# PROTOCOL

**TRICS IV: Transfusion Requirements in Younger Patients Undergoing Cardiac Surgery**
An international, multi-centre, randomized controlled trial to assess transfusion thresholds in younger patients undergoing cardiac surgery **(pages 1 - 44)**

**Hepcidin and Iron Storage Sub-Study**
The relationship of hepcidin on patient outcomes after cardiac surgery
A sub-study of the Australian participants in the TRICS IV study **(pages 1 – 15)**

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| **Protocol version:** | **TRICS IV**<br>Version 1.0, 05 March 2021<br><br>**Hepcidin and Iron Storage Sub-Study**<br>Protocol Addendum 1_AUS<br>Version 1.0, 10 August 2021 |
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| **Website:** | https://medicine.unimelb.edu.au/research-groups/medicine-and-radiology-research/critcare/about-us/trics-iv-trial |
| **Trial Coordinating Centre:** | The University of Melbourne<br>Department of Critical Care<br>Melbourne Medical School |
| **Sponsor:** | The University of Melbourne, Parkville, VIC, 3010, Australia |
| **Endorsement:** | Australian and New Zealand College of Anaesthetists Clinical Trials Network (ANZCA CTN) |


# Clinical Study Protocol

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| Study Title: | **TRICS IV: Transfusion Requirements in Younger Patients Undergoing Cardiac Surgery**<br>An international, multi-centre, randomized controlled trial to assess transfusion thresholds in younger patients undergoing cardiac surgery |
| Principal Investigators: | Dr. David Mazer, St. Michael's Hospital (Toronto, ON, Canada)<br>Dr. Nadine Shehata, Mount Sinai Hospital (Toronto, ON, Canada) |
| Coordination Centre: | Applied Health Research Centre (AHRC) of St. Michael's Hospital, Unity Health Toronto (Toronto, ON, Canada), at the University of Toronto |
| Version: | Version 1.0 |
| Date: | 05-Mar-2021 |

## Signature Page for Sponsor

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| Version: | Version 1.0 |
| Date: | 05-Mar-2021 |

Approved by the following:

Dr. David Mazer, Principal Investigator — Date (dd-mmm-yyyy) March 8/2021

N. Shehata — March 8,2021

Dr. Nadine Shehata, Principal Investigator — Date (dd-mmm-yyyy)

## Signature Page for Site Investigator

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I have read this protocol and agree to conduct this trial in accordance with all stipulations of the protocol and in accordance with Good Clinical Practice (GCP) and relevant local regulatory requirements that govern the conduct of clinical research.

| Site Investigator Name | Signature | Date |
|---|---|---|
| Please print | | (dd-mmm-yyyy) |

## Abbreviations

| | |
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| AHRC | Applied Health Research Centre |
| AKI | Acute Kidney Injury |
| CABG | Coronary Artery Bypass Graft Surgery |
| CAC | Central Adjudication Committee |
| CAM/CAM-ICU | Confusion Assessment Method (Intensive Care Unit) |
| CC | Coordination Centre |
| CCU | Cardiac Care Unit (Intensive care) |
| CIHR | Canadian Institutes of Health Research |
| CK-MB | Creatine Kinase - MB |
| CO | Cardiac Output |
| CPB | Cardiopulmonary Bypass |
| EC | Executive Committee |
| ECG | Electrocardiogram |
| eCRF | Electronic Case Report Form |
| EEG | Electroencephalogram |
| ER | Emergency Room |
| DSMB | Data and Safety Monitoring Board |
| GCP | Good Clinical Practice |
| Hb | Hemoglobin |
| HCN | Health Card Number |
| HCT | Hematocrit |
| HIF | Hypoxia Inducible Factor |
| HR | Hazard Ratios |
| ICDSC | Intensive Care Delirium Screening Checklist |
| ICH | International Conference on Harmonization |
| ICU | Intensive Care Unit |
| INR | International Normalized Ratio |
| IRB | Institutional Review Board |
| ITT | Intention to Treat |
| IV | Intravenous |
| KDIGO | Kidney Disease Improving Global Outcomes |
| MI | Myocardial Infarction |
| NHMRC | National Health and Medical Research Council |
| nNOS | Neuronal Nitric Oxide Synthase |
| OR | Operating Room |
| PCI | Percutaneous Coronary Intervention |
| PP | Per-protocol |
| aPTT | Activated Partial Thromboplastin Time |
| RBC | Red Blood Cell |
| RCT | Randomized Controlled Trial |
| REB | Research Ethics Board |
| rFVIIa | Recombinant factor VIIa |
| RR | Risk Ratio |
| SC | Steering Committee |
| TCPS2 | Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (version 2) |
| TRALI | Transfusion-Related Acute Lung Injury |
| ULN | Upper Limit of Normal |

## TABLE OF CONTENTS

# 1 PROTOCOL SUMMARY

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|---|---|
| **Title** | **TRICS IV: Transfusion Requirements in Younger Patients Undergoing Cardiac Surgery**<br>An international, multi-centre, randomized controlled trial to assess transfusion thresholds in younger patients undergoing cardiac surgery |
| **Principal Investigators** | Dr. David Mazer (St. Michael's Hospital), Toronto, ON Canada<br>Dr. Nadine Shehata (Mount Sinai Hospital), Toronto, ON Canada |
| **Funding** | Funded primarily through peer reviewed operating grants from the Canadian Institutes of Health Research (CIHR) and Australian National Health and Medical Research Council (NHMRC) |
| **Hypothesis** | A higher hemoglobin (Hb) concentration for red blood cell (RBC) transfusion (liberal transfusion strategy) will be superior to a restrictive strategy in terms of vital organ function (heart, brain and kidney) and mortality 6 months after cardiac surgery |
| **Primary Endpoint** | Composite score of any one of the following events occurring 6 months after cardiac surgery: (1) all-cause mortality; (2) myocardial infarction; (3) new onset renal failure requiring dialysis; or (4) new focal neurological deficit (stroke) |
| **Secondary Endpoints** | Incidence of each individual component of the primary outcome: all-cause mortality, myocardial infarction, new onset renal failure requiring dialysis, and new focal neurological deficit (stroke) within 6 months<br>Composite and individual all-cause mortality, myocardial infarction, new onset renal failure requiring dialysis, and new focal neurological deficit (stroke) with index hospitalization or after 28 days postoperatively, whichever comes first<br>Length of stay in the Intensive Care Unit (ICU) and hospital (index hospitalization)<br>Prolonged low output state defined as the need for two or more inotropes for 24 hours or more, intra-aortic balloon pump or ventricular assist device postoperatively (index hospitalization)<br>Duration of mechanical ventilation (index hospitalization)<br>Infection: defined as septic shock with positive blood cultures; pneumonia defined as autopsy diagnosis or roentgenographic infiltrate and at least two out of three of the following criteria: fever, leukocytosis, and positive sputum culture; and/or deep sternal or leg wound infection requiring intravenous antibiotics and/or surgical debridement (index hospitalization) |

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| | Acute kidney injury (AKI), defined by the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline - a 50% increase in serum creatinine within 1 week or a 26.5 µmol/L increase within 48 hours (index hospitalization)<br><br>Delirium, based on one of the following criteria: Confusion Assessment Method (CAM) or CAM-ICU (even on 1 occasion), or Intensive Care Delirium Screening Checklist (ICDSC) >3, or 3D-CAM, or 4AT ≥4, or more than one dose of haloperidol or similar antipsychotic drug, or documented delirium by neurologist or neurosurgeon or psychiatrist consultation (index hospitalization)<br><br>Gut infarction confirmed by imaging (e.g. angiography), autopsy, or through surgical means (index hospitalization)<br><br>Hospital visits (hospitalization and/or emergency visits and coronary revascularization at 6 months)<br><br>The proportion of patients transfused and the number of blood products utilized (RBCs, plasma, platelets) (index hospitalization)<br><br>Seizures, defined as generalized or focal tonic-clonic movements consistent with seizure; or electroencephalogram (EEG) demonstrating epileptiform discharges; or diagnosis of seizures by neurologist or neurosurgeon consultation (index hospitalization)<br><br>Encephalopathy, defined as unexpected delayed awakening or severely altered mental status (unconscious despite no sedative medication for more than 5 days), or encephalopathy documented by neurologist or neurosurgeon or psychiatrist consultation (index hospitalization) |
| **Study Population** | Patients at moderate to high risk of death (EuroSCORE I ≥ 6) that are 65 years of age or younger undergoing cardiac surgery on cardiopulmonary bypass (CPB) |
| **Study Design** | Multi-centre open-label randomized controlled trial (RCT) of a restrictive versus liberal transfusion strategy in higher risk patients ≤65 years of age having cardiac surgery on cardiopulmonary bypass, using a superiority trial design |
| **Sample Size** | N = 1,440 randomized patients (720 per group) |
| **Accrual Period** | 24 months recruitment + 6 months follow up |
| **Study Duration** | March 2021 – February 2024 (estimated) |
| **Study Intervention** | *Restrictive transfusion strategy:* patients will receive a RBC transfusion if their Hb concentration is <75 g/L (<7.5 g/dL; <4.7mmol/L) intraoperatively and/or postoperatively. |

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| | *Liberal transfusion strategy:* patients will receive a RBC transfusion if their Hb concentration is <95 g/L (<9.5 g/dL; <5.9mmol/L) intraoperatively, or postoperatively in the ICU; and/or <85 g/L (< 8.5 g/dL; <5.3mmol/L) on the ward. |
| **Assessments** | Both investigational arms will be followed during their index hospitalization or to postoperative day 28, whichever comes first; thereafter for 6 months postoperatively to assess clinical outcomes and mortality. |

# 2 INTRODUCTION, BACKGROUND & SCIENTIFIC RATIONALE

## *2.1 Background and Rationale*

Cardiac surgery is one of the most frequent surgical procedures, with approximately 35,000-40,000 procedures conducted in Canada each year.[1] Fifty percent of patients undergoing cardiac surgery are <65 years.[2] Anemia in these patients is common due to underlying conditions and hemodilution during cardiopulmonary bypass (CPB). Perioperative anemia has been associated with a significant increase in cardiac (myocardial infarction) and non-cardiac (renal failure and stroke) adverse events and mortality[3,4]. Patients undergoing cardiac surgery receive a high proportion of RBCs to decrease the risks associated with anemia (approximately 14% to 20% of the blood supply is consumed by cardiac surgical patients[5-7]); although there are potential benefits to transfusion, transfusion is costly, often in short supply and may also lead to considerable mortality and morbidity. Restrictive transfusion triggers have been suggested to be preferred by RCTs in many patient populations including critically ill patients[8], patients undergoing hip fracture surgery[9], and with acute upper gastrointestinal bleeding[10].

Recently, we completed a multicentered multinational RCT, Transfusion Requirements in Cardiac Surgery III (TRICS III) funded by CIHR, the National Health and Medical Research Council of Australia, and the Health Research Council of New Zealand comparing a restrictive transfusion threshold to a liberal threshold in 5243 patients undergoing high risk cardiac surgery. The study was completed ahead of schedule, within budget, and with approximately 30% more patients than initially anticipated. The TRICS III trial outcomes were published in the New England Journal of Medicine[11,12]. Overall, TRICS III demonstrated that a restrictive transfusion strategy was not inferior to a liberal strategy with respect to a composite outcome of death, myocardial infarction (MI), stroke, or new-onset renal failure to day 28, and at 6 months after surgery[11,12]. Predefined subgroup analysis, however, showed a highly significant interaction between the transfusion threshold and age[12]. Whereas restrictive transfusion was favored in elderly patients, the odds ratios for the primary composite outcome showed an incremental rise in risk with each decade <65 years of age[12]. There is thus an urgent need to settle the question of whether restrictive transfusion practices in younger patients expose them to significant unnecessary harm.

Transfusion Requirements in Younger Patients Undergoing Cardiac Surgery IV (TRICS IV) is the fourth RCT conducted by an experienced and dedicated group of international investigators to determine whether a liberal transfusion strategy is superior to a restrictive strategy in patients ≤65 years.

TRICS IV is an extension of our experience to understand the mechanisms of anemia induced organ dysfunction and our clinical studies to determine optimal transfusion thresholds in cardiac surgery. The problem we wish to answer is whether a high hemoglobin concentration (Hb) trigger for transfusion (liberal strategy) is superior to a low trigger (restrictive strategy) in higher risk patients (EuroSCORE I ≥ 6) undergoing cardiac surgery who are ≤65 years. Currently, it is not known the extent to which anemia in younger patients is a risk factor for adverse outcomes and whether RBC transfusion is required to maintain vital organ function and reduce adverse outcome.

## 2.2 Physiological Effects of Anemia

Acute severe anemia has been associated with increased mortality likely due to impaired oxygen delivery and tissue hypoxia[13]. Since Hb contributes to more than 99% of blood oxygen content, severe anemia leads to inadequate tissue oxygen delivery, resulting in tissue hypoxia, organ failure, and death. As defined by studies in animals and humans, acute reduction in Hb is sensed at the cellular level and leads to adaptive cardiovascular responses to optimize tissue oxygen delivery. These responses include 1) a characteristic increase in cardiac output (CO) that is proportional to the degree of anemia; 2) a reduction in systemic vascular resistance with organ-specific vasodilation to facilitate preferential perfusion of vital organs, including the heart and brain; and 3) an increase in tissue oxygen extraction. In addition, anemia results in the activation of hypoxic cellular mechanisms, including neuronal nitric oxide synthase (nNOS) and hypoxia inducible factor (HIF), with the purpose of maintaining oxygen homeostasis and sustaining organism survival[13].

Acute hemodilution that occurs during CPB has been shown to reduce oxygenation in the brain, heart, kidney, intestine, and muscle[14-16]. The heart, under normal conditions extracts 60 to 70% of oxygen delivered[17]. Other organs can increase oxygen extraction to compensate for reduced oxygen delivery; however, increasing myocardial blood flow by coronary vasodilatation is the only compensatory method available for the heart. This increased susceptibility to adversities secondary to anemia[18-20] is particularly problematic when there is a limited capacity to increase blood flow such as in patients who have stenosed coronary arteries, in the presence of myocardial hypertrophy, aortic valve disease, or other circulatory abnormalities[21,22].

## 2.3 What is the Evidence that the Optimal Hemoglobin Threshold for Transfusion Differs According to the Age of the Patient?

Younger aged patients are susceptible to anemia. Isovolemic removal of blood to reduce the Hb concentration to 50 g/L in 21 healthy subjects aged 19-33 years resulted in inadequate systemic oxygen in two female subjects who developed transient ST segment changes that were asymptomatic (at a Hb of 62 g/L and 46 to 53 g/L)[23]. Isovolemic removal of blood to reduce the Hb to 50 to 60 g/L in nine other healthy subjects (mean age 29 ± 5 years) also resulted in mild cognitive impairment demonstrating inadequacy of cerebral perfusion[24].

Anemia may be a physiological response to aging. A systematic review found that the prevalence of anemia increased with age in both men and women[25]. The prevalence of anemia observed in individuals more than 85 years was at least 2-3-fold greater than younger individuals. In one study of community dwelling individuals, anemia occurred in 43% of men 70-74 years to 60% of men >85 years. In a RCT comparing liberal vs. restrictive transfusion strategies following cardiac surgery, transfused patients were generally older than non-transfused patients, suggesting that they reach Hb triggers earlier because of age[26]. With age, systemic metabolic requirements are reduced[27]. Heart failure occurs more frequently in the elderly - one study suggested that 50% of heart failure occurs in patients >70 years[28,29]; these patients may not tolerate the volume of RBCs and thus restrictive strategies may then be associated with better outcomes. In TRICS III, the subgroup

analysis demonstrated a significant interaction with age (p=0.004) for the primary composite outcome after 6 months. The unadjusted odds ratio for the primary outcome for age 65-74 years was 1.18 (95% CI 0.91–1.54), for 55-64 years 1.47 (95% CI 0.89–2.44), for 45-54 years, 1.79 (95% CI 0.88– 3.67) and for patients <45 years, 2.37 (95% 0.87–6.43)[12]. Since the majority of patients in TRICS III were over 65 years of age, the signal for harm may have been further diluted in younger patients. In TITRe2, the age interaction was directionally similar to TRICS III with the odds ratio for the primary outcome showing a trend favoring liberal transfusion in patients <75 years: 1.3 (95% CI 0.91-1.84) for the restrictive strategy[30]. The mean age of the 502 patients in the TRACS trial, another RCT comparing liberal and restrictive transfusion strategies was 60 years and a trend for harm was also apparent with restrictive strategies: hazard ratio for mortality, 1.28 (95% CI, 0.60-2.73) and the incidence of cardiogenic shock was 5% in the liberal group (95% CI, 2%-7%) vs 9% in the restrictive group (95% CI, 5%-12%)[2].

### *2.4 What are the Adverse Events Associated with Transfusion?*

There are a number of complications related to transfusion that are associated with considerable morbidity and mortality. Non-infectious risks from transfusion such as transfusion related acute lung injury (TRALI), which tends to occur more frequently in patients having cardiac surgery[31], perhaps because of increased systematic and pulmonary inflammation with cardiac surgery[32,33], has a high case fatality rate, 5 to 13%[34,35]. Additionally, administrative errors resulting in hemolytic transfusion reactions can be life-threatening[36]. Pulmonary edema, due to the volume of RBCs occurs more frequently in patients with critical illness and cardiovascular disease transfused at a Hb of 100 g/L compared to a Hb of 70 g/L[8]. Although the risk of acquiring transfusion transmitted viruses is low[37], new emerging pathogens constantly threaten the blood supply. Unnecessary transfusions also have an impact on the care of patients. Optimum utilization of blood components is essential as there is a continuous strain on blood systems because of increased blood utilization. Additionally, increased transfusion results in increased resource utilization[38,39] and the cost of blood is increasing[40]. In an era where there are new emerging pathogens and blood shortages, it is essential for patients to be transfused appropriately.

### *2.5 What Systemic Reviews Have Been Conducted in Cardiac Patients?*

Our recently published meta-analysis of 13 RCTs comparing restrictive and liberal transfusion strategies in patients undergoing cardiac surgery demonstrated that restrictive transfusion strategies did not result in an overall increased risk of anemia-induced, tissue hypoxia-associated events[41]. The risk ratio (RR) of mortality from 4545 patients assigned to a restrictive transfusion strategy and 4547 transfused according to a liberal strategy was 0.96 (95% CI 0.8, 1.2), myocardial infarction RR 1.0 (95% CI 0.8, 1.3), renal failure RR 0.96 (95% CI 0.8, 1.2), stroke 0.93 (95% CI 0.7, 1.3), or infection RR 1.1 (95% CI 0.98, 1.3). We are unaware of any meta-analyses specifically evaluating the effect of age and transfusion strategies. However, in our meta-analysis, the risk ratios for mortality, stroke and infection were all numerically higher with restrictive transfusion in pediatric studies[41].

*The lack of well conducted randomized controlled trials evaluating the effect of age as well as the suggestion of increased risk associated with restrictive transfusion strategies in younger patients further substantiates the need for this RCT.*

### *2.6 What Randomized Controlled Trials are being Conducted Addressing Hemoglobin Thresholds in Cardiac Surgery According to Age?*

TRICS III[11] and TITRe2[30] were recently completed and compared restrictive and liberal transfusion strategies. TITRe2, a superiority trial, randomized 2003 cardiac surgical patients with a postoperative Hb <90 g/L to receive RBC transfusions to maintain a Hb of >75 g/L or >90 g/L. A statistically significant difference was not apparent in the primary composite outcome of infection and ischemic events three months after surgery (35.1% in the restrictive group and 33.0% in the liberal group odds ratio, 1.1 (95% CI 0.9, 1.3)); but there was a 1.6% absolute difference in the secondary outcome of 90-day mortality favoring the liberal strategy. Subgroup analysis according to decades of age was not conducted[30].

Although TRICS III established non-inferiority of the restrictive transfusion strategy in the 4860 patients analyzed (Hb <75 g/L intraoperatively and postoperatively, or a liberal strategy of RBC transfusion for Hb <95 g/L intraoperatively and postoperatively while in an intensive care unit (ICU), and <85 g/L on a non-ICU ward), a trend of poorer outcomes with a restrictive strategy in younger patients was demonstrated as described above[11].

No other trials assessing the effect of age on transfusion thresholds in cardiac surgery are listed on clinicaltrials.gov.

### *2.7 Why is a Trial Needed Now?*

If restrictive transfusion strategies in younger cardiac surgery patients are associated with harm, a trial is needed to ensure that these patients are transfused according to the appropriate threshold. If younger patients are not experiencing harm from restrictive transfusion strategies, they should not be exposed to the morbidity and mortality of transfusions if transfused unnecessarily.

### *2.8 Hypothesis*

A higher Hb concentration for RBC transfusion (liberal transfusion strategy) will be superior to a restrictive strategy in terms of vital organ function (heart, brain and kidney) and mortality 6 months after cardiac surgery.

# 3 STUDY OBJECTIVES

## *3.1 Primary Objective*

The primary objective is to determine whether a liberal RBC transfusion strategy is superior to a restrictive strategy in terms of vital organ function (heart, brain and kidney) and all-cause mortality 6 months after surgery.

## *3.2 Secondary Objectives*

The secondary objectives are to determine whether the liberal strategy is superior to a restrictive strategy in terms of:

1. Incidence of each individual component of the primary outcome: all-cause mortality, myocardial infarction, new onset renal failure requiring dialysis, and new focal neurological deficit (stroke) within 6 months
2. Composite and individual all-cause mortality, myocardial infarction, new onset renal failure requiring dialysis, and new focal neurological deficit (stroke) with index hospitalization or after 28 days postoperatively, whichever comes first
3. Length of stay in the Intensive Care Unit (ICU) and hospital (index hospitalization)
4. Prolonged low output state defined as the need for two or more inotropes for 24 hours or more, intra-aortic balloon pump or ventricular assist device postoperatively (index hospitalization)
5. Duration of mechanical ventilation (index hospitalization)
6. Infection: defined as septic shock with positive blood cultures; pneumonia defined as autopsy diagnosis or roentgenographic infiltrate and at least two out of three of the following criteria: fever, leukocytosis, and positive sputum culture; and/or deep sternal or leg wound infection requiring intravenous antibiotics and/or surgical debridement (index hospitalization)
7. Acute kidney injury (AKI), defined by the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline[46] - a 50% increase in serum creatinine within 1 week or a 26.5 µmol/L increase within 48 hours (index hospitalization)
8. Delirium, based on one of the following criteria: Confusion Assessment Method (CAM) or CAM-ICU (even on 1 occasion), or Intensive Care Delirium Screening Checklist (ICDSC) > 3, or 3D-CAM, or 4AT ≥4, or more than one dose of haloperidol or similar antipsychotic drug, or documented delirium by neurologist or neurosurgeon or psychiatrist consultation (index hospitalization)
9. Gut infarction confirmed by imaging (e.g. angiography), autopsy, or through surgical means (index hospitalization)
10. Hospital visits (hospitalization and/or emergency visits and coronary revascularization at 6 months)

11. The proportion of patients transfused and the number of blood products utilized (RBCs, plasma, platelets) (index hospitalization)
12. Seizures, defined as generalized or focal tonic-clonic movements consistent with seizure; or electroencephalogram demonstrating epileptiform discharges; or diagnosis of seizures by neurologist or neurosurgeon consultation (index hospitalization)
13. Encephalopathy, defined as unexpected delayed awakening or severely altered mental status (unconscious despite no sedative medication for more than 5 days), or encephalopathy documented by neurologist or neurosurgeon or psychiatrist consultation (index hospitalization)

# 4 STUDY POPULATION

## *4.1 Inclusion Criteria*

1. ≥18 and ≤65 years of age
2. Planned cardiac surgery using cardiopulmonary bypass
3. Informed consent obtained
4. Preoperative European System for Cardiac Operative Risk Evaluation (EuroSCORE I) of 6 or more (using the standard additive EuroSCORE I available at www.euroscore.org/calcold.html or refer to the study Manual of Operations)

## *4.2 Exclusion Criteria*

1. Patients who refuse participation
2. Patients who are unable to receive or who refuse blood products
3. Patients who are involved in a preoperative autologous pre-donation program
4. Patients who are having a heart transplant or having surgery solely for an insertion of a ventricular assist device
5. Pregnancy or lactation (a negative pregnancy test must be obtained prior to randomization for women of childbearing potential)

# 5 STUDY DESIGN

## *5.1 Study Overview*

TRICS IV is an international, multi-centre, open-label randomized controlled trial of two commonly used RBC transfusion strategies in higher risk patients ≤65 years of age having cardiac surgery, using a superiority trial design. Patients will be identified from the cardiac surgical schedule, or in the preoperative anesthesia or cardiac surgery clinics or ward at each hospital where informed consent will be obtained.

## *5.2 Randomization Procedure*

Randomization will be centralized, web-based and generated by the Applied Health Research Centre (AHRC) at the Li Ka Shing Knowledge Institute of St. Michael's Hospital, Unity Health Toronto. Randomization will have a 1:1 ratio, will be based on random permuted blocks of varying sizes, and will be stratified by centre and age. We will use web-based electronic case report forms (eCRFs), which can be accessed by each site, to randomly allocate participants to interventions.

## *5.3 Blinding*

The study allocations cannot feasibly be blinded, and therefore this is designed as an open label trial. Adjudication of postoperative myocardial infarction, cerebrovascular accident, infectious and renal outcomes will be blinded, nonetheless.

## *5.4 Premature Discontinuation*

Patients have the right to discontinue from the study or study transfusion strategy for any reason, at any time.

All participants who are randomized and undergo surgery will be followed for the remainder of the trial up to the 6-month outcome, unless the participant withdraws consent for follow up.

Any participant who is randomized but for any reason does not undergo surgery will not be followed and data will not be collected longitudinally. In this situation, the participant will be replaced to achieve the required sample size.

## *5.5 Concomitant Treatment*

There are no other study related restrictions on concomitant medications, treatments or procedures.

# 6 STUDY INTERVENTION

## *6.1 Description of the Study Intervention/Transfusion Strategies*

Study participants will be randomized to one of the following transfusion strategies in a 1:1 manner:

- ***Restrictive transfusion strategy:*** patients will receive a RBC transfusion if their Hb concentration is <75 g/L (<7.5 g/dL; <4.7 mmol/L) intraoperatively and/or postoperatively.
- ***Liberal transfusion strategy:*** patients will receive a RBC transfusion if their Hb concentration is <95 g/L (<9.5 g/dL; <5.9 mmol/L) intraoperatively, or postoperatively in the ICU; or <85 g/L (<8.5 g/dL; <5.3 mmol/L) on the ward.

The study period for intervention will commence on induction of anesthesia for the index cardiac surgery. When the appropriate Hb trigger is reached, patients in each group will have one unit of RBCs administered followed by repeat determination of the Hb concentration. Each group will be transfused only if their Hb concentration falls below the transfusion threshold. The Hb should be checked before each unit of blood.

The RBC transfusion must take place within the following time frame after the time that the trigger Hb was measured: 2 hours (for patients in the operating room (OR)), 18 hours (in the ICU/Cardiac Care Unit (CCU) or other intensive care environment, including step-down units), or 40 hours (on the hospital ward).

If the investigator decides to perform another intervention, for example re-measuring the Hb or hemoconcentration, and a second Hb value is measured above the trigger within the protocol-defined time period (2, 18 or 40 hours, as above), then a transfusion is not required and this would still be considered an adherent "trigger event" (see section 6.4).

Although it is desirable to adhere to the standard triggers, the assigned Hb trigger can be increased to 80 g/L (8.0 g/dL; 5.0 mmol/L) in the restrictive transfusion group or 100 g/L [(10.0 g/dL; 6.2 mmol/L) intraoperative/ICU] / 90 g/L [(9.0 g/dL; 5.6 mmol/L) on the ward] in the liberal transfusion group during an episode of any of the following severe physiologic derangements:

- Mixed/central venous or cerebral oxygen saturation <50%
- Lactate > 4 mmol/L in the absence of the use of epinephrine
- Definitive clinical evidence of active myocardial ischemia as defined by the 4$^{th}$ Universal Definition[42]
- Severe organ dysfunction or failure (heart, kidney, or brain) despite use of two or more inotropic agents or a mechanical circulatory assist device (examples include refractory heart failure or cardiogenic shock, anuria or impending dialysis, or severe neurologic dysfunction requiring acute intervention).

The Hb trigger can be increased due to physiologic derangements above for a maximum of 48 hours or until the physiologic derangement improves, whichever occurs first. After 48 hours, the originally assigned trigger will be resumed and all RBC transfusion administered outside of the assigned strategy will be considered non-adherent.

## *6.2 Duration of Intervention*

The assigned transfusion strategy will be applied starting from the time the patient enters the OR for the index cardiac surgical procedure, and will be continued until hospital discharge (based on the index cardiac surgery).

Patients who return to the ICU or the OR after being transferred to the ward in the liberal strategy group will be transfused according to the transfusion strategy for those settings (i.e.<95 g/L; <9.5 g/dL; <5.9 mmol/L).

## *6.3 Measurement of Hemoglobin*

While central laboratory measurement of Hb is preferred, any validated method for determining Hb (using co-oximetry, spectrophotometry) may be used for the purpose of determining transfusion triggers and measuring post-transfusion Hb values (e.g. central laboratory, blood gas machine, approved point-of-care.) Sites may check with the coordination centre (CC) if there is any uncertainty about approved point-of-care testing.

## *6.4 Adherence to Transfusion Strategy and "Trigger Events"*

A **"trigger event"** is defined as follows: an occurrence which starts when a Hb value is measured below the assigned trigger for the first time since any previous event, and ending either (1) when a RBC transfusion is administered, or (2) a Hb value is recorded above the assigned threshold, or (3) after the protocol-defined time period of 2 hours (in the OR), 18 hours (in the ICU/CCU), or 40 hours (on the ward), **whichever comes first.**

All "trigger events" will be recorded in the eCRF.

At the trigger event level, **adherence** will be considered to have occurred if (1) a RBC transfusion is started within the protocol-defined time period, or (2) a Hb value above the threshold is measured within the protocol-defined period.

**Non-adherence** will be considered to have occurred if (1) a RBC transfusion is given without a protocol-defined Hb trigger being met, or (2) a RBC transfusion is <u>not</u> given subsequent to a "trigger event", and the Hb remains below the threshold at the end of the protocol-defined period (or a repeated Hb value was not performed during the protocol-defined time period).

In the event that two (or more) units of RBCs are administered in parallel or immediately consecutively, without measuring the Hb value between units, and if the first Hb value measured after the RBC transfusion of these multiple units is still lower than the transfusion trigger, then the administration of each unit will be considered adherent. If the first Hb value after the transfusion of these multiple RBC units is above the trigger, then the initial RBC transfusion would be considered adherent, and any additional units given *after* the initial unit (and before any subsequent Hb measurements) would be considered non-adherent.

Adherence to transfusion strategies will be closely monitored throughout the study, and it is expected that sites will maintain an acceptable adherence rate.

### *6.5 Temporary Protocol Suspensions*

Patients who have rapid blood loss, or are hemodynamically unstable (e.g. a systolic blood pressure <80 mmHg or the need of two or more inotropes) due to blood loss, can be transfused RBCs at the discretion of the attending physician, and the protocol may be temporarily suspended. These patients will not be withdrawn from the study. The protocol will be resumed immediately after such an event and RBC transfusions administered during this time will not be considered a breach of protocol or non-adherent (although all transfusions will be recorded in the eCRF). The RBC transfusion protocol can be suspended for acute bleeding for a maximum of 24 hours or until surgical hemostasis, whichever occurs first. After 24 hours, the protocol will be resumed and all RBC transfusion administered outside of the assigned strategy will be considered non-adherent.

### *6.6 Other (non-red-cell) Transfusions*

Other blood products may be administered based on institutional protocols but should require the presence of ongoing bleeding and/or documented measurement of abnormal coagulation. The non-RBC product transfusions should be targeted toward the hemostatic abnormality in accordance with published guidelines and generally accepted practice. Plasma (15 ml/kg) or Prothrombin Complex Concentrate should be considered in the presence of hemorrhage with an international normalized ratio (INR) >1.5 if there is ongoing bleeding. Cryoprecipitate or fibrinogen concentrate is suggested in the presence of bleeding and a fibrinogen concentration <1.5 g/L. Platelet transfusion should be considered when there is bleeding with a platelet count less than 80,000 x $10^9$/L or in the presence of documented abnormalities of platelet function. Information about non-red-cell and hemostatic factor concentrate transfusions will be collected in the eCRF (e.g. platelets, rFVIIa, fibrinogen concentrate, prothrombin complex concentrates).

# 7 CONDUCT OF THE STUDY

After eligibility is confirmed and informed consent is obtained, the following assessments will occur. See "Schedule of Events" (section 15).

## *7.1 Screening (-90 days to 0 days before the index surgical event)*

- Written informed consent
- Eligibility criteria including EuroSCORE I and pregnancy test (dipstick or beta HCG) for women of childbearing potential

## *7.2 Preoperative/baseline assessments (-30 days to 0 days before the index surgical event)*

- Recheck eligibility if >30 days since initial screening
- EuroSCORE I
- Standard 12-lead Electrocardiogram (ECG)
- Demographics
- Medical/Cardiac History
- Height and Weight measurements
- Concomitant Medications
- Preoperative laboratory values (preoperative value closest to the index surgical date, no more than 30 days prior to the surgery date)
- Randomization through the eCRF; *whenever feasible, randomization will occur no more than 24 hours prior to the scheduled surgery to minimize the risk of losing randomized patients due to unplanned surgery cancellations*

## *7.3 Index Cardiac Surgery (Day 0)*

- Surgical/procedure details
- Perioperative medications (anti-fibrinolytics, vasoactive medications) and intravenous (IV) fluids
- Reporting of all protocol-defined "trigger events" (see section 6.4) and transfusions
- Clinical events (see section 8.12.1)
- Intraoperative laboratory values

## *7.4 Postoperative period (Day of surgery until hospital discharge or postoperative day 28, whichever comes first)*

- Reporting of all protocol-defined "trigger events" (see section 6.4) and transfusions
- Clinical events (see section 8.12.1)
- Standard 12-lead ECGs will be performed on the day after surgery and on day 4-6 postoperatively or at hospital discharge, whichever comes first
- Postoperative laboratory values

### *7.5 6 month Follow-up*

- Clinical events (see section 8.12.2)
- Emergency room (ER) visits and hospital admissions since discharge from the index hospitalization
- Obtain the last available standard 12-lead ECG done for clinical purposes
- Concomitant Medications

# 8 CLINICAL PROCEDURES AND SAFETY EVALUATIONS

## *8.1 Informed Consent*

Once deemed an appropriate candidate for the study, the patient will be informed of the possibility of study participation. The benefits and risks of participating in the study will be explained to the patient, and the patient will be provided an opportunity to read the informed consent form and ask any questions he/she may have. Prior to conducting any study-related procedures, the patient must provide consent to participate by signing the Institutional Review Board/Research Ethics Board (IRB/REB) approved consent form.

## *8.2 Demography*

This includes date of birth, sex and ethnicity.

**In Canada**, participants will be asked to provide their provincial Health Card Number (HCN) for future linkage with administrative health data (through the Canadian Institute of Health Information) to perform more in-depth analyses of health care utilization (hospitalizations and ER visits) within the 6 months after the index cardiac surgery.

## *8.3 Medical/Cardiac History*

Data will be collected from patients at baseline consisting of demographics, previous cardiac investigations, and previous cardiac and non-cardiac history.

## *8.4 Pregnancy test*

A negative pregnancy test (dipstick or beta HCG) is required to be obtained prior to randomization for women of childbearing potential.

## *8.5 Standard 12-lead ECG*

A 12-lead ECG will be performed preoperatively, on the day after surgery and on day 4-6 postoperatively or at hospital discharge (whichever comes first). We will also collect the last ECG performed for clinical purposes up to the 6 month follow-up visit.

## *8.6 Body weight and height*

Body weight and height will be recorded at baseline.

## *8.7 EuroSCORE I*

The preoperative EuroSCORE I will be collected in the eCRF.

## *8.8 Surgical details*

Details about the type of surgical procedure, interventions, duration and complications will be recorded including type of cardioplegia, blood loss, use of hemofiltration, duration of cardiopulmonary bypass and aortic cross-clamp, presence of arrhythmias, and the use of intra-

aortic balloon pump/ventricular assist device. In addition, the type and volume of IV fluids administered in the first 24 hours after induction of anesthesia will be collected in the eCRF.

### *8.9 Medications*

Anti-fibrinolytic agents, vasoactive medications and type and volume of IV fluid administered will be recorded in the eCRF for the intraoperative and postoperative periods.

### *8.10 Laboratory tests*

The following laboratory values are standard of care at most clinical institutions, and the laboratory data will be captured in the eCRF as follows:

Preoperative laboratory values: Hb, hematocrit (HCT), platelets, red cell distribution width, INR, activated partial thromboplastin time (aPTT), fibrinogen, creatinine, and creatine kinase–MB (CK-MB) or troponin (preferred)

Intraoperative laboratory values: Lowest Hb (pre/during CPB and chest closure), lowest HCT (pre/during CPB and chest closure), lowest arterial and mixed venous $pO_2$ (pre/during CPB and chest closure), lowest arterial and mixed venous $O_2$ saturation (pre/during CPB and chest closure), lowest cardiac index (pre-CPB and chest closure) if measured, minimum pump flow (during-CPB)

Postoperative laboratory values:

- **Hb** is required to be assessed on postoperative days 1, 2, 5 ±2, and 11 ±3 while in hospital. Hb measurements that are done for clinical purposes will be collected, if more than one Hb is measured on a particular day, the lowest value for that day will be recorded in the eCRF. Any patient who is transfused, is required to have a pre- and post- transfusion Hb measurement.
- **CK-MB or troponin** (preferred) is required to be assessed on the first postoperative day, approximately 24 hours after the end of surgery.
- **Creatinine** is required to be measured on postoperative days 1, 2, 3 and 5, and otherwise as clinically indicated.
- **Other laboratory values** will be performed as clinically indicated or as per local guidelines and collected in the eCRF up to hospital discharge/postoperative day 28: HCT, platelets (within 2 hours of chest closure), INR, aPTT and fibrinogen (within 2 hours of chest closure), CK-MB or troponin (preferred), arterial and mixed venous $O_2$ saturation, arterial and mixed venous $pO_2$.

### *8.11 Transfusions and "Trigger Events"*

All protocol defined "trigger events" (per section 6.4) and all RBC transfusions will be recorded in the eCRF from the start of the index surgical procedure until hospital discharge, as well as the pre- and post-event/transfusion Hb times and values.

All non-RBC transfusions administered during the index hospitalization period will be reported in the eCRF.

## *8.12 Clinical Events*

### *8.12.1 Clinical events from the start of surgery until hospital discharge or postoperative day 28, whichever comes first*

The following clinical events will be assessed through review of the patient medical records/chart, and reported in the eCRF from the start of the index cardiac surgery until discharge or postoperative day 28, whichever comes first. All definitions are standard definitions.[42-46]

- **All-cause mortality**
- **Myocardial infarction** which will be defined in accordance with the 4th Universal Definition of Myocardial Infarction[42]

a) **Early perioperative MI** (intraoperatively or within 48 hours after surgery): Detection of cardiac troponin (preferred) or CK-MB values more than 10 times the 99th percentile of the upper limit from a normal baseline* (i.e. the 99th percentile or less of the upper limit of normal) during the first 48 hours following surgery, and at least one of the following:
   - development of new pathological Q waves on the ECG
   - imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
   - identification of a coronary dissection, new intracoronary thrombus of the new graft or native coronary artery(ies) by angiography or autopsy
   - Although a new left bundle branch block is not part of the 4th Universal Definition, we will include the collection of left bundle branch block to permit comparison to data collected in TRICS III that complied with the 3rd Universal Definition, which included left bundle branch block

*or, if preoperative cardiac enzymes are elevated, the postoperative enzymes are increased by more than 10 times the upper limit of normal (ULN) from the baseline value (i.e. greater than baseline plus 10 x the ULN).

b) **Late perioperative MI** (more than 48 hours after surgery): Detection of a rise and/or fall of cardiac troponin (preferred) or CK-MB values, with at least one value above the upper limit of normal, and with at least one of the following:
   - symptoms of ischemia, as long as the symptoms/signs are not explained by another proven clinical condition (pulmonary embolism, myocarditis, etc.)
   - new, or presumed new, significant ST-T wave changes or new left bundle branch block
   - development of new Q waves on the ECG
   - imaging evidence of new loss of viable myocardium or new regional wall motion abnormality
   - identification of a new intracoronary thrombus of the new graft or native coronary artery(ies) by angiography or autopsy

*Percutaneous coronary intervention (PCI) related MI* is included in the late MI group, and is arbitrarily defined by elevation of troponin values more than 5 times the 99th percentile of the upper limit from a normal baseline, or a rise of troponin values more than 20% if the baseline values are elevated and are stable or falling. In addition, either (i) symptoms suggestive of myocardial ischemia, or (ii) new ischemic ECG changes, or (iii) angiographic findings consistent with a procedural complication or (iv) imaging demonstration of new loss of viable myocardium or new regional wall motion abnormality are required.

  - **New onset renal failure requiring dialysis** (excluding dialysis during CPB)
  - **New focal neurological deficit (stroke)** lasting more than 24 hours confirmed by clinical assessment and brain imaging

- **Infection:** infection will be defined as septic shock with positive blood cultures; pneumonia defined as autopsy diagnosis or roentgenographic infiltrate and at least two of the following three criteria: fever, leukocytosis, and positive sputum culture; and/or deep sternal or leg wound infection requiring intravenous antibiotics and/or surgical debridement[44]
- **Acute kidney injury:** defined by the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline[46] - a 50% increase in serum creatinine within 1 week or a 26.5 µmol/L increase within 48 hours
- **Delirium:** based on one of the following criteria: CAM/CAM-ICU (even on 1 occasion), or ICDSC > 3, or 3D-CAM, or 4AT ≥4, or more than one dose of haloperidol or similar antipsychotic drug, or documented delirium by neurologist or neurosurgeon or psychiatrist consultation
- **Gut infarction:** confirmed by imaging (e.g. angiography), autopsy, or through surgical means
- **Seizures:** defined as generalized or focal tonic-clonic movements consistent with seizure; or EEG demonstrating epileptiform discharges; or diagnosis of seizures by neurologist or neurosurgeon consultation
- **Encephalopathy**: defined as unexpected delayed awakening or severely altered mental status (unconscious despite no sedative medication for more than 5 days), or encephalopathy documented by neurologist or neurosurgeon or psychiatrist consultation

### *8.12.2 Clinical events at 6 month follow-up visit*

At 6 months, the following clinical outcomes will be collected based on a follow-up visit:

- All-cause mortality
- MI
- New onset renal failure requiring dialysis
- Stroke
- Surgical or non-surgical coronary revascularization
- COVID-19 test result (if done for clinical purposes)

# 9 EVALUATION OF STUDY RESULTS

## *9.1 Primary Endpoints*

The primary outcome is primary composite endpoint of all-cause mortality, myocardial infarction defined in accordance with the 4th Universal Definition of Myocardial Infarction, new-onset renal failure with dialysis, or new focal neurological deficit (stroke) lasting more than 24 hours confirmed by clinical and/or computed tomographic scan, occurring within 6 months of the initial surgery.

## *9.2 Secondary Endpoints*

The secondary endpoints are as follows; see section 8.12 for definitions and details:

1) Incidence of each individual component of the primary outcome: all-cause mortality, myocardial infarction, new onset renal failure requiring dialysis, and new focal neurological deficit (stroke) within 6 months
2) Composite and individual all-cause mortality, myocardial infarction, new onset renal failure requiring dialysis, and new focal neurological deficit (stroke) with index hospitalization or after 28 days postoperatively, whichever comes first
3) Length of stay in the Intensive Care Unit (ICU) and hospital (index hospitalization)
4) Prolonged low output state defined as the need for two or more inotropes for 24 hours or more, intra-aortic balloon pump or ventricular assist device postoperatively (index hospitalization)
5) Duration of mechanical ventilation (index hospitalization)
6) Infection: defined as septic shock with positive blood cultures; pneumonia defined as autopsy diagnosis or roentgenographic infiltrate and at least two out of three of the following criteria: fever, leukocytosis, and positive sputum culture; and/or deep sternal or leg wound infection requiring intravenous antibiotics and/or surgical debridement (index hospitalization)
7) Acute kidney injury (AKI), defined by the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guideline[46] - a 50% increase in serum creatinine within 1 week or a 26.5 µmol/L increase within 48 hours (index hospitalization)
8) Delirium, based on one of the following criteria: Confusion Assessment Method (CAM) or CAM-ICU (even on 1 occasion), or Intensive Care Delirium Screening Checklist (ICDSC) > 3, or 3D-CAM, or 4AT ≥4, or more than one dose of haloperidol or similar antipsychotic drug, or documented delirium by neurologist or neurosurgeon or psychiatrist consultation (index hospitalization)
9) Gut infarction confirmed by imaging (e.g. angiography), autopsy, or through surgical means (index hospitalization)
10) Hospital visits (hospitalization and/or emergency visits and coronary revascularization at 6 months)

11) The proportion of patients transfused and the number of blood products utilized (RBCs, plasma, platelets) (index hospitalization)

12) Seizures, defined as generalized or focal tonic-clonic movements consistent with seizure; or electroencephalogram demonstrating epileptiform discharges; or diagnosis of seizures by neurologist or neurosurgeon consultation (index hospitalization)

13) Encephalopathy, defined as unexpected delayed awakening or severely altered mental status (unconscious despite no sedative medication for more than 5 days), or encephalopathy documented by neurologist or neurosurgeon or psychiatrist consultation (index hospitalization)

# 10 STATISTICAL METHODS

## *10.1 Sample Size Estimation*

TRICS IV is a superiority trial powered for the primary composite endpoint of all-cause mortality, myocardial infarction, new-onset renal failure with dialysis, or new focal neurological deficit (stroke), within 6 months after the initial surgery. A RR of 0.70, with an incidence rate for the primary endpoint of 14% in the liberal and 20% in the restrictive transfusion arm was used for the sample size calculation, which is in line with observed rates in the modified intention-to-treat (ITT) analysis of TRICS III in individuals younger than 69 years old[12]. As observed in TRICS III, we assumed that 1.5% of the patients would be lost to follow-up by 6 months. With a 1:1 allocation ratio and a two-sided $\alpha$=0.05, we found that enrolment of a total of 1,440 patients (720 per group) would provide 85% power to detect a RR of 0.70 for the liberal as compared with the restrictive transfusion strategy while accounting for a 1.5% attrition rate and one interim analysis of the primary outcome. To ensure that the main analysis of the primary outcome has at least 80% power, we will conduct a conditional power analysis after 50% of the recruited participants have their primary outcome data available. If the conditional power is ≥60% but <80%, we will re-estimate the sample size needed so that the main analysis of the primary outcome has 80% power, and recruitment of participants will continue until the re-estimated sample size is reached. For all other values of conditional power, we will continue recruitment until the originally planned target sample size of 1,440 patients is reached.

We may include the first 100 randomized participants in an internal pilot assessment of the adherence to Hb triggers that utilize physiological changes for initiation of RBC transfusion.

## *10.2 Analysis population*

The ITT population consists of all patients who underwent randomization and subsequent cardiac surgery with available outcome data.

The per-protocol (PP) population consists of all participants who underwent randomization and subsequent cardiac surgery with cardiopulmonary bypass with available outcome data, except for patients who had a protocol adherence of less than 90%, patients who were withdrawn from the trial by the treating physician at any time, and patients who withdrew consent.

## *10.3 Baseline characteristics*

The baseline characteristics of the patients will be summarized by group. Continuous variables will be summarized using mean and standard deviation, or median and inter-quartile range if data do not have a symmetric distribution. Categorical variables will be summarized with counts and percentages. No statistical comparisons of participant characteristics at baseline will be performed between groups.

## *10.4 Primary Outcome Analyses*

The primary analysis of the primary outcome will be based on a chi-squared test. Superiority of liberal over restrictive transfusion will be declared with a p-value<0.05. A secondary analysis of the primary outcome will be based on a Cox regression model to derive hazard ratios (HR) with 95% confidence intervals, with the time to event presented per group using Kaplan Meier curves.

## *10.5 Secondary and Tertiary Outcomes and Safety Analyses*

Separate analysis plans will be prepared for the renal outcomes and health economic outcomes. A separate plan will also be prepared to examine alternative ways to analyze adherence to the transfusion strategies, as well as any future exploratory analyses. For the secondary outcomes, the treatment effect and two-sided 95% CI for each outcome will be estimated by unadjusted analyses using the same variables as for the primary outcome unless otherwise stated, as appropriate. Both PP and ITT analyses of the secondary outcomes will be performed.

For dichotomous outcomes, risk differences and odds ratios along with 95% confidence intervals will be reported for the unadjusted analyses while the odds ratio will be used for adjusted analyses employing logistic regression. For continuous outcomes, mean differences and adjusted mean differences (from linear regression models) will be reported along with 95% confidence intervals. Other outcomes such as length of hospital and ICU stay, and duration of mechanical ventilation may be inappropriate for standard linear regression models, in which case a transformation (e.g. logarithm) or alternate model (e.g. time-to-event) will be considered. Adjusted analyses will be performed using the appropriate regression framework. Time to death at 6 months will be summarized by Kaplan-Meier curves.

Adherence will be summarized by group; we will also describe the distribution of the location of the non-adherent events (operating room, ICU, ward). The mean hemoglobin value (a) prior to non-adherent RBC transfusion events and (b) for non-adherent trigger events will be reported, by group.

## *10.6 Interim Analyses*

One interim analysis will be undertaken after 50% of patients have reached determination of the primary outcome. A group sequential design has been employed that applies a one-sided boundary. The boundary is based on a Hwang-Shih-DeCani spending function for efficacy. If the boundary is crossed, there will be a non-binding recommendation to stop the trial for benefit. We will only conduct additional safety reviews if otherwise directed by the Data and Safety Monitoring Board (DSMB). The following table shows the Z-statistics for the non-binding recommendation to stop the trial due to benefit.

| **Analysis** | **Z-statistic** |
|---|---|
| **Interim** | 2.75 |
| **Final** | 1.98 |

## *10.7 Planned Subgroup Analyses*

We will conduct subgroup analyses of the primary outcome to determine if the effect of the transfusion strategy varies according to the subgroups below. These analyses will be exploratory only. Subgroup analyses will be accompanied by a test for a treatment by subgroup interaction. Additionally, subgroup specific treatment effects and treatment by subgroup interactions will be estimated with an adjusted model using all of the covariates listed below and a single term for interaction between treatment and sub group defined by each covariate of interest. This procedure

will be repeated for each covariate to model adjusted subgroup specific estimates. Tests for trend across ordered subgroups of left ventricular function will be derived in an analogous manner. P values, effect estimates and 95% confidence intervals will be reported as estimated from crude and adjusted models.

Subgroup analyses will be performed based on the following criteria:

1. Age
2. Sex
3. Diabetes (with and without insulin)
4. Baseline creatinine >200 (as collected for baseline EuroSCORE I)
5. Baseline preoperative hemoglobin
6. Preexisting pulmonary disease (as collected for baseline EuroSCORE I)
7. Type of surgery: Coronary artery bypass graft (CABG) only, CABG with another procedure, other procedure only (non-CABG)
8. Left ventricular function (Good, Moderate, Poor, Very Poor)

### *10.8 Sensitivity Analysis*

We will fit a random-effects model that accounts for the variation between sites in the between-group difference of the primary composite outcome. We will also conduct an analysis of the primary composite outcome adjusted for variables that have a clinically significant difference between groups at baseline. In additional sensitivity analyses, we will determine whether analyses of specific patient subsets, as defined below, yield similar results for the primary outcome:

1. Excluding patients with any non-adherence to the assigned transfusion strategy
2. Excluding patients whose hemoglobin was never measured below 95g/L from the time of randomization to the end of the index hospitalization period
3. Excluding patients who did not receive any RBC transfusions from the time of randomization to the end of the index hospitalization period
4. Excluding patients who had at least one protocol suspension from the time of randomization to the end of the index hospitalization period
5. Excluding patients randomized according to physiologic triggers
6. Including only patients of the PP population

### *10.9 Missing Values*

While we anticipate that the primary outcome will be available in >95% of patients, if the primary outcome is missing in more than 5% of patients we will perform analyses to mitigate the effect of missing data as follows: we will develop a missing data model and perform a multiple imputation analysis. We will also carry out inverse-probability weighting. This is a two-stage model where a logistic regression model is first used to estimate the probability of not being missing. Then the analysis of the outcome proceeds on complete cases through a weighted analysis where the inverse probability of "completeness" forms the weights. The inference of the imputed model and inverse-probability weighting will be compared to that of the model with missing data.

# 11 ORGANIZATIONAL STRUCTURE

## *11.1 Participating Sites*

A list of participating sites will be maintained by the CC (Coordination Centre). Sites will be located within Canada and internationally, and it is estimated that approximately 65 clinical sites will be involved.

## *11.2 Coordination Centre*

The CC is located in the AHRC, Li Ka Shing Knowledge Institute of St. Michael's Hospital, Unity Health Toronto (www.ahrconline.ca) at the University of Toronto. The CC will be responsible for developing and programming the electronic eCRFs, trial procedure manuals, trial documentation, data monitoring, data management and analysis, and providing progress and data reports to the Executive Committee (EC), DSMB, and participating sites.

## *11.3 Executive Committee*

The EC will include the Principal Investigators and co-Principal Investigators. The EC will be responsible for providing clinical and methodological guidance, including overall study design, execution, analysis, and publication of the main study results. The EC will oversee the management of the clinical trial sites and will also provide guidance around the publication strategy. While the study is ongoing, the EC will approve any protocol amendment that may become necessary and is responsible for maintaining the scientific integrity of the study.

## *11.4 Steering Committee*

The trial Steering Committee (SC) will consist of the EC and at least one representative of each of the geographic regions of Investigators (e.g. Canada, Australia, United States, United Kingdom, South America, Europe, Asia, and Africa). The Steering Committee will meet by teleconference regularly throughout the trial period to discuss enrollment rates and non-adherence, and at the completion of the study to provide operational insight and assist with issue resolution. A list of committee members will be maintained by the CC.

## *11.5 Clinical Adjudication Committee (CAC)*

This committee consists of at least three members of appropriate clinical background (cardiac surgeons, cardiologists, hematologists, anesthesiologists, or intensivists) who have been appointed by the EC. The members of the CAC will review and adjudicate clinical outcomes in a blinded manner according to a manual of procedures approved by the CAC and the EC. Sites will be requested to provide de-identified clinical documents for review by the CAC.

## *11.6 Data and Safety Monitoring Board*

An independent DSMB will be assembled to ensure patient safety, receive and review interim analyses, provide feedback to the SC, and ensure the study follows the highest ethical standards. The DSMB will be provided data on safety regularly throughout the trial period, in accordance

with the DSMB Charter. The safety data will include all clinical events (per section 8.12.) We will only conduct additional safety reviews if otherwise directed by the DSMB.

The DSMB will consider clinical and statistical significance, consistency of data over time, consistency of the direction of risk and benefit-risk ratios if there is consideration for recommendation for early trial discontinuation. The DSMB will have the ability to request additional safety analyses or additional interim analyses, and to convene a full committee meeting and make any further recommendations to the steering committee about the safe conduct of the trial after considering all the available data and any new external data from relevant studies. The DSMB will have access to the randomization codes, if needed.

# 12 DATA COLLECTION AND MONITORING

## *12.1 Case Report Forms*

Electronic data capture (REDCap™) will be used for this trial, meaning that all study data will be entered in eCRFs at the investigational site. Data collection will be completed by authorized study site personnel designated by the site Investigator. Appropriate training and security measures will be completed with the site Investigator and all authorized study site personnel prior to the study being initiated and any data being entered into the system for any study subjects. The study data will be housed on a secure in-house server at St. Michael's Hospital in Toronto throughout the duration of study, and up to 15 years after the study is complete.

## *12.2 Data Collection and Cleaning*

All eCRF corrections are to be made by an Investigator or other authorized study site personnel. Prior to database lock, the site Investigator/co-Investigator must confirm that he/she has reviewed the data, and that the data are complete and accurate. Data validation procedures will be described in detail in the Data Management Plan.

# 13 INVESTIGATOR RESPONSIBILITIES AND OBLIGATIONS

## *13.1 Local Ethics Review Board*

This study will be carried out in accordance with the current International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (GCP), Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans, version 2 (TCPS2) and applicable local laws and regulations.

According to local laws and regulations, the study protocol and the patient informed consent form (in the local language) must be approved by a local IRB/REB for each participating centre.

It is the responsibility of the site Investigator to submit the protocol for institutional review. A copy of the letter of approval from the local IRB/REB must have been received by the CC prior to activation of the investigational site. Major changes to the protocol, as well as a change of a site Investigator, must be approved by the local IRB/REB and documentation of this approval must be provided. Records of the local IRB/REB review and approval of all documents pertaining to this study must be kept on file by the site Investigator.

## *13.2 Informed Consent and Patient Protection*

It is an obligation of the site Investigator to obtain informed consent from every study patient by means of a dated and signed informed consent form before any study related procedure is performed.

'Informed consent' also implies individual discussion with the patient about the nature of study interventions to be conducted in a language that is easy to comprehend. The patient should fully understand that his/her refusal to participate in the study will not affect the quality of medical care. In addition, the patient must be informed that, without disclosing his/her name, relevant medical data will be disclosed to the CC and that his/her medical records may be inspected during on-site monitoring.

The patient should be informed in writing that his/her medical data relevant to this study will be stored and analyzed while maintaining confidentiality in accordance with local data protection laws. All data transferred to the eCRF and any process derived from the eCRF will be de-identified.

The study patient should also be informed in writing about the possibility of audits by authorized representatives of the CC or the Sponsor or a designee in which case a review of those parts of the hospital records relevant to the study may be required.

## *13.3 Study Protocol Adherence and Modifications*

Site Investigators must read, understand and follow the study protocol. The same applies to instructions given in the eCRF and to any additional instructions issued through the CC. Changes to the protocol should only be made by the EC in the form of protocol amendments. The CC is responsible for the distribution of any protocol amendments to site Investigators. Site Investigators are responsible for the distribution of an amendment to all staff involved in the study and to the local IRB/REB.

### *13.4 Data Collection and Documentation*

For every patient, the hospital or clinic file must clearly indicate that the patient has given informed consent and participated in the study. All entries in the eCRFs must be backed up by source data. Source data must be made available if requested by the EC or CC. The eCRFs must be completed in a timely manner. All study records should be kept in accordance with applicable national laws and regulations.

### *13.5 Records Retention*

The site Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. The investigator study file will contain the protocol/amendments, REB/IRB and governmental approval (if required) with correspondence, sample informed consent, staff curriculum vitae and authorization forms and other appropriate documents/correspondence, etc. Should the site Investigator wish to assign the study records to another party or move them to another location, the CC must be notified in advance. Trial records at each site should be stored as per local requirements or for 7 years after publication of the trial, whichever is longer.

### *13.6 Confidentiality of Trial Documents and Patient Records*

The site Investigator must assure that patient confidentiality will be maintained and that participant identities shall be protected from unauthorized parties. In eCRFs or other documents submitted to the CC, patients should not be identified by their names, but by their subject identification code, which is assigned in the eCRF. The Investigator should keep a patient enrolment log relating codes to the names of patients. The Investigator should maintain study documents that are not for submission to the CC (e.g. patients' signed consent forms), in strict confidence.

### *13.7 Direct Access to Source Data/Documents*

The site Investigator shall supply the EC, CC or delegate on request with any required background data from the study documentation or clinic records.

### *13.8 Trial Network Registration*

The study is registered on clinicaltrials.gov (NCT04754022).

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# 15 SCHEDULE OF EVENTS*

| Assessment | Screening | Preoperative | Index Cardiac Surgery | Postoperative (ICU, Ward) | 6 months |
|---|---|---|---|---|---|
| Study Day/Month | -90 to 0 | -30 to 0 | Day 0 | Postoperative period: day of surgery until hospital discharge or postoperative day 28 | 6 month +/- 21 days after Day 0 |
| **Observation/Procedure** | | | | | |
| Informed Consent | X | | | | |
| Inclusion/Exclusion | X | | | | |
| Pregnancy Test[1] | X[1] | | | | |
| Demographics | | X | | | |
| Medical/Cardiac History | | X | | | |
| Height and Weight | | X | | | |
| EuroSCORE I | X | X | | | |
| Randomization | | X | | | |
| Surgical Details | | | X | | |
| Intervention (transfusion strategy) Applied[2] | | | XXXXXXXXXXXXXXXX[2] | | |
| Trigger Event & Transfusion Details Collected[2] | | | XXXXXXXXXXXXXXXX[2] | | |
| Preoperative Laboratory Values[3] | | X[3] | | | |
| Intraoperative Laboratory Values[4] | | | X[4] | | |
| Postoperative Laboratory Values[5] | | | | X[5] | |
| ECG[6] | | X[6] | | X[6] | X[6] |
| Concomitant Medications | | X | | | X |
| Clinical Events | | | XXXXXXXXXXXXXXX | | X[7] |

[1]Women of childbearing potential.

[2]The assigned transfusion strategy will be applied from the time of index surgery until hospital discharge from the index surgery.

[3]Hb, hematocrit, platelets, INR, aPTT, fibrinogen, creatinine, and CK-MB or troponin (preferred).

[4]Lowest Hb (pre/during CPB and chest closure), lowest HCT (pre/during CPB and chest closure), lowest arterial and mixed venous $pO_2$ (pre/during CPB and chest closure), lowest arterial and mixed venous $O_2$ saturation (pre/during CPB and chest closure), lowest cardiac index (pre-CPB and chest closure) if measured, minimum pump flow (during-CPB).

[5]Hb must be assessed on postoperative days 1, 2, 5±2, and 11±3 while in hospital. Hb measurements that are done for clinical purposes will be collected, if more than one Hb is measured on a particular day, the lowest value for that day will be recorded in the eCRF. Any patient who is transfused, is required to have a pre- and post- transfusion Hb measurement. CK-MB or troponin (preferred) is required to be assessed on the first postoperative day, 24 hours after the end of surgery. Serum creatinine is required to be measured on postoperative days 1, 2, 3 and 5 and otherwise as clinically indicated. Other laboratory values will be performed as clinically indicated or as per local guidelines and collected in the eCRF up to hospital discharge/postoperative day 28: HCT, platelets (within 2 hours of chest closure), INR, aPTT and fibrinogen (within 2 hours of chest closure), CK-MB or troponin (preferred), arterial and mixed venous $O_2$ saturation, arterial and mixed venous $pO_2$.

[6]A 12-lead ECG will be performed preoperatively, on the day after surgery and on day 4-6 postoperatively or at hospital discharge, whichever comes first. We will also collect the last ECG performed for clinical purposes up to the 6 month follow-up visit.

[7]Includes ER visits and hospital re-admissions.

*For sites participating in the Hepcidin and Iron Storage Sub-Study, refer to the Schedule of Events in Protocol Addendum 1.

# TRICS IV Protocol Addendum 1_AUS:
# Hepcidin and Iron Storage Sub-Study

| | |
|---|---|
| Title: | **The relationship of hepcidin on patient outcomes after cardiac surgery**<br>A sub-study of the Australian participants in the TRICS IV study |
| Principal Investigators: | Dr Raymond Hu (Austin Health), Heidelberg, Vic, Australia<br>Prof David Scott (St Vincent's Health Melbourne), Fitzroy, Vic, Australia<br>Prof James Isbister (University of Sydney), NSW, Australia<br>Prof Alistair Royse (University of Melbourne), Parkville, Vic, Australia |
| Coordination Centre: | Applied Health Research Centre (AHRC) of St. Michael's Hospital, Unity Health Toronto (Toronto, ON, Canada), at the University of Toronto |
| Version: | Version 1.0 |
| Date: | 10-August-2021 |

## Signature Page for Lead Investigators

| | |
|---|---|
| Title: | **The relationship of hepcidin on patient outcomes after cardiac surgery**<br>A sub-study of the Australian participants in the TRICS IV study |
| Version: | Version 1.0 |
| Date: | 10-August-2021 |

Approved by the following:

10 August 2021

Dr Raymond Hu, Principal Investigator — Date (dd-mmm-yyyy)

10 August 2021

Prof David Scott, Principal Investigator — Date (dd-mmm-yyyy)

## Signature Page for Site Investigator

| | |
|---|---|
| Title: | **The relationship of hepcidin on patient outcomes after cardiac surgery**<br>A sub-study of the Australian participants in the TRICS IV study |
| Version: | Version 1.0 |
| Date: | 10-August-2021 |

I have read this protocol addendum and agree to conduct this sub-study in accordance with all stipulations of the protocol addendum and in accordance with Good Clinical Practice (GCP) and relevant local regulatory requirements that govern the conduct of clinical research.

| Site Investigator Name | Signature | Date |
|---|---|---|
| Please print | | (dd-mmm-yyyy) |

# TABLE OF CONTENTS

# 1 SUB-STUDY SUMMARY

| | |
|---|---|
| **Title** | **The relationship of hepcidin on patient outcomes after cardiac surgery**<br>A sub-study of the Australian participants in the TRICS IV study |
| **Principal Investigators** | Dr Raymond Hu (Austin Health), Heidelberg, Vic, Australia<br>Prof David Scott (St Vincent's Health Melbourne), Fitzroy, Vic, Australia<br>Prof James Isbister (University of Sydney), NSW, Australia<br>Prof Alistair Royse (University of Melbourne), Parkville, Vic, Australia |
| **Funding** | Funded primarily through Medical Research Future Fund (MRFF) |
| **Major Hypothesis** | Baseline hepcidin concentration is associated with clinical outcomes after cardiac surgery, when adjusted for other associated risk factors |
| **Minor Hypothesis** | Hemoglobin concentration at 30 days is associated with clinical outcomes after cardiac surgery, when adjusted for other associated risk factors |
| **Primary Endpoint** | Days Alive and Out of Hospital (DAOH) at 30 days (for major and minor hypothesis). |
| **Secondary Endpoints** | Composite outcome as for TRICS IV (all-cause mortality, myocardial infarction, new onset renal failure requiring dialysis or new focal neurological deficit (stroke) at 6 months (for major and minor hypothesis)<br>Hemoglobin concentration at 30 days (only for major hypothesis) |
| **Study Population** | All Australian participants in the TRICS IV trial |
| **Study Design** | Observational nested cohort sub-study |
| **Sample Size** | Target N = 500 |
| **Accrual Period** | 24 months recruitment + 6 months follow up |
| **Study Duration** | June 2021 – February 2024 (estimated) |
| **Study Intervention** | *None* |
| **Assessments** | As for TRICS IV |

# 2 SUB-STUDY DESCRIPTION

## *2.1 Background, Rationale and Hypothesis*

The iron regulatory protein, hepcidin, is responsible for controlling dietary iron absorption and body iron distribution. A group of 165 anaemic cardiac surgical patients was studied in the UK and plasma hepcidin concentration was the only hematological variable associated with outcome, with mean days alive and out of hospital 2.7 (95% CI 0.4 to 5.1) days less if hepcidin was ≥20 ng/mL compared with <20 ng/mL (p=0.024)[1]. However, other biomarkers that are intricately related to hepcidin have also been associated with patient outcomes in cardiac surgery such as iron storage markers, transfusion and anaemia[2,3,4,5]. Nevertheless, the precise relationship between these factors is not understood and has never been investigated in a transfusion randomised controlled trial. Additionally, many of these observational studies have not taken into account more complex biochemical analysis of iron status based on novel understandings of the significance of hemoglobin content of reticulocytes, soluble transferrin/log ferritin ratio and C-Reaction Protein[6,7,8] and other acute phase reactants such as fibrinogen.

Cardiac surgery is associated with a high incidence of prolonged postoperative anaemia[9], which has also been linked to adverse patient outcomes[9]. The contribution of baseline hepcidin status together with other hematinic variables including iron storage on prolonged postoperative anaemia has not been studied in this population. The opportunity to examine these relationships within a prospective cohort that are randomised to different protocolised transfusion thresholds allows for significant confounders to be removed.

The primary outcome metric for patient outcomes will be Days Alive and Out of Hospital (DAOH). This metric has been chosen as it encompasses both morbidity, mortality and patient functionality, and has utility as a clinically relevant patient-centred outcome[10]. Secondary outcome metrics are hemoglobin concentration at 30 days and pooled primary composite outcomes reported for TRICS IV all-cause mortality, myocardial infarction, new onset renal failure requiring dialysis or new focal neurological deficit at 6 months).

The investigation of hepcidin levels in our cohort together with a more complex biochemical analysis of iron status will enable us to test the hypothesis that hepcidin levels are associated with adverse outcomes after adjustment for hematological variables (iron status, transfusion status and hemoglobin level) and other clinical variables (EuroSCORE I). It will also allow us to explore the association of baseline hepcidin values with hemoglobin recovery 30 days after cardiac surgery. Additionally, the relationship between hemoglobin concentration at 30 days and DAOH at 30 days will also be explored.

## *2.2 Objectives*

Major hypothesis: To determine if baseline hepcidin values together with a more complete analysis of iron status is associated with clinical outcomes (see primary and secondary endpoints below) after cardiac surgery, when adjusted for other associated factors.

Minor hypothesis: To determine whether hemoglobin concentration at 30 days is associated with clinical outcomes (see primary and secondary endpoints below) after cardiac surgery, when adjusted for other associated factors.

### *2.3 Study Population*

All Australian participants in the TRICS IV trial.

### *2.4 Study Design*

Observational nested cohort sub-study.

### *2.5 Conduct of the Study*

All of the Australian arm of TRICS IV will be co-recruited to this sub-study. This will involve consenting to additional blood tests at -7 to 0 days to index surgery and blood tests at 30 days (see laboratory tests below). Consent will also be required for the collection of additional data: perioperative hemopoietic interventions, incidental laboratory values related to Patient Blood Management, estimated venesected blood loss in hospital (up to 30 days) and DAOH at 30 days. These will be derived from the medical records or from direct patient inquiry.

### *2.6 Clinical Procedures: Additional Data*

A table of the additional data that will be included is provided in section 5.

### *2.7 Evaluation of Study Results*

- Primary endpoints
    - Days Alive and Out of hospital (DAOH) at 30 days
- Secondary endpoints
    - Composite outcome as for TRICS IV (all-cause mortality, myocardial infarction, new onset renal failure requiring dialysis or new focal neurological deficit) at 6 months
    - Hemoglobin concentration at 30 days (not relevant for minor hypothesis)

### *2.8 Statistical Methods*

The Australian contribution is planned with recruitment of 500 patients, recruited over 3 years from up to 12 sites. With 50% of anaemic patients having elevated hepcidin levels[1], 150 anaemic patients would provide 80% power to detect a mean difference of 3 days alive and out of hospital (DAOH) between patients with functional iron deficiency (assuming an SD of 6.5 DAOH)[1]. If 30% of cardiac surgical patients are anaemic[11,] then 500 patients will confirm this finding whilst also substantially supporting recruitment for TRICS IV.
Secondary analysis will involve multivariable linear regression modelling to explore the relationship between hepcidin levels and DAOH. The results of our study may be combined with those from other regions following this identical protocol to enable a pooled analysis to increase power and generalisability.

### *2.9 Organizational Structure*

Data management will be supported by the Coordination Centre for TRICS IV, based in Canada (AHRC, Li Ka Shing Knowledge Institute of St. Michael's Hospital, Unity Health Toronto at the University of Toronto).

The project will be sponsored in Australia by the Department of Critical Care, Faculty of Medicine, University of Melbourne and the lead site will be St. Vincent's Hospital Melbourne. Trial management will be significantly aided by the Clinical Trials Network of the Australian and New Zealand College of Anaesthetists (ANZCA) which has formally endorsed the TRICS IV Australian project.

# 3 REFERENCES


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# 4 SCHEDULE OF EVENTS

| Assessment | Screening | Preoperative | Index Cardiac Surgery | Postoperative (ICU, Ward) | Day 30 | 6 months |
|---|---|---|---|---|---|---|
| Study Day/Month | -90 to 0 | -30 to 0 | Day 0 | Postoperative period: day of surgery until hospital discharge or postoperative day 28 | Day 30 | 6 month +/- 21 days after Day 0 |
| **Observation/Procedure** | | | | | | |
| Informed Consent | X | | | | | |
| Inclusion/Exclusion | X | | | | | |
| Pregnancy Test[1] | X[1] | | | | | |
| Demographics | | X | | | | |
| Medical/Cardiac History | | X | | | | |
| Height and Weight | | X | | | | |
| EuroSCORE I | X | X | | | | |
| Randomization | | X | | | | |
| Surgical Details | | | X | | | |
| Intervention (transfusion strategy) Applied[2] | | | XXXXXXXXXXXXXXXX[2] | | | |
| Trigger Event & Transfusion Details Collected[2] | | | XXXXXXXXXXXXXXXX[2] | | | |
| Preoperative Laboratory Values[3] | | X[3] | | | | |
| Intraoperative Laboratory Values[4] | | | X[4] | | | |
| Postoperative Laboratory Values[5] | | | | X[5] | | |
| ECG[6] | | X[6] | | X[6] | | X[6] |
| Concomitant Medications | | X | | | | X |
| For Hepcidin and Iron Storage Sub-Study: hemopoietic interventions[7] | | X[7] | | X[7] | | |
| For Hepcidin and Iron Storage Sub-Study: incidental laboratory values related to Patient Blood Management or work up for anaemia[8] | | X[8] | | X[8] | | |
| For Hepcidin and Iron Storage Sub-Study: specific timed laboratory values[9, 10] | | X[9] | | | X[10] | |
| For Hepcidin and Iron Storage Sub-Study: additional data[11] | | | | X[11] | X[11] | |
| Clinical Events | | | XXXXXXXXXXXXXXX | | | X[12] |

[1]Women of childbearing potential

[2]The assigned transfusion strategy will be applied from the time of index surgery until hospital discharge from the index surgery

[3]Hb, hematocrit, platelets, INR, aPTT, fibrinogen, creatinine, and CK-MB or troponin (preferred)

[4]Lowest Hb (pre/during CPB and chest closure), lowest HCT (pre/during CPB and chest closure), lowest arterial and mixed venous $pO_2$ (pre/during CPB and chest closure), lowest arterial and mixed venous $O_2$ saturation (pre/during CPB and chest closure), lowest cardiac index (pre-CPB and chest closure) if measured, minimum pump flow (during-CPB)

[5]Hb must be assessed on postoperative days 1, 2, 5±2, and 11±3 while in hospital. Hb measurements that are done for clinical purposes will be collected, if more than one Hb is measured on a particular day, the lowest value for that day will be recorded in

the eCRF. Any patient who is transfused, is required to have a pre- and post- transfusion Hb measurement. CK-MB or troponin (preferred) is required to be assessed on the first postoperative day, 24 hours after the end of surgery. Serum creatinine is required to be measured on postoperative days 1, 2, 3 and 5 and otherwise as clinically indicated. Other laboratory values will be performed as clinically indicated or as per local guidelines and collected in the eCRF up to hospital discharge/postoperative day 28: HCT, platelets (within 2 hours of chest closure), INR, aPTT and fibrinogen (within 2 hours of chest closure), CK-MB or troponin (preferred), arterial and mixed venous $O_2$ saturation, arterial and mixed venous $pO_2$.

[6]A 12-lead ECG will be performed preoperatively, on the day after surgery and on day 4-6 postoperatively or at hospital discharge, whichever comes first. We will also collect the last ECG performed for clinical purposes up to the 6 month follow-up visit.

[7,8,9,10,11]For further details related to Hepcidin and Iron Storage Sub-study, see section 5 "additional data to collect"

- [7]From -30 to -7 days before surgery: cause of any anaemia, iron therapy, use of erythropoietin stimulating agents, ACE inhibitor or Angiotension II receptor blockers. From -7 to 0 days before surgery: cause of any anaemia, iron therapy, use of erythropoietin stimulating agents. From 0-30 days: iron therapy, use of erythropoietin stimulating agents, ACE inhibitor or Angiotension II receptor blockers.

- [8]Hemoglobin, Blood film, Serum iron, Ferritin, Transferrin, Transferrin saturation, Soluble transferrin receptor (sTfR), sTfR assay type, C-reactive protein, Vitamin B12, Folate, Thyroid stimulating hormone, Direct antiglobulin test, Haptoglobin, Lactate Dehydrogenase, Hemoglobin electrophoresis (preop only), Erythropoietin level.

- [9]Hemoglobin, Blood film, Reticulocyte Hemoglobin, Serum iron, Ferritin, Transferrin, Transferrin saturation, Soluble transferrin receptor (sTfR), sTfR assay type, Hepcidin, C-reactive protein, Fibrinogen.

- [10]Hemoglobin, Blood film, Reticulocyte Hemoglobin, C-reactive protein to be measured on Day 30 (+14) days

- [11]Estimated venesected blood loss in hospital (up to 30 days); and Days Alive and Out of Hospital (DAOH) at 30 days.

[12]Includes ER visits and hospital re-admissions.

# 5 ADDITIONAL DATA TO BE COLLECTED

| | -30 to -7 days to Index Surgery* | -7 to 0 days to Index Surgery** | Postop Day 0 to Day 30*** | Postop Day 30 |
|---|---|---|---|---|
| Laboratory tests | | | | |
| Hemoglobin | (x)[a] | x | (x)[a] | x[b] |
| Blood film | (x) | x | (x) | x[b] |
| Reticulocyte Hemoglobin | | x[c] | | x[b,c,d] |
| Serum iron | (x) | x | (x) | |
| Ferritin | (x) | x | (x) | |
| Transferrin | (x) | x | (x) | |
| Transferrin saturation | (x) | x | (x) | |
| Soluble transferrin receptor (sTfR) | (x) | x | (x) | |
| sTfR assay type[e] | (x) | x | (x) | |
| Hepcidin[f] | | x | | |
| C-reactive Protein | (x) | x | (x) | x |
| Fibrinogen | (x) | (x) | (x) | |
| Vitamin B12 | (x) | | (x) | |
| Folate | (x) | | (x) | |
| Thyroid Stimulating Hormone | (x) | | (x) | |
| Direct antiglobulin Test | (x) | | (x) | |
| Haptoglobin | (x) | | (x) | |
| Lactate Dehydrogenase | (x) | | (x) | |

| | | | | |
|---|---|---|---|---|
| Hemoglobin electrophoresis | (x) | | | |
| Erythropoietin level | (x) | | (x) | |
| Clinician diagnosis | | | | |
| Cause of anaemia (if present)[g] | x | x | | |
| Therapies that could alter Hemoglobin | | | | |
| Iron therapy (Y/N) | x | x | x | |
| Iron therapy type (oral, IV) | x | x | x | |
| Erythropoietin stimulating agent use | x | x | x | |
| Relevant medications | | | | |
| Angiotensin Converting Enzyme inhibitor or Angiotensin II receptor blocker use (>1 week) | x | | x | |
| | | | | |
| Estimated venesected blood loss in hospital[h] | | | x | |
| | | | | |
| Days Alive and Out of Hospital | | | | x |

* = record value furthest away from index surgery
** = record value closest to index surgery
*** = record value furthest away from index surgery

x = mandatory data collection
(x) = record if performed as part of existing Patient Blood Management strategies or as part of clinical workup of newly diagnosed anaemia.

a) May already be available from TRICS IV database.
b) May be taken at 30 days (+14 days).
c) See note on Data collection/storage below.
d) Record only if able to be performed in same facility as -7 to 0 bloods.
e) sTfR will be analysed by Roche or Siemens only according to local referral pathways.
f) Hepcidin assayed by enzyme-linked immunosorbent assay (ELISA) using DRG Hepcidin 25 (bioactive) HS ELISA kit[1].
g) Cause of anaemia will be assigned by two hematologists and adjudicated by a third if necessary.
h) As per Salisbury et al.[2]

### *5.1 Data Collection / Storage*

- Reticulocyte mean hemoglobin content will be estimated by:
    - Reticulocyte hemoglobin content (CHr) on Siemens Advia 2120 machines; reticulocyte hemoglobin (Ret-He) on Sysmex XE/XN machines; mean reticulocyte hemoglobin content (MCHr) on Abbott Sapphire machines; reticulocyte hemoglobin expression (RHE) on Mindray BC 6800 machines; or reticulocyte hemoglobin cellular content (RHCc) on ABX-Horiba Pentra Nexus DX machines[3].
    - Correlating values for Red Cell Size Factor (RSf) on Beckman Coulter analysers to CHr values of Siemens Advia machines[4].
- Hepcidin samples will be batch analysed after serum separation and freezing to -70℃.
- All other laboratory samples are to be collected by facilities accredited by the National Association of Testing Authority and according to standards.

### *5.2 References for Additional Data Collection*


1. Uelker B, Cushman I, Dudek T, Herkert DM, Geacintov DCE. High Sensitivity Hepcidin-25 Bioactive Elisas: Manual and Fully Automated System for the Quantification of Hepcidin-25 in Human Serum and Plasma. Blood 2016;128:4820-.
2. Salisbury AC, Reid KJ, Alexander KP, et al. Diagnostic blood loss from phlebotomy and hospital-acquired anemia during acute myocardial infarction. Arch Intern Med 2011;171:1646-53.
3. Buttarello M. Laboratory diagnosis of anemia: are the old and new red cell parameters useful in classification and treatment, how? International Journal of Laboratory Hematology 2016;38:123-32.
4. Urrechaga E. Clinical utility of the new beckman-coulter parameter red blood cell size factor in the study of erithropoiesis. International Journal of Laboratory Hematology 2009;31:623-9.