**Activation of the Renin–Angiotensin system and myocardial injury in noncardiac surgery: pre-specified analysis of a phase-II, randomised controlled multi-centre trial (SPACE-AXIS)**

**Statistical Analysis Plan: SPACE-AXIS**

Version 1.0

Date: 11.12.2023

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| --- |
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| **Date** | 11.12.2023 |

# **Administrative information**

**Trial Information**

|  |  |
| --- | --- |
| **REC number:** | 16/LO/1495 |
| **Trial Sponsor:** | Queen Mary University of London |
| **Trial Sponsor reference:** | 011368 |
| **Trial Funder:** | British Oxygen Company research chair award in Anaesthesia, administered by the National Institute for Academic Anaesthesia |
| **ISRCTN number:** | 17251494 |
| **NIHR CRN Portfolio ID number:** | 31645 |
| **Protocol version (date):** | Version 7.0 (14/12/2020) |

Version 1.0 of the SAP for SPACE-AXIS was written after TA had access to unblinded data (i.e. trial dataset with the variables for treatment allocation included). Note that all contributors are blinded to the primary outcome as samples will be processed by an independent laboratory at the end of the trial (defined as when the last patient leaves hospital).

**Remit of the SAP**

The purpose of this document is to provide details of the statistical analyses and presentation of results to be reported for the SPACE-AXIS sub study of the main SPACE trial. It is important to set these out and to agree them in advance of inspecting the outcome data for the trial, so that data derived decisions in the analysis are avoided. Any exploratory, post hoc or unplanned analysis will be clearly identified as such in the respective study analysis report. This SAP does not include in its remit the health economic analysis which will be planned in a separate document.

# **Background and study design**

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| --- | --- |
| **Study objectives** | **Primary Objective**Examine which preoperative characteristics of the renin-angiotensin-aldosterone (RAAS) axis are associated with myocardial injury.**Secondary Objectives*** Examine which preoperative characteristics of the RAAS axis are associated with myocardial injury, all-cause complications and/or pressor use and/or hypotension.
 |
| **Study design** | Secondary analysis of data from a phase II, multi-centre, two-arm, parallel group randomised controlled trial- pre-specified analysis. |
| **Setting** | Surgical services of hospitals undertaking major elective surgery |
| **Participants** | **Inclusion criteria*** Informed consent (no incapacitated or vulnerable adult or minors will be included)
* Age 60 years and over
* Undergoing major surgery (e.g. major joint replacement or vascular or gastrointestinal) requiring general and/or regional anaesthesia with sedation
* Currently taking ACE-I or combined ACE-I and ARB therapy or combination therapy where medication includes ACE-I or ARB
* Expected duration of surgery longer than 120 minutes
* American Society of Anaesthesiologists physical status grade 3 or above
* All female subjects must be postmenopausal, as demonstrated by clinical history, or demonstrated not to be pregnant through a preoperative pregnancy test

**Exclusion criteria*** Current participation in any other trials where care or treatment is being altered
* Recent myocardial infarction (within 3 months)
* Any condition, which in the opinion of the treating clinician, would result in the patient being harmed by the cessation of the ACE-I and/or ARB therapy.
 |
| **Statistical treatise** | Clusters of similar RAAS activation are identified by unsupervisedagglomerative hierarchical clustering, an agnostic technique that pools similar groups of data from a large dataset to identify common biological features associated with a particular outcome.  |
| **Primary outcome measure** | The primary outcome is myocardial injury defined by elevations in troponin-T, comparing across clusters of patients who have similar RAAS activation characteristics before surgery. |

# **Outcome measures**

**Primary outcome measure**

The primary outcome is myocardial injury, a binary variable based on plasma high sensitivity Troponin-T measured in blood samples collected immediately before the induction of anaesthesia, and then postoperative day 1± 6 hours and day 2 ± 6 hours after surgery. The primary outcome is met under the following conditions:

* Troponin-T ≥15 ng/L within 48 hours after surgery with a pre-operative value <15 ng/L *OR*
* Troponin-T increase ≥5 ng/L within 48 hours after surgery with a pre-operative value ≥15ng/L

**Secondary outcome measures**

* Peak level of Troponin-T measured within 48 hours of surgery. Peak Troponin-T level (ng/L) will be calculated as the highest Troponin-T from the blood samples collected at 24 hours and 48 hours after surgery, within each cluster
* All-cause complications within each cluster
* Pressor use and/or hypotension

Full definitions of secondary outcome measures can be found in the SPACE protocol.

**Methodology:** Clusters of similar RAAS activation. Clusters of similar RAAS activation are identified by agglomerative hierarchical clustering, displayed as a dendrogram. The algorithms begin with each object in a separate cluster. At each step, the two clusters that are most similar are joined into a single new cluster, using the unweighted pair-group method, the most widely used of all the hierarchical cluster techniques. The distance between two groups is defined as the average distance between each of their members. The [Euclidean] distance value that will yield an appropriate number of clusters will be determined by dendogram. At each generation of clusters, samples are merged into larger clusters to minimize the within-cluster sum of squares or to maximize the between-cluster sum of squares.

 Input variables for cluster analysis are absolute immediate preoperative values for key components of the RAAS including renin, aldosterone, angiotensin-converting enzyme 2, dipeptidyl peptidase-3[[1](#_ENREF_1)], plus NT-proBNP as a measure of integrated cardiovascular health. This analysis will have a single principal exposure variable, i.e. the cluster within which a particular patient enrolled into SPACE resides. Absolute values for each RAAS component will be presented in a figure. To compare differences between clusters, analysis of variance, Kruskal-Wallis, and chi-square tests are

used for parametric continuous, nonparametric continuous, and categorical variables, respectively.

**Sample size and randomisation**

**Sample size calculation**

For the main SPACE trial, assuming an incidence of postoperative myocardial injury of 50% in patients undergoing major surgery in the cessation group, a sample size of 248 patients will provide 90% power to detect as statistically significant (p<0.05) an 20% absolute risk reduction to 30% [[2](#_ENREF_2), [3](#_ENREF_3)]. Allowing for 5% withdrawal/loss to follow up, we will aim to recruit a total of 260 patients.

**Randomisation procedure**

Randomisation will occur after the participant has provided informed consent 72 hours before the surgical procedure is due to start. Participants are randomised to a treatment group in a 1:1 ratio using a computer-generated dynamic procedure (minimisation) with a random component. Minimisation variables are trial centre, surgical procedure category (surgery involving the gut and all other surgery) and ACE-I and/or ARB category. Each participant will be allocated with 80% probability to the treatment group that minimises between group differences in these factors among all participants recruited to the trial to date, and to the alternative group with 20% probability. To enter a patient into the SPACE trial, research staff at the site will log on to a secure web-based randomisation and data entry platform hosted by Queen Mary University of London and complete the patient’s details to obtain a unique patient identification number and allocation to a treatment group. A patient’s treatment group allocation will only be revealed to the person performing randomisation.

# **Analysis methods for SPACE-AXIS**

**Summary of cluster characteristics**

Baseline characteristics will be summarised for each cluster by the mean and standard deviation or median and interquartile range for continuous variables, and the number and percent for categorical variables. The following baseline characteristics will be summarised by treatment group:

* Demographic: age (years), gender (male/female)
* Co-morbid disease: (a) COPD; (b) asthma; (c) interstitial lung disease or pulmonary fibrosis; (d) ischaemic heart disease; (e) diabetes mellitus; (f) heart failure; (g) liver cirrhosis; (h) active cancer; (i) previous stoke or TIA; (j) peripheral vascular disease; (k) Hypertension; (l) any treated infections within the previous month
* Current smoker
* ASA grade (III/IV)
* Pre-operative blood test results (within 4 weeks before surgery or most recent): (a) haemoglobin (g/L); (b) creatinine (µmol/L); blood pressure before and after randomisation.
* Minimisation criteria:
	+ Planned surgical procedure: (a) surgery involving the gut; (b) all other surgery
	+ Class of drug routinely taken: (a) ACE-I; (b) ARB
	+ Trial centre: (a) County Durham and Darlington NHS foundation trust; (b) Plymouth hospitals NHS trust; (c) Barts Health NHS trust; (d) University college London hospitals; (e) University hospitals Bristol NHS foundation trust
	+ Randomisation arm [stop/continue ACEi/ARB]
* Surgical procedure performed: (a) surgery involving the gut; (b) all other surgery
* Cardiovascular medication: (a) beta-blocker; (b) calcium channel antagonist; (c) Doxazosin; (d) Diuretic; (e) Statin; (f) Nitrate; (g) Anti-platelet agents (h) ACE-I/ARB drugs

**General analysis principles**

Analyses will follow the intention-to-treat principle: all randomised patients with a recorded outcome will be included in the analysis and analysed according to whichever cluster they reside in [[4](#_ENREF_4), [5](#_ENREF_5)]. Patients will be included in the analysis, regardless of whether the treatment they received was compliant with the protocol. Definitions of what constitutes a recorded outcome for each outcome can be found in Appendix 1 of the original analysis plan for SPACE. Patients with missing outcome data will be excluded from the analysis. Missing data for baseline covariates to be included in the analysis model will be accounted for using mean imputation for continuous variables and the missing indicator approach will be used for missing data for categorical variables [[6](#_ENREF_6), [7](#_ENREF_7)].

For the analysis of the primary outcome, each secondary outcome, and all process measures, we will present the following information:

* The number of patients included in each analysis, by cluster.
* A summary statistic of the outcome (e.g. mean (SD), number (%)), by cluster.
* A 95% confidence interval for the estimated treatment effect
* A two-sided p-value

For all analyses, a significance level of 5% will be used.

**Representativeness of patients**

All participating sites have been asked to keep a log of eligible patients not recruited to the trial. Reasons for non-participation will be categorised and summarised. Participation in the trial, treatment allocation and completeness of follow-up will be illustrated by a CONSORT flow diagram [[8](#_ENREF_8)].

**Analysis software**

All analyses will be conducted in NCSS 2023 (NCSS 2023 Statistical Software (2023). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/ncss)

**Analysis of primary outcome**

Primary analysis

The primary outcome, proportion of patients with myocardial injury within 48 hours after surgery in each cluster will be analysed, under the ITT principle using Fisher’s exact test. [[9](#_ENREF_9)].

**Analysis of secondary outcomes**

Peak level of Troponin-T measured within 48 hours of surgery: The mean (SD) peak level of Troponin-T measured within 48 hours of surgery will be reported within each identified cluster. Differences between clusters in the mean peak level troponin-t will be analysed using ANOVA.

All-cause complications within 30 days of surgery: Infection, myocardial infarction, acute heart failure stroke and death within 30 days of surgery will be reported within each identified cluster. Differences between clusters will be analysed using Chi-squared test.

Pressor use and hypotension: The number (%) will be presented in each identified cluster.

**Sensitivity analysis**

Because beta-blockers also alter RAAS activation,[[10](#_ENREF_10)] a sensitivity analysis excluding patients on beta-blockers will be undertaken.

# **Other analyses, data summaries and graphs**

**Clinical management**

Clinical management for clusters will be summarised but not subjected to statistical testing. Numbers (%) and means (SD) or medians (IQR) will be provided separately for each identified cluster:

* Surgical technique
* Anaesthetic technique
* Planned and actual level of care on the first night after surgery
* Blood pressure during surgery
* Intravenous fluids during surgery

**Process measures**

Summary measures will be presented separately for each identified cluster, in accord with StEP-COMPAC guidelines for perioperative outcomes.

**Complications within 30 days after surgery**

The number and percentage of patients experiencing each of the following complications will be presented for each identified cluster.

* Cardiac complications
* Respiratory complications
* Infective complications
* Other complications
* Acute kidney injury

**References**

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8. Schulz, K.F., et al., *CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials.* BMC Medicine, 2010. **8**(1): p. 18.

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10. Chen, L., et al., *Stimulation of renin secretion by angiotensin II blockade is Gsalpha-dependent.* J Am Soc Nephrol, 2010. **21**(6): p. 986-92.

# **Dummy tables**

**Table 1: Baseline Characteristics**

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| --- |
| **Baseline Characteristics** |
| Cluster 1 | Cluster 2 to xx |
| Gender - no. (%) |   |   |
| Male |   |   |
| Female |   |   |
| Age (years) |   |   |
| Mean (SD) |   |   |
| Median (IQR) |   |   |
| Current Smoker - no. (%) |   |   |
| American Society of Anaesthesiology grade - no. (%) |   |   |
| III |   |   |
| IV |   |   |
| Chronic comorbid disease - no. (%) |   |   |
| COPD |   |   |
| Asthma |   |   |
| Interstitial lung disease or pulmonary disease |   |   |
| Ischaemic heart disease  |   |   |
| Diabetes mellitus |   |   |
| Heart failure |   |   |
| Liver cirrhosis |   |   |
| Active cancer  |   |   |
| Stroke or transient ischaemic attack (TIA) |   |   |
| Peripheral vascular disease  |   |   |
| Hypertension |   |   |
| Any treated infections within the previous month  |   |   |
| Planned surgical procedure - no. (%) |   |   |
| Surgery involving the gut |   |   |
| All other surgery  |   |   |
| Class of drug routinely taken - no. (%) |   |   |
| ACE-I |   |   |
| ARB |   |   |
| Trial Centre - no. (%) |   |   |
| County Durham and Darlington NHS Foundation Trust |   |   |
| Plymouth Hospitals NHS Trust |   |   |
| Barts Health NHS Trust |   |   |
| University College London Hospitals |   |   |
| University Hospitals Bristol NHS Foundation Trust |   |   |
| Surgical procedure performed - no. (%) |   |   |
| Surgery involving the gut |   |   |
| All other surgery  |   |   |
| Pre-operative blood tests results |   |   |
| Haemoglobin (d/DL) |   |   |
| Mean (SD) |   |   |
| Median (IQR) |   |   |
| Creatinine (μmol/L)  |   |   |
| Mean (SD) |   |   |
| Median (IQR) |   |   |
| Cardiovascular medication - no. (%) |   |   |
| Beta-blocker |   |   |
| Calcium channel antagonist |   |   |
| Doxazosin |   |   |
| Diuretic |   |   |
| Statin |   |   |
| Nitrate |   |   |
| Anti-platelet agents |   |   |
| ACE-I/ARB drugs |  |  |
| Systolic Blood pressure: pre-admission clinic |  |  |
|  Day of surgery |  |  |
| Diastolic Blood pressure: pre-admission clinic |  |  |
|  Day of surgery |  |  |
| Mean arterial blood pressure: pre-admission clinic |  |  |
|  Day of surgery |  |  |
| RCRI Score - no. (%) |   |   |
| 0 - 2 |   |   |
| 3 - 6 |   |   |

Abbreviations: SD, standard deviation; IQR, Interquartile range; COPD, chronic obstructive pulmonary disease.

**Table 2: Clinical management**

|  |  |
| --- | --- |
| **Clinical management characteristics** | **Number of patients with available data - no. (%)** |
| Cluster 1-xx |
| Surgical technique - no. (%) |  |  |
| Open surgical technique used during surgery |  |  |
| Laparoscopic or laparoscopic assisted technique |  |  |
| Laparoscopic converted to open |  |  |
| Anaesthetic technique - no. (%) |   |   |
| General anaesthesia alone  |   |   |
| General anaesthesia + epidural  |   |   |
| General anaesthesia + spinal  |   |   |
| General anaesthesia + other regional  |   |   |
| Regional anaesthesia + sedation |   |   |
| Endotracheal tube inserted  |   |   |
| Planned level of care on the first night after surgery - no. (%) |   |   |
| Critical care unit level 3 |   |   |
| Critical care unit level 2 |   |   |
| Post-anaesthesia care unit |   |   |
| Surgical ward |   |   |
| Actual level of care on the first night after surgery - no. (%) |  |  |
| Critical care unit level 3 |  |  |
| Critical care unit level 2 |  |  |
| Post-anaesthesia care unit |  |  |
| Surgical ward |  |  |
| Blood pressure during surgery |   |   |
| Systolic blood pressure <90 mmHg - no. (%) |   |   |
| Phenylephrine, total dose during surgery (mcg) |   |   |
| Median (IQR) |   |   |
| Ephedrine, total dose during surgery (mg) |   |   |
| Median (IQR) |   |   |
| Metaraminol, total dose during surgery (mg) |   |   |
| Median (IQR) |   |   |
| Other pressor support - no. (%) |   |   |
| Arrhythmias - no. (%) |   |   |
| Intravenous fluids during surgery  |   |   |
| Total volume of intravenous fluid administered excl. blood products (Ml/kg/h) |   |   |
| Median (IQR) |   |   |
| Total volume of blood products administered (mL) |   |   |
| Median (IQR) |   |   |
| Lactate end of surgery mmol/L |   |   |
| Median (IQR) |   |   |

Abbreviations: SD, standard deviation; IQR, Interquartile range

**Table 3: Primary and secondary outcomes.**

|  |  |  |
| --- | --- | --- |
| **Outcomes** |  |  |
| Cluster 1-xx | **OR (95% CI)** | **P-value** |
| **Primary outcome** |  |  |  |  |
| Myocardial injurya |   |   |   |   |
| **Secondary outcomes** |  |  |  |  |
| Peak hsTnTa |   |   |   |   |
| Infectionb |   |   |   |   |
| Myocardial infarctionb |   |   |   |   |
| Acute heart failureb |   |   |   |   |
| Strokeb |   |   |   |   |
| Deathb |  |  |  |  |

a Within 48 hours of surgery

b Within 30 days of surgery

**Table 4: Complications within 30 days of surgery**

|  |  |
| --- | --- |
| **Complication** | **Number of patients with available data and included in analysis - no. (%)** |
| Cluster 1 - XX |
| Cardiac - no. (%) |   |   |   |   |
| Arrhythmia |   |   |   |   |
| Cardiac arrest with resuscitation  |   |   |   |   |
| Respiratory - no. (%) |   |   |   |   |
| Pneumonia |   |   |   |   |
| Pleural effusion  |   |   |   |   |
| Pneumothorax |   |   |   |   |
| Bronchospasm  |   |   |   |   |
| Aspiration pneumonitis |   |   |   |   |
| Acute lung injury |   |   |   |   |
| Acute respiratory distress syndrome  |   |   |   |   |
| Infection - no. (%) |   |   |   |   |
| Surgical site infection (superficial) |   |   |   |   |
| Surgical site infection (deep) |   |   |   |   |
| Surgical site infection (organ space) |   |   |   |   |
| Urinary tract infection  |   |   |   |   |
| Infection, source uncertain |   |   |   |   |
| Laboratory confirmed blood stream infection |   |   |   |   |
| Other - no. (%) |   |   |   |   |
| Acute kidney Injury - no. (%) |   |   |   |   |

**Table 5: Adverse events**

|  |  |
| --- | --- |
| **Adverse Events - no. (%)** | Cluster 1 - XX |
| Patients with ≥ 1 adverse event |   |   |  |  |
| Type of adverse event |   |   |  |  |
| Hypertensiona |   |   |  |  |
| Hypotensionb |   |   |  |  |
| Acute kidney injuryc |   |   |  |  |
| Other |   |   |  |  |

a Defined as Systolic BP>180mmHg from randomisation until 48 hours after surgery, Diastolic BP> 100mmHg from randomisation until 48 hours after surgery

b Defined as requiring pressor via central venous access from randomisation until 48 hours after surgery

c Defined as in the absence of haemorrhage/sepsis (KIDIGO grades 1-4) within 30 days after surgery

**Dummy figure**

RAAS components – absolute values per identified cluster [expressed as median/25-75th box plots]