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St Vincent's HREC Ref: HREC 051/17

Protocol amendment to "OPTIMISE II protocol v1.0" Protocol version date: 02/11/2016 – Biomarker collection and analysis, Australian sites

Introduction
The OPtimisation of Perioperative Cardiovascular Management to Improve Surgical outcomE II trial (Optimise II) is large multi-centre randomised trial, which seeks to establish whether targeted fluid optimisation with minimally invasive cardiac output monitoring and a low dose inotrope infusion can improve outcomes in patients having elective gastrointestinal surgery. Collected blood samples will also be analysed for biomarkers of endothelial and myocardial injury.

Aims and significance
The aim is to compare two biomarkers of endothelial and myocardial damage, Syndecan-1 and troponin respectively, in a group of participants and to correlate these with both primary and secondary outcomes of the trial.

Hypotheses:

1. Syndecan-1, a marker of endothelial glycocalyx damage, will be lower in participants randomised to GDT as compared with standard care.
2. Troponin, as a measure of myocardial damage, will not be different in participants randomised to GDT as compared with standard care.
3. Syndecan-1 levels will be correlated to both primary and secondary outcomes of the main study in both GDT and standard care arms.

Significance:
The proposed biomarker analysis will provide novel data on the mechanisms of harms and/or benefits of GDT. The findings of the overall trial and biomarker analysis will almost certainly lead to influential publications.

Background: Biomarker Analysis
We also propose to measure two biomarkers (Syndecan-1 and Troponin) in the Australian cohort that have close mechanistic links to the intervention of goal-directed therapy (GDT), the primary outcome of surgical site infection and secondary outcomes related to mortality and morbidity.

Syndecan-1 is a promising breakdown product of the glycocalyx, which lines endothelial cells. The glycocalyx has anatomical and functional significance with a role in modulating inflammation and maintaining vascular permeability. Damage of the glycocalyx and shedding of syndecan-1 is associated with inflammatory states, sepsis and hypervolemia (13). Comparison of glycocalyx breakdown between treatment arms and correlation of glycocalyx breakdown to primary or secondary outcomes may reveal insights into mechanisms of injury. A study of syndecan-1 showed elevation in patients in sepsis and major surgery compared to control(14). Syndecan-1 has also been shown to increase after fluid bolus administration (15) .

Troponin TnT (>0.02ng/mL) has been shown in the VISION study to be strongly associated with 30-day mortality for patients undergoing non-cardiac surgery with an adjusted hazard ratio of 2.41 or higher (16). Since troponin assays are highly sensitive we may be able to detect differences between control and invention arms with regards to subclinical myocardial injury. This is especially important since OPTIMISE I found a higher rate of severe cardiovascular events in the GDT arm (1.4% compared to 0%). Measurement of troponin would help establish the safety of GDT and pre-emptive use of low dose inotropes.

The VISION study found troponin to be elevated in 11.9% of patients, however the OPTIMISE II population is older and undergoing higher risk surgery and thus we expect a higher rate of troponin elevation.

Methods:

Recruitment will be in conjunction with the Optimise II trial and follow fully the clinical and ethical guidelines governing this study. The consent for the collection and storage of blood samples is detailed in the study documents PICF "Master Participant Information Sheet/Consent Form V 2 25/10/2017".

Troponin assays will be performed on all 200 Australian participants. Troponin samples will be collected at time 0, 6, 24 and 48 hours after commencement of surgery. Where possible, the samples will be taken in conjunction with routine blood samples related to perioperative care of the patient to minimize unnecessary venipuncture.

Syndecan-1 and troponin samples will be collected at time 0, 6, 24 and 48 hours after commencement of surgery. Syndecan-1 samples will be collected from 50 participants and centrifuged, aliquoted and stored at minus 80^{o}C for later testing. The smaller cohort of 50 patients reflects limitations of the logistics of laboratory support (centrifugation and deep freezing), sample processing time, and the cost of an ELISA assay.

Additional biomarkers may be explored on collected samples dependent on availability of assays and clinical significance. In this event, they will utilize existing collected samples.

Analysis:

1. Syndecan-1 levels between the two groups, usual care vs. GDT, will be compared and analyzed for a significant difference.
2. Troponin levels between the two groups, usual care vs. GDT, will be compared and analyzed for a significant difference.
3. Logistic regression modelling for the primary outcome will be used to explore associations with biomarkers.
4. Secondary outcomes such as renal injury or cardiovascular events may also be explored for association with biomarkers