



# Myocardial protection: comparing histological effects of single-dose cardioplegic solutions – study protocol for a secondary analysis of the CARDIOPLEGIA trial

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**Background:** Myocardial protection is crucial for successful cardiac surgery, as it prevents heart muscle damage that can occur during the procedure. Prolonged hypoxia without proper protection can lead to adenosine triphosphate consumption, microvilli loss, blister formation, and edema. Custodiol, del Nido, and modified del Nido are single-dose cardioplegic solutions with proven safety and significance in modern surgery. While each has been independently assessed for patient outcomes, limited research directly compares them. This study aims to compare their myocardial protection using histological analysis.

**Methods:** In a double-blind clinical trial, at least 90 patients will be randomly assigned to receive one of the three cardioplegic solutions. Myocardial biopsies will be collected before cardiopulmonary bypass and 15 minutes after reperfusion. The surgical, anesthetic and perfusion techniques will be the same for all patients, following the Institution's standard protocols.

**Discussion:** The ideal cardioplegic solution does not exist, and its selection remains challenging for surgeons. In modern surgical practice, understanding the behavior of these solutions and the ischemic tissue damage caused during induced cardiac arrest allows for safer surgical procedures. The results of this clinical trial can help in understanding the behavior of cardioplegic solutions and their tissue effects. Thus, by selecting the best cardioplegic solution, ischemic damage can be minimized, enhancing the effectiveness

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of this essential technique in cardiac procedures. The study may aid in implementing clinical protocols in several institutions, aiming to choose the solution with a superior myocardial protection profile, increasing safety, and reducing expenses.

**Trial Registration:** Brazilian Clinical Trials Registry (ReBEC, <http://ensaiosclinicos.gov.br/>): RBR-997tqhh. Registered: January 26th, 2022.

**Keywords:** Myocardial protection; cardioplegic solutions; custodiol solution; Bretschneider cardioplegic solution; del Nido solution

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## Introduction

### Background

Most cardiac surgeries require cardiopulmonary bypass (CPB) with induced cardiac arrest. In this context, myocardial protection with cardioplegia is essential, limiting the metabolic activity of the heart and enhancing the myocardium's ability to resist ischemia for prolonged periods, optimizing surgical outcomes.

Single-dose cardioplegic solutions were introduced seeking the ideal solution, with the following specific benefits: (I) avoiding procedure interruptions, reducing aortic cross-clamping and CPB times in more complex or minimally invasive surgeries; and (II) more effective protection from postoperative cardiac dysfunction (1).

Histidine-tryptophan-ketoglutarate (HTK) solution, first introduced by Bretschneider in the 1970s (2), was developed as an alternative to hyperkalemic solutions (3,4), and classified as intracellular crystalloid cardioplegia due to its low potassium, sodium, and calcium content. Custodiol has found widespread acceptance in numerous European countries as the first choice in standard cardiac interventions and, mainly, for the preservation of myocardium in cardiac transplants (3).

Pedro del Nido and his team developed del Nido cardioplegia in the 1990s, as described by Matte and del Nido himself in 2012 (5), being used since 1994 for pediatric cardiac surgery and also used successfully in adults since 2003 (6-15). It is a mixed extracellular solution of blood and crystalloid that allows a longer duration of safe myocardial ischemic arrest. The crystalloid base of the solution includes Plasma-Lyte, characterized by an electrolyte formulation analogous to human physiological

plasma and free of calcium, added to mannitol, lidocaine, magnesium sulfate, bicarbonate, and potassium.

Certain surgeons have endorsed (16) the adoption of modified del Nido cardioplegia, replacing Plasma-Lyte with Lactated Ringer as base solution, with myocardial protection similar or superior to the blood cardioplegia strategy. All of these single-dose solutions are linked with secure administration and capable of effective myocardial protection over prolonged intervals during CPB, allowing uninterrupted procedures to be performed (5,13,17-22).

### Rationale and knowledge gap

The search for an ideal cardioplegic solution remains elusive. The need for postoperative hemodynamic support (inotropic, assistive devices, intra-aortic balloon pump) indicates that myocardial damage after cardiac surgery remains prevalent with the usual cardioplegic techniques and multi-dose solutions. The existing literature does not definitively establish the superiority of any specific method, and no studies directly compare solutions, especially single-dose ones.

### Objective

Therefore, this study is a secondary analysis of the Cardioplegia Trial and aims to compare the effects of Custodiol, del Nido, and modified del Nido solutions on myocardial protection through histological examination to analyze of cellular structural changes, in patients undergoing elective cardiac surgery. We present this article in accordance with the SPIRIT reporting checklist (available at <https://jtd.amegroups.com/article/view/10.21037/jtd-23->

1442/rc) (8).

## Methods

This is an independently registered Cardioplegia Trial substudy (Brazilian Clinical Trials Registry - ReBEC, <http://ensaiosclinicos.gov.br/>: RBR-997tqhh). Details of this trial have been previously reported (1). This study will adhere to the guidelines outlined in the Consolidated Standards of Reporting Trials (CONSORT) (23). Methods relevant to this report are described below.

### *Study design*

A double-blind, randomized clinical trial to compare the effects of single-dose cardioplegia solutions (Custodiol, del Nido, and modified del Nido) on myocardial protection through myocardial cytological evaluation.

### *Eligibility criteria*

The selection of the study site was based on convenience, with eligibility criteria pre-determined by the researchers. All patients undergoing elective valve replacement or coronary artery bypass graft (CABG) surgery with CPB at Nossa Senhora da Conceição Hospital, Porto Alegre, Brazil, will be included. Patients with prior cardiac surgery, chronic renal failure (serum creatinine greater than 2.0 mg/dL), severe psychiatric illness, emergency surgeries, and combined surgeries will be excluded from the study.

### *Surgical technique*

General anesthesia will be administered to all patients. The surgical approach will involve a median sternotomy. CPB will be implemented through arterial cannulation in the ascending aorta or femoral artery and double-stage venous cannulation in the right atrium, or alternatively, single-stage in the superior and inferior vena cava, depending on the type of surgery. Cardiac arrest due to cardioplegia will be performed in CPB and after aortic cross-clamping.

### *Cardioplegic solutions: preparation and administration*

The perfusionist will prepare the solutions. Myocardial protection will be performed by infusing cardioplegic solution into the aortic root or coronary ostia anterogradely, as specifically indicated.

Custodiol solution is sterile and does not require preparation for administration. Each 1,000 mL of Custodiol contain: sodium chloride (15.0 mmol/L), potassium chloride (9.0 mmol/L), potassium hydrogen 2-ketoglutarate (1.0 mmol/L), magnesium chloride hexahydrate (4.0 mmol/L), histidine hydrochloride monohydrate (18.0 mmol/L), histidine (180.0 mmol/L), tryptophan (2.0 mmol/L), mannitol (30.0 mmol/L), and calcium chloride dihydrate (0.015 mmol/L). Its physical properties are: pH 7.02–7.20 at 25 °C (77.0 °F) [pH 7.40–7.45 at 4 °C (39.2 °F)] and osmolality 290 mosmol/kg (17). It will be administered in a single dose of 25 mL/kg within a timeframe of 6 to 8 min, at a temperature ranging from 4 to 8 °C, with infusion pressure maintained between 150 to 200 mmHg. Additionally, there is the option of administering supplementary doses after 3 hours from the initial dose.

According to the routine, the del Nido and modified del Nido solutions will be handled in the Service. The del Nido cardioplegia composition includes a base solution Plasma-Lyte A (1,000 mL), mannitol 20% (16.3 mL), magnesium sulfate 50% (4.0 mL), sodium bicarbonate 1 mEq/mL (13.0 mL), potassium chloride 2 mEq/mL (13.0 mL) and lidocaine 1% (13.0 mL). In modified del Nido cardioplegia, the base solution is replaced by Lactated Ringer's solution. These crystalloid bases are mixed with autologous blood in a ratio of 4:1. They will be administered at a dose of 20 mL/kg (for patients weighing over 50 kg, the maximum dosage will be capped at 1,000 mL), at a temperature of 4 °C, with a system pressure maintained between 100 to 200 mmHg, and the administration flow will be set between 200 to 300 mL/min. If necessary, additional doses may be infused after 90 minutes of the initial dose (7).

### *Tissue analysis: myocardial biopsy*

During the surgical procedure, a biopsy of a myocardial fragment of the anterior wall of the right ventricle with a dermatological punch of 1 mm will be performed at two different times: (I) B1 (first biopsy): before the CPB (moment 1); and (II) B2 (second biopsy): 15 minutes after the opening of the aortic cross-clamping (moment 2), still in CPB. Suturing will be performed only in cases of persistent bleeding. The biopsied material will be immediately placed in a vial with formaldehyde and sent to the hospital's Pathology Service for analysis under optical microscopy.

Inflammatory cells such as leukocytes (neutrophils, macrophages, and lymphocytes) and events such as margination, diapedesis, edema, and tissue necrosis will

be analyzed. The analysis of the myocardial biopsy will be performed in the pathology laboratory of the hospital by two specialized pathologists, independently blinded to any and all interventions performed. The standardized scale of the International Society of Heart and Lung Transplantation 2005 (ISHLT 2005) (24,25) will be used to evaluate structural changes: Grade 0R, no or minimal inflammation; Grade 1R, inflammation with no muscle damage or a single focus of muscle damage; Grade 2R, multifocal infiltrate with muscle damage; and Grade 3R, diffuse infiltrate with muscle damage.

### ***Main outcome and participant timeline***

The strength of this analysis is that it compares the histological findings of the myocardial tissue of patients undergoing elective CPB surgery using the cardioplegic solutions Custodiol, del Nido, and modified del Nido.

Initially, eligibility criteria will be assessed. Once approved for the study, written informed consent will be obtained and baseline clinical, echocardiographic, and laboratory data will be collected. During surgery, patients will receive cardioplegia according to the allocation groups, blood samples will be taken (cardiac enzymes, blood gas analysis, glycemic profile, electrolytes, and complete blood count) and myocardial biopsies will be taken. During the 24 hours after surgery, three additional blood samples will be taken for the study. Relevant outcomes during and after surgery will be collected and another echocardiogram will be performed up to the 7th postoperative day. Patients will be followed up until hospital discharge or 30 days after surgery. The initiation of participant enrollment took place on January 26th, 2022.

### ***Sample***

Adult patients undergoing elective valve replacement or CABG surgery in a high-complexity public hospital in Cardiovascular Surgery in southern Brazil, randomized to receive Del Nido solution, modified Del Nido, or Custodiol during CPB. Monitoring will be conducted to assess the efficacy of the initially proposed treatment.

Based on the study by Talwar *et al.* (26), the sample size calculation was performed for the Cardioplegia Trial using the G-Power software version 3.1.9.4, considering an effect  $f=0.466$  on troponin levels. It would be necessary to investigate 75 patients to maintain a significance level of  $\alpha=0.05$  and a power of 95%. Considering possible losses

of up to 20% and maintenance of statistical power in the multivariate analysis, a minimum of 90 individuals will be selected for the study, with a distribution of at least 30 individuals randomized to each group.

### ***Recruitment and allocation***

Potential candidates for cardiac surgery will be identified when referred to the Service or from the surgical waiting list and approached by a team researcher regarding possible participation in the research. Patients meeting the eligibility criteria will be randomized to receive Custodiol, del Nido, or modified del Nido following their consent and confirmation of eligibility.

### ***Randomization***

Patients will be randomly assigned in blocks to receive the solutions of Custodiol, del Nido, or modified del Nido during CPB through the randomizer.org website. The numbers will be placed within opaque and individually sealed envelopes.

### ***Blinding and adjudication of independent events***

On the day of surgery, and prior to anesthetic induction, the perfusionist will be provided with the opaque and sealed envelope specifying the cardioplegic solution to be prepared and administered in accordance with its specific instructions. All the patients and family members, anesthesiologist, pathologists, nurses and laboratory personnel will be blinded to the nature of the intervention. The surgical team will be excluded from participating in the analysis of the results to prevent measurement biases, as they will be blinded only for intervention involving del Nido or modified del Nido, as the Custodiol infusion requires a different administration time and time lapse between doses compared to the del Nido solution.

All researchers engaged in the adjudication process will maintain blindness regarding patient allocation to the intervention type. The adjudicated data will be used in the final safety and efficacy analysis.

### ***Data collection***

Right ventricular myocardial biopsy will be collected, measuring approximately 1 mm in its largest diameter, in 2 moments: before the beginning of CPB and 15 minutes

after opening the aortic cross-clamping.

### *Statistical analysis*

Quantitative variables will be described using mean and standard deviation for symmetric distributions, or median and interquartile amplitude for asymmetric distributions. Absolute and relative frequencies will describe qualitative variables. The Shapiro-Wilk test will be used to evaluate the normality of continuous variables. Pearson's chi-square test will be used to compare categorical variables. A multivariate multinomial logistic regression model will be used to adjust for potential confounding factors. The significance level adopted will be 5%, and the data will be analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0.

The agreement among pathologists in myocardial evaluation will be assessed using kappa statistics, calculated with WinPEPI version 11.65. The agreement strength was categorized as: light (0.00 to 0.20), fair (0.21 to 0.40), moderate (0.41 to 0.60), substantial (0.61 to 0.80), or near perfect (0.81 to 1.00) (27).

### *Data monitoring and safety*

While the del Nido and Custodiol cardioplegic solutions differ in their mechanisms for inducing cardiac arrest, both exhibit the ability for safe administration in a single dose. They are equally proficient in maintaining myocardial preservation over extended periods during ischemia within CPB procedures (5,17,18).

The main investigator will conduct audits of the outcomes after every five interventions or sooner in instances of documenting severe postoperative adverse events. The study investigators will assess adverse events and have the authority to terminate the study prematurely in event of a discernible and clinically significant increase in risk.

All collected data will undergo evaluation by at least two independent authors, with rigorous quality control measures implemented during data entry to ensure accuracy and coherence. To ensure the team's performance quality, the main investigator will randomly review 20% of the protocols for quality assessment.

### *Ethical aspects and dissemination*

The study will follow the Declaration of Helsinki (as revised in 2013). The study protocol and its related documents

were approved by the Research Ethics Committee of the Nossa Senhora da Conceição Hospital, registered under No. 4,029,545, and informed consent will be obtained from all individual participants.

Any modification that may impact the conduct of the study, including changes to the design, objective, sample, or significant administrative aspects, will require a formal amendment to the Institutional Research Ethics Committee.

Concerning the matters of privacy and confidentiality, it is ensured that patient identities will remain anonymous, and the collected data will solely serve the research's intended objectives. The study's outcomes will undergo submission to a peer-reviewed journal for publication, alongside presentations at pertinent surgical conferences.

### *Data availability statement*

The datasets generated and used during this study are available from the corresponding author upon reasonable request.

The technical appendix, statistical code, and dataset may be available upon the completion of the trial from the Dryad or a similar repository.

## **Discussion**

Custodiol, del Nido, and modified del Nido are all cardioplegic solutions used for myocardial protection during cardiac surgery. A secondary analysis of the randomized controlled cardioplegia trial will compare the efficacy of these solutions in terms of histological analysis.

### *Potential impact and significance of the clinical trial*

Despite the distinct nature of the cardioplegic solutions del Nido and Custodiol, both are correlated with secure administration in a single dose and can preserve the myocardium for a long period during ischemia in the CPB (5,17,18). However, the mechanisms involved in this process are not yet completely known. The available literature does not clearly establish the superiority of any specific cardioplegic solution, and there are no direct comparison studies between single-dose solutions.

In a clinical trial conducted by Talwar and colleagues (26), 100 pediatric patients undergoing elective tetralogy of Fallot surgery, between July 2017 and January 2018, were randomized to receive del Nido or Custodiol cardioplegic

solution. The group that received del Nido showed better preservation of cardiac index and output, lower inotropic scores, and a reduced release of troponin-I, shorter duration of mechanical ventilation, and stay in the intensive care unit and hospital. Electron microscopy revealed reduced myocardial edema and improved preservation of myofibrillar architecture and glycogen storage. Other clinical trial (28), comparing del Nido with its modified version, in a similar population, observed that myocardial protection was equivalent between groups. Mehrabian and colleagues (29) conducted a randomized trial involving 40 patients assigned to receive one of these cardioplegic solutions, concluding that both provide effective and comparable myocardial protection during CPB in adults.

The need for hemodynamic support in the postoperative period suggests that myocardial damage after cardiac surgery persists with the multi-dose cardioplegias. Few studies have compared Cardioplegia Custodiol or del Nido with conventional blood (6,22,30-37), all showing the superiority of single-dose ones, often without histological analysis.

The del Nido cardioplegia solution seems to be highly efficient in terms of myocardial protection and economic aspects. Nevertheless, the limited number of randomized trials in pediatric patients, and even fewer in adult patients, most of which are retrospective, constrains their overall value (7,14,38). Hence, there is a need for prospective and randomized studies to confirm the hypothesis regarding the viability or superiority of this cardioplegia in relation to others concerning myocardial protection.

This study directly compares the three cardioplegic solutions regarding myocardial histological changes in patients during cardiac surgery. However, the main limitation of our research is the calculation of the sample size, performed for the Cardioplegia Trial, based on the evaluation of cardiac enzymes.

### **Clinical applicability**

The ideal cardioplegic solution does not exist, and its selection remains challenging for surgeons. In modern surgical practice, understanding the behavior of these solutions and the ischemic tissue damage caused during induced cardiac arrest allows for safer surgical procedures. The results of this clinical trial can help in understanding the behavior of cardioplegic solutions and their tissue effects. Thus, by selecting the best cardioplegic solution, ischemic damage can be minimized, enhancing the

effectiveness of this essential technique in cardiac procedures. The study may aid in implementing clinical protocols in several institutions, aiming to choose the solution with a superior myocardial protection profile, enhancing safety and lowering costs. Furthermore, despite being conducted at a single center, our hospital is one of the largest public general hospitals in Brazil, a reference center in the highly complex care of the public health system in the country, reflecting the national standard of practice.

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study will follow the Declaration of Helsinki (as revised in 2013). The study was approved by the Research Ethics Committee of the Nossa Senhora da Conceição Hospital, registered under No. 4,029,545, and informed consent will be obtained from all individual participants.

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## References

- Almeida AS, Ceron RO, Anschau F, et al. Comparison between Custodiol, del Nido and modified del Nido in the myocardial protection - Cardioplegia Trial: a study protocol for a randomised, double-blind clinical trial. *BMJ Open* 2021;11:e047942.
- Bretschneider HJ, Hübner G, Knoll D, et al. Myocardial resistance and tolerance to ischemia: physiological and biochemical basis. *J Cardiovasc Surg (Torino)* 1975;16:241-60.
- Groenewoud AF, Buchholz B, Gubernatis F, et al. First results of the multicenter study of HTK protection for kidney transplants. *Transplant Proc* 1990;22:2212.
- Hölscher M, Groenewoud AF. Current status of the HTK solution of Bretschneider in organ preservation. *Transplant Proc* 1991;23:2334-7.
- Matte GS, del Nido PJ. History and use of del Nido cardioplegia solution at Boston Children's Hospital. *J Extra Corpor Technol* 2012;44:98-103. Erratum in: *J Extra Corpor Technol* 2013;45:262.
- Ad N, Holmes SD, Massimiano PS, et al. The use of del Nido cardioplegia in adult cardiac surgery: A prospective randomized trial. *J Thorac Cardiovasc Surg* 2018;155:1011-8.
- Sanetra K, Pawlak I, Cisowski M. Del Nido cardioplegia - what is the current evidence? *Kardiochir Torakochirurgia Pol* 2018;15:114-8.
- Chan AW, Tetzlaff JM, Altman DG, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. *Ann Intern Med* 2013;158:200-7.
- Kim K, Ball C, Grady P, et al. Use of del Nido Cardioplegia for Adult Cardiac Surgery at the Cleveland Clinic: Perfusion Implications. *J Extra Corpor Technol* 2014;46:317-23.
- Yerebakan H, Sorabella RA, Najjar M, et al. Del Nido Cardioplegia can be safely administered in high-risk coronary artery bypass grafting surgery after acute myocardial infarction: a propensity matched comparison. *J Cardiothorac Surg* 2014;9:141.
- Najjar M, George I, Akashi H, et al. Feasibility and safety of continuous retrograde administration of Del Nido cardioplegia: a case series. *J Cardiothorac Surg* 2015;10:176.
- Ota T, Yerebakan H, Neely RC, et al. Short-term outcomes in adult cardiac surgery in the use of del Nido cardioplegia solution. *Perfusion* 2016;31:27-33.
- Mongero LB. Del Nido Cardioplegia-Not Just Kids Stuff. *J Extra Corpor Technol* 2016;48:P25-8.
- Guajardo Salinas GE, Nutt R, Rodriguez-Araujo G. Del Nido cardioplegia in low risk adults undergoing first time coronary artery bypass surgery. *Perfusion* 2017;32:68-73.
- Sanetra K, Domaradzki W, Cisowski M, et al. The impact of del Nido cardioplegia solution on blood morphology parameters. *Perfusion* 2023;38:277-84.
- Kantathut N, Cherntanomwong P, Khajarern S, et al. Lactated Ringer's as a Base Solution for del Nido Cardioplegia. *J Extra Corpor Technol* 2019;51:153-9.
- Edelman JJ, Seco M, Dunne B, et al. Custodiol for myocardial protection and preservation: a systematic review. *Ann Cardiothorac Surg* 2013;2:717-28.
- Siddiqi S, Blackstone EH, Bakaeen FG, Bretschneider and del Nido solutions: Are they safe for coronary artery bypass grafting? If so, how should we use them? *J Card Surg* 2018;33:229-34.
- An KR, Rahman IA, Tam DY, et al. A Systematic Review and Meta-Analysis of del Nido Versus Conventional Cardioplegia in Adult Cardiac Surgery. *Innovations (Phila)* 2019;14:385-93.
- Orak Y, Kocarlan A, Boran OF, et al. Comparison of the operative and postoperative effects of del Nido and blood cardioplegia solutions in cardiopulmonary bypass surgery. *Braz J Cardiovasc Surg* 2020;35:689-96.
- Pérez-Andreu J, Fernández-Doblas J, Sao Avilés A, et al. Myocardial protection in the arterial switch operation: Custodiol versus cold blood cardioplegia. *Interact Cardiovasc Thorac Surg* 2020;30:136-43.
- Chan J, Oo S, Butt S, et al. Network meta-analysis comparing blood cardioplegia, Del Nido cardioplegia and custodiol cardioplegia in minimally invasive cardiac surgery. *Perfusion* 2023;38:464-72.
- Schulz KF, Altman DG, Moher D, et al. CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials. *Ann Intern Med* 2010;152:726-32.
- Stewart S, Winters GL, Fishbein MC, et al. Revision of the 1990 working formulation for the standardization of nomenclature in the diagnosis of heart rejection. *J Heart Lung Transplant* 2005;24:1710-20.
- Neil DAH. *Transplant Pathology*. In: Suvarna SK. editor. *Cardiac Pathology: A Guide to Current Practice*. London: Springer, 2013:163-181.
- Talwar S, Chatterjee S, Sreenivas V, et al. Comparison

- of del Nido and histidine-tryptophan-ketoglutarate cardioplegia solutions in pediatric patients undergoing open heart surgery: A prospective randomized clinical trial. *J Thorac Cardiovasc Surg* 2019;157:1182-1192.e1.
27. Altman DG. *Practical Statistics for Medical Research*. London: Chapman & Hall, 1991:404.
  28. Talwar S, Harshavardhan N, Kapoor PM, et al. Plasmalyte-A Based del Nido Cardioplegia Versus Plain Ringer Based del Nido Cardioplegia: Double-Blind Randomized Trial. *World J Pediatr Congenit Heart Surg* 2022;13:187-95.
  29. Mehrabani MJ, Firoozabadi MD, Tafti SHA, et al. Clinical outcomes and electrolyte balance factors in complex cardiac operations in adults; del nido versus custodiol cardioplegia solutions: a randomized controlled clinical trial. *Iran Red Crescent Med J* 2018;20:e64648.
  30. George G, Varsha AV, Philip MA, et al. Myocardial protection in cardiac surgery: Del Nido versus blood cardioplegia. *Ann Card Anaesth* 2020;23:477-84.
  31. Ler A, Sazzad F, Ong GS, et al. Comparison of outcomes of the use of Del Nido and St. Thomas cardioplegia in adult and paediatric cardiac surgery: a systematic review and meta-analysis. *Perfusion* 2020;35:724-35.
  32. Misra S, Srinivasan A, Jena SS, et al. Myocardial Protection in Adult Cardiac Surgery With del Nido Versus Blood Cardioplegia: A Systematic Review and Meta-Analysis. *Heart Lung Circ* 2021;30:642-55.
  33. Ali I, Hassan A, Shokri H, et al. Efficacy of Histidine-Tryptophan-Ketoglutarate Solution Versus Blood Cardioplegia in Cardiac Surgical Procedures: A Randomized Controlled | Parallel Group Study. *Heart Surg Forum* 2021;24:E170-6.
  34. Sen O, Aydin U, Kadirogullari E, et al. Custodiol versus Blood Cardioplegia: Comparison of Myocardial Immunohistochemical Analysis and Clinical Outcomes. *Braz J Cardiovasc Surg* 2022;37:680-7.
  35. Albadrani M. Histidine-tryptophan-ketoglutarate solution versus multidose cardioplegia for myocardial protection in cardiac surgeries: a systematic review and meta-analysis. *J Cardiothorac Surg* 2022;17:133.
  36. Bradić J, Andjić M, Novaković J, et al. Cardioplegia in Open Heart Surgery: Age Matters. *J Clin Med* 2023;12:1698.
  37. Zhai K, Cheng X, Zhang P, et al. Del Nido cardioplegia for myocardial protection in adult cardiac surgery: a systematic review and update meta-analysis. *Perfusion* 2023;38:6-17.
  38. Fresilli S, Labanca R, Monaco F, et al. Del Nido Cardioplegia in Adult Cardiac Surgery: Meta-Analysis of Randomized Clinical Trials. *J Cardiothorac Vasc Anesth* 2023;37:1152-9.

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