

STUDY PROTOCOL

Title: Platelets and complement activation in coronary artery bypass graft surgery (CABG)

Protocol Code: PAC

Version, Date: Version 2.1, 27.08.2018

Sponsor: **Medical University Innsbruck**
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CONFIDENTIALITY

The present study, including archiving of study documents, will be conducted according to this protocol, and in compliance with Good Clinical Practices, the Declaration of Helsinki in its latest version, the local laws and regulations and the applicable regulatory requirements.

CONFIDENTIAL

The information provided in this document is strictly confidential and is available for review to Investigators, potential Investigators, health authorities and appropriate ethics committees. No disclosure should take place without written authorization from the Sponsor, except to the extent necessary to obtain informed consent from potential subjects. Once signed, the terms of this protocol are binding for all parties.

DECLARATION OF THE SPONSOR

Study Title: **Platelets and complement activation in coronary artery bypass graft surgery (CABG) factor assays**

Protocol Code Number: **PAC**

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The present study protocol was subject to critical review. Its content is consistent with the current risk/benefit evaluation as well as with the moral, ethical and scientific principles of good clinical practice, the latest version of the Declaration of Helsinki, the local laws and the regulations and the applicable regulatory requirements.

Univ.-Prof. Dr. Karl Lindner

Department of Anesthesiology and
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Place, Date, Signature

The signatories above confirm that they have read this study protocol and agree that it contains all information required for study performance. They also agree to conduct the study as set out in this protocol. It has been understood that all documentation previously not published will be kept in strictest confidence.

DECLARATION OF THE PRINCIPAL INVESTIGATOR AND THE SUB INVESTIGATOR, STUDY AUTHORS AND THE STATISTICIAN

Study Title: **Platelets and complement activation in CABG**

I have read the study protocol and agree that it contains all information required for study performance. I agree to conduct the study as set out in the protocol. In particular, I agree to adhere to the moral, ethical and scientific principles of good clinical practice, the latest version of the declaration of Helsinki, the local laws and regulations and the applicable regulatory requirements.

In case of any changes which may concern the responsibility of each of the signatories, I will notify them.

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DECLARATION OF THE PRINCIPAL INVESTIGATOR

Study Title: **Platelets and complement activation in CABG ("PAC")**

I have read this study protocol and agree that it contains all the information required for study performance. I agree to conduct the study as set out in the protocol. In particular, I agree to adhere to the moral, ethical and scientific principles of good clinical practice, the latest version of the declaration of Helsinki, the local laws and regulations and the applicable regulatory requirements.

Name

Place, Date

Signature

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SYNOPSIS

Sponsor	Medical University Innsbruck
Center	University Hospital Innsbruck
Title	Platelets and complement activation in CABG
Protocol Code	PAC

DESIGN OF THE CLINICAL STUDY	
Indication	elective coronary artery bypass graft surgery (CABG)
Study Design	Prospective, observational, single center study
Objective	<p><u>Primary:</u></p> <ul style="list-style-type: none"> -To assess the impact of complement activation on platelet count. <p><u>Secondary:</u></p> <ul style="list-style-type: none"> - To assess the specific pathway of complement activation, mtDNA levels and their effects on platelet count and platelet function using mean platelet volume to platelet count ratio (MPV/PTC) and specific platelet function by fluorescence-activated cell sorting (FACS). - To explore whether these variables correlate with transfusion requirements, postoperative morbidity (need for revision surgery, cardiovascular events including thromboembolic events, sepsis, single or multiple organ failure according to SOFA Score) and in hospital mortality.
Endpoints and Evaluation Parameters	<p><u>Primary endpoint:</u></p> <ul style="list-style-type: none"> - Correlation of the change of C5b-9 and change of platelet count from baseline (T0) to after 15 minutes of administration of protamine (T2). <p><u>Secondary endpoints:</u></p> <ul style="list-style-type: none"> - correlation between levels of complement factors [c5b-9, C1q (C1r/C1s), C3a, C5a, MBL, Factor B, Factor D], mtDNA level [human NADH dehydrogenase 1 gene] and platelet function [MPV/PTC ratio and FACS for platelet activation factor 4 (PAF-4)] - correlation between levels of complement [see above] and concentration of coagulation factors [F I – F XIII, FXa, FXIIa, Kallikrein, Bradykinin, endogenous thrombin potential (ETP)] and transfusion requirements - correlation of levels of complement, mtDNA and platelet function to postoperative morbidity (need for revision surgery, cardiovascular events including thromboembolic events, sepsis, single or multiple organ failure according to SOFA Score) and in hospital mortality
Number of Patients	190

Duration of Patient Participation	- day of surgery to postoperative day three (POD 3) - Follow-Up Period "Medical Monitoring": until discharge or day 30
Dates of Beginning and End of Study	Date of beginning of study: 1.July 2018 Date of end of study: 1.July 2020
Inclusion Criteria	I.1. Male and female subjects ≥ 18 years and ≤ 85 years I.2. elective coronary artery bypass graft surgery on cardiopulmonary bypass (CPB) with or without valve surgery (max. 2 valves) and elective valve surgery (max. 2 valves) I.3. ASA I - IV I.4. written informed consent
Exclusion Criteria	E.1. emergency CABG with cardiac valve surgery and emergency valve surgery and emergency aortic dissection E.2. preexisting complement deficiency syndromes E.3. preexisting thrombocytopathy or thrombocytopenia (platelet count below 100 G/L) E.4. Known history of congenital coagulopathy E.5. Patients that are known to be pregnant E.6. Known participation in another interventional clinical trial
Study-related Measurements and Data collection	<u>Study related measurements:</u> complement factors, platelet function (FACS), mtDNA, concentrations of coagulation factors and thrombin generation will be obtained at baseline (T0) and at several time points (T1-T8) during and after CABG until POD3. <u>Data collection:</u> Details on patient's condition before surgery, as well as all clinical data until discharge or day 30
Study Procedure	Except study related blood sampling patient's care is not influenced by the study and follows clinical routine. The Visit time points are: T0 baseline: after insertion of arterial cannula beginning surgery T1 after re-opening aortic clamp on CPB T2 15 minutes after protamine infusion T3 end of surgery = admission lab at the postoperative intensive care unit T4 4 hours after end of surgery T5 12 hours after end of surgery T6 24 hours after surgery begin T7 48 hours after surgery begin T8 72 hours after surgery begin T9 30 days after surgery or hospital admission <u>Study related blood sampling (T0-T8):</u> <ul style="list-style-type: none"> Levels of complement factors: c5-b9, C1q (C1r/C1s), C3a, C5a, MBL, Factor B, Factor D

	<ul style="list-style-type: none"> ▪ mtDNA (human NADH dehydrogenase 1 gene) ▪ Platelet count, mean platelet volume, fluorescence-activated cell sorting (FACS) ▪ Coagulation factors: FI – FXIII, FXa, FXIIa, Kallikrein, Bradykinin, endogenous thrombin potential (ETP) <p><u>Routine laboratory blood sampling (T0-T9):</u></p> <p>activated clotting time (ACT), Rotem parameters, Multiplate test analysis, whole blood cell count, standard plasmatic coagulation tests,</p> <p>blood gas analysis, CRP, Procalcitonin (PCT), renal and liver function parameters, cardiac enzymes (CK, CK-MB, Myoglobin, Trop T, NTpro BNP)</p> <p><u>Clinical Data:</u></p> <p><i>The following parameters will be collected at baseline(T0):</i></p> <p>age, gender, body weight(kg) and size (cm), medical history (chronic renal impairment, Diabetes, Hypertension, previous myocardial infarction, previous stroke, previous percutaneous coronary intervention, previous CABG), pre-medication, Euroscore II.</p> <p><i>During surgery and until POD 3 following data will be collected if available:</i></p> <p>Details on CPB, Cell Saver blood volume, blood pressure (BP), hear rate (HR), central venous pressure (CVP), mean pulmonary artery pressure (MPAP),cardiac index (C.I.), cardiac output (C.O.), Oxygen Saturation (SpO2), Horowitz, Fraction of inspired Oxygen (FiO2), Diuresis per hour, Concomitant Medication, transfusion and coagulation products, need for haemofiltration , need for extracorporeal membrane oxygenation (ECMO) and/or need for inhalative NO.</p>
Risk-Benefit Analysis	There is no risk for pain and infection since the blood is drawn from a routinely done cannula. The total amount of blood sampling is 150 ml within 3 days and will not harm the patients. The patients benefit from the intensified diagnostics.
Statistics	Primary endpoint: Correlation of the change of C5b-9 and change of platelet count from baseline (T0) to after 15 minutes of administration of protamine (T2).

STUDY FLOW CHART

	T0 Baseline Day of surgery Day 0	T1 CPB Opening Aortic crossclamp Day0	T2 CPB 15min after Protamin Day0	T3 End of surgery Admission post Op ICU Day0	T4 PACU 4h post surgery Day 0	T5 PACU 12h post surgery Day 0	T6 PACU 08:00 Day1	T7 ICU 08:00 Day2	T8 ICU 08:00 Day3	T9 Ward/ICU Day 30
Special laboratory ¹	x	x	x	x	x	x	x	x	x	
Routine laboratory ²	x	x	x	x	x	x	x	x	x	x
Clinical data ³	x	x	x	x	x	x	x	x	x	x
Concomitant Medication	x	x	x	x	x	x	x	x	x	
Transfusions/ Coagulation products		x	x	x	x	x	x	x	x	
Scores	Euroscore II				SOFA*	SOFA*	SOFA*	SOFA*	SOFA*	
Final survey										x

*calculation of SOFA if patient stays at ICU

¹ c5-b9, C1q (C1r/C1s), C3a, C5a, MBL, Factor B, Factor D, mtDNA, Platelet count, mean platelet volume, fluorescence-activated cell sorting (FACS), FI – FXIII, FXa, FXIIa, Kallikrein, Bradykinin, endogenous thrombin potential (ETP)

² activated clotting time (ACT), Rotem parameters, Multiplate test analysis, whole blood cell count, standard plasmatic coagulation tests, blood gas analysis, CRP, Procalcitonin (PCT), renal and liver function parameters, cardiac enzymes (CK, CK-MB, Myoglobin, TROP T, NTpro BNP)

³ age, gender, body weight(kg) and size (cm), medical history (chronic renal impairment, Diabetes, Hypertension, previous myocardial infarction, previous stroke, previous percutaneous coronary intervention, previous CABG), pre-medication, Euroscore II, Details on CPB, Cell Saver blood volume, blood pressure (BP), heart rate (HR), central venous pressure (CVP), mean pulmonary artery pressure (MPAP), cardiac index (C.I.), cardiac output (C.O.), Oxygen Saturation (SpO₂), Horowitz, Fraction of inspired Oxygen (FI_{O2}), Diuresis per hour, Concomitant Medication, transfusion and coagulation products, , need for extracorporeal membrane oxygenation (ECMO) and/or need for inhalative NO.

ABBREVIATIONS

ASA	American Society of Anesthesiologists (ASA) Score is a global score that assesses the physical status of patients before surgery.
BGA	Blood gas analysis
CA	Competent Authority
CABG	Coronary Artery Bypass Graft
CPB	Cardiopulmonary Bypass
CRF	Case Report Form
ECMO	extracorporeal membrane oxygenation
ETP	endogenous thrombin potential
FI - FXIII	coagulation factor I - XIII
FACS	fluorescence-activated cell sorting
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
mtDNA	mitochondrial DNA
MPV	mean platelet volume
MPV ratio	Mean platelet volume/mean platelet count
NA	not applicable
ND	not done
POD	postoperative day
RIFLE Score	Risk Injury Failure Loss Endstage kidney injury score
SOFA Score	sequential organ failure assessment score

PLATELETS AFTER COMPLEMENT ACTIVATION IN CARDIAC SURGERY - PAC

1. INTRODUCTION

1.1. Background

Patients undergoing coronary artery bypass graft surgery (CABG) frequently exhibit postoperative bleeding complications which are still a major cause for morbidity and mortality (1). One major contributing factor is the loss of platelets and impaired platelet function. During cardiopulmonary bypass (CPB) blood comes in close contact with foreign surfaces which induces a series of reactions; especially the complement system as part of the innate immunity is highly activated (2). Due to the strong crosslink between complement system, platelet function and the plasmatic coagulation it is likely that complement activation during CPB has an impact on the overall process of clot formation. (3). Besides the activation of the complement system there is growing evidence that the occurrence of mitochondrial DNA (mtDNA) during CPB might be related to further platelet activation (4). Activated platelets may enhance micro-thrombosis leading to organ failure and thereby contributing to postoperative morbidity.

Several studies investigated the effect of complement activation in cardiac surgery patients (2;5;6-7) however, it is still unclear whether the activation of the complement system further increases platelet loss.

1.2. Need for the Study

One major complication during and after CABG surgery is bleeding requiring transfusion and even reoperation in about 2%- 8% of patients.

A recent study shows that impaired hemostasis was observed in 9,5 - 21,5% of these cases (1). As bleeding complications increase patient morbidity and mortality, this study is designed to investigate, the possible mechanisms of platelet loss during CABG.

We hypothesize that increased complement activation during CPB leads to platelet activation and loss of platelets. We further hypothesize that the degree of complement activation and levels of mtDNA correlate with postoperative bleeding, transfusion requirements and clinical outcome.

1.3. Risk Benefit analysis

For blood withdrawal no additional arterial/venous puncture is needed.

Blood samples will all be withdrawn from preexisting arterial cannulas. Only if there is no arterial line available, blood will be withdrawn of an also preexisting central venous catheter.

The total amount of blood collected over the entire study period will be 150 ml, which seems to be a negligible risk compared to the average blood loss in CABG surgery.

The findings of this study will help to understand further details of the mechanism of platelet drop and complement activation during and after CABG surgery. Furthermore, the results could help to develop new therapeutic strategies to avoid activation of the complement and hemostatic systems during CPB which may decrease bleeding complications in CABG patients.

2. STUDY OBJECTIVES

2.1. Primary Study Objective

The aim of the study is to examine the correlation between complement activation (c5b-9) and platelet decline during elective coronary artery bypass graft (CABG) **with or without valve surgery (max. 2 valves) and elective valve surgery (max. 2 valves)** on cardiopulmonary bypass (CPB).

2.2. Further Study Objectives

A secondary aim of the study is to investigate the specific pathway of complement activation, the mtDNA levels and the interaction with platelet function.

Therefore we search for correlations between levels of complement factors [c5b-9, C1q (C1r/C1s), C3a, C5a, MBL, Factor B, Factor D], mtDNA level [human NADH dehydrogenase 1 gene] and platelet function [MPV/PTC ratio and FACS for platelet activation factor 4 (PFA)].

Further it will be explored whether the level of complement activation and levels of mtDNA and the plasmatic coagulation system correlate with transfusion requirements, postoperative morbidity (need for revision surgery, cardiovascular events including thromboembolic events, sepsis, single or multiple organ failure according to SOFA Score), and in hospital mortality.

We will correlate levels of complement [see above] and concentration of coagulation factors [F I – F XIII, FXa, FXIIa, Kallikrein, Bradykinin, endogenous thrombin potential (ETP)] and transfusion requirements and correlate levels of complement, mtDNA and platelet function to postoperative morbidity (need for revision surgery, cardiovascular events including thromboembolic events, sepsis, single or multiple organ failure according to SOFA Score) and in hospital mortality.

3. INVESTIGATIONAL PLAN

3.1. General Design of the Study

This is a prospective observational single center study in elective cardiac surgery patients undergoing coronary artery bypass grafts with or without valve surgery (max. 2 valves) and elective valve surgery (max. 2 valves).

Patient care is not influenced by the study and follows clinical routine. Patient follow up will be until hospital discharge or day 30 after surgery.

3.2. Timetable

T0	baseline: after insertion of arterial cannula beginning surgery
T1	after re-opening aortic clamp on CPB
T2	15 minutes after protamine infusion
T3	end of surgery = admission lab at the postoperative intensive care unit
T4	4 hours after surgery/admission
T5	12 hours after surgery/admission
T6	24 hours after surgery begin
T7	48 hours after surgery begin
T8	72 hours after surgery begin
T9	30 days after surgery or hospital admission

3.3. Blood sampling

3.3.1 Study related blood samples (T0-T8)

- Levels of complement factors: c5-b9, C1q (C1r/C1s), C3a, C5a, MBL, Factor B, Factor D
- mtDNA (human NADH dehydrogenase 1 gene)
- Platelet count, mean platelet volume, fluorescence-activated cell sorting (FACS)
- Coagulation factors: FI – FXIII, FXa, FXIIa, Kallikrein, Bradykinin, endogenous thrombin potential (ETP)
- Residual plasma and serum from blood samples will be stored at -80°C for further differentiated complement and mtDNA assays if useful

3.3.2. Collection of routinely measured laboratory parameters, and clinical data (T0-T9)

The following routine laboratory parameters will be collected if available:

activated clotting time (ACT), Rotem parameters, Multiplate test analysis, whole blood cell count, standard plasmatic coagulation tests, blood gas analysis, CRP, Procalcitonin (PCT), renal and liver function parameters, cardiac enzymes (CK, CK-MB, Myoglobin, Trop T, NTpro BNP)

Study related blood sampling will overlap with routine blood sampling.

Clinical Data

The following parameters will be collected at baseline(T0):

age, gender, body weight(kg) and size (cm), medical history (chronic renal impairment, Diabetes, Hypertension, previous myocardial infarction, previous stroke, previous percutaneous coronary intervention, previous CABG), pre-medication, Euroscore II.

During surgery following data will be collected if available:

Details on CPB, Cell Saver blood volume, blood pressure (BP), hear rate (HR), central venous pressure (CVP), mean pulmonary artery pressure (MPAP),cardiac index (C.I.), cardiac output (C.O.), Oxygen Saturation (SpO₂), Horowitz, Fraction of inspired Oxygen (FiO₂), Diuresis per hour, Concomitant Medication, transfusion and coagulation products, , need for extracorporeal membrane oxygenation (ECMO), need for inhalative NO

As appropriate, the above mentioned clinical data will be collected until POD3 (T1-T9).

3.4. Primary Study Endpoint

The primary endpoint is the correlation of the change of C5b-9 and change of platelet count from baseline (T0) to after 15 minutes of administration of protamine (T2).

3.5. Secondary Study Endpoints

- levels of complement activation: c5b-9, C1q (C1r/C1s), C3a, C5a, MBL, Factor B, Factor D
- Levels of mtDNA
- platelet function as measured by FACS
- Levels of coagulation factors FI – FXIII, FXa, FXIIa, Kallikrein, Bradykinin, endogenous thrombin potential (ETP)
- transfusion requirements (red blood cell concentrates, fresh frozen plasma, platelet concentrate) and dosages of coagulation products administered during surgery, at ICU and until POD 3

- Need for revision surgery because of re-bleeding or hematoma formation within the first 24 hours at ICU
- hours/days on mechanical ventilation
- Oxygenation index (Horowitz quotient) during surgery, at ICU until POD 3 if applicable
- length of ICU stay
- length of hospital stay
- postoperative complications:
 - single and multiple organ failure according to SOFA score
 - acute kidney injury (RIFLE score)
 - diagnosed infection and sepsis
- incidence and type of cardiovascular thromboembolic events
- in hospital mortality

3.6. Number of patients

190 patients will be included after written informed consent.

3.7. Timetable

The duration of the planned recruitment phase is 2 years. For the single subject the duration of the participation 72 hours in the active phase and further 27 days (until 30 days after inclusion) in the Follow-Up phase.

ISSUE	Time schedule
Submission to the EC:	
Study Conduction:	
Laboratory Analysis:	
Final Report / Publication:	

4. STUDY POPULATION

All adult patients undergoing elective coronary artery bypass graft surgery with written informed consent meeting the following criteria:

4.1. Inclusion criteria:

- I.1. Male and female subjects > 18 years and < 86 years
- I.2. elective coronary artery bypass graft surgery with or without valve surgery (max. 2 valves) and elective valve surgery (max. 2 valves) on cardiopulmonary bypass (CPB)
- I.3. ASA I - IV
- I.4. written informed consent

4.2. Exclusion criteria:

- E.1. emergency CABG with or without cardiac valve surgery and emergency valve surgery and emergency aortic dissection
- E.2. preexisting complement deficiency syndromes
- E.3. preexisting thrombocytopathy or thrombocytopenia (platelet count below 100 G/L)
- E.4. Known history of congenital coagulopathy
- E.5. Patients that are known to be pregnant
- E.6. Known participation in another interventional clinical trial

5. FURTHER STUDY PROCEDURES

5.1. Patient Withdrawal (Drop-out)

Patients can drop-out of the study by their own request or the request of the patient's authorized person or the patient's legal guardian (whenever applicable) without mentioning any reasons and without any consequences for the subsequent treatment. Moreover, patients can be withdrawn from the study by the Investigator for health risk reasons.

If the investigator decides to withdraw the patient from the study the reason will be documented in the CRF. It includes, but is not limited to:

- Withdrawal of consent/ not giving consent
- Lost to Follow-Up, etc

If the patient decides to be withdrawn from the study the patient will be asked to agree to use the data collected in course of the study. The patient's decision will be recorded in the patient's medical file.

5.2. Discontinuation of the Study

The Sponsor is authorized to discontinue the study because of relevant medical/administrative causes. The reasons for the discontinuation of the study have to be documented in detail. Patients who are still enrolled at the time of discontinuation, have to be examined for a final investigation, which will be documented in the CRF. If the Investigator has any ethical qualms concerning the continuation of the study, this has to be immediately reported to the Sponsor.

The Sponsor is authorised to discontinue the study, if:

- The recruitment rate of patients is not sufficient
- Serious, non-resolvable problems of quality of the collected data evolve
- New scientific findings during the study's run-time do not allow a continuation of the study

5.3. Closure of the Study

The study must be closed after completion and the ethics committees will be informed when the study is closed.

6. DOCUMENTATION AND DATA MANAGEMENT

The accomplishments of the study in agreement with the GCP-guidelines as well as the trueness of all data documented in the CRF are the responsibility of the Investigator. All collected data of this study have to be recorded in the CRF by appropriate authorized persons.

The Investigator records the participation in a special identification list of patients. Additionally, the participation of the patient in this clinical study has to be recorded in the patient chart.

6.1. Data Entry Form (CRF)

Data of patients and investigation results will be recorded into Case Report Forms (CRF), which are especially developed for this study.

Only the use of black ball pens is allowed. Corrections have to be made in such a way, that previous entries remain readable. Corrections have to be signed and dated by the authorized person, who made the corrections. Data which are not available or were not collected, have to be clearly identifiable as such (NA and ND). The reasons should be documented, if necessary.

6.2. Data Management

Data items from the CRF are entered centrally into the study database by authorized data management staff using appropriate entry techniques and probability check. Entered data are systematically checked by data management staff, using error messages printed from validation programs and database listings. Obvious errors or omissions are corrected by the data management personnel. After recording of all entries and clarification of all queries, the database will be closed at the completion of the study. This closure has to be documented.

7. STATISTICS

Primary analysis

The primary endpoint of this study is the correlation of the change in C5b-9 and the change in platelet count from baseline (T0) to 15 minutes after administration of protamine (T2). The analyzed study population will consist of all patients with available measurements necessary to assess the primary endpoint.

The two changes (C5b-9 and platelet count) are continuous variables and their correlation (Spearman's rank-order correlation denoted by ρ) will be assessed. Therefore, the hypotheses

$$H_0: \rho = 0 \quad \text{vs.} \quad H_1: \rho \neq 0$$

will be tested using the Spearman rank-order correlation test, i.e. the null hypothesis that the Spearman correlation is zero against the alternative that it is not equal to zero. As C5b-9 levels and platelet count are assumed to be predictive for the clinical course of the patients, the association with secondary endpoints will be assessed. Moreover, as C5b-9 and platelet count will be documented at the study time points T0 to T8, their evolution over time will be analyzed using a linear mixed-effects model.

Sample size estimation

To the best of our knowledge, no study is available reporting the association of complement activity and change of platelets in cardiac patients. However, Qin et al. (4) reported a strong correlation (Pearson's correlation coefficient) of $r=0.683$ between platelets (MPV/PTC ratio) and mtDNA levels in 68 patients who underwent CABG. As mtDNA levels are strongly associated with complement activation (9), we expect to observe a comparable correlation between the change of platelets and complement activation. Power analysis was performed to determine the sample size for Spearman's correlation using G*Power (version 3.1.9.2). Assuming an effect size corresponding to $r=0.683$, a sample size of 162 has a power of 95% to assess a significant difference from a large effect of $r=0.5$ with a type I error at a global 5% level (two-tailed). To account for drop-outs, a total of 190 patients will be included.

Secondary analysis

All secondary parameters will be summarized using descriptive statistics, i.e. no./total no. (%) for categorical variables and median (25th to 75th percentile) or mean (SD), however appropriate.

Explorative data analyses will be performed as applicable to investigate the relationship between the comprehensive measurements. These might involve standard explorative techniques such as factor analysis (FA), principal component analysis (PCA), multiple factor analysis (MFA), homogeneity analysis and different clustering approaches as well as more recently developed techniques arising in the context of machine learning.

8. ETHICAL, LEGAL AND ADMINISTRATIVE ISSUES

8.1. Good Clinical Practice and Declaration of Helsinki

The procedures set out in this study protocol are designed to ensure that the Sponsor and the Investigator abide the principles of the ICH guidelines on GCP (E6) recommended for adaptation on 1st of May 1996 by the ICH Steering Committee and the Declaration of Helsinki concerning the conduct, evaluation and documentation of the study.

8.2. Approval of the Study Protocol

Prior to study start, the study protocol and/or other appropriate documents will be approved by the appropriate ethics committee and competent authorities.

8.3. Obtaining Informed Consent

Latest at the day before surgery the patient will be informed about the aim of the study and the need of study related blood sampling and collection of routine laboratory measurements and clinical data.

The patient will be asked to agree on processing the data collected during their participation in the observational clinical study. The patient's decision will be recorded in the patient's medical file.

8.4. Confidentiality

All local legal requirements regarding data protection will be adhered to. All study findings and documents will be regarded as confidential. The Investigator and members of the research team must not disclose any information without prior written approval from the Sponsor.

The anonymity of patients participating must be maintained. Throughout documentation and evaluation, the patients will be recognized on CRFs and other documents by identification number. Documents that identify the patient personally (e.g., the signed informed consent) will be maintained confidential by the Investigator. The patients will be told that all study findings will be handled in strictest confidence.

8.5. Archiving of Study Records

Essential documents will be retained for a minimum of 10 years after completion or discontinuation of the study.

9. AMENDMENTS

After the protocol has been submitted to an ethics committee (EC), any substantial change will require a formal amendment. Once the study has started, amendments will be made only in exceptional cases. The ethics committees must be informed of all amendments.

10. SCORES

SOFA

The SOFA is a six-organ dysfunction/failure score which is daily assessed. Each organ is graded from 0 (normal) to 4 (the most abnormal), providing a daily score of 0 to 24 points. Single organ failure is defined as SOFA score >2 for one organ and multiple organ daily arises with at least 2 organ systems with >2 points.

EUROSCORE II

The abbreviation stands for European System for Cardiac Operative Risk Evaluation and calculate the estimated pre-operative mortality by using the following factors which can be seen in the graphic below.

The screenshot displays the EuroSCORE II mobile application interface on an iPad. The app is titled "EuroSCORE II" and shows various input fields for patient and cardiac factors, operation-related factors, and a section for estimated mortality. The interface is organized into columns and rows with buttons for selection and calculation.

Patient Related Factors

- Patient Age (years): 18-95
- Gender: Male Female
- Creatinine Clearance (ml/min) or Dialysis: > 85 51 - 85 < 51 Dialysis
- Calculate Creatinine Clearance
- Extracardiac Arteriopathy: Yes No
- Poor Mobility: Yes No
- Previous Cardiac Surgery: Yes No
- Chronic Lung Disease: Yes No
- Active Endocarditis: Yes No
- Critical Preop State: Yes No
- Diabetes on Insulin: Yes No

Cardiac Related Factors

- NYHA Class: I II III IV
- CCS Class 4 Angina: Yes No
- LV Function (EF): < 21% 21 - 30% 31 - 50% > 50%
- Recent MI: Yes No
- PA Systolic Pressure (mm Hg): < 31 31 - 55 > 55

Operation Related Factors

- Urgency: Elective Urgent Emergent Salvage
- Thoracic Aortic Surgery: Yes No
- Weight of the Intervention: (see key below) 1 2 3 4

Key for Weight of the Intervention:

- 1. Isolated CABG
- 2. Single non CABG
- 3. Two procedures
- 4. Three + procedures

Estimated Mortality:

Buttons at the bottom: Calculate Risk, Add to Database, Stored Patients, Email Patient Data.

11. REFERENCES

1. Vivacqua, A., Koch, C. G., Yousuf, A. M., Nowicki, E. R., Houghtaling, P. L., Blackstone, E. H., & Sabik, J. F. (2011). Morbidity of bleeding after cardiac surgery: Is it blood transfusion, reoperation for bleeding, or both? *Annals of Thoracic Surgery*, 91(6), 1780–1790. <https://doi.org/10.1016/j.athoracsur.2011.03.105>
2. Oliver, W. C., Nuttall, G. A., Barash, P. G., Warren, O. J., Eng, M., Smith, A. J., ... Athanasiou, T. (2009). The Inflammatory Response to Cardiopulmonary Bypass : Part 1 — Mechanisms of Pathogenesis. *YJCAN*, 23(2), 223–231. <https://doi.org/10.1053/j.jvca.2008.08.007>
3. Oikonomopoulou, K., Ricklin, D., Ward, P. A., & Lambris, J. D. (2012). Interactions between coagulation and complement - Their role in inflammation. *Seminars in Immunopathology*. <https://doi.org/10.1007/s00281-011-0280-x>
4. Qin, C., Gu, J., Hu, J., Qian, H., Fei, X., Li, Y., ... Meng, W. (2016). Platelets activation is associated with elevated plasma mitochondrial DNA during cardiopulmonary bypass. *Journal of Cardiothoracic Surgery*, 11(1), 1–9. <https://doi.org/10.1186/S13019-016-0481-4>
5. Bruins, P., Velthuis, H. te, Yazdanbakhsh, A. P., Jansen, P. G. M., van Hardevelt, F. W. J., de Beaumont, E. M. F. H., ... HackHome, C. E. (1997). Activation of the Complement System During and After Cardiopulmonary Bypass Surgery. *Circulation*, 96(10), 1–14. <https://doi.org/http://dx.doi.org/10.1161/01.CIR.96.10.3542>
6. Pağowska-Klimek, I., Świerzko, A. S., Michalski, M., Głowacka, E., Szala-Poździej, A., Sokołowska, A., ... Cedzyński, M. (2016). Activation of the lectin pathway of complement by cardiopulmonary bypass contributes to the development of systemic inflammatory response syndrome after paediatric cardiac surgery. *Clinical and Experimental Immunology*, 184(2), 257–263. <https://doi.org/10.1111/cei.12763>
7. Hoedemaekers, C., van Deuren, M., Sprong, T., Pickkers, P., Mollnes, T. E., Klasen, I., & van der Hoeven, J. (2010). The Complement System Is Activated in a Biphasic Pattern After Coronary Artery Bypass Grafting. *Annals of Thoracic Surgery*, 89(3), 710–716. <https://doi.org/10.1016/j.athoracsur.2009.11.049>
8. Ricklin, D., Hajishengallis, G., Yang, K., & Lambris, J. D. (2010). Complement: A key system for immune surveillance and homeostasis. *Nature Immunology*. Nature Publishing Group. <https://doi.org/10.1038/ni.1923>
9. Sandler, N., Kaczmarek, E., Itagaki, K., Zheng, Y., Otterbein, L., Khabbaz, K., ... Hauser, C. J. (2018). Mitochondrial DAMPs Are Released During Cardiopulmonary Bypass Surgery and Are Associated With Postoperative Atrial Fibrillation. *Heart Lung and Circulation*, 27(1), 122–129. <https://doi.org/10.1016/j.hlc.2017.02.014>