BMJ Open

Study Protocol: NITric oxide during cardiopulmonary bypass to improve Recovery in Infants with Congenital heart defects (NITRIC trial): A Randomised Controlled Trial.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-026664
Article Type:	Protocol
Date Submitted by the Author:	19-Nov-2018
Keywords:	infant, Cardiac surgery < SURGERY, mortality, nitric oxide, cardiopulmonary bypass, inflammation

Complete List of Authors:

Schlapbach, Luregn; The University of Queensland, Paediatric Critical Care Research Group
Horton, Steve; Royal Children's Hospital M, Cardiac Surgical Unit; University of Melbourne, Faculty of Medicine
Long, Debbie; Queensland Health, Lady Cilento Children's Hospital; Menzies Health Institute Queensland, Alliance for Vascular Access Teaching and Research Group
Beca, John; Starship Children's Hospital, Paediatric Intensive Care Unit
Erickson, Simon; Perth Children's Hospital, Paediatric Critical Care
Festa, Marino
d'Udekem, Yves; Royal Children's Hospital M, Cardiac Surgical Unit; University of Melbourne, Faculty of Medicine
Alphonso, Nelson; The University of Queensland, Paediatric Critical Care Research Group; Queensland Health, Lady Cilento Children's Hospital
Winlaw, David; The Children's Hospital at Westmead, Sydney Children`s Hospital Network, Heart Centre for Children
Johnson, Kerry; Queensland Health, Lady Cilento Children's Hospital
Delzoppo, Carmel; Royal Children's Hospital M, Cardiac Surgical Unit
van Loon, Kim; University Medical Center Utrecht
Gannon, B; The University of Queensland, Centre for Business and Economics of Health
Fooken, Jonas; The University of Queensland, Centre for Business and Economics of Health
Blumenthal, Antje; The University of Queensland, Diamantina Institute
Young, Paul ; Wellington Hospital,
Jones, Mark; Bond University
Butt, Warwick; Royal Children's Hospital M, Cardiac Surgical Unit
Schibler , Andreas; Queensland University of Technology; Queensland Health, Lady Cilento Children's Hospital

Title Page:

Study Protocol: NITric oxide during cardiopulmonary bypass to improve Recovery in Infants with Congenital heart defects (NITRIC trial): A Randomised Controlled Trial.

Authors, highest degree and affiliations:

- Luregn J Schlapbach, MD, FCICM, Paediatric Critical Care Research Group, Child Health Research Institute, The University of Queensland; Paediatric Intensive Care Unit, Queensland Children`s Hospital, Children`s Health Queensland, Brisbane, Australia. Email: l.schlapbach@uq.edu.au
- Steve B Horton PhD, Cardiac Surgical Unit, Royal Children's Hospital, Melbourne. Faculty of Medicine, Department of Paediatrics, University of Melbourne. Honorary Research Fellow, Murdoch Children's Research Institute, Melbourne, Australia. Email: Steve.Horton@rch.org.au
- Debbie Long, PhD, Paediatric Critical Care Research Group, Child Health Research Institute, The University of Queensland; Paediatric Intensive Care Unit, Queensland Children`s Hospital, Children`s Health Queensland, Brisbane, Australia. Email: Debbie.Long2@health.qld.gov.au
- John Beca, MBChB, FCICM. FRACP, Paediatric Intensive Care Unit, Starship Children's Hospital, Auckland, New Zealand. Email: JohnBeca@adhb.govt.nz
- Simon Erickson, MBBS FCICM, Paediatric Critical Care, Perth Children's Hospital, Western Australia and The University of Western Australia. Email: Simon.Erickson@health.wa.gov.au
- Marino Festa, MD, FCICM, Kids Critical Care Research, Paediatric Intensive Care Unit, Children's Hospital at Westmead, Sydney Children's Hospital Network, Sydney, New South Wales, Australia. Email: marino.festa@health.nsw.gov.au

- Yves d'Udekem, MD, PhD, Department of Cardiac Surgery, Royal Children's Hospital, Melbourne, Australia; Heart Research, Murdoch Childrens Research Institute, Melbourne, Australia; Department of Paediatrics, The University of Melbourne, Melbourne, Australia. Email: Yves.Dudekem@rch.org.au
- Nelson Alphonso, FRCS (C.Th), FRACS, Cardiac Surgery, Queensland Children`s Hospital, and Associate Professor, School of Medicine, Children's Health Clinical Unit, University of Queensland, Brisbane, Queensland, Australia. Email: nelson.alphonso@health.qld.gov.au
- David Winlaw MBBS, MD, FRACS, Heart Centre for Children, The Children's Hospital at Westmead, Sydney Children`s Hospital Network, and Faculty of Medicine and Health, University of Sydney, Sydney, Australia. Email: david.winlaw@health.nsw.gov.au
- Kerry Johnson, Paediatric Critical Care Research Group, Child Health Research Institute, The University of Queensland; Paediatric Intensive Care Unit, Queensland Children`s Hospital, Children`s Health Queensland, Brisbane, Australia. Email: Kerry.Johnson3@health.qld.gov.au
- Carmel Delzoppo, BHS, Paediatric Intensive Care Unit Royal Children's Hospital Melbourne, Murdoch Children's Research Institute. Email: Carmel.Delzoppo@rch.org.au
- Kim van Loon, MD, PhD, Division of Anaesthetics, University Medical Center Utrecht Utrecht, the Netherlands. Email: k.vanloon1@gmail.com
- Brenda Gannon, PhD, Professor of Health Economics, Centre for the Business and Economics of Health, The University of Queensland, Brisbane, Australia. brenda.gannon@uq.edu.au

- Jonas Fooken, PhD, Centre for the Business and Economics of Health, The University of Queensland, Brisbane, Australia. Email: j.fooken@uq.edu.au
- Antje Blumenthal, Dr rer. nat., Associate Professor and Head of the Infection and Inflammation Group at The University of Queensland Diamantina Institute, The University of Queensland, Translational Research Institute, Brisbane, Australia. Email: a.blumenthal@uq.edu.au
- Paul Young, MBChB, FCICM, PhD, Director of the Intensive Care Research Programme, Medical Research Institute of New Zealand, Wellington, New Zealand Email: paul.young@ccdhb.org.nz
- Mark Jones, PhD, School of Public Health, Bond University, South Port, Australia. Email: majones@bond.edu.au
- Warwick Butt, Associate Professor, MB B.S, FRACP, FJFICM, FCICM. Paediatric Intensive Care Unit Royal Children's Hospital Melbourne, Murdoch Children's Research Institute, Melbourne University. Email: Warwick.Butt@rch.org.au
- Andreas Schibler, MD, FCICM, Paediatric Critical Care Research Group, Child Health Research Institute, The University of Queensland; Paediatric Intensive Care Unit, Queensland Children`s Hospital, Children`s Health Queensland, Brisbane, Australia. Email: a.schibler@uq.edu.au

On behalf of the NITRIC Study Group, the Australian and New Zealand Intensive Care Society Clinical Trials Group (ANZICS CTG), the Paediatric Critical Care Research group (PCCRG) and the ANZICS Paediatric Study Group (PSG)

Correspondence

A/Prof Andreas Schibler, MD, FCICM

Paediatric Critical Care Research Group (PCCRG), Child Health Research Centre, Queensland Children's Hospital Precinct and Mater Research Institute, The University of Queensland Level 7, 62 Graham St, South Brisbane, Queensland, 4101, Australia

Email: a.schibler@uq.edu.au

Phone: ++61 (0) 7 3068 11 11

Alternative correspondence:

A/Prof Luregn J. Schlapbach, MD, FCICM

Paediatric Critical Care Research Group, Child Health Research Institute, The University of Queensland; and Queensland Children`s Hospital, Children`s Health Queensland, The University of Queensland Level 7, 62 Graham St, South Brisbane, Queensland, 4101, Australia

Email: l.schlapbach@uq.edu.au

Phone: ++61 (0) 7 3068 11 11

Conflict of Interest Statement:

Yves d'Udekem is a consultant for MSD and Actelion.

LJS, SH, DL, KJ, NA, DW, MF, SE, JB, CD, KL, MJ, AF, BG, AB, PY, WB, AS have no conflicts of interest to declare.

Word count: 3441

Key words: cardiopulmonary bypass; child; congenital heart disease; infant; inflammation; ventilation; nitric oxide; mortality

Abbreviations:

CPB	Cardio Pulmonary Bypass
CHD	Congenital Heart Disease
ECLS	Extracorporeal Life Support
FiO_2	Fraction of inspired oxygen
CO_2	Carbon dioxide
NO	Nitric oxide
SaO_2	Arterial oxygen saturation
$ScvO_2$	Central venous oxygen saturation
PICU	Paediatric Intensive Care Unit

Study Protocol: NITric oxide during cardiopulmonary bypass to improve Recovery in Infants with Congenital heart defects (NITRIC trial): A Randomised Controlled Trial.

Trial registration: ACTRN12617000821392

Protocol Version 1.3, dated 17th July 2018

Funding sources: This work was supported by grants from the Heart Kids Foundation, Australia; the Children`s Health Foundation, Brisbane, Australia; and by a grant from the Australian National Health and Medical Research Council (1140322).

Luregn Schlapbach, Yves d'Udekem, Andreas Schibler hold a NHMRC Clinical Practitioner Fellowship. The Victorian Government's Operational Infrastructure Support Program

supported this research project. Paul Young holds a Clinical Practitioner Fellowship from the Health Research Council of New Zealand

The funding sources had no involvement in study design, analyses, nor interpretation of the results.

Mallinckrodt Pharmaceuticals will provide nitric oxide delivery devices to study centres but has no involvement in study design, conduct, nor analyses.

Author contributorship statement:

The study protocol first draft was designed by LJS and AS based on the previous pilot study design established by SH, and WB. MJ wrote the section on statistical analyses. JF and BG wrote the section on health economic analyses The present study protocol has been revised with input from SH, DL, JB, SE, MF, YdU, NA, DW, KJ, CD, KvL, BG, JF, PY, AB, MJ and WB. LJS prepared the final protocol manuscript which was reviewed and approved by all authors.

Roles and Responsibilities:

The study protocol first draft was designed by LJS and AS based on the previous pilot study design established by SH, and WB. The present study protocol has been revised with input from SH, DL, KJ, CJ, CD, WB, SE, MF, BG, JF, PY, AB, MJ and JB.

The study steering group consists of LJS, SH, DL, WB and AS.

LJS, AS, DL, KJ, NA, SE, WB, SH, CdZ, KL, YdU, MF, JB are responsible for local study setup conduct, and recruitment.

AB and LJS are responsible for host inflammation analyses.

MJ is responsible for statistical analyses.

JF and BG are responsible for health economic analyses.

Abstract

Introduction: Congenital heart disease (CHD) is a major cause of infant mortality in industrialised countries. Many infants with CHD require corrective surgery with most operations requiring cardiopulmonary bypass (CPB). CPB triggers a systemic inflammatory response which is associated with low cardiac output syndrome (LCOS), post-operative morbidity and mortality. Delivery of nitric oxide (NO) into CPB circuits can provide myocardial protection and reduce bypass-induced inflammation, leading to less LCOS and improved recovery. We hypothesized that using NO during CPB increases ventilator-free days (the number of days patients spend alive and free from invasive mechanical ventilation up until day 28) compared with standard care. Here we describe the NITRIC trial protocol.

Methods and Analysis: The NITRIC trial is a randomised, double blind, controlled, parallel-group, two-sided superiority trial to be conducted in at least five paediatric cardiac surgical centres. 1320 infants below two years of age undergoing cardiac surgery with CPB will be randomly assigned to NO at 20ppm administered into the CPB oxygenator for the duration of CPB or standard care (no NO) in a 1:1 ratio with stratification by age (<six weeks ≥six weeks), single ventricle physiology (Y/N), and study centre. The primary outcome will be ventilator-free days to day 28. Secondary outcomes include a composite of LCOS, need for extracorporeal membrane oxygenation, or death within 28 days of surgery; length of stay in intensive care and in hospital; and, health care costs. Analyses will be conducted on an intention to treat basis. Pre-planned secondary analyses will investigate the impact of NO on host inflammatory profiles post surgery.

Ethics and dissemination: The study has ethical approval (HREC/17/QRCH/43) and is registered in the Australian New Zealand Clinical Trials Registry (ACTRN12617000821392). The NITRIC trial commenced recruitment in 2017. The primary manuscript will be submitted for publication in a peer reviewed journal.

Strengths and limitations of this study:

- This study tests the efficacy and safety of a simple intervention during cardiopulmonary bypass to improve early postoperative morbidity.
- The intervention aims to reduce patient-centred adverse outcomes after a common high-risk procedure for the most common congenital condition.
- The study includes follow-up of neurodevelopmental outcome and quality of life which will allow assessment of the long-term impact of the intervention.
- The study includes biobanking to investigate the biological mechanisms underlying the clinical findings in nested studies.
- The study will be the largest randomised controlled trial performed in paediatric cardiac surgery to date.

Introduction

Congenital heart disease (CHD) is the most common congenital condition, affecting around one in a hundred live born children[1]. Up to 50% need cardiac surgery to correct the underlying abnormality at some stage during their life, with the majority of procedures requiring cardiopulmonary bypass (CPB). Substantial reductions in perioperative mortality in children following cardiac surgery have been achieved[2], and adult survivors of CHD now outnumber paediatric patients with CHD in most high income countries[3, 4]. Despite these advances, major postoperative morbidity remains common and is associated with increased rate of long term mortality, morbidity, and disability[5]. The exposure of host blood to large artificial organ surfaces combined with myocardial injury during surgery, results in a strong systemic inflammatory response of the host, which is further aggravated by reperfusion injury and the release of damage-associated molecular patterns during surgery[6]. Endotoxin release, leukocyte and complement activation, widespread activation of inflammatory mediators, and endothelial leak[7] postoperatively contribute to low cardiac output syndrome (LCOS) [8, 9]. Postoperative LCOS is clinically defined by a need for inotropes to maintain end organ perfusion, an increased arterial-venous oxygen extraction, lactataemia, and oliguria. LCOS may lead to multi-organ failure and a need for extracorporeal life support (ECLS)[10]. Several studies have shown that the presence and severity of LCOS, which affects 25-40% of children post CPB in the first hours following heart surgery[11], is strongly associated with postoperative morbidity and mortality. CPB-related side effects are most pronounced in infants and young children[12] due to their higher metabolic requirement, altered inflammatory response, and higher CPB circuit to patient blood volume. At the same time, this age group is exposed to CPB during a vulnerable phase of brain development [13-15] and remains at highest risk of suffering neurological impairments[16] due to acute brain injury occurring within the context of LCOS. Recent trials to reduce LCOS during cardiac surgery for CHD testing

interventions such as steroids[17] have not demonstrated consistent benefit[18]. Given the adverse effects of cardiopulmonary bypass on early recovery and long-term neurodevelopment there remains an urgent need for clinical trials evaluating novel therapies to address these problems[5].

Nitric oxide (NO) is an endogenous anti-inflammatory mediator[19] and is essential to regulate endothelial function and microvascular inflammation[20]. Several studies have demonstrated that exogenous NO can reduce myocardial damage in clinical and experimental settings of ischaemia and reperfusion[21-24]. A previous small single centre study in 16 children reported a reduction in bypass-induced inflammation using gaseous NO delivered at 20ppm to CPB circuits[25]. The duration of mechanical ventilation was significantly shorter (8.4 versus 16.3 hours; P< .05) and so was ICU length of stay (53.8 versus 79.4 hours; P < .05) in children receiving NO compared to the placebo group. We have previously reported the feasibility and safety of NO delivery to CPB in a single centre randomized controlled pilot study in 198 infants and children (0-16 years) undergoing cardiac surgery[26]. This pilot study demonstrated a statistically significantly lower proportion of children with a LCOS in the intervention arm, a reduced requirement for ECLS, and a trend to reduced length of stay, and shorter duration of mechanical ventilation. The effect was greatest in children under two years of age, with the greatest treatment benefit observed in children under six weeks.

Accordingly, we designed the NITRIC trial to test the primary hypothesis that, in infants under two years having cardiac surgery, using NO during CPB increases ventilator-free days (the number of days patients spend alive and free from invasive mechanical ventilation up until post-operative day 28) compared with standard care. Here we describe the NITRIC trial protocol.

METHODS

The NITRIC trial is a 1320-patient multicentre, randomised, double-blind, standard care-controlled, parallel-group, trial in infants and children < 2 years of age undergoing open heart surgery on CPB (**Figure 1**).

Study setting. Tertiary/quaternary paediatric cardiac surgical services in Australia and New Zealand, including Cardiac and Paediatric Intensive Care Services of Royal Children's Hospital, Melbourne; Starship Children's Hospital Auckland NZ; The Children's Hospital at Westmead, Sydney; Princess Margaret Hospital for Children, Perth; and Queensland Children's Hospital, Brisbane. Addition of a further international study site is in progress.

Participants. Eligible children will be identified in the pre-operative clinics, in the general cardiac wards or in the neonatal or paediatric intensive care unit. ***Included*** will be infants and children < 2 years of age undergoing elective open heart surgery on CPB where consent of parents/guardian is obtained prior to surgery. The ***exclusion criteria*** relate to patient characteristics that will interfere with consent, with the intervention, or with measurement of the primary and secondary outcomes. Inclusion and exclusion criteria are shown in **Table 1**.

Enrolment of patients undergoing repeated surgery during the first two years of life: In order to assess the impact of the intervention on long-term outcomes, patients who were previously enrolled and randomised into the study who require a second or subsequent surgical procedure (such as a patient with single ventricle physiology requiring a staged palliation) will undergo the same treatment allocation for subsequent surgeries requiring CPB, unless parents opt out. Patients who were not recruited into the study during their first procedure, but are scheduled for a subsequent procedure requiring CPB prior to their second birthday, will be eligible for recruitment.

Randomisation and Blinding. Treatment assignment will be performed using a secure, centralised, web-based randomisation interface (REDCap [27], The University of Queensland). The allocation sequence will be generated by the study statistician using computer-generated random numbers using a variable block size stratified by age (< six weeks, six weeks to 24 months), univentricular versus biventricular lesions, and by site. Of the investigating team, only the study perfusionist will be aware of the randomisation and NO delivery. Parents and caregivers will be blinded for the intervention. *Rationale for stratification:* The age group under six weeks represents the cohort at highest surgical risk. In the pilot study the effect size of the NO delivery was greatest in these infants. Cardiac physiology (univentricular versus biventricular) is a major determinant of surgical complexity, risk, and outcome.

Blinding of the Intervention. Blinding arrangements in the operating theatre will be achieved by covering the NO delivery system with drapes. The dedicated study NO delivery system will be connected to the CPB oxygenator at all times, independent of randomisation. The family, surgeons, anaesthetists and PICU staff will be not aware of the treatment arm a patient is allocated to. The perfusionists will be advised that all aspects of CPB except for provision of NO (or not) should be performed according to standard institutional practice for study participants.

Interventions. Infants will be randomly assigned to NO or standard care. Those allocated to the *NO arm* will receive NO during CPB blended into the fresh gas flow of the CPB oxygenator, which is kept at 3L/min. NO levels are maintained at 20 ppm using a NO delivery system (Ikaria INOmax DSIR, Ikaria, NJ, USA) or similar device. Continuous sampling of NO and NO_2 concentration will be undertaken from an access port before the oxygenator. NO will be started immediately when the patient is placed on CPB and ceased once weaned off CPB. Patients allocated to the *standard study arm* will receive the standard gase oxygen-air mix into the CPB oxygenator at a flow rate of 3L/min. If patients require several CPB runs during the

same procedure, the study treatment will be provided for each CPB run using the same treatment allocation for every CPB run.

Relevant concomitant perioperative care. Arterial partial pressures of CO_2 will be maintained constantly in both study arms as per institutional practice (following each centre's protocols on alpha/pH stat and temperatures). The FiO2 of the fresh gas flow will be set between 21% and 100%, according to centre specific CPB protocols. Techniques of anaesthesia and surgery will not be specified to allow site specific individual practice. The decision whether a patient requires treatment with inhaled NO (iNO) into the ventilator circuit prior to, during, or after CPB remains at the discretion of the treating physicians (anaesthetists, cardiac surgeons, or intensivists) independent of treatment allocation. The postoperative care and decisions on inotropes and other vasoactive drug delivery, fluid management, renal replacement therapy, iNO therapy or indication for ECLS will be performed as per site specific standard protocols of care.

Study outcomes (Table 2). The **Primary outcome** is ventilator free days (VFD) for the first 28 days post randomisation. The primary outcome will be measured using duration of invasive respiratory support for all episodes with an endotracheal tube in situ for the first 28 days post randomisation. A systematic zero value will be assigned for patients who die to weigh death as the most pejorative outcome. **Secondary outcomes** are defined as the composite outcome compromising LCOS, need for postoperative ECLS, or 28-day mortality; ICU and hospital length of stay; and health care costs. In addition, the long-term outcome of patients will be followed up at twelve months post procedure.

LCOS[10] is defined as a blood lactate level greater than 4 mmol/l with an oxygen extraction of greater than 35% (SaO_2-$ScvO_2$ gradient >35%) within the first 48 hours postoperatively, or a high inotrope requirement defined as Vasoactive-Inotrope Score ≥15 (VIS)[28, 29].

Rationale for primary and secondary outcomes: Ventilator free days represents one of the strongest predictors of short- and long-term outcomes[10], including length of intensive care unit (ICU) stay, morbidity (impaired neurodevelopment, hospital-acquired infections) and mortality. VFD directly reflect intensive care resource use and health care costs[30]. The composite of LCOS, ECLS use, and mortality as a secondary end point, is a strong patient-centred outcome and directly relates to the intervention in terms of biological plausibility.

Adverse Events: It is recognised that the postoperative paediatric cardiac surgical patient population will experience a number of common aberrations in laboratory values, signs and symptoms due to the severity of the underlying disease and the impact of standard therapies. Intensive care patients will frequently develop life-threatening organ failure(s) unrelated to study interventions and despite optimal management. Therefore, consistent with established practice in academic ICU trials[31], events that are part of the natural history of the primary disease process or expected complications of critical illness will not be reported as serious adverse events in this study. All adverse events which are considered to be potentially causally related to the study intervention or are otherwise of concern in the investigator's judgement will be reported unless they are pre-specified study outcomes. Specific adverse events related to NO delivery during CBP include air embolism, severe hypotension during bypass and increased MetHb (MetHb >3%). Of note, in previous studies, methaemoglobin values using NO at 20 ppm were similar in both the control and intervention groups (1.4%)[25, 26]. Events that are collected as study outcomes will not be reported as adverse events.

Safety Data Monitoring. The Data Safety Monitoring Board (DSMB) consists of a general and a cardiac paediatric intensivist, a cardiac surgeon, and a statistician. None of the DSMB members will be involved in recruitment of study patients at their site. DSMB members will not be supervised by any study investigator or participate as investigators in any study currently

under review by this DSMB. The primary objective of the DSMB is to monitor the safety of the intervention and the validity and integrity of the data from the NITRIC study. Additionally, the DSMB will evaluate the pace of recruitment and will make recommendations to the NITRIC Chief investigator(s) and Steering Board regarding the continuation, modification, or termination of the study. The DSMB will evaluate on an ongoing basis, the accumulating safety assessments to ensure the ongoing safety of study subjects. The DSMB will meet via teleconference call after recruitment of 660, and of 1000 children, respectively, and upon trial completion.

The DSMB can request unblinding in the event of a serious adverse event defined as a cardiac arrest, need for ECLS, or other incident leading to permanent harm considered to be likely related to the study intervention.

Sample size. A pilot study showed an approximate 66 hours (0.33 SD) increase in ventilator-free days (VFD) associated with the study intervention [26]. Based on the primary outcome measure VFD, 1,320 patients (660 per group) would be required to demonstrate a significant increase in VFD assuming a minimally clinically significant small effect size (0.2 SD), 90% power, two-sided alpha level of significance of 5%, 10% withdrawals, and 15% increase in sample size to account for a non-normal distribution of VFD. In Australia and New Zealand approximately 800 of children < 2 years of age undergo surgery for a congenital heart defect requiring CPB each year, including patients with multiple procedures. The consent rate of eligible patients was 78% in the pilot trial[26]. With an expected conservative estimate 60% enrolment rate of eligible patients, we expect a three year recruitment period for the study.

Data collection, management, and analysis

Data collection: Baseline variables (demographics, primary cardiac diagnosis, comorbidities including syndromes), pre-operative disease severity, surgical data (length of CPB and cross-clamp, type of surgery and complexity score, other CPB characteristics, blood product usage), primary end points, secondary end points, pre-determined physiological variables of interest, and process of care measures will be prospectively recorded into a study REDCap online database. Plausibility and range checks are implemented. Paired arterial and venous gases will be collected postoperatively at 0, 6, 12, 24, and 48 hrs (in PICU until discharge to the ward or removal of arterial and central venous lines, whichever occurs first) to assess for lactate and SaO_2-$ScvO_2$ gradient. Key physiological and blood parameters and Paediatric Logistic Organ Dysfunction-2 (PELOD-2) scores[32] will be measured at 0, 6, 12, 24, and 48 hours post admission or until PICU discharge whichever occurs earlier. Delayed chest closure, use of inhaled NO, and duration of circulatory, renal, and ventilatory support postoperatively will be recorded. Gross functional performance assessment will be recorded on admission to PICU and upon discharge from hospital[33]. Neurological and functional outcome (including phone interviews with parents/caregivers) will be assessed at 12 months postoperatively using Ages and Stages questionnaires (ASQ)[34, 35] and assessment of quality of life using paediatric quality of life inventory (PedsQL)[36]. Details on the long-term follow-up will be published separately.

Biobanking: Blood markers for myocardial injury and inflammatory response will be collected on induction of anaesthesia (baseline – pre-bypass), at admission to PICU (0 hours – post bypass) and at 12, and 24 hours. Blood will be collected pre-surgery as preoperative baseline (before onset of cardiac surgery, done by anaesthetist during the induction of the patient once the arterial line has been inserted): 1-2ml of EDTA blood (for DNA), 2.5ml of PAXgene blood (for gene expression markers), 1-2ml of serum; and postoperatively at 0,12,24 hours (in PICU

until discharge to the ward): 2.5ml of PAXgene blood (time point 0), and 1-2ml of serum. The samples will be processed, stored and shipped according to accepted international standards and batch analysed.

The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. The investigators will maintain adequate case histories of study participants, including accurate case report forms (CRFs), and source documentation. Data will be prospectively entered into a secure web-based database (REDCap https://redcap.health.uq.edu.au/), hosted by the University of Queensland. Printed paper CRFs will be available if required. All study information and documentation will be securely stored for a period of 15 years after the date of the child's eighteenth birthday.

Statistical Analysis Plan

Analysis plan. Analyses will apply the intention to treat principle. Descriptive statistics will be used to describe baseline characteristics of the study cohort and each subgroup by treatment group. The primary outcome measure will be analysed using a Mann-Whitney test as ventilator-free days is non-normally distributed variable. Analysis of secondary outcomes includes both comparisons of measurements and proportions, using confidence intervals of differences as the major method of presentation where possible, otherwise standard techniques such as Mann-Whitney U tests, t-tests and chi-squared tests will be utilised. Survival outcomes will be compared between treatment groups using Kaplan-Meier product limit method and log-rank test. Statistical significance will be set at the 0.05 level for the primary outcome.

A safety and efficacy interim analysis after 660 (the half-way point), and after 1000 enrolled patients will be performed by an independent statistician to evaluate for safety endpoints, to assess the predictive probability of reaching the study goals, and compare VFD between

treatment groups. Consideration to stopping the trial early by an independent Data and Safety Monitoring Board (DSMB) will be based on safety concerns, futility, or strong evidence of a difference between groups for VFD (based on a Haybittle–Peto boundary P=0.001). A detailed analysis plan specifying statistical analyses including health economic analyses will be placed in the public domain prior to recruitment of the last participant[37].

Biomarker measurements. Nested sub-studies will be performed in selected samples at sites performing biobanking (i) to test the impact of the intervention on markers of systemic and myocardial inflammation; (ii) to compare treatment response between patients depending on pre-intervention severity assessed by markers of inflammation and organ failure; and (iii) to biochemically define responders to the intervention (to identify patient subgroups pre-randomisation that are more likely to respond to a specific treatment). The use of samples/data will be governed by the study steering board and resulting publications must appropriately acknowledge the study. Combining a large RCT with a nested biobank is recommended to maximize scientific knowledge[38].

Health Economic Evaluation. A within trial economic evaluation will be used to determine if providing NO is cost-saving compared to usual care from the health system perspective. A comprehensive cost-effectiveness analysis will be undertaken to determine the level of cost savings (if any). Length of stay (in PICU and in non-intensive stay) will be the main outcome variable. Resources used before first discharge will be compared between treatments. Resources will subsequently be costed, based on hospital cost centre or standard national sources (e.g. Independent Hospital Pricing Authority).

Monitoring. The study leadership team is responsible for 100% monitoring of investigator and study nurse credentials, training records, and delegation of responsibility logs, and will review 100% of Consent Forms. CRFs will be compared to source documentation to ensure data are

accurate and complete. 100% of source data verification of eligibility criteria and the primary outcome and the composite secondary outcome of LCOS, ECLS or death will be performed. An independent monitoring per each site will monitor the data fields required for eligibility, primary, and secondary study endpoints, and SAEs using primary source verification. In addition, site visits and regular monitoring of the blood sample storage will be performed.

Patient and Public Involvement. Consumers and the public were consulted to design a video informing parents about the study. Patients and the public had no other involvement in study design.

Ethics. This protocol and the informed consent document and any subsequent modifications have been reviewed and approved by the human research ethics committee (Children`s Health Queensland HREC/17/QRCH/43). This study will be conducted in compliance with the current version of the protocol. Any change to the protocol document or Informed Consent Form that affects the scientific intent, study design, patient safety, or may affect a participants' willingness to continue in the study is considered an amendment, and therefore will be written and filed as an amendment to this protocol and/or informed consent form. All protocol deviations must be recorded in the patient record (source document) and on the CRF and must be reported to the PI. Protocol deviations will be assessed for significance by the Principal Investigator.

Consent will be sought from the parents of every child <2 years of age undergoing CPB for elective cardiac surgery over the study period. When the family is seen by the surgeons in pre-assessment clinic (usually days prior to surgery), the study will be mentioned to them by the surgeon. In addition, the study team will provide study information prior to hospitalisation to families, including printed study flyers, and links to online study documentation (media release). Participant confidentiality is strictly held in trust by the participating investigators,

research staff, and the sponsoring institution and their agents. The study protocol, documentation, data and all other information generated will be held in strict confidence.

Significance: Postoperative paediatric cardiac surgical patients have a high consumption of intensive care resources and remain at very high risk of major complications, including cardiac arrest, death, and long-term neurological impairment. Approximately 10% of children with CHD survive with major neurological sequelae postoperatively, resulting in a massive lifelong burden for patients, families, healthcare systems, and the society[13]. An attempt to reduce LCOS and hence perioperative morbidity has the potential to translate not only to a reduction in intensive care resource utilisation, but also to impact positively on long-term outcomes. Side-effects from heart surgery for CHD translate into long-term morbidity, persisting into adult life with a major impact on other family members and society. This study will deliver the high-level randomised evidence with the potential to show a reduction in postoperative morbidity and mortality in children with CHD.

Acknowledgements: The authors would like to thank the parents and children participating in this trial and the the medical and nursing and research teams in the participating sites for their help in study setup, recruitment, data collection, and monitoring of study data.

NITRIC Study Group:

Warwick Butt, Steve Horton, Johnny Millar, Carmel Delzoppo, Yves D`Udekem: Cardiac and Paediatric Intensive Care Services of Royal Children's Hospital, Melbourne, Australia

John Beca, David Buckley, Taryn Evans, Claire Sherring, John Artrip: Starship Children's Hospital Auckland, New Zealand

Marino Festa, Killian O`Shaughnessy, David Winlaw, Rebecca Fletcher, Simon Byrne, The Children's Hospital at Westmead, Sydney, Australia

Simon Erickson, Sam Barr, Rae Kelly, David Andrews: Perth Children`s Hospital, Perth, Australia

Luregn J Schlapbach, Andreas Schibler, Deborah Long, Kerry Johnston, Carla Zuzak, Nelson Alphonso, Benjamin Anderson: Queensland Children's Hospital, Brisbane, Australia

Antje Blumenthal: The University of Queensland Diamantina Institute, Translational Research Institute, The University of Queensland, Brisbane, Australia

Jonas Fooken, Brenda Gannon: Centre for the Business and Economics of Health, The University of Queensland, Brisbane, Australia

Paul Young: Medical Research Institute of New Zealand, Wellington, New Zealand

Mark Jones: School of Public Health, The University of Queensland, Brisbane, Australia

Kim van Loon, Nicole JCW van Belle-van Haaren, Bram van Wijk, Erik Koomen, Wilhelmina Children's Hospital Utrecht, Utrecht, The Netherlands

REFERENCES

1. Triedman JK, Newburger JW: **Trends in Congenital Heart Disease: The Next Decade**. *Circulation* 2016, **133**(25):2716-2733.
2. Erikssen G, Liestol K, Seem E, Birkeland S, Saatvedt KJ, Hoel TN, Dohlen G, Skulstad H, Svennevig JL, Thaulow E *et al*: **Achievements in congenital heart defect surgery: a prospective, 40-year study of 7038 patients**. *Circulation* 2015, **131**(4):337-346; discussion 346.
3. Marelli AJ, Ionescu-Ittu R, Mackie AS, Guo L, Dendukuri N, Kaouache M: **Lifetime prevalence of congenital heart disease in the general population from 2000 to 2010**. *Circulation* 2014, **130**(9):749-756.
4. Marelli AJ, Mackie AS, Ionescu-Ittu R, Rahme E, Pilote L: **Congenital heart disease in the general population: changing prevalence and age distribution**. *Circulation* 2007, **115**(2):163-172.
5. Kaltman JR, Andropoulos DB, Checchia PA, Gaynor JW, Hoffman TM, Laussen PC, Ohye RG, Pearson GD, Pigula F, Tweddell J *et al*: **Report of the pediatric heart network and national heart, lung, and blood institute working group on the perioperative management of congenital heart disease**. *Circulation* 2010, **121**(25):2766-2772.

6. Zahler S, Massoudy P, Hartl H, Hahnel C, Meisner H, Becker BF: **Acute cardiac inflammatory responses to postischemic reperfusion during cardiopulmonary bypass**. *Cardiovasc Res* 1999, **41**(3):722-730.

7. Levy JH, Tanaka KA: **Inflammatory response to cardiopulmonary bypass**. *Ann Thorac Surg* 2003, **75**(2):S715-720.

8. Duval EL, Kavelaars A, Veenhuizen L, van Vught AJ, van de Wal HJ, Heijnen CJ: **Pro- and anti-inflammatory cytokine patterns during and after cardiac surgery in young children**. *Eur J Pediatr* 1999, **158**(5):387-393.

9. Domanski MJ, Mahaffey K, Hasselblad V, Brener SJ, Smith PK, Hillis G, Engoren M, Alexander JH, Levy JH, Chaitman BR *et al*: **Association of myocardial enzyme elevation and survival following coronary artery bypass graft surgery**. *JAMA* 2011, **305**(6):585-591.

10. Hoffman TM, Wernovsky G, Atz AM, Kulik TJ, Nelson DP, Chang AC, Bailey JM, Akbary A, Kocsis JF, Kaczmarek R *et al*: **Efficacy and safety of milrinone in preventing low cardiac output syndrome in infants and children after corrective surgery for congenital heart disease**. *Circulation* 2003, **107**(7):996-1002.

11. Duke T, Stocker C, Butt W: **Monitoring children after cardiac surgery: a minimalist approach might be maximally effective**. *Crit Care Resusc* 2004, **6**(4):306-310.

12. McElhinney DB, Wernovsky G: **Outcomes of neonates with congenital heart disease**. *Curr Opin Pediatr* 2001, **13**(2):104-110.

13. Ballweg JA, Wernovsky G, Gaynor JW: **Neurodevelopmental outcomes following congenital heart surgery**. *Pediatr Cardiol* 2007, **28**(2):126-133.

14. Marino BS: **New concepts in predicting, evaluating, and managing neurodevelopmental outcomes in children with congenital heart disease**. *Curr Opin Pediatr* 2013, **25**(5):574-584.

15. Marino BS, Lipkin PH, Newburger JW, Peacock G, Gerdes M, Gaynor JW, Mussatto KA, Uzark K, Goldberg CS, Johnson WH, Jr. *et al*: **Neurodevelopmental outcomes in children with congenital heart disease: evaluation and management: a scientific statement from the American Heart Association**. *Circulation* 2012, **126**(9):1143-1172.

16. Brown MD, Wernovsky G, Mussatto KA, Berger S: **Long-term and developmental outcomes of children with complex congenital heart disease**. *Clin Perinatol* 2005, **32**(4):1043-1057, xi.

17. Bronicki RA, Backer CL, Baden HP, Mavroudis C, Crawford SE, Green TP: **Dexamethasone reduces the inflammatory response to cardiopulmonary bypass in children**. *Ann Thorac Surg* 2000, **69**(5):1490-1495.

18. Elhoff JJ, Chowdhury SM, Zyblewski SC, Atz AM, Bradley SM, Graham EM: **Intraoperative Steroid Use and Outcomes Following the Norwood Procedure: An Analysis of the Pediatric Heart Network's Public Database**. *Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies* 2016, **17**(1):30-35.

19. Hataishi R, Rodrigues AC, Neilan TG, Morgan JG, Buys E, Shiva S, Tambouret R, Jassal DS, Raher MJ, Furutani E *et al*: **Inhaled nitric oxide decreases infarction size and improves left ventricular function in a murine model of myocardial ischemia-reperfusion injury**. *American journal of physiology Heart and circulatory physiology* 2006, **291**(1):H379-384.

20. Chello M, Mastroroberto P, Perticone F, Celi V, Colonna A: **Nitric oxide modulation of neutrophil-endothelium interaction: difference between arterial and venous coronary bypass grafts**. *J Am Coll Cardiol* 1998, **31**(4):823-826.

21. Jones SP, Bolli R: **The ubiquitous role of nitric oxide in cardioprotection**. *J Mol Cell Cardiol* 2006, **40**(1):16-23.

22. Jones SP, Girod WG, Palazzo AJ, Granger DN, Grisham MB, Jourd'Heuil D, Huang PL, Lefer DJ: **Myocardial ischemia-reperfusion injury is exacerbated in absence of endothelial cell nitric oxide synthase**. *Am J Physiol* 1999, **276**(5 Pt 2):H1567-1573.

23. Minamishima S, Kida K, Tokuda K, Wang H, Sips PY, Kosugi S, Mandeville JB, Buys ES, Brouckaert P, Liu PK *et al*: **Inhaled nitric oxide improves outcomes after successful cardiopulmonary resuscitation in mice**. *Circulation* 2011, **124**(15):1645-1653.

24. Schulz R, Kelm M, Heusch G: **Nitric oxide in myocardial ischemia/reperfusion injury**. *Cardiovasc Res* 2004, **61**(3):402-413.

25. Checchia PA, Bronicki RA, Muenzer JT, Dixon D, Raithel S, Gandhi SK, Huddleston CB: **Nitric oxide delivery during cardiopulmonary bypass reduces postoperative morbidity in children--a randomized trial**. *J Thorac Cardiovasc Surg* 2013, **146**(3):530-536.

26. James C, Millar J, Horton S, Brizard C, Molesworth C, Butt W: **Nitric oxide administration during paediatric cardiopulmonary bypass: a randomised controlled trial**. *Intensive Care Med* 2016, **42**(11):1744-1752.

27. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG: **Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support**. *Journal of biomedical informatics* 2009, **42**(2):377-381.

28. Gaies MG, Jeffries HE, Niebler RA, Pasquali SK, Donohue JE, Yu S, Gall C, Rice TB, Thiagarajan RR: **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):529-537.

29. Gaies MG, Gurney JG, Yen AH, Napoli ML, Gajarski RJ, Ohye RG, Charpie JR, Hirsch JC: **Vasoactive-inotropic score as a predictor of morbidity and mortality in infants after cardiopulmonary bypass**. *Pediatr Crit Care Med* 2010, **11**(2):234-238.

30. Contentin L, Ehrmann S, Giraudeau B: **Heterogeneity in the definition of mechanical ventilation duration and ventilator-free days**. *Am J Respir Crit Care Med* 2014, **189**(8):998-1002.

31. Cook D, Lauzier F, Rocha MG, Sayles MJ, Finfer S: **Serious adverse events in academic critical care research**. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2008, **178**(9):1181-1184.

32. Leteurtre S, Duhamel A, Salleron J, Grandbastien B, Lacroix J, Leclerc F, Groupe Francophone de Reanimation et d'Urgences P: **PELOD-2: an update of the PEdiatric logistic organ dysfunction score**. *Crit Care Med* 2013, **41**(7):1761-1773.

33. Pollack MM, Holubkov R, Funai T, Clark A, Moler F, Shanley T, Meert K, Newth CJ, Carcillo J, Berger JT *et al*: **Relationship between the functional status scale and the pediatric overall performance category and pediatric cerebral performance category scales**. *JAMA Pediatr* 2014, **168**(7):671-676.

34. Pierrat V, Marchand-Martin L, Arnaud C, Kaminski M, Resche-Rigon M, Lebeaux C, Bodeau-Livinec F, Morgan AS, Goffinet F, Marret S *et al*: **Neurodevelopmental outcome at 2 years for preterm children born at 22 to 34 weeks' gestation in France in 2011: EPIPAGE-2 cohort study**. *Bmj* 2017, **358**:j3448.

35. Noeder MM, Logan BA, Struemph KL, Condon N, Mueller I, Sands B, Davies RR, Sood E: **Developmental screening in children with CHD: Ages and Stages Questionnaires**. *Cardiology in the young* 2017, **27**(8):1447-1454.

36. Desai AD, Zhou C, Stanford S, Haaland W, Varni JW, Mangione-Smith RM: **Validity and responsiveness of the pediatric quality of life inventory (PedsQL) 4.0 generic core scales in the pediatric inpatient setting**. *JAMA Pediatr* 2014, **168**(12):1114-1121.

37. Billot L, Venkatesh B, Myburgh J, Finfer S, Cohen J, Webb S, McArthur C, Joyce C, Bellomo R, Rhodes A *et al*: **Statistical analysis plan for the Adjunctive Corticosteroid Treatment in Critically Ill Patients with Septic Shock (ADRENAL) trial**. *Critical care and resuscitation : journal of the Australasian Academy of Critical Care Medicine* 2017, **19**(2):183-191.

38. Gustafsson F, Atar D, Pitt B, Zannad F, Pfeffer MA, participants in 10th Cardiovascular Clinical Trialists W: **Maximizing scientific knowledge from randomized clinical trials**. *Am Heart J* 2010, **159**(6):937-943.

Figures

Figure 1. Study Flow Diagram.

Table 1: Inclusion and Exclusion criteria

Patient group	Criterium	Definition
Inclusion	*Age*	• Postnatal age below 2 years
	Procedure	• Elective Heart surgery
		• Cardiopulmonary bypass used during surgery
	Consent	• Parental/caregiver consent available prior to surgery
Exclusion	*Age*	• Age ≥2 years
	Procedure	• emergency cardiac surgery which may preclude obtaining informed consent (acutely required life-saving procedure in a patient unlikely to survive the next hours without the surgery)
		• Heart surgery not requiring cardiopulmonary bypass
	Consent	• Lack of parental/caregiver consent
	Pulmonary hypertension	• persistently elevated pulmonary vascular resistance preoperatively receiving inhaled NO or preoperative intravenous use of drugs involved in the NO pathway such as glyceryl trinitrate within 48 hours prior to CPB (oral sildenafil treatment alone is not an exclusion)
	Pre-operative disease	• ECLS immediately prior to surgery
		• Receiving ongoing treatment with antimicrobials for confirmed or suspected sepsis or septic shock diagnosed within 48hours prior to the time of surgery
		• Treated with high doses of vasoactive drugs defined as a Vasoactive-Inotrope Score (VIS) ≥15 within 24 hours prior to surgery[a]
		• cardiac arrest within one week (7d) prior to surgery
		• Acute respiratory distress syndrome requiring high frequency oscillatory ventilation within 48 hours prior to surgery
		• chronic ventilator dependency
		• pre-existing methaemoglobinemia (MetHb>3%)

a) Gaies MG, Gurney JG, Yen AH, *et a*l: Vasoactive-inotropic score as a predictor of morbidity and mortality in infants after cardiopulmonary bypass. *Pediatr Crit Care Med* 2010, **11**(2):234-238.

Table 2: Study outcomess

Outcome	Criterium	Definition
Primary	*Ventilator-free days (VFD)*	• duration of respiratory support for all episodes for the first 28 days post randomisation • zero value for patients dying within 28 days post randomisation • refers to invasive respiratory support with an endotracheal tube in situ
Secondary	*Composite outcome of low cardiac output syndrome (LCOS), Extracorporeal life support (ECLS), or death*	• ***LCOS*** is defined as one or more of the following[a]: - Blood lactate level greater than 4 mmol/l with an oxygen extraction of greater than 35% (SaO_2-$ScvO_2$ gradient >35%) within the first 48 hours postoperatively - a high inotrope requirement defined as Vasoactive-Inotrope Score ≥15 (VIS)[b] where VIS = dopamine dose (mcg/kg/min) + dobutamine dose (mcg/kg/min) + 100 x adrenaline dose (mcg/kg/min) + 100 x noradrenaline dose (mcg/kg/min) + 10 x milrinone dose (mcg/kg/min) + 10,000 x vasopressin dose (U/kg/min). • ECLS is defined as treatment with ECLS during the first 48 hours post randomisation • Death is defined as death occurring within the first 28 days post randomisation
	Length of stay	• Length of stay in paediatric intensive care unit (PICU) • Length of stay in hospital
	Costs	• Health-care related costs (starting at time of admission to PICU postoperatively)
	Neurodevelopmental and functional outcome at 12 months	• Ages and Stages Questionnaire (ASQ) scores below threshold for at least one of the five domains measured 12 months post randomisation
Process of care measures	*Severity indicators*	• Treatment with ECLS postoperatively • Duration of postoperative time spent with open chest including unplanned chest reopening • Treatment and duration of treatment using inhalational nitric oxide postoperatively • Treatment and duration of treatment of postoperative renal replacement therapy
Physiological descriptors	*Host inflammation*	• Serum cytokine levels measured during the first 24 hours • Inflammation markers measured during the first 24 hours
	Myocardial injury	• Levels of postoperative serum troponin levels measured during the first 24 hours
	Organ dysfunction	• Severity and duration of postoperative organ dysfunction measured by PELOD-2 • postoperative Acute Kidney Injury and serum creatinine levels measured during the first 24 hours

- severity and duration of postoperative delirium

a) Hoffman TM, Wernovsky G, Atz AM, *et al*: Efficacy and safety of milrinone in preventing low cardiac output syndrome in infants and children after corrective surgery for congenital heart disease. *Circulation* 2003, 107(7):996-1002.
b) Gaies MG, Gurney JG, Yen AH, *et al*: Vasoactive-inotropic score as a predictor of morbidity and mortality in infants after cardiopulmonary bypass. *Pediatr Crit Care Med* 2010, 11(2):234-238.

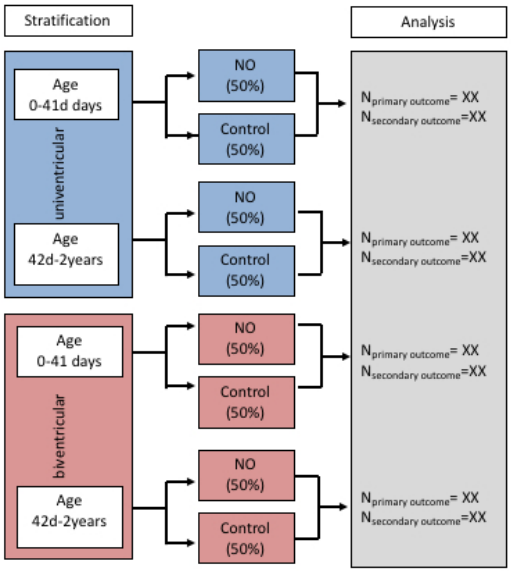


Figure 1

254x190mm (72 x 72 DPI)

BMJ Open

Study Protocol: NITric oxide during cardiopulmonary bypass to improve Recovery in Infants with Congenital heart defects (NITRIC trial): A Randomised Controlled Trial.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-026664.R1
Article Type:	Protocol
Date Submitted by the Author:	15-May-2019
Complete List of Authors:	Schlapbach, Luregn; The University of Queensland, Paediatric Critical Care Research Group Horton, Stephen; Royal Children's Hospital M, Cardiac Surgical Unit; University of Melbourne, Faculty of Medicine Long, Debbie; Queensland Health, Lady Cilento Children's Hospital; Menzies Health Institute Queensland, Alliance for Vascular Access Teaching and Research Group Beca, John; Starship Children's Hospital, Paediatric Intensive Care Unit Erickson, Simon; Perth Children's Hospital, Paediatric Critical Care Festa, Marino d'Udekem, Yves; Royal Children's Hospital M, Cardiac Surgical Unit; University of Melbourne, Faculty of Medicine Alphonso, Nelson; The University of Queensland, Paediatric Critical Care Research Group; Queensland Health, Lady Cilento Children's Hospital Winlaw, David; The Children's Hospital at Westmead, Sydney Children`s Hospital Network, Heart Centre for Children Johnson, Kerry; Queensland Health, Lady Cilento Children's Hospital Delzoppo, Carmel; Royal Children's Hospital M, Cardiac Surgical Unit van Loon, Kim; University Medical Center Utrecht Gannon, B; The University of Queensland, Centre for Business and Economics of Health Fooken, Jonas; The University of Queensland, Centre for Business and Economics of Health Blumenthal, Antje; The University of Queensland, Diamantina Institute Young, Paul ; Wellington Hospital, Jones, Mark; Bond University Butt, Warwick; Royal Children's Hospital M, Cardiac Surgical Unit Schibler , Andreas; Queensland University of Technology; Queensland Health, Lady Cilento Children's Hospital
Primary Subject Heading:	Intensive care
Secondary Subject Heading:	Cardiovascular medicine, Health economics, Paediatrics
Keywords:	infant, Cardiac surgery < SURGERY, mortality, nitric oxide, cardiopulmonary bypass, inflammation

Title Page:

Study Protocol: NITric oxide during cardiopulmonary bypass to improve Recovery in Infants with Congenital heart defects (NITRIC trial): A Randomised Controlled Trial.

Authors, highest degree and affiliations:

- Luregn J Schlapbach, MD, FCICM, Paediatric Critical Care Research Group, Child Health Research Institute, The University of Queensland; Paediatric Intensive Care Unit, Queensland Children`s Hospital, Children`s Health Queensland, Brisbane, Australia. Email: l.schlapbach@uq.edu.au
- Stephen B Horton PhD, Cardiac Surgical Unit, Royal Children's Hospital, Melbourne. Faculty of Medicine, Department of Paediatrics, University of Melbourne. Honorary Research Fellow, Murdoch Children's Research Institute, Melbourne, Australia. Email: Steve.Horton@rch.org.au
- Debbie Long, PhD, Paediatric Critical Care Research Group, Child Health Research Institute, The University of Queensland; Paediatric Intensive Care Unit, Queensland Children`s Hospital, Children`s Health Queensland, Brisbane, Australia. Email: Debbie.Long2@health.qld.gov.au
- John Beca, MBChB, FCICM. FRACP, Paediatric Intensive Care Unit, Starship Children's Hospital, Auckland, New Zealand. Email: JohnBeca@adhb.govt.nz
- Simon Erickson, MBBS FCICM, Paediatric Critical Care, Perth Children's Hospital, Western Australia and The University of Western Australia. Email: Simon.Erickson@health.wa.gov.au
- Marino Festa, MD, FCICM, Kids Critical Care Research, Paediatric Intensive Care Unit, Children's Hospital at Westmead, Sydney Children's Hospital Network, Sydney, New South Wales, Australia. Email: marino.festa@health.nsw.gov.au

- Yves d'Udekem, MD, PhD, Department of Cardiac Surgery, Royal Children's Hospital, Melbourne, Australia; Heart Research, Murdoch Childrens Research Institute, Melbourne, Australia; Department of Paediatrics, The University of Melbourne, Melbourne, Australia. Email: Yves.Dudekem@rch.org.au
- Nelson Alphonso, FRCS (C.Th), FRACS, Cardiac Surgery, Queensland Children`s Hospital, and Associate Professor, School of Medicine, Children's Health Clinical Unit, University of Queensland, Brisbane, Queensland, Australia. Email: nelson.alphonso@health.qld.gov.au
- David Winlaw MBBS, MD, FRACS, Heart Centre for Children, The Children's Hospital at Westmead, Sydney Children`s Hospital Network, and Faculty of Medicine and Health, University of Sydney, Sydney, Australia. Email: david.winlaw@health.nsw.gov.au
- Kerry Johnson, Paediatric Critical Care Research Group, Child Health Research Institute, The University of Queensland; Paediatric Intensive Care Unit, Queensland Children`s Hospital, Children`s Health Queensland, Brisbane, Australia. Email: Kerry.Johnson3@health.qld.gov.au
- Carmel Delzoppo, BHS, Paediatric Intensive Care Unit Royal Children's Hospital Melbourne, Murdoch Children's Research Institute. Email: Carmel.Delzoppo@rch.org.au
- Kim van Loon, MD, PhD, Division of Anaesthetics, University Medical Center Utrecht Utrecht, the Netherlands. Email: k.vanloon1@gmail.com
- Brenda Gannon, PhD, Professor of Health Economics, Centre for the Business and Economics of Health, The University of Queensland, Brisbane, Australia. brenda.gannon@uq.edu.au

- Jonas Fooken, PhD, Centre for the Business and Economics of Health, The University of Queensland, Brisbane, Australia. Email: j.fooken@uq.edu.au
- Antje Blumenthal, Dr rer. nat., Associate Professor and Head of the Infection and Inflammation Group at The University of Queensland Diamantina Institute, The University of Queensland, Translational Research Institute, Brisbane, Australia. Email: a.blumenthal@uq.edu.au
- Paul Young, MBChB, FCICM, PhD, Director of the Intensive Care Research Programme, Medical Research Institute of New Zealand, Wellington, New Zealand Email: paul.young@ccdhb.org.nz
- Mark Jones, PhD, School of Public Health, Bond University, South Port, Australia. Email: majones@bond.edu.au
- Warwick Butt, Associate Professor, MB B.S, FRACP, FJFICM, FCICM. Paediatric Intensive Care Unit Royal Children's Hospital Melbourne, Murdoch Children's Research Institute, Melbourne University. Email: Warwick.Butt@rch.org.au
- Andreas Schibler, MD, FCICM, Paediatric Critical Care Research Group, Child Health Research Institute, The University of Queensland; Paediatric Intensive Care Unit, Queensland Children`s Hospital, Children`s Health Queensland, Brisbane, Australia. Email: a.schibler@uq.edu.au

On behalf of the NITRIC Study Group, the Australian and New Zealand Intensive Care Society Clinical Trials Group (ANZICS CTG), the Paediatric Critical Care Research group (PCCRG) and the ANZICS Paediatric Study Group (PSG)

Correspondence

A/Prof Andreas Schibler, MD, FCICM

Paediatric Critical Care Research Group (PCCRG), Child Health Research Centre, Queensland Children's Hospital Precinct and Mater Research Institute, The University of Queensland Level 7, 62 Graham St, South Brisbane, Queensland, 4101, Australia

Email: a.schibler@uq.edu.au

Phone: ++61 (0) 7 3068 11 11

Alternative correspondence:

A/Prof Luregn J. Schlapbach, MD, FCICM

Paediatric Critical Care Research Group, Child Health Research Institute, The University of Queensland; and Queensland Children`s Hospital, Children`s Health Queensland, The University of Queensland Level 7, 62 Graham St, South Brisbane, Queensland, 4101, Australia

Email: l.schlapbach@uq.edu.au

Phone: ++61 (0) 7 3068 11 11

Conflict of Interest Statement:

Yves d'Udekem is a consultant for MSD and Actelion.

LJS, SH, DL, KJ, NA, DW, MF, SE, JB, CD, KL, MJ, AF, BG, AB, PY, WB, AS have no conflicts of interest to declare.

Word count: 3441

Key words: cardiopulmonary bypass; child; congenital heart disease; infant; inflammation; ventilation; nitric oxide; mortality

Abbreviations:

CPB	Cardio Pulmonary Bypass
CHD	Congenital Heart Disease
ECLS	Extracorporeal Life Support
FiO_2	Fraction of inspired oxygen
CO_2	Carbon dioxide
NO	Nitric oxide
SaO_2	Arterial oxygen saturation
$ScvO_2$	Central venous oxygen saturation
PICU	Paediatric Intensive Care Unit

Study Protocol: NITric oxide during cardiopulmonary bypass to improve Recovery in Infants with Congenital heart defects (NITRIC trial): A Randomised Controlled Trial.

Trial registration: ACTRN12617000821392

Protocol Version 1.3, dated 17th July 2018

Funding sources: This work was supported by grants from the Heart Kids Foundation, Australia; the Children`s Health Foundation, Brisbane, Australia; and by a grant from the Australian National Health and Medical Research Council (1140322).

Luregn Schlapbach, Yves d'Udekem, Andreas Schibler hold a NHMRC Clinical Practitioner Fellowship. The Victorian Government's Operational Infrastructure Support Program

supported this research project. Paul Young holds a Clinical Practitioner Fellowship from the Health Research Council of New Zealand.

The funding sources had no involvement in study design, analyses, nor interpretation of the results.

Mallinckrodt Pharmaceuticals will provide nitric oxide delivery devices to study centres but has no involvement in study design, conduct, nor analyses.

Author contributorship statement:

The study protocol first draft was designed by LJS and AS based on the previous pilot study design established by SH, and WB. MJ wrote the section on statistical analyses. JF and BG wrote the section on health economic analyses The present study protocol has been revised with input from SH, DL, JB, SE, MF, YdU, NA, DW, KJ, CD, KvL, BG, JF, PY, AB, MJ and WB. LJS prepared the final protocol manuscript which was reviewed and approved by all authors.

Abstract

Introduction: Congenital heart disease (CHD) is a major cause of infant mortality. Many infants with CHD require corrective surgery with most operations requiring cardiopulmonary bypass (CPB). CPB triggers a systemic inflammatory response which is associated with low cardiac output syndrome (LCOS), post-operative morbidity and mortality. Delivery of nitric oxide (NO) into CPB circuits can provide myocardial protection and reduce bypass-induced inflammation, leading to less LCOS and improved recovery. We hypothesized that using NO during CPB increases ventilator-free days (the number of days patients spend alive and free from invasive mechanical ventilation up until day 28) compared with standard care. Here we describe the NITRIC trial protocol.

Methods and Analysis: The NITRIC trial is a randomised, double blind, controlled, parallel-group, two-sided superiority trial to be conducted in at least five paediatric cardiac surgical centres. One thousand three-hundred and twenty infants below two years of age undergoing cardiac surgery with CPB will be randomly assigned to NO at 20ppm administered into the CPB oxygenator for the duration of CPB or standard care (no NO) in a 1:1 ratio with stratification by age (<six weeks ≥six weeks), single ventricle physiology (Y/N), and study centre. The primary outcome will be ventilator-free days to day 28. Secondary outcomes include a composite of LCOS, need for extracorporeal membrane oxygenation, or death within 28 days of surgery; length of stay in intensive care and in hospital; and, health care costs. Analyses will be conducted on an intention to treat basis. Pre-planned secondary analyses will investigate the impact of NO on host inflammatory profiles post-surgery.

Ethics and dissemination: The study has ethical approval (HREC/17/QRCH/43, dated 26th April 2017), is registered in the Australian New Zealand Clinical Trials Registry (ACTRN12617000821392), and commenced recruitment in July 2017. The primary manuscript will be submitted for publication in a peer reviewed journal.

Strengths and limitations of this study:

- This study tests the efficacy and safety of a simple intervention during cardiopulmonary bypass to improve early postoperative morbidity.
- The intervention aims to reduce patient-centred adverse outcomes after a common high-risk procedure for the most common congenital condition.
- The study includes follow-up of neurodevelopmental outcome and quality of life which will allow assessment of the long-term impact of the intervention.
- The study includes biobanking to investigate the biological mechanisms underlying the clinical findings in anxillary studies.
- The study will be the largest randomised controlled trial performed in paediatric cardiac surgery to date.

Introduction

Congenital heart disease (CHD) is the most common congenital condition, affecting around one in a hundred live born children[1]. Up to 50% need cardiac surgery to correct the underlying abnormality at some stage during their life, with the majority of procedures requiring cardiopulmonary bypass (CPB). Substantial reductions in perioperative mortality in children following cardiac surgery have been achieved[2], and adult survivors of CHD now outnumber paediatric patients with CHD in most high income countries[3, 4]. Despite these advances, major postoperative morbidity remains common and is associated with increased rate of long term mortality, morbidity, and disability[5]. The exposure of host blood to large artificial organ surfaces combined with myocardial injury during surgery, results in a strong systemic inflammatory response of the host, which is further aggravated by reperfusion injury and the release of damage-associated molecular patterns during surgery[6]. Endotoxin release, leukocyte and complement activation, widespread activation of inflammatory mediators, and endothelial leak[7] postoperatively contribute to low cardiac output syndrome (LCOS) [8, 9]. Postoperative LCOS is clinically defined by a need for inotropes to maintain end organ perfusion, an increased arterial-venous oxygen extraction, lactataemia, and oliguria. LCOS may lead to multi-organ failure and a need for extracorporeal life support (ECLS)[10]. Several studies have shown that the presence and severity of LCOS, which affects 25-40% of children post CPB in the first hours following heart surgery[11], is strongly associated with postoperative morbidity and mortality. CPB-related side effects are most pronounced in infants and young children[12] due to their higher metabolic requirement, altered inflammatory response, and higher CPB circuit to patient blood volume. At the same time, this age group is exposed to CPB during a vulnerable phase of brain development [13-15] and remains at highest risk of suffering neurological impairments[16] due to acute brain injury occurring within the context of LCOS. Recent trials to reduce LCOS during cardiac surgery for CHD testing

interventions such as steroids[17] have not demonstrated consistent benefit[18]. Given the adverse effects of cardiopulmonary bypass on early recovery and long-term neurodevelopment there remains an urgent need for clinical trials evaluating novel therapies to address these problems[5].

Nitric oxide (NO) is an endogenous anti-inflammatory mediator[19] and is essential to regulate endothelial function and microvascular inflammation[20]. Several studies have demonstrated that exogenous NO can reduce myocardial damage in clinical and experimental settings of ischaemia and reperfusion[21-24]. A previous small single centre study in 16 children reported a reduction in bypass-induced inflammation using gaseous NO delivered at 20ppm to CPB circuits[25]. The duration of mechanical ventilation was significantly shorter (8.4 versus 16.3 hours; P< .05) and so was ICU length of stay (53.8 versus 79.4 hours; P < .05) in children receiving NO compared to the placebo group. We have previously reported the feasibility and safety of NO delivery to CPB in a single centre randomized controlled pilot study in 198 infants and children (0-16 years) undergoing cardiac surgery[26]. This pilot study demonstrated a statistically significantly lower proportion of children with a LCOS in the intervention arm, a reduced requirement for ECLS, and a trend to reduced length of stay, and shorter duration of mechanical ventilation. The effect was greatest in children under two years of age, with the greatest treatment benefit observed in children under six weeks.

Accordingly, we designed the NITRIC trial to test the primary hypothesis that, in infants under two years having cardiac surgery, using NO during CPB increases ventilator-free days (the number of days patients spend alive and free from invasive mechanical ventilation up until post-operative day 28) compared with standard care. Here we describe the NITRIC trial protocol.

METHODS

The NITRIC trial is a 1320-patient multicentre, randomised, double-blind, standard care-controlled, parallel-group, trial in infants and children < 2 years of age undergoing open heart surgery on CPB (**Figure 1**).

Study setting. Tertiary/quaternary paediatric cardiac surgical services in Australia and New Zealand, including Cardiac and Paediatric Intensive Care Services of Royal Children's Hospital, Melbourne; Starship Children's Hospital Auckland NZ; The Children's Hospital at Westmead, Sydney; Princess Margaret Hospital for Children, Perth; and Queensland Children's Hospital, Brisbane. Addition of a further international study site is in progress.

Participants. Eligible children will be identified in the pre-operative clinics, in the general cardiac wards or in the neonatal or paediatric intensive care unit. ***Included*** will be infants and children < 2 years of age undergoing elective open heart surgery on CPB where consent of parents/guardian is obtained prior to surgery. The ***exclusion criteria*** relate to patient characteristics that will interfere with consent, with the intervention, or with measurement of the primary and secondary outcomes. Inclusion and exclusion criteria are shown in **Table 1**.

Enrolment of patients undergoing repeated surgery during the first two years of life: In order to assess the impact of the intervention on long-term outcomes, patients who were previously enrolled and randomised into the study who require a second or subsequent surgical procedure (such as a patient with single ventricle physiology requiring a staged palliation) will undergo the same treatment allocation for subsequent surgeries requiring CPB, unless parents opt out. Patients who were not recruited into the study during their first procedure, but are scheduled for a subsequent procedure requiring CPB prior to their second birthday, will be eligible for recruitment.

Randomisation and Blinding. Treatment assignment will be performed using a secure, centralised, web-based randomisation interface (REDCap [27], The University of Queensland). The allocation sequence will be generated by the study statistician using computer-generated random numbers using a variable block size stratified by age (< six weeks, six weeks to 24 months), univentricular versus biventricular lesions, and by site. Of the investigating team, only the study perfusionist will be aware of the randomisation and NO delivery. Cardiologists, cardiac surgeons, anesthesiologists, intensivists, PICU nurses, research assistants, data analysts, and parents and caregivers will be blinded for the intervention. *Rationale for stratification:* The age group under six weeks represents the cohort at highest surgical risk. In the pilot study the effect size of the NO delivery was greatest in these infants[26]. Cardiac physiology (univentricular versus biventricular) is a major determinant of surgical complexity, risk, and outcome[1, 5].

Blinding of the Intervention. Blinding arrangements in the operating theatre will be achieved by covering the NO delivery system with drapes. The dedicated study NO delivery system will be connected to the CPB oxygenator at all times, independent of randomisation. The family, surgeons, anaesthetists and PICU staff will be not aware of the treatment arm a patient is allocated to. The perfusionists will be advised that all aspects of CPB except for provision of NO (or not) should be performed according to standard institutional practice for study participants.

Interventions. Infants will be randomly assigned to NO or standard care. Those allocated to the *NO arm* will receive NO during CPB blended into the fresh gas flow of the CPB oxygenator, which is kept at 3L/min. NO levels are maintained at 20 ppm using a NO delivery system (Ikaria INOmax DSIR, Ikaria, NJ, USA) or similar device. Continuous sampling of NO and NO_2 concentration will be undertaken from an access port before the oxygenator. NO will be started immediately when the patient is placed on CPB and ceased once weaned off CPB.

Patients allocated to the *standard study arm* will receive the standard gase oxygen-air mix into the CPB oxygenator at a flow rate of 3L/min. If patients require several CPB runs during the same procedure, the study treatment will be provided for each CPB run using the same treatment allocation for every CPB run.

Relevant concomitant perioperative care. Arterial partial pressures of CO_2 will be maintained constantly in both study arms as per institutional practice (following each centre's protocols on alpha/pH stat and temperatures). The FiO2 of the fresh gas flow will be set between 21% and 100%, according to centre specific CPB protocols. Techniques of anaesthesia and surgery will not be specified to allow site specific individual practice. The decision whether a patient requires treatment with inhaled NO (iNO) into the ventilator circuit prior to, during, or after CPB remains at the discretion of the treating physicians (anaesthetists, cardiac surgeons, or intensivists) independent of treatment allocation. The postoperative care and decisions on inotropes and other vasoactive drug delivery, fluid management, renal replacement therapy, iNO therapy or indication for ECLS will be performed as per site specific standard protocols of care.

Study outcomes. The **Primary outcome** is ventilator free days (VFD) for the first 28 days post randomisation (**Table 2**). The primary outcome will be measured using duration of invasive respiratory support for all episodes with an endotracheal tube in situ for the first 28 days post randomisation. A systematic zero value will be assigned for patients who die to weigh death as the most pejorative outcome. Treatment with non-invasive ventilation and high-flow nasal cannulae will not be considered as ventilator days. **Secondary outcomes** are defined as the composite outcome compromising LCOS, need for postoperative ECLS, or 28-day mortality; ICU and hospital length of stay; and health care costs. In addition, the long-term outcome of patients will be followed up at twelve months post procedure.

LCOS[10] is defined as a blood lactate level greater than 4 mmol/l with an oxygen extraction of greater than 35% (SaO_2-$ScvO_2$ gradient >35%) within the first 48 hours postoperatively, or a high inotrope requirement defined as Vasoactive-Inotrope Score ≥15 (VIS)[28, 29].

Rationale for primary and secondary outcomes: Ventilator free days represents one of the strongest predictors of short- and long-term outcomes[10], including length of intensive care unit (ICU) stay, morbidity (impaired neurodevelopment, hospital-acquired infections) and mortality. VFD directly reflect intensive care resource use and health care costs[30]. VFD fulfills SMART criteria (Specific, Measurable, Achievable, Realistic and Timely). LCOS is directly related to VFD, as infants usually do not tolerate weaning or fail extubation if LCOS and organ dysfunction have not resolved. The composite of LCOS, ECLS use, and mortality as a secondary end point, is a strong patient-centred outcome and directly relates to the intervention in terms of biological plausibility.

Adverse Events: It is recognised that the postoperative paediatric cardiac surgical patient population will experience a number of common aberrations in laboratory values, signs and symptoms due to the severity of the underlying disease and the impact of standard therapies. Intensive care patients will frequently develop life-threatening organ failure(s) unrelated to study interventions and despite optimal management. Therefore, consistent with established practice in academic ICU trials[31], events that are part of the natural history of the primary disease process or expected complications of critical illness will not be reported as serious adverse events in this study. All adverse events which are considered to be potentially causally related to the study intervention or are otherwise of concern in the investigator's judgement will be reported unless they are pre-specified study outcomes. Specific adverse events related to NO delivery during CBP include air embolism, severe hypotension during bypass and increased MetHb (MetHb >3%). Of note, in previous studies, methaemoglobin values using

NO at 20 ppm were similar in both the control and intervention groups (1.4%)[25, 26]. Events that are collected as study outcomes will not be reported as adverse events.

Safety Data Monitoring. The Data Safety Monitoring Board (DSMB) consists of a general and a cardiac paediatric intensivist, a cardiac surgeon, and a statistician. None of the DSMB members will be involved in recruitment of study patients at their site. DSMB members will not be supervised by any study investigator or participate as investigators in any study currently under review by this DSMB. The primary objective of the DSMB is to monitor the safety of the intervention and the validity and integrity of the data from the NITRIC study. Additionally, the DSMB will evaluate the pace of recruitment and will make recommendations to the NITRIC Chief investigator(s) and Steering Board regarding the continuation, modification, or termination of the study. The DSMB will evaluate on an ongoing basis, the accumulating safety assessments to ensure the ongoing safety of study subjects. The DSMB will meet via teleconference call after recruitment of 660, and of 1000 children, respectively, and upon trial completion.

The DSMB can request unblinding in the event of a serious adverse event defined as a cardiac arrest, need for ECLS, or other incident leading to permanent harm considered to be likely related to the study intervention.

Sample size. A pilot study of 134 patients aged < 2 years showed a 2.74 days (66 hours) increase in ventilator-free days (VFD) associated with the study intervention [26]. This includes patients who died, who were considered as zero VFDs. The VFD increase associated with the intervention represents an effect size of 0.33 standard deviations (SD) based on a SD of 8.1 days in the pilot study control group. Based on the primary outcome measure VFD, 1,320 patients (660 per group) will be required to demonstrate a significant increase in VFD assuming a minimally clinically significant effect size (0.2 SD; 1.66 days or 40 hours), 90% power, two-

sided alpha level of significance of 5%, 10% withdrawals, and 15% increase in sample size to account for a non-normal distribution of VFD. In Australia and New Zealand approximately 800 of children < 2 years of age undergo surgery for a congenital heart defect requiring CPB each year, including patients with multiple procedures. The consent rate of eligible patients was 78% in the pilot trial[26]. With an expected conservative estimate 60% enrolment rate of eligible patients, we expect a 3.5-year recruitment period for the study.

Data collection, management, and analysis

Data collection: Baseline variables (demographics, primary cardiac diagnosis, comorbidities including syndromes), pre-operative disease severity, surgical data (length of CPB and cross-clamp, type of surgery and complexity score, other CPB characteristics, blood product usage), primary end points, secondary end points, pre-determined physiological variables of interest, and process of care measures will be prospectively recorded into a study REDCap online database. Plausibility and range checks are implemented. Paired arterial and venous gases will be collected postoperatively at 0, 6, 12, 24, and 48 hrs (in PICU until discharge to the ward or removal of arterial and central venous lines, whichever occurs first) to assess for lactate and SaO_2-$ScvO_2$ gradient. Key physiological and blood parameters and Paediatric Logistic Organ Dysfunction-2 (PELOD-2) scores[32] will be measured at 0, 6, 12, 24, and 48 hours post admission or until PICU discharge whichever occurs earlier. Delayed chest closure, use of inhaled NO, and duration of circulatory, renal, and ventilatory support postoperatively will be recorded. Gross functional performance assessment will be recorded on admission to PICU and upon discharge from hospital[33]. Neurological and functional outcome (including phone interviews with parents/caregivers) will be assessed at 12 months postoperatively using Ages and Stages questionnaires (ASQ)[34, 35] and assessment of quality of life using paediatric

quality of life inventory (PedsQL)[36]. Details on the long-term follow-up will be published separately.

Biobanking: Blood markers for myocardial injury and inflammatory response will be collected on induction of anaesthesia (baseline – pre-bypass), at admission to PICU (0 hours – post bypass) and at 12, and 24 hours, in patients where parents consent to biobanking. Blood will be collected pre-surgery as preoperative baseline (before onset of cardiac surgery, done by anaesthetist during the induction of the patient once the arterial line has been inserted): 1-2ml of EDTA blood (for DNA), 2.5ml of PAXgene blood (for gene expression markers), 1-2ml of serum; and postoperatively at 0,12,24 hours (in PICU until discharge to the ward): 2.5ml of PAXgene blood (time point 0), and 1-2ml of serum. The samples will be processed, stored and shipped according to accepted international standards and batch analysed.

The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. The investigators will maintain adequate case histories of study participants, including accurate case report forms (CRFs), and source documentation. Data will be prospectively entered into a secure web-based database (REDCap https://redcap.health.uq.edu.au/), hosted by the University of Queensland. Printed paper CRFs will be available if required. All study information and documentation will be securely stored for a period of 15 years after the date of the child's eighteenth birthday.

Statistical Analysis Plan

Analysis plan. Analyses will apply the intention to treat principle. Descriptive statistics will be used to describe baseline characteristics of the study cohort and each subgroup by treatment group. The primary outcome measure will be analysed using a Mann-Whitney U test as ventilator-free days is non-normally distributed variable. Analysis of secondary outcomes

includes both comparisons of measurements and proportions, using confidence intervals of differences as the major method of presentation where possible, otherwise standard techniques such as Mann-Whitney U tests, t-tests and chi-squared tests will be utilised. Survival outcomes will be compared between treatment groups using Kaplan-Meier product limit method and log-rank test. Statistical significance will be set at the 0.05 level for the primary outcome.

A safety and efficacy interim analysis after 660 (the half-way point), and after 1000 enrolled patients will be performed by an independent statistician to evaluate for safety endpoints, to assess the predictive probability of reaching the study goals, and compare VFD between treatment groups. Consideration to stopping the trial early by an independent Data and Safety Monitoring Board (DSMB) will be based on safety concerns, futility, or strong evidence of a difference between groups for VFD (based on a Haybittle–Peto boundary P=0.001). A detailed analysis plan specifying statistical analyses including health economic analyses will be placed in the public domain prior to recruitment of the last participant[37].

Biomarker measurements. Nested sub-studies will be performed in selected samples at sites performing biobanking (i) to test the impact of the intervention on markers of systemic and myocardial inflammation; (ii) to compare treatment response between patients depending on pre-intervention severity assessed by markers of inflammation and organ failure; and (iii) to biochemically define responders to the intervention (to identify patient subgroups pre-randomisation that are more likely to respond to a specific treatment). The use of samples/data will be governed by the study steering board and resulting publications must appropriately acknowledge the study. Combining a large RCT with a nested biobank is recommended to maximize scientific knowledge[38].

Health Economic Evaluation. A within trial economic evaluation will be used to determine if providing NO is cost-saving compared to usual care from the health system perspective. A comprehensive cost-effectiveness analysis will be undertaken to determine the level of cost savings (if any). Length of stay (in PICU and in non-intensive stay) will be the main outcome variable. Resources used before first discharge will be compared between treatments. Resources will subsequently be costed, based on hospital cost centre or standard national sources (e.g. Independent Hospital Pricing Authority).

Monitoring. The study leadership team is responsible for 100% monitoring of investigator and study nurse credentials, training records, and delegation of responsibility logs, and will review 100% of Consent Forms. CRFs will be compared to source documentation to ensure data are accurate and complete. 100% of source data verification of eligibility criteria and the primary outcome and the composite secondary outcome of LCOS, ECLS or death will be performed. An independent monitoring per each site will monitor the data fields required for eligibility, primary, and secondary study endpoints, and SAEs using primary source verification. In addition, site visits and regular monitoring of the blood sample storage will be performed.

Patient and Public Involvement. Consumers and the public were consulted to design a video informing parents about the study. Patients and the public had no other involvement in study design.

Ethics. This protocol and the informed consent document and any subsequent modifications have been reviewed and approved by the human research ethics committee (Children`s Health Queensland HREC/17/QRCH/43). This study will be conducted in compliance with the current version of the protocol. Any change to the protocol document or Informed Consent Form that affects the scientific intent, study design, patient safety, or may affect a participants'

willingness to continue in the study is considered an amendment, and therefore will be written and filed as an amendment to this protocol and/or informed consent form. All protocol deviations must be recorded in the patient record (source document) and on the CRF and must be reported to the PI. Protocol deviations will be assessed for significance by the Principal Investigator.

Consent will be sought from the parents of every child <2 years of age undergoing CPB for elective cardiac surgery over the study period. When the family is seen by the surgeons in pre-assessment clinic (usually days prior to surgery), the study will be mentioned to them by the surgeon. In addition, the study team will provide study information prior to hospitalisation to families, including printed study flyers, and links to online study documentation (media release). Participant confidentiality is strictly held in trust by the participating investigators, research staff, and the sponsoring institution and their agents. The study protocol, documentation, data and all other information generated will be held in strict confidence.

Significance: Postoperative paediatric cardiac surgical patients have a high consumption of intensive care resources and remain at very high risk of major complications, including cardiac arrest, death, and long-term neurological impairment. Approximately 10% of children with CHD survive with major neurological sequelae postoperatively, resulting in a massive lifelong burden for patients, families, healthcare systems, and the society[13]. An attempt to reduce LCOS and hence perioperative morbidity has the potential to translate not only to a reduction in intensive care resource utilisation, but also to impact positively on long-term outcomes. Side-effects from heart surgery for CHD translate into long-term morbidity, persisting into adult life with a major impact on other family members and society. This study will deliver the high-level randomised evidence with the potential to show a reduction in postoperative morbidity and mortality in children with CHD.

Acknowledgements: The authors would like to thank the parents and children participating in this trial and the the medical and nursing and research teams in the participating sites for their help in study setup, recruitment, data collection, and monitoring of study data.

NITRIC Study Group:

Warwick Butt, Steve Horton, Johnny Millar, Carmel Delzoppo, Yves D`Udekem: Cardiac and Paediatric Intensive Care Services of Royal Children's Hospital, Melbourne, Australia

John Beca, David Buckley, Taryn Evans, Claire Sherring, John Artrip: Starship Children's Hospital Auckland, New Zealand

Marino Festa, Killian O`Shaughnessy, David Winlaw, Rebecca Fletcher, Simon Byrne, The Children's Hospital at Westmead, Sydney, Australia

Simon Erickson, Sam Barr, Rae Kelly, David Andrews: Perth Children`s Hospital, Perth, Australia

Luregn J Schlapbach, Andreas Schibler, Deborah Long, Kerry Johnston, Carla Zuzak, Nelson Alphonso, Benjamin Anderson: Queensland Children's Hospital, Brisbane, Australia

Antje Blumenthal: The University of Queensland Diamantina Institute, Translational Research Institute, The University of Queensland, Brisbane, Australia

Jonas Fooken, Brenda Gannon: Centre for the Business and Economics of Health, The University of Queensland, Brisbane, Australia

Paul Young: Medical Research Institute of New Zealand, Wellington, New Zealand

Mark Jones: School of Public Health, The University of Queensland, Brisbane, Australia

Kim van Loon, Nicole JCW van Belle-van Haaren, Bram van Wijk, Erik Koomen, Wilhelmina Children's Hospital Utrecht, Utrecht, The Netherlands

Roles and Responsibilities:

The study protocol first draft was designed by LJS and AS based on the previous pilot study design established by SH, and WB. The present study protocol has been revised with input from SH, DL, KJ, CJ, CD, WB, SE, MF, BG, JF, PY, AB, MJ and JB.

The study steering group consists of LJS, SH, DL, WB and AS.

LJS, AS, DL, KJ, NA, SE, WB, SH, CdZ, KL, YdU, MF, JB are responsible for local study setup conduct, and recruitment.

AB and LJS are responsible for host inflammation analyses.

MJ is responsible for statistical analyses.

JF and BG are responsible for health economic analyses.

REFERENCES

1. Triedman JK, Newburger JW: **Trends in Congenital Heart Disease: The Next Decade**. *Circulation* 2016, **133**(25):2716-2733.
2. Erikssen G, Liestol K, Seem E, Birkeland S, Saatvedt KJ, Hoel TN, Dohlen G, Skulstad H, Svennevig JL, Thaulow E *et al*: **Achievements in congenital heart defect surgery: a prospective, 40-year study of 7038 patients**. *Circulation* 2015, **131**(4):337-346; discussion 346.
3. Marelli AJ, Ionescu-Ittu R, Mackie AS, Guo L, Dendukuri N, Kaouache M: **Lifetime prevalence of congenital heart disease in the general population from 2000 to 2010**. *Circulation* 2014, **130**(9):749-756.

4. Marelli AJ, Mackie AS, Ionescu-Ittu R, Rahme E, Pilote L: **Congenital heart disease in the general population: changing prevalence and age distribution**. *Circulation* 2007, **115**(2):163-172.

5. Kaltman JR, Andropoulos DB, Checchia PA, Gaynor JW, Hoffman TM, Laussen PC, Ohye RG, Pearson GD, Pigula F, Tweddell J *et al*: **Report of the pediatric heart network and national heart, lung, and blood institute working group on the perioperative management of congenital heart disease**. *Circulation* 2010, **121**(25):2766-2772.

6. Zahler S, Massoudy P, Hartl H, Hahnel C, Meisner H, Becker BF: **Acute cardiac inflammatory responses to postischemic reperfusion during cardiopulmonary bypass**. *Cardiovasc Res* 1999, **41**(3):722-730.

7. Levy JH, Tanaka KA: **Inflammatory response to cardiopulmonary bypass**. *Ann Thorac Surg* 2003, **75**(2):S715-720.

8. Duval EL, Kavelaars A, Veenhuizen L, van Vught AJ, van de Wal HJ, Heijnen CJ: **Pro- and anti-inflammatory cytokine patterns during and after cardiac surgery in young children**. *Eur J Pediatr* 1999, **158**(5):387-393.

9. Domanski MJ, Mahaffey K, Hasselblad V, Brener SJ, Smith PK, Hillis G, Engoren M, Alexander JH, Levy JH, Chaitman BR *et al*: **Association of myocardial enzyme elevation and survival following coronary artery bypass graft surgery**. *JAMA* 2011, **305**(6):585-591.

10. Hoffman TM, Wernovsky G, Atz AM, Kulik TJ, Nelson DP, Chang AC, Bailey JM, Akbary A, Kocsis JF, Kaczmarek R *et al*: **Efficacy and safety of milrinone in preventing low cardiac output syndrome in infants and children after corrective surgery for congenital heart disease**. *Circulation* 2003, **107**(7):996-1002.

11. Duke T, Stocker C, Butt W: **Monitoring children after cardiac surgery: a minimalist approach might be maximally effective**. *Crit Care Resusc* 2004, **6**(4):306-310.

12. McElhinney DB, Wernovsky G: **Outcomes of neonates with congenital heart disease**. *Curr Opin Pediatr* 2001, **13**(2):104-110.

13. Ballweg JA, Wernovsky G, Gaynor JW: **Neurodevelopmental outcomes following congenital heart surgery**. *Pediatr Cardiol* 2007, **28**(2):126-133.

14. Marino BS: **New concepts in predicting, evaluating, and managing neurodevelopmental outcomes in children with congenital heart disease**. *Curr Opin Pediatr* 2013, **25**(5):574-584.

15. Marino BS, Lipkin PH, Newburger JW, Peacock G, Gerdes M, Gaynor JW, Mussatto KA, Uzark K, Goldberg CS, Johnson WH, Jr. *et al*: **Neurodevelopmental outcomes in children with congenital heart disease: evaluation and management: a scientific statement from the American Heart Association**. *Circulation* 2012, **126**(9):1143-1172.

16. Brown MD, Wernovsky G, Mussatto KA, Berger S: **Long-term and developmental outcomes of children with complex congenital heart disease**. *Clin Perinatol* 2005, **32**(4):1043-1057, xi.

17. Bronicki RA, Backer CL, Baden HP, Mavroudis C, Crawford SE, Green TP: **Dexamethasone reduces the inflammatory response to cardiopulmonary bypass in children**. *Ann Thorac Surg* 2000, **69**(5):1490-1495.

18. Elhoff JJ, Chowdhury SM, Zyblewski SC, Atz AM, Bradley SM, Graham EM: **Intraoperative Steroid Use and Outcomes Following the Norwood Procedure: An Analysis of the Pediatric Heart Network's Public Database**. *Pediatric critical care medicine : a journal of the Society of Critical Care Medicine and the World Federation of Pediatric Intensive and Critical Care Societies* 2016, **17**(1):30-35.

19. Hataishi R, Rodrigues AC, Neilan TG, Morgan JG, Buys E, Shiva S, Tambouret R, Jassal DS, Raher MJ, Furutani E *et al*: **Inhaled nitric oxide decreases infarction size and improves left ventricular function in a murine model of myocardial ischemia-reperfusion injury**. *American journal of physiology Heart and circulatory physiology* 2006, **291**(1):H379-384.

20. Chello M, Mastroroberto P, Perticone F, Celi V, Colonna A: **Nitric oxide modulation of neutrophil-endothelium interaction: difference between arterial and venous coronary bypass grafts**. *J Am Coll Cardiol* 1998, **31**(4):823-826.

21. Jones SP, Bolli R: **The ubiquitous role of nitric oxide in cardioprotection**. *J Mol Cell Cardiol* 2006, **40**(1):16-23.

22. Jones SP, Girod WG, Palazzo AJ, Granger DN, Grisham MB, Jourd'Heuil D, Huang PL, Lefer DJ: **Myocardial ischemia-reperfusion injury is exacerbated in absence of endothelial cell nitric oxide synthase**. *Am J Physiol* 1999, **276**(5 Pt 2):H1567-1573.

23. Minamishima S, Kida K, Tokuda K, Wang H, Sips PY, Kosugi S, Mandeville JB, Buys ES, Brouckaert P, Liu PK *et al*: **Inhaled nitric oxide improves outcomes after successful cardiopulmonary resuscitation in mice**. *Circulation* 2011, **124**(15):1645-1653.

24. Schulz R, Kelm M, Heusch G: **Nitric oxide in myocardial ischemia/reperfusion injury**. *Cardiovasc Res* 2004, **61**(3):402-413.

25. Checchia PA, Bronicki RA, Muenzer JT, Dixon D, Raithel S, Gandhi SK, Huddleston CB: **Nitric oxide delivery during cardiopulmonary bypass reduces postoperative morbidity in children--a randomized trial**. *J Thorac Cardiovasc Surg* 2013, **146**(3):530-536.

26. James C, Millar J, Horton S, Brizard C, Molesworth C, Butt W: **Nitric oxide administration during paediatric cardiopulmonary bypass: a randomised controlled trial**. *Intensive Care Med* 2016, **42**(11):1744-1752.

27. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG: **Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support**. *Journal of biomedical informatics* 2009, **42**(2):377-381.

28. Gaies MG, Jeffries HE, Niebler RA, Pasquali SK, Donohue JE, Yu S, Gall C, Rice TB, Thiagarajan RR: **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):529-537.

29. Gaies MG, Gurney JG, Yen AH, Napoli ML, Gajarski RJ, Ohye RG, Charpie JR, Hirsch JC: **Vasoactive-inotropic score as a predictor of morbidity and mortality in infants after cardiopulmonary bypass**. *Pediatr Crit Care Med* 2010, **11**(2):234-238.

30. Contentin L, Ehrmann S, Giraudeau B: **Heterogeneity in the definition of mechanical ventilation duration and ventilator-free days**. *Am J Respir Crit Care Med* 2014, **189**(8):998-1002.

31. Cook D, Lauzier F, Rocha MG, Sayles MJ, Finfer S: **Serious adverse events in academic critical care research**. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* 2008, **178**(9):1181-1184.

32. Leteurtre S, Duhamel A, Salleron J, Grandbastien B, Lacroix J, Leclerc F, Groupe Francophone de Reanimation et d'Urgences P: **PELOD-2: an update of the PEdiatric logistic organ dysfunction score**. *Crit Care Med* 2013, **41**(7):1761-1773.

33. Pollack MM, Holubkov R, Funai T, Clark A, Moler F, Shanley T, Meert K, Newth CJ, Carcillo J, Berger JT *et al*: **Relationship between the functional status scale and the**

pediatric overall performance category and pediatric cerebral performance category scales. *JAMA Pediatr* 2014, **168**(7):671-676.

34. Pierrat V, Marchand-Martin L, Arnaud C, Kaminski M, Resche-Rigon M, Lebeaux C, Bodeau-Livinec F, Morgan AS, Goffinet F, Marret S *et al*: **Neurodevelopmental outcome at 2 years for preterm children born at 22 to 34 weeks' gestation in France in 2011: EPIPAGE-2 cohort study**. *Bmj* 2017, **358**:j3448.

35. Noeder MM, Logan BA, Struemph KL, Condon N, Mueller I, Sands B, Davies RR, Sood E: **Developmental screening in children with CHD: Ages and Stages Questionnaires**. *Cardiology in the young* 2017, **27**(8):1447-1454.

36. Desai AD, Zhou C, Stanford S, Haaland W, Varni JW, Mangione-Smith RM: **Validity and responsiveness of the pediatric quality of life inventory (PedsQL) 4.0 generic core scales in the pediatric inpatient setting**. *JAMA Pediatr* 2014, **168**(12):1114-1121.

37. Billot L, Venkatesh B, Myburgh J, Finfer S, Cohen J, Webb S, McArthur C, Joyce C, Bellomo R, Rhodes A *et al*: **Statistical analysis plan for the Adjunctive Corticosteroid Treatment in Critically Ill Patients with Septic Shock (ADRENAL) trial**. *Critical care and resuscitation : journal of the Australasian Academy of Critical Care Medicine* 2017, **19**(2):183-191.

38. Gustafsson F, Atar D, Pitt B, Zannad F, Pfeffer MA, participants in 10th Cardiovascular Clinical Trialists W: **Maximizing scientific knowledge from randomized clinical trials**. *Am Heart J* 2010, **159**(6):937-943.

Figures

Figure 1. Study Flow Diagram.

Table 1: Inclusion and Exclusion criteria

Patient group	Criterium	Definition
Inclusion	*Age*	• Postnatal age below 2 years
	Procedure	• Open elective Heart surgery
		• Cardiopulmonary bypass used during surgery
	Consent	• Parental/caregiver consent available prior to surgery
Exclusion	*Age*	• Age ≥2 years
	Procedure	• Emergency cardiac surgery which may preclude obtaining informed consent (acutely required life-saving procedure in a patient unlikely to survive the next hours without the surgery)
		• Heart surgery not requiring cardiopulmonary bypass
	Consent	• Lack of parental/caregiver consent
	Pulmonary hypertension	• Persistently elevated pulmonary vascular resistance preoperatively receiving inhaled NO or preoperative intravenous use of drugs involved in the NO pathway such as glyceryl trinitrate within 48 hours prior to CPB (oral sildenafil treatment alone is not an exclusion)
	Pre-operative disease	• ECLS immediately prior to surgery
		• Receiving ongoing treatment with antimicrobials for confirmed or suspected sepsis or septic shock diagnosed within 48hours prior to the time of surgery
		• Treated with high doses of vasoactive drugs defined as a Vasoactive-Inotrope Score (VIS) ≥15 within 24 hours prior to surgery[a]
		• Cardiac arrest within one week (7d) prior to surgery
		• Acute respiratory distress syndrome requiring high frequency oscillatory ventilation within 48 hours prior to surgery
		• Chronic ventilator dependency (patients treated with non-invasive or invasive ventilation continuously for >28 days prior to cardiopulmonary bypass)
		• Pre-existing methaemoglobinemia (MetHb>3%)

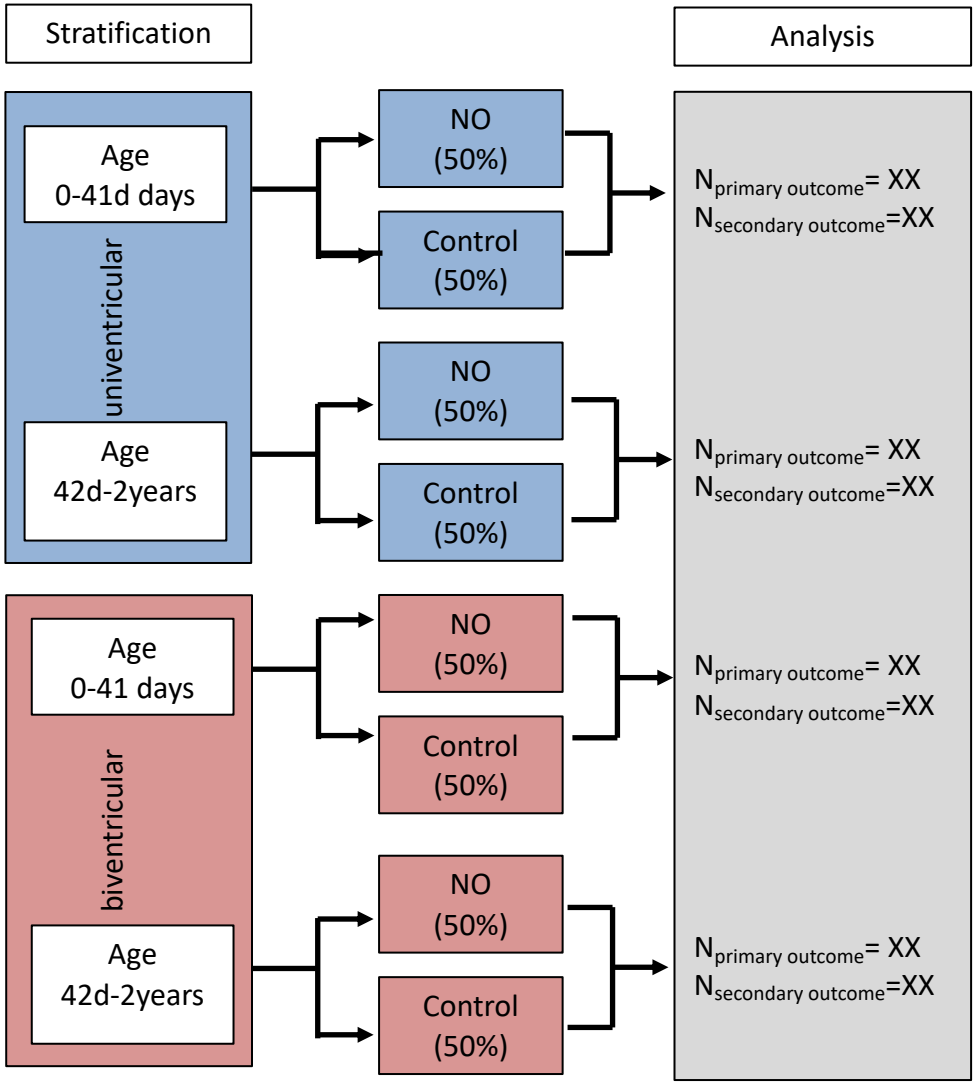
a) Gaies MG, Gurney JG, Yen AH, *et a*l: Vasoactive-inotropic score as a predictor of morbidity and mortality in infants after cardiopulmonary bypass. *Pediatr Crit Care Med* 2010, **11**(2):234-238.

Table 2: Study outcomess

Outcome	Criterium	Definition
Primary	*Ventilator-free days (VFD)*	• duration of respiratory support for all episodes for the first 28 days post randomisation • zero value for patients dying within 28 days post randomisation • refers to invasive respiratory support with an endotracheal tube in situ • treatment with non-invasive ventilation and high-flow nasal cannulae will not be considered as ventilator days
Secondary	*Composite outcome of low cardiac output syndrome (LCOS), Extracorporeal life support (ECLS), or death*	• ***LCOS*** is defined as one or more of the following[a]: - Blood lactate level greater than 4 mmol/l with an oxygen extraction of greater than 35% (SaO_2-$ScvO_2$ gradient >35%) within the first 48 hours postoperatively - a high inotrope requirement defined as Vasoactive-Inotrope Score ≥15 (VIS)[b] where VIS = dopamine dose (mcg/kg/min) + dobutamine dose (mcg/kg/min) + 100 x adrenaline dose (mcg/kg/min) + 100 x noradrenaline dose (mcg/kg/min) + 10 x milrinone dose (mcg/kg/min) + 10,000 x vasopressin dose (U/kg/min). • ECLS is defined as treatment with ECLS during the first 48 hours post randomisation • Death is defined as death occurring within the first 28 days post randomisation
	Length of stay	• Length of stay in paediatric intensive care unit (PICU) • Length of stay in hospital
	Costs	• Health-care related costs (starting at time of admission to PICU postoperatively)
	Neurodevelopmental and functional outcome at 12 months	• Ages and Stages Questionnaire (ASQ) scores below threshold for at least one of the five domains measured 12 months post randomisation and Quality of Life
Process of care measures	*Severity indicators*	• Treatment with ECLS postoperatively • Duration of postoperative time spent with open chest including unplanned chest reopening • Treatment and duration of treatment using inhalational nitric oxide postoperatively • Treatment and duration of treatment of postoperative renal replacement therapy

Physiological descriptors	*Host inflammation*	• Serum cytokine levels measured during the first 24 hours • Inflammation markers measured during the first 24 hours
	Myocardial injury	• Levels of postoperative serum troponin levels measured during the first 24 hours
	Organ dysfunction	• Severity and duration of postoperative organ dysfunction measured by PELOD-2 • Postoperative Acute Kidney Injury and serum creatinine levels measured during the first 24 hours • Severity and duration of postoperative delirium

a) Hoffman TM, Wernovsky G, Atz AM, *et al*: Efficacy and safety of milrinone in preventing low cardiac output syndrome in infants and children after corrective surgery for congenital heart disease. *Circulation* 2003, 107(7):996-1002.
b) Gaies MG, Gurney JG, Yen AH, *et al*: Vasoactive-inotropic score as a predictor of morbidity and mortality in infants after cardiopulmonary bypass. *Pediatr Crit Care Med* 2010, 11(2):234-238.


Stratification
Analysis
Age
0-41d days
univentricular
Age
42d-2years
NO
(50%)
Control
(50%)
NO
(50%)
Control
(50%)
Age
0-41 days
biventricular
Age
42d-2years
NO
(50%)
Control
(50%)
NO
(50%)
Control
(50%)
$N_{primary\ outcome}$= XX
$N_{secondary\ outcome}$=XX
$N_{primary\ outcome}$= XX
$N_{secondary\ outcome}$=XX
$N_{primary\ outcome}$= XX
$N_{secondary\ outcome}$=XX
$N_{primary\ outcome}$= XX
$N_{secondary\ outcome}$=XX

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	7
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	9,10
	2b	Specific objectives or hypotheses	10
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	11
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	11, Table 1
	4b	Settings and locations where the data were collected	11
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	12,13
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	13, 14, Table 2
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	15
	7b	When applicable, explanation of any interim analyses and stopping guidelines	17,18
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	12
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	12
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	12
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	12
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	12

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	12,13
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	17,18
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	17,18, Table 2
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1, page 16
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	7
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	NA
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	NA
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	NA
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	14,15
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	8, 20
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	NA
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	NA
Other information			
Registration	23	Registration number and name of trial registry	7
Protocol	24	Where the full trial protocol can be accessed, if available	This submission
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.