Study Title:

The effect of integrating the biomarker Copeptin into the process of managing patients with suspected ACS

Acronym:

Biomarkers in Cardiology-8 Study (BIC-8)

Protocol

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Abbreviations

ACS	Acute coronary syndrome
ADH	Antidiuretic hormone
AE	Adverse Event
AMI	Acute Myocardial Infarction
ASAP	As soon as possible
CA	Coronary angiography
CC	Cardiac catheterization
CABG	Coronary arteries bypass grafting
CPU	Chest Pain Unit
ED	Emergency Department
SAE	Serious Adverse Event
ITT	Intent to Treat Population
MACE	Major adverse cardiovascular event
NSTEACS	Non-ST-elevation acute coronary syndrome
NSTEMI	Non-ST-elevation myocardial infarction
PCI	Percutaneous coronary intervention
POCT	Point-of-care testing
RCT	Randomized clinical trial
STEMI	ST-elevation myocardial infarction
TnI	Troponin I
TnT	Troponin T

1 Study Participants

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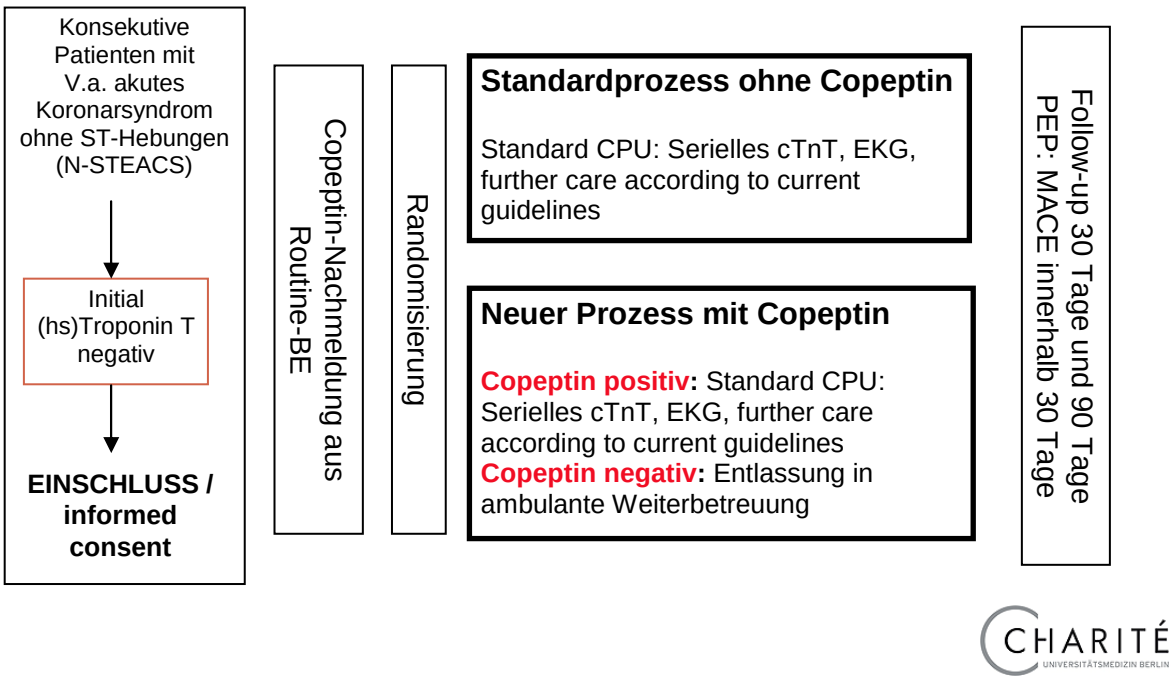
2 Synopsis

Study Title	***The effect of integrating the biomarker Copeptin into the process of managing patients with suspected ACS***
Sponsor and Principal Investigator	Prof. Dr. Martin Möckel Charité – Universitätsmedizin Berlin Augustenburger Platz 1 / 13353 Berlin Tel.: 030 450 553472 Fax: 030 450 7 553203 Email: martin.moeckel@charite.de
Objectives	To quantify the benefit of integrating the new biomarker Copeptin into the process of managing patients with suspected non ST-elevation acute coronary syndrome (N-STEACS) with a negative baseline Troponin test result in the Emergency Department. Hypothesis : Patients with suspected ACS with a negative baseline Troponin who test negative for Copeptin at admission are at low risk of ACS and can safely be discharged into ambulant care. Secondary hypotheses: Patients with suspected ACS with a negative baseline Troponin who test positive for Copeptin at admission are at risk for ACS and should be evaluated as by standard of care. The Integration of Copeptin into the CPU process will shorten the patient's length of Emergency Department/ CPU and hospital stay and will improve patient satisfaction.
Study Design	Multicentre, open, randomized, controlled clinical trial (RCT) Interventional diagnostic biomarker trial 1:1 Randomization in two study arms with differing process management: a) interventional/experimental arm (Integration of Copeptin) b) control/ standard arm (Copeptin not available to treating staff)
Number of Sites	3 sites in Germany, 1 site in Switzerland and 1 site in Austria
Experimental Diagnostic Process	Experimental Arm: Patients with the above described characteristics who test negative for Copeptin at admission will be considered low-risk and will be discharged into ambulant care without further interventions. Patients with the above described characteristics who test positive for Copeptin at admission will be evaluated as by standard of care according to current guidelines..
Standard Diagnostic	Control Arm:

Process	Copeptin result will not be revealed to the treating physicians. Patients will be evaluated as by standard of care according to current guidelines.
Gold Standard	DGK / ESC / AHA Guidelines for the management of patients with suspected Non-ST-elevation ACS (NSTEACS)
Time Schedule	Date of study / recruitment start: February 2011 Date of termination of recruitment: February 2012 Date of study termination: Atumn 2012
Total Number of Patients	n=446 per treatment group (total number of patients 892)
Study Population	Men and women of full age with suspected Non-ST-elevation acute coronary syndrome (NSTEACS) and negative baseline Troponin.
Inclusion Criteria	Admission to the Emergency Department with symptoms consistent with ACS: - Typical chest pain (with or without ECG-changes, but no ST-elevation) suggestive of unstable angina or non-ST-elevated myocardial infarction (NSTEMI) - Troponin negative at admission according to the current clinical practice Patient willing and able to give written informed consent
Exclusion Criteria	Patients with ST-elevation myocardial infarction (STEMI) Continuing chest pain or recurrent episodes of chest pain under therapy High-risk patients with suspected ACS who need to be hospitalized for reasons independent of their initial troponin result Patients who need to be hospitalized for other medical reasons Patients in need of urgent life-saving interventions Patients under 18 years of age Patients with a life expectancy < 6 months Patients with any condition that leads the treating physician to not consider the patient eligible for the trial
Study endpoints	Primary Endpoint: Rate of MACE (all- cause death or survived sudden cardiac arrest, myocardial infarction, re-hospitalisation for acute coronary syndrome, acute unplanned PCI, coronary artery bypass grafting (CABG) and documented life-threatening arrhythmias (VF, VT, AV-block III)) within 30 days Copeptin vs. Control group Secondary endpoints: Efficacy endpoint of rate of Patients in whom coronary angiography is performed Rate of Patients requiring PCI due to their findings in cardiac catheterization. Rate of bleeding events at 30 and 90 days defined as non-CABG intracranial, intraocular, or retroperitoneal bleeding; access-site hemorrhage requiring intervention or resulting in a ≥5 cm diameter hematoma; reduction in hemoglobin concentration of ≥4 g/dL without or ≥3 g/dL with an overt bleeding source; reoperation for bleeding; or blood product transfusion. Further secondary endpoints evaluate efficacy, safety, cost effectiveness and patient satisfaction of the new process including length of stay in the hospital
Safety	All major cardiac adverse events (MACE) as defined in the protocol and bleeding complications as defined in the protocol will be assessed and documented for the duration of the study.
Withdrawal Criteria	• Patients who are eligible for discharge from their cardiac situation but cannot be discharged for other medical reasons will be excluded from the trial • Patients who withdraw their consent will be excluded from the trial • Patients who violate the protocol will be excluded from the trial

3 Study-Design



4 Introduction

4.1 Introduction and background

The term “acute coronary syndrome (ACS)” summarizes a set of acute, life-threatening stages of coronary artery disease, namely acute myocardial infarction with ST-segment elevations in the ECG (STEMI), acute myocardial infarction without ST-segment elevations in the ECG (NSTEMI) and unstable angina (UA). The latter two are also referred to as Non-ST-elevation acute coronary syndrome (NSTEACS). Cardinal symptom of ACS is acute chest-pain which, together with the patient’s medical history and nature of physical findings leads to the first diagnosis of “suspected acute coronary syndrome” with admission to Emergency Departments (EDs) and Chest Pain Units (CPUs). Despite the general knowledge that patients with ACS present themselves to the ED with chest pain, epidemiological data reveals that only about 50% of patients with chest pain do suffer from ACS, the rest of these patients are diagnosed with other cardiac or extra-cardiac, e.g. gastro-oesophageal, musculoskeletal, pleura-pulmonal, mediastinal or psychogenic conditions [1]. Further evaluation and decision-making in these patients largely depends on their Troponin blood level as it identifies high risk patients. Myocardial infarction has recently been defined in an expert consensus paper by the ESC/ACCF/AHA/WHF task force for the redefinition of myocardial infarction [2]. The diagnosis is based on a rise or fall of cardiac biomarkers together with either symptoms of ischemia, ECG-changes or imaging evidence of new loss of viable myocardium. The preferred biomarker is Troponin I or T drawn on first assessment

and 6-9 hours later. Thus, patients with a positive Troponin at baseline belong to a well defined patient group which can be treated according to standard guidelines. Unfortunately, in a population of patients with suspected acute coronary syndrome less than a quarter of the patients is tested positive for Troponin at presentation to the Emergency Department. In a chest pain evaluation by Newby et al. 1005 patients without ST-segment elevation in 6 chest pain units were tested for various cardiac biomarkers. At baseline, 13.7% of patients were positive for Troponin I, after serial testing this number grew to 26% [3]. In a trial performed by our site we enrolled 429 patients with suspected acute coronary syndrome of which 23.8% were initially Troponin I positive, including patients with ST-segment elevation [1]. In both trials the majority of patients were Troponin negative. It is one of the big challenges in Emergency Medicine to decide on whether a Troponin negative chest pain patient should be admitted to hospital for urgent catheterization, admitted to hospital for further evaluation and possibly later cardiac catheterization or should be discharged home for further ambulatory evaluation and care.
Pope et al. assessed the incidence of missed diagnoses of acute cardiac ischemia in the Emergency Department by analysing data from 10.689 patients with chest pain suggestive of acute coronary syndrome. 2.1 % of patients with acute myocardial infarction and 2.3% of patients with unstable angina were mistakenly discharged from the ED [4]. Given the huge impact this may have on the patient's life this is an unacceptably high number. On the other hand, fear of this potentially fatal error means that a large number of patients are being classified as suspected acute coronary syndrome and are therefore admitted to hospital for serial biomarker testing, monitoring and initialisation of medical treatment despite the absence of an acute illness. These patients are put at an unnecessary risk of side effects from the antithrombotic and anticoagulant medication which they receive as standard care for the duration of their ACS evaluation.
A new cardiac biomarker that provides "rule-out" information in a sense that patients at low risk can be identified straight after admission to the Emergency Department and can safely be transferred to ambulant care is highly sought after.

4.2 Rationale

Copeptin, a 39 amino acid glycopeptide, is the C-terminal portion of Pro-Vasopressin. It is co-secreted from the posterior pituitary gland together with Vasopressin and mirrors the amount of Vasopressin in the circulation. Vasopressin is primarily known as Anti-Diuretic Hormone (ADH), which acts in the kidney to regulate the body's retention of water and in high concentration causes arterial vasoconstriction.
Vasopressin is, as a central hormone, also a crucial part of the hypothalamo-pituitary-adrenal axis, which responds to severe, life-threatening "stress inputs"; its levels reflect the body's individual stress level [5 6].
Vasopressin itself has a half-life of 5-10 minutes and is therefore difficult to measure in-vivo. Copeptin is secreted stoichiometrically with Vasopressin, it remains stable for days after blood withdrawal and can therefore easily be measured [7]. Copeptin has been studied as a diagnostic and prognostic marker since 2006. In acute myocardial infarction Copeptin levels have been shown to increase early after the onset of symptoms (0-4 hours) and start decreasing after 4-5 hours [8]. In acute myocardial infarction two trials have investigated the prognostic value of Copeptin. Khan et al. assessed the value of Copeptin and NT-proBNP for the prediction of death and heart failure after 60 days in 980 patients with acute myocardial

infarction. Both biomarkers predicted outcome independently with an area under the curve of 0.75 for Copeptin and 0.76 for NT-proBNP. Copeptin plus NT-proBNP assessment had the highest predictive value with an AUC of 0.84 [7]. In a similar trial Voors et al. found Copeptin to have an even stronger predictive value than NT-proBNP and BNP in a study on 224 patients assessed 3 days after their acute myocardial infarction [9].
The first trial assessing the diagnostic value of Copeptin in acute myocardial infarction was conducted by Reichlin et al. They enrolled 487 patients in the Emergency Department with symptoms suggestive of myocardial infarction to measure Copeptin at presentation. Of these 487 patients 17% had a final diagnosis of myocardial infarction (37% STEMI and 63% NSTEMI), 17% of unstable angina, 13% of cardiac symptoms due to other cardiac causes, 43% had a non-cardiac disease and in 11% the symptoms were of unknown origin. Copeptin levels in this study were significantly higher in patients with acute myocardial infarction than in patients with other diagnoses (median 20.8 pmol/L vs. 6.0 pmol/L, $p<0.001$). Copeptin significantly increased the diagnostic accuracy of Troponin from an area under the receiver operating curve (AUC) of 0.86 for Troponin T alone to 0.97 in a dual marker approach of Troponin T plus Copeptin which demonstrates that due to their different pathomechanisms Troponin, a marker of cardiac injury and Copeptin, a marker of stress, both identify patients that the other does not or to a lesser extend.
Further analysis by Reichlin et al. revealed that Copeptin in conjunction with Troponin T was particularly useful as a rule-out marker in acute myocardial infarction (AMI). TnT below 0.01 µg/L plus Copeptin below 14 pmol/L ruled out AMI at presentation with a sensitivity of 98.8% and a specificity of 77.1% (negative predictive value 99.7%) [8].
The most recent publication on Copeptin in acute myocardial infarction by Keller et al. validated these data. They tested the early diagnostic value of Copeptin, in addition to Troponin, in 1293 patients of which 244 had a discharge diagnosis of acute myocardial infarction and 211 of unstable angina. In this trial the combined measurement of Troponin and Copeptin again improved the identification of patients with AMI and provided a negative predictive value of 94.8 % (Copeptin cut-off 13 pmol/l), emphasising the value for a safe-rule out of AMI [10].
Copeptin is an extremely promising new cardiac biomarker that has the potential to improve the process of identifying high and low risk patients with suspected acute coronary syndrome. It is an optimal complement to Troponin regarding both pathomechanism and timing of elevation. Both markers combined could accelerate further patient management by bypassing the time that is required for serial Troponin testing and further diagnostic evaluation.

5 Study objectives

This is a multicentre biomarker randomized controlled trial to quantify the benefit of integrating the new biomarker Copeptin into the process of managing patients with suspected non ST-elevation acute coronary syndrome (N-STEACS) with a negative baseline Troponin I test result in the Emergency Department and Chest Pain Unit.
Hypothesis:
Patients with suspected ACS with a negative baseline Troponin who test negative for Copeptin at admission are at low risk of ACS and can safely be discharged into ambulant

care. These patients will not experience a higher rate of MACE within 30 days after their admission than patients who are managed by standard practise of care.
Secondary hypotheses:
Patients with suspected ACS with a negative baseline Troponin T who test positive for Copeptin at admission are at risk for ACS and should be evaluated as by standard of care.
The Integration of Copeptin into the CPU process will shorten the patient's length of Emergency Department/ CPU and hospital stay and will improve patient satisfaction

5.1 Primary endpoint

Rate of MACE (all- cause death or survived sudden cardiac arrest, myocardial infarction, re-hospitalisation for acute coronary syndrome, acute unplanned PCI, coronary artery bypass grafting (CABG) and documented life-threatening arrhythmias (VF, VT, AV-block III)) within 30 days Copeptin vs. Control group

5.2 Secondary endpoints

5.2.1 Secondary efficacy endpoints
Rate of Patients in whom coronary angiography is performed
Rate of Patients with PCI after Index CA
Rate of Patients with CABG after Index CA
Other cardiovascular diagnoses found in index CA
5.2.2 Secondary safety endpoints
Safety endpoint of all major adverse cardiac events (MACE) defined as all- cause death or survived sudden cardiac arrest, myocardial infarction, re-hospitalisation for acute coronary syndrome, acute unplanned PCI, coronary artery bypass grafting (CABG) and documented life-threatening arrhythmias (VF, VT, AV-block III)at 30 days.
MACE at 90 days
All-cause death at 30 days
Re-hospitalisation for suspected acute coronary syndrome at 30 days
Acute Myocardial Infarction (AMI)
Acute unplanned PCI
CABG
Documented life-threatening arrhythmias or survived sudden cardiac arrest
Rate of bleeding events defined as non-CABG intracranial, intraocular, or retroperitoneal bleeding; access-site hemorrhage requiring intervention or resulting in a ≥5 cm diameter hematoma; reduction in hemoglobin concentration of ≥4 g/dL without or ≥3 g/dL with an overt bleeding source; reoperation for bleeding; or blood product transfusion.
5.2.3 Patient satisfaction endpoints
Patient satisfaction at discharge from ED/CPU
5.2.4 Health economy endpoints
Length of stay at the Emergency Room
Length of hospital stay in the CPU
Length of stay in an intensive care unit (ICU)
Total length of hospital stay including time as an inpatient on other wards
5.2.5 Predefined subgroup analysis
Male/female
GRACE-Score strata

Age strata

5.3 Endpoints Committee

The Endpoints Committee will be composed of cardiologists and ED physicians.
This Committee is responsible for assessing the primary and secondary efficacy endpoints.

5.4 Study design

National, multi-centre, prospective open randomized controlled clinical trial. The treating physicians will be blinded to the test result of the investigational marker for the control group.

5.5 Timetable

Start of recruitment / Initiation

- February 2011

Estimated trial duration:

- 12/18 months

Recruitment will stop at each site when the proposed sample size has been reached.

6 Study population

Eligible men and women of full age, who are patients of the Emergency Departments and Chest Pain Units of the participating sites, will be enrolled in this trial. There will be no specific gender distribution as gender specific differences concerning efficacy and safety of the investigational diagnostic process are not to be expected.

6.1 Inclusion criteria

- Admission to the Emergency Department with symptoms consistent with ACS:
  - Typical chest pain (with or without ECG-changes, but no ST-elevation) suggestive of unstable angina or non-ST-elevated myocardial infarction (NSTEMI)
  - Cardiac Troponin negative at admission according to the current clinical practice
- Patient willing and able to give written informed consent

6.2 Exclusion criteria

- Patients with ST-elevation myocardial infarction (STEMI)
- Continuing chest pain or recurrent episodes of chest pain under appropriate therapy
- High-risk patients with suspected ACS who need to be hospitalized for reasons independent of their initial Troponin result
- Patients who need to be hospitalized for other medical reasons
- Patients in need of urgent life-saving interventions
- Patients under 18 years of age
- Patients with a life expectancy < 6 months
- Patients with any condition that leads the treating physician to not consider the patient eligible for the trial

6.3 Justification for the inclusion of minors and persons unable to consent (if applicable)

Not applicable

7 Description of the investigational process

See also BIC-8 Flowcharts (attachment 1)

7.1 General Patient Flow

Consecutive N-STEACS patients of the Chest Pain Unit with a negative Troponin T at admission will be invited to participate. Troponin T is tested as part of the standard management of patients with suspected acute coronary syndrome. Patients who give their written informed consent will be randomized into one of two study arms (experimental and standard management).

Experimental Arm: Further management dependent on Copeptin result

1. Patients of the above described characteristics who test **negative for Copeptin** at admission will be considered low-risk. In these patients we abstain from further ACS evaluation and instead discharge them into ambulant care. Before discharge patients will have a final visit to secure their well-being. The visit includes a physical examination, an ECG and an assessment for MACE and bleeding events.
Patients who show any signs and symptoms of an acute illness requiring in-hospital treatment will not be discharged (see withdrawal criteria 5.3). This decision is at the discretion of the attending physician.
To secure the patients safety they will be discharged into each co-operating network of resident cardiologists . Patients are to see a cardiologist within a maximum of 3 days after discharge (if discharge falls on a Friday), preferably on the next day after discharge. Further appointments will be at the discretion of the resident cardiologist (see 7.2).

2. Patients with the above described characteristics who test **positive for Copeptin** at admission will be managed as by standard practice i.e. patients will be evaluated as by standard of care abiding current guidelines for the management of patients with suspected ACS

7.2 Safety assurance: Praxis-connect

Praxis connect is an internet-based portal for the management of patient referrals between hospitals and private practices.
It can be used for exchanging patient data between different hospitals and private practises using an encoded SSL-connection between the clinical institutions and a data processing centre, securing data privacy.

Through Praxis connect the Emergency Department physicians can directly book appointments into co-operating cardiologist's appointment calendars. Additionally they can grant the cardiologist access to important patient documents.
This system will be used at Charité study sites and can be offered to the other centers if necessary.

7.3 Description of investigational diagnostic test / Cut-off

Copeptin will be measured using the homogeneous sandwich fluoro-immunoassay B·R·A·H·M·S Copeptin KryptorPlus. The test has a detection limit of 0.5 pmol/L and a functional assay sensitivity (detected by inter-assay precision of 20% CV) of < 2 pmol/L. Test results are available after 14 minutes of incubation. In contrast to mature Vasopressin, Copeptin is stable in all matrices at room temperature for at least 7 days and at 4°C for at least 14 days. The assay therefore offers considerable advantages for the exact measurement of the Vasopressin production. Reichlin et al. used a Copeptin cut-off level of14 pmol/L. Copeptin levels below this cut-off in combination with a troponin T level < 0.01 µg/L correctly ruled out acute myocardial infarction with a sensitivity of 98.8% and a negative predictive value of 99.7% [8]. To assure patient safety, we will use a cut-off level of 10 pmol/l to decide between negative and positive test results. In the Gutenberg Heart study Copeptin testing in 5000 individuals revealed a 95th percentile for Copeptin of 9.8 pmol/L. In the Copeptin trial by Keller et al., the highest sensitivity (88.2%) and NPV (94.6%) were achieved with 9.8 pmol/L as the cut-off [10]. The excellent negative predictive value at this cut-point is the basis for the safety of patients who are discharged based on their Copeptin levels.

7.4 Description of the standard diagnostic tests /Cutoff

Troponin I or T or high-sensitive Troponin T will be tested as by standard practice of care at the individual study sites. The initial Troponin test can be performed on Point-of-care devices to assure timely randomization.
Standard Troponin T will be considered positive at a level of or above 0.03µg/L.
High sensitive Troponin T will be considered positive at a cut-off level of above 0.014 µg/L

7.5 Standard process

Copeptin results will not be revealed to the treating physicians. Patients will be managed as by standard practice i.e. patients will be evaluated as by standard of care abiding current guidelines for the management of patients with suspected ACS.

7.6 Procedures in case of emergency

The Emergency Departments of the study sites are available on 7 days for 24 hours. All physicians of the Emergency Departments will be informed of the trial and will re-admit study patients to the ED if necessary.

8 Study procedures

See also BIC-8 Flowcharts

8.1 Recruitment / screening procedures

Screening:

The screening for eligible patients will take place in the Emergency Departments with adjacent CPU of the 3 study centers.

All patients with suspected acute coronary syndrome must be screened for eligibility and listed in a screening-log, stating their enrolment in the trial or reasons why a patient was not enrolled. Unless patients are enrolled in the trial this list will only contain the patients age, gender, Troponin result and reason(s) for not enrolling.

The screening process involves a check of the patient's medical history, the results of his/her physical examination , an ECG and a routine blood draw including the routine Troponin test on a Point-of-care-(POCT) device to be able to assess the patient for the fulfillment of the **Inclusion and Exclusion Criteria (see 6.1 and 6.2).**

8.2 Informed consent

The investigator ensures that each study subject is provided with full oral and written information about the nature, purpose, expected advantages and possible risks of the study, has been given an opportunity to ask questions about the study and the involvement and has received answers to all such questions. Further, the investigator ensures that no patient will be enrolled into the study without prior obtaining of written informed consent which is to be dated and signed by the patient and by the investigator or sub-investigator who provided the information. The final versions of patient information and consent will be presented to the Ethics Committee. Both the patient information and the patient consent form are prepared in duplicate. One of each form for the Investigator, a duplicate will be handed to the patient.

8.3 Copeptin testing

Copeptin will be requested from the laboratory to be tested in the surplus routine blood samples after the informed consent has been signed.

8.4 Enrolment and randomization

Patients will be assigned to their study group by randomization via sealed envelopes as soon as the written informed consent has been signed.

The Copeptin test result will not automatically be communicated to the Emergency Department but will need to be requested via telephone after the randomization. It will only be released for patients in the experimental group. To assure this, the randomization letter for patients in the experimental group will contain a code which will be noted down in the central laboratory before releasing the Copeptin value.

The code can be compared with the code in the patient's randomization letter to proof adherence to this process.

In patients who are randomized to the control group, the Copeptin result will only be documented at the laboratory and will not be available to the investigators during the recruitment period.

8.5 Registration

Patients, who meet all inclusion criteria, who have given their written informed consent and have been randomized will be reported to the trial centre via Fax.:

- BIC-Trials
- 0049 (0)30 450 7 553 307

The following data are to be included in this registration FAX (attachment 6)

- Study site, Investigator
- Pseudonym of patient
- Gender
- Randomization group

The trial centre will regularly (1x/week) update the members of the steering committee on the numbers and basic characteristics (s. above) of patients enrolled.

8.6 Clinical processes

Procedure	Screening	Enrolment	Randomization	Discharge	30d	90d
Screening Log	x					
Medical history	x					
Physical exam.	x					
Routine -ECG	x					
Blood routine*	x					
Copeptin		x**				
Informed consent		x				
Copeptin result request			(x) ***			
Questionnaire contentedness				x		
MACE and bleeding event assessment				x	x	x

- Icluding Troponin and CK

**request from routine blood sample from screening period

*** only for patients in experimental group

All in-hospital examinations are standard practice of care in patients with suspected ACS. The deviations concern the process of patient management – early discharge of Copeptin negative patients and further evaluation of high-risk ACS of Copeptin positive patients - rather than in-hospital examinations. All CPU patients are under constant monitoring and risk assessment.

8.7 Follow-up

All patients will have a 30-day and a 90-day follow-up. Both follow-ups are telephone visits to assess:

- Survival
- MACE and bleeding complications

In case of death or hospitalization during the study period medical records will be requested. The patient will consent to this in the informed consent form.

8.8 Individual trial duration

Patients will be considered study-patients until the 90 day telephone visit has been completed.

8.9 Additional 3 hour blood test

Patients can, on an optional basis, agree to an additional, study-related blood draw at 3 hours after their initial admission. This blood sample will be used to further evaluate the kinetics of Copeptin. Additionally, Troponin will be measured to compare the two markers in these samples.
Patients who agree to the blood draw have to give their separate written consent in a special box of the informed consent form (s. attachment).

9 Risk-benefit-assessment

Copeptin negative patients might experience major adverse cardiac events after discharge, which could have been prevented in case of hospital admission, due to unrecognized acute coronary syndrome.
Precaution:
Patients for this trial are very carefully selected. Patients with concomitant diseases or conditions which require a hospitalisation as well as patients with instable angina despite maximal therapy are excluded from participation. Thus, only stable, symptom-free patients will be discharged. We cannot exclude that of these patients, some will be at risk for ACS-related complications. As a precaution, patients will only be discharged after an appointment with one of our co-operating resident cardiologists has been made via the praxis-connect system as explained in section 7.1. Thus, the onward specialist care of these patients is guaranteed. The co-operating cardiologists will be well informed and trained in all trial-matters. All patients will be re-admitted to the Emergency Department upon any suspicion of risk for complications.

Copeptin positive patients might be regarded as high-risk patients and receive more intensive treatment.
Precaution:
Management of all patients will be closely monitored by the principal investigator of each site to assure suitable care and therapy.

Potential advantages for patients in the experimental arm:
Copeptin negative patients bypass further ACS evaluation and are prevented from hospitalization. Patients might be spared an unnecessary hospital admission and antithrombotic and anticoagulant treatment with their related hazards and side-effects. Patients will profit from the personal care by the resident co-operating cardiologist.

All patients will profit from the close and intense monitoring and follow-up by an experienced clinical research team.

Potential advantages for the general public:

If the new processes are shown to be efficient, safe, cost effective and/or to improve patient contentedness this trial is a great benefit to the general public.

Additional 3 hour blood draw:
The 3 hour additional blood draw is a study related blood draw. If a venous catheter is in place, it can be used to draw the blood for this sample. Otherwise a new venous puncture is necessary which holds the small risk of pain at the puncture site, local nerve irritation, hematoma and very rarely vasovagal collapse. The risk will be minimized by putting well trained staff in charge of the blood draws.

10 Termination and subsequent treatment

10.1 Premature termination of the individual participant

- Patients who are eligible for discharge from their cardiac situation but cannot be discharged for other medical reasons will be excluded from the trial
- Patients who withdraw their consent will be excluded from the trial
- Patients who violate the protocol will be excluded from the trial

10.2 Premature termination of the clinical study

- Unjustifiable risk in risk-benefit analysis (decision taken by principal Investigator)
- New scientific evidence provided during the study that could affect the patient's safety (benefit-risk analysis no longer positive)

10.3 Follow-up and continuing treatment after regular / premature termination

All patients who have not been excluded from participation will be contacted via telephone for a 30 day follow-up to assess MACE and bleeding events.
All patients will be contacted via telephone after 90 days to assess MACE and bleeding events. All efforts should be made to contact all patients for the 90 day follow-up telephone visit, if the consent to do so is granted.

11 Assessment of MACE and bleeding events

11.1 Definition

Major adverse cardiac event (MACE) is defined as

- All- cause death or survived sudden cardiac arrest
- myocardial infarction
- Re-hospitalisation for acute coronary syndrome
- Acute unplanned PCI
- Coronary artery bypass grafting (CABG)
- Documented life-threatening arrhythmias (VF, VT, AV-block III)

Major bleeding is defined as

- Non-CABG intracranial, intraocular, or retroperitoneal bleeding
- Access-site hemorrhage requiring intervention or resulting in a ≥5 cm diameter hematoma
- Reduction in hemoglobin concentration of ≥4 g/dL without or ≥3 g/dL with an overt bleeding source
- Reoperation for bleeding
- Blood product transfusion

11.2 Documentation of MACE and major bleeding events

All MACE and major bleeding events will be documented, no matter if the Investigator suspects a causal connection to the investigational process or not.
The event will be recorded in the CRF.

11.3 Reporting of MACE and major bleeding events

The Investigator will report any MACE or major bleeding event within 1 week after his/her knowledge of the event to the Sponsor. This announcement will be done via telephone or fax to:
BIC-Trials
Tel.: 0049 (0)30 450 665406
Fax: 0049 (0)30 450 7 553 307

The Investigator will write a written record describing the event, its cause, treatment and outcome.

Exceptional rules:
In this clinical trial the following Serious Adverse Events are excluded from the notification requirement:

- Severe or unexpected events which occur after enrolment, but before the experimental process was initiated

11.4 Investigator Site File (ISF)

All essential documents will be kept in the Investigator Site File which will be stored at the study site in accordance with ICH GCP.

12 Quality management

12.1 Control of trial progress and data quality

1. Quality Assurance (QA) is maintained to meet practice standards required by the ethics committee.

2. Identification of Deficiencies- QA standards are set to insure that patient safety and protocol mandated procedures are being met, and that any variances are identified, reported to the responsible committees and investigators for remedial action.

3. Corrective Action Plan- A plan to correct for deficiencies will be initiated when such deficiencies are deemed of a severity requiring remediation. Such an action plan will be governed by the guidelines of the QA practice standards maintained as stated in section 1.

12.1.1 Monitoring

The sponsor will contact the sites prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to satisfy regulatory and ethical, requirements. When reviewing data collection procedures, the discussion will include identification, agreement and documentation of data items for which the CRF will serve as the source document.
The sponsor will monitor the study to ensure that the:
• Data are authentic, accurate, and complete.
• Safety and rights of subjects are being protected.
• Study is conducted in accordance with the currently approved protocol and any other study agreements, GCP, and all applicable regulatory requirements.
The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

12.1.2 Audits / inspections

At its discretion, the sponsor may conduct a quality assurance audit of this trial. If such an audit occurs, the investigator agrees to allow the auditor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor to discuss findings and any relevant issues. In addition, regulatory agencies may conduct a regulatory inspection of this trial. If such an inspection occurs, the investigator agrees to allow the inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the inspector to discuss findings and any relevant issues.

13 Data entry und data management

All patient related data will be recorded under a pseudonym. Every patient will receive a patient number / pseudonym which will be unique for this individual patient. The Investigator will compile a confidential list, which relates these patient numbers to the patient's full name. This list will only be accessible to the study team and the monitor. Original patient files may be viewed by monitors, auditors and inspectors.

13.1 Data collection / documentation form

All data will be recorded in an electronic Case Report Form.
All data will be recorded online and will be transmitted directly to the database of the study centre. Data will be transferred between the workstation computer at the study site and the study server via a secure connection (secure socket layer /SSL) so that the data cannot be manipulated.

13.2 Data processing

The Sponsor will file all data electronically. To verify accuracy of the data, range, validity and consistency checks will be performed. Implausible or missing data can be corrected or added after consulting the Investigator. Documentation for these corrections will be stored with the CRFs.
All validated data will be stored in the eCRF. After termination of the study and after completion of all entries, the database will be closed (frozen) for further entries. This process will be documented.

13.3 Generation of pseudonym

All patient data will be saved under a pseudonym. The study software will automatically generate a pseudonym for every new patient/the pseudonym for new patients can be typed into the study software. A document for clear patient allocation will be printed and must be kept in a safe place.

14 Statistical Analysis

14.1 Sample size estimation

See appendix 2

14.2 Statistical analysis

14.2.1 Definition of population for analysis

First a check of the success of randomisation will be conducted: Bivariate comparisons of basic demographics and baseline risk-markers between experimental group and control group .
Exact Fisher tests will be conducted for comparison of proportions of the main outcome in case of a successful randomisation. But logistic regression analysis will also be employed to adjust for potential confounding.
If more than 10% of patients are lost during follow-up, additional intention to treat analyses will be conducted.

14.2.2 Analysis of endpoint

Safety of discharging patients with negative Troponin and negative Copeptin at baseline We chose a primary safety endpoint to prove that discharge of these patients does not increase their mortality or their risk for cardiac events like survived sudden cardiac arrest, myocardial infarction, re-hospitalisation for acute coronary syndrome, acute unplanned PCI, coronary artery bypass grafting (CABG) and documented life-threatening arrhythmias (VF, VT, AV-block III). Möckel et al. saw a 6 week MACE rate of 6.4% in 266 patients with a serial negative Troponin result, who were evaluated for an acute coronary syndrome and a 7.8% MACE rate in all patients who were sent home after judgment of the attending physician.

14.2.3 Methods against Bias

Selection bias should be minimized by inviting ALL consecutive patients in all three centers who fulfill the stringent inclusion / exclusion criteria. A volunteer bias seems unlikely in this context (no completely new highly advertised treatment or similar).
Confounding bias for known and unknown confounders should be minimized by a successful randomization. Additionally, even if the randomization proved to be successful, the main findings (see hypotheses) will in any case be validated by multivariate logistic regression modeling adjusting for all potential confounders (demographics, risk factors and risk markers).
Information bias will be minimized by standardized testing procedures for biomarkers and by training of the involved centers for standardization of management protocols. Blinding cannot be performed on the patient side because the management procedures of the arms tested differ; the same is true for the treating physician. However the major outcomes are "hard" data and it is hard to imagine information bias in the form of measurement bias may occur here. Additionally, any conflict of interest from treating physicians will be excluded.

15 Reporting

15.1 Statistical report

The statistical analysis and composition of a biometrical report will be performed by Prof. Reinhold Müller in cooperation with the Sponsor and Principal Investigator. All data in this report is confidential.

15.2 Final report

The composition of a final integrated report will be conducted.

15.3 Publication (policy)

The study results will be published irrespective of the study outcome.

16 Ethical, legal and regulatory aspects

16.1 Legal requirements of the study

- **Approval of Ethics Committee**

Prior to patient enrolment each principal investigator ensures that the protocol and the patient information and consent form will be reviewed by an appropriate Institutional Review Board ("IRB") / Ethics Committee ("EC"). If during the study, it is necessary to amend either the protocol or the Informed Consent form the investigator will be responsible for IRB/EC review once again.

- **Patient insurance**

Patient insurance will be covered by the employer's liability insurance. Each site will provide a written statement of the respective insurance to guarantee insurance coverage.

- **Data protection**

The participants' data will be saved in a pseudonymous form, which will neither contain initials nor full date of birth. All regulative requirements applying to data protection will be met. Re-identification of a participant subject's name is possible from the patient identification log, which is kept in a locked research office at the Trial site where access is only possible by the principal Investigator or persons authorised by the principal Investigator.
Patients will be informed that their disease-related data will be saved for scientific purpose (Publication, etc.) using a pseudonym. Consenting patients have got the right to be informed about the data recorded.

16.2 Archiving of data / access to records

Originals of all study-related report forms will be stored in the study headquarters at the trial site for at least 10 years after completion of the trial.
The Principle Investigator will store all administrative documents (correspondence with the Ethics Committee, the Supervising Authority, trial centre, study site), patient identification log, the signed patient consent forms, copies of the data documentation form and common study documentation (protocol, amendments) for the duration mentioned above. Original data of study patients (medical records) will be stored for at least 10 years.
A list allowing patient identification will be kept for 15 years.

17 References

1 Mockel M, Muller R, Vollert JO, et al. Lipoprotein-associated phospholipase A2 for early risk stratification in patients with suspected acute coronary syndrome: a multi-marker approach: the North Wuerttemberg and Berlin Infarction Study-II (NOBIS-II). *Clin Res Cardiol* 2007 Sep;**96**(9):604-12.

2 Thygesen K, Alpert JS, White HD. Universal definition of myocardial infarction. *Eur Heart J* 2007 Oct;**28**(20):2525-38.

3 Newby LK, Storrow AB, Gibler WB, et al. Bedside multimarker testing for risk stratification in chest pain units: The chest pain evaluation by creatine kinase-MB, myoglobin, and troponin I (CHECKMATE) study. *Circulation* 2001 Apr 10;**103**(14):1832-7.

4 Pope JH, Aufderheide TP, Ruthazer R, et al. Missed diagnoses of acute cardiac ischemia in the emergency department. *N Engl J Med* 2000 Apr 20;**342**(16):1163-70.

5 Itoi K, Jiang YQ, Iwasaki Y, et al. Regulatory mechanisms of corticotropin-releasing hormone and vasopressin gene expression in the hypothalamus. *J Neuroendocrinol* 2004 Apr;**16**(4):348-55.

6 Katan M, Morgenthaler N, Widmer I, et al. Copeptin, a stable peptide derived from the vasopressin precursor, correlates with the individual stress level. *Neuro Endocrinol Lett* 2008 Jun;**29**(3):341-6.

7 Khan SQ, Dhillon OS, O'Brien RJ, et al. C-terminal provasopressin (copeptin) as a novel and prognostic marker in acute myocardial infarction: Leicester Acute Myocardial Infarction Peptide (LAMP) study. *Circulation* 2007 Apr 24;**115**(16):2103-10.

8 Reichlin T, Hochholzer W, Stelzig C, et al. Incremental value of copeptin for rapid rule out of acute myocardial infarction. *J Am Coll Cardiol* 2009 Jun 30;**54**(1):60-8.

9 Voors AA, von HS, Anker SD, et al. C-terminal provasopressin (copeptin) is a strong prognostic marker in patients with heart failure after an acute myocardial infarction: results from the OPTIMAAL study. *Eur Heart J* 2009 May;**30**(10):1187-94.

10 Keller T, Tzikas S, Zeller T, et al. Copeptin improves early diagnosis of acute myocardial infarction. *J Am Coll Cardiol* 2010 May 11;**55**(19):2096-106.

11 Morgenthaler NG, Struck J, Alonso C, et al. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. *Clin Chem* 2006 Jan;**52**(1):112-9.

18 Appendices

Study Flowcharts
Power calculation