy of myocardial protection was changed in the Clinic for Pediatric Cardiology and Cardiac Surgery. When the cold crystalloid cardioplegia was used for myocardial protection and introduced with inappropriately high pressure it definitely may have had increased the ischemic damage of immature myocardium.

Ensuring optimal cardioplegia infusion pressure is still an issue, especially in neonatal cardiac surgery. It could be due to the reason that it is very difficult to do researches in this field because of many technical and ethical problems. Recomendations can be found in literature. However, they are more based on the practical experience of different centres of pediatric cardiac surgery or different animal models. As a result, for the first time there has been done a research *in vivo* establishing the delivery pressure of cardioplegia, which is not harmful for neonates, taking into account biomechanical and structural properties of coronary arteries of neonates.

Nowadays in the Clinic for Pediatric Cardiology and Cardiac Surgery the infusion pressure of cardioplegic solution is strongly monitored following the recomendation based on the scientific results of this research. The desired pressure is 60 – 80 mmHg. However, it should not exceed 100 mmHg , especially in neonates.

One of the main goal in modern pediatric bypass equipment is to reduce the size of the extracorporeal circuit in order to minimize the prime volume. High priming volume can produce a low hematocrit on CPB in small infants, which results in electrolyte imbalance and decreased tissue oxygenation, therefore the donor blood should be used in the prime.

The use of donor blood itself has several disadvantages, including complement activation, induction of a transfusion reaction, infusion of lactate, glucose and potassium, therefore this should be avoided as much as possible. When blood is stored, many alterations occur in its constinuents, in particular an increase in potassium and lactate levels, and decrease in pH, which have been associated with severe complications, especially in neonates with severe congenital cardiac lession.

As it was very hard to convince the department responsible for obtaining the blood products in Children's University Hospital for necessity of having maximally fresh PRBC's available for pediatric cardiac surgery, the decission was made to do a scientific research regarding this topic. After publishing the scientific results and producing the recomendations based on them we finally convinced these specialists and started to include in the priming of CPB as fresh PRBC as possible with maximally short storage time before use.

Structure and amount of the promotional work

The promotional work has been written in Latvian. It consists of introduction, aim and objectives of the study, scientific and practical novelty, summary of literature, materials and methods, results, discussion, conclusions, recomendations. The total amount of work is 139 pages, including 21 tables and 28 pictures. There are 170 references included.

MATERIALS AND METHODS

Myocardial protection

To compare the strategy of cold crystalloid and cold blood cardioplegia used for intraoperative protection of myocardium in pediatric cardiac surgery and to evaluate the effectiveness of both methods, after receiving the permission of the Ethical Committee of Children's University Hospital, Riga, Latvia, indicating that there is no conflict with the ethical standards on human experimentation, the research was done from 2007 until 2011. We collected the blood samples from artery and coronary sinus before and after aortic occlusion, the data from 100 operations altogether. We decided to analyze lactates from coronary sinus before and after aortic occlusion to evaluate the influence of local ischemia on myocardium itself and the effectiveness of both methods used for the protection of myocardium. In addition, we analyzed lactates from artery before and after aortic occlusion to evaluate the influence of global ischemia, and used these results also as a control group.

The methodology of the research was based on the analysis of literature. Four blood samples altogether were taken intraoperatively – two at the beginning of aortic occlusion and two directly after the declamping of aorta. At first blood sample with syringe was taken from the right atrium at the beginning of aortic occlusion at the same time taking the blood sample also

from CPB pump as a control. Later blood sample of the first blood coming from the coronary sinuss after aortic occlusion was taken with syringe at the same time taking the blood sample also from CPB pump as a control. The blood samples were analysed with GEM PREMIER 3000 *(Fig. 1. 1)*.

Fig.1.1. GEM PREMIER 3000

The results were compared with the echocardioscopic grading of left ventricular function in early postoperative period (Fraction of shortening or FS, Ejection fraction or EF).

Taking into account references, we decided to include in our research also analysis of Troponin I taken in the early postoperative period and 12 hours after the cardiac operation. Unfortunately, at the beginning of our research there were technical difficulties to do this analysis in the Children's University Hospital. Therefore this analysis had been done only in 21 cases included in this study.

Blood samples from artery for the analysis of Troponin I was taken in the intensive care unit and sent to the Laboratory of Children's University Hospital for the analysis of Troponin I.

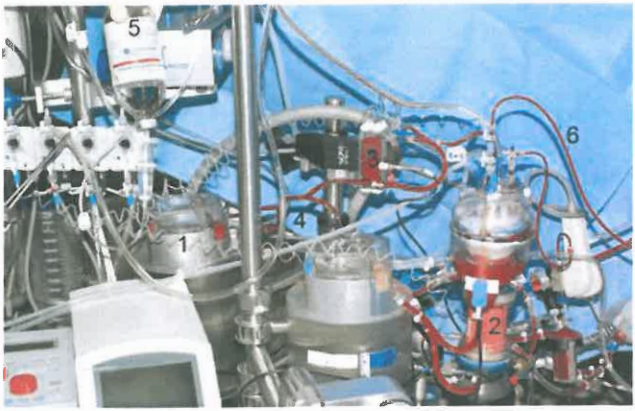


Fig.1.2. Standart setup for cardiopulmonary bypass and delivery system of cardioplegic solution

The pump (1) takes oxygenated blood from the oxygenator (2), where oxygenation takes place during CPB, to the heat exchanger (3). There is an Y-connector (4), where the cardioplegic solution (5) has been mixed with the oxygenated blood. After the cooling of the blood in the heat exchanger (3) cold blood cardioplegia through the line (6) has been delivered into the coronary arteries of patients

All patients had been divided into two groups depending on the strategy used for intraoperative myocardial protection – **cold blood (Group 1)** and **cold crystalloid (Group 2)**. In Group 1 the concentration of *Martindale* solution (*Martindale Pharmaceuticals, Romford, UK*) was used for myocardial protection. 20 ml was diluted in 500 ml Ringer solution beforehand. With the special system for delivering the cardioplegia (*Sorin Group Vanguard Blood Cardioplegia 4:1*) and CPB pump the solution was mixed with the oxygenated blood from oxygenator (4 parts blood, 1 part solution), cooled until temperature

of 4°C and delivered into the aortic root with the pressure 60 – 80 mmHg. The initial dose was calculated 30 ml/kg. Additionaly cardioplegia was repeated every 25 to 30 minutes with half of initial dose or 15 ml/kg. *„Hot shot"* or warm blood without cardioplegia solution was delivered into the aortic root before declamping aorta with the dose of 10 ml/kg *(Fig.1.2)*.

Tab.1.1

Values of parameters in Group 1

	Mean values +/- SD	Median	Mode	25^{th} percentile	75^{th} percentile
Age (month)	18 +/- 28	10	12	7,5	13,5
Weight (kilograms)	9.4 +/- 8.9	7.7	8.5	6.45	8.95
Time of CPB (minutes)	141 +/- 49	129	100	108.5	171
Time of aortic occlusion (minutes)	84 +/- 37	75	86	62	99

Tab.1.2

Values of parametrs in Group 2

	Mean values +/- SD	Median	Mode	25^{th} percentile	75^{th} percentile
Age (month)	50 +/- 53	24	24	12	78
Weight (kilograms)	20 +/- 19	12.5	9.5	9.5	22
Time of CPB (minutes)	95 +/- 64	70.5	55	55	101.75
Time of aortic occlusion (minutes)	46 +/- 42	25	16	16.75	58.5

In Group 2 the solution of St.Thomas Nr 1 (*London, UK*) was used for myocardial protection. It was introduced into the aortic root with the system of manual inflation without the control of delivering pressure. The initial dose was calculated 20 ml/kg. Additionaly cardioplegia was repeated every 25 to 30 minutes with the half of initial dose or 10 ml/kg. To gain hypothermia the solution of St.Thomas had been preserved in refrigerator before use.

There was observed better returning of the sinuss rythm and hemodynamic stability even after the longer periods of aortic cross-clamping, which made possible the correction of more complicated congenital heart defects in lower temperature. Therefore main criteria for choosing the strategy of intraoperative myocardial protection were age and weight of patients as well as the time of aortic occlusion and CPB expected – cold crystalloid cardioplegia had been used whenever there were older patients with bigger weight and shorter duration of aortic occlusion and CPB. In Group 1 there had been included patients with more complex congenital heart lession such as tetralogy of Fallot, atrioventricular septal defect, transposition of great arteries, double outlet right ventricule, total anomal pulmonary vein return, ventricular septal defect. Besides these patients were younger with smaller weight. In Group 2 there had been included patients with relatively not as complex congenital heart lessions such as atrial and ventricul septal defect, and older with bigger weight. All mean values of parameters including standartdeviation, median, mode, 25th and 75th pecentile are presented in Table 1.1. and Table 1.2.

There had been determined also correlation between the coronary lactates after aortic occlusion and echocardioscopic grading of left ventricular function in the early postoperative period (Fraction of shortening or FS, Ejection fraction or EF).

The mathematical processing of data had been done with the computer programme Microsoft Excel 2007. All results of research as well as values of

different parameters of both groups were expressed as mean plus standard deviation. In case there was a big standartdeviation, median, mode, 25th and 75th percentile had been calculated. When two groups were compared, data had been analyzed using Mann-Whitney U test and student *t* test. A *p* and *z* values were calculated. A *p* value of less than 0.05 was considered statistically significant.

To estimate the correlation between different variable values the Pearson correlation coeficient *r* was calculated.

Biomechanical properties of coronary arteries

Between May, 2009 and December, 2011 twelve samples of neonatal coronary arteries and seven samples of adult coronary artery, retrieved during autopsies, were used as experimental materials. The research was done after receiving the permission of the Ethical Committee of Children's University Hospital, Riga, Latvia, indicating that there is no conflict with the ethical standards on human experimentation. The rather small number of experimental material is attributable to the relatively small number of deaths amongst newborn babies in Latvia. In addition, autopsies are not always performed due to the reluctance of parents. Therefore it proved to be a challenge to obtain a sufficient amount of experimental materials.

The length of the specimens used in this study was approximately 4 cm. After resection the specimens were stored in Custodiol Perfusion Solution (Bretschneider's Solution) no longer than 24 hours until the mechanical tests were performed.

The aim of this study was to investigate the biomechanical and structural properties of normal and non-hypoxic neonatal coronary artery. As a result, in order for a sample to be eligible for the study, the age at the time of death could not exceed one month (neonatal period). In addition, only samples with no

history of any kind of cardiac pathology, including atypical anatomy of coronary arteries were accepted. An additional exclusion criterion was prematurity. The cause of death for neonates used in this study included infant respiratory distress syndrome, meconium aspiration syndrome, intraventricular hemorrhage, neonatal sepsis, birth trauma.

The mean age for neonates was 9.3 ± 9.7 days (median 4.5; 95% CI ± 5.49) and the mean weight 3.99 ± 0.7 kg (median 4.2; 95% CI ± 0.39).

The main criterion for adult specimens to be included in this study was no signs of atherosclerosis after the careful inspection of the experimental material. This was necessary to ensure that atherosclerosis in the sample does not affect the results of biomechanical experiments and analysis of the wall of coronary arteries. Younger age was preferred, although not compulsory. The cause of death included criminal acts, suicide and car accident.

A special device was used to measure the internal pressure, axial force, longitudinal and circumferential deformation of coronary arteries (*Fig.1.3*).

One end of the artery was clamped to the support to which a pressure transducer and specially designed inductive force transducer were connected. The other end was clamped to the support to which a pressure bottle containing fluid was connected. The force transducer recorded the force necessary to maintain the vessel at its *in situ* length. Axial stretch was introduced by a slide mechanism to which the balance arms were fixed. The axial deformation of the artery was measured with a specially designed inductive strain transducer connected to one of the arms of the balance. Diameter changes in the specimen were sensed optically with a video-dimensional analyzer coupled with a suitable lightning system for high contrast. The changes in diameter were tracked and recorded continuously.

Figure 1.3. The view of experimental strand: 1 – sample, 2 – optical camera

A sample of coronary artery was gradually loaded by internal pressure from 0 to 220 mmHg while the length of the sample was maintained constant at L_o, the length *in situ*. The pressure was elevated in 20-mmHg steps. The initial external diameter at inner pressure p = 0 mmHg and at *in situ* axial length L_o was noted as D_o. The diameter D was recorded at each pressure level (*Fig.1.4A, Fig.1.4B*).

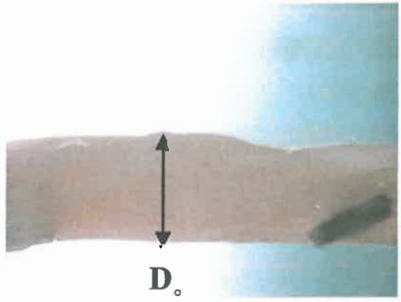


Fig.1.4A. Neonatal coronary artery pressurized with the inner pressure of 0 mmHg (D_o)

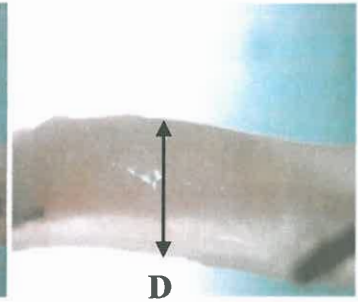


Fig.1.4B. Neonatal coronary artery pressurized with the inner pressure of 220 mmHg (D)

The value of wall thickness h was calculated as follows:

$$h = h_0 \, x \, \lambda_3, \qquad (1)$$

where

$$\lambda_3 = 1/(\lambda_1 x \lambda_2), \quad (2)$$

$$\lambda_2 = (D/D_0), \qquad (3)$$

The circumferencial stress was calculated as:

$$\sigma = (p \, x \, R) / h, \quad (4)$$

where p - inner pressure, R - radius.

$$\lambda_1 = (L/L_0) = 1.0 \quad (5)$$

In these equations, h_o is the initial thickness of the specimen and λ_1, λ_2 and λ_3 are, respectively, the stretch ratios in the axial, circumferential, and radial directions respectively. Because the length of the artery was maintained constant at L_o, the value of λ_1 is always one.

The initial wall thickness h_o was measured with a cathetometer to ± 0.0001 mm accuracy.

The flexibility and stiffness of arteries are frequently characterized by the values of compliance, pressure-strain elastic modulus and stiffness parameters. Compliance is the fractional change in external diameter with the change in pressure.

Because the pressure-diameter relation of an arterial wall is generally nonlinear, compliance and pressure-strain elastic modulus are not usually material constants but change with the internal pressure

The mathematical processing of data had been done with the computer programme Microsoft Excel 2007.Age and weight in neonatal group as well as ultimate stress and ultimate strain were expressed as mean values plus standard deviation. In addition, the median value and 95% confidence interval was

calculated, where appropriate. When two groups were compared, data had been analyzed using Mann-Whitney U test and student *t* test. A *p* value of less than 0.05 was considered statistically significant.

Structural properties of coronary arteries

After the biomechanial experiments samples of coronary arteries were examined histologically in the Institute of Anatomy and Anthropology of Riga Stradins University to evaluate the influence of infusion pressure on the ultrastructure of coronary artery. During each biomehanical experiment two samples of tissue of coronary arteries cut perpendicularly the longitudinal axis of blood vessel were fixed in formaline solution. The first sample had been taken before and the second after the influence of infusion pressure on coronary artery. Samples from two coronary arteries were used basically for trying to establish the more precise infussion pressure, which might be dangerous for the ultrastructure of neonatal coronary arteries.

Structural changes of the coronary wall were estimated using conventional sample fixation, embedding and sectioning. Consecutive sections were used as negative controls of immunohistochemical reactions, hematoxylin and eosin (H&E). In addition, they were also used for Masson's trichrome staining to confirm the occurrence and distribution of constituents of the vascular wall. Immunohistochemistry was performed using the EnVision Detection System (Dako, Glostrup, Denmark). Paraffin sections were deparaffinized in xylene, immersed in absolute ethanol at first and then in graded alcohols afterwards. Endogenous peroxidase activity was blocked with 0.1% H_2O_2 in methanol for 20 minutes. Antigen retrieval was accomplished with the sections of specimens placed in 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. for 30 minutes. After antigen retrieval, specimens were allowed to cool for 30 minutes after which they were incubated at 4°C overnight with the following primary

antibodies: monoclonal mouse anti-human smooth muscle actin and desmin (DakoCytomation, Glostrup, Denmark, 1:100 dilution, clone 1A4 and clone D33, respectively), which both label smooth muscle cells; monoclonal mouse anti-human vimentin (DakoCytomation, Glostrup, Denmark, 1:100 dilution, clone Vim 3B4), which labels cells of mesenchymal origin; monoclonal mouse anti-human CD34 class II antibody ((DakoCytomation, Glostrup, Denmark, 1:50 dilution, clone QBEnd10), which stains endothelial cells through recognition of a single-chain transmembrane protein expressed on these cells. The staining procedure was achieved by the EnVision technique using Dako ready-to-use, peroxidase-conjugated, rabbit/mouse EnVision reagents and peroxidase substrate solution (diaminobenzidine-H_2O_2). Finally, sections of specimens were washed, counterstained with hematoxylin, washed with tap water, mounted, and covered with coverslips. Immunohistochemical controls included omission of the primary antibody or substitution of it with nonimmune IgG or phosphate buffered saline solution (pH 7.4). The sections of specimens were photographed in a Leitz DMRB brightfield microscope using a digital camera DC 300F.

The metabolic changes in stored packed red blood cells

To evaluate the effect of length of storage of PRBC on the concentration of potassium, sodium, lactate, glucose and pH in PRBC's used in pediatric cardiac surgery from October,2006 until June,2009 blood samples were drawn from 118 PRBC used in cardiopulmonary bypass before they were added to the priming solution. Fresh PRBCs were preferred and patients would receive old ones only if no fresh PRBC was available. The research was done after receiving the permission of the Ethical Committee of Children's University Hospital, Riga, Latvia, indicating that there is no conflict with the ethical standards on human experimentation.

All PRBC's were splited in two groups depending on the age of blood: ≤5 days (Group 1), n=69 and >5 days (Group 2), n=49. Blood samples were drawn from the PRBC, stored at 4°C in preservative solution, consisting of citrate, dextrose, phosphate and adenine (CP2 and SAGM), and were analyzed by GEM PREMIER 3000.

The mathematical processing of data had been done with the computer programme Microsoft Excel 2007. All results of research were expressed as mean plus standard deviation. When two groups were compared, data had been analyzed using Mann-Whitney U test and student *t* test. A *p* and *z* values were calculated. A *p* value of less than 0.05 was considered statistically significant.

To estimate the correlation between different variable values the Pearson correlation coeficient *r* was calculated. It was used to evaluate the correlation between the change in concentration of different parameters and the storage time of PRBC.

RESULTS

Myocardial protection

Characteristics of both groups

There were 82 patients included in the Group 1. All data from operations and postoperative period had been collected and summarized. The mean age was18 ± 28 (median 10; 75.percentile 13.5) month, the mean weight was 9.4 ± 8.9 (median 7.7; 75.percentile 8.95) kilograms, the mean time of CPB was 141 ± 49 (median 129; 75.percentile 171) minutes, and the mean time of aortic occlusion was 84 ± 37 (median 75; 75.percentile 99) minutes.

The mean increase in the level of lactates in arterial blood was 0.4 ± 0.7 (median 0.3) mmol/l, but in the blood comming from coronary sinuss – 2.8 ± 2.1 mmol/l. The mean echocardioscopic grading of left ventricular function in early postoperative period - FS = 39 ± 6, EF = 70 ± 8 %.

The mean level of Troponin I in the early postoperative was 11.2 ± 6.4 ug/L, but 12 hours after the operation – 4.2 ± 1.8 ug/L. This analysis was made only in 18 patients in Group 1.

The correlation was observed between the level of coronary lactates after aortic occlusion and the echocardioscopic grading of left ventricular function in early postoperative period in Group 1.

There were 18 patients included in the Group 2. All data from operations and postoperative period had been collected and summarized. The mean age was 50 ± 53 (median 24; 75.percentile 78) month, the mean weight was 20 ± 19 (median 12.5; 75.percentile 22) kilograms, the mean time of CPB was 95 ± 64 (median 71; 75.percentile 102) minutes, and the mean time of aortic occlusion was 46 ± 42 (median 25; 75.percentile 59) minutes.

The mean increase in the level of lactates in arterial blood was 0.2 ± 0.5 (median 0.15) mmol/l, but in the blood comming from coronary sinuss - 0.4 ± 0.7 mmol/l (median 0.25) mmol/l. The mean echocardioscopic grading of left ventricular function in early postoperative period - FS = 42 ± 6, EF = 73 ± 8 %.

The mean level of Troponin I in the early postoperative was 4.6 ± 0.3 ug/L, but 12 hours after the operation – 1.6 ± 0.06 ug/L. This analysis was made only in 3 patients in Group 2.

The correlation was observed between the level of coronary lactates after aortic occlusion and the echocardioscopic grading of left ventricular function in early postoperative period in Group 2.

Comparison of both groups

Mean age 18 ± 28 (median 10) vs 50 ± 53 (median 24) month ($z=3.59$, $p<0.001$); mean weight 9.4 ± 8.9 (median 7.7) vs 20 ± 19 (median 12.5) kg ($z=4.1$, $p<0.001$); mean time of CPB 141 ± 49 vs 95 ± 64 (median 70.5)

minutes (z=3.84, p<0.001); time of aortic occlusion 84 ± 37 vs 46 ± 42 (median 25) minutes (z=3.95, p<0.001).

The mean increase of lactates in arterial blood was 0.4 ± 0.7 (median 0.3) mmol/l in Group 1 vs 0.2 ± 0.5 (median 0.15) mmol/l in Group 2 (z=1.45, p=0.1283), but in the blood comming from coronary sinuss - 2.8 ± 2.1 mmol/l vs 0.4 ± 0.7 (median 0.25) mmol/l (z=5.13, p<0.0001). The mean echocardioscopic grading of left ventricular function in early postoperative period – FS = 39 ± 6 vs 42 ± 6 (z=1.82, p=0.0511); EF = 70 ± 8 % vs 73 ± 8% (z=1.56, p=0.0747).

The mean level of Troponin I in the early postoperative was 11.2 ± 6.4 vs 4.6 ± 0.3 (p<0.0001) ug/L, but 12 hours after the operation – 4.2 ± 1.8 vs 1.6 ± 0.06 (p<0.0001).

The correlation was observed between the level of coronary lactates after aortic occlusion and the echocardioscopic grading of left ventricular function in early postoperative period in both groups.

Biomechanical and structural properties of coronary arteries

Analysis of biomechanical properties

We observed that in neonates the relationship between pressure and strain on the one hand, and stress and strain on the other hand was non-linear. There was a significant difference between neonates and adults in terms of elastic modulus (*Fig.2.1.*).

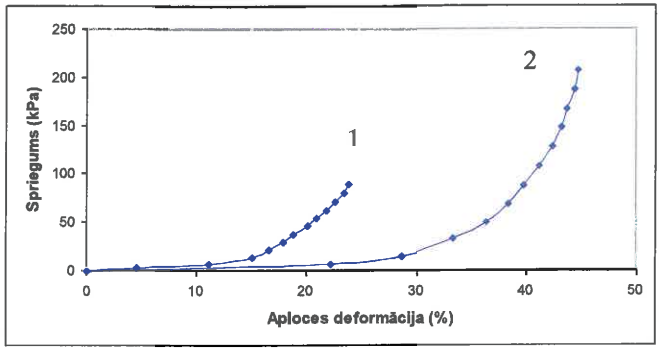


Figure 2.1. Relationship between stress and strain in coronary arteries

1- adults, 2- neonates

We observed a fast and significant increase of strain until the inner pressure reached 80 – 100 mmHg (10.66 – 13.33 kPa) whilst the increase of stress in the arterial wall was less rapid. When the internal pressure exceeded 100 mmHg (13.33 kPa), the strain of the arterial wall increased much slower whilst the wall stress and modulus of elasticity began to increase rapidly.

When the inner pressure was 80 mmHg (10.66 kPa), the strain in the wall of neonatal coronary artery reached 32.71 ± 6.59%, which is more than two times higher than that in the coronary artery of adults – 18.44 ± 1.51% ($z=3.416$, $p<0.001$) respectively. The stress in neonatal coronary artery reached 68.17 ± 10.65 kPa with the same inner pressure and increased rapidly as the pressure was gradually increased *(Tab.2.1.,Tab.2.2.)*.

When the inner pressure reached 120 mmHg (15.99 kPa), the strain in the wall of neonatal coronary artery was 107.48 ± 15.05 kPa, which is more than two times higher than the strain with inner pressure of 80 mmHg (10.66 kPa). The strain in the wall of adult coronary artery was 53.36 ± 8.03 kPa ($z=3.416$, $p<0.001$).

Table 2.1.

Stress in neonatal and adult coronary arteries when applying different inner pressure

	ε (%) (60 mmHg)	ε (%) (80 mmHg)	ε (%) (100 mmHg)	ε (%) (120 mmHg)	ε (%) (220 mmHg)
Neonates	30.44 ± 6.13	32.71 ± 6.59	34.42 ± 6.54	35.82 ± 6.41	45.66 ± 6.47
Adults	14.31 ± 1.52	15.86 ± 1.45	17.16 ± 1.5	18.44 ± 1.51	20.59 ± 2.27

Tabula 2.2.

Strain in neonatal and adult coronary arteries when applying different inner pressure

	σ (kPa) (60 mmHg)	σ (kPa) (80 mmHg)	σ (kPa) (100 mmHg)	σ (kPa) (120 mmHg)	σ (kPa) (220 mmHg)
Neonates	48.94 ± 7.37	68.17 ± 10.65	87.89 ± 11.34	107.48 ± 15.05	201.95 ± 35.17
Adults	27.85 ± 4.43	37.73 ±5.77	47.48 ± 7.04	53.36 ± 8.03	111.07 ± 18.02

Table 2.3.

Modulus of elasticity in neonatal and adult coronary arteries when applying different inner pressure

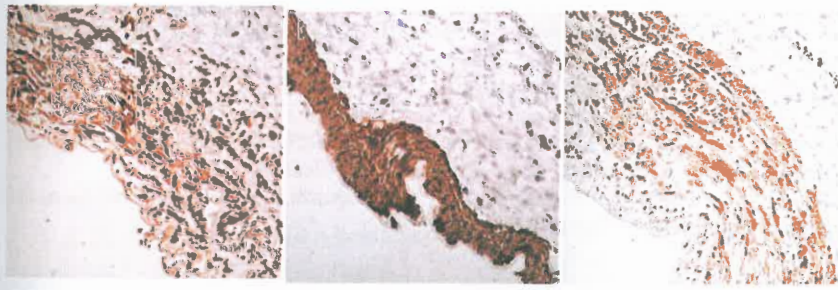
	E (kPa) (60-80)mmHg	E (kPa) (80-100)mmHg	E (kPa) (100-120)mmHg
Neonates	867.08 ± 199.43	1176.42 ± 215.42	1494.49 ± 331.01
Adults	641.42 ± 36.14	781.8 ± 103.66	825.25 ± 108.64

When the inner pressure exceeded 80 mmHg (10.66 kPa) the stiffness in the wall of neonatal coronary artery increased rapidly in comparison to the stiffness in the wall of adult coronary artery (*Tab.2.3.*). The modulus of elasticity of the wall of neonatal coronary artery was 867.08 ± 199.43 kPa as long as the inner pressure had been less than 80 mmHg. As soon as the pressure reached 100 - 120 mmHg (13.33-15.99 kPa), the modulus of elasticity in neonates begun to increase rapidly -1176 ± 215.42 kPa. In the same pressure the modulus of elasticity in adults was only 781.8 ± 103.66 kPa ($z=3.025$, $p<0.001$). It was observed that the modulus of elasticity increases much faster in the neonatal coronary artery (867.08 ± 199.43 to 1494.49 ± 331.01 kPa) than in the adult one (641.42 ± 36.14 to 825.25 ± 106.64 kPa), when the inner pressure increases from 60 mmHg to 120 mmHg ($z=2.06$, $p<0.05$) (*Tab.2.3.*). This may indicate the possible damage in the wall of neonatal coronary artery, when the inner pressure exceeds 100 – 120 mmHg (13.33-15.99 kPa).

Analysis of structural properties

Conventional histological examination revealed overall thickening of the coronary wall, occasional and partial split and/or interruption of internal elastic lamina, and redistribution of constituents of the vascular bed in neonates after exposure of 120 mmHg. By contrast, we observed rather moderate vascular changes observed in adults. When the inner pressure was elevated up to 120 mmHg, analysis of contractile and tensile properties of the vascular wall showed prominent affection of the coronary artery wall with medial edema, redistribution of myocytes and injury of adventitial *vasa vasorum*. All alterations presented with medial thickening appeared after the application of load *(Fig.2.2B, Fig.2.2E)*.

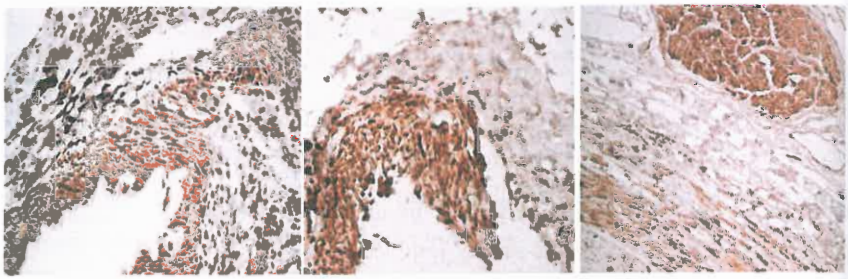
Medial myocytes presented loss of compactness and circumferential orientation in neonates, forming groups, which displayed haphazard distribution *(Fig.2.2A, Fig.2.2B)*. Due to the development of edema and redistribution of smooth muscular cells the medial thickness in neonates sometimes revealed the range equal to the medial coat of the coronary arteries in adults enrolled in this study *(Fig.2.2C)*.



2.2A. Strong medial positivity for α-smooth muscle actin before load of internal pressure in neonate. Original magnification x 250
2.2B. Immunohistochemical reaction with the anti-actin antibody revealing marked thickening of media and changes in structural integrity of myocytes appearing after load of internal pressure in neonate. Original magnification x 200
2.2C. Moderate medial positivity for α-smooth muscle actin after load of internal pressure in adult, occasional intimal positivity. Original magnification x 200

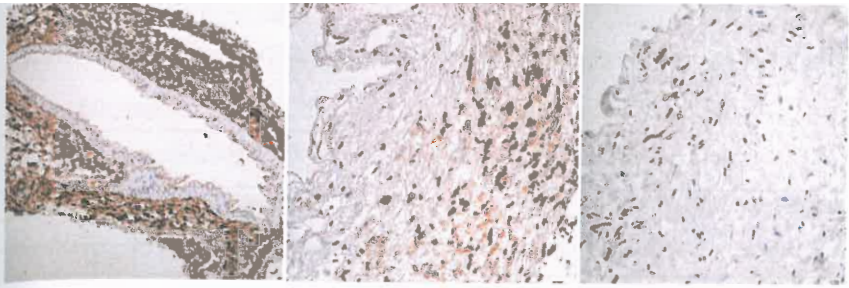
Marked redistribution of cellular constituents within the medial-adventitial coats under the exposure of load in neonates had been proved again when supportive and tensile vimentin containing cytoplasmic intermediate filaments were stained. Decoration with the anti-vimentin antibody revealed that the expression of this antigen after load was almost nil in neonates *(Fig.2.2D, Fig.2.2E)* thus resembling the immunostaining in adults *(Fig.2.2F)*.

Regarding anti-desmin decoration, the positivity appeared at the medial-adventitial interface of neonates prior and after load, but the same tendency did not show in adults. Orientation of these occasional smooth muscular cells displaying desmin positivity had changed from linear up to haphazard and even zigzag *(Fig.2.2I)*.



2.2D. Medial-adventitial positivity for vimentin before load of internal pressure in neonate. Original magnification x 250
2.2E. Immunohistochemical reaction with the anti-vimentin antibody demonstrating marked thickening of vascular *tunica media* detectable by smooth muscular decoration with disappearance of immunostaining from adventitia coat in neonate after application of load. Original magnification x 250
2.2F. Weak to moderate medial positivity for vimentin in adult, positivity in nerve fiber bundle. Original magnification x 250

Finally, a strong decoration of the endothelial cells within adventitial microvascular beds assessed using anti-CD34 antibody demonstrated a decrease of it and loss of microvascular density in neonates when applying the inner pressure *(Fig2.2H)*.



2.2G. CD34-positive endothelial cells heavily impregnating capillary wall of the adventitial coat of neonate before application of pressure. Original magnification x 100

2.2H. Immunohistochemical reaction with the anti-CD34 antibody a remarcable decrease of the density of microvascular network in the adventitial coat of coronary artery of neonate appearing under experimental conditions. Original magnification x 250

2.2I. Haphazardly distributed desmin-positive smooth muscular cells after application of pressure to the coronary artery of neonate. Original magnification x 200

The metabolic changes in stored packed red blood cells

A hundred and eight PRBC's used in pediatric cardiac surgery were included in the study. In 69 blood samples the age of PRBC was under 5 days (Group 1) and in 49 blood samples the age of PRBC was over 5 days (Group 2).

All mean values of different parameters in both groups including standartdeviation as well as results of p and z analysis are reflected in the Table 2.4.

There was an intermediate correlation in both groups in terms of storage time and level of potassium (correlation coefficient 0.42 vs 0.17), storage time and level of lactate (correlation coefficient 0.43 vs 0.5), pH and level of potassium (correlation coefficient 0.34 vs 0.68), pH and level of lactate (correlation coefficient 0.49 vs 0.53), and the levels of potassium and lactate (correlation coefficient 0.7 vs 0.6). There was a linear increase in the levels of potassium and lactate depending on the storage age of PRBC.

Table 2.4.
Mean values +/- SD of parameters showing the difference between Group 1 and Group 2

	Group 1 (n=69)	Group 2 (n=49)	p value	z value
Storage time	4 ± 1	8 ± 5	<0.0001	9.233
pH	6.7 ± 0.1	6.5 ± 0.3	<0.0001	5.02
Potassium	7.4 ± 2.9	10.2 ± 3.3	<0.0001	4.28
Sodium	136.5 ± 5.2	133.1 ± 8.3	<0.05	2.16
Glucose	366.9 ± 35.3	349.4 ± 67.7	0.1583	1.25
Lactate	7.7 ± 2.2	10.5 ± 2.2	<0.0001	5.19

DISCUSSION

Myocardial protection

One of the most important questions in pediatric cardiac surgery is myocardial protection, which is needed for an adequate heart function after aortic occlussion and cardiopulmonary bypass that in turn is directly influencing the patient's perioperative morbidity. A special care must be taken when operating children and neonates because congenital heart diseases are various and most surgical repairs are complex. Besides it is proved that the immature myocardium has structural and functional characteristics different from those of the adult myocardium. It is widely accepted that the immature heart has a greater tolerance to ischemia than the adult or mature heart, but it is more vulnerable to the increased amount of water, which is an issue for those using the crystalloid cardioplegia [*Jones et al.,2006; Milerova et al.,2010*].

Cardioplegia is full dyastolic electromechanical standstill of the heart when cardioplegic solution is injected into coronary arteries with a pump. Tissue preservation is achieved by conserving energy stores through rapid arrest due to the high concentration of potassium. It decreases the ongoing metabolic rate and minimises changes induced by ischemia. Potassium induces depolarisation of the membranes of myocites making them non-excitable as long as it remains

in tissues. As the non-coronary collaterals flushes potassium out of the tissues after a period of time electromechanical activity of the heart recovers, which often can not be seen, therefore potassium usually goes together with magnesium in cardioplegic solutions to increase the effectiveness.

It is known that immature myocardium is vulnerable to high concentration of calcium, so the influx of it induced by electromechanical activity of the heart should be prevented. Because of ischemia myocardial necrosis starts to take place and after the reperfusion contracture of heart can be seen induced by non-controlled influx of calcium into cells. This is called „stone heart" [*Katz et al.;1977*] and can be prevented by using magnesium in cardioplegic solutions.

It is often very difficult to estimate the proper concentration of potassium as too high can be deleterious to myocardium. Therefore there are always hyperpolarizing agents and hypothermia included when using cardioplegia to decrease it [*Vinten-Johansen et al.,2000*].

The most important goal of cardioplegia is to protect the heart against ischemic damage of myocardium. Within long period of time there has been introduced many different methods to prevent myocardium from ischemic lessions in cardiac surgery. However, there are two strategies basically used worldwide nowadays in pediatric cardiac surgery – cold crystalloid and cold blood cardioplegia. The main diference imbetween is that in second case cardioplegic solution is given into coronary arteries mixed with oxygenated blood beforehand. The main advantage of this strategy is that the use of fluids can be considerably decreased. There are still many discussions regarding the choise of the best method for myocardial protection and in majority of cases it depends mainly on the surgeon and experience of the institution. However, the most popular has become the cold blood cardioplegia with or without „hot shot" in pediatric cardiac surgery, which is more physiologic for immature heart and improves the early postoperative period by reducing the input of excessive fluids into myocardium and reducing the risk of edema.

Notwithstanding many researches available regarding the myocardial protection there is still not one common strategy for all pediatric cardiac centres worldwide. This is basically due to the fact that congenital heart diseases are various and most surgical repairs are complex, and the immature myocardium has structural and functional characteristics different from those of the adult myocardium. However, the main principles – complete dyastolic standstill of the heart, decompretion of chambers, hypothermia for reducing metabolic necessities – remains unchangeable.

It is widely accepted that the immature heart has a greater tolerance to ischemia-reperfusion damage than the adult heart. This is due to the fact that the main energetical resource in mature heart are fatty acids comparing to neonates, where there are more reserves of glycogen making it possible to produce energy also anaerobically. This is an advantage when talking about ischemia and remains for neonates as long as they reach the age of two month. There are also more amino acids in neonatal myocardium making it possible to produce additional energy. Besides the reserves of myocardial ATP in neonates ceases much slower comparing to adults due to the fact that the need for energy in neonatal heart is lower [*Vinten-Johansen et al.,2000*].

Taking into account many references of literature, normal immature myocardium has a greater tolerance to ischemia than the adult or mature heart. But what is characteristic for normal myocardium, might not be same in case of congenital heart disease and surgical operation needs to be done when myocardium is weakened due to cyanotic pathology and heart failure. It is known that mycardium has functional depression after ischemia comparing to normal one. That means it can be more sensitive to global ischemia [*Jones et al.,2006*] and manifestation of ischemia-reperfusion injury in the early postoperative period might be clinically more severe [*Oliveira et al.,2011*] due to immature antioxydant system [*Saugstad,2005*; *Solberg et al.,2010*].

Normally heart has relativelly big functional reserves and can compensate an essential damage. However, this ability mainly depends on individual characteristics of each patient and disease. There are four major factors needs to be done by cardioplegia: (1) rapid induction of electromechanical standstill, (2) prolonged period of electromechanical standstill, (3) minimalisation of ischemia, (4) reperfusion control. Each of above mentioned factors is important, therefore there is no method of cardioplegia, which is most perfect. In general, the best strategy of intraoperative myocardial protection is the one, which maximally minimises the toxic influence of ischemia on myocardium. If the goal is reached, it can be asserted that also the reperfusion injury is minimilized and a good myocardial protection is achieved. In addition, it has to be remembered that myocardium suffers less from hypoxia comparing to ischemia, which rapidly decreases amount of ATP. That causes different psysiologic and morphologic reactions leading to the irreversible damage of myocardium.

As there are still many discussions regarding the choise of best method for myocardial protection among different researchers and institutions of congenital heart surgery, there are a number of studies available in literature. However, the most popular has become the cold blood cardioplegia with or without „hot shot" in pediatric cardiac surgery, which is more physiologic for the immature heart and improves the early postoperative period. Therefore there are more references about positive influence of blood cardioplegia on immature myocardium. It is observed that blood cardioplegia is associated with smaller metabolic ischemic stress of myocardium and reperfusion injury [*Caputo et al.,2002*]; it protects metabolism and function of myocardium more effectively and the cardiac index is more than 20% higher at the end of CPB [*Amark et al.,2006*]; it preserves the endothelium dependent heart function in the left and the right ventricule showing good microvascular protection of endothelium [*Charles et al.,1995*]; improves heart function in the early

postoperative period and decreases the amount of arrhythmias by protecting reserves of high energy phophates (ATP) [*Ibrahim et al.,1999*]; reduces myocardial acidosis in the time of electromechanical standstill due to physiologic buffering capacity of blood and ability to transport oxygen to myocardium [*Warner et al.,1987*]. There has been stressed the role of oxygenated blood in myocardial protection due to the oxygen it contains [*Hearse et al.,1976*] as well as free radical scavengers of blood minimizing the reperfusion injury [*Julia et al.,1991*]. The additional positive effect on immature myocardium of *„hot shot"* has been described by many authors [*Modi et al.,2006; Nomura et al.,2001; Vinten-Johansen et al.,2000*]. It recovers energetic reserves and aerobic metabolism of myocardium at the beginning of reperfusion without electromechanical activity of the heart, and it is of great importance in preventing myocardium from reperfusion injury.

In a long period of time there was crystalloid cardioplegia used with *St.Thomas* solution in Clinic for Pediatric Cardiology and Cardiac Surgery, Children's University Hospital. The success had been variable. In general crystalloid cardioplegia is good for myocardial protection during congenital heart surgery. However, taking into account different specific aspects of immature myocardium (e.g., myocardial edema as the reaction on excessive fluid input) and relatively long aortic crossclamp time needed for the correction of more complex congenital heart lessions, often the results of surgery were not satisfactory due to the heart failure. During this period of time the overall experience of clinic was not sufficient, and long aortic occlusion and CPB time was needed for correction of congenital heart lessions leading to relatively high perioperative morbidity. To improve this situation the decission was made to analyse the experience of different congenital heart centres regarding the strategies used for myocardial protection.

Taking into account the experience of many leading pediatric cardiosurgery centres worldwide and many reports available, the conclusion

was made that the most popular method of protecting the pediatric and neonatal heart is cold blood cardioplegia with or without „hot shot". In many researches there has been proved the blood cardioplegia superiority over the crystalloids. However, it was never used before in Latvia.

Using the above mentioned experience and knowledge, we started to employ the cold blood cardioplegia in pediatric cardiac surgery in the Clinic for Pediatric Cardiology and Cardiosurgery, Children's University Hospital . What we have observed already from first operations was better returning of the sinuss rhythm and hemodynamic stability even after the longer periods of aortic cross-clamping. Arrhythmias were observed significantly less and echocardioscopic grading of the left heart function in the early postoperative period showed good results. That made possible the correction of more complicated congenital heart defects in lower temperature for smaller patients.

At the beginning it was crucial to develop proper methodology for using blood cardioplegia. Therefore we had taken into account the theoretical basis as well as practical experience gained in different cardiac centres in Germany, United Kingdom, France, Canada and Sweden. With the special system for delivering the cardioplegia and CPB pump the solution was mixed with the oxygenated blood from oxygenator (4 parts blood, 1 part solution). The initial dose was calculated 30 ml/kg. Additionaly cardioplegia was repeated every 25 to 30 minutes with the half of initial dose or 15 ml/kg. *„Hot shot"* or warm blood without cardioplegia solution was delivered into the aortic root before declamping aorta with the dose of 10 ml/kg. Initially it was made mixing the warm blood with cardioplegic solution. As often the complete electromechanical standstill had remained even after the end of aortic occlusion, decission was made to take into account the recomendations of Lund hospital and make *„hot shot"* without cardioplegic solution not to increase the concentration of systemic potassium.

To gain objective data to prove the blood cardioplegia strategy superiority over crystalloid cardioplegia the decission was made to summarize the perioperative data whenever both methods were used.

Taking into account references available in the literature, an increase in lactate level usually indicates that there is an activation of ATP being produced anaerobically due to the hypoxia and is considered to be an objective criterium for judging how much ischemia affects locally vital organs or globally the organism altogether. *Amark et al.* had analysed arterial and coronary sinuss blood, where the lactate level and oxygen before and after aortic occlusion was evaluated. In addition, the echocardioscopic grading of left ventricular function was made. They concluded that blood cardioplegia preserves more myocardial metabolism comparing to crystalloid cardioplegia and the cardiac index is more than 20% higher at the end of CPB. After the evaluation of technical possibilities in the Clinic for Pediatric Cardiology and Cardiosurgery, Children's University Hospital we decided to analyze the lactates from coronary sinus before and after aortic occlusion to evaluate the influence of local ischemia on myocardium itself and the effectiveness of both methods used for the protection of myocardium. In addition, we analyzed lactates from artery before and after aortic occlusion to evaluate the influence of global ischemia, and used these results also as a control group. Later the results were compared with the echocardioscopic grading of left ventricular function in early postoperative period (*Fraction of shortening* or FS, *Ejection fraction* or EF).

Taking into account references in the literature [*Angeli et al.,2011, Durandy,2008*], where as the only objective criterium indicating the ischemic damage of myocardium and the effectiveness of intraoperative protection, the level of Troponin I in the early postoperative period as well as the speed of decreasing of this level in the late postoperative period has been stated. As a result we decided to include in our research also analysis of Troponin I taken in

the early postoperative period and 12 hours after the cardiac operation. Unfortunately, at the beginning of our research there were technical difficulties to do this analysis in the Children's University Hospital. Therefore this analysis has been done only in 21 cases included in this study. Besides it has to be admitted that the level of Troponin I is not absolutely specific in the case of evaluating the effectiveness of myocardial protection because it is influenced also by surgical trauma.

After the analysis of the results of our research the statistically significant difference between both groups had been found in terms of age, weight, time of aortic occlusion and CPB. It can be explained due to the fact that, as mentioned before, main criteria for choosing the strategy of myocardial protection from the point of ethical and clinical aspects were weight and age of the patient as well as the predicted time of aortic occlusion and CPB. That means cold blood cardioplegia was used whenever younger patient with smaller weight had been operated. Therefore straight from the beginning it was clear that comparison of both groups might not be objective and the criterion proving the blood cardioplegia superiority over crystalloid cardioplegia should have been found.

The statistically significant difference was found in terms of the mean increase in lactate level in arteries and coronary sinuss. It could be explained due to the distinction of both groups in aortic occlusion and CPB time. However, taking into account that an increase in lactate level usually indicates an activation of ATP being produced anaerobically because of hypoxia and is considered to be an objective criterion for judging how much ischemia affects locally vital organs or globally the organism altogether, significant difference between groups was not observed. We can speculate that this is due to the fact that there had been well-runned myocardial protection and CPB. Besides the mean increase in coronary lactates was not significant in the blood cardioplegia

group comparing to the mean aortic occlusion time. This can testify good myocardial protection done by the blood cardioplegia.

Analysing the results of our research and searching for an objective criterion proving the blood cardioplegia superiority over crystalloid cardioplegia, we observed that the echocardioscopic grading of left ventricular function in early postoperative period was similar in both groups. Therefore it can be asserted that the myocardial protection in both groups was adequate. However, it has to be remembered that in the blood cardioplegia group there had been patients with younger age, smaller weight and more complex congenital heart lessions associated with longer aortic occlusion and CPB time. So we can speculate that blood cardioplegia provides better myocardial protection. This conclusion is based mainly on echocardioscopic grading of the left ventricular function in the early postoperative period showing almost no difference between both groups.

After the analysis of results of our research statistically significant difference between both groups had been found in terms of the concentration of Troponin I in early postoperative perioda s well as 12 hours after the operation. Taking into consideration that the level of Troponin I is not absolutely specific in case of evaluating the effectiveness of myocardial protection because it is influenced also by surgical trauma, intraoperative myocardial protection and aortic occlusion time, statistically significant difference between both groups in early postoperative period can be explained. However, it has to be stated that the concentration of Troponin I in the blood cardioplegia group was not very high comparing to relatively long aortic occlusion time and complexity of congenital heart disease. Besides we observed remarkable decrease in this concentration within first 12 hours in arterial blood in Group 1 unlike the crystalloid cardioplegia group showing the good ability of myocardium to recover. Unfortunately it has to be admitted that there had been only three patients included in Group 2, which might not be a sufficient number to judge

the influence of myocardial protection strategy on the concentration of Troponin I properly.

The similar correlation was observed between the level of coronary lactates after aortic occlusion and the echocardioscopic grading of left ventricular function in early postoperative period in both groups.

Summarizing all results of the research, it can be stated that similarly to many studies found in literature the strategy of cold blood cardioplegia provides better myocardial protection whenever longer aortic cross-clamp and CPB time is expected. Therefore we can speculate that one of the reasons why lately the perioperative morbidity has remarkably decreased in the Clinic for Pediatric Cardiology and Cardiosurgery, Children's University Hospital is radical change of intraoperative myocardial protection strategy. Besides it has to be admitted that we experienced difficulties to finish our research due to the fact that, taking into account early results of the research, we have completely switched to the strategy of cold blood cardioplegia and within the last year there had not been a congenital heart operation where the crystalloid cardioplegia was used. This is the reason why in the Group 2 when analysing Troponin I concentration had been included only three patients.

In addition, basing on our positive experience and references of literature, since 2011 the strategy of cold blood cardioplegia with good results has been employed for intraoperative myocardial protection also in aquired cardiac surgery in the Latvian Centre for Aquired Cardiac Surgery.

Biomechanical and structural properties of coronary arteries

One of the most important goals in pediatric cardiac surgery is an adequate myocardial protection. It is achieved by conserving energy stores through rapid arrest which decreases the metabolic rate and minimises ischemic changes with specific protective agents. However, also the completeness of the delivery of

cardioplegic solution plays an important role in preventing the intraoperative myocardial damage. Therefore it is mandatory to monitor the infusion pressure of cardioplegic solution. A greater number of regions of myocardium receive limited amounts of cardioplegic solution at low perfusion pressure, which may explain the patchy nature of subendocardial damage seen with inadequate myocardial protection [*Aldea et al.,1990*]. On the other hand, too high perfusion pressure would damage coronary arteries and the myocardium. In any case this injury manifests by postbypass myocardial and vascular dysfunction, increased edema and decreased ATP levels [*Buckberg et al.,1993*]. So the „safe" infusion pressure has been recomended between 50 and 100 mmHg [*Vinten-Johansen et al.,2000*].

It is known that ischemia and reperfusion impairs normal physiologic mechanisms responsible for the normal balance between intra- and extracellular fluid. Besides the composition of cardioplegic solutions (onconicity, hemodilution) and the condition of delivery (hypothermia, high delivery pressure) are known to exaggerate the development of edema resulting from ischemia or systemic inflammatory responses. Furthermore, edema increases the microvascular resitence until degree, which impairs the blood flow in myocardium leading to increased distance of diffusion between myofibrils. It can result in impaired supply of myocites with oxygen. This pathologic process can be started by the cardioplegic solution infused in ischemic myocardium, especially if introduced with inadequatelly high pressure as well as by hemodilution and hypoosmolarity, which is induced by crystalloid cardioplegia. In addition, there are also physiologic changes in ion-pump systems started by hypothermia [*Castaneda et al.,1994; Jones et al,2006*].

Applying high delivery pressure can severely damage the myocardium, especially in the ischemic myocardium. It is known that a normal myocardium tolerates relatively high infusion pressures, but the myocardium within and

surrounding ischemic segments is vulnerable to edema induced by high delivery pressure [*Irtun et al., 1997*].

A number of studies in this area have stressed the importance of measuring the delivery pressure of cardioplegic solution, when injecting it into coronary arteries. Very often the cardioplegic solution is infused into coronary arteries with high pressure mistakenly believing that fast electromechanical standstill of the heart preserves high energy phosphates. even though this high delivery pressure gives a fast cardiac arrest, it can also result in a faster breakdown of high-energy nucleotids at the beginning of reperfusion [*Lindal et al, 1990*]. As a result, the myocardium may have less energy to work properly after aortic occlusion and contractility would be much worse [*Irtun et al., 1997*].

It is known that coronary arteries has their own autoregulation providing relatively constant blood flow there [*Buckberg et al., 1993*]. Precapillary sphincters regulates movement of blood vessels and the capillary pressure remains almost constant. If they are partly relaxed and there is inadequately high pressure, the injury of vascular endothelium and myocardium may take place. But the most important fact is that autoregulation in neonates is immature and too high or too low perfusion pressure could be deleterious [*Ishiyama et al., 2006*].

Very few researches have investigated the influence of delivery pressure of cardioplegic solution even in adult hearts not to mention neonates. It might be due to many technical and ethical problems. On the other hand, ensuring optimal cardioplegia infusion pressure remains an issue, especially in neonatal cardiac surgery. Due to the structural, physiologic, biomechanical and metabolic differences the myocardium of neonates may be more prone the pressure injury in pediatric cardiac surgery.

Antegrade cardioplegia is often injected without directly monitoring the delivery pressure, which can result in cardioplegia being delivered at a too high or too low pressure than desired. In addition, the preffered procedure in some

centers of cardiac surgery is to deliver cardioplegia as quickly as possible in order to induce a swift, diastolic cardiac arrest without transitorial fibrillation and with, presumably, maximum conservation of the high-energy nucleotids. Unfortunately, it has to be admitted that also in the Clinic for Pediatric Cardiology and Cardiosurgery, Children's University Hospital the infusion pressure had not been measured until the change of strategy for myocardial intraoperative protection. Before then the crystalloid cardioplegic solution was introduced in aortic root by manual inflation without controlling infusion pressure probably intensifying ischemic damage in the immature myocardium.

The recommendation can be found in literature based more on the experience of different centres of congenital heart surgery and less on studies employing animal models or human tissues. The recommended infusion in aortic root is 30 – 40 mmHg for neonates below 10 kg and 40 – 70 mmHg for children above 10 kg [*Jones et al,2006; Vinten-Johansen et al.,2000*].

Kronon et al. on animal model showed that the infusion pressure in neonatal cardiac surgery must not exceed 100 mmHg. Besides it has to be remembered that neonatal patients requiring the primary repair of a congenital heart disease are often hypoxic and this profoundly alters the effect of different cardioplegic infusion pressures on myocardium. Low cardioplegia infusion pressure allows the cardioplegia to facilitate repair of the injury caused by hypoxia and reoxygenation, which results in complete preservation of myocardium as well as the vascular endothelial function. This supports the safety of a cardioplegic infusion pressure of 30 to 50 mmHg and implies that it is high enough to ensure adequate myocardial distribution. Infusion pressure of less than 30 mmHg is too low to ensure adequate myocardial protection [*Kronon et al.,1998*] . Slightly higher cardioplegic infusion pressure (80 to 100 mmHg) may result in an increased cellular injury because hypoxia alters the myocardium. Such injury results in postbypass myocardial and vascular dysfunction, increased edema and decreased ATP levels [*Buckberg et al.,1993*].

Similar results were found also in our research. We concluded that infusion pressure exceeding 100 mmHg can be deleterious for the immature heart, taking into account biomechanical and structural properties of neonatal coronary arteries.

The mechanical properties of the wall of blood vessels are very important because they influence the arterial physiology. They depend on collagen and elastic fibres as well as smooth muscle cells and ground substances. Furthermore, stress and strain in the arterial wall are significant factors in understanding the pathophysiology and mechanics of the cardiovascular system [*Hayash et al.,2001; Ozolanta et al.,1998*].

The pressure-diameter relation plays an important role in the pressure-flow relationship of blood flow through the blood vessel. The compliance of the vasculature is an important determinant of the non-linearity of the pressure-flow relationship. Furthermore, the pressure-diameter-length can be transformed into biaxial (circumferential and longitudinal) stress–strain relation where the mean circumferential Cauchy stress is computed from pressure, diameter and wall thickness as per Laplace's equation. Finally, the strain is computed from circumference (or diameter) measurements in reference to the zero-stress state.

Several researches have observed that the wall thickness-to-radius ratio increases in proportion to the increase in pressure. As a result, the circumferential average wall stress is restored after some period of growth and remodelling. In addition, the strain reaches its peak sooner and normalizes faster than stress, thereforethe vessel appears to be more sensitive to changes in strain than in stress [*Kassab et al,2006*].

Furthermore, a number of studies have shown that the physical nonlinearity of arterial material is characterized by an increasing stiffness as the strain increases. The origin of this behavior is the mechanical properties of the

basic structural components of arteries, i.e. elastin and collagen, as well as the architecture of the arterial wall as explained above [*Hayash et al,2001*].

In our experiments we observed a fast and significant increase of strain until the inner pressure reached 80 – 100 mmHg whilst the increase of stress in the arterial wall was less rapid. When the internal pressure exceeded 100 mmHg, the strain of the arterial wall increased much slower whilst the wall stress and modulus of elasticity began to increase rapidly.

It has to be taken into account that in neonates coronary arteries are immature after birth. The wall of the vessels is much thinner comparing to the coronary arteries of adults and this influences the biomechanical properties of blood vessels. These arteries are much more fragile and easier to damage. In early life of a child, they increase in length, diameter and wall thickness in proportion to changes in body weight and length. The intima becomes thicker due to migration and proliferation of vascular smooth muscle cells followed by synthesis of extracellular matrix molecules.

As very few references can be found in literature regarding this topic, the decission was made to establiblish „safe" infussion pressure of cardioplegia, taking into account biomechanical and structural properties of neonatal coronary arteries. The first difficulties we experienced was rather small number of experimental material, which is is attributable to the relatively small number of deaths amongst newborn babies in Latvia. In addition, autopsies are not always performed due to the reluctance of parents. Besides only samples with no history of any kind of cardiac pathology, including atypical anatomy of coronary arteries were accepted for neonates and no signs of atherosclerosis – for adults .Therefore it proved to be a challenge to obtain a sufficient amount of experimental materials. The other problem was to gain experimental without damaging it, taking into account that the diameter of neonatal coronary artery is arround 2 mm. Nevertheless, we succeeded in performing 19 experiments altogether.

Results showed that in neonates the relationship between pressure and strain on the one hand, and stress and strain on the other hand was non-linear (*Fig.2.1.*). There was observed a fast and significant increase of strain until the inner pressure reached 80 – 100 mmHg whilst the increase of stress in the arterial wall was less rapid. When the internal pressure exceeded 100 mmHg, the strain of the arterial wall increased much slower whilst the wall stress and modulus of elasticity began to increase rapidly (*Tab.2.1.,Tab.2.2.*). When the inner pressure exceeded 80 mmHg the stiffness in the wall of neonatal coronary artery increased rapidly in comparison to the stiffness in the wall of adult coronary artery (*Tab.2.3.*). The structural elements of the arterial wall have been straightened and possible damage in the wall of coronary arteries of neonates may appear when applyed infusion pressure of 100 – 120 mmHg.

When speaking about structural properties, the references can be found about influence of infussion pressure on the ultrastructure of neonatal coronary arteries. It was observed that the pressure of 60 mmHg and reperfusion afterwards makes no damage of cells in arterial wall. On the contrary, if applyed higher pressure without reperfusion, endothelium was partly separated from extracellular matrix. In addition, intracellular vacuolisation and lateral splitting between cells was observed. This damage did not enter in the deeper layers. The worst situation stated was high infusion pressure followed by reperfusion. In that case the layer of smooth myocytes also had been damaged – there was vacuolisation observed. In addition, the mitochondria of endotheliocytes had been spoilt. There was a massive damage and separation of the cells in endothelium as well as cell death of 30% in the layer of smooth myocytes [*Katayama et al., 1997*].

Conventional histological examination revealed partial damage and rupture of intimal and medial constituents of the wall of neonatal coronary artery under exposure to pressure of 100 mmHg. This was in stark contrast with the rather intact vascular wall in adults. There was prominent affection of the

coronary artery wall with medial edema, redistribution of myocytes and injury of adventitial *vasa vasorum (Fig.2.2B,Fig.2.2E)*; medial myocytes presented loss of compactness and circumferential orientation in neonates, forming groups, which displayed haphazard *(Fig.2.2A,Fig.2.2B);* the medial thickness in neonates sometimes revealed the range equal to the medial coat of the coronary arteries in adults enrolled in this study *(Fig.2.2C)*. Marked redistribution of cellular constituents within the medial-adventitial coats under the exposure of load in neonates had been proved again when supportive and tensile vimentin containing cytoplasmic intermediate filaments were stained. Decoration with the anti-vimentin antibody revealed that the expression of this antigen after load was almost nil in neonates *(Fig.2.2D, Fig.2.2E)* thus resembling the immunostaining in adults *(Fig.2.2F)*. Orientation of occasional smooth muscular cells displaying desmin positivity had changed from linear up to haphazard and even zigzag *(Fig.2.2I)*, and a strong decoration of the endothelial cells within adventitial microvascular beds assessed demonstrated a decrease of it and loss of microvascular density in neonates when applying the inner pressure *(Fig2.2H)*.

It is known that the wall becomes thicker after the damage of neonatal coronary artery [*Rapola et al.,1977*]. In that case it can be spoken of proatherosclerotic changes, as this may be a possible place to form the atherosclerotic plaque later on in the life of patient. *Nakashima et al.*,2007 reports that the diffuse intimal thickening has been observed in the very early age of life of patients. Later on fatty acids and proteoglicanes accumulates in the outer layer of the thickening and form atherosclerotic plague. *Milei et all.,2010* has reached similar conclusions. Therefore we can speculate that the damage of neonatal coronary arteries when applying inappropriately high delivery pressure of cardioplegic solution may also increase the risk of myocardial infarction in patient's future life. However, to reach a definitive conclusion in this topic more researches need to be performed in this area.

The recommendations had been developed based on results of our research. The infusion pressure of cardioplegia has been monitored in the Clinic for Pediatric Cardiology and Cardiosurgery, Children's University Hospital. The goal is 60 – 80 mmHg strictly not exceeding 100 mmHg, especially in neonates.

The metabolic changes in stored packed red blood cells

One of the main goal in modern pediatric bypass equipment is to reduce the size of the extracorporeal circuit in order to minimize the prime volume, which can actually exceed the blood volume of a neonate by as much as 200% to 300%, whereas in an adult patient, the priming volume accounts for only 25% to 30% of the blood volume. Even though nowadays there has been developed smaller tubing and oxygenators, which reduces priming volume significantly, it is still an issue in pediatric CPB.

High priming volume can produce a low hematocrit on CPB in small infants, which results in reduction of plasma proteins and clotting factors, decrease of the colloid osmotic pressure (interstitial edema), electrolyte imbalance, exaggerated release of stress hormones with activation of complement, white blood cells and platelets, therefore it is compulsory to use the donor blood in the prime. The use of donor blood itself has several disadvantages, including transmission of viral particles, complement activation, induction of a transfusion reaction leading to acute renal failure in neonates after cardiac surgery [*Bojan et al.,2012*]. In addition, there is an infusion of lactate, glucose, potassium and citrate-phosphate-dextrose, therefore this should be avoided as much as possible. However, for the reason of hemodilution most institutions use packed red blood cells in their priming solutions

Although packed red blood cells (PRBC) are essential to maintain appropriate hematocrit level and adequate oxygen delivery in children, they are associated with significant metabolic imbalances (acid-base, glucose,

electrolyte) leading to a number of severe complications. When blood is stored, many alterations occur in its constinuents. In particular, there is an increase in potassium and lactate levels, and decrease in pH. The most important changes from the physiological range pointed out by *Ratcliffe et al.,1986* are in concentration of sodium, potassium, glucose and lactate. They experienced increase in the level of potassium and lactate, and decrease in the level of sodium and glucose. Besides they pointed out a strong correlation between the duration of storage and the level of sodium, potassium and lactate.

The biggest problem appears to be hyperkalaemia. Because red blood cell membranes are only slightly permeable to potassium, their movement is largely dependent on energy-dependent transport mechanisms (glycolytically derived adenosine triphosphate). During storage, red blood cell membranes age, adenosine triphosphate synthesis and potassium pumping decrease, and intracellular potassium leaks into the supernatant, which leads to hyperkalaemia [*Smith et al,2008*]. As there is no oxygen in PRBC, the energy is mainly produced in the way of anaerobic glycolysis, therefore there is an increase in the level of lactate and decrease in the level of glucose in the supernatant leading to acidosis [*Jaggers et al.,2000; Keidan et al.,2004; Schroeder et al.,2005*]. It is observed that hyperlactatemia is strongly correlated with postoperative morbidity after the correction of complex congenital heart lessions due to the bad perfusion of tissues and cardiac output, especially in neonates [*Hatherill et al,1997; Siegel et al,1996*]. Therefore an additional infusion of lactates may increase acidosis.

Hyperkalaemia and hyperlactatemia related to transfusion itself is not a big problem. What makes it an important issue in pediatric cardiac surgery are many other mechanisms contributing to the risks of PRBC transfusion-induced hyperkalaemia or increased potassium cardiotoxicity include hyperglycemia, hypocalcemia, hypothermia and acidosis. First, surgical stress and shock are associated with hyperglycemia, which induces an increase in serum osmolality

causing potassium to exit cells. Second, massive transfusion of citrated blood is associated with hypocalcemia, which predisposes to cardiac membrane instability at lower potassium levels. Hypothermia also slows the metabolism of citrate, which exacerbates hypocalcemic states. Third, in hypothermia heart becomes more sensitive to the toxic effects of potassium [*Smith et al.,2008*].

Therefore many institutional protocols advocate limiting the use of PRBC in priming solution for the pediatric CPB to relatively fresh stored PRBC to avoid such complications. To evaluate the effect of length of storage of PRBC on the concentration of potassium, sodium, lactate, glucose and pH in PRBC used in pediatric cardiac surgery for priming of CPB in the Clinic for Pediatric Cardiology and Cardiac Surgery, Children's University Hospital as well as to improve the quality of CPB, decision was made to do a research about this topic.

Our results showed statistically significant difference between both groups in terms of potassium and lactate concentration, but not as significant in terms of sodium. There was no difference between groups in terms of glucosis (*Tab. 2.4.)*. In addition, similarly as *Ratcliffe et al.,1986*, a linear increase in the levels of potassium and lactate depending on the storage age of PRBC was observed.

The recommendations had been developed in the Clinic for Pediatric Cardiology and Cardiac Surgery, Children's University Hospital based on results of our research to include in priming of CPB as fresh PRBC as possible.

CONCLUSIONS

1. Both blood and crystalloid cardioplegia provides good myocardial protection in pediatric cardiac surgery.
2. Blood cardioplegia is better for younger patients with smaller weight, and whenever longer aortic occlussion and cardiopulmonary bypass time is

expected, and should be used in operations for correction of more complex congenital heart lessions.

3. There is an increase of the stress and modulus of elasticity in the arterial wall of neonatal coronary arteries when the internal pressure exceeds 100 mmHg (13,33 kPa) comparing to adults. The structural elements of the arterial wall have been straightened and a possible damage in the wall of coronary arteries of neonates may appear.
4. There is an overall thickening of the coronary wall, occasional and partial split and/or interruption of internal elastic lamina, and redistribution of constituents of the vascular bed in neonates after exposure of 120 mmHg (15,99 kPa) revealed in conventional histological examination. By contrast, there are rather moderate vascular changes in adults.
5. The delivery pressure of cardioplegic solution in neonatal coronary arteries should not exceed 100 mmHg (13,33 kPa), taking into account biomechanical and structural properties of neonatal coronary artery, because higher increases the risk of structural damage of the vascular wall leading to the injury of myocardium.
6. There is a direct correlation between the concentration of potassium and lactate, and the the storage age of PRBC. In addition, there is a linear increase in the levels of potassium and lactate depending on the storage age of PRBC.
7. PRBC's used for the priming of CPB in pediatric cardiac surgery should be as fresh as possible with a shorter storage age to avoid unexpected complications.

PRACTICAL RECOMMENDATIONS

1. As both methods – cold blood and cold crystalloid - used for the intraoperative myocardial protection in pediatric cardiac surgery are safe and provides good myocardial protection, they can be used without any limitations when operating on congenital heart lessions.
2. Blood cardioplegia provides better myocardial protection for younger patients with smaller weight, and whenever longer aortic occlussion and cardiopulmonary bypass time is expected, therefore it should be used in operations for correction of more complex congenital heart lessions. There is not significant difference between both methods, if the patient is older with bigger weight and the congenital heart lession is not complex.
3. Taking into account biomechanical and structural properties of neonatal coronary artery, the delivery pressure of cardioplegic solution must be strictly monitored. The desired pressure is 60 – 80 mmHg. However, it should not exceed 100 mmHg not to increase the risk of structural damage of the vascular wall.
4. All PRBC's used for the priming of CPB in pediatric cardiac surgery should be as fresh as possible with a shorter storage age to avoid unexpected complications. However, they should not exceed five days of age.

PUBLICATIONS AND PRESENTATIONS

Publications

1. **The proper delivery pressure for cardioplegic solution in neonatal cardiac surgery – an investigation of biomechanical and structural properties of neonatal coronary arteries.** N.Sikora, A.Lācis, V.Kasyanov, V.Groma, V.Ozoliņš, L.Šmits, E.Ligere. Perfusion, March 2013; 10.1177/0267659113481487

2. **Biomechanical Properties of Coronary Arteries Neonates: Preliminary Results.** N.Sikora, A.Lācis, E.Teivāne, V.Ozoliņš, L.Šmits, I.Bergmane, V.Kasyanov. ANALYSIS AND DESIGN OF BIOLOGICAL MATERIALS AND STRUCTURES Advanced Structured Materials, 2012, Volume 14, Part 2, 111 – 124, DOI: 10. 1007/978-3-642-22131-6_9

3. **Biomechanical properties of coronary arteries neonates – first results.** N.Sikora, E.Teivāne, A.Lācis, V.Ozoliņš, L.Šmits, I.Bergmane, V.Kasyanov. RSU Collection of Scientific Papers: Research articles in medicine&pharmacy, 2010: Internal Medicine. Surgery. Medical Basic Sciences. Stomatology. Pharmacy. – Rīga: RSU, 2011: 85 – 94

4. **Miokarda aizsardzība iedzimto sirdskaišu operācijās – etapa pētījuma rezultāti.** N.Sikora, A.Lācis, L.Šmits, V.Ozoliņš. RSU Collection of Scientific Papers: Research articles in medicine&pharmacy, 2009: Internal Medicine. Surgery. Medical Basic Sciences. Stomatology. Pharmacy. – Rīga: RSU, 2010: 157 – 162

5. **The metabolic changes in fresh versus old stored blood used in priming of extracorporeal circuit in cardiopulmonary bypass for pediatric patients-first results.** N.Sikora, L.Šmits, V.Ozoliņš, E.Teivāne, A.Lācis. ACTA CHIRURGICA LATVIENSIS 2009; 9: 24-27

6. **Myocardial protection in pediatric cardiac surgery.** N.Sikora. Work of residency. RSU, 2008

6. **Myocardial protection in pediatric cardiac surgery.** N.Sikora, L.Šmits, V.Ozoliņš, A.Lācis. ACTA CHIRURGICA LATVIENSIS, 2007; 7: 48 – 52

Abstracts

1. **The proper delivery pressure for cardioplegic solution in neonatal cardiac surgery – an investigation of biomechanical and structural properties of neonatal coronary arteries.** N.Sikora, A.Lacis, V.Ozolins, E.Ligere, L.Smits, V.Kasyanov, V.Groma. 22nd World Congress of the World Society of Cardio-Thoracic Surgeons, September 9 – September 11, 2012

2. **The proper delivery pressure for cardioplegic solution in neonatal cardiac surgery – an investigation of biomechanical and structural properties of neonatal coronary arteries.** N.Sikora, A.Lacis, V.Ozolins, E.Ligere, L.Smits, V.Kasyanov, V.Groma. 13th Annual Congress of the South African Heart Association, Structural Heart Disease 2012, Sun City, South Africa, July 19 – July 22, 2012

3. **Myocardial protection in pediatric cardiac surgery.** N.Sikora, L.Šmits, V.Ozoliņš, E.Ligere, A.Lācis. 14th European Congress on Extracorporeal Circulation Technology, Dubrovnik, Croatia, June 15 – June 18, 2011

4. **The metabolic changes in fresh versus old stored blood used in priming of extracorporeal circuit in cardiopulmonary bypass for pediatric patients.** N.Sikora, L.Šmits, V.Ozoliņš, E.Ligere, A.Lācis. 14th European Congress on Extracorporeal Circulation Technology, Dubrovnik, Croatia, June 15 – June 18, 2011

5. **Miokarda aizsardzības metožu efektivitātes novērtējums iedzimto sirdskaišu operācijās.** N.Sikora, A.Lācis, L.Šmits, V.Ozoliņš, E.Ligere. RSU Scientific conference, April 14 - April 15, 2011

6. **Biomechanical properties of coronary arteries neonates.** N.Sikora, E.Teivāne, A.Lācis, V.Ozoliņš, L.Šmits, I.Bergmane, V.Kasyanov. 4th

International Conference on Advanced Computational Engineering and Experimenting, Paris, France, 08-09 July, 2010

7. **Myocardial protection in pediatric cardiac surgery – first results of the research.** N.Sikora, L.Šmits, V.Ozoliņš, E.Teivāne, I.Bergmane, A.Lācis. 8th International Congress of the Croatian Society of Extracorporeal Circulation Technology, Malinska, Croatia, May 06 – May 09, 2010

8. **The metabolic changes in fresh versus old stored blood used in priming of extracorporeal circuit in cardiopulmonary bypass for pediatric patients – first results.** N.Sikora, L.Šmits, V.Ozoliņš, E.Teivāne, I.Bergmane, A.Lācis. 8th International Congress of the Croatian Society of Extracorporeal Circulation Technology, Malinska, Croatia, May 06 – May 09, 2010

9. **Sirds vainagartēriju biomehāniskās īpašības jaundzimušajiem: pirmie rezultāti.** N.Sikora, L.Šmits, V.Ozoliņš, E.Teivāne, A.Lācis, V.Kasjanovs. RSU Scientific conference, March 18 - March 19, 2010

10. **Miokarda aizsardzība iedzimto sirdskaišu operācijās – agrīnie pētījuma rezultāti.** N.Sikora, A.Lācis, L.Šmits, V.Ozoliņš, E.Teivāne. RSU Scientific conference , 2009

11. **Miokarda aizsardzība iedzimto sirdskaišu korekcijas operācijās.** N.Sikora, A.Lācis, L.Šmits, V.Ozoliņš. RSU Scientific conference., 2008

International and local presentations

1. **The proper delivery pressure for cardioplegic solution in neonatal cardiac surgery – an investigation of biomechanical and structural properties of neonatal coronary arteries.** N.Sikora, A.Lacis, V.Ozolins, E.Ligere, L.Smits, V.Kasyanov, V.Groma. Oral and Poster presentation. 22nd

World Congress of the World Society of Cardio-Thoracic Surgeons, September 9 – September 11, 2012

2. **The proper delivery pressure for cardioplegic solution in neonatal cardiac surgery – an investigation of biomechanical and structural properties of neonatal coronary arteries.** N.Sikora, A.Lacis, V.Ozolins, E.Ligere, L.Smits, V.Kasyanov, V.Groma. Poster presentation. 13th Annual Congress of the South African Heart Association, Structural Heart Disease 2012, Sun City, South Africa, July 19 – July 22, 2012

3. **Myocardial protection in pediatric cardiac surgery.** N.Sikora, L.Šmits, V.Ozoliņš, E.Ligere, A.Lācis. Oral presentation. 14th European Congress on Extracorporeal Circulation Technology, Dubrovnik, Croatia, June 15 – June 18, 2011

4. **The metabolic changes in fresh versus old stored blood used in priming of extracorporeal circuit in cardiopulmonary bypass for pediatric patients.** N.Sikora, L.Šmits, V.Ozoliņš, E.Ligere, A.Lācis. Oral presentation. 14th European Congress on Extracorporeal Circulation Technology, Dubrovnik, Croatia, June 15 – June 18, 2011

5. **Miokarda aizsardzības metožu efektivitātes novērtējums iedzimto sirdskaišu operācijās.** N.Sikora, A.Lācis, L.Šmits, V.Ozoliņš, E.Ligere. Oral presentation. RSU Scientific conference, April 14 - April 15, 2011

6. **Myocardial protection in pediatric cardiac surgery – first results of the research.** N.Sikora, L.Šmits, V.Ozoliņš, E.Teivāne, I.Bergmane, A.Lācis. Oral presentation. 8th International Congress of the Croatian Society of Extracorporeal Circulation Technology, Malinska, Croatia, May 06 – May 09, 2010

7. **The metabolic changes in fresh versus old stored blood used in priming of extracorporeal circuit in cardiopulmonary bypass for pediatric patients – first results.** N.Sikora, L.Šmits, V.Ozoliņš, E.Teivāne, I.Bergmane, A.Lācis. Oral presentation. 8th International Congress of the Croatian Society of Extracorporeal Circulation Technology, Malinska, Croatia, May 06 – May 09, 2010

8. **Sirds vainagartēriju biomehāniskās īpašības jaundzimušajiem: pirmie rezultāti.** N.Sikora, L.Šmits, V.Ozoliņš, E.Teivāne, A.Lācis, V.Kasjanovs. Poster presentation. RSU Scientific conference, March 18 - March 19, 2010

9. **Miokarda aizsardzība iedzimto sirdskaišu operācijās – agrīnie pētījuma rezultāti.** N.Sikora, A.Lācis, L.Šmits, V.Ozoliņš, E.Teivāne. Poster presentation. RSU Scientific conference , 2009

10. **Miokarda aizsardzība iedzimto sirdskaišu korekcijas operācijās.** N.Sikora, A.Lācis, L.Šmits, V.Ozoliņš. Poster presentation. RSU Scientific conference., 2008

ABBREVIATIONS

ATP – adenosine triphosphate

CPB – cardiopulmonary bypass

FS or *Fraction of shortening* – echocardioscopic grading of left ventricular function

EF or *Ejection fraction* – echocardioscopic grading of left ventricular function

„Hot shot" – delivering of warm blood without cardioplegia solution into the aortic root before declamping aorta

PRBC – packed red blood cells