Statistical Analysis Plan

(SAP)

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Author: Sebastian Roed Rasmussen (MD, Dept. Cardiothoracic Anaesthesiology, Rigshospitalet)

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Protocol: <Persimune_suPAR_study1> Statistical Analysis Plan

Title: Predictive value of suPAR and hsCRP on postoperative mortality in 951 patients undergoing elective on-pump cardiac surgery.

CONTENTS

Outcome after cardiac surgery is currently built on risk stratification based on patient demographics, comorbidities, physiological reserve and procedural variables, but these parameters explain only a minor part of the observed variation in perioperative complications between patients. A recent trend in risk modelling has been the addition of various biomarkers to supplement clinical risk predictors. By identifying predictive parameters of postoperative outcome such as biomarkers, future prevention- and treatment strategies for high risk patients may be developed and could potentially reduce morbidity and mortality after cardiac surgery and possibly in the future introduce the great opportunity of personalized medicine. One of the most important contributors to morbidity after cardiac surgery is the systemic inflammatory response syndrome (SIRS). The inflammatory response is triggered by the combination of surgical trauma, activation of blood components in the extracorporeal circuit on cardiopulmonary bypass, ischemia/reperfusion injury and endotoxin release. This inflammation most likely plays a central role in the development of all vital organ failures postoperatively including acute kidney injury, perioperative myocardial infarction, respiratory failure and stroke (Laffey et al. 2002, Karkouti et al. 2009, Kertai et al. 2015).

The fundamental role of inflammation in cardiovascular disease has prompted interest in the predictive capability of numerous biomarkers that detect subclinical levels of inflammation. Formation of atherosclerotic plaques is proven to be influenced by inflammation, and soluble urokinase plasminogen activating receptor (suPAR) and High-sensitivity C-Reactive Protein (hsCRP) highlight different elements of inflammatory biochemical pathways linked to cardiovascular risk (Desmedt et al. 2017). CRP is involved in the trigger process of vascular remodelling and is positively associated with anthropometric measures where suPAR is linked to endothelial dysfunction, subclinical organ damage and atherosclerotic disease burden. SuPAR and hsCRP have been studied in infectious disease as well as in cardiovascular disease but the relationship to cardiovascular surgery outcome has only recently been explored. CRP is an acute-phase protein produced by the liver in response to several cytokines released from leukocytes during inflammation in response to infection or trauma. High-sensitivity CRP (hsCRP) measures low levels of CRP with increased sensitivity. SuPAR is a novel biomarker that correlates significantly with cardiovascular events and outperforms traditional markers of inflammation in prognosticating a range of cardiovascular events. SuPAR is released into the circulation by cleavage of the membrane-bound uPAR from various cells, including inflammatory and endothelial cells (Hodges et al. 2015). HsCRP has received a lot of attention regarding screening, reclassification and prediction of treatment response in patients suffering from cardiovascular disease and elevated levels of hsCRP preoperatively was found to be associated with increased risk of postoperative cardiovascular events after on pump coronary artery bypass grafting surgery (Balciunas et al., 2009).

2 DATA SOURCE

PATS: BMI, procedure codes, CPB time and aorta clamp time, procedure date, date and time for admission and discharge ICU.

ICCA: length of stay ICU (if missing in PATS)

Biochemical data: creatinine

The Danish National Patient Register: age, sex, date of death

Perfusionist charts: CPB time and aorta clamp time (if missing in PATS)

Sundhedsplatformen: type of surgery, previous PCI, AMI within 90 days of surgery, hypertension, diabetes mellitus, smoking status, left ventricle ejection fraction (LVEF), NYHA, CCS, date of discharge hospitalisation, length of stay ICU (if missing in PATS), and the following defined according to the EuroSCORE II; extracardiac arthropathy, mobility status, previous cardiac surgery, chronic lung disease, active endocarditis, critical preoperative state and pulmonary hypertension,

The blood samples for the study were drawn preoperatively or within the first 24 hours of surgery (only preoperative data for hsCRP included) and stored in the Persimune Biobank or two other biobanks at Dept. Cardiothoracic Anaesthesiology and Dept. Cardiothoracic Surgery, Rigshospitalet (registered Katrine Buggeskov, MD, PhD and Anne Vedel, MD, respectively). The analysis is performed using commercially available analyses (suPARnostic® kit (validated to measure suPAR concentrations between 0.6 and 22 ng/mL) (ViroGates)). HsCRP will be measured by high sensitivity CRP assays (Tina-quant hsCRP latex assay (validated to measure CRP concentrations between 0.3 – 20 mg/L) (Roche/Hitachi)). In patients with CRP>20 mg/L a regular CRP-measurement will be performed.

3 ANALYSIS OBJECTIVES

This study aims to investigate whether preoperative levels of suPAR and hsCRP are associated with death after cardiac surgery. Further, to assess whether suPAR and hsCRP provides increased predictive accuracy of the clinical risk model EuroSCORE II. The purpose of the study is to gain knowledge on whether these inflammatory biomarkers might be able to reveal a pro-inflammatory disease state that represents a significant risk in patients undergoing cardiovascular surgery. Hence, these biomarkers may assist clinicians to gain more accurate risk identification and potentially compassionate treatment for high risk patients.

4 ANALYSIS SETS/ POPULATIONS/SUBGROUPS

The population includes adult patients (≥18 years) undergoing cardiac surgery at department of Cardiothoracic Surgery at Rigshospitalet, Copenhagen University hospital, Denmark.

Inclusion Criteria:

- Patients planned for elective on-pump cardiac surgery (isolated coronary artery bypass graft (CABG), single and multiple valvular procedures, combined CABG and valvular surgery, and others) and have given informed consent on delivering a blood sample for the biobank.

Exclusion Criteria:

- Peroperatively cancelling the surgery due to anatomic challenging findings, sudden change to off-pump coronary artery bypass (OPCAB) surgery, death prior to surgery and project blood samples not available.

5 ENDPOINTS AND COVARIATES

Primary objective

1. Association of preoperative suPAR values in relation to the censored time-to-event outcome "death from any cause" [Time Frame: from index surgery to date of data collection]

Secondary objectives:

2. Association of preoperative hsCRP values in relation to the censored time-to-event outcome "death from any cause" [Time Frame: from index surgery to date of data collection]
3. Assess whether adding suPAR, hsCRP or combined suPAR+hsCRP measurements improves predictive accuracy of EuroSCORE II [Time Frame: from index surgery to 30 days postoperative]
4. Sensitivity of the models; EuroSCORE II, suPAR, hsCRP, suPAR+hsCRP, EuroSCOREII+suPAR, EuroSCOREII+hsCRP and EuroSCOREII+suPAR+hsCRP in relation to the time-to-event outcome "death from any cause" [Time Frame: from index surgery to 30 days postoperative]
5. Specificity of the models; EuroSCORE II, suPAR, hsCRP, suPAR+hsCRP, EuroSCOREII+suPAR, EuroSCOREII+hsCRP and EuroSCOREII+suPAR+hsCRP in relation to the time-to-event outcome "death from any cause" [Time Frame: from index surgery to 30 days postoperative]
6. Asses association between suPAR and hsCRP measurements

Other outcome measures:

1. 30 days mortality measured by a yes/no question of "all-cause mortality" [Time Frame: from index surgery to 30 days]
2. 1-year mortality measured by a yes/no question of "all-cause mortality" [Time Frame: from index surgery to 1 year]

Covariates to be used in the analysis in order of priority according to number of events:

1. Sex (female/male), age (continuous), current smoker (status preoperative: yes/no), diabetes mellitus (status preoperative: yes/no), creatinine (preoperative: continuous), CPB time (continuous) and aorta clamp time (continuous), AMI within 90 days (status preoperative: yes/no)

6 HANDLING OF MISSING VALUES AND OTHER DATA CONVENTIONS

Missing data is expected to be at random and due to device fault or misplaced registration. No data are extracted from questionnaires or physical follow-up by the patients. Missing values will be handled using pairwise deletion, still analysing all cases with the variable of interest.

7 STATISTICAL METHODOLOGY

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7.1 STATISTICAL PROCEDURES

A table 1 with patient characteristics for the entire study population will be generated presenting frequencies (percent), mean ± standard deviation (SD), and median (25–75% quartile), where appropriate. This table will include the following variables: age (continuous (years)), sex (male), body-mass-index/BMI (continuous (kg/m^2), diabetes mellitus (NIDDM/IDDM), arterial hypertension (yes), EuroSCORE II value (continuous (%)), smoking status (never/previous/active), precious PCI (yes), NYHA (I-IV), CCS class 4 (yes), left ventricle ejection fraction/LVEF (continuous (%)), Previous cardiac surgery (yes), chronic lung disease (yes), acute myocardial infarction (AMI) within 90 days of surgery (yes), pulmonary hypertension (no, moderate, severe), weight on the intervention (isolated CABG, single non CABG, 2 procedures, 3 procedures), surgery on thoracic aorta (yes), baseline creatinine (continuous (µmol/L)), hsCRP (continuous (mg/L)), suPAR (continuous (ng/mL)),
In addition, the following peri- and postoperative variables will be used for analysis: cardiopulmonary by-pass/CPB time (continuous (min)), aorta clamp time (continuous (min)), length of stay – ICU (continuous (hours)), length of stay – Hospitalisation (continuous (days)).
A table 2 will be generated including relevant tests for association between the individual variables mentioned above and continuous values of suPAR and hsCRP, respectively.

We will fit Cox proportional hazards models to assess the association between preoperative suPAR and hsCRP-values and death from any cause. The goodness-of-fit of the Cox model will be investigated and the model will be adapted as required to fulfil the model assumptions. The model will be adjusted for sex, age, current smoker, AMI within 90 days, diabetes mellitus, creatinine, CPB time and aorta clamp time. 30-days survival will be modelled using logistic regression. If we cannot find Cox and/or logistic models that fit the data, alternative models will be considered.

Receiver operating characteristics (ROC) curve and area under the curve (AUC) analysis will be performed comparing EuroSCORE II, suPAR, hsCRP, suPAR+hsCRP, EuroSCOREII+suPAR, EuroSCOREII+hsCRP and EuroSCOREII+suPAR+hsCRP. Sensitivity and specificity values will be included.

Regression analysis between suPAR and hsCRP measurement will be performed.

Kaplan-Meier survival plots will be generated for the 4 quartiles of suPAR and hsCRP, respectively.

Two supplementary tables will be generated presenting the pre-, peri- and postoperative variables divided into the 4 quartiles of suPAR and hsCRP, respectively.

A two-sided p value <0.05 will be considered significant.

8 QC PLANS

An internal quality control of the reliability of the biochemical analyses of the study biomarkers will be performed at the department of clinical biochemistry. Since the remaining clinical data is extracted from well-established electronical databases, no internal validation on the data will be performed.