del Nido versus St. Thomas' blood cardioplegia in the young: a multi-centre randomised controlled trial in children undergoing cardiac surgery

The DESTINY Trial

Trial Registration: ISRCTN 13638147

Statistical Analysis Plan

SAP Version Number	Protocol Version Number
2.0	5.0

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Statistical Analysis Plan (SAP) Amendments

SAP version number	SAP section number	Description of and reason for change	Timing of change with respect to interim analysis/ final analysis/ database lock	Blind Reviewer	
2.0	5.3	Clarification of sentence regarding the exclusion of participants who do not undergo surgery.	Before final database lock	Name:	Rebecca Woolley
	9	Plasma high-sensitivity troponin-I (µg.h/L) value removed as a covariate adjustment for the primary outcome. This is not the baseline data used for the calculation of the primary outcome.		Signature:	
	9.3	Clarification on sentence regarding analyses being based on received data in the first instance.			
	9.4	Clarification of which data to use for the VIS secondary outcome.		Date:	
	9.7	Removal of metabolomic sub-study as this is no longer being analysed. Addition of three further exploratory analyses relating to systemic target temperature and topical cooling of the heart during surgery			

Abbreviations & Definitions	
Abbreviation / Acronym	**Meaning**
AUC	Area under the time-concentration curve
BCTU	Birmingham Clinical Trials Unit
CONSORT	Consolidated Standards of Reporting Trials
DMC	Data Monitoring Committee
ECLS	Extra-corporeal life support
eGFR	Estimated glomerular filtration rate
ISRCTN	International Standard Randomised Controlled Trial Number
ITT	Intention to Treat
PICU	Paediatric Intensive Care Unit
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SUSAR	Suspected Unexpected Serious Adverse Reaction
TSC	Trial Steering Committee
VIS	Vasoactive Inotrope Score
Term	**Definition**
International Standard Randomised Controlled Trial Number	A clinical trial registry
Protocol	Document that details the rationale, objectives, design, methodology and statistical considerations of the study
Randomisation	The process of assigning trial subjects to intervention or control groups using an element of chance to determine the assignments in order to reduce bias.
Statistical Analysis Plan	Pre-specified statistical methodology documented for the trial, either in the protocol or in a separate document.

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1. Introduction

This document is the Statistical Analysis Plan (SAP) for the DESTINY trial, and should be read in conjunction with the current trial protocol. This SAP details the proposed analyses and presentation of the data for the main paper(s) reporting the results for the DESTINY trial.

The results reported in these papers will follow the strategy set out here. Subsequent analyses of a more exploratory nature will not be bound by this strategy, though they are expected to follow the broad principles laid down here. The principles are not intended to curtail exploratory analysis (e.g. to decide cut-points for categorisation of continuous variables), nor to prohibit accepted practices (e.g. transformation of data prior to analysis), but they are intended to establish rules that will be followed, as closely as possible, when analysing and reporting data.

Any deviations from this SAP will be described and justified in the final report or publication of the trial (using a table as shown in Appendix A). The analysis will be carried out by an appropriately qualified statistician, who should ensure integrity of the data during their data cleaning processes.

2. Background and rationale

The background and rationale for the trial are outlined in detail in the protocol. A brief summary is given here.

During most surgery for congenital heart disease, it is necessary to stop the heart, allowing access to a still and bloodless field to enable repair of intracardiac defects. Cardioplegia has been fundamental to arresting the heart and protecting against ischaemia-reperfusion injury during surgery for over 40 years, with approx. 3,500 cardiac surgical operations with cardioplegic arrest performed in children in the UK & Ireland each year. Whilst on cardiopulmonary bypass, a cross-clamp is placed across the proximal aorta and cardioplegia injected into the coronary arteries via the aortic root, leading to electro-mechanical arrest. This reduces myocardial oxygen uptake to only 10% of that of the perfused beating heart, and progressive hypothermia leads to a further stepwise reduction. However, myocardial injury still occurs routinely following aortic cross-clamping in children, as demonstrated by the ubiquitous release of troponin after surgery. Myocardial protection therefore is a key determinant of heart function and outcome following cardiac surgery.

Current paediatric cardioplegia techniques are primarily derived from adult or laboratory models; however, the immature myocardium has significant structural, physiological, and metabolic differences from the adult heart. Myocardial protection that is effective in adults therefore may not be optimal for young children, especially neonates and those with chronic preoperative cyanosis.

Many types of cardioplegia solution are available and there is wide variation in their use worldwide. In the US, del Nido cardioplegia is used most commonly in children with a recent survey finding it is preferred by 76% of centres performing complex surgery in neonates. On the other hand, St. Thomas' blood cardioplegia is used by most surgeons in most centres in the UK where del Nido solution is not currently commercially available. There are significant theoretical and practical differences between these two autologous blood cardioplegia solutions; identifying the best cardioplegia for specific patient groups will enable the care of the child undergoing surgery to be individualised, improve outcomes by reducing myocardial injury, morbidity, and costs, and may improve long-term cardiac function.

3. Trial objectives

The primary objective is to evaluate whether, in children undergoing cardiac surgery with cardioplegic arrest, the use of del Nido cardioplegia, compared with St. Thomas' blood cardioplegia reduces myocardial injury, as determined by AUC for plasma troponin following surgery.

Secondary objectives aim to evaluate whether the use of del Nido cardioplegia, compared with St. Thomas' blood cardioplegia:

- reduces the duration of ischaemia (aortic cross-clamp time), the volume of cardioplegia given, and the need for internal defibrillation during reperfusion,
- improves myocardial protection, reducing the frequency or severity of LCOS, and makers of reduced tissue perfusion (lactate and omega),
- improves other clinical outcomes, including duration of mechanical ventilation, length of stay on the Paediatric Intensive Care Unit, length of stay in hospital, and 30-day survival.

4. Trial methods

4.1. Trial design

DESTINY is a phase II/III, 2 arm multi-centre, participant-blinded, assessor-blinded, parallel group, individually randomised pilot trial with allocations on a 1:1 basis.

Participants will be recruited from 4 paediatric cardiac surgery centres in the UK (Birmingham, Bristol, Great Ormond Street and Leeds), with the potential to expand to more centres if required.

As the technique for delivery of cardioplegia and interval between doses necessarily differs between treatment groups, patient safety may be compromised by blinding those administering the cardioplegia. The operating surgeon, perfusionist, anaesthetist, theatre scrub team and research nurse therefore will not be blinded to the intervention. However, patients, parents/guardians and outcome assessors such as, cardiologists, other surgeons, PICU medical and nursing staff, and ward staff will be blinded to the allocation. For further details on blinding procedures, please see the protocol.

4.2. Trial interventions

The investigational intervention is del Nido cardioplegia in a 1:4 blood:crystalloid preparation, given at 4-8°C, with an initial dose of 20ml/kg and subsequent doses every 60-90 minutes if required, at the discretion of the surgeon, as required.

The control intervention is St. Thomas' Hospital blood cardioplegia in a 4:1 blood:crystalloid using Harefield Hospital preparation, given at 4-8°C, with an initial dose of 20-30ml/kg, subsequent doses of 15 ml/kg every 20-30 minutes at the discretion of the surgeon, as required.

4.3. Primary outcome measure

The primary outcome is the area under the time-concentration curve (AUC) for plasma high-sensitivity troponin-I (μg.h/L) in the first 24 hours after aortic cross-clamp release (reperfusion).

For details on the derivation of the primary outcome measure, please see section 9.4.

4.4. Secondary outcome measures

The secondary outcomes are as follows:

- Low cardiac output syndrome (LCOS) defined as either of the following in the first 48 hours after reperfusion: Vasoactive Inotrope Score (VIS) ≥15, or major cardiac event (cardiac arrest, ECLS or death) (n)
- Duration of mechanical ventilation (hours), defined as the number of hours from termination of index CPB to extubation
- Length of postoperative stay on Paediatric Intensive Care (hours), defined as number of hours from admission to PICU from theatre following index procedure to discharge from PICU
- Max VIS by thresholds: ≥10, ≥15 and ≥20 in the first 48 hours (n)
- Total VIS in the first 4 hours after PICU admission following the index procedure (score)
- Arterial lactate (mmol/L) in the first 12 hours
- Omega, determined by [SaO2]/[SaO2-ScvO2] in the first 12 hours
- Total aortic cross-clamp time (mins)
- Total volume of cardioplegia given (ml)
- Need for internal defibrillation during reperfusion (n)
- Delayed sternal closure, incidence (n) and duration (days)
- Unplanned reoperation, including chest re-opening on PICU (n)
- Need for new renal replacement therapy (n)
- Lowest estimated glomerular filtration rate (eGFR), calculated using the bedside Schwartz equation and the peak postoperative creatinine on routine monitoring during

the first 7 days following the index procedure (ml/min/1.73m^2), and according to the paediatric RIFLE categories (n)
- Length of postoperative stay in the hospital (days), defined as number of days from day of surgery to discharge from hospital or death, whichever is sooner
- 30-day survival (n)

4.5. Timing of outcome assessments

The schedule of trial procedures and outcome assessments are given in the protocol.

4.6. Randomisation

Participants will be randomised in a 1:1 ratio to either del Nido cardioplegia or to St. Thomas' blood cardioplegia.

Randomisation will be performed centrally at the Birmingham Clinical Trials Unit (BCTU) using a minimisation algorithm to ensure balance in the treatment allocation over the following variables:
- Age: neonate (0-30 days), infant (31 days to <1 year), child (1 to <7 years), older child (7 to <16 years)
- Incision or resection of ventricular myocardium anticipated (yes/no)
- Surgical centre

A 'random element' will be included in the minimisation algorithm, so that each participant has a probability (unspecified here), of being randomised to the opposite treatment that they would have otherwise received.

Full details of the randomisation specification will be stored in a confidential document at BCTU.

4.7. Sample size

It is hypothesised that del Nido cardioplegia reduces myocardial injury during surgery compared with St. Thomas' blood cardioplegia. The DESTINY trial will use postoperative hs-troponin-I release to measure the level of myocardial injury which has been shown to strongly correlate with clinical outcomes including inotropic support, duration of ventilation, ventricular dysfunction, and early death.

The justification for the sample size is based on data from the BRICC trial. In a similar cohort of participants to those in the control group for this study, a mean area under curve for postoperative hs-troponin release value of approximately 64.0 µg.h/L and a standard deviation of approximately 42.0 µg.h/L were observed.

To detect a difference of 30% (relative reduction, 19.2 µg.h/L absolute reduction) between groups using the standard method of a two-sample t-test and assuming equal variance with 90%

power and a type I error rate of 0.05, 102 participants per group will need to be randomised, 204 in total. Assuming and adjusting for a 3% loss to follow-up/drop-out rate (a low rate of drop-out is expected as the primary outcome is measured at 24 hours and it is anticipated that all children undergoing heart surgery would still be in hospital at this point), 220 participants will need to be recruited.

4.8. Framework

The objective of the trial is to test the superiority of one intervention to another.

The null hypothesis is that there is no difference in mean postoperative hs-troponin release values between the intervention groups. The alternative hypothesis is that there is a difference between the groups.

4.9. Interim analyses and stopping guidance

A separate Data Monitoring Committee (DMC) reporting template will be drafted and agreed by the DMC including an agreement on which outcomes will be reported at interim analyses. The statistical methods stated in this SAP will be followed for the outcomes included in the DMC report, where possible.

4.10. Internal Pilot Progression Rules

Not applicable

4.11. Timing of final analysis

The final analysis for the trial will occur after all participants have passed 30 days following the index surgery and the corresponding outcome data has been entered onto the trial database and validated as being ready for analysis. This is provided that the trial has not been stopped early for any reason (e.g. DMC advice or funding body request). This analysis will include data items up to and including 30 days and no further.

4.12. Timing of other analyses

Longer term data will form part of the follow-up imaging sub-study but will be analysed separately once participants have completed the corresponding assessments.

A further SAP will be written for these additional analyses.

4.13. Trial comparisons

All references in this document to 'group' refer to del Nido cardioplegia or St. Thomas blood cardioplegia.

5. Statistical Principles

5.1. Confidence intervals and p-values

All estimates of differences between groups will be presented with two-sided 95% confidence intervals, unless otherwise stated. P-values will be reported from two-sided tests at the 5% significance level.

5.2. Adjustments for multiplicity

No correction for multiple testing will be made.

5.3. Analysis populations

All primary analyses (primary and secondary outcomes including safety outcomes) will be by intention-to-treat (ITT). Participants will be analysed in the intervention group to which they were randomised, and all participants shall be included whether or not they received the allocated intervention. This is to avoid any potential bias in the analysis.

In the event a participant does not undergo surgery, they will be excluded from the analyses. No data will have been collected from these participants given they have not undergone surgery. In this instance we will refer to the ITT cohort as 'modified ITT' to be clear that all randomised patients have not been included in the analysis.

A per protocol analysis for the primary outcome will also be carried out but only as a sensitivity analysis. Refer to section 5.4 for definition of adherence and definition of the per protocol group. Refer to section 9.10 for further details on any sensitivity analyses.

5.4. Definition of adherence

Adherence to allocated intervention will be monitored by completion of the Perfusion CRF by the Clinical Perfusionist who will be administering the intervention. We will define adherence as receiving only the allocated intervention during the index procedure. The per-protocol population is made up of only those adherent to the allocated intervention.

5.5. Handling protocol deviations

A protocol deviation is defined as a failure to adhere to the protocol such as errors in applying the inclusion/exclusion criteria, the incorrect intervention being given, or incorrect data being collected or measured. We will apply a strict definition of the ITT principle and will include all participants as per the ITT population described in section 5.3 in the analysis, in some form, regardless of deviation from the protocol.[1] This does not include those participants who have specifically withdrawn consent for the use of their data in the first instance; however these outcomes will be explored as per other missing responses. This also does not include instances where a participant does not receive surgery, in which case we will assume a modified ITT analysis as described in section 5.3.

5.6. Unblinding

For double-blind studies, the unblinding of the Trial Statistician to the intervention code will take place once the database is locked for final analysis, unless the DMC request that they review the interim data with knowledge of the intervention groups or the DMC request to be unblinded at an interim analysis.

6. Trial population

6.1. Recruitment

A flow diagram (as recommended by CONSORT[2]) will be produced to describe the participant flow through each stage of the trial. This will include information on the number (with reasons) of losses to follow-up (drop-outs and withdrawals) over the course of the trial. A template for reporting this is given in Appendix D.

6.2. Baseline characteristics

The trial population will be tabulated as per Appendix D. Categorical data will be summarised by number of participants, counts and percentages. Continuous data will be summarised by the number of participants, mean and standard deviation if deemed to be normally distributed or number of participants, median and interquartile range if data are skewed, and ranges if appropriate. Tests of statistical significance will not be undertaken, nor confidence intervals presented.[3]

7. Intervention(s)

7.1. Description of the intervention(s)

A template for reporting information on the intervention(s) is given in Appendix D.

7.2. Adherence to allocated intervention

A cross-tabulation of allocated intervention by the adherence categories stated in section 5.4 will be produced (proportions and percentages). A template for reporting adherence is given in Appendix D.

8. Protocol deviations

Frequencies and percentages by group will be tabulated for the protocol deviations as per Appendix D.

9. Analysis methods

Intervention groups will be compared using appropriate statistical models, to adjust for all covariates as specified in section 9.1, where possible.

9.1. Covariate adjustment

In the first instance, intervention effects between groups for all outcomes will be adjusted for the minimisation parameters listed in section 4.6 and baseline values if applicable. Categorised continuous variables (e.g. age) will be treated as continuous variables in this adjustment. All included covariates will be treated as fixed effects in the model (including centre).

If covariate adjustment is not possible (e.g. the model does not converge), then unadjusted estimates or estimates adjusted for baseline values if applicable will be produced, and it will be made clear in the final report why this occurred (e.g. not possible due to low event rate/lack of model convergence).

If a log-binomial model fails to converge, a Poisson regression model with robust standard errors will be used to estimate the same parameters.[4] If this also fails to converge, unadjusted estimates will be produced from the log-binomial model. It will be made clear in the final report why this occurred (e.g. not possible due to low event rate/lack of model convergence).

9.2. Distributional assumptions and outlying responses

Distributional assumptions (e.g. normality of regression residuals for continuous outcomes) will be assessed visually prior to analysis, although in the first instance the proposed primary method of estimation in this analysis plan will be followed. If responses are considered to be particularly skewed and/or distributional assumptions violated, the impact of this will be examined through sensitivity analysis; this will consist of transformation of responses prior to analysis (e.g. log transformation) in the first instance. If extreme values are apparent and considered to be affecting the integrity of the analysis, a sensitivity analysis consisting of removing the outlying

response(s) and repeating the analysis will be performed. Output from these analyses, if performed, will be described and presented alongside the original analysis (or included, e.g. in appendices) with the excluded values clearly labelled. See section 9.10 for further details regarding sensitivity analyses.

9.3. Handling missing data

All analyses will in the first instance be completed on received data only with every effort made to follow-up participants to minimise any potential for bias. To examine the possible impact of missing data on the results, and to make sure we are complying with the intention-to-treat principle, sensitivity analysis will be performed on the primary outcome measure.[5] See section 9.10 for further details regarding sensitivity analyses.

9.4. Data manipulations

The Trial Statistician will derive all responses from the raw data recorded in the database.

Primary outcome

The area under the curve (AUC) for hs-troponin-I release in the first 24 hours (ng/L) will be calculated from samples taken at baseline, 3, 6, 9, 12 and 24 hours after aortic cross-clamp release using the trapezoid rule: i.e. (6 hours hs-troponin value + 3 hours hs-troponin value)*(6 hours – 3 hours)*0.5 will calculate the AUC between time 3 hours and 6 hours. This is repeated for each of the five intervals, and then all five AUCs are summed to calculate the total AUC for each participant. See section 9.5 for further information on the participants who contribute to the primary analysis population.

Missing baseline troponin values will be imputed using the median value of the participant's treatment group. Due to the low variability of the baseline measurements this should not impact on the validity of the results.

Secondary outcomes

- Vasoactive Inotrope Score (VIS): dopamine dose (µg/kg/min) + dobutamine dose (µg/kg/min) + (100 x adrenaline dose (µg/kg/min)) + (10 x milrinone dose (µg/kg/min) + (10,000 x vasopressin dose (U/kg/min) + (100 x noradrenaline dose (µg/kg/min).[6,7] The scores for each hour are either summed to calculate the total VIS for a given period, ie. the first 4 hours after PICU admission, or identify the maximum VIS to determine whether a defined threshold has been reached during a given period, ie. in the first 48 hours after reperfusion. If a drug is not given, then this essentially means a score of zero for that dose. If a dose is not provided, it will be assumed that the participant did not receive that drug and a score of zero will be given for that dose. Note that the data collected for "Hour 1" reflects the inotropic support upon arrival to ICU and therefore may be affected by any lability related to the process of transfer from theatre to ICU. We will therefore exclude Hour 1 values from all calculations, with Hours

2-5 used to calculate 'Total VIS in in the first 4 hours after PICU admission', and Hours 2-48 used to assess thresholds for 'LCOS' and 'Max VIS thresholds'.

When a participant switches between inotropic agents on ICU, values for two or more agents may be entered for the same hour, artificially increasing the calculated VIS. We will therefore use clinical judgement to exclude the VIS calculation for a single hour that is clearly associated with switching agents, in the absence of a cardiac arrest or other major event.

- Duration of mechanical ventilation (hours), defined as the number of hours from termination of index CPB to extubation. If a participant dies following surgery without being extubated, the duration will be measured until the time that mechanical ventilation is stopped.
- Length of postoperative stay on Paediatric Intensive Care (hours), defined as number of hours from admission to PICU from theatre following index procedure to discharge from PICU. Note that both the hour of admission/discharge is collected along with minute of admission/discharge. The length of stay will be calculated using the following steps:
    - calculate time in hours = discharge in hours – admission in hours;
    - calculate time in minutes = discharge in minutes – admission in minutes;
    - calculate total time in minutes = (time in hours*60)+time in minutes;
    - calculate total time in hours = total time in minutes/60.

  Then the total time in hours will be formatted to 2 decimal places. Participants who remain in PICU at 30 days (end of follow-up) will be classed as not having the event of interest. Participants who withdraw or die in the PICU before 30 days will be censored at date of withdrawal or date of death.
- Omega, determined by the arterial oxygen saturation divided by the difference between arterial oxygen saturation and central venous oxygen saturation ie. [SaO2]/[SaO2-ScvO2].[8]
- Delayed sternal closure, duration (days) defined as number of days from day of surgery to sternal closure or death, whichever is sooner
- Lowest estimated glomerular filtration rate (eGFR), calculated using the bedside Schwartz equation and the peak postoperative creatinine on routine monitoring during the first 7 days following the index procedure (ml/min/1.73m^2), and according to the paediatric RIFLE categories (n).[9]
- Length of postoperative stay in the hospital (days), defined as number of days from day of surgery to discharge from hospital or death, whichever is sooner

9.5. Analysis methods – primary outcome(s)

A template for reporting the primary outcome is given in Appendix D.

AUC for troponin release in the first 24 hours (µg.h/L) will be calculated for each participant using the trapezoidal rule from samples taken at six time-points: baseline (pre-surgery), and at 3, 6, 9, 12 and 24 hours after aortic cross-clamp release.

To be included in the primary analysis, the participant must provide a troponin value at all timepoints after aortic cross-clamp release. Missing baseline troponin values will be imputed using the median value of the participant's treatment group.

The adjusted mean difference between groups along with 95% confidence intervals will be estimated using a linear regression model. Statistical significance of the treatment group parameter will be determined from the p-value generated by the model.

See section 9.1 for covariate adjustment and model convergence.

9.6. Analysis methods – secondary outcomes

A template for reporting the secondary outcomes is given in Appendix D.

Continuous data items (e.g. eGFR) will be analysed using a linear regression model. Results will be presented as adjusted mean differences with 95% confidence intervals.

Continuous outcomes measured across more than 3 time points (e.g. arterial lactate and omega) will be analysed using mixed effect repeated measures models using all available data. Baseline value of the measure (if available) and time as a continuous variable will be included in the model. In the initial model, a treatment by time cross-term will be included in the model, to test whether the intervention has modified the response trajectory over time. If this is not significant, it will be considered that the treatment effect is constant over time, and models without the treatment by time cross-term will be fitted. Results will be presented as adjusted mean differences and 95% confidence intervals.

Time to event data outcomes (such as time to PICU and hospital discharge) will be analysed using a Cox regression model. Results will be presented as Hazard ratio and 95% confidence intervals. Kaplan Meier plots will also be presented for visual interpretation of the data.

Binary outcomes (e.g. need for internal defibrillation during reperfusion) will be analysed using a logistic regression model. Adjusted risk ratios and risk differences along with 95% confidence intervals will be derived using marginal standardisation method.

9.7. Analysis methods – exploratory outcomes and analyses

Any other data that does not form a pre-specified outcome will be presented using simple summary statistics by intervention group (i.e. numbers and percentages for binary data and

means (or medians) and standard deviations (or inter-quartile ranges) for continuous normal (or non-normal) data.

Two exploratory subgroup analyses of the primary outcome will be performed relating to the age of the cardioplegia solution at the point of use. The age of the solution will be calculated as the difference between the surgery date and the batch manufacture date of the cardioplegia. Once the age of the solution has been calculated, two binary variables will be derived

- Cardioplegia age <=3 months vs. cardioplegia age >3 months
- Cardioplegia age <=6 months vs. cardioplegia age >6 months

The subgroup analyses will then be conducted as per the description in section 9.9.

Exploratory subgroup analyses of the primary outcome will be performed relating to systemic target temperature and topical cooling of the heart during surgery. Three analyses will be performed:

- Systemic target temperature <=32 degrees vs. systemic target temperature >32 degrees
- Topical cooling of the heart = "yes" vs. topical cooling of the heart = "no"
- The 4-way combination of the above two categories:
  - Temperature <=32 degrees, topical cooling = "yes"
  - Temperature <=32 degrees, topical cooling = "no"
  - Temperature >32 degrees, topical cooling = "yes"
  - Temperature >32 degrees, topical cooling = "no"

9.8. Safety data

The number and percentage of participants experiencing any adverse events, serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs) will be presented by intervention group. A table listing the SAEs defined in the protocol will be provided. A template for reporting this safety data is given in Appendix D.

9.9. Planned subgroup analyses

Interpretation of subgroup analysis will be treated with caution (output will be treated as exploratory rather than definitive).[10] Analysis will be limited to the primary outcome only, and will be restricted to those variables for which the randomisation was minimised (see section 4.6) and one additional subgroup:

- pre-operative oxygen saturations: <90% (cyanotic); or ≥90% (acyanotic);

The effects of these subgroups will be examined by including a treatment group by subgroup interaction parameter in the regression model. A threshold of p=0.05 will be used to determine statistical significance of the interaction. A template for reporting the subgroup analyses for the primary outcome is given in Appendix D9.

9.10.Sensitivity analyses

Sensitivity analyses will be limited to the primary outcome and will consist of:

- A complete case analysis including only those participants used in the primary analysis, but the natural log transformation of the AUC will be used before performing linear regression and the results from this model will be interpreted in the original scale appropriately.
- Per-protocol analysis (population described in sections 5.3 and 5.4);.
- An analysis to assess the effect of missing responses post baseline using trapezoidal rule to impute the missing data, i.e. the adjacent data points that are available will be connected to form straight lines. Missing baseline troponin values will be imputed as before. To be included in the analysis, the participant must have at least two troponin values provided after aortic cross-clamp release.
- An analysis to assess the effect of missing responses post baseline using percentile value imputation of the participant's treatment group at the same time point, in which 25th percentile values will be imputed for missing values in St. Thomas' group and 75th percentile values for those in del Nido group. To be included in the analysis, the participant must have undergone the index surgery.
- The same analysis as above, using percentile value imputation, but including all randomised participants, even if they withdrew before the index operation.

10. Analysis of sub-randomisations

Not applicable

11. Health economic analysis

No health economic analysis is planned for this trial.

12. Statistical software

Statistical analysis will be undertaken in the following statistical software packages:

- SAS software
- Stata.

13. References

1. Gupta SK. Intention-to-treat concept: A review. Perspect Clin Res. 2011;2(3):109-112.
2. Schulz KF, Altman DG, Moher D, for the CONSORT Group. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. BMJ. 2010;340:c332.
3. Altman DG, Dore CJ. Randomisation and baseline comparisons in clinical trials. Lancet. 1990;335:149–53.
4. Zou G. A modified Poisson regression approach to prospective studies with binary data. Am J Epidemiol. 2004;159(7):702-6.

5. White IR, Horton NJ, Carpenter J, Pocock SJ. Strategy for intention to treat analysis in randomised trials with missing outcome data. BMJ. 2011;342:d40.
6. Gaies MG, Gurney JG, Yen AH, Napoli ML, Gajarski RJ, Ohye RG, et al. Vasoactive-inotropic score as a predictor of morbidity and mortality in infants after cardiopulmonary bypass. Pediatr Crit Care Med. 2010;11(2):234-8.
7. Gaies MG, Jeffries HE, Niebler RA, Pasquali SK, Donohue JE, Yu S, et al. Vasoactive-inotropic score is associated with outcome after infant cardiac surgery: an analysis from the Pediatric Cardiac Critical Care Consortium and Virtual PICU System Registries. Pediatr Crit Care Med. 2014;15(6):529-37.
8. Tibby SM, Murdoch IA. Monitoring cardiac function in intensive care. Arch Dis Child. 2003;88(1):46-52.
9. Soler YA, Nieves-Plaza M, Prieto M et al. Pediatric Risk, Injury, Failure, Loss, End-Stage renal disease score identifies acute kidney injury and predicts mortality in critically ill children: a prospective study. Pediatr Crit Care Med. 2013;14(4):e189-95.
10. Wand R, Lagakos SW, Ware JH, Hunter DJ, Drazen JM. Reporting of subgroups analyses in clinical trials. NEJM. 2007;357:2189-94.
11. Sterne JAC, White IR, Carlin JB, Spratt M, Royston P, Kenward MG et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *Br Med J* 2009; 338:b2393.

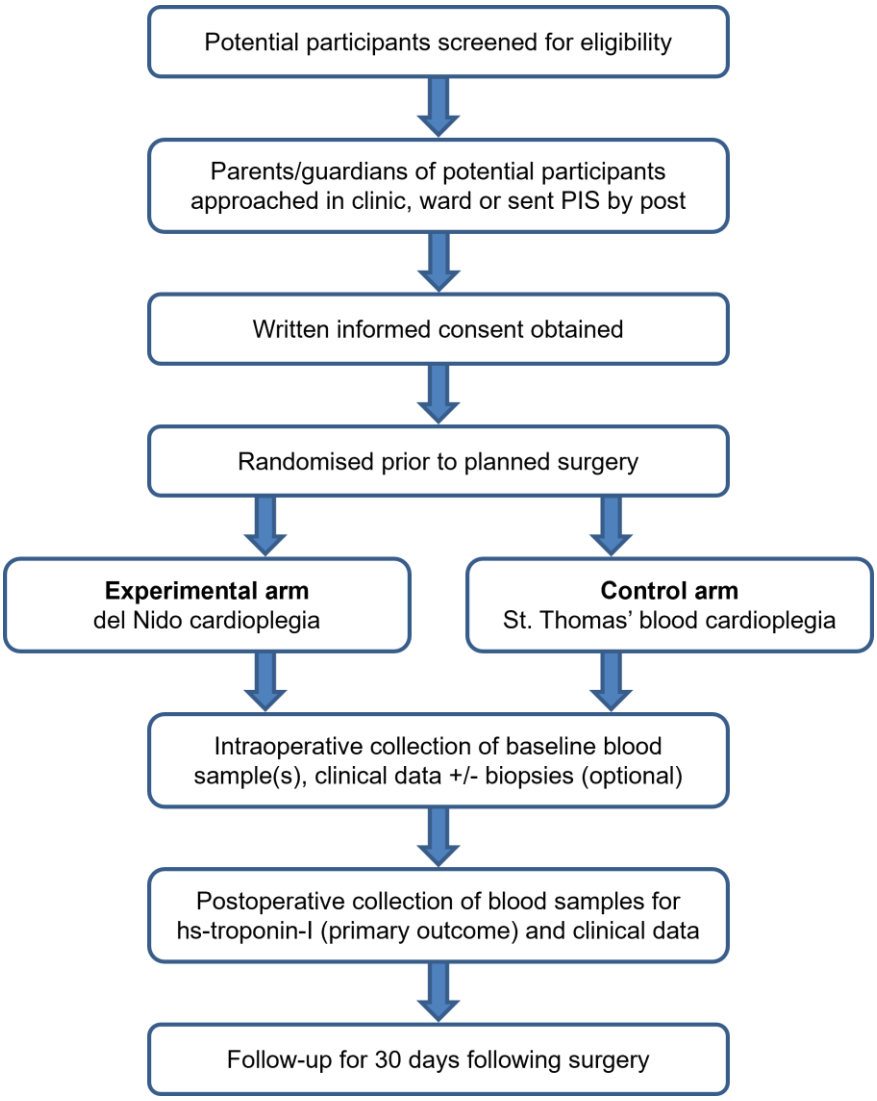
Appendix A: Deviations from SAP

This report below follows the statistical analysis plan dated *<insert effective date of latest SAP>* apart from following:

Section of report not following SAP	Reason
<insert section >	<insert, e.g. exploratory analyses request by TMG>

https://stats.oarc.ucla.edu/stata/code/using-xtreg/

Appendix B: Trial schema



Appendix C: Schedule of assessments

See trial protocol.

Appendix D: Template report

A template report for the final analyses will be provided in a separate document.