STUDY PROTOCOL

Efficacy and safety of Haemocomplettan® P in patients experiencing microvascular bleeding while undergoing elective complex cardiac surgery

Haemocomplettan® P during elective complex cardiac surgery


Principal investigator: A. P. Nierich, MD, PhD. Department of Cardiothoracic Anesthesia and Intensive Care, Isala Clinics, Zwolle, The Netherlands.

EudraCT number: 2009-018086-12

Version date: 22-02-2011

Version number: Version 1.5
8. **Page 19. Drug accountability**
   All supplies (Haemocomplettan P and placebo solution) will be accounted for throughout the study using the drug inventory log, which will be provided to the Monitor prior the first patient enrolled in the study.

9. **Page 21: Renal failure**
   - Recalculation of criteria reveals: serum creatinine >354\(\mu\)mol/L.

10. **Page 22. Additional variables**
    Addition of multiplate measurement: TRAP − platelet stimulation via the thrombin receptor (using TRAP-6), sensitive to IIbIIIa receptor antagonists.

11. **Page 23. Randomization**
    Addition of the following text: The patients will be randomized by the research-team using a computerized web-based randomization protocol designed by the Julius Center for Health Sciences and Primary Care. This protocol uses block randomization of 4 balanced outcomes per block (2x placebo and 2x IMP). The blocks themselves are also randomized. Based on the randomization result, the circulating anesthesia-nurse will either prepare a placebo solution or an active medication solution (IMP). The circulating anesthesia-nurse preparing the study medication is not involved in the operation with the study subject. The prepared syringes of both treatment groups are of the same size and colour. The fluid inside the bottle is of the same volume (50ml), same colour and viscosity. The bottles are labelled with the “study kit number”. This method ensues an adequate blinding of the research team (i.e. surgical team) for the allocated treatment.

12. **Page 24: Study blinding**
    The bottles with study medication will be labelled with the kit and study identification number before delivery to the operating room.
    The circulating anesthesia-nurse, who is not involved with the surgery of the study, will perform the measurements with ROTEM ® and Multiplate ® . All anesthesia-nurses are trained by the representatives of both POC monitors for correct use. The results of these POC monitoring devices are blinded for the operating team. Study unblinding will take place following closure of the study database.

13. **Page 24-25: Baseline on Day 0**
    - Excluded in protocol:
      Other coagulation (fibrinogen, aPIT, PT)
      Biochemistry measurements will include AST, ALT, Ca, CK, CK-MB, creatinine, CRP, glucose, K, Na, pH, pCO2, pO2, troponin T, and urea.
    - Included in protocol: Multiplate ® TRAP-test and Spijtserum

14. **Page 25-26: Removal of cardiopulmonary bypass**
    - Included in protocol: Multiplate ® TRAP-test and Spijtserum
    - The 5-minute bleeding mass test is changed to 5 minute bleeding volume.

15. **Page 28: Replacement of individual subjects after withdrawal**
    Text replaced by: Randomisation of subjects will continue until at least 106 subjects provide the primary efficacy endpoint data.
## 1. STUDY SYNOPSIS

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<th>Efficacy and safety of Haemocomplettan P in patients experiencing microvascular bleeding while undergoing elective complex cardiac surgery.</th>
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<td><strong>Study code</strong></td>
<td>BI1401_2010</td>
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<tr>
<td><strong>Date</strong></td>
<td>22-02-2011</td>
</tr>
<tr>
<td><strong>Principal investigator</strong></td>
<td>A. P. Nierich, MD, PhD. Anesthesiologist/Intensivist. Department of Cardiothoracic Anesthesia and Intensive Care, Isala Klinieken, Zwolle, The Netherlands.</td>
</tr>
<tr>
<td><strong>Pharmacy</strong></td>
<td>A. Wieringa. Pharmacist. Hospital Pharmacy Isala Klinieken Zwolle.</td>
</tr>
</tbody>
</table>
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- J.W.A. Romijn, MD. Department of Anesthesiology. Institute for Cardiovascular Research, VU University Medical Center, Amsterdam. |
| **Monitor** | Athena Care Zwolle, The Netherlands. |
| **Steering committee** | - Professor K.G.M. Moons, PhD. Professor of Clinical Epidemiology. Julius Center for Health Sciences and Primary Care. UMC Utrecht.  
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| **Coordinating Investigator** | A. P. Nierich, MD, PhD. Anesthesiologist/Intensivist. Department of Cardiothoracic Anesthesia and Intensive Care, Isala Klinieken, Zwolle.  
Date: 30-07-2010  
Signature: |
### Background
Fibrinogen concentrate is increasingly used in cardiac surgery to reverse coagulopathy. Whether its use reduces blood loss, transfusion and occurrence of clinical adverse events remains unknown.

### Domain
Domain: Complex cardiac surgery. Defined as: CABG and valve(s) or aortic surgery (root, ascending or arch) or multiple valve surgery.

### Study design
Single center, prospective, randomized, double-blind, placebo-controlled, phase II study.

### Study medication
1. Intervention: Haemocomplettan P (human fibrinogen concentrate, pasteurized).

### Effect
**Intended effect:** Enhancement of coagulation; hemostasis.

**Unintended effect:**
- Type A: Thromboembolic event
- Type B: Allergic reaction

### Study objectives
**Primary objective:**
1. To determine whether fibrinogen concentrate infusion reduces perioperative blood loss during elective complex cardiac surgery.

**Secondary objective:**
1. To determine whether fibrinogen concentrate infusion reduces blood loss and transfusion of allogenic blood products in patients experiencing microvascular bleeding during elective complex cardiac surgery.
2. To determine whether fibrinogen concentrate infusion is safe and well-tolerated.

### Study population
Patients who are at least 18 years old and experience microvascular bleeding while undergoing elective complex cardiac surgery.

### Inclusion criteria
- Eighteen years of age or older.
- Undergoing elective complex cardiac surgery (*combined* CABG and valve(s) or multiple valves or aortic root, aorta ascendens or aortic arch surgery).
- Understood and willingly given written informed consent (Dutch language) to participate following an explanation of study background, restrictions and procedures.
- Experience clinically relevant non-surgical microvascular bleeding following removal of CPB during surgery, defined as a 5-minute bleeding volume between 60 and 250 ml AND the intraoperative conditions prior to administration of study medication are:
  - Body temperature > 36°C
  - Blood pH > 7.3
  - Hb > 5.3 mmol/L or Ht > 0.25
  - ACT < 140 seconds
- **Dosing formula has a positive outcome**
| Exclusion criteria | o Positive pregnancy test, pregnancy or lactation.  
o Women of child-bearing age not using a medically approved method of contraception during the study.  
o Undergoing an emergency operation.  
o Proof or suspicion of a congenital or acquired coagulation disorder (e.g. VWD or via severe liver disease).  
o Apoplexy in the 2 months preceding study surgery.  
o Manifest venous or arterial thrombosis.  
o Medication:  
  o Clopidogrel use in the 5 days preceding surgery.  
  o Tirofiban use in the 2 days preceding surgery.  
  o INR >1.4 if on coumadines.  
o Sensitivity to any of the components of study medication.  
o Participation in another clinical study in the 4 weeks preceding this study.  
o Any indication that the restrictions or procedures of the study may not be adhered to (e.g. an uncooperative attitude).  
o Any indication that the study restrictions, procedures, or consequences therein have not been considered or understood, such that informed consent cannot be convincingly given.  
o Multiple morbidities, with a notably constrained remaining length of life. |
| Dose schedule and mode of administration | Single intravenous infusion after the first clinically relevant bleeding following removal of the subject from cardiopulmonary bypass (CPB) and after antagonizing with protamine. Dosing will be individually determined based upon plasma fibrinogen levels measured according to Clauss method during the reperfusion-rewarming phase on CPB which is prior to removal of CPB.  
Formula for Haemocomplettan®P infusion:  
\[(2.5 - \text{plasma fibrinogen on CPB g/L}) \times (1-\text{Ht on CPB}) \times 0.07 \times \text{body weight (kg)} = \text{whole g Haemocomplettan P to be infused.}\]  
Infusion will be calculated as total gram, allowing adequate preparing of the commercially available vials of Haemocomplettan P.  
For placebo an equivalent volume of placebo will be infused. |
| Study endpoints | Primary efficacy endpoint:  
1. Perioperative blood loss measured as blood loss in ml between infusion of fibrinogen concentrate and closure of chest.  
Secondary endpoints:  
1. Postoperative blood loss, measured as blood loss in the ICU between closure of chest and:  
   • 1st hour  
   • 2nd hour  
   • 3rd hour  
   • 6 hours  
   • 12 hours  
   • 24 hours  
   • At the actual time of chest tube removal.  
2. Number of units of allogenic blood products (platelets + FFP +
RBCs) administered to subjects, between administration of study medication and closure of chest.
3. Number of units of allogenic blood products administered to subjects, between administration of study medication and 24 hours thereafter.
4. Number of units of allogenic blood products (platelets + FFP + RBCs) administered to subjects, from admission to the ICU to discharge to the ward.
5. Number of units PPSB or Novoseven given in peri- and postoperative period.
6. Duration of post CPB phase, from infusion of study medication to transfer to ICU.
7. Ventilation-time in hours during ICU stay.
8. Duration of stay in hours in the ICU following last suture of the initial surgery.
9. Duration of hospital stay in hours following last suture of the initial surgery.
10. Proportion of subjects that receive a follow-on surgery to correct unacceptable bleeding within 5 days of last suture.
11. Wound, sternal or other types of infection.
12. Major clinical events:
   - Mortality at 30 days post-surgery
   - MACE (major adverse cardiac event)
   - Cerebrovascular accident/transient ischemic attack
   - Renal insufficiency or failure
   - Venous thromboembolism/pulmonary embolism
   - Allergic or other systemic reaction to study medication

<table>
<thead>
<tr>
<th>Additional efficacy variables</th>
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<tbody>
<tr>
<td>- ROTEM®, including MCF via FIBTEM and EXTEM.</td>
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<td>- Multiplate®, including ADP-test, ASPI-test and TRAP-test.</td>
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<tr>
<td>- Blood fibrinogen via Clauss method.</td>
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<tr>
<td>- Other coagulation parameters: APTT, PT, platelets.</td>
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<tr>
<th>Drug concentration measurements</th>
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<td>Fibrinogen determination via the Clauss assay (i.e. for activity).</td>
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<th>Sample size</th>
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<td>60 patients in intervention (fibrinogen) group and 60 patients in control (placebo) group. Total of 120 patients to be included in study.</td>
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<thead>
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<th>Study center and location</th>
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<tr>
<td>Department of Cardiothoracic Anesthesia and Intensive Care, Isala Clinics, Zwolle, The Netherlands.</td>
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<th>Study duration</th>
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<td>Approximately 12 months including all subjects. Recruiting should require approximately 9 months. For each individual subject the study begins with signing of the informed consent document. The data for the primary efficacy endpoint should be collected within 24 hours of administration of study medication. The last follow-up for each subject will be at 30 days post-surgery.</td>
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# Version 1.5

d.d. 22/02/2011
Confidential. Author: Nierich

## STUDY SYNOPSIS

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### 1.1 List of abbreviations

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<th>Description</th>
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<tr>
<td>ABR</td>
<td>ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)</td>
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<tr>
<td>ACT</td>
<td>Activated clotting time</td>
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<td>ADP</td>
<td>Adenosindiphosphate</td>
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<tr>
<td>AE</td>
<td>Adverse Event</td>
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<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
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<tr>
<td>APTT</td>
<td>Activated partial thromboplastin time</td>
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<tr>
<td>AR</td>
<td>Adverse Reaction</td>
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<td>ASPI</td>
<td>Arachidonic acid</td>
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<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
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<tr>
<td>CA</td>
<td>Competent Authority</td>
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<tr>
<td>Ca</td>
<td>Calcium</td>
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<td>CABG</td>
<td>Coronary artery bypass</td>
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<tr>
<td>CCMO</td>
<td>Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek</td>
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<tr>
<td>CPB</td>
<td>Cardiopulmonary bypass</td>
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<tr>
<td>CK</td>
<td>Creatine kinase</td>
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<tr>
<td>CK-MB</td>
<td>Creatine kinase, muscle and brain subunits</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CSP</td>
<td>Clinical study protocol</td>
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<tr>
<td>CT</td>
<td>Computer tomography</td>
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<tr>
<td>CV</td>
<td>Curriculum Vitae</td>
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<tr>
<td>DSMB</td>
<td>Data Safety Monitoring Board</td>
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<tr>
<td>EU</td>
<td>European Union</td>
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<tr>
<td>EudraCT</td>
<td>European drug regulatory affairs Clinical Trials</td>
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<tr>
<td>EXTEM®</td>
<td>Extrinsic pathway Thromboelastometry</td>
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<tr>
<td>FFP</td>
<td>Fresh frozen plasma</td>
</tr>
<tr>
<td>FIBTEM®</td>
<td>Fibrinogen Thromboelastometry</td>
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<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
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<tr>
<td>HA</td>
<td>Human albumin</td>
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<tr>
<td>Hb</td>
<td>Hemoglobin</td>
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<tr>
<td>HEPTEM®</td>
<td>Heparinase modified Thromboelastometry</td>
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HRQoL   Health-related quality of life
Ht      Hematocrit
IB      Investigator’s Brochure
IC      Informed Consent
ICU     Intensive Care Unit
IMP     Investigational Medicinal Product
IMPD    Investigational Medicinal Product Dossier
INR     International normalized ratio
INTEM®  Intrinsic pathway thromboelastometry
K       Potassium
MCF     Maximum clot firmness
METC    Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
MP      Medicinal Product
MRI     Magnetic resonance imaging
Na      Sodium
pCO2    Plasma carbon dioxide tension
pH      Potential for hydrogen ion concentration
pO2     Plasma oxygen tension
POC     Point of Care
PPSB    Prothromin complex
PT      Partial thromboplastin time
RBC     Red blood cell concentrate
ROTEM®  Rotation Thrombo-elastometry
(S)AE   (Serious) Adverse Event
SF-36   Short-Form 36
SPC     Summary of Product Characteristics (Dutch: officiële productinformatie IB1-tekst)
SUSAR   Suspected Unexpected Serious Adverse Reaction
TRAP    Thrombin receptor activation of platelet
VWD     Von Willebrand disease
Wbp     Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)
WMO     Medical Research Involving Human Subjects Act (Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)
1.2 SUMMARY:
In an attempt to further reduce the use of allogenic blood products in cardiac surgery, coagulation factor replacement therapies have been considered as substitutes. Fibrinogen concentrate is increasingly used in surgical patients suffering excessive bleeding refractory to conventional hemostasis treatment. However, these studies published so far comprise small study groups with questionable study designs. Therefore a large randomized trial is needed to determine the effect of fibrinogen concentrate on blood loss and transfusion. The aim of this study is to determine whether fibrinogen concentrate reduces blood loss and transfusion in patients undergoing elective complex cardiac surgery.

From retrospective analysis of the data of the Isala Clinics Zwolle, the procedures combined CABG and valve(s), aortic surgery and multiple valves are associated with excessive blood loss and transfusion. As shown by our results of the use of fibrinogen in this type of cardiac surgery patients, the use of fibrinogen tends to reduce postoperative blood loss and tends to improve patient outcome, but the effect was limited due to probably inadequate dosing and timing of this blood component.

By choosing the domain of complex cardiac surgery which is prone for excessive blood loss and transfusion, we will focus on subjects undergoing high risk procedures. Hereby we hypothesize that administration of fibrinogen concentrate improves hemostasis in patients experiencing microvascular bleeding during complex cardiac surgery. This improvement of the hemostasis is measured by reduced blood loss and transfusion of blood products during surgery and the postoperative period. Furthermore, we hypothesize that fibrinogen concentrate is safe and well tolerated in patients undergoing complex cardiac surgery. This hypothesis will be investigated by a detailed study of clinical outcomes.

Furthermore, we will evaluate whether POC monitoring with thromboelastometry (ROTEM®) and platelet aggregation analyzer (Multiplate®) might provide faster and more specific conditions for diagnosis and management of coagulation disorders during cardiothoracic procedures.
2. INTRODUCTION

Complex cardiac surgery is often complicated by excessive peri-operative bleeding, most frequently due to insufficient surgical hemostasis or impairments of the coagulation system [1]. Excessive bleeding increases the risk of peri-operative transfusions, follow-on thoracic surgery and myocardial infarction, which can ultimately lead to increased morbidity and mortality [2]. Although improvement and standardization of the surgical techniques have resulted in better patient outcomes in recent years, the risk of bleeding and consumption of blood products during surgery remain high [3-7].

In addition to the bleeding potential brought about by the large wound area intrinsic to cardiac surgery, cardiopulmonary bypass (CPB) is associated with temporary dysfunction of the maintenance of hemostasis, both of the hemostatic system and of the fibrinolytic system [8-11]. The result is platelet dysfunction, coagulation factor activation, depletion and fibrinolysis [12]. Strategies to reduce the bleeding potential during cardiac surgery have included collection and reinfusion of autologous blood products, alterations in heparin and protamine dosing and prophylactic use of anti-fibrinolytic therapy [13-15]. These strategies have reduced mediastinal tube drainage and transfusion requirements. However, microvascular bleeding still occurs and transfusion of allogenic blood products is still very much necessary [16]. Cardiac surgeries consume notable amounts of allogenic blood products; in the United States for example, these surgeries consume 10 to 20% of the nation’s supply of blood products [17]. This high consumption translates into resource challenges for hospitals via fluctuations in blood donation, and due to notable storage and processing costs [18].

One of the promising new developments in blood preservation techniques is the use of coagulation factor concentrate fibrinogen, which is the key substrate for plasmatic blood coagulation. Commercially available fibrinogen concentrate is labelled for reversing coagulopathy found in congenital hypo-, dys- and afibrinogenaemia and in acquired hypofibrinogenaemia. Acquired hypofibrinogenaemia as a result of dilution and/or consumption, is the most common cause of low fibrinogen levels during surgery inducing coagulation disorders which eventually may lead to severe bleedings [19]. In the past years, there is increasing interest in the use of fibrinogen concentrate in patients with severe coagulopathic bleeding in various fields of surgery [20-24]. This interest is supported by the publication of several in-vitro and in-vivo studies which have shown that fibrinogen concentrate reverses dilutional coagulopathy caused by colloid fluid infusions [25-29].

However, these studies were not performed in the domain of cardiac surgery. Only recently, two studies addressed the use of fibrinogen concentrate in cardiac surgery with both reporting a reduction in postoperative blood loss. However, these studies were conducted in a small number of patients [30-31].

Since a few years, fibrinogen concentrate is increasingly used in our hospital to treat coagulopathy in complex cardiac surgery patients with excessive bleeding. In this domain, we conducted a non-randomized intervention study to quantify whether fibrinogen concentrate reduced postoperative blood loss and need for transfusion. In this study, we included all 658 patients who underwent complex cardiac surgery in years 2007 and 2008 in the Isala Clinics Zwolle, The Netherlands. Of this group, 153 (23%) received fibrinogen during surgery while 505 patients did not receive fibrinogen concentrate and were controls. After adjusting for patient- and procedure characteristics, fibrinogen reduced blood loss at ICU by 9% (ratio of geometric means 0.91 (0.76-1.09)). Transfusion during ICU stay reduced with 11% (odds ratio (OR) 0.89 (0.58-1.37)). Thirty-day mortality and prolonged ventilation were lower in the fibrinogen group (OR 0.59 (0.23-1.52) and 0.76 (0.38-1.50) respectively), whereas myocardial infarction and renal insufficiency occurred more frequently (OR 1.21 (0.51-2.89) and 1.16 (0.47-2.87) respectively).

Although our findings did not reach statistical significance, we found a trend to a reduction in postoperative blood loss and need for transfusion in complex cardiac surgery patients who received fibrinogen concentrate. Until now this is the largest (non-randomized) study on the topic (submitted for publication). It is imperative that these results support the need for randomized trials (such as this
presented protocol) to properly quantify the effect of fibrinogen concentrate in the bleeding cardiac surgery patient.

A recent approach to reduce allogenic blood-product transfusions during cardiac surgery uses thromboelastometric measurements like TEG®/ROTEM® to diagnose intra-operatively acquired coagulation disturbances. TEG® and ROTEM® are viscoelastic point-of-care (POC) measures of clot formation and dissolution, which provide data on thrombin generation and the contribution of fibrin and platelets to clot strength [8, 32]. A randomized and blinded prospective study compared thromboelastometry guided transfusion algorithm with routine transfusion therapy. This study showed a 75% decrease in number of patients receiving fresh frozen plasma and a 50% decrease of patients receiving platelet transfusion in the thromboelastometry guided transfusion group. This reduction may have been due to improved hemostasis in these patients because of earlier and more specific identification of the hemostasis abnormality which probably led to a more appropriate intraoperative transfusion therapy [8]. A study of Royston et al. did show a threefold reduction in the use of haemostatic products without reduction in blood loss. These results suggest that intra-operative POC monitoring of coagulation in the anticoagulated patient can be used to guide treatment of coagulation disorders [33]. A study conducted by Avidan et. compared three groups; a thromboelastometry guided transfusion algorithm, a routine laboratory measurements guided transfusion algorithm and a group with no transfusion algorithm but with transfusion at clinician’s discretion. This resulted in a reduction in transfusion of red blood cell concentrate and blood components between the algorithm-guided groups and the control group (no algorithm). However, between two algorithm-guided groups there was no difference in transfusion in red blood cell concentrate and blood components. This suggests that implementation of a transfusion algorithm alone might reduce allogenic blood transfusion. It also suggests that there is no added value of POC monitoring compared to routine laboratory tests [34]. Although point-of-care monitoring with thromboelastometry offers the potential for early and more accurate diagnosis (vs PT, aPTT) of coagulation disorders, the studies conducted so far show contrasting results with respect to its usefulness in the domain of cardiac surgery.

Furthermore, these studies had small number of subjects and were of a questionable study design making the result difficult to interpret. In an additional efficacy analysis we will evaluate whether POC monitoring with thromboelastometry and a platelet aggregation analyzer might provide faster and more specific conditions for diagnosis and treatment of coagulation disorders compared to conventional laboratory measurements.
3. **STUDY OBJECTIVES**

**Primary objective**
To determine whether fibrinogen concentrate infusion reduces perioperative blood loss in patients experiencing clinically relevant microvascular bleeding during elective complex cardiac surgery.

**Secondary objective**
To determine whether fibrinogen concentrate infusion reduces transfusion of allogenic blood products in patients experiencing clinically relevant microvascular bleeding during elective complex cardiac surgery and to determine whether its use is safe and well-tolerated.

4. **STUDY DESIGN**
This phase II study is a single-center, investigator initiated, randomized, double-blind, placebo-controlled study. This study will be conducted in subjects who are undergoing elective complex cardiac surgery. Complex cardiac surgery is defined as combined CABG and valve(s), aortic surgery (root, ascending or arch) or multiple valve surgery. This study will be performed at a single center in Zwolle, the Netherlands, by the departments of Cardiothoracic Surgery and Cardiothoracic Anesthesia and Intensive Care. The study comprises an intervention group of 60 subjects and a control group of 60 subjects totalling 120 study subjects. Recruitment should require approximately 9 months for the 120 subjects to be enrolled in the study. Baseline examination (including signing of the Informed Consent Document (ICD)) of potential subjects will begin in February 2011 and the data of the last included patient should be completed including follow-up at the end of November 2011 (10 months). Including analysis of the results, the study duration will be approximately 12 months. Observation of each individual subject will include detailed evaluation from the day of baseline examination (Day −3 to −1) to 30 days post-surgery for SF-36, EuroQoL and SAE recordings. Data for the primary efficacy endpoint will be collected within 24 hours of administration of study medication.

5. **STUDY POPULATION**

5.1 **Population base**
The Isala Clinics Zwolle is a high volume cardiac surgery center with approximately 1400 to 1500 cardiothoracic surgery procedures performed each year, of which 400 to 450 cases comprise complex cardiac surgery procedures. Patients undergoing complex cardiac surgery are prone to develop clinically relevant microvascular bleeding. Therefore, it is likely that the study population of 60 cases per arm is to be completed within 1 year, with expected study duration of 9 months. All subjects will be screened prior to randomization to confirm eligibility for the study, to ensure informed consent and to record a detailed medical history. Days prior to surgery, eligibility will be determined based upon the inclusion and exclusion criteria listed below. After written informed consent, the patient will be included in the study only when clinically relevant microvascular bleeding is experienced after removal of CPB. The subject will then be randomized to either receive placebo or fibrinogen concentrate. Each subject can be enrolled only once. There are no other restrictions than the inclusion and exclusion criteria for eligibility of the patients. Considering the results of our retrospective analysis, we expect population will be male for 60% and have a median age of 70 years. For inclusion of the study, there are no restrictions on age, gender and ethnic background.
5.2 Inclusion criteria
1. Eighteen years of age or older.
2. Undergoing elective complex cardiac surgery (combined CABG and valve(s) or multiple valves or aortic root, aorta ascendens or aortic arch surgery).
3. Understood and willingly given written informed consent (Dutch language) to participate following an explanation of study background, restrictions, and procedures.
4. Experience clinically relevant bleeding of the microvasculature following removal of CPB during surgery (clinically relevant microvascular bleeding defined as a 5-minute bleeding volume between 60 and 250 ml)
5. After removal of CPB; intraoperative conditions prior to infusion of study medication are:
   — Body temperature > 36°C
   — Blood pH > 7.3
   — Hb > 5.3 mmol/L or Ht > 0.25
   — ACT < 140 seconds
   **All 5 points must be met for inclusion.**
6. The dosing formula has a positive outcome.

5.3 Exclusion criteria
1. Positive pregnancy test, pregnancy or lactation.
2. Women of child-bearing age not using a medically approved method of contraception during the study.
3. Undergoing an emergency operation.
4. Proof or suspicion of a congenital or acquired coagulation disorder (e.g. VWD or via severe liver disease).
5. Myocardial Infarction (MI) or apoplexy in the 2 months preceding study surgery.
6. Manifest venous or arterial thrombosis.
7. Medication:
   o Clopidogrel administration in the 5 days preceding surgery.
   o Tirofiban administration in the 2 days preceding surgery.
   o INR >1.4 if on coumadines.
8. Participation in another clinical study in the 4 weeks preceding this study.
9. Sensitivity to any of the components of study medication.
10. Any indication that the restrictions or procedures of the study may not be adhered to (e.g. an uncooperative attitude).
11. Any indication that the study restrictions, procedures, or consequences therein have not been considered or understood, such that informed consent cannot be convincingly given.
12. Multiple morbidities, with a notably constrained remaining length of life.

5.4 Sample size calculation
The primary outcome in this study is the amount of blood loss in the operation theatre after infusion of the study drug or placebo. In 2006, in which fibrinogen concentrate was not yet available for this type of patients, median blood loss during surgery (including blood loss as measured through the cell saver) was 2200 ml. We expect that this can be reduced by about 40% to 1350 ml. The analyses will be based on a log10 transformation of blood loss. On this scale, the expected reduction is from 3.34 to 3.13, with a standard deviation (SD) of 0.385. One interim analysis at 50% availability of outcomes will be performed, and one final analysis. The Type I error for these analyses will be controlled through an α-spending function with O’Brien-Fleming type criteria. This means that the interim analysis will be done at a two-sided α-level of 0.003 (0.3%) and the final analysis at a level of 0.049 (4.9%). Based on these criteria and using a power of 80% and an overall level of significance of 0.05, 53 patients are required in each arm, making 106 patients in total. Taking into account potential loss-
to-follow up, 120 patients will be included into the study. The total population eligible for inclusion is approximately 350 patients.

### TREATMENT OF SUBJECTS

<table>
<thead>
<tr>
<th>Formulation</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Formulation</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Active substance</strong></td>
<td>Human fibrinogen concentrate, pasteurized</td>
</tr>
<tr>
<td><strong>Trade name, if available</strong></td>
<td>Haemocomplettan® P, CSL Behring</td>
</tr>
<tr>
<td><strong>Dosage form</strong></td>
<td>Powder and diluent (water for injection)</td>
</tr>
<tr>
<td><strong>Dosing formula</strong></td>
<td></td>
</tr>
<tr>
<td>• Fibrinogen concentrate dose for infusion with formula:</td>
<td></td>
</tr>
<tr>
<td>((2.5 - \text{[plasma fibrinogen on CPB g/L]}) \times (1 - \text{Ht on CPB}) \times 0.07 \times \text{body weight (kg)} = \text{whole g fibrinogen to be dosed via Haemocomplettan® P.}</td>
<td></td>
</tr>
<tr>
<td>Infusion will be calculated as total gram, allowing adequate preparing of the commercially available vials of Haemocomplettan® P.</td>
<td></td>
</tr>
<tr>
<td><strong>Placebo</strong></td>
<td>An equivalent volume human albumin will be infused</td>
</tr>
<tr>
<td><strong>Duration of treatment</strong></td>
<td>Single infusion administered over approximately 10 minutes</td>
</tr>
<tr>
<td><strong>Mode of application</strong></td>
<td>Intravenous route</td>
</tr>
</tbody>
</table>

#### 5.5 Intervention drug

The infusion of study medication will be performed after the patient was found to fulfil the aforementioned inclusion criteria following removal of CPB during surgery.

Patients randomized to the intervention group will receive a single IV infusion of study medication within 10 minutes after the first clinically relevant bleeding, following removal of the subject from CPB. Dosing will be individually determined based on plasma fibrinogen concentrations (measured with Clauss method during the reperfusion period on CPB) and body weight, calculated using the following formula:

\[
(2.5 - \text{[plasma fibrinogen g/L]}) \times 0.07 \times (1 - \text{Ht on CPB}) \times \text{body weight (kg)} = \text{whole g fibrinogen to be dosed via Haemocomplettan® P (or equivalent volume of placebo).}
\]

**Note:** 2.5 g/L is the target (endogenous) plasma fibrinogen concentration.

**Note:** 0.07 x body weight x (1-Ht on CPB) = circulating plasma volume based on weight.

Since no dosing regime is available, above described dosing regimen is based on current available in vitro and in vivo literature [23, 26, 30, 35-39]. Normal plasma levels of fibrinogen are between 2-4 g/L. After surgery these levels raise up to 4-6 g/L since it is an acute phase response protein. Our results in cardiac surgery patients show a decreasing bleeding tendency, if plasma fibrinogen levels measured during CPB are well above 2.5 g/L. A clear cut-off level in patients that do not show a bleeding tendency cannot easily be determined, but preliminary data show that this level will range between 2.5 and 3.0 g/L (unpublished data).
Based on this formula, a maximum of 8 g of fibrinogen is expected to be administered. Normally, the level measured during CPB towards the end of surgery is not below 1.0 g/L, and Ht = 0.25.

If the patient weighs 70kg and has a plasma fibrinogen of 1.0g/L, the abovementioned formula shows that in this case \((2.5-1.0) \times 0.07 \times (1-0.25) \times 70=5.55\), meaning 6g fibrinogen concentrate will be infused.

Infusion will be calculated as total gram, allowing adequate preparing of the commercially available vials of Haemocomplettan \(^\text{P}\). Every 1 gram of fibrinogen concentrate is reconstituted with 50 ml water for injection, so e.g., 6 gram fibrinogen means 300ml fluid infusions.

If for example, a study patient has a plasma fibrinogen concentration of 2.9 g/L, the formula becomes negative: \((2.5-2.9) \times 0.07 \times (1-0.25) \times 70=-1.47\). This patient has enough fibrinogen concentration in plasma. Consequently this patient is excluded from the study and will not be randomized. A 5-minute bleeding volume test will also not be performed.

Dosing adjustments or other modifications of this treatment scheme are not planned as the repetitive therapeutic steps with allogenic blood products will provide sufficient coagulation therapy to achieve the required hemostasis intra- and post-operatively. The Haemocomplettan \(^\text{P}\) lyophilizate contains no preservatives. The placebo solution for injection will consist of human albumin and will also be provided by the hospital pharmacy.

5.6 Placebo: Human albumin

Human Albumin (HA) is chosen as a placebo since it resembles the characteristics of fibrinogen in terms of color, viscosity and transparency of the fluid. HA is already standard used at the Isala Clinics as a priming solution of the CPB system since 20 years without signs of adverse effects.

**Albumin infusion solution (Cealb, Sanquin 200g/L, bottle of 100ml)**

The bottle of albumin has a volume of 100ml and is concentrated with 200g/L albumin. The study bottles of albumin will be diluted with saline and will contain 2g in 50ml in total. This concentration resembles the total protein load in the bottles with Haemocomplettan \(^\text{P}\). The placebo solution for injection will also be prepared under aseptic conditions. For patients randomized to the placebo arm, the amount of fluid to be infused is calculated based on the amount that would have been infused if the patient were in the intervention group. This avoids noticing and identifying the randomization group based on the amount of fluid infusion. So for the aforementioned example, if the patient were randomized to the placebo group, he/she would receive 300ml of placebo infusion.

5.7 Prior and concomitant treatment

All medications being taken by subjects at baseline examination and all products given in addition to study medication during the study, are regarded as concomitant treatments and will be documented on the CRF. Antifibrinolytic prophylaxis will be given to all subjects as part of the surgical procedures. Altogether, subjects will receive 30 mg/kg tranexamic acid at start of the CPB and 15mg/kg at the end of CPB. Desmopressin will be administered to all patients participating in the study with a dose of 0.3µg/kg after CPB.

5.8 Escape medication

After the infusion of study medication, the standard transfusion protocol of the Isala Clinics for thoracic surgery will be followed (see addendum). Dosing adjustments or other modifications of this treatment scheme are not planned as the repetitive therapeutic steps with allogenic blood products will provide sufficient coagulation therapy to achieve the required hemostasis intra and post-operatively.
In case of emergency, per example uncontrollable bleeding, the infusion of escape medication is at the discretion of the attending anesthesiologist. If the anesthesiologist decides to abandon the treatment protocol, this will be documented in the CRF. The attending anesthesiologist will document in detail the reason for diversion, the used intervention with attention to dose and time points of intervention. Possible escape medication may be: PPSB (Cofact Sanquin, CLB), dose at the discretion of the anesthesiologist. Recombinant factor VIIa (Novoseven, Novo Nordisk Farma bv), dose at the discretion of the anesthesiologist.

6. **INTERVENTION DRUG**

6.1 **Description of study medication**

Investigator’s brochure (addendum)

Name of the medicinal product: Haemocomplettan® P 1g.
Manufacturer:
CSL Behring GmbH
Emil-von-Behring-Str. 76
35041 Marburg, Germany

Haemocomplettan® P is a heat-treated, lyophilized fibrinogen (coagulation factor I) powder made from pooled human plasma. Each vial contains 900 to 1300 mg fibrinogen, 400 to 700 mg human albumin, 375 to 660 mg L-arginine hydrochloride, 200 to 350 mg sodium chloride and 50 to 100 mg sodium citrate. Sodium hydroxide and hydrochloric acid may have been used to adjust the pH. All plasma used in the manufacture of Haemocomplettan® P is tested using FDA-licensed serological assays for hepatitis B surface antigen and antibodies to HIV-1/2 and HCV. Additionally, the plasma is tested with FDA-licensed Nucleic Acid Testing (NAT) for HCV and HIV-1 and found to be non-reactive (negative). For HBV, an investigational NAT procedure is used; however, the significance of a negative result has not been established. In addition, the plasma has been tested by NAT for HAV and B19V. Only plasma that passed virus screening is used for production, and the limit for B19V in the fractionation pool is set not to exceed 104 IU of B19V DNA per ml.

Haemocomplettan® P is manufactured from cryoprecipitate into a glycine precipitate, which is then further purified by multiple precipitation/adsorption steps. The manufacturing process has been demonstrated to reduce the risk of virus transmission in an additive manner: Cryoprecipitation, Al(OH)3 adsorption/glycine precipitation/Al(OH)3 adsorption, heat treatment (+60°C for 20 hours in an aqueous solution), and two subsequent glycine precipitation steps (initial and main glycine precipitation steps). These steps have been validated independently in a series of in vitro experiments for their capacity to inactivate and/or remove both enveloped and non-enveloped viruses.

Pharmacotherapeutic group: Haemostyptics/antihaemorrhagics. ATC code: B02B B01

**Drug formulation**

<table>
<thead>
<tr>
<th>Active substance</th>
<th>Human fibrinogen concentrate, pasteurized, lyophilized</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trade name, if available</td>
<td>Haemocomplettan® P</td>
</tr>
<tr>
<td>Dosage form</td>
<td>Powder and diluent (water for injection)</td>
</tr>
<tr>
<td>Duration of treatment</td>
<td>Single infusion administered over approximately 10 minutes</td>
</tr>
<tr>
<td>Mode of application</td>
<td>Intravenous</td>
</tr>
</tbody>
</table>

The Haemocomplettan® P contains no preservatives.
Qualitative and quantitative composition:

Active ingredients

<table>
<thead>
<tr>
<th>Haemocomplettan P</th>
<th>1 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder</td>
<td>1925 - 3010 mg</td>
</tr>
<tr>
<td>human fibrinogen</td>
<td>900 - 1300 mg</td>
</tr>
<tr>
<td>total protein</td>
<td>1300 - 1900 mg</td>
</tr>
</tbody>
</table>

Other ingredients

<table>
<thead>
<tr>
<th>Haemocomplettan</th>
<th>1 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Albumin</td>
<td>400-700 mg</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>200-350 mg</td>
</tr>
<tr>
<td>L-arginine hydrochloride</td>
<td>375-660 mg</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>50-100 mg</td>
</tr>
</tbody>
</table>

Sodium hydroxide and hydrochloric acid may have been used to adjust the pH.

Additional information on Haemocomplettan P can be found in the Haemocomplettan P Investigator’s Brochure (addendum).

6.2 Summary of findings from clinical studies
For information see the Investigator’s Brochure (addendum) sections 4 and 5.

6.3 Description of route of administration
The preparation will be warmed to room or body temperature before administration. The injection or infusion rate will not exceed approximately 50 ml per minute. The patient will be observed for any immediate reaction. If any reaction takes place that might be related to the administration of Haemocomplettan P, the rate of infusion will be decreased or the infusion stopped, as required by the clinical condition of the patient. Study medication will be administered through a separate injection site (i.e. intravenous line).

6.4 Description of dosage
For patients randomized to the intervention group, dosing will be individually determined based upon plasma fibrinogen levels measured with Clauss method at the end of CPB.

- Formula:
  \[(2.5 - \text{[plasma fibrinogen]}) \times 0.07 \times (1-\text{Ht on CPB}) \times \text{body weight (kg)} = \text{whole g fibrinogen to be dosed via Haemocomplettan P. Infusion will be calculated as total-gram, allowing adequate preparing of the commercially available vials of Haemocomplettan P.}\]

- In placebo an equivalent volume human albumin will be prepared for infusion.

6.5 Preparation and labelling
Reconstitution of Haemocomplettan P will be carried out under aseptic conditions. Solutions that are cloudy or contain residues (deposits or particles) cannot be used. Both the diluent (i.e. water for injection) and the Haemocomplettan P powder (i.e. lyophilizate) will be warmed to room or body temperature (maximum: 37°C) in unopened bottles. Each gram of Haemocomplettan P will be reconstituted with 50 ml water for injection.

The cap of the Haemocomplettan P infusion bottle (USP [United States Pharmacopeia] and PharmEUR [European pharmacopeia] conform type II glass infusion bottle of 100 ml capacity) will
be removed to expose the central portions of the infusion stoppers, the surfaces of which will be treated with an antiseptic solution and allowed to dry. The diluent will be transferred into the infusion bottle with an appropriate transfer device, ensuring complete wetting of the powder. The bottle will be gently swirled until the powder is reconstituted, avoiding strong shaking. The powder will be completely reconstituted within a maximum of 15 minutes (generally within 5 to 10 minutes). When reconstituted as recommended, a colourless to yellowish, clear to slightly opalescent solution of neutral pH will be obtained. After reconstitution Haemocomplettan® P is chemically and physically stable for 24h at room temperature, but will not be refrigerated and should be administered within this time period since it contains no preservatives.

For the current study the medication will be transferred to orange syringes of 50 ml to ensure that the study medication is not distinguishable from the placebo. The syringes will be labelled with “CLOTS and study number” and “for clinical research only” and will be stored in an authorized refrigerator.

The placebo solution will be supplied by the study center pharmacy and will also be labeled “CLOTS and study number” and “for clinical research only”.

6.6 Drug accountability

The hospital pharmacy will supply the lyophilized Haemocomplettan® P, which will be stored at +2 to +8°C. It will not be frozen. The medication will be stored in the bottles, and accompanying cartons, in which it is delivered. It is stable for at least 60 months when stored at +2 to +8°C. The prepared Haemocomplettan® P and placebo will be stored separately from normal hospital or medical practice stocks. The study drug will be stored at the pharmacy of the Isala Clinics Zwolle according to the Haemocomplettan® P product information. All supplies (Haemocomplettan® P and placebo solution) will be accounted for throughout the study using the drug inventory log, which will be provided to the Monitor prior the first patient enrolled in the study. At the end of the study, the dated and signed original drug inventory log is to be maintained at the study center as verification of final drug accountability.

After the drug accountability documentation has been checked by the Monitor, partially used and empty IMP infusion bottles (Haemocomplettan® P and placebo solution) will be destroyed by the study center. Unused IMP bottles are to be returned to the hospital pharmacy.
7. **METHODS**

7.1 **Primary efficacy endpoint**
1. Perioperative blood loss measured as blood loss in ml between infusion of study medication and closure of chest.

7.2 **Secondary efficacy endpoints**
1. Postoperative blood loss, measured as blood loss at the ICU between closure of chest and:
   - 1st hour
   - 2nd hour
   - 3rd hour
   - 6th hours
   - 12th hours
   - 24th hours
   - At the actual time of chest tube removal.
2. Number of units of allogenic blood products (platelets + FFP + RBCs) administered to subjects, between administration of study medication and closure of chest.
3. Number of units of allogenic blood products administered to subjects, between administration of study medication and 24 hours thereafter.
4. Number of units of allogenic blood products (platelets + FFP + RBCs) administered to subjects, from admission to the ICU to discharge to the ward.
5. Number of units PPSB or Novoseven given in the peri- and postoperative period.
6. Duration of post CPB phase, from infusion of study medication to transfer to ICU
7. Ventilation-time in hours during ICU stay.
8. Duration of stay in hours in the ICU following last suture of the initial surgery.
9. Duration of hospital stay in hours following last suture of the initial surgery.
10. Proportion of subjects that receive a follow-on surgery to correct unacceptable bleeding within 5 days of last suture.
11. Wound, sternal or other types of infection.
12. Major clinical events:
   - Mortality at 30 days post-surgery
   - MACE (major adverse cardiac event)
   - Cerebrovascular accident/ transient ischemic attack
   - Renal insufficiency or failure
   - Venous thromboembolism/ pulmonary embolism
   - Allergic or other systemic reaction to study medication
7.3 Safety Variables:

30-day mortality
Thirty-day mortality is defined as death of any cause within 30 days after the elective complex cardiac surgery. Reporting on 30-day follow-up.

Cerebrovascular accident (CVA):
Cerebrovascular accident is defined as a new motor or sensory deficit with its origin in the central nervous system or an unexplained coma status lasting longer than 24 hours. Diagnosis by consulting neurologist or confirmed by positive finding on CT or MRI.

Transient ischemic attack (TIA):
TIA is defined as brief episode of neurological dysfunction resulting from focal temporary cerebral ischemia lasting less than 24 hours, not associated with cerebral infarction. Diagnosis by a consulting neurologist.

Myocardial infarction:
Myocardial infarction is defined as myocardial specific creatine kinase (CKMB) >120 U/L (five times upper reference limit) plus a peak CKMB/CK ratio >10%, or pathological new Q waves on a postoperative electrocardiogram (ECG) [40].

To avoid false-positive diagnoses in the complex cardiac surgery procedure of this cohort, CKMB value ≥180 U/L (7.5 times upper reference limit) and a peak CKMB/creatine kinase ratio >10% plus either new pathological Q waves or new LBBB (left bundle branch block), or angiographically documented new graft or native coronary artery occlusion, or imaging evidence of new loss of viable myocardium have been designated as procedure related myocardial infarction. Preoperative CK and CKMB will be measured and repeated in all patients on arrival in the ICU and 4 hours thereafter. Measurements are repeated at 4 hour intervals when a CKMB level in the ICU is >50 U/L, the percentage is >10% of total CK, and when CKMB is increasing between the first and second measurement. These measurements are continued until the CKMB level is decreasing. In the ICU, all patients are continuously monitored with 12-lead ECG with a printed version on arrival, at discharge and with suspected myocardial injury.

Renal injury:
Renal injury is defined as increase in postoperative serum creatinine of at least two times the preoperative value or a decrease in glomerular filtration rate (GFR) of more than 50% [41].

Renal failure:
Acute renal failure is defined as an increase in postoperative serum creatinine of at least three times the preoperative value and a decrease in GFR of at least 75% or a serum creatinine >354µmol/L associated with an acute increase of serum creatinine of at least 50µmol/l. Creatinine is measured in all patients before surgery and once daily as long as they are in the ICU.

Venous thrombosis:
Deep vein thrombosis (DVT) of the lower extremity is divided in two types; distal (calf vein) or proximal (thigh) vein thrombosis. By comparison, distal calf vein thrombosis is felt to be of lesser importance compared to proximal vein thrombosis. Proximal deep vein thrombosis is more commonly associated with serious disease and possibly fatal outcomes. Proximal deep venous thrombosis can be identified by non-invasive testing in 20 to 50 percent of patients with acute pulmonary embolism when only a single study is performed [42]. Prospective studies have demonstrated that lack of compressibility of a vein with the ultrasound probe is highly sensitive (>95 percent) and specific (>95 percent) for proximal vein thrombosis [43-45]. All patients included in this study will undergo a compression ultrasound testing prior to surgery and at day 3 after surgery. Proven DVT will be treated...
accordingly. In case of an inconclusive test result, a venography will be performed. When test result is normal, the ultrasound test will be repeated when symptoms of DVT re-appear.

**Pulmonary embolism:**
If a patient is suspected to have a pulmonary embolism, a spiral CT will be performed to rule out or confirm pulmonary embolism. Spiral CT for diagnosing pulmonary embolism has a high sensitivity of 83% and a much higher specificity of 96%. [46]

### 7.4 Additional variables

**ROTEM®**
Rotem is a viscoelastic point-of-care device that measures clot formation. Its coagulation measurements include:

- **EXTEM®** (extrinsic pathway thromboelastometry) – via factors I, II, V, VII, and X, including the effects of fibrinolysis and platelets
- **FIBTEM®** (fibrinogen thromboelastometry) – as in EXTEM®, however a platelet blockade is induced; it is used to estimate the amount of functional fibrinogen
- **INTTEM®** (intrinsic pathway thromboelastometry) – via factors I, II, V, VIII, IX, X, and XI, including the effects of fibrinolysis and platelets
- **HEPTEM®** (heparinase thromboelastometry) – as in INTEM®, however a heparin-degrading enzyme is included, whereby a heparin-induced coagulation disorder can be detected.

ROTEM® tracings can be analyzed for coagulation time, clot formation time, clot propagation (alpha angle), MCF, and firmness of the fibrinogen-fibrin part of the clot. Approximately 1.5 ml blood will be required for these measurements, prepared as citrated plasma. During this study, the ROTEM® variables will be measured at 3 time-points; start of surgery, end-warming and at skin closure before leaving the operation theatre.

**MULTIPLATE®**
Multiplate is a platelet aggregation analyzer. It analyses whether the use of ASA, clopidogrel and tirofiban effects platelet aggregation. The test that will be used: ASPI (aspirin effect), ADP (clopidogrel effect) and TRAP (thrombin receptor activation). These measurements will be performed at 3 time-points: start of surgery, end-warming and at skin closure before leaving the operation theatre.

Multiplate® measurements provide comprehensive information on platelet function and antiplatelet therapy via the following reagents:

- **ADP** – platelet activation via ADP receptors, most important being P2Y12, which is blocked by clopidogrel, prasugrel, and ticlopidinesensitive
- **ASPI** – platelet activation via ASPI, which is converted to thromboxane A2, a potent platelet activator
- **TRAP** – platelet stimulation via the thrombin receptor (using TRAP-6), sensitive to IIbIIIa receptor antagonists.

**Fibrinogen**
Fibrinogen in blood will be determined via the Clauss assay (i.e. for activity). This measurement will be made from 1.5 ml of the 3.0 ml blood taken for other coagulation evaluations.

**Other coagulation**
Other coagulation measurements will include PT and APTT in plasma.
Approximately 1.5 ml of the 3.0 ml blood taken for coagulation analysis will be required for these measurements, prepared as citrated plasma.
HRQoL
Two validated, standardized questionnaires will be used to evaluate QoL:
  - SF-36: Generic health survey consisting of 36 questions, relying on subject self-reporting of their perceived state of health.
  - EuroQoL-5D: Generic health survey consisting of 5 domains, relying on subject self-reporting of their perceived state of health.
Both questionnaires will be filled in by the patients at baseline examination, 30 day and 1 year follow-up. Examples of each of these questionnaires are included in this protocol.

7.5 Randomization
The inclusion and randomization procedure in this study is influenced by the following two important premises:
- Eligibility for the study can only be determined when the patient does have microvascular bleeding after removal of CPB, i.e., during the procedure.
- Once the patient is found to be eligible, study medication has to be administered as soon as possible.

The inclusion and randomization procedure will be as follows:
1. Before start of the surgery, it will be determined whether the patient could become eligible by the research nurse visiting the patient at the ward between 3 and 1 day prior to day of surgery. When the patient meets the inclusion criteria that can already be established at this time point (regarding age, type of surgery) and exclusion criteria and the ICD (informed consent document) is signed, the patient is theoretically eligible for the study. Each of these patients is assigned a screening number in sequential order (i.e. 001, 002, 003). This number will be used for subject identification throughout the study and should be mentioned on every CRF page.
2. After removal of CPB, final eligibility of the patient is determined based on the intraoperative characteristics and the presence of microvascular bleeding. If the patient fulfils these criteria, the patient is included in the study.
3. The patients will be randomized by the research-team using a computerized web-based randomization protocol designed by the Julius Center for Health Sciences and Primary Care. This protocol uses block randomization of 4 balanced outcomes per block (2x placebo and 2x IMP). The blocks themselves are also randomized. Based on the randomization result, the circulating anesthesia-nurse will either prepare a placebo solution or an active medication solution (IMP). The circulating anesthesia-nurse preparing the study medication is not involved in the operation with the study subject. The prepared syringes of both treatment groups are of the same size and colour. The fluid inside the bottle is of the same volume (50ml), same colour and viscosity. The bottles are labelled with the “study kit number”. This method ensues an adequate blinding of the research team (i.e. surgical team) for the allocated treatment.
4. Based on the plasma fibrinogen level determined just before removal of CPB, the required amount of study medication is calculated based on the formula presented in sections 5.5 and 6.4. If for example, 6gram of study medication is to be infused, 6 syringes of study medication will be prepared. The anesthesia-nurse at the operation room will record the kit number on the CRF before infusion of the fluid.
5. If the patient does not fulfill the inclusion criteria and consequently is not included in the study, the surgical procedure proceeds according to current standard care. The kit with bottles
of study medication or placebo remains in the refrigerator, and will be used with the first patient who fulfills the inclusion criteria within a time span of 24 hours after preparation. Otherwise, the prepared medication will be returned to the pharmacy for destruction.

Randomization will continue until at least 60 subjects in the placebo group and 60 subjects in the intervention group deliver primary endpoint data.

7.6 Study blinding
Unblinded preparation of study medication will be performed by the circulating anesthesia-nurse of the Isala Clinics Zwolle according to the web-based randomization protocol. The bottles with study medication will be labelled with the kit and study identification number before delivery to the operating room.

The circulating anesthesia-nurse, who is not involved with the surgery of the study, will perform the measurements with ROTEM® and Multiplate®. All anesthesia-nurses are trained by the representatives of both POC monitors for correct use. The results of these POC monitoring devices are blinded for the operating team. Study unblinding will take place following closure of the study database. In case the investigator considers a SAE to be unexpected, severe, and warrant specific knowledge of the identity of the study medication for a given subject, the investigator may break the blind via communication with the study center. The subject should then be discontinued from the study. An independent Data Safety and Monitoring Board (DSMB) is installed for this purpose.

8. STUDY PROCEDURES

8.1 BEFORE SURGERY
For the purpose of this study’s visit schedule, Day 0 will be defined as the day of elective complex cardiac surgery. Time zero will be defined as administration of study medication.

Baseline examination
Baseline examination will take place from Day −3 to Day −1 at the study center, at which point subjects will be evaluated for study eligibility. Eligibility will be determined via the inclusion and exclusion criteria. Each subject will give written informed consent (enrolment), and in the context of evaluating eligibility, the following examinations will be performed:
  o Medical history
  o Physical examination
  o Neurological systems
  o Vital signs
  o Urine pregnancy test, if applicable
  o Blood tests
  o Ultrasound of lower extremity for deep venous thrombosis
  o EuroQoL: EQ-5D and SF-36
  o Concomitant medications, especially those for the purpose of blood volume management, starting at informed consent and continuously recorded
  o AEs recorded starting at informed consent and continuously recorded

Vital signs include systolic and diastolic blood pressure and pulse rate and will be recorded using standard clinical procedures.
8.2 DAY OF SURGERY

8.2.1 Baseline on Day 0
Subjects will generally report to the study center on the evening before Day 0 (i.e. the evening before the day of surgery).
On the morning of Day 0 minutes after induction of anesthesia, the following examinations will be performed:
- Hematology (Hb, Ht, platelet, plasma fibrinogen concentration)
- ROTEM® (EXTEM, INTEM, FIBTEM)
- Multiplate® (ADP-test, ASPI-test, TRAP-test)
- Plasma fibrinogen concentration (Clauss method)
- Spijtserum
  - Each patient will receive 30mg/kg of tranexamic acid before start of CPB

8.2.2 Start of cardiopulmonary bypass
- Hematology, Hb, Ht, Thrombocyte count and plasma fibrinogen concentration

8.2.3 Removal of cardiopulmonary bypass
Approximately 20 minutes before subjects are removed from CPB (i.e. the heart-lung machine) the following examinations will be performed:
- ROTEM® (EXTEM, FIBTEM, HEPTEM)
- Multiplate® (ADP-test, TRAP-test)
- Plasma fibrinogen concentration (Clauss method)
- Hematology
- Spijtserum
- Recording of concomitant medications
- Recording of AEs
- Subjects will then be removed from CPB and heparin is antagonized with protamine 1:1 and all patients will receive 15mg/kg tranexamic acid
- All patients receive demopressin 0.3µg/kg iv before removal of CPB

The 5-minute bleeding volume test.
Following removal of the CPB, after administration of protamine and an ACT < 140 seconds, the period of drying of the surgical area starts by applying fresh surgical cloths and compresses in the thoracic cavity, allowing 5 minutes to pass before removal and evaluation of the blood volume taken up into the cloths. As the surgical area is shaped like a cave and will be covered completely with surgical cloths and compresses, nearly all blood will be effectively absorbed. Additionally, no blood will be suctioned from the surgical area and no liquids will be applied during this 5-minute period. Following removal of the surgical cloths, the volume of the blood soaked into the cloths will be wrenched out of these gauzes and together with the fluid still into the surgical area, it will be suctioned into a separate reservoir and both this volume will be weighted and measured.
- A 5-minute bleeding volume of:
  - < 60ml indicates that the bleeding is not clinically relevant.
  - 60 to 250ml indicates that the bleeding of the microvasculature is clinically relevant.
  - > 250ml indicates that bleeding of the macro-vasculature has occurred, being considered clinically relevant surgical bleeding.
If the patient has a 5-minute bleeding volume of 60 to 250 ml, and meets the following intraoperative criteria:

- Body temperature > 36°C
- Blood pH > 7.3
- Hb > 5.3 mmol/L or Ht > 0.25
- ACT < 140 seconds

The patient is included into the study and study medication will be administered over approximately 10 minutes via an IV infusion. The 5-minute bleeding volume will again be evaluated to confirm that an appropriate hemostatic condition is being maintained. If clinically relevant bleeding of the microvasculature is experienced, then the transfusion guideline of the Isala Clinics will be followed (addendum).

In summary the transfusion guideline implies:

- Ht< 0.23, then 1 U RBC is administered.
- Thrombocyte count <100x10^9/L in bleeding patient, then 1 U platelet concentrate is administered.
- Plasma loss, both cell-saver and blood-loss >1L, then 2 U FFP is administered.
- Plasma loss, both cell-saver and blood-loss >2L, then 4 U FFP is administered.

### 8.2.4 End of surgery

Following last suture, the following examinations will be performed:

- Patient transfer to the ICU
- ROTEM® (EXTEM, INTEM, HEPTEM, FIBTEM)
- Recording of AEs

### 8.2.5 Arrival ICU after surgery

- Blood tests
- Plasma fibrinogen concentration (Claus method)
- Other coagulation (APTT, PT)
- Hematology
- Biochemistry
- Recording of concomitant medications
- Measurement of blood drainage will take place from arrival ICU up to 24 hours following start of infusion of study medication.
- Recording of transfusion during ICU stay
- Recording of AE’s

### 8.3 First day post-surgery

On Day 1, approximately 24 hours (+2h) following administration of study medication, the following examinations will be performed:

- Blood tests
- Plasma fibrinogen concentration (Claus method)
- Hematology
- Biochemistry
- Recording of concomitant medications
- Recording of AEs
8.4 Day of discharge form ICU
The day of discharge from ICU will ideally be on Day 1 or 2, but may vary depending on the clinical condition of the given subject. Before discharge from the ICU, the following examinations will be performed:
- Physical examination
- Vital signs
- Recording of concomitant medications
- Recording of AEs

8.5 Stay at the ward
- Plasma fibrinogen concentration (Claus method)
- Physical examination
- Vital signs
- Recordings of concomitant medications
- Recording of AEs
- Ultrasound of lower extremity for deep venous thrombosis

8.6 Day of discharge form hospital
The day of discharge from hospital will ideally be on Day 7 to 10, but may vary depending on the clinical condition of the given subject. Before discharge from the hospital, the following examinations will be performed:
- Physical examination
- Vital signs
- Recordings of concomitant medication
- Recording of AEs

8.7 Day 30 follow-up
On Day 30, subjects will report to the study center and the following examinations will be performed:
- EuroQoL; EQ-5D and SF-36
- Reporting of SAEs
- Reporting of morbidity and mortality

8.8 Withdrawal and replacement of individual subjects
In accordance with ICH principles of GCP, the investigator always has the option to advise a subject to withdraw from the study if the subject’s safety or well-being is compromised by his or her further participation in the study. Concern for the interests of the subject must always prevail over the interest of the study. Additionally, subjects can decide to discontinue from the clinical study at any time without indicating a reason and without any consequences to future therapy.
The reason and date of withdrawal for all subjects withdrawn from the clinical study must be recorded in detail on the CRF and in the subject's medical records (e.g., adverse event, lost to follow-up). If possible, the subject should confirm her/his decision in writing.
8.9 **Replacement of individual subjects after withdrawal**
Randomisation of subjects will continue until at least 106 subjects provide the primary efficacy endpoint data.

8.10 **Follow-up of subjects withdrawn from treatment**
Data collected until the subject’s decision to withdraw from the study will be retained in a protected form under the provisions of the respective data privacy regulations for the purpose of the study. Further information will be collected if any adverse events or other discomforts were experienced. The investigator will complete all procedures usually required at the end of the study at the time a subject is discontinued from the clinical study. A complete final examination must be performed on all subjects who do not complete the study according to the protocol.

8.11 **Premature termination of the study**
The investigator has the right to discontinue this study at any time for medical or administrative reasons. The decision to discontinue the study will be based upon the risk associated with study medication outweighing its benefit. Furthermore, the planned group sequential interim analyses will guide the decision to terminate the study prematurely (section 10.5) in case of overwhelming evidence of efficacy. The serious adverse effects will also be reported to the responsible authorities (section 9.3). Following emergency unblinding of individual subjects experiencing unexpected, severe SAEs, so as to minimize the risk to other study subjects, it should be determined if Haemocomplettan® P was indeed the study medication given to these subjects. If the study is prematurely terminated for any reason, the investigator will promptly inform the study subjects and should ensure appropriate therapy and follow-up.

9. **SAFETY REPORTING**

9.1 **Section 10 WMO event**
In accordance to section 10, subsection 1 of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects’ health. The investigator will take care that all subjects are kept informed.

9.2 **Adverse and serious adverse events**
An adverse event (AE) is any untoward medical occurrence in a subject administered a medicinal product (MP), which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a MP, whether or not considered related to the MP. All AE’s reported spontaneously by the subject or observed by the investigator or his staff will be recorded. An increase in severity or frequency of a sign or symptom of a concomitant disease in a given subject would also be reported as an AE.

AEs can also be recognized from changes in the subject’s physical examinations from baseline. In general such changes should be considered as AEs and need to be reported subsequent to the following scrutiny:

1. If the change from baseline is considered by the investigator to be part of the normal daily fluctuations of an underlying disease process, this shall not be reported as an AE
2. If the change from baseline is considered by the investigator to be an untoward medical occurrence different from point 1, this medical occurrence shall be reported as an AE.

All AEs, including any new, intercurrent illnesses or worsening of existing illnesses, will be reported to the DSMB and documented. The period of observation for all AEs extends from the time the subject gives informed consent to the final examination procedures at discharge. SAEs will be reported up through the Day 30 follow-up. AEs are divided into the categories "serious" and "nonserious". This determines the procedure, which must be used to report and document the AE.

A SAE is any untoward medical occurrence that at any dose:
- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is according to investigator’s judgment another medically important condition.
- Is a new event likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction.
- Major safety finding from a newly completed animal study.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. "Life-threatening" means that the subject was at immediate risk of death from the AE as it occurred. It does not include an AE that, had it occurred in a more severe form, might have caused death. AEs, which do not fall into these categories, are defined as nonserious. Expected AEs are determined by this protocol and the current Investigator's Brochure.

Regardless of the classification of an AE as serious or nonserious (see above), its severity will be assessed as mild, moderate or severe, according to medical criteria alone:
- **Mild**: Does not interfere with routine activities.
- **Moderate**: Interferes somewhat with routine activities.
- **Severe**: Impossible to perform routine activities.

Subjects will be instructed by the investigator to report the occurrence of any AE. The following algorithm will be used to assess the causality of all AEs:

- **Not related**: The event can readily be explained by factors not involving the MP, and a temporal relationship with the MP does not exist.
- **Possibly related**: A clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the MP, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.
- **Probably related**: The temporal relationship between the administration of the IMP is compelling, and the event cannot be explained by the subject’s medical condition or other therapies.
- **Related**: The event follows a reasonable temporal sequence from administration of the IMP, follows a known or suspected response pattern to the IMP, is confirmed by improvement upon stopping the IMP (dechallenge) and reappears upon repeated exposure (rechallenge).

A dechallenge-rechallenge test will not be performed with the studied IMP (Haemocomplettan P) described in this study protocol.
All AEs, regardless of severity, will be followed-up by the investigator until satisfactory resolution. All subjects experiencing AEs (whether considered associated with the use of the IMP or not) will be monitored until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report should be supplied, if possible. All findings will be reported on an "AE / SAE" page in the CRF and in the subject's medical records.
In the event the investigator considers an AE to be serious enough to require specific knowledge of the identity of the study substance, the investigator may, only if necessary for the subject's care, break the blind for that subject only, after approval by the DSMB.

9.2.1 Events (AEs/SAEs) that are specific to the common underlying disease, the surgery process, and hospitalization in the ICU

Adverse events recording related to the surgical procedure are part of the quality assessment process peri-operatively and will be monitored by the surgical team itself. These data are also available for the DSMB.

Problems related to the use of CPB
Caused by different factors such as air in blood circulation, cardiac failure at end of surgery, atheromatous disease of the aorta, prior surgery or type of cardioplegia. Adverse drug reactions such as protamin response are also recorded.

Problems of renal function
Based on a post-pump syndrome or caused by pre-surgery or temporary intra-surgery interruption of perfusion with CPB.

Problems of intestinal or liver functions
Caused by pre-surgery or temporary intra-surgery interruption of perfusion with CPB.

Infections (either local or systemic, i.e. sepsis with multi-organ failure)
Can never be ruled out for surgery accompanied with ICU hospitalization.

Post-pump syndrome or SIRS
Generalized, inflammatory syndrome with inner-vessel (intravascular) volume loss caused by increased capillary permeability; subsequent to the surgical trauma or intra-operative employment of the CPB without detectable accompanying infection. Therapy is high dose inotropic support.

Impaired lung function or lung failure
Caused by artificial ventilation, intra-operative mechanical damage especially of the left lung in aortic surgery, and caused by infections due to ICU hospitalization.

Allergic reaction towards non-coagulation therapy/medication
If observed immediately after administration of medications other than platelets, FFP, or fibrinogen concentrate (Haemocomplettan P).

Specific thrombosis / embolism when underlying disease exists
Myocardial infarction, apoplexy, or peripheral embolism within intra- or post-operative course.

Common, acute left or right ventricular load
Based on large intra- and postoperative volume changes through crystalloid (Ringer or NaCl) or colloid solution.
Dysfunctions of medicinal treated underlying diseases
Due to surgical and intensive care medicinal stress, dysfunction of metabolism (e.g. diabetes mellitus), of circulation (e.g. hypertension), or of other systems may arise.

Surgical hemorrhage plus revision surgery
Post operative hemorrhage clearly due to surgical reasons.

Events (AEs/SAEs) that are specific to coagulation therapy
The below listed events are often accompanying coagulation therapy and shall therefore be assessed as ‘expected’.

Allergic reaction towards coagulation therapy/medication
If observed immediately after administration of coagulation therapy (platelets, FFP, RBC, or Haemocompletan®) and no other evident reason existing.

Systemic thrombo-embolism
If observed immediately after administration of coagulation therapy, i.e. allogenic blood products or fibrinogen concentrate (Haemocompletan®).

Specific thrombosis / embolism without underlying disease
Myocardial infarction, apoplexy, or peripheral embolism within intra-operative or postoperative course that are closely connected with administration of coagulation therapy (platelets, FFP, or fibrinogen concentrate (Haemocompletan®)).

Transfusion Related Acute Lung Injury (TRALI)
Closely connected to administration of coagulation therapy (FFP) and no other evident reason existing.

Non-surgical hemorrhage plus revision surgery
Post-operative hemorrhage not related to surgical intervention and therefore related to a coagulopathy.

9.2.2 Suspected unexpected serious adverse reactions (SUSAR)
Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered. Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (Investigator’s Brochure).

The principal investigator will report expedited the following SUSARs through the web portal ToetsingOnline to the METC:
- SUSARs that have arisen in the clinical trial that was assessed by the METC.
- SUSARs that have arisen in other clinical trials with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.
9.2.3 Annual safety report

In addition to the expedited reporting of SUSARs, the principal investigator will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, Medicine Evaluation Board and competent authorities of The Netherlands (CCMO). This safety report consists of:

- A list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study.
- A report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.3 Documentation

Regardless of severity, all AE/SAEs, which occur during the period of observation from giving informed consent up through the Day 30 follow-up, will be reported to the Monitor, Steering Committee and DSMB within 24 hours by telephone or by fax. Next to this, all AE/SAEs will also be reported through the web portal ToetsingOnline (toetsingonline.ccmo.nl) to the accredited METC that approved the protocol. SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days for completion of the report. After receipt of the initial report, the METC will review the information and contact the investigator, if necessary, to obtain further information for assessment of the AE/SAE. When follow-up information is obtained by the investigator, a follow-up report including the new information will be prepared and sent to the Monitor, Steering Committee and DSMB. The report should be marked "Follow-up report".

Any AE/SAE occurring at any time after the end of the study and considered to be caused by the IMP and therefore a possible adverse drug reaction will be reported as described.

All AEs, including intercurrent illnesses and an increase in severity or frequency of a sign or symptom of a concomitant disease, will be reported and documented as described. AEs are to be documented on the "AE / SAE” page of the CRF. All findings relating to AEs experienced by a subject will be reported on this form and included in the subject’s medical records.

9.4 Follow-up of adverse events

All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
10. STATISTICAL ANALYSIS

10.1 Descriptive statistics
Statistical analysis of the data will be performed by SPSS version 17.0 (SPSS Inc. Illinois, USA) and the R statistical package. For base-line characteristics, categorical data will be presented using counts and percentages. Continuous variables which are normally distributed will be summarized with the mean and standard deviation (SD), or the median and interquartile range where appropriate. The key patient characteristics in this trial are:

1. Age
2. Gender
3. Euroscore
4. Body surface area (BSA)
5. Left ventricle ejection fraction
6. Renal insufficiency
7. Diabetes mellitus
8. Preoperative ROTEM variables
9. Preoperative Multiplate variables
10. Preoperative coagulation laboratory variables (INR, APTT, PT, fibrinogen concentration)
11. Preoperative haemoglobin, hematocrit and platelet count.

The procedure characteristics in this trial are:

1. Type of procedure (CABG+valve(s), multiple valves, aortic surgery (root, ascendens, arch))
2. CPB time
3. Aortic occlusion time
4. Lowest core temperature
5. Use of cell-saver
6. All ROTEM and Multiplate variables
7. Perioperative fibrinogen concentration levels in plasma
8. Perioperative haemoglobin, hematocrit and platelet count (towards end of CPB)

10.2 Primary analysis
The primary outcome in this study is the amount of blood loss in the operation theatre after infusion of the study drug or placebo. The primary analysis will be based on all subjects for which the primary outcome was observed, according to the treatment as randomized. Analysis will be based on the log transformed values of the observed blood loss. The two treatment groups will be compared by means of a t-test, ensuring an overall two-sided α-level of 5% across the analysis performed (see interim analysis).

10.3 Related analyses
As an explorative analysis, analyses of covariance of the primary outcome variable with potential predictors of response will be performed. Age, gender, euroscore, lowest core temperature and cardiopulmonary bypass time may be considered as such covariates. Observed imbalance between the two treatment groups for other baseline characteristics may lead to include these for further sensitivity analyses. ROTEM® tracings will be analyzed descriptively for coagulation time, clot formation time, clot propagation (alpha angle), MCF, and clot firmness of the fibrinogen-fibrin part of the clot. Data of Quality of Life questionnaires (SF-36 and EQ 5-D) will be analyzed descriptively.
10.4 Safety analysis
Safety analyses will be performed for the safety analysis set, i.e., including all patients according to the treatment they received.
All reported adverse events (AEs) will be categorized as:
o Screening
o Treatment-emergent
o Follow-up adverse events

Screening AEs are defined as follows:
All adverse events with onset or worsening during screening or baseline period (before start of infusion of study medication).

Treatment-emergent AEs (TEAEs) are defined as follows:
All adverse events with onset or worsening after start of infusion of study medication up to and including day 10 after surgery.

Follow-up AEs are defined as follows:
All adverse events with onset or worsening from day 11 after surgery until end of follow-up

10.5 Interim analysis
According to the ICH E9 (International guideline) section Statistical principles for clinical trials, the goal of an interim analysis is to stop the trial early:
- If the superiority of the treatment under study is clearly established.
- If the demonstration of a relevant treatment difference has become unlikely.
- If unacceptable adverse effects are apparent.

One planned formal interim analysis of efficacy will be performed after 50% of outcomes have been obtained. The overall Type I error for these analyses will be controlled through an α-spending function with O’Brien-Fleming type criteria. This means that the interim analysis will be done at a two-sided α-level of 0.003 (0.3%) and the final analysis at a level of 0.049 (4.9%).

The Julius Center for Primary Care and Health Sciences will perform the interim analysis, under direction of the statistician in the DSMB. The investigators and study team members remain blinded during the complete conduct of the study. The interim analysis will be performed on unblinded data by the DSMB to ensure adequate interpretation of the result. The O’Brien-Fleming criteria will be used as guidance by the DSMB to consider discontinuation of the study. The advice of the DSMB will be presented to the sponsor/principal investigator and the Steering Committee, without revealing any details of actual intermediate results.

The DSMB will further assess at regular intervals the progress of this clinical trial, safety data, and critical efficacy variables and recommends to the sponsor/principal investigator whether to continue, modify or terminate a trial. The DSMB will have written operating procedures and will maintain records of all its meetings, including interim results; these should be available for review when the trial is complete. The independence of the DSMB is intended to control the sharing of important comparative information and to protect the integrity of the clinical trial from adverse impact resulting from access to trial information. The DSMB will chair at the Julius Center for Primary Care and Health Sciences and its composition include clinical trial scientists knowledgeable in the appropriate disciplines and without conflicts of interests.

The interim-analysis report is strict confidential and should include:
- Patients recruitment progress
- Data quality
- Baseline characteristics
- Patients compliance
- Primary and secondary outcomes
- Adverse events (SUSAR, SAE, AE)
- Other safety measures
- Statement on continuation/discontinuation of the study

Possible consequences of the interim analyses are:
- Continuation study
- Discontinuation study
- Modification study
- Modification of Patient Information letter

Notification of the Ethics Committee in case of the DSMB advice warrants this, remains the responsibility of the sponsor/principal investigator

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement
The procedures set out in this CSP are designed to ensure that the investigating team abides by the principles of the Guideline for GCP of ICH and the Declaration of Helsinki in the version amended by the 59th WMA General Assembly October 2008 in Seoul and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts Copies of the ICH GCP Guidelines are included in the investigator's study file.
Before the start of the study, the CSP, written ICD(s), and other appropriate documents will be submitted to and approved by the METC Zwolle, and by the authorities, in accordance with local legal requirements.

11.2 Recruitment and consent
Patients who are scheduled for elective surgery and complex cardiac surgery (CABG and valve(s), aortic surgery (root, ascendens and arch) and multiple valves) are recruited for participation in the study. Patients undergoing complex cardiac surgery are admitted to the hospital 1 to 2 days prior to the scheduled surgery.
The investigator is responsible for obtaining informed consent in accordance with local laws. Trained research nurses will visit the patient at the ward to provide information about the study. The information will be given orally and in an understandable form by a physician or medically qualified person (according to local laws), well informed about the nature, scope and possible consequences of the clinical study. Additionally, written information about the study will also be provided.
The date of providing the information and by whom it was done will be documented on the ICD.
If a subject is unable to read, an impartial witness will be present during the entire informed consent discussion. After the written ICD and the written information is read and explained to the subject or the subject’s legally authorized representative, and after the subject or the subject’s legally authorized representative has orally consented to the subject’s participation in the clinical study and, if capable of doing so, has signed and personally dated the ICD, the witness should sign and personally date the ICD. By signing the ICD, the witness attests that the information in the ICD and any other written information was accurately explained to and apparently understood by the subject or the subject’s legally authorized representative, and that informed consent was freely given by the subject or the subject’s legally authorized representative.
The subjects or their legally authorized representatives will be given a copy of the personally dated and signed ICD. The original signed ICD should be filed in the investigator’s study file. The terms of the consent and when it was obtained will also be documented in the CRF and the medical records.

The written ICD and any other written information to be provided to subjects should be revised whenever important new information becomes available that may be relevant to the subject’s consent. Any revised written ICD, and written information should receive the independent ethics committee’s approval in advance of its use. The subject or the subject’s legally authorized representative should be informed in a timely manner if new information becomes available that may be relevant to the subject’s willingness to continue participation in the clinical study. The communication of this information should be documented.

11.3 Benefits and risks assessment.
Our retrospective analysis, supported by previous studies on fibrinogen concentrate in cardiac surgery, show that the benefits outweigh the risks associated with its use. The control of haemostasis and reduction of blood loss and transfusion is improved with fibrinogen concentrate, with reductions in morbidity, mortality and ICU stay. There are indication of an increased incidence of renal insufficiency and myocardial infarction however these data were not significant. Due to lack of RCT investigating the effects of fibrinogen concentrate in cardiac surgery, its effect in this domain remains unclear. With the RCT described in this protocol, the effect of fibrinogen concentrate infusion on blood loss in cardiac surgery, especially complex cardiac surgery, will be determined.

The use of Haemocomplettan in non-cardiac surgery is extensive for more than 20 years without apparent side effects. Therefore it is expected that current protocol will not lead to serious unexpected side-effects of the medication.

11.4 Compensation for injury
The investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO. Insurance will be provided by Marketform Insurance. See addendum,
12. **Administrative Aspects and Publication**

12.1 **Handling and storage of data and documents**
All subject names will be kept confidential. Subjects will be identified throughout documentation and evaluation by the number allotted to them during the study. The subjects will be told that all study findings will be handled in strictest confidence. The signed ICDs remain with the investigator. By signing the “signature page for investigators” of this CSP, the investigator agrees to obtain a correctly completed ICD for each subject included in the study. He also agrees to allow these to be inspected on request. The investigator will maintain a personal list of subject numbers and subject names (subject identification list) to enable records to be found at a later date. Subject numbers will be codes used for the studied subjects and are not the patient ID numbers used in the Isala Clinics. The handling of patient data complies with the Dutch Personal Data Protection Act (De Wet Bescherming Persoonsgegevens, Wbp).

12.2 **Amendments**
A substantial amendment is any written description of change(s) to or formal clarification of a CSP which may impact on the conduct of the clinical study, potential benefit of the clinical study, or may affect subject safety, including changes of study objectives, study design, subject population, sample sizes, study procedures, or significant administrative aspects.

A ‘substantial amendment’ is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:
- The safety or physical or mental integrity of the subjects of the trial;
- The scientific value of the trial;
- The conduct or management of the trial; or
- The quality or safety of any intervention used in the trial.
All substantial amendments will be notified to the METC and to the competent authority. The independent ethics committee or institutional review board, and the authority, will be informed of all substantial amendments. Prior to implementation, approval will be obtained from the independent ethics committee or institutional review board, and the authority, if necessary according to applicable regulations. A non-substantial amendment of a CSP includes minor correction or clarification that has no significant impact on the way the clinical study is to be conducted and no effect on subject safety (i.e. administrative changes like change of telephone number(s), logistical changes, etc).

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed. Any changes of the CSP (substantial amendments and non-substantial amendments) will be integrated into an updated CSP, if the changes are numerous or if required by applicable law. The independent ethics committee or institutional review board should be notified of all non-substantial amendments, if necessary according to applicable regulations.

12.3 **Annual progress report**
The principal investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/serious adverse reactions, other problems, and amendments.

12.4 **End of study report**
The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient’s last visit.
In case the study is ended prematurely, the investigator will notify within 15 days the accredited METC, including the reasons for the premature termination.

Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.5 Public disclosure and publication policy
CRF’s will be supplied by Athena Care. The bottom copy will be retained by the investigator.

All entries in the CRFs will be made legibly in blue ball-point pen (not pencil, felt tip, or fountain pen). All findings collected during the study will be entered by the investigator on these CRFs. If the investigator authorizes other persons to make entries on the CRF, the names, positions, signatures and initials of these persons will be written on the CRF.

All data required by the CSP should be adequately documented in source documents, e.g. hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical study.

Each page of the CRFs that contains any data will be initialled and dated by the person entering the data. The “AE / SAE” pages as well as the additional pages for SAEs have to be dated and signed by a study investigator. The completed set of CRFs will be reviewed, signed and dated by the investigator named in the CSP. CRFs will be completed immediately after the final examination. Arrangements will be made by the Monitor to collect the CRFs on completion.

A reasonable explanation will be given by the investigator or authorized staff for all missing data.

If corrections are made to entries in the CRF by the investigator or authorized staff, the words or figures will be crossed through, leaving the initial entry legible. The correction will then be dated and initialised. Incorrect entries will not be covered with correcting fluid, obliterated, or made illegible in any way.

12.6 Record retention
The medical records upon which the CRFs are based as well as the investigator's study file including all CRFs have to be kept according to legal requirements.
13. REFERENCES


Amendment 08-06-2011

METC nr: 10.0662  
CCMO nr: NL32188.075  
EUDRA-CT nr: 2009-018086-12  

Study: Efficacy and safety of Haemocomplettan® P in patients experiencing microvascular bleeding while undergoing elective complex cardiac surgery

1. **Page 2. DSMB: modification in participants**  
M.J.C Eijkemans, PhD. Academic staff, Associate professor of Biostatistics. Julius Center for Health Sciences and Primary Care. UMC Utrecht.  
L. Noyez, MD, PhD. Cardiothoracic Surgeon. Department of Cardiothoracic Surgery. Heart Center, Radboud University Nijmegen.  
J.W.A. Romijn, MD. Department of Anesthesiology. Institute for Cardiovascular Research, VU University Medical Center, Amsterdam.

2. **Page 2. Steering committee: modification in participants**  
Professor K.G.M. Moons, PhD. Professor of Clinical Epidemiology. Julius Center for Health Sciences and Primary Care. UMC Utrecht.  
Professor K.C.B. Roes, PhD. Professor of Biostatistics. Julius Center for Health Sciences and Primary Care. UMC Utrecht.  
J.W.M. Heemskerk, PhD. Professor of Biochemistry. Department of Biochemistry, CARIM. Maastricht University. MUMC, Maastricht.  
Marcus D. Lancé, MD. Anesthesiologist. University hospital Maastricht.

3. **Page 3. Inclusion criteria**  
Modification in text for clarity, adding “**combined**” in the sentence: Undergoing elective complex cardiac surgery (**combined** CABG and valve(s) or multiple valves or aortic root, aorta ascendens or aortic arch surgery).  
“**Dosing formula has a positive value**” added as inclusion criteria.

4. **Page 13. Study design: Change of study schedule for completion**  
Baseline examination (including signing of the Informed Consent Document (ICD)) of potential subjects will begin in **February 2011** and the data of the last included patient should be completed including follow-up at the end of **February 2012** (12 months).

5. **Page 14. Sample size calculation**  
Sample size is recalculated to 106 patients in total instead of 102.

6. **Page 16. Example added**  
“If for example, a study patient has a plasma fibrinogen concentration of 2.9 g/L, the formula becomes negative: (2.5-2.9) x 0.07 x (1-0.25) x 70= -1.47. This patient has enough fibrinogen concentration in plasma. Consequently this patient is excluded from the study en will not be randomized. A 5-minute bleeding test will also not be performed.”

Cealb of Sanquin is used instead of Baxter. Placebo remains same concentration of albumin.
8. **Page 19. Drug accountability**
   All supplies (Haemocomplettan P and placebo solution) will be accounted for throughout the study using the drug inventory log, which will be provided to the **Monitor** prior the first patient enrolled in the study.

9. **Page 21: Renal failure**
   - Recalculation of criteria reveals: serum creatinine >354µmol/L.

10. **Page 22. Additional variables**
    Addition of multiplate measurement: TRAP – platelet stimulation via the thrombin receptor (using TRAP-6), sensitive to IIbIIIa receptor antagonists.

11. **Page 23. Randomization**
    Addition of the following text: The patients will be randomized by the research-team using a computerized web-based randomization protocol designed by the Julius Center for Health Sciences and Primary Care. This protocol uses block randomization of 4 balanced outcomes per block (2x placebo and 2x IMP). The blocks themselves are also randomized. Based on the randomization result, the circulating anesthesia-nurse will either prepare a placebo solution or an active medication solution (IMP). The circulating anesthesia-nurse preparing the study medication is not involved in the operation with the study subject. The prepared syringes of both treatment groups are of the same size and colour. The fluid inside the bottle is of the same volume (50ml), same colour and viscosity. The bottles are labelled with the “study kit number”. This method ensues an adequate blinding of the research team (i.e. surgical team) for the allocated treatment.

12. **Page 24: Study blinding**
    The bottles with study medication will be labelled with the kit and study identification number before delivery to the operating room.
    The circulating anesthesia-nurse, who is not involved with the surgery of the study, will perform the measurements with ROTEM® and Multiplate®. All anesthesia-nurses are trained by the representatives of both POC monitors for correct use. The results of these POC monitoring devices are blinded for the operating team. Study unblinding will take place following closure of the study database.

13. **Page 24-25: Baseline on Day 0**
    - **Excluded in protocol:**
      Other coagulation (fibrinogen, APTT, PT)
      Biochemistry measurements will include AST, ALT, Ca, CK, CK-MB, creatinine, CRP, glucose, K, Na, pH, pCO2, pO2, troponin T, and urea.
    - **Included in protocol:** Multiplate® TRAP-test and Spijtserum

14. **Page 25-26: Removal of cardiopulmonary bypass**
    - Included in protocol: Multiplate® TRAP-test and Spijtserum
    - The 5-minute bleeding mass test is changed to 5 minute bleeding volume.

15. **Page 28: Replacement of individual subjects after withdrawal**
    Text replaced by: Randomisation of subjects will continue until at least 106 subjects provide the primary efficacy endpoint data.