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

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3 **Title Page:**
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7 **Infants with Congenital heart defects (NITRIC trial): A Randomised Controlled Trial.**
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**On behalf of the NITRIC Study Group, the Australian and New Zealand Intensive Care
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40 **Conflict of Interest Statement:**
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42 Yves d'Udekem is a consultant for MSD and Actelion.
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58 ventilation; nitric oxide; mortality
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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

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Trial registration: ACTRN12617000821392

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10 The funding sources had no involvement in study design, analyses, nor interpretation of the
11
12 results.
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14 Mallinckrodt Pharmaceuticals will provide nitric oxide delivery devices to study centres but
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16 has no involvement in study design, conduct, nor analyses.
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33 design established by SH, and WB. MJ wrote the section on statistical analyses. JF and BG
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35 wrote the section on health economic analyses The present study protocol has been revised
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37 with input from SH, DL, JB, SE, MF, YdU, NA, DW, KJ, CD, KvL, BG, JF, PY, AB, MJ and
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For peer review only

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 \geq 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

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3 interventions such as steroids[17] have not demonstrated consistent benefit[18]. Given the
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5 adverse effects of cardiopulmonary bypass on early recovery and long-term neurodevelopment
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7 there remains an urgent need for clinical trials evaluating novel therapies to address these
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9 problems[5].
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12 Nitric oxide (NO) is an endogenous anti-inflammatory mediator[19] and is essential to regulate
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14 endothelial function and microvascular inflammation[20]. Several studies have demonstrated
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16 that exogenous NO can reduce myocardial damage in clinical and experimental settings of
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18 ischaemia and reperfusion[21-24]. A previous small single centre study in 16 children reported
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20 a reduction in bypass-induced inflammation using gaseous NO delivered at 20ppm to CPB
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22 circuits[25]. The duration of mechanical ventilation was significantly shorter (8.4 versus 16.3
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24 hours; $P < .05$) and so was ICU length of stay (53.8 versus 79.4 hours; $P < .05$) in children
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26 receiving NO compared to the placebo group. We have previously reported the feasibility and
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28 safety of NO delivery to CPB in a single centre randomized controlled pilot study in 198 infants
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30 and children (0-16 years) undergoing cardiac surgery[26]. This pilot study demonstrated a
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32 statistically significantly lower proportion of children with a LCOS in the intervention arm, a
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34 reduced requirement for ECLS, and a trend to reduced length of stay, and shorter duration of
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36 mechanical ventilation. The effect was greatest in children under two years of age, with the
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38 greatest treatment benefit observed in children under six weeks.
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44 Accordingly, we designed the NITRIC trial to test the primary hypothesis that, in infants under
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46 two years having cardiac surgery, using NO during CPB increases ventilator-free days (the
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48 number of days patients spend alive and free from invasive mechanical ventilation up until
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50 post-operative day 28) compared with standard care. Here we describe the NITRIC trial
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52 protocol.
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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.

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

14
15 **Blinding of the Intervention.** Blinding arrangements in the operating theatre will be achieved
16 by covering the NO delivery system with drapes. The dedicated study NO delivery system will
17 be connected to the CPB oxygenator at all times, independent of randomisation. The family,
18 surgeons, anaesthetists and PICU staff will be not aware of the treatment arm a patient is
19 allocated to. The perfusionists will be advised that all aspects of CPB except for provision of
20 NO (or not) should be performed according to standard institutional practice for study
21 participants.

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23 **Interventions.** Infants will be randomly assigned to NO or standard care. Those allocated to
24 the *NO arm* will receive NO during CPB blended into the fresh gas flow of the CPB
25 oxygenator, which is kept at 3L/min. NO levels are maintained at 20 ppm using a NO delivery
26 system (Ikaria INOmax DSIR, Ikaria, NJ, USA) or similar device. Continuous sampling of NO
27 and NO₂ concentration will be undertaken from an access port before the oxygenator. NO will
28 be started immediately when the patient is placed on CPB and ceased once weaned off CPB.
29 Patients allocated to the *standard study arm* will receive the standard gase oxygen-air mix into
30 the CPB oxygenator at a flow rate of 3L/min. If patients require several CPB runs during the
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3 same procedure, the study treatment will be provided for each CPB run using the same
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5 treatment allocation for every CPB run.
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9 **Relevant concomitant perioperative care.** Arterial partial pressures of CO₂ will be
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11 maintained constantly in both study arms as per institutional practice (following each centre's
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13 protocols on alpha/pH stat and temperatures). The FiO₂ of the fresh gas flow will be set
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15 between 21% and 100%, according to centre specific CPB protocols. Techniques of anaesthesia
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17 and surgery will not be specified to allow site specific individual practice. The decision whether
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19 a patient requires treatment with inhaled NO (iNO) into the ventilator circuit prior to, during,
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21 or after CPB remains at the discretion of the treating physicians (anaesthetists, cardiac
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23 surgeons, or intensivists) independent of treatment allocation. The postoperative care and
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25 decisions on inotropes and other vasoactive drug delivery, fluid management, renal
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27 replacement therapy, iNO therapy or indication for ECLS will be performed as per site specific
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29 standard protocols of care.
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37 **Study outcomes (Table 2).** The **Primary outcome** is ventilator free days (VFD) for the first
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39 28 days post randomisation. The primary outcome will be measured using duration of invasive
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41 respiratory support for all episodes with an endotracheal tube in situ for the first 28 days post
42
43 randomisation. A systematic zero value will be assigned for patients who die to weigh death as
44
45 the most pejorative outcome. **Secondary outcomes** are defined as the composite outcome
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47 compromising LCOS, need for postoperative ECLS, or 28-day mortality; ICU and hospital
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49 length of stay; and health care costs. In addition, the long-term outcome of patients will be
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51 followed up at twelve months post procedure.
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55 **LCOS**[10] is defined as a blood lactate level greater than 4 mmol/l with an oxygen extraction
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57 of greater than 35% (SaO₂-ScvO₂ gradient >35%) within the first 48 hours postoperatively, or
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59 a high inotrope requirement defined as Vasoactive-Inotrope Score ≥ 15 (VIS)[28, 29].
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3 **Rationale for primary and secondary outcomes:** Ventilator free days represents one of the
4 strongest predictors of short- and long-term outcomes[10], including length of intensive care
5 unit (ICU) stay, morbidity (impaired neurodevelopment, hospital-acquired infections) and
6 mortality. VFD directly reflect intensive care resource use and health care costs[30]. The
7 composite of LCOS, ECLS use, and mortality as a secondary end point, is a strong patient-
8 centred outcome and directly relates to the intervention in terms of biological plausibility.
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19 **Adverse Events:** It is recognised that the postoperative paediatric cardiac surgical patient
20 population will experience a number of common aberrations in laboratory values, signs and
21 symptoms due to the severity of the underlying disease and the impact of standard therapies.
22 Intensive care patients will frequently develop life-threatening organ failure(s) unrelated to
23 study interventions and despite optimal management. Therefore, consistent with established
24 practice in academic ICU trials[31], events that are part of the natural history of the primary
25 disease process or expected complications of critical illness will not be reported as serious
26 adverse events in this study. All adverse events which are considered to be potentially causally
27 related to the study intervention or are otherwise of concern in the investigator's judgement
28 will be reported unless they are pre-specified study outcomes. Specific adverse events related
29 to NO delivery during CBP include air embolism, severe hypotension during bypass and
30 increased MetHb (MetHb >3%). Of note, in previous studies, methaemoglobin values using
31 NO at 20 ppm were similar in both the control and intervention groups (1.4%)[25, 26]. Events
32 that are collected as study outcomes will not be reported as adverse events.
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51 **Safety Data Monitoring.** The Data Safety Monitoring Board (DSMB) consists of a general
52 and a cardiac paediatric intensivist, a cardiac surgeon, and a statistician. None of the DSMB
53 members will be involved in recruitment of study patients at their site. DSMB members will
54 not be supervised by any study investigator or participate as investigators in any study currently
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3 under review by this DSMB. The primary objective of the DSMB is to monitor the safety of
4 the intervention and the validity and integrity of the data from the NITRIC study. Additionally,
5 the DSMB will evaluate the pace of recruitment and will make recommendations to the
6 NITRIC Chief investigator(s) and Steering Board regarding the continuation, modification, or
7 termination of the study. The DSMB will evaluate on an ongoing basis, the accumulating safety
8 assessments to ensure the ongoing safety of study subjects. The DSMB will meet via
9 teleconference call after recruitment of 660, and of 1000 children, respectively, and upon trial
10 completion.

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

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

42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 **Data collection, management, and analysis** 58 59 60

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3 **Data collection:** Baseline variables (demographics, primary cardiac diagnosis, comorbidities
4 including syndromes), pre-operative disease severity, surgical data (length of CPB and cross-
5 clamp, type of surgery and complexity score, other CPB characteristics, blood product usage),
6 primary end points, secondary end points, pre-determined physiological variables of interest,
7 and process of care measures will be prospectively recorded into a study REDCap online
8 database. Plausibility and range checks are implemented. Paired arterial and venous gases will
9 be collected postoperatively at 0, 6, 12, 24, and 48 hrs (in PICU until discharge to the ward or
10 removal of arterial and central venous lines, whichever occurs first) to assess for lactate and
11 SaO₂-ScvO₂ gradient. Key physiological and blood parameters and Paediatric Logistic Organ
12 Dysfunction-2 (PELOD-2) scores[32] will be measured at 0, 6, 12, 24, and 48 hours post
13 admission or until PICU discharge whichever occurs earlier. Delayed chest closure, use of
14 inhaled NO, and duration of circulatory, renal, and ventilatory support postoperatively will be
15 recorded. Gross functional performance assessment will be recorded on admission to PICU
16 and upon discharge from hospital[33]. Neurological and functional outcome (including phone
17 interviews with parents/caregivers) will be assessed at 12 months postoperatively using Ages
18 and Stages questionnaires (ASQ)[34, 35] and assessment of quality of life using paediatric
19 quality of life inventory (PedsQL)[36]. Details on the long-term follow-up will be published
20 separately.

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47 **Biobanking:** Blood markers for myocardial injury and inflammatory response will be collected
48 on induction of anaesthesia (baseline – pre-bypass), at admission to PICU (0 hours – post
49 bypass) and at 12, and 24 hours. Blood will be collected pre-surgery as preoperative baseline
50 (before onset of cardiac surgery, done by anaesthetist during the induction of the patient once
51 the arterial line has been inserted): 1-2ml of EDTA blood (for DNA), 2.5ml of PAXgene blood
52 (for gene expression markers), 1-2ml of serum; and postoperatively at 0,12,24 hours (in PICU
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3 until discharge to the ward): 2.5ml of PAXgene blood (time point 0), and 1-2ml of serum. The
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5 samples will be processed, stored and shipped according to accepted international standards
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7 and batch analysed.
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11 The investigators are responsible for ensuring the accuracy, completeness, legibility, and
12
13 timeliness of the data reported. The investigators will maintain adequate case histories of study
14
15 participants, including accurate case report forms (CRFs), and source documentation. Data will
16
17 be prospectively entered into a secure web-based database (REDCap
18
19 <https://redcap.health.uq.edu.au/>), hosted by the University of Queensland. Printed paper CRFs
20
21 will be available if required. All study information and documentation will be securely stored
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23 for a period of 15 years after the date of the child's eighteenth birthday.
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30 **Statistical Analysis Plan**

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32 **Analysis plan.** Analyses will apply the intention to treat principle. Descriptive statistics will
33
34 be used to describe baseline characteristics of the study cohort and each subgroup by treatment
35
36 group. The primary outcome measure will be analysed using a Mann-Whitney test as
37
38 ventilator-free days is non-normally distributed variable. Analysis of secondary outcomes
39
40 includes both comparisons of measurements and proportions, using confidence intervals of
41
42 differences as the major method of presentation where possible, otherwise standard techniques
43
44 such as Mann-Whitney U tests, t-tests and chi-squared tests will be utilised. Survival outcomes
45
46 will be compared between treatment groups using Kaplan-Meier product limit method and log-
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48 rank test. Statistical significance will be set at the 0.05 level for the primary outcome.
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55 A safety and efficacy interim analysis after 660 (the half-way point), and after 1000 enrolled
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57 patients will be performed by an independent statistician to evaluate for safety endpoints, to
58
59 assess the predictive probability of reaching the study goals, and compare VFD between
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3 treatment groups. Consideration to stopping the trial early by an independent Data and Safety
4 Monitoring Board (DSMB) will be based on safety concerns, futility, or strong evidence of a
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6 difference between groups for VFD (based on a Haybittle–Peto boundary $P=0.001$). A detailed
7
8 analysis plan specifying statistical analyses including health economic analyses will be placed
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10 in the public domain prior to recruitment of the last participant[37].
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17 **Biomarker measurements.** Nested sub-studies will be performed in selected samples at sites
18 performing biobanking (i) to test the impact of the intervention on markers of systemic and
19 myocardial inflammation; (ii) to compare treatment response between patients depending on
20 pre-intervention severity assessed by markers of inflammation and organ failure; and (iii) to
21 biochemically define responders to the intervention (to identify patient subgroups pre-
22 randomisation that are more likely to respond to a specific treatment). The use of samples/data
23 will be governed by the study steering board and resulting publications must appropriately
24 acknowledge the study. Combining a large RCT with a nested biobank is recommended to
25 maximize scientific knowledge[38].
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38 **Health Economic Evaluation.** A within trial economic evaluation will be used to determine
39 if providing NO is cost-saving compared to usual care from the health system perspective. A
40 comprehensive cost-effectiveness analysis will be undertaken to determine the level of cost
41 savings (if any). Length of stay (in PICU and in non-intensive stay) will be the main outcome
42 variable. Resources used before first discharge will be compared between treatments.
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55 **Monitoring.** The study leadership team is responsible for 100% monitoring of investigator and
56 study nurse credentials, training records, and delegation of responsibility logs, and will review
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58
59
60 100% of Consent Forms. CRFs will be compared to source documentation to ensure data are

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3 accurate and complete. 100% of source data verification of eligibility criteria and the primary
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5 outcome and the composite secondary outcome of LCOS, ECLS or death will be performed.
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7 An independent monitoring per each site will monitor the data fields required for eligibility,
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9 primary, and secondary study endpoints, and SAEs using primary source verification. In
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11 addition, site visits and regular monitoring of the blood sample storage will be performed.
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16 **Patient and Public Involvement.** Consumers and the public were consulted to design a video
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18 informing parents about the study. Patients and the public had no other involvement in study
19
20 design.
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25 **Ethics.** This protocol and the informed consent document and any subsequent modifications
26
27 have been reviewed and approved by the human research ethics committee (Children`s Health
28
29 Queensland HREC/17/QRCH/43). This study will be conducted in compliance with the current
30
31 version of the protocol. Any change to the protocol document or Informed Consent Form that
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33 affects the scientific intent, study design, patient safety, or may affect a participants`
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35 willingness to continue in the study is considered an amendment, and therefore will be written
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37 and filed as an amendment to this protocol and/or informed consent form. All protocol
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39 deviations must be recorded in the patient record (source document) and on the CRF and must
40
41 be reported to the PI. Protocol deviations will be assessed for significance by the Principal
42
43 Investigator.
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47
48 Consent will be sought from the parents of every child <2 years of age undergoing CPB for
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50 elective cardiac surgery over the study period. When the family is seen by the surgeons in pre-
51
52 assessment clinic (usually days prior to surgery), the study will be mentioned to them by the
53
54 surgeon. In addition, the study team will provide study information prior to hospitalisation to
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56 families, including printed study flyers, and links to online study documentation (media
57
58 release). Participant confidentiality is strictly held in trust by the participating investigators,
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3 research staff, and the sponsoring institution and their agents. The study protocol,
4 documentation, data and all other information generated will be held in strict confidence.
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10 **Significance:** Postoperative paediatric cardiac surgical patients have a high consumption of
11 intensive care resources and remain at very high risk of major complications, including cardiac
12 arrest, death, and long-term neurological impairment. Approximately 10% of children with
13 CHD survive with major neurological sequelae postoperatively, resulting in a massive lifelong
14 burden for patients, families, healthcare systems, and the society[13]. An attempt to reduce
15 LCOS and hence perioperative morbidity has the potential to translate not only to a reduction
16 in intensive care resource utilisation, but also to impact positively on long-term outcomes.
17 Side-effects from heart surgery for CHD translate into long-term morbidity, persisting into
18 adult life with a major impact on other family members and society. This study will deliver the
19 high-level randomised evidence with the potential to show a reduction in postoperative
20 morbidity and mortality in children with CHD.
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4 this trial and the the medical and nursing and research teams in the participating sites for their
5 help in study setup, recruitment, data collection, and monitoring of study data.
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12 **NITRIC Study Group:**
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3 **Figures**
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8 **Figure 1.** Study Flow Diagram.
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For peer review only

Table 1: Inclusion and Exclusion criteria

Patient group	Criterion	Definition
Inclusion	<i>Age</i>	<ul style="list-style-type: none"> • Postnatal age below 2 years
	<i>Procedure</i>	<ul style="list-style-type: none"> • Elective Heart surgery • Cardiopulmonary bypass used during surgery
	<i>Consent</i>	<ul style="list-style-type: none"> • Parental/caregiver consent available prior to surgery
Exclusion	<i>Age</i>	<ul style="list-style-type: none"> • Age ≥ 2 years
	<i>Procedure</i>	<ul style="list-style-type: none"> • 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
	<i>Consent</i>	<ul style="list-style-type: none"> • Lack of parental/caregiver consent
	<i>Pulmonary hypertension</i>	<ul style="list-style-type: none"> • 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)
<i>Pre-operative disease</i>	<ul style="list-style-type: none"> • 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 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.

Table 2: Study outcomes

Outcome	Criterion	Definition
Primary	<i>Ventilator-free days (VFD)</i>	<ul style="list-style-type: none"> 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	<i>Composite outcome of low cardiac output syndrome (LCOS), Extracorporeal life support (ECLS), or death</i>	<ul style="list-style-type: none"> LCOS is defined as one or more of the following^a: <ul style="list-style-type: none"> Blood lactate level greater than 4 mmol/l with an oxygen extraction of greater than 35% (SaO₂-ScvO₂ 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
	<i>Length of stay</i>	<ul style="list-style-type: none"> Length of stay in paediatric intensive care unit (PICU) Length of stay in hospital
	<i>Costs</i>	<ul style="list-style-type: none"> Health-care related costs (starting at time of admission to PICU postoperatively)
	<i>Neurodevelopmental and functional outcome at 12 months</i>	<ul style="list-style-type: none"> 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	<i>Severity indicators</i>	<ul style="list-style-type: none"> 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	<i>Host inflammation</i>	<ul style="list-style-type: none"> Serum cytokine levels measured during the first 24 hours Inflammation markers measured during the first 24 hours
	<i>Myocardial injury</i>	<ul style="list-style-type: none"> Levels of postoperative serum troponin levels measured during the first 24 hours
	<i>Organ dysfunction</i>	<ul style="list-style-type: none"> 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.

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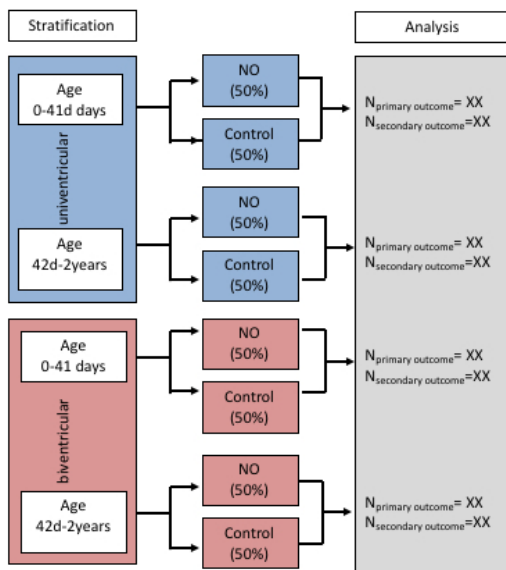


Figure 1

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:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-026664.R1
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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 Fooker, 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

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3 **Title Page:**
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5 **Study Protocol: NITric oxide during cardiopulmonary bypass to improve Recovery in**
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7 **Infants with Congenital heart defects (NITRIC trial): A Randomised Controlled Trial.**
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**On behalf of the NITRIC Study Group, the Australian and New Zealand Intensive Care
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group (PCCRG) and the ANZICS Paediatric Study Group (PSG)**

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40 **Conflict of Interest Statement:**
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42 Yves d'Udekem is a consultant for MSD and Actelion.
43

44 LJS, SH, DL, KJ, NA, DW, MF, SE, JB, CD, KL, MJ, AF, BG, AB, PY, WB, AS have no
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46 conflicts of interest to declare.
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51 **Word count:** 3441
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56 **Key words:** cardiopulmonary bypass; child; congenital heart disease; infant; inflammation;
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58 ventilation; nitric oxide; mortality
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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

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4
5 Health Research Council of New Zealand.
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10 The funding sources had no involvement in study design, analyses, nor interpretation of the
11
12 results.
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14 Mallinckrodt Pharmaceuticals will provide nitric oxide delivery devices to study centres but
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16 has no involvement in study design, conduct, nor analyses.
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28 **Author contributorship statement:**
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30 The study protocol first draft was designed by LJS and AS based on the previous pilot study
31
32 design established by SH, and WB. MJ wrote the section on statistical analyses. JF and BG
33
34 wrote the section on health economic analyses The present study protocol has been revised
35
36 with input from SH, DL, JB, SE, MF, YdU, NA, DW, KJ, CD, KvL, BG, JF, PY, AB, MJ and
37
38 WB. LJS prepared the final protocol manuscript which was reviewed and approved by all
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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 \geq 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 ancillary 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

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

11
12 Nitric oxide (NO) is an endogenous anti-inflammatory mediator[19] and is essential to regulate
13 endothelial function and microvascular inflammation[20]. Several studies have demonstrated
14 that exogenous NO can reduce myocardial damage in clinical and experimental settings of
15 ischaemia and reperfusion[21-24]. A previous small single centre study in 16 children reported
16 a reduction in bypass-induced inflammation using gaseous NO delivered at 20ppm to CPB
17 circuits[25]. The duration of mechanical ventilation was significantly shorter (8.4 versus 16.3
18 hours; $P < .05$) and so was ICU length of stay (53.8 versus 79.4 hours; $P < .05$) in children
19 receiving NO compared to the placebo group. We have previously reported the feasibility and
20 safety of NO delivery to CPB in a single centre randomized controlled pilot study in 198 infants
21 and children (0-16 years) undergoing cardiac surgery[26]. This pilot study demonstrated a
22 statistically significantly lower proportion of children with a LCOS in the intervention arm, a
23 reduced requirement for ECLS, and a trend to reduced length of stay, and shorter duration of
24 mechanical ventilation. The effect was greatest in children under two years of age, with the
25 greatest treatment benefit observed in children under six weeks.

26
27 Accordingly, we designed the NITRIC trial to test the primary hypothesis that, in infants under
28 two years having cardiac surgery, using NO during CPB increases ventilator-free days (the
29 number of days patients spend alive and free from invasive mechanical ventilation up until
30 post-operative day 28) compared with standard care. Here we describe the NITRIC trial
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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.

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

16
17 **Blinding of the Intervention.** Blinding arrangements in the operating theatre will be achieved
18 by covering the NO delivery system with drapes. The dedicated study NO delivery system will
19 be connected to the CPB oxygenator at all times, independent of randomisation. The family,
20 surgeons, anaesthetists and PICU staff will be not aware of the treatment arm a patient is
21 allocated to. The perfusionists will be advised that all aspects of CPB except for provision of
22 NO (or not) should be performed according to standard institutional practice for study
23 participants.

24
25 **Interventions.** Infants will be randomly assigned to NO or standard care. Those allocated to
26 the *NO arm* will receive NO during CPB blended into the fresh gas flow of the CPB
27 oxygenator, which is kept at 3L/min. NO levels are maintained at 20 ppm using a NO delivery
28 system (Ikaria INOmax DSIR, Ikaria, NJ, USA) or similar device. Continuous sampling of NO
29 and NO₂ concentration will be undertaken from an access port before the oxygenator. NO will
30 be started immediately when the patient is placed on CPB and ceased once weaned off CPB.

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3 Patients allocated to the *standard study arm* will receive the standard gase oxygen-air mix into
4 the CPB oxygenator at a flow rate of 3L/min. If patients require several CPB runs during the
5 same procedure, the study treatment will be provided for each CPB run using the same
6 treatment allocation for every CPB run.
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13 **Relevant concomitant perioperative care.** Arterial partial pressures of CO₂ will be
14 maintained constantly in both study arms as per institutional practice (following each centre's
15 protocols on alpha/pH stat and temperatures). The FiO₂ of the fresh gas flow will be set
16 between 21% and 100%, according to centre specific CPB protocols. Techniques of anaesthesia
17 and surgery will not be specified to allow site specific individual practice. The decision whether
18 a patient requires treatment with inhaled NO (iNO) into the ventilator circuit prior to, during,
19 or after CPB remains at the discretion of the treating physicians (anaesthetists, cardiac
20 surgeons, or intensivists) independent of treatment allocation. The postoperative care and
21 decisions on inotropes and other vasoactive drug delivery, fluid management, renal
22 replacement therapy, iNO therapy or indication for ECLS will be performed as per site specific
23 standard protocols of care.
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41 **Study outcomes.** The **Primary outcome** is ventilator free days (VFD) for the first 28 days
42 post randomisation (**Table 2**). The primary outcome will be measured using duration of
43 invasive respiratory support for all episodes with an endotracheal tube in situ for the first 28
44 days post randomisation. A systematic zero value will be assigned for patients who die to weigh
45 death as the most pejorative outcome. Treatment with non-invasive ventilation and high-flow
46 nasal cannulae will not be considered as ventilator days. **Secondary outcomes** are defined as
47 the composite outcome compromising LCOS, need for postoperative ECLS, or 28-day
48 mortality; ICU and hospital length of stay; and health care costs. In addition, the long-term
49 outcome of patients will be followed up at twelve months post procedure.
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3 **LCOS**[10] is defined as a blood lactate level greater than 4 mmol/l with an oxygen extraction
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5 of greater than 35% ($\text{SaO}_2\text{-ScvO}_2$ gradient >35%) within the first 48 hours postoperatively, or
6
7 a high inotrope requirement defined as Vasoactive-Inotrope Score ≥ 15 (VIS)[28, 29].
8
9

10 **Rationale for primary and secondary outcomes:** Ventilator free days represents one of the
11
12 strongest predictors of short- and long-term outcomes[10], including length of intensive care
13
14 unit (ICU) stay, morbidity (impaired neurodevelopment, hospital-acquired infections) and
15
16 mortality. VFD directly reflect intensive care resource use and health care costs[30]. VFD
17
18 fulfills SMART criteria (Specific, Measurable, Achievable, Realistic and Timely). LCOS is
19
20 directly related to VFD, as infants usually do not tolerate weaning or fail extubation if LCOS
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22 and organ dysfunction have not resolved. The composite of LCOS, ECLS use, and mortality
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24 as a secondary end point, is a strong patient-centred outcome and directly relates to the
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26 intervention in terms of biological plausibility.
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33 **Adverse Events:** It is recognised that the postoperative paediatric cardiac surgical patient
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35 population will experience a number of common aberrations in laboratory values, signs and
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37 symptoms due to the severity of the underlying disease and the impact of standard therapies.
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39 Intensive care patients will frequently develop life-threatening organ failure(s) unrelated to
40
41 study interventions and despite optimal management. Therefore, consistent with established
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43 practice in academic ICU trials[31], events that are part of the natural history of the primary
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45 disease process or expected complications of critical illness will not be reported as serious
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47 adverse events in this study. All adverse events which are considered to be potentially causally
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49 related to the study intervention or are otherwise of concern in the investigator's judgement
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51 will be reported unless they are pre-specified study outcomes. Specific adverse events related
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53 to NO delivery during CBP include air embolism, severe hypotension during bypass and
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55 increased MetHb (MetHb >3%). Of note, in previous studies, methaemoglobin values using
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3 NO at 20 ppm were similar in both the control and intervention groups (1.4%)[25, 26]. Events
4 that are collected as study outcomes will not be reported as adverse events.
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8 **Safety Data Monitoring.** The Data Safety Monitoring Board (DSMB) consists of a general
9 and a cardiac paediatric intensivist, a cardiac surgeon, and a statistician. None of the DSMB
10 members will be involved in recruitment of study patients at their site. DSMB members will
11 not be supervised by any study investigator or participate as investigators in any study currently
12 under review by this DSMB. The primary objective of the DSMB is to monitor the safety of
13 the intervention and the validity and integrity of the data from the NITRIC study. Additionally,
14 the DSMB will evaluate the pace of recruitment and will make recommendations to the
15 NITRIC Chief investigator(s) and Steering Board regarding the continuation, modification, or
16 termination of the study. The DSMB will evaluate on an ongoing basis, the accumulating safety
17 assessments to ensure the ongoing safety of study subjects. The DSMB will meet via
18 teleconference call after recruitment of 660, and of 1000 children, respectively, and upon trial
19 completion.
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36 The DSMB can request unblinding in the event of a serious adverse event defined as a cardiac
37 arrest, need for ECLS, or other incident leading to permanent harm considered to be likely
38 related to the study intervention.
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46 **Sample size.** A pilot study of 134 patients aged < 2 years showed a 2.74 days (66 hours)
47 increase in ventilator-free days (VFD) associated with the study intervention [26]. This
48 includes patients who died, who were considered as zero VFDs. The VFD increase associated
49 with the intervention represents an effect size of 0.33 standard deviations (SD) based on a SD
50 of 8.1 days in the pilot study control group. Based on the primary outcome measure VFD, 1,320
51 patients (660 per group) will be required to demonstrate a significant increase in VFD assuming
52 a minimally clinically significant effect size (0.2 SD; 1.66 days or 40 hours), 90% power, two-
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3 sided alpha level of significance of 5%, 10% withdrawals, and 15% increase in sample size to
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5 account for a non-normal distribution of VFD. In Australia and New Zealand approximately
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7 800 of children < 2 years of age undergo surgery for a congenital heart defect requiring CPB
8
9 each year, including patients with multiple procedures. The consent rate of eligible patients
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11 was 78% in the pilot trial[26]. With an expected conservative estimate 60% enrolment rate of
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13 eligible patients, we expect a 3.5-year recruitment period for the study.
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19 **Data collection, management, and analysis**

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21 **Data collection:** Baseline variables (demographics, primary cardiac diagnosis, comorbidities
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23 including syndromes), pre-operative disease severity, surgical data (length of CPB and cross-
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25 clamp, type of surgery and complexity score, other CPB characteristics, blood product usage),
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27 primary end points, secondary end points, pre-determined physiological variables of interest,
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29 and process of care measures will be prospectively recorded into a study REDCap online
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31 database. Plausibility and range checks are implemented. Paired arterial and venous gases will
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33 be collected postoperatively at 0, 6, 12, 24, and 48 hrs (in PICU until discharge to the ward or
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35 removal of arterial and central venous lines, whichever occurs first) to assess for lactate and
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37 SaO₂-ScvO₂ gradient. Key physiological and blood parameters and Paediatric Logistic Organ
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39 Dysfunction-2 (PELOD-2) scores[32] will be measured at 0, 6, 12, 24, and 48 hours post
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41 admission or until PICU discharge whichever occurs earlier. Delayed chest closure, use of
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43 inhaled NO, and duration of circulatory, renal, and ventilatory support postoperatively will be
44
45 recorded. Gross functional performance assessment will be recorded on admission to PICU
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47 and upon discharge from hospital[33]. Neurological and functional outcome (including phone
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49 interviews with parents/caregivers) will be assessed at 12 months postoperatively using Ages
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51 and Stages questionnaires (ASQ)[34, 35] and assessment of quality of life using paediatric
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3 quality of life inventory (PedsQL)[36]. Details on the long-term follow-up will be published
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5 separately.
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10 **Biobanking:** Blood markers for myocardial injury and inflammatory response will be collected
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12 on induction of anaesthesia (baseline – pre-bypass), at admission to PICU (0 hours – post
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14 bypass) and at 12, and 24 hours, in patients where parents consent to biobanking. Blood will
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16 be collected pre-surgery as preoperative baseline (before onset of cardiac surgery, done by
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18 anaesthetist during the induction of the patient once the arterial line has been inserted): 1-2ml
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20 of EDTA blood (for DNA), 2.5ml of PAXgene blood (for gene expression markers), 1-2ml of
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22 serum; and postoperatively at 0,12,24 hours (in PICU until discharge to the ward): 2.5ml of
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24 PAXgene blood (time point 0), and 1-2ml of serum. The samples will be processed, stored and
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26 shipped according to accepted international standards and batch analysed.
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32 The investigators are responsible for ensuring the accuracy, completeness, legibility, and
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34 timeliness of the data reported. The investigators will maintain adequate case histories of study
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36 participants, including accurate case report forms (CRFs), and source documentation. Data will
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38 be prospectively entered into a secure web-based database (REDCap
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40 <https://redcap.health.uq.edu.au/>), hosted by the University of Queensland. Printed paper CRFs
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42 will be available if required. All study information and documentation will be securely stored
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44 for a period of 15 years after the date of the child's eighteenth birthday.
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50 **Statistical Analysis Plan**

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52 **Analysis plan.** Analyses will apply the intention to treat principle. Descriptive statistics will
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54 be used to describe baseline characteristics of the study cohort and each subgroup by treatment
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56 group. The primary outcome measure will be analysed using a Mann-Whitney U test as
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58 ventilator-free days is non-normally distributed variable. Analysis of secondary outcomes
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3 includes both comparisons of measurements and proportions, using confidence intervals of
4 differences as the major method of presentation where possible, otherwise standard techniques
5 such as Mann-Whitney U tests, t-tests and chi-squared tests will be utilised. Survival outcomes
6 will be compared between treatment groups using Kaplan-Meier product limit method and log-
7 rank test. Statistical significance will be set at the 0.05 level for the primary outcome.
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17 A safety and efficacy interim analysis after 660 (the half-way point), and after 1000 enrolled
18 patients will be performed by an independent statistician to evaluate for safety endpoints, to
19 assess the predictive probability of reaching the study goals, and compare VFD between
20 treatment groups. Consideration to stopping the trial early by an independent Data and Safety
21 Monitoring Board (DSMB) will be based on safety concerns, futility, or strong evidence of a
22 difference between groups for VFD (based on a Haybittle–Peto boundary $P=0.001$). A detailed
23 analysis plan specifying statistical analyses including health economic analyses will be placed
24 in the public domain prior to recruitment of the last participant[37].
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38 **Biomarker measurements.** Nested sub-studies will be performed in selected samples at sites
39 performing biobanking (i) to test the impact of the intervention on markers of systemic and
40 myocardial inflammation; (ii) to compare treatment response between patients depending on
41 pre-intervention severity assessed by markers of inflammation and organ failure; and (iii) to
42 biochemically define responders to the intervention (to identify patient subgroups pre-
43 randomisation that are more likely to respond to a specific treatment). The use of samples/data
44 will be governed by the study steering board and resulting publications must appropriately
45 acknowledge the study. Combining a large RCT with a nested biobank is recommended to
46 maximize scientific knowledge[38].
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3 **Health Economic Evaluation.** A within trial economic evaluation will be used to determine
4 if providing NO is cost-saving compared to usual care from the health system perspective. A
5 comprehensive cost-effectiveness analysis will be undertaken to determine the level of cost
6 savings (if any). Length of stay (in PICU and in non-intensive stay) will be the main outcome
7 variable. Resources used before first discharge will be compared between treatments.
8 Resources will subsequently be costed, based on hospital cost centre or standard national
9 sources (e.g. Independent Hospital Pricing Authority).

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20 **Monitoring.** The study leadership team is responsible for 100% monitoring of investigator and
21 study nurse credentials, training records, and delegation of responsibility logs, and will review
22 100% of Consent Forms. CRFs will be compared to source documentation to ensure data are
23 accurate and complete. 100% of source data verification of eligibility criteria and the primary
24 outcome and the composite secondary outcome of LCOS, ECLS or death will be performed.
25 An independent monitoring per each site will monitor the data fields required for eligibility,
26 primary, and secondary study endpoints, and SAEs using primary source verification. In
27 addition, site visits and regular monitoring of the blood sample storage will be performed.
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40 **Patient and Public Involvement.** Consumers and the public were consulted to design a video
41 informing parents about the study. Patients and the public had no other involvement in study
42 design.
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50 **Ethics.** This protocol and the informed consent document and any subsequent modifications
51 have been reviewed and approved by the human research ethics committee (Children's Health
52 Queensland HREC/17/QRCH/43). This study will be conducted in compliance with the current
53 version of the protocol. Any change to the protocol document or Informed Consent Form that
54 affects the scientific intent, study design, patient safety, or may affect a participants'
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3 willingness to continue in the study is considered an amendment, and therefore will be written
4 and filed as an amendment to this protocol and/or informed consent form. All protocol
5 deviations must be recorded in the patient record (source document) and on the CRF and must
6 be reported to the PI. Protocol deviations will be assessed for significance by the Principal
7 Investigator.
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15 Consent will be sought from the parents of every child <2 years of age undergoing CPB for
16 elective cardiac surgery over the study period. When the family is seen by the surgeons in pre-
17 assessment clinic (usually days prior to surgery), the study will be mentioned to them by the
18 surgeon. In addition, the study team will provide study information prior to hospitalisation to
19 families, including printed study flyers, and links to online study documentation (media
20 release). Participant confidentiality is strictly held in trust by the participating investigators,
21 research staff, and the sponsoring institution and their agents. The study protocol,
22 documentation, data and all other information generated will be held in strict confidence.
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36 **Significance:** Postoperative paediatric cardiac surgical patients have a high consumption of
37 intensive care resources and remain at very high risk of major complications, including cardiac
38 arrest, death, and long-term neurological impairment. Approximately 10% of children with
39 CHD survive with major neurological sequelae postoperatively, resulting in a massive lifelong
40 burden for patients, families, healthcare systems, and the society[13]. An attempt to reduce
41 LCOS and hence perioperative morbidity has the potential to translate not only to a reduction
42 in intensive care resource utilisation, but also to impact positively on long-term outcomes.
43 Side-effects from heart surgery for CHD translate into long-term morbidity, persisting into
44 adult life with a major impact on other family members and society. This study will deliver the
45 high-level randomised evidence with the potential to show a reduction in postoperative
46 morbidity and mortality in children with CHD.
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3 **Acknowledgements:** The authors would like to thank the parents and children participating in
4 this trial and the the medical and nursing and research teams in the participating sites for their
5 help in study setup, recruitment, data collection, and monitoring of study data.
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12 **NITRIC Study Group:**
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16
17 Warwick Butt, Steve Horton, Johnny Millar, Carmel Delzoppo, Yves D`Udekem: Cardiac and
18 Paediatric Intensive Care Services of Royal Children`s Hospital, Melbourne, Australia
19

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5 Children's Hospital Utrecht, Utrecht, The Netherlands
6

7 8 **Roles and Responsibilities:** 9

10 The study protocol first draft was designed by LJS and AS based on the previous pilot study
11 design established by SH, and WB. The present study protocol has been revised with input
12 from SH, DL, KJ, CJ, CD, WB, SE, MF, BG, JF, PY, AB, MJ and JB.
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14

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16 The study steering group consists of LJS, SH, DL, WB and AS.
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18 LJS, AS, DL, KJ, NA, SE, WB, SH, CdZ, KL, YdU, MF, JB are responsible for local study
19 setup conduct, and recruitment.
20
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22 AB and LJS are responsible for host inflammation analyses.
23

24 MJ is responsible for statistical analyses.
25

26 JF and BG are responsible for health economic analyses.
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Figures

Figure 1. Study Flow Diagram.

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Table 1: Inclusion and Exclusion criteria

Patient group	Criterion	Definition
Inclusion	<i>Age</i>	<ul style="list-style-type: none"> • Postnatal age below 2 years
	<i>Procedure</i>	<ul style="list-style-type: none"> • Open elective Heart surgery • Cardiopulmonary bypass used during surgery
	<i>Consent</i>	<ul style="list-style-type: none"> • Parental/caregiver consent available prior to surgery
Exclusion	<i>Age</i>	<ul style="list-style-type: none"> • Age ≥ 2 years
	<i>Procedure</i>	<ul style="list-style-type: none"> • 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
	<i>Consent</i>	<ul style="list-style-type: none"> • Lack of parental/caregiver consent
	<i>Pulmonary hypertension</i>	<ul style="list-style-type: none"> • 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)
	<i>Pre-operative disease</i>	<ul style="list-style-type: none"> • ECLS immediately prior to surgery • Receiving ongoing treatment with antimicrobials for confirmed or suspected sepsis or septic shock diagnosed within 48 hours 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$>3\%$)

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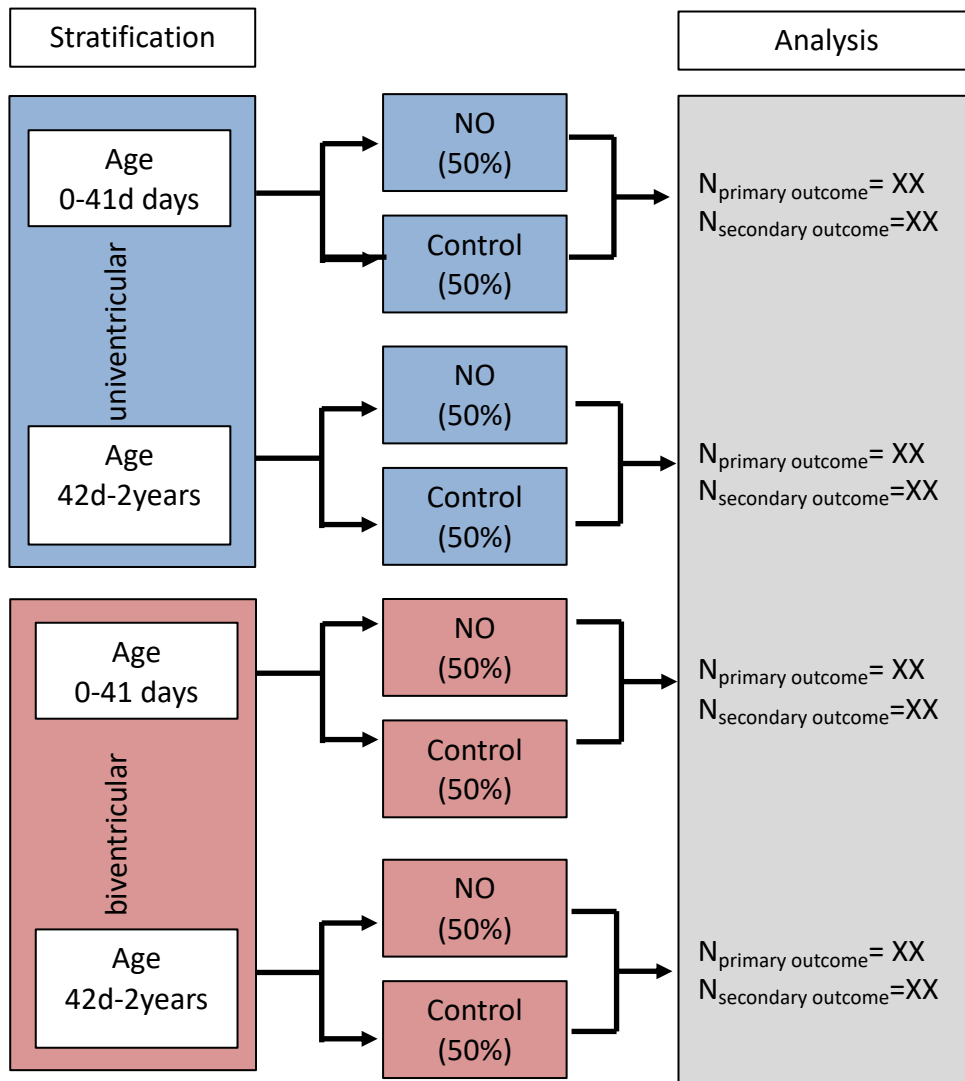
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Table 2: Study outcomes

Outcome	Criterion	Definition
Primary	<i>Ventilator-free days (VFD)</i>	<ul style="list-style-type: none"> • 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	<p><i>Composite outcome of low cardiac output syndrome (LCOS), Extracorporeal life support (ECLS), or death</i></p> <p><i>Length of stay</i></p> <p><i>Costs</i></p> <p><i>Neurodevelopmental and functional outcome at 12 months</i></p>	<ul style="list-style-type: none"> • LCOS is defined as one or more of the following^a: <ul style="list-style-type: none"> - Blood lactate level greater than 4 mmol/l with an oxygen extraction of greater than 35% (SaO₂-ScvO₂ 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 in paediatric intensive care unit (PICU) • Length of stay in hospital • Health-care related costs (starting at time of admission to PICU postoperatively) • 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	<i>Severity indicators</i>	<ul style="list-style-type: none"> • 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

<i>Physiological descriptors</i>	<i>Host inflammation</i>	<ul style="list-style-type: none"> • Serum cytokine levels measured during the first 24 hours • Inflammation markers measured during the first 24 hours
	<i>Myocardial injury</i>	<ul style="list-style-type: none"> • Levels of postoperative serum troponin levels measured during the first 24 hours
	<i>Organ dysfunction</i>	<ul style="list-style-type: none"> • 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

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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	

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*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.

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