The impact of miniaturized extracorporeal circulation on thrombin generation and postoperative blood loss

A randomized controlled clinical trial

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ABBREVIATIONS

aPTT	= activated partial thromboplastin times
CABG	= Coronary Artery Bypass Grafting
CAT	= Calibrated Automated Thrombography ()
cECC	= conventional ExtraCorporeal Circulation
CK-MB	= Creatine Kinase-MB
CRF	= Case Report File
CRP	= C-Reactive Protein
ECC	= ExtraCorporeal Circulation
ECG	= ElectroCardioGram
F 1+2	= Prothrombin fragment 1+2
GCP	= Good Clinical Practice
IL-panel	= Interleukin panel
INR	= International Normalized Ratio
miECC	= miniaturized ExtraCorporeal Circulation
PI	= Principle investigator
PT	= Prothrombin
RCT	= Randomized Controlled Trial
SvO2	= Mixed venous oxygen saturation
TMF	= Trial Monitoring File
TNF-α	= Tumor Necrosis Factor alpha

SYNOPSIS

Study Title	The impact of miniaturized extracorporeal circulation on thrombin generation and postoperative blood loss - A randomized controlled clinical trial
Primary Study Objectives	To assess the impact of miECC compared to cECC on thrombin generation after CABG
Secondary Study Objectives	To evaluate the impact of miECC versus cECC on: • Blood loss and transfusion requirement • Coagulation and fbrinolysis • Inflammatory response • Haemodilution and haemolysis • Endorgan protection • Feasibility and safety
Study Design	A single-center, double-blind, parallel-group randomized controlled trial
Primary outcome measures	• Postoperative thrombin generation
Secondary outcome measures	Effects on: • Postoperative blood loss and transfusion requirement • Fibrinolysis: – Clot lysis – Fibrin D-dimer • Coagulation tests – Platelet count – aPTT, INR – Antithrombin – Fibrinogen – Prothrombin fragment 1+2 • Inflammatory response – TNF-α, Interleukin panel – CRP, white blood count • Haemodilution (Nadir intraoperative haematocrit)

	• Haemolysis (LDH) • End-organ protection : – Postoperative CK-MB for myocardial injury – Postoperative creatinine clearance for renal injury – Intraoperative blood lactate for inadequate tissue perfusion • Efficacy/safety and feasibility parameters – Perioperative myocardial infarction – In-hospital neurological events (TCI/stroke) – Postoperative requirement of renal replacement therapy – Re-exploration because of bleeding – Repeat revascularization – Length of ICU stay – Duration of inotropic support and mechanical ventilation – Incidence of atrial fibrillation in-hospital – Incidence of infection (requiring antibiotic therapy, wound revision for graft leg infection, superficial or deep sternal wound infection) – Feasibility of miECC as measured by conversion to cECC and intraoperative complications – 30-day MACCE (Death, MI, cerebrovascular accident, repeat revascularization, deep sternal wound infection)
Study Intervention	Non-emergent CABG with (1) miECC (2) cECC
Duration	Planned enrollment period: 09/2017 - 09/2018 Data analysis: 10/2018 - 02/2019
Sample Size	60 Patients undergoing CABG are randomized 1:1 to receive either miECC or cECC.
Study Population	Elective primary CABG -patients with ECC

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1. HYPOTHESIS AND STUDY OBJECTIVES

1.1. Hypothesis

We propose that miniaturized extracorporeal circulation (miECC) is associated with higher thrombin generation as an indicator of preserved haemostatic capacity early after coronary artery bypass grafting (CABG) compared to conventional extracorporeal circulation (cECC). We hypothesize that the preserved haemostatic capacity will be associated with reduced postoperative bleeding.

1.2. Primary objective

The primary objective of this study is to investigate whether the use of miECC will result in preserved overall haemostatic capacity early after CABG as measured by thrombin generation potential compared with the use of cECC.

1.3. Secondary objectives

The secondary objectives of this study are to compare miECC with cECC in the following:

- Postoperative bleeding
- Transfusion requirement
- Fibrinolysis
- Coagulation
- Inflammatory response
- Haemodilution and haemolysis
- End-organ protection
  - CK-MB as surrogate marker for myocardial injury
  - Creatinine clearance for renal injury
  - Plasma lactate as surrogate marker for inadequate tissue perfusion
- Feasibility and safety

2. BACKGROUND AND RATIONALE

2.1. Background

CABG is the standard treatment of complex ischemic heart disease according to current guidelines (1). In Denmark, more than 70 CABG operations are conducted per 100 000 inhabitants (2). The vast majority of CABG procedures are performed using conventional extracorporeal circulation (cECC). The haemodynamic support, the arrested heart and the bloodless field allow optimal conditions for a complete myocardial revascularization with a low mortality. In Denmark, 30-day mortality for isolated CABG is below 2% (3). However, several adverse systemic effects leading to a significant morbidity can be attributed to cECC. The contact of blood with air and foreign surfaces causes platelet activation, activation and consumption of coagulation factors and a systemic inflammatory response (4,5).

The miECC concept was developed during the last 15 years with the aim to attenuate the adverse systemic effects of cECC. It is based on a closed circuit without a venous cardiotomy reservoir to prevent blood-air contact. Many circuits are furthermore treated with a coating to increase the haemocompatibilty of the blood in contact with the foreign surfaces. Haemodilution is minimized by a low priming volume and low-volume cardioplegia solution. Mechanical stress is reduced by use of a centrifugal pump instead of a roller pump and avoidance of a cardiotomy suction device. Shed blood from the operative field is removed by means of a cell-saver with a washing step to remove debris, activated coagulation factors, and free haemoglobin. Strong evidence supports, that the utilization of miECC in CABG can attenuate perioperative morbidity. MiECC has been shown to be associated with reduced blood loss and transfusion requirements, preserved renal function, reduced incidence of atrial fibrillation, and improved myocardial protection (6). No single large multicenter RCT has so far been sufficiently powered to demonstrate a survival benefit. However, metaanalyses and a propensity score analysis have previously shown a trend towards reduced mortality associated with miECC (7–9) and the most recent metaanalysis including 24 RCTs comprising 2770 patients undergoing heart surgery demonstrated a significant decrease in mortality in miECC compared with cECC (10). The implementation of the miECC concept into clinical practice remains extremely low worldwide despite strong evidence of its safety and beneficial effects (6). The standard treatment in Denmark is to perform CABG with cECC. No Danish center is currently using miECC. Implementation of the miECC technique appears to be limited by the concerns regarding safety and feasibility of miECC raised by few small-scale human and experimental studies (11,12). The lack of a major survival benefit and impending additional

costs may contribute to the reluctance within the cardiothoracic community despite the promising perspective of the miECC.

2.2. Rationale

The suggested mechanisms behind the beneficial effect of miECC are manifold. There is compelling evidence that miECC significantly reduces postoperative bleeding and the need for perioperative blood transfusion (6). Blood transfusion has been shown to increase mortality after CABG independently of the preoperative risk profile (13). Blood transfusion and reexploration for bleeding are independently associated with increased risk of major morbidity and mortality (14). The reduced need for transfusion of red blood cells is mainly attributed to the reduced haemodilution as a consequence of the reduced priming volume of the circuit (6). Few studies have focused on the coagulation changes induced by miECC compared to cECC (11,15–17) or off-pump CABG (18). Significantly decreased platelet counts and significantly increased standard coagulation tests like prothrombin (PT) and activated partial thromboplastin times (aPTT) were demonstrated early after CABG with cECC as compared to miECC in one randomized controlled trial (16). These findings were confirmed in a small non-randomized study (15). Others reported only marginal differences in standard coagulation tests (11,17). Postoperative impairment of platelet aggregation was comparable between miECC and cECC as measured by multiple electrode aggregometry (17), and postoperative rotation thromboelastometry showed no significant differences between cECC and miECC (17). Recent studies have suggested decreased thrombin generation as assessed by calibrated automated thrombography (CAT) early after CABG with cECC to be associated with increased postoperative bleeding (19–21). Thrombin generation assays reflect the plasma haemostatic capacity. Multiple pro- and anticoagulant reactions in the coagulation process result in thrombin as the final enzyme and major effector of the coagulation cascade.

We intend to examine the impact of miECC in comparison to cECC on the early postoperative thrombin generation potential and whether preservation of the coagulation system integrity with miECC is associated with reduced postoperative bleeding and transfusion requirements.

3 STUDY DESIGN AND OUTCOMES

3.1. Study design

Prospective controlled randomized clinical study at the Department of Cardiothoracic Surgery at Aarhus University Hospital, Denmark

3.2. Primary outcome measure

Primary outcome measures for the randomized comparison between miECC and cECC are:

- Thrombin generation after weaning from ECC (post protaminization)

3.3. Secondary outcomes measures

Secondary outcome measures for the randomized comparison between miECC and cECC are:

- Postoperative bleeding as assessed by
  - Cumulated blood loss from the mediastinal and pleural drains 24 hours postoperative
  - Transfusion requirement (packed red blood cells, fresh frozen plasma and platelet concentrates) during surgery and in-hospital
- Postoperative coagulation as assessed by platelet count, aPTT, International Normalized Ratio (INR), antithrombin, fibrinogen, prothrombin fragment 1+2 (F 1+2)
- Postoperative fibrinolytic activity as assessed by clot lysis, and fibrin D-dimer
- Inflammatory response as assessed by Tumor Necrosis Factor alpha (TNF-α), Interleukin-panel (IL-panel), C-reactive protein (CRP), white blood count
- Haemodilution as measured by nadir haematocrit during extracorporeal circulation
- Haemolysis as assessed by lactate dehydrogenase (LDH) levels
- Reexploration because of bleeding in-hospital
- End-organ protection:
  - Postoperative peak creatine kinase-MB (CK-MB) as a surrogate marker for myocardial injury
  - Postoperative renal function as reflected by nadir estimated glomerular filtration (eGFR)
  - Intraoperative blood lactate as a surrogate marker for inadequate tissue perfusion
- Efficacy/safety and feasibility outcomes in-hospital as measured by:
  - Perioperative myocardial infarction as defined in study evaluations
  - Cerebrovascular accident as defined in study evaluations
  - Requirement of renal replacement therapy
  - Repeat revascularization (Reexploration because of ischemia, PCI)

  - Length of intensive care unit stay (days)
  - Duration of inotropic support and mechanical ventilation (hours)
  - Incidence of atrial fibrillation
  - Infection (requiring antibiotic therapy, wound revision for graft leg infection, superficial or deep sternal wound infection)
  - Length of postoperative stay in hospital (days)
  - Intraoperative feasibility of miECC versus cECC
- 30-day MACCE according to the electronic patient record
  - Death from any cause (cardiovascular and non-cardio-vascular mortality).
  - Myocardial infarction
  - Cerebrovascular accident
  - Repeat revascularization
  - Deep sternal wound infection

4 SELECTION AND ENROLLMENT OF PARTICIPANTS

4.1. Inclusion criteria

- Age > 40 years
- Non-emergent CABG with ECC
- Current use of low-dose acetylsalicylic acid
- Agreement of eligibility by the multidisciplinary heart team

4.2. Exclusion criteria

- Inability to give informed consent
- Emergent treatment required (< 24 hours)
- Concomitant cardiac surgery
- Previous cardiac surgery
- Severely reduced kidney function (eGFR < 30ml/min/1.73m^2 or on dialysis)
- Severely reduced ejection fraction (EF < 45%)
- Diagnosis of bleeding disorders
- Non-aspirin antiplatelet drugs stopped < 5 days preoperatively (Clopidogrel, Prasugrel, Ticagrelor, Ticlopidine)
- Current use of systemic glucocorticoid therapy

- Current use of vitamin K antagonists or new oral non-vitamin K anticoagulants
- Platelet count > 450 or <100 x 10^9/l prior to surgery
- Pregnant women or women of child bearing potential without negative pregnancy test
- Active participant in any other intervention trial

4.3. Study enrollment procedures and informed consent

Consecutive patients with non-emergent multivessel coronary artery disease scheduled for primary isolated CABG with ECC will be screened for eligibility by the multidisciplinary heart team. Eligible patients will together with the information regarding their scheduled CABG receive written information regarding the study (*Deltagerinformation om deltagelse i et videnskabeligt forsøg*) and a brochure regarding volunteer's rights in a health science research project (*Forsøgspersoners rettigheder i et sundhedsvidenskabeligt forskningsprojekt*). Information will include the recommendation to bring a companion on admission and emphasize that participation is voluntary and that she/he can any time without reason withdraw their consent and drop out of the project. Patients are admitted one to three days prior to surgery. Patients interested in participation will receive verbal information about the study by the consultant cardiac surgeon in a quiet and undisturbed environment. The investigator will ensure that the patient is adequately informed both verbally and in writing about the study, background, design, risks, side effects, disadvantages, benefits and usefulness. Written consent is signed on the day of surgery giving the patient minimum 24 hours opportunity to ask questions and to reconsider. Copies of the consent form will be given to the patients. After informed consent has been obtained, the REDCap Randomization Module will allocate the patients to the miECC- or cECC-cohort in a 1:1 ratio (22). Patients will be asked permission to store blood samples in two kinds of biobank located at Department of Clinical Biochemistry, Aarhus University Hospital. This is stated clearly in the written information received by all patients. The first research biobank is essential for the study, as it will store frozen plasma samples for batch analysis of thrombin generation, F 1+2, and inflammatory markers. Eventual excessive material will be transferred to the biobank for future unspecified research. This biobank will be used for future, but not yet specified, projects focusing on coagulation research. Its content will only be used after approval from The Central Denmark Region Committees on Health Research Ethics. The samples will be destroyed ten years after completion of the project.

If a study participant wishes his blood sample to be removed from the biobank after termination of the study, this will be granted, but previously produced results will not be deleted.

4.4. Study duration and follow-up

To adequately project the expected enrolment rate, we performed a retrospective analysis of all patients who underwent elective CABG in 2016 at the Department of Cardiothoracic and Vascular Surgery of Aarhus University Hospital based on our quality database (Western Denmark Heart Registry). In 2016, we identified 149 patients, who met the major in-and exclusion criteria of the current study (elective CABG with ECC, age > 40 years, no prior heart surgery, no severely reduced kidney function, no severely reduced ejection fraction, no current use of systemic glucocorticoid therapy). We expect approximately 80% of patients to fulfil the additional in-and exclusion criteria. We presume at the most 60% of eligible patients to give informed consent. To include 60 patients, the approximate duration of the enrolment period is one year.

5. MATERIALS AND METHODS

5.1. Study treatment

In both groups, the same anticoagulation regimen and surgical procedure is employed. After median sternotomy, a pedicled left internal mammary artery and a no-touch saphenous vein graft are harvested. Unfractionated heparin is administered with a target activated coagulation time of >400 seconds. Additional boluses of heparin are administered as required during the procedure.

Heparin and protamine doses are assessed by the HMS Plus® Hemostasis Management System (Medtronic International, Tolochenaz, CH). Tranexamic acid as an antifibrinolytic agent is given on induction of anaesthesia (2 g), on commencement of ECC (1 g), and after weaning from ECC (1 g).

A 24-F arterial cannula, a 29/29 F dual-stage venous cannula, and a combined vent-/cardioplegia cannula in the ascending aorta are utilized. Normothermia and goal-directed perfusion management are applied (maintaining mixed venous oxygen saturation (SvO2) >65% as measured by in-line blood gas monitoring on the venous return line and the oxygen delivery (DO_2) level above >270 ml/min/m^2).

After completion of the distal anastomoses and removal of the aortic cross-clamp, the proximal anastomosis is performed with an aortic partial clamp. Protamine is administered to neutralize heparin after weaning from ECC. Chest tubes 32F are inserted to drain the left pleural cavity and the mediastinum.

MiECC

The miECC closed circuit consists of polyvinylchloride and silicone tubing coated with a hydrophilic biosurface (Balance® Biosurface) to reduce platelet adhesion and activation. It contains a centrifugal pump (Affinity™ CP centrifugal blood pump AP40, Medtronic International, Tolochenaz, CH), an automatic venous air removal device (Affinity® VARD, Medtronic International, Tolochenaz, CH), and an oxygenator with integrated arterial filter and a membrane surface of 2.5 m^2 (Affinity Fusion®, Medtronic International, Tolochenaz, CH). Ante- and retrograde autologous priming is aimed for in all patients to reduce the effective priming volume of the system to 400 to 700 ml. The shed blood is collected and processed with a cell-saving device applying Ringer's solution (Autolog®, Medtronic International, Tolochenaz, CH). Myocardial protection is accomplished using antegrade intermittent cold modified blood Calafiore cardioplegia every 20 minutes. A soft-shell reservoir bag is used to collect blood from the vent and to regulate the volume.

cECC

The cECC circuit consists of uncoated polyvinylchloride and silicone tubing (Costumpack M450311F, Medtronic International, Tolochenaz, CH), a hard-shell venous reservoir, an oxygenator with integrated arterial filter (Affinity Fusion®, Medtronic International, Tolochenaz, CH). A roller pump (Stöckert S5, Munich, Germany), and an oxygenator with integrated arterial filter and a membrane surface of 2.5 m2 (Affinity Fusion®, Medtronic International, Tolochenaz, CH). The flow rate for the cECC system is set at 2.4 L/min/m^2. The priming volume of the system is 1400 mL of Ringer's solution. Myocardial protection is accomplished using antegrade intermittent cold blood Harefield cardioplegia every 20 minutes.

Anaesthesia

All preoperative cardiac medication including low-dose aspirin is continued until the day prior to surgery. Other medication affecting haemostasis is discontinued according to institutional guidelines. β-blocking agents are continued until and including the day of operation.

Anaesthesia is induced according to local standards (Propofol, Sufentanil, and Rocuronium in a weight-adapted dose), maintained with Propofol infusion and Sevoflurane as well as repeated injections of Sufentanil and Rocuronium according to the clinical need. In the operating room, patients are monitored with five-lead electrocardiography, radial artery pressure, a central venous catheter in internal jugular vein, a thermo dilution pulmonary artery catheter (Swan-Ganz CCOmbo CCO/SvO2, Edwards Lifesciences LLC, Irvine, CA), oxygen pulse oximetry, transoesophageal echocardiography, and temperature monitoring. The patient's temperature is maintained at 37 °C by means of a heater cooler unit during ECC and a forced-air warming device off ECC. Excessive fluid administration is avoided to reduce haemodilution. Intraoperative positioning of the patient and low-dose vasoactive agents are used to keep mean arterial pressure between 50 and 60 mmHg during ECC.

Postoperative management

Patient sedation is maintained with an intravenous Propofol infusion, until arrival at intensive care unit (ICU). Upon arrival at ICU, a focus-assessed transthoracic echo (FATE) is performed by the attending anaesthesiologist to exclude pericardial or pleural effusions, and the patient is extubated and observed overnight at the ICU. All patients receive oral Paracetamol 1g and additional non-steroidal anti-inflammatory drugs (Ketorolac) and/ or morphine-like analgesics (morphine or oxycodone) as required. During the postoperative period, the chest tubes are removed at the earliest ten hours postoperative, following mobilization, on the condition that total drainage is decreasing and less than 200 mL over a four hour period, and without air leakage. Perioperative glucocorticoids given to prevent post-operative nausea and vomiting are registered. Low-dose acetylsalicylic acid and statin therapy is resumed on the first postoperative day and continued lifelong. Dalteparin 5000 IE are given once daily from the first postoperative day to prevent deep venous thrombosis until fully mobilized.

5.2. Laboratory measurements

Blood sampling

All analyses are performed at the Department of Clinical Biochemistry at Aarhus University Hospital, Denmark. Blood is collected into vacuum test tubes containing the following additives: 3.2% sodium citrate for thrombin generation and other coagulation tests as well as inflammatory markers; lithium heparin for CRP, LDH, CK-MB, creatinine/ eGFR, lactate and intraoperative hæmatocrit, and EDTA for haematological analyses. Blood samples for

haematological analyses, the majority of coagulation tests (aPTT, INR, antithromin, fibrinogen), CRP, LDH, lactate, creatinine, eGFR and CK-MB analyses are analyzed within 90 minutes of sampling.

Blood samples during surgery and on ICU (T0-T4) are taken through the sheath of the central venous line, through which no heparin is administered. After flushing the catheter, fluid is withdrawn until blood is visualized in the syringe. Additional 2ml (minimum waste) are discarded prior to blood sampling.

Thrombin generation

Thrombin is an essential part of the coagulation cascade and is responsible for converting fibrinogen to fibrin. Thrombin generation is a measure of the ability to generate thrombin in platelet poor plasma. We use the Fluoroskan Ascent plate reader (Thermo Scientific, Helsinki, Finland) for measurement of fluorogenic activity in the microtitre wells. Data are transformed to a computer and processed by the Thrombinoscope® software package (Thrombinoscope BV, Maastricht, The Netherlands). From the thrombograms, we derive numerical values for the following parameters: a) lagtime in minutes (start of thrombin activity), b) peak thrombin activity in nmol, c) time-to-peak in minutes, and d) total endogenous thrombin generation potential (area under the curve) in nmol/minute.

Coagulation tests

APTT, INR, antitrombin and fibrinogen are measured by CS2100i (Sysmex, Kobe, Japan). F1+2 is determined by ELISA (Enzygnost, Siemens Healthcare Diagnostics)

Fibrinolysis tests

Clot lysis is evaluated using an in-house dynamic turbidimetric assay. Briefly, in a 96-well microplate, citrated platelet poor plasma is mixed with a reaction solution including tissue factor and tissue plasminogen activator. Plates are read using a Victor X4 (Perkin Elmer, Turku, Finland), and the following parameters are recorded: a) clot maximum absorbance (reflecting clot density), b) clot lysis time (reflecting fibrinolysis time), and c) area under the curve (a measure integrating clot formation and degradation).

Fibrin d-dimer is determined employing the CS2100i (Sysmex, Kobe, Japan).

Inflammatory markers

TNFα, and the interleukin panel are measured according to the manufacturers' instruction with enzyme-linked immunosorbent assays (V-PLEX Plus Proinflammatory Panel 1 (human) kit, MSD, Rockville, MA, USA).

Standard Biochemical Parameters

- Complete blood counts (haemoglobin, white blood cell count, platelet count, immature platelet count, and immature platelet fraction) are performed using a Sysmex XE-5000 haematology analyzer (Sysmex, Kobe, Japan)
- Plasma CRP, LDH, CK-MB, creatinine are measured using the Cobas® 6000 (Roche, Basel, Switzerland). eGFR per 1.73 m^2 body surface area (eGFR/1,73m^2) is calculated according to the Chronic Kidney Disease-Epidemiology Collaboration equation (CKD-EPI)
- Intraoperative haematocrit and blood lactate are determined using the ABL800 FLEX (Radiometer Medical A/S, Denmark).

The total amount of blood for the project from each participant is specified below

Research biobank

Plasma for thrombin generation, F1+2, inflammatory markers and clot lysis is stored in a biobank (-80°C freezer) and analyzed as batch analyses.

Biobank for future unspecified research

It will store blood samples in coded form at minus 80°C for later analysis (2 ml serum and 4 ml EDTA plasma). Additional analyses will only be performed after approval from the Central Denmark Region Committees on Biomedical Research Ethics.

6 STUDY EVALUATIONS

6.1 Description of evaluations

Screening evaluation

Eligibility will be assessed by the Heart Team at the multidisciplinary conference, based on clinical information and laboratory analyses. On admission, the predicted perioperative mortality is calculated with the European System for Cardiac Operative Risk Evaluation (23). Cardiac, anti-inflammatory (NSAID), statin and anticoagulation medications are recorded.

Blood sampling

Blood samples including serological parameters will be obtained at the following time points:

- T0; preoperative after induction of anaesthesia (after insertion of central venous line)
- T1; after weaning of the ECC prior to protaminization
- T2; 10 minutes after full protaminization
- T3; six hours after the end of the ECC
- T4; 1. postoperative day (16-20 hours following end of surgery)

There will be drawn 12 ml blood per blood sample for analysis of prespecified study parameters, in total a volume of 60 ml blood obtained solely for the project. Provided the patients' consent, additional 6 ml per blood samples will be drawn for the research biobank, in total a volume of 30 ml blood. Thus, the total volume of blood required for the current study is 100 ml. Results of routine blood samples to monitor organ function and inflammation on the second (T5), third (T6), and fourth (T7) postoperative day will be included in the study.

Evaluation of cerebrovascular accidents

Any neurological dysfunction consistent with a cerebrovascular accident will prompt clinical quantification of the severity of the impairment by the NIHSS: 0= No stroke symptoms; 1-4 = Minor stroke; 5-15= Moderate stroke; 16-20= Moderate to severe stroke; 21-42= Severe stroke (24). Any case of suspected stroke is verified by CT scanning to exclude bleeding. Patients with clinical signs of stroke without corresponding findings on CT scanning will be examined by MRI. Symptoms indicative of stroke with resolution within 24 hours without imaging evidence of ischemic injury defined TCI.

Evaluation of perioperative MI

A perioperative MI is defined according to the new definition of clinically relevant MI of the Society for Cardiovascular Angiography and Interventions (25). In patients with normal baseline CK-MB, the peak CK-MB measured within 48 hours of the procedure rises to ≥10 × the local laboratory upper limit of normal, or to ≥5 × upper limit of normal with new pathologic Q-waves in ≥2 contiguous leads or new persistent left bundle branch blok. Electrocardiograms (ECG) are performed by routine at arrival on ICU, on the first postoperative day and on discharge from hospital.

Intraoperative feasibility of miECC versus cECC

The feasibility of CABG procedure with miECC will be assessed in comparison to cECC. The operating team will evaluate the following aspects at the end of the procedure:

Serious adverse device events like air lock, dissection, bleeding that exceeds the capacity of the cell saver, air emboli, stop of the circuit, and conversion to cECC are registered as described in safety aspects.

In addition, technical aspects will be registered: postoperative fluid gain in milliliters, and venous drainage, visibility due to blood in the operative field, and the ability to maintain SvO2 >65% graded into the categories “perfect”, “impaired” or “insufficient”.

30 day assessment

The electronic patient record is reviewed 30 days after CABG with regards to adverse events:

- Death of any cause
- Death due to cardiac causes (MI, arrhythmia congestive heart failure)
- Death due to other vascular causes (cerebrovascular accident, pulmonary embolus, dissection, or bleeding event)
- Death due to non-cardiovascular causes including any othercause of death
- Myocardial infarction
- Cerebrovascular accident
- Repeat revascularization
- Major bleeding
- Deep sternal wound infection

Other non-specified serious adverse events will be captured

6.2 Schedule of evaluations

	Admission	Induction anaesthesia	Prior protamin	After protamin	6 hours po	Day 1 po	Day 2 po	Day 3 po	Day 4 po	Discharge	Day 30 po
		T0	T1	T2	T3	T4	T5	T6	T7		
Initiation procedures											
Eligibility	●										
Demographics		●									
Medical history		●									
Informed consent		●									
Laboratory tests											
Thrombin generation		●	●	●	●	●					
Coagulation tests		●	●	●	●	●					
Inflammatory marker TNFα, IL-6, IL-1β, IL-8		●	●	●	●	●					
Hæmatocrit, lactate*		●	●	●	●						
LDH, CK-MB*					●	●					
CRP, WBC*		●				●	●			●	
Creatinin, eGFR*		●			●	●	●	●	●	●	
ECG*	●				●	●				●	
Medication NSAID, glucocorticoids						●				●	
Efficacy/safety outcomes											
Serious adverse events		---------- Continious reporting ----------									●
Adverse events				●		●				●	●

* Results of routine laboratory and electrocardiogram (ECG) analyses to be included in the study

7. SAFETY ASPECTS

7.1. Insurance

The investigators will be covered by the liability and work injury insurance taken out by Aarhus University Hospital. Participants are covered by the act on complaints and compensation within the health care system.

7.2. Adverse events and serious adverse events

Adverse events

Adverse events are defined as unfavorable and unintended symptoms or signs (including abnormal laboratory findings), which occur during the study up to 30 days after CABG.

Adverse events are recorded regardless of their relationship to the study intervention.

- Periprocedural myocardial infarction
- Other intraoperative complications
- Atrial fibrillation requiring pharmacological therapy or defibrillation
- Reexploration for bleeding
- Requirement of transfusion
- Prolonged stay on intensive care unit (> 24 hours)
- Prolonged requirement of mechanical ventilation
- Requirement of pharmacological haemodynamic support
- Reintervention for graft occlusion or myocardial ischaemia (Surgery or PCI)
- Deep sternal wound infection
- Superficial wound infection requiring revision
- Postoperative infection requiring antibiotic therapy

Adverse events will be recorded after surgery, on discharge from the ICU, on discharge from hospital, and after 30 days based upon the electronic patient record. Unsolicited events will be captured

Serious adverse events

Serious adverse events are defined, as any untoward medical occurrence that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, or results in persistent or significant disability/incapacity. The following events within 30 days are defined as serious adverse events:

- Any death occurring in-hospital
- Stroke
- Postoperative requirement of renal replacement therapy
- Requirement of temporary mechanical circulatory support

Serious adverse device effects

Serious intraoperative complications are defined as air lock, dissection, bleeding exceeding the capacity of the cell-saver, air emboli, stop of the circuit, and conversion to cECC.

All serious adverse events will be recorded immediately after occurrence.

7.3. Reporting procedures and follow-up

The assessment of the causal relationship between an adverse event and the study intervention is a clinical decision made by the steering committee. The assessment is based on whether there is a reasonable possibility that the study intervention has caused the event. The outcome of an adverse will be documented as: resolved/recovered, resolved/recovered with sequelae, not resolved/recovered, fatal, or unknown. Stopping rules are described under statistics considerations.

8. STATISTICS CONSIDERATIONS

8.1. General design issues

Treatment group allocation cannot be concealed for the operating team, but participants, other members of the treatment team, laboratory personal and other practitioners administering postoperative care will be blinded regarding the allocation to ensure unbiased ascertainment of outcomes.

8.2. Sample size and randomization

Our primary outcome measure is the difference in thrombin generation expressed as endogenous thrombin generation potential between miECC and cECC patients after weaning of the ECC (post protaminization). Thrombin generation data (measured by the same method as ours) has recently been published on patients undergoing CABG with cECC just prior to surgery (19). According to Moorlag et al., mean endogenous thrombin generation potential in platelet poor plasma is 1138.1 mol/L x min with a standard deviation at 268.2 mol/L x min in patients undergoing CABG with cECC prior to surgery (19). Choosing a minimal relevant difference (MIREDIF) of 250 mol/L x min, a significance level of 5% (2a), and a statistical power of 90% (1-b) we have to include 25 participants in each group. In order to ensure a complete dataset and assuming a dropout rate of about 20%, we aim to include a total of 60 participants.

8.3. Stopping rules

Intraoperative death will temporarily suspend enrollment and study intervention until a safety review by the steering commitee is convened. Other adverse events such as air lock of the ECC circuit, perioperative myocardial infarction and stroke trigger a safety review by groups by the steering committee. The findings are used to determine whether the study should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Cases of Suspected Unexpected Severe Adverse Reactions (SUSAR) will be reported to the Central Denmark Region Committees on Biomedical Research Ethics according to *"Vejledning til indberetning af mistænkte uventede og alvorlige bivirkninger set i kliniske forsøg"*.

Patients enrolled may withdraw voluntarily from study participation at any time and for any reason. If voluntary withdrawal occurs, the subject will be asked for permission to be followed for clinical outcome.

8.4. Data analyses

All primary and secondary end points will be analyzed both on an intention-to-treat and on a per protocol principle. For per protocol analyses, only patients who received the appropriate treatment according to their randomization and have a complete dataset available at all five perioperative time points (T0-T4) will be included. Tracking of outcome measures will begin at randomization and continue until death or end of follow-up (30 days after CABG). No formal interim analysis will be performed. Continuous variables will be presented as mean ±

standard deviation (SD) if normally distributed, otherwise as median with interquartile range (25th and 75th percentile), minimum and maximum values. Discrete variables will be presented in frequencies and percentages. Kaplan-Meier plots will be constructed to visualize time to event variables and log-rank tests will be used.

All statistical analyses will be done using Stata 10.1 SE (Stata Corp., College Station, USA). Statistical consultation is provided by the Department of Biostatistics, Aarhus University.

9 DATA COLLECTION AND QUALITY ASSURANCE

9.1. Data collection forms

Study data are collected and managed using REDCap electronic data capture tools hosted at Aarhus University Hospital (22). REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources. An electronic Case Report Form (CRF) will be constructed for data registration.

9.2 Data management

Data will be handled, processed, and archived according to the guidelines of the Data Protection Agency. Each participant will be given an identification number at enrolment. Each identification number will have a Case Report Form (CRF). The list over identification codes will be deleted at the end of the study. All trial data including Trial Master File, CRF, the source datasheet of the CRF, and the list over identification codes will be stored in the external server REDCap with continuous backup. REDCap data are kept for 10 years. Information on study participants will be protected according to the Act of Processing of Personal Data, and the Health Act.

9.3. Training

The sponsor Ivy S. Modrau and co-investigator Debbie Richards Halle will provide GCP and NIHSS training certificate prior to start of the study.

9.4. Protocol deviations

Conversions from miECC to cECC will be documented and reviewed as described in safety aspects. Data from these patients will be analyzed according to the intention-to-treat principle but will be excluded from the per protocol analysis.

10 ECONOMY

The Department of Cardiothoracic and Vascular Surgery at Aarhus University Hospital covers the running expenses for both surgical interventions. Funding for publication of the protocol, laboratory analysis, statistical assistance, and expenses for presentation at scientific meetings, data retrieval will be continuously applied for at non-commercial private and public foundations.

11 ETHICAL CONSIDERATIONS

11.1. Risk of study intervention

Few publications have raised concerns regarding miECC as compared to cECC due to ventricular dilatation, loss of a bloodless field and the risk of air lock of the closed circuit during perfusion (11,12). However, these reports are anecdotal, and large-scale controlled clinical trials have confirmed the safety of modern miECC systems (6). We apply a miECC system with the following integrated safety measures: 1) an integrated venous air removing device to prevent air lock, 2) an electric clamp system to stop circulation if air should have passed the bubble trap, 3) an integrated of collapsible soft-shell reservoir to make blood volume management easier 4) an aortic root vent to reduce blood in the surgical field, 5) a cell-saving device to collect and process shed blood from the operating field, and 6) a conventional heart lung machine on stand-by to allow rapid conversion to a conventional open circuit in case of unexpected emergent intraoperative scenarios as the ultimate safety measure.

We believe that the risk of study participation is minimal and outweighed by the value of the knowledge gained through this study, which will be of potential benefit for future patients undergoing coronary bypass surgery. Participants will not derive any individual benefit from study participation.

The current study involves no increased risk of discomfort or bruising due to blood sampling,

as the blood samples are taken through the central venous line during at the first 5 time points (T0-T4). The results of routine blood samples regarding organ dysfunction and inflammation at the following time points (T5-T7) are included in the study. The total volume of 60 ml for prespecified blood samples and 30 ml for research biobank drawn over a period of 5 days is considered of minimal risk for the participants

11.2. Conflict of interest

The study has been initiated solely by the investigators. The investigators have no conflict of interest to declare and no commercial interest to conduct the study.

11.3. Approval

The study is approved by the Central Denmark Region Committees on Biomedical Research Ethics (Nr 1-10-72-150-17) and the Danish Data Protection Agency (Nr. 1-16-02-188-17). The study is registered at www.clinicaltrials.gov (ClinicalTrials.gov ID: NCT03216720). The protocol follows the Declaration of Helsinki-II and Consort guidelines.

12 STUDY ORGANIZATION

12.1 Steering committee

The main role of the steering committee is to monitor and supervise the progress of the trial. The steering committee consists of the sponsor Ivy S. Modrau, Department of Cardiothoracic and Vascular Surgery, the co-investigator professor Anne-Mette Hvas, Department of Clinical Biochemistry and professor J. Michael Hasenkam, Principal of the Scandinavian School of Cardiovascular Technology, all Aarhus University Hospital.

12.2 Delegation of study-related tasks

The principal investigator (PI) is responsible for personally conducting or supervising the study. Certain study-related tasks delegate to co-investigators and study staff.

- Screening of patients regarding eligibility for the study will be conducted by the multidisciplinary Heart Team consisting of interventional cardiologist and cardiac surgeon based on electronic patient report including laboratory data, coronary angiogram and other imaging evaluations.
- The PI arranges that eligible patients receive written information regarding the study together with the information regarding their scheduled CABG.

- The medical histories and assessment of inclusion/exclusion criteria are collected by the PI and the co-investigator perfusionist aspirant Debbie Richards Halle.
- Oral information about the study is given to the patient at admission one to three days prior to surgery by one of the licensed physician investigators listed in the protocol.
- The PI will ensure the participant has received sufficient oral and written information regarding the trial and obtain informed consent on the morning of surgery.
- The operation team (perfusionist, anaesthesiologist, surgeon) will collect the intraoperative data including adverse events on respective CRFs.
- The primary study endpoints cumulated postoperative blood loss from drains and transfusion requirement are assessed by the PI or the co-investigator perfusionist aspirant Debbie Richards Halle.
- The adverse events in-hospital are recorded by the PI or the co-investigator perfusionist aspirant Debbie Richards Halle.
- The Department of Clinical Biochemistry at Aarhus University Hospital is responsible for collection and analyses of laboratory data throughout the study
- NIHSS will be performed by certified co-investigators.
- Any co-investigator will record serious adverse events as soon as they occur
- The PI evaluates adverse events 30 days postoperatively by review of the electronic patient record and signs the CRF
- The Department of Clinical Biochemistry at Aarhus University Hospital is responsible for collection, anonymization and appropriate use of blood samples for the biobank. They guarantee the complete destruction of the biobank after 10 years

13 COOPERATION AND PUBLICATION POLICY

The study is performed in collaboration between the Department of Cardiothoracic and Vascular Surgery, the Department of Clinical Biochemistry, Center for Haemophilia and Thrombosis, and the Department of Anaesthesiology and Intensive Care, Aarhus University Hospital. Department of Clinical Biochemistry has all the equipment and knowledge needed for carrying out the biochemical analyses.

The technique of miECC was implemented at the Department of Cardiothoracic and Vascular Surgery, Aarhus University Hospital in 2016 following a learning visit of the operating team (surgeon, perfusionist, anaethesiologist, operating room nurse) to Doctor Aschraf El-Essawi at the Department of Thoracic and Cardiovascular Surgery, Klinikum Braunschweig, Germany. In addition, the Scandinavian School of Cardiovascular Technology at Aarhus has close collaboration with chief perfusionist Adrien Bauer at the Department of Cardiothoracic Surgery at the MediClin Heart Centre Coswig, Germany Hospital. Perfusionist aspirant Debbie Richards will visit the center in Coswig for exchange of experience in May 2017. Both centers' have long-term experience with miECC and participated in the largest multicenter RCT regarding miECC so far (26).

We intend to publish the protocol in the BioMed Central Journal of Cardiothoracic Surgery. Results, whether positive, negative or inconclusive, will be published in the best peer-reviewed scientific cardiothoracic journals possible and be presented at international conferences meetings in the field of cardiothoracic surgery. The part of the study concerning inflammatory markers will serve as the basis for the master thesis of perfusionist aspirant Debbie Richards Halle at the Scandinavian School of Cardiovascular Technology. The results of the master thesis are to be published as an article in a peer-reviewed journal by Debbie Richards as first author. All above-mentioned investigators are expected to receive authorships according to the Vancouver declaration.

Participants will be able to request a copy of the results of the study from the principle investigator at the end of the study.

14 PLANNED TIMELINE

Protocol complete			May 2017
Approval from Science Ethics committee			August 2017
Enrollment	September 2017	-	September 2018
Data collection	September 2017	-	Oktober 2018
Data analysis	Oktober 2018	-	February 2019
Publication			2019

15 PERSPECTIVES

MiECC has emerged as a promising treatment to attenuate several adverse systemic effects that are attributed to extracorporeal circulation leading to a significant morbidity. However, many questions regarding the underlying mechanisms remain unanswered. The present study will clarify the effect of different ECC systems on the coagulation system and its proposed link to postoperative blood loss and transfusion requirement. In addition, analysis of inflammatory markers in the postoperative period will contribute to the understanding of the systemic inflammatory response following ECC. Finally, the current study will add to the overall clinical evidence regarding clinical efficacy, safety and feasibility of miECC in CABG.

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