Statistical analysis plan (SAP): A-TRAK

1. Administrative information:

Title: Angiotensin-II for Reduction of Acute Kidney Injury after cardiac surgery – a pilot to determine feasibility of a definitive double blind randomized controlled trial (A-TRAK)
Trial registration number: ACTRN12621000195853
SAP version: 1.0
Protocol version: 1.8
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2. Introduction:

Each year more than 25,000 Australians have cardiac surgery. While the mortality rate is relatively low (around 1-2%), the rate of other complications such as acute kidney injury (AKI) is higher (up to 40%). Acute kidney injury (AKI) is a reduction in kidney function over a short period of time. In many cases it resolves, but patients who have had AKI have higher rates of long-term kidney problems and mortality rates. A few will need dialysis. Maintaining blood flow and pressure for the kidney may help prevent injury. Angiotensin II is produced by the body naturally but can also be given as a drug. It has effects that increase blood pressure. Studies in patients with infection have shown that angiotensin II may have a beneficial effect in kidney injury. Animal experiments suggest it may be superior to noradrenaline in maintenance of medullary oxygenation. Our hypothesis is that angiotensin II given to maintain blood pressure in the immediate perioperative period may reduce kidney injury compared to noradrenaline. In this pilot study we will determine the feasibility of a definitive study comparing angiotensin II to noradrenaline.

Objectives:
To determine the feasibility of a double-blinded randomized controlled trial (RCT) comparing an infusion of angiotensin II to noradrenaline to maintain mean arterial blood pressure (MAP) greater than 70mmHg during and for up to 48 hours after cardiac surgery. Specifically, our primary objectives were to determine:

1) The duration and dose of study drug required intraoperatively and postoperatively for up to 48 hours to maintain MAP>70mmHg
2) Whether the rate of consent would be sufficient for recruitment
3) Whether the study protocol would be acceptable to clinicians (protocol compliance) and safe for patients
4) Whether both drugs were effective in maintaining blood pressure in the target range for the majority of patients in the study population

Additional feasibility objectives included:
a) To determine the rate of AKI in the study population

b) Exploratory outcomes including any difference in the maximum change in creatinine between groups, and differential drug effects related to renin-angiotensin-aldosterone system baseline status as determined by biochemical analysis

3. Study methods:

Trial design:
A two-center pilot of a double-blinded, parallel-group randomized controlled trial comparing an infusion of angiotensin II to noradrenaline to maintain MAP > 70mmHg intraoperatively and for up 48 hours postoperatively (or ICU discharge, whichever is sooner) in cardiac surgical patients. 1:1 allocation ratio.

Randomization:
Consenting patients will be randomly assigned from a computer-generated list (1:1, blocks of 2,4 and 6), stratified by centre and emergency status, to either Angiotensin II or Noradrenaline groups. Randomisation will be completed by trained and dedicated research staff in the online research electronic data capture (REDCap Consortium, Vanderbilt University, Nashville, TN database).

Sample size:
Feasibility study – sample size of 60 was chosen pragmatically to give sufficient data to inform a future study, including precision of overall outcome rate

Safety and interim analysis:
A safety monitor reviewed the data unblinded at one point during the study (outcome data available for >30 patients)

Timing of final analysis:
Outcomes will be recorded up to hospital discharge or 30 days from the procedure date, whichever occurs sooner

4. Statistical analysis principles:

- This SAP was finalised before the end of data collection and database locking
- A two-sided p-value of 0.05 will be considered significant
- 95% confidence intervals will reported where appropriate
- Due to the pilot nature of the trial adjustment for multiplicity of outcomes will not be pre-specified and secondary outcomes should be considered exploratory
- Adherence to treatment will be assessed as a primary outcome (discussed later)
- Protocol deviation will be defined as stopping the study drug early or changing the MAP target
- Analysis will be completed on an intention to treat basis for all patients randomised who undergo cardiac surgery
- Statisticians carrying out the analysis will be blinded to treatment group
- Due to the pilot nature of the study, we will not adjust for covariate imbalance between groups
- Whenever appropriate the centre will be included as a random effect in the model

- Where comparisons are made between groups the following statistical tests will be used where appropriate:
    - **Binary outcomes:**
      Generalized linear model with binomial distribution and identity link, and reported as absolute difference.
    - **Continuous outcomes:**
      Generalized linear model with Gaussian distribution reported as mean difference or quantile model with T of 0.50 (median regression) and reported as median difference for skewed data.
    - **Ordinal outcomes :**
      Cumulative logistic model reported as common odds ratio.
    - **Trend over time**
      Mixed-effect generalized linear model with Gaussian distribution, including an interaction between group and time as fixed effect, and considering patients as random effects to account for repeated measurements.

5. Trial population:

Screening: the number of patients screened, the number eligible, the number approached and the number who consent to participate will be reported
Recruitment: a CONSORT diagram will include the number of patients screened, the number eligible, the number approached, the number consented, those randomly allocated to each treatment group, completed treatment and those followed up for the primary outcome
Withdrawal: any patients withdrawn will be recorded
Changes in eligibility criteria: will be reported and explained
Patient characteristics and baseline comparisons:

- A description of the baseline characteristics will be presented by treatment group
- Categorical variables will described in terms of frequencies and percentages
- Continuous variables will be described in terms of mean and standard deviation (SD) or median and IQR (Q1-Q3)
- Baseline measures for all patients will be tabulated for the following variables:
    - AKI risk factors:
        - Age
        - BMI
        - Preoperative creatinine
        - Preoperative haemoglobin concentration
        - NYHA classification
        - AKI risk score (as defined in the protocol)
    - Additional risk factors:
        - Sex
        - Peripheral vascular disease
        - Previous cardiac surgery
        - Hypercholesterolaemia
        - Diabetes
        - Hypertension
        - Ejection fraction estimate
            - Normal (>60%)

                - Mild impairment (45-59%)
                - Moderate impairment (30-44%)
                - Severe impairment (<30%)
            - Urgency of procedure
                - Elective
                - Urgent
                - Emergency
            - Type of surgery
                - Coronary artery bypass grafting (CABG)
                - Valve procedure
                - Combined CABG/valve procedure
                - Other
            - Duration of surgery, cross clamp time, bypass time
    - Preoperative ACEi/ARB usage
        - Preoperative ACEi – yes/no
        - Preoperative ARB – yes/no
        - Time from ACEi/ARB last administration to induction of anaesthesia

6. Analysis

Outcome definitions and analysis methods

Primary feasibility outcomes – *key feasibility outcomes in bold:*

- **A) Study drug utilisation will be measured. Successful drug administration will be defined as study drug infusion duration greater than 4 hours in greater than 50% of individuals, and greater than 12 hours in greater than 25% of patients in both groups (as per study protocol).**
- Subsequently drug utilisation will be tabulated by treatment group, mean (SD) or median (IQR Q1-3) for continuous outcomes and compared between groups:
    - Duration of study drug infusion intraoperatively
        - A) Absolute (minutes)
        - B) As a percentage of intraoperative time (from time of connection of study drug to patient IV line to transfer to ICU)
    - Duration of study drug infusion during cardiopulmonary bypass
        - A) Absolute (minutes)
        - B) As a percentage of cardiopulmonary bypass time
    - Duration of study drug infusion in intensive care (ICU)
        - A) Absolute (hours)
        - B) As a percentage of ICU time (first 48 hours from arrival, or until ICU discharge, whichever is sooner)
    - Maximum study drug administration rate intraoperatively (mls/hr)
    - Maximum study drug administration rate in ICU (mls/hr)
    - Total study drug intraoperatively (mls)
    - Total study drug in ICU (mls)
- **B) Recruitment rate**

    - **A greater than 40% consent rate from approached patients will be defined as successful (as per the study protocol).** Data on screening, eligibility and consent rate will be presented in the CONSORT diagram.
- **C) Protocol adherence**
    - **Success will be defined as greater than 80% protocol adherence overall**
    - Protocol deviations will be summarised and events surrounding deviation described.
- **D) Efficacy of maintaining MAP > 70mmHg using study drug:**
    - **The following will be tabulated according to treatment group ((frequency (percentage)) and comparisons made between groups:**
        - **Blood pressure maintained greater than a mean arterial pressure of 70mmHg for 50% of the time using study drug as per protocol – yes/no (open label vasopressor will result in negative response)**
            - **Intraoperatively – assessed by medical record**
            - **In ICU – assessed by medical record**
    - Additional vasopressor, inotrope and fluid use tabulated by treatment group (frequency (percentage) for categorical outcomes, mean (SD) or median (IQR Q1-3) for continuous outcomes) and compared between groups:
        - Open label noradrenaline use:
            - Intraoperatively – yes/no
            - Maximum intraoperative dose if used (mcg/kg/min)
            - Postoperative in first 48 hours – yes/no
            - Postoperative in first 48 hours – maximum dose if used (mcg/kg/min)
        - Vasopressin use:
            - Intraoperatively – yes/no
            - Maximum intraoperative dose if used (IU/kg/min)
            - Postoperative in first 48 hours – yes/no
            - Postoperative in first 48 hours – maximum dose if used (IU/kg/min)
        - Adrenaline use:
            - Intraoperatively – yes/no
            - Maximum intraoperative dose if used (mcg/kg/min)
            - Postoperative in first 48 hours – yes/no
            - Postoperative in first 48 hours – maximum dose if used (mcg/kg/min)
        - Milrinone use:
            - Intraoperatively – yes/no
            - Maximum intraoperative dose if used (mcg/kg/min)
            - Postoperative in first 48 hours – yes/no
            - Postoperative in first 48 hours – maximum dose if used (mcg/kg/min)
        - Methylene blue at any time – yes/no
        - Total volume of non-blood product IV fluids in operating theatre (mls)
        - Total number of red cell units used in operating theatre (n)

        - Total number of other blood products units (non red cell) used in operating theatre (n)
        - Total volume of non-blood product IV fluids in ICU (mls)
        - Total number of red cell units used in ICU (n)
        - Total number of other blood products units (non red cell) used in operating theatre (n)
- E) **Major adverse events:**
    - Hospital mortality within 30 days of procedure
    - Unplanned extracorporeal circulatory support postoperatively
    - Permanent stroke (as defined by ANZSCTS criteria) postoperatively
    - Requirement for renal replacement therapy postoperatively

Secondary efficacy outcomes:

- **The following will be tabulated according to treatment group**
- **Description will be in terms of frequency (percentage) for categorical outcomes, mean (SD) or median (IQR Q1-3) for continuous outcomes.**
- **Inferential statistics will be used to compare between groups as described above**
    - Maximum change in creatinine from preoperative to up to 7 days postoperatively
    - Acute kidney injury (AKI) of any stage by KDIGO criteria
    - AKI by stage 1-3

Other exploratory outcomes and adverse events:

Drug equivalency outcomes:

- The volume of drug given in each group will be compared between groups
    - Intraoperatively – as a mean dose per unit time (mls/hr)
    - In ICU – as a mean dose per unit time (mls/hr)
- A second analysis will be carried out for comparative dose as above stratified by the following:
    - ACEi dose within 48 hours of commencement of surgery
    - ARB dose within 48 hours of commencement of surgery
    - Neither of the above

Other outcomes including adverse outcomes common after cardiac surgery, or previously noted in angiotensin II studies:

- The following additional outcomes will be tabulated and compared between treatment groups:
    - Intraoperative urine output (mls/hr)
    - Urine output for first 24 hours after surgery (mls/hr)
    - Urine output for 24-48 hours (mls/hr)
    - Cardiac index (pre-bypass, post-bypass and maximum and minimum in ICU) (l/min/m2)

- Mean pulmonary artery pressure, mean systemic arterial pressure, central venous pressure and systemic vascular resistance - pre-surgery and post-surgery (mmHg and dynes)
- ICU and hospital length of stay (hours and days respectively)
- New onset atrial fibrillation
- Delirium (as described in notes or defined by confusion assessment method (CAM)-ICU
- Deep venous thrombosis
- Arterial thrombosis
- Thrombocytopaenia (platelets <50 x 10^9/l)
- Any complication relating to severe hypertension (MAP>120mHg), for example left ventricular failure, aortic dissection or major haemorrhage

Additional biochemical analysis:

- The following additional outcomes will be tabulated and compared between treatment groups:
  - 6 Hour creatinine clearance post operatively
  - Difference in creatinine clearance post operatively (6 hour creatinine clearance minus preoperative eGFR) (mls/min)
  - Delta creatinine from preoperative to immediate postoperative
  - Delta creatinine from preoperative to Day 1 postoperative
  - Delta creatinine from preoperative to Day 2 postoperative
  - Maximum serum sodium in first 72 hours
  - Minimum serum sodium in first 72 hours
  - Maximum serum potassium in first 72 hours
  - Minimum serum potassium in first 72 hours
  - Maximum glucose in first 72 hours
  - Maximum lactate in first 48 hours
  - Minimum pH in first 48 hours

Renin-angiotensin-aldosterone system analysis:

The following will be used for exploratory analyses as part of as separate manuscript. The results will be tabulated and comparisons made between between treatment groups. Changes in biomarker levels in response to treatment groups will be explored.

- Preoperative, immediate postoperative and 24 hours postoperatively:
  - Renin levels (serum)
  - Aldosterone levels (serum)
  - Angiotensin I and II levels (serum)
  - ACE-2 levels (serum)
  - ACE-2 levels (urine)
  - Neutrophil gelatinase-associated lipocalin (NGAL) (serum/urine)

Other analysis details:

Missing data: Where data are missing groups will be analysed by available data (only likely to be relevant for secondary and exploratory outcomes)

Software: Analysis will primarily by conducted in Stata 16 or “R”