CLINICAL STUDY PROTOCOL

Study Title:	Impact of EMpagliflozin on cardiac function and biomarkers of heart failure in patients with acute MYocardial infarction (EMMY-Trial) – a phase III Study
Protocol Number:	HS-2017-01
Product:	Empagliflozin
Sponsor:	Medical University of Graz
Protocol Date:	17 October 2022
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Ethics number:	29-179 ex 16/17
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Financial support: Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria

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Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the investigator team, regulatory authorities and members of the Research Ethics Committee.

Trials Sponsor: Medical University of Graz, Auenbruggerplatz 2-4, 8036 Graz

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Abbreviations

3-point MACE	Major acute cardiovascular events (cardiovascular death, non-fatal myocardial infarction, non-fatal stroke)
ACR	Albumin/Creatinine Ratio
ADA	American Diabetes Association
AESI	Adverse events of Special Interest
ALAT	Alanine-Aminotransferase
ASAT	Aspartate-Aminotransferase
BASG	Bundesamt für Sicherheit im Gesundheitswesen
BMI	Body mass index
CHF	Congestive Heart Failure
CLTR	Department for Clinical Trials
CRF	Case Report Form
DKA	Diabetic Ketoacidosis
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eGFR	Estimated Glomerular Filtration Rate
EMEA	European Medicine Agency
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FDA	Food and Drug Administration
FPFV	First Patient First Visit
GCP	Good Clinical Practice
GGT	Gamma-Glutamyl-Transferase
HDL	High Density Lipoprotein
IMI	Institute of Medical Informatics, Statistics and Documentation
ISF	Investigator Site File
INS	Institute Surveillance
KKS	Koordinierungszentrum für Klinische Studien
LBBB	Left bundle branch block
LDL	Low Density Lipoprotein
LPLV	Last Patient Last Visit

MCI	Myocardial Infarction
nt-proBNP	N-terminal pro b-type natriuretic peptide
NYHA	New York Heart Association
pH	Pondus Hydrogenii
RR	Riva Rocci Blood Pressure Measurement
SGLT-2	Sodium-dependent glucose cotransporter 2
SUSAR	Suspected Unexpected Serious Adverse Reaction
T2DM	Type 2 Diabetes Mellitus
TAPSE	Tricuspid Annular Plane Systolic Excursion
TMF	Trial Master File
TMG	Trial Management Group
TSC	Trial Steering Committee
UKPDS	United Kingdom Prospective Study
ULN	Upper Limit of Normal
UTI	Urinary Tract Infection
WMSI	Wall Motion Score Index

Synopsis

Sponsor	Medical University of Graz Auenbruggerplatz 2-4 8036 Graz, Austria
Principal Investigators	Harald Sourij, MD, Associate Professor Dirk von Lewinski, MD, Associate Professor
Indication	Patients with acute myocardial infarction
Study design and phase	Randomized, prospective, placebo-controlled, double blind, multicenter study in patients with acute myocardial infarction – a Phase III study.
Study Short Title	Empagliflozin in acute myocardial infarction
Keyword	SGLT-2 inhibition, acute myocardial infarction, heart failure
Aims of the trial	*Primary objective:* The primary objective is to investigate the impact of Empagliflozin on biomarkers of heart failure in patients with myocardial infarction with and without type 2 diabetes mellitus within 6 months after the event. *Secondary objective:* To investigate - Short term changes of nt-proBNP levels - Short term and intermediate term changes in echocardiography parameters - Change in levels of ketone body concentrations - Change in HbA1c levels - Change in body weight - Number of hospital re-admissions due to heart failure or other causes - Duration of hospital stay - All-cause mortality
Outcome measures of the trial	*Primary outcome measures:* - Difference in the change of nt-proBNP levels between treatment groups from randomization to week 26 *Secondary outcomes measures:* - -Difference in the change of ejection fraction between treatment groups from randomization to week 26 - -Difference in the change of ejection fraction between treatment groups from randomization to week 6

	- Difference in the change of left ventricular diastolic function from randomization to week 26 - Difference in the change of left ventricular diastolic function from randomization to week 6 - Difference in the change of nt-proBNP levels between treatment groups from randomization to week 6 - Difference in the change of HbA1c between treatment groups from randomization to week 26 (in subjects with known T2DM) - Difference in the change of body weight between treatment groups from randomization to week 6 - Difference in the change of body weight between treatment groups from randomization to week 26 - Difference in the change of blood beta-hydroxybutyrate levels between the treatment groups from randomization to week 6 - Difference in the change of blood beta-hydroxybutyrate levels between the treatment groups from randomization to week 26 - Difference in the number of hospital re-admissions due to heart failure between the treatment groups - Difference in the number of hospital re-admissions for any cause between the treatment groups - Difference in the duration of hospital stay between the treatment groups after initiation of the study treatment
Number of patients	476 patients
Time schedule	*With reference to the trial:* EC Submission: NOV/2016 Local Authority Submission.: NOV/2016 First Patient In (FPFV): 11/MAY/2017 Last Patient In: 10/DEC/2021 Last Patient Out (LPLV): 03/MAY/2022 Data Base Lock: 17/MAY/2022 First Results available: JUL/2022 Clinical Study Report: SEP/2022 *With reference to patients:* Duration of the treatment: 6 months
Main inclusion criteria	1)Myocardial infarction with evidence of significant myocardial necrosis defined as a rise in creatinkinase >800 U/l and a troponin T- or I level >10x ULN. In addition at least 1 of the following criteria must be met:

	- Symptoms of ischemia - ECG changes indicative of new ischemia (new ST-T changes or new LBBB) - Imaging evidence of new regional wall motion abnormality 2)18 – 80 years of age 3)Informed consent has to be given in written form. 4)eGFR > 45 ml/min/1.73m^2 5)Blood pressure before first drug dosing: $RR_{systolic}$ >110mmHg 6) Blood pressure before first drug dosing: $RR_{diastolic}$ >70mmHg 7) First intake of study medication ≤72h after myocardial infarction after performance of a coronary angiography
Main exclusion criteria	1)Any other form of diabetes mellitus than type 2 diabetes mellitus, history of diabetic ketoacidosis 2)Blood pH < 7,32 3)Known allergy to SGLT-2 inhibitors 4)Haemodynamic instability as defined by intravenous administration of catecholamine, calciumsensitizers or phosphodiesterase inhibitors 5)>1 episode of severe hypoglycemia within the last 6 months under treatment with insulin or sulfonylurea 6) Patients currently being treated with any SGLT-2 inhibitor (dapagliflozin, canagliflozin, empagliflozin) or having received treatment with any SGLT-2 inhibitor within the 4 weeks prior to the screening visit
Study medication	Active substance: Empagliflozin Commercial name: Jardiance Manufacturer: Boehringer Ingelheim
Treatment plan	Empagliflozin, 10mg once daily orally administered or matched placebo

1. INTRODUCTION AND RATIONALE

Type 2 diabetes mellitus (T2DM) is associated with an about two to three-fold increased risk for cardiovascular events as compared to subjects without diabetes[1]. The United Kingdom Prospective Study (UKPDS) was the first to demonstrate a reduction of macrovascular and microvascular complications in subjects with T2DM by an intensified glucose lowering treatment regimen with either insulin/sulfonylurea or metformin in the long term follow up[2]. After Rosiglitazone was removed from the European market due to remaining uncertainty about the cardiovascular safety of this drug, the Federal Drug Administration (FDA) and the European Medicine Agency (EMEA) have issued new guidelines for new antihyperglycemic drugs to be licensed, requiring a thorough assessment of cardiovascular safety and in most cases the performance of a large cardiovascular outcome trial[3].

Sodium-dependent glucose cotransporter 2 (SGLT-2) is mainly expressed in human kidneys and small intestinal cells. In the proximal tubule of the nephron SGLT-2 is responsible for the reabsorption of approximately 90% of the filtrated glucose[4]. Inhibition of SGLT-2 was shown to increase renal glucose excretion and to lower glucose. Subsequently, a number of SGLT-2 inhibitors were developed and are currently approved for the treatment of type 2 diabetes.

Recently, Zinman B. et al published the results of the EMPA-REG-OUTCOME trial where the cardiovascular impact of a glucose lowering regimen including Empagliflozin as compared to usual glucose control without an SGLT-2 inhibitor was investigated[5]. The trial demonstrated an unexpected reduction in the primary composite endpoint, comprising cardiovascular death, non-fatal myocardial infarction and non-fatal stroke. The reduction was mainly driven by a 38% relative risk reduction in cardiovascular deaths; moreover they demonstrated an impressive 35% relative risk reduction in the secondary endpoint hospitalization for heart failure. Of note, the beneficial effects observed in the Empagliflozin group seem to occur very rapidly after commencing the treatment, as suggested by the early separation of the Kaplan-Meier curves. However, the mechanisms responsible for this finding remain unclear. Diuretic effects with subsequent impact on hemodynamics or potential cardioprotective effects of glucagon, which levels rise under the treatment with SGLT-2 inhibitors and the resulting rise in ketone bodies or a small increase in hematocrit have been suggested [6, 7].

We hypothesize that Empagliflozin beneficially influences the increased pre- and afterload as well as it reduces the increased sodium reabsorption following myocardial damage with subsequent reduced cardiac output. Whether or not Empagliflozin has direct effects on myocardial remodelling has not been elucidated, yet. Moreover, recent animal data suggest that in failing heart ketone bodies seems to be increasingly important as energy source[8]. Empagliflozin has been shown to increase levels of ketone bodies, which might has a beneficial impact on the metabolism and energy supply of the failing heart[9].

In addition, although SGLT-2 is not expressed in human myocardium, direct, non-SGLT-2 mediated effects on the heart muscle cannot be excluded, given that the molecule is small in size.

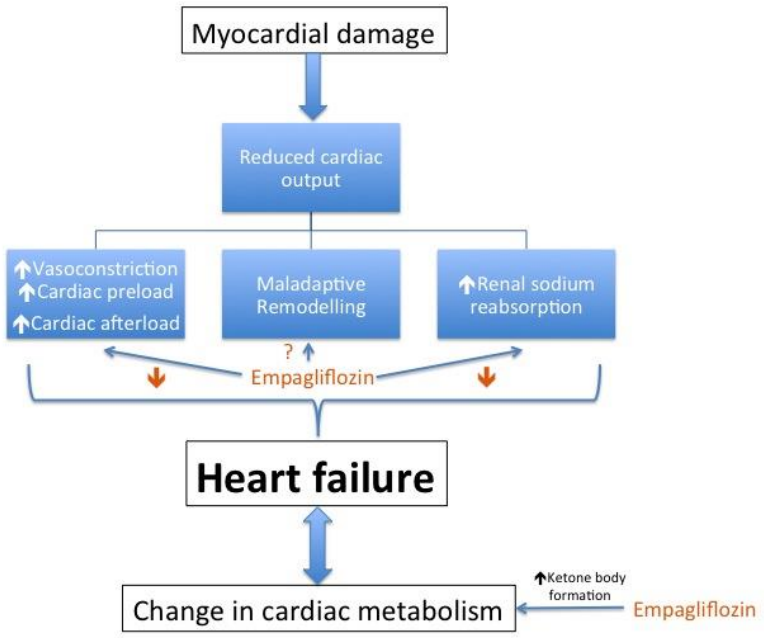

Figure 1 Summary of potential beneficial mechanisms of Empagliflozin used after MCI

The aim of this mechanistic study is to investigate the impact of Empagliflozin on cardiac function and biomarkers for heart failure in patients with acute myocardial infarction. Since previous trials in acute myocardial infarction using aldosterone blockers demonstrated that an early intervention might be crucial for a beneficial outcome[10], we have chosen to start treatment within 72 hours after performance of a coronary angiography for acute myocardial infarction.

1.1 Aim of the study

The aim of this mechanistic study is to investigate the impact of Empagliflozin on cardiac function and biomarkers of heart failure in patients with acute myocardial infarction.

1.2 Study hypothesis

Treatment with the SGLT-2 inhibitor Empagliflozin, commenced early after acute myocardial infarction will reduce the biomarker for heart failure nt-proBNP more effectively than placebo within 6 months after the event.

2. OBJECTIVES AND OUTCOMES

2.1 Primary objective

The primary objective is to investigate the impact of Empagliflozin on biomarkers of heart failure in patients with myocardial infarction with and without type 2 diabetes mellitus within 6 months after the event.

2.2 Secondary objectives

To investigate

- Short term changes of nt-proBNP levels
- Short term and intermediate term changes in echocardiography parameters
- Change in levels of ketone body concentrations
- Change in HbA1c levels
- Change in body weight
- Number of hospital re-admissions due to heart failure or other causes
- Duration of hospital stay
- All-cause mortality

2.3 Safety objectives

- All-cause mortality
- Number of serious adverse events
- Number of hypoglycemic events
- Number of genital infections
- Number of ketoacidotic events
- Changes in liver function parameters (AST, ALT, GGT)
- Changes in renal function parameters (creatinine, eGFR)

2.4 Primary outcomes

Difference in the change of nt-proBNP levels between treatment groups from randomization to week 26

2.5 Secondary outcomes

- Difference in the change of ejection fraction between treatment groups from randomization to week 26
- Difference in the change of ejection fraction between treatment groups from randomization to week 6
- Difference in the change of left ventricular diastolic function from randomization to week 26
- Difference in the change of left ventricular diastolic function from randomization to week 6
- Difference in the change of nt-proBNP levels between treatment groups from randomization to week 6
- Difference in the change of HbA1c between treatment groups from randomization to week 26 (in subjects with known diabetes mellitus Type 2)
- Difference in the change of body weight between treatment groups from randomization to week 6
- Difference in the change of body weight between treatment groups from randomization to week 26
- Difference in the change of blood beta-hydroxybutyrate levels between the treatment groups from randomization to week 6
- Difference in the change of blood beta-hydroxybutyrate levels between the treatment groups from randomization to week 26
- Difference in the number of hospital re-admissions due to heart failure between the treatment groups
- Difference in the number of hospital re-admissions for any cause between the treatment groups

- Difference in the duration of hospital stay between the treatment groups after initiation of the study treatment

3. STUDY DESCRIPTION

3.1 Design

This is a randomized, double-blind (patients and physicians), placebo controlled multi-center study to evaluate the effect of Empagliflozin 10mg once daily (p.o.) for 26 weeks on cardiac function and biomarkers for heart failure in patients with acute myocardial infarction (Figure 1). The study will be conducted in 11 sites in Austria (Appendix B) with an aim to enrol 476 patients (Figure 2) to evaluate the overall study hypothesis.

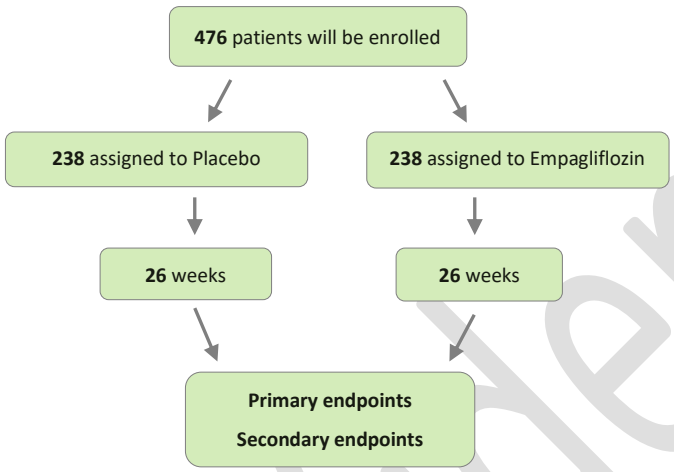

Figure 2 Study Flow Chart

3.2 Duration of study

It is anticipated that the study will run for 30 months.

4. STUDY POPULATION

We will study subjects after an acute myocardial infarction. We aim to recruit 150 subjects with established diabetes mellitus type 2.

4.1 Inclusion criteria

1) Myocardial infarction with evidence of significant myocardial necrosis defined as a rise in creatinkinase >800 U/l and a troponin T-level (or troponin I-level) >10x ULN. In addition at least 1 of the following criteria must be the met:
   - Symptoms of ischemia
   - ECG changes indicative of new ischemia (new ST-T changes or new LBBB)
   - Imaging evidence of new regional wall motion abnormality
2) 18 – 80 years of age

3) Informed consent has to be given in written form
4) eGFR > 45 ml/min/1.73m^2
5) Blood pressure before first drug dosing: $RR_{systolic}$>110mmHg
6) Blood pressure before first drug dosing: $RR_{diastolic}$>70mmHg
7) First intake of study medication ≤72h after myocardial infarction after performance of a coronary angiography

4.2 Exclusion criteria

1) Any other form of diabetes mellitus than type 2 diabetes mellitus, history of diabetic ketoacidosis
2) Blood pH < 7,32
3) Known allergy to SGLT-2 inhibitors
4) Haemodynamic instability as defined by intravenous administration of catecholamine, calciumsensitizers or phosphodiesterase inhibitors
5) >1 episode of severe hypoglycaemia within the last 6 months under treatment with insulin or sulfonylurea
6) Females of child bearing potential without adequate contraceptive methods (i.e. sterilisation, intrauterine device, vasectomised partner; or medical history of hysterectomy)
7) Acute symptomatic urinary tract infection (UTI) or genital infection
8) Patients currently being treated with any SGLT-2 inhibitor (dapagliflozin, canagliflozin, empagliflozin) or having received treatment with any SGLT-2 inhibitor within the 4 weeks prior to the screening visit

4.3 Withdrawal criteria

Subjects may be withdrawn from the study at the discretion of the Investigator or Sponsor due to a safety concern or if judged non-compliant with trial procedures. A subject must be withdrawn from treatment of the following applies:

- Subject chooses to withdraw from the study at any time
- Adverse event requires unblinding of the study medication
- Pregnancy or intention of becoming pregnant
- Intolerable adverse effects
- Major violation of the study protocol
- Occurrence of an exclusion criterion
- Other circumstances that would endanger the health of the subject if he/she were to continue his/her participation in the trial.

Reasons for withdrawals and discontinuation of any subject from the protocol have to be recorded.

4.4 Termination of the entire trial

Premature termination of the clinical trial will be considered when the risk-benefit ratio changes markedly for the patient, the use of the study medication is no longer justifiable, the sponsor believes it is necessary to terminate the clinical trial for safety reasons, when early evidence of the superiority or inferiority of a treatment group is obtained by an interim analysis or by other research results, or when the clinical trial proves to be impracticable.

4.5 Unblinding

In the case of a requirement to unblind study medication, one of the chief investigators needs to be informed to discuss the unblinding. The unblinding list is held by the Institute of Medical Informatics, Statistics and Documentation (IMI), Medical University of Graz, which is not involved in study investigations.

4.6. Tracking of drop out patients

In order to guarantee a 100% follow-up of the mortality in this study, we would like to apply to be able to call up the mortality data of the study participants from Statistics Austria for the period of study participation. This can increase the quality of the data even further and thus improves the significance of the study.

4.7. Query of mortality data

In the beginning of the year 2023, the study team would like to request a query from Statistic Austria regarding the mortality of the participating study patients.

5. VISIT PROCEDURES, MEASUREMENTS AND ASSESSMENTS

5.1 Recruitment and screening

Sites will recruit competitively and sites will receive compensation for their work based on a per-patient fee. The clinic staff of the intensive care unit informs patients about the possibility of being enrolled in this study. No study-related procedures are undertaken before obtaining informed consent. The study team in detail explains the study procedures and asks the participant about their willingness to participate in this research study. After informed consent is signed and obtained, participants are given a signed copy of the informed consent document and are assigned a screening identification number.

After obtaining informed consent at the screening visit, subject´s eligibility will be further assessed and documented by using a source data form with a list of inclusion and exclusion criteria, medical history (including all medications past and current) will be acquired and the following measurements will be performed: body weight, height, blood pressure, ECG and blood parameters (see Table 2). Laboratory results up to 2 days before screening can be used to test eligibility for the trial.

Subjects unable to provide written informed consent will not be included in the study.

5.2 Randomisation

After screening, all eligible patients for the trial will be randomised into one of the two arms of the study via Randomiser Software (Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz, http://www.randomizer.at), which will be programmed with a randomisation schedule provided by an independent statistician. The randomisation will be stratified by site, type 2 diabetes and by sex.

Only the subject number and subject initials will be recorded in the case report form (CRF). The investigator will maintain a personal subject identification list (subject numbers with the corresponding subject names) to enable records to be identified.

A summary of all visits and procedures has been outlined below (Table 1). A more detailed description of each visit and accompanying procedures can be found later in the protocol (5.4 - 5.5)

5.3 Visit schedule

	Visit 1 (V1) Screening / Baseline	Visit 2 (V2) (week 6 ± 2)	Visit 3 (V3) (week 12 ± 2)	Visit 4 (V4) (Week 26 ± 2)	Visit 5 (V5) (4 weeks ±1w after V4)
Informed Consent	X				
Inclusion/exclusion criteria	X				
Randomisation	X				
Demography, medical history	X				
Concomitant medication	X	X	X	X	
Vital signs	X	X	X	X	
Height	X				
Weight	X	X	X	X	
Physical examination	X	X		X	
ECG	X				
Cardiac ultrasound	X	X		X	
Biobank sampling	X	X		X	
nt-proBNP (local)	X	X	X	X	
Blood sampling	X	X	X	X	
Liver function parameters (AST, ALT, GGT)	X	X	X	X	
Renal function parameters (creatinine, eGFR)	X	X	X	X	
Adverse Events		X	X	X	X

Dispense medication	X				
Drug accountability				X	
Safety assessment before discharge	X				

Table 1 Visit schedule

5.4 Visit procedures

Visit 1 - Screening/Enrolment Visit (Day 0 – hospital stay)

- Obtain informed consent of potential participant verified by signature on study informed consent form (10.5)
- Collect blood to check inclusion criteria (creatinkinase, troponin T (or troponin I), eGFR, blood pH, nt-proBNP) (5.5.7)
- Collect and prepare blood samples for biobank (5.5.6)
- Perform echocardiography (5.5.7)
- Obtain demographic information, medical history, medication history, alcohol and tobacco use history (5.5.1)
- Record vital signs (5.5.3)
- Measure body weight and body height (5.5.4)
- Verify inclusion/exclusion criteria (4.2 and 4.3)
- Randomize the subject (5.2)
- Dispense the study medication for trial period (9.4)
- Adjust concomitant antihyperglycemic medication
- Assessment of side effects and patient safety before discharge (5.5.8)

Visit 2 - Follow up 1 Visit (Week 6 ± 2 weeks)

- Record adverse events as reported by participant or observed by investigator (6.1 – 6.6)
- Record vital signs (5.5.3)
- Measure body weight (5.5.4)
- Collect blood for routine biochemistry (5.5.5)
- Perform echocardiography (5.5.7)
- Record changes in medication
- Collect and prepare blood samples for biobank (5.5.8)

Visit 3 - Safety Visit (Week 12 ± 2 weeks)

- Record adverse events as reported by participant or observed by investigator (6.1 – 6.6)
- Record vital signs (5.5.3)
- Measure body weight (5.5.4)
- Collect blood for routine biochemistry (5.5.5)
- Record changes in medication
- Record participant's adherence to treatment program

Visit 4 - Follow-up 2 Visit (Week 26 ± 2 weeks)

- Record adverse events as reported by participant or observed by investigator (6.1 – 6.6)
- Record vital signs (5.5.3)
- Measure body weight (5.5.4)
- Collect blood for routine biochemistry (5.5.5)
- Perform echocardiography (5.5.7)
- Record changes in medication
- Collect and prepare blood samples for biobank (5.5.6)
- Record participant's adherence to treatment program
- Drug accountability (5.5.11)

Visit 5 - Telephone assessment (4weeks ± 1week after V4)

- Record adverse events as reported by participant (6.1 – 6.6)

5.5 Description of procedures and measurements

5.5.1 Medical history and physical examination

A medical history will be performed at the screening visit to record illnesses, disorders and medications. This information needs to be updated on all follow-up visits.

Physical examination will be performed at the Screening visit (study visit 1) according to local procedure. During this visit the physician will perform a physical examination with the focus on cardiac, lung and abdominal examination. Any abnormal, clinical significant findings must be recorded in the SDF (Source Data Form) as well as in the eCRF (Clincase). Any changes in subsequent visits as compared to the screening visit which fulfils the criteria of an AE must be recorded as an AE. Any changes in concomitant illness will be recorded as changes in medical history. Any changes in medications will be recorded in the eCRF.

5.5.2 ECG

An ECG will be performed at the screening visit. The ECG will be interpreted, signed and dated by the investigator before randomisation.

5.5.3 Vital signs

Pulse should be recorded at all visits after resting for five minutes in a sitting position. Systolic and diastolic blood pressure will be measured in sitting position at all visits.

5.5.4 Body weight and height

Weight should be measured at all visits. The same and calibrated pair of scales should preferably be used throughout the trial. Height will be recorded at the screening visit. BMI (body mass index) will be calculated as follows: BMI = weight (kg)/height^2 (m^2).

5.5.5 Routine biochemistry

Blood samples will be obtained at all visits after an 8 hour fast and processed by the local laboratory using standard methods' for routine tests. Patients can take their regular morning medications but are asked that they do not take any of their diabetes medications on the morning of their study visit. Patients should bring their regular medication along to their study visits to be further advised by the doctor. See table 2 below for a detailed description of blood collection at each visit:

Blood test	Visit 1	Visit 2	Visit 3	Visit 4
Hematology (full blood count)	X	X	X	X
HbA1c	X	X	X	X
Sodium, Potassium, Chloride	X	X	X	X
Calcium	X	X	X	X
Phosphate	X	X	X	X
Magnesium	X	X	X	X
Iron, Ferritin, Transferrin	X	X	X	X
Creatinkinase	X	X	X	X
LDH (Lactatdehydrogenase)	X	X	X	X
AST (Aspartate-Aminotransferase)	X	X	X	X
CK MB	X	X	X	X
ALT (Alanine-Aminotransferase)	X	X	X	X
GGT (Gamma-Glutamyl-Transferase)	X	X	X	X
CRP (C-reactive Protein)	X	X	X	X
Creatinine	X	X	X	X
eGFR	X	X	X	X
Total cholesterol	X	X	X	X
Triglycerides	X	X	X	X
HDL (high density lipoprotein)	X	X	X	X
LDL (low density lipoprotein)	X	X	X	X
Troponin T (Troponin I)	X			
NT-pro BNP	X	X	x	X

Fasting glucose	X	X	X	X
Myoglobin	X			
pH (arterial/venous)	X			
Urine Albumin/Creatinine Ratio (ACR)	X	X		X

Table 2 Summary of blood tests at each study visit

5.5.6 Blood sample collection and plasma extraction for biobanking

Blood will be collected via venous puncture into 16ml serum, 6ml EDTA and 4ml sodium citrate vacutainers and centrifuged within 30 min of collection. Plasma should be transferred into Eppendorf tubes and stored at -80°C locally. In regular intervals these samples will be shipped on dry ice to the Biobank of the Medical University of Graz, where they will be stored at -80°C for future biomarker analyses. NT-proBNP and blood ketone bodies will be measured in batches from these stored biomarker samples.

5.5.7 Echocardiography

Echocardiography will be performed using locally available ultrasound devices. Left ventricular wall thickness and chamber diameter will be assessed. Left ventricular systolic function will be assessed in 4- and 2-chamber views using the modified Simpson`s rule. Regional systolic dysfunction will be classified using the WMSI (wall motion score index) and/or strain measurements. Left ventricular diastolic function will be measured using E as well as lateral and septal e`. Right ventricular function will be evaluated using Tricuspid Annular Plane Systolic Excursion (TAPSE). Left atrial volume index will be calculated from LA dimensions. Colour flow Doppler as well as continuous as well as pulsed wave Doppler will be used for detection of valve diseases. A detailed overview on the EMMY trial echocardiography sub-study is given in the Echo Manual (please see Appendix C).

5.5.8 Assessment of side effects and patient safety before discharge

Physical examination and a check of possible side effects will be performed before discharge at study visit 1.

5.5.9 Faeces sampling

Sampling will be performed using stool collection tubes by Sarstedt, Nümbrecht, Germany, using a stool collector (Süsse Stuhlfänger, Gudensberg, Germany). Directions for safe and hygienic fecal collection will be delivered to the probands in words as well as in pictograms. Bacterial DNA will be extracted from stool samples using the MagNA Pure LC DNA Isolation Kit III (Roche). The 16S rRNA gene will be amplified in a PCR reaction and sequenced with next-generation sequencing technology (Roche Genome Sequencer FLX or Illumina MiSeq) and interpreted by the respective software analyzers.

5.6 Benefit-Risk Assessment

Empagliflozin has been studied in subjects with type 2 diabetes and demonstrated cardiovascular benefit in terms of reduction of a 3-point MACE composite endpoint as well as of hospitalization for heart failure[5]. However, it has not been studied shortly after myocardial infarction yet. Given that the drug is not causing hypoglycemic events per se, hypoglycemia appears not to be a major issue in subjects without diabetes, that are studied in this trial as well. When

Empagliflozin is used together with sulfonylurea or insulin, hypoglycemia might occur and therefore participants will be instructed to reduce concomitant antihyperglycemic medication accordingly.

Fungal genital infections are the most common side effect of Empagliflozin and participants will be informed about this and instructed on prevention measures. Rarely, serious infections of the genitals and the area around the genitals have been reported. This infection, called necrotizing fasciitis of the perineum, is also referred to as Fournier's gangrene.

In people with type 2 diabetes mellitus, the risk for ketoacidosis is increased, in particular in situations such as insulin deficiency, alcohol abuse, exsiccosis or infections.

There will be no direct beneficial effect for all participants, however, those participants with known diabetes mellitus type 2 randomised to the Empagliflozin group will benefit from the glucose lowering effect of the drug.

Overall this trial aims to develop novel future strategies for heart failure prevention in subjects with acute myocardial infarction.

6. ADVERSE EVENTS (AE)

An AE is any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, including an exacerbation of a pre-existing condition, or disease temporally associated with the use of the trial device/procedure, whether or not considered related to the treatment.

All adverse events that occur during this study will be recorded on the adverse event case report forms.

6.1 Adverse Event description

For the purposes of the study, AEs will be followed up according to local practice until the event has stabilised or resolved, or the Follow-up Visit, whichever is the sooner. SAEs will be recorded throughout the study.

6.2 Severity of Adverse Events

Mild: Awareness of event(s) or sign(s) but easily tolerated

Moderate: Discomfort enough to cause some interference with usual activity

Severe: Incapacitating or causing inability to carry out usual activity

6.3 Causality of Adverse Events

Medical judgment should be used to determine the relationship, considering all relevant factors, including pattern of reaction, temporal relationship, de-challenge or re-challenge, confounding factors such as concomitant medication, concomitant diseases and relevant history. Assessment of causal relationship should be recorded in the case report forms.

Yes: There is a reasonable causal relationship between the investigational product administered and the AE.

No: There is no reasonable causal relationship between the investigational product administered and the AE.

6.4 Abnormal Laboratory Test Results

All clinically significant abnormal laboratory test results besides Troponin T (Troponin I), creatinkinase, nt-proBNP occurring during the study will be recorded as adverse events.

6.5 Adverse events of Special Interest (AESIs)

The term AESI relates to any specific AE that has been identified at the project level as being of particular concern for prospective safety monitoring and safety assessment within this trial, e.g. the potential for AEs based on knowledge from other compounds in the same class. AESIs need to be reported to the Pharmacovigilance Department of Boehringer Ingelheim within the same timeframe that applies to SAEs.

Patients with AESIs need to be followed up appropriately, regardless of the origin of the laboratory data (e.g. central, local etc.). The Investigator should consider which, if any, concomitant therapies should not be taken during evaluation. Discontinued treatments can be reintroduced per Investigator discretion.

The following are considered as AESIs:

6.5.1 Hepatic injury

A hepatic injury is defined by the following alterations of hepatic laboratory parameters after randomisation:

- an elevation of AST and/or ALT ≥ 3 fold ULN combined with an elevation of total bilirubin ≥ 2 fold ULN measured in the same blood sample
- an isolated elevation of ALT and/or AST ≥ 5 fold ULN

These laboratory findings constitute a hepatic injury alert and the patients showing these abnormalities need to be followed up according to medical judgement.

In case of clinical symptoms of hepatic injury (icterus, unexplained encephalopathy, unexplained coagulopathy, right upper quadrant abdominal pain, etc.) without laboratory results (ALT, AST, total bilirubin) available, the Investigator should make sure these parameters are analysed, if necessary in an unscheduled blood test.

6.5.2 Decreased renal function

Decreased renal function is defined by a creatinine value showing a ≥ 2 fold increase from baseline and is above the ULN.

For the AESI “decreased renal function” the Investigator shall collect an unscheduled laboratory sample for creatinine as soon as possible and initiate follow-up laboratory tests of creatinine according to medical judgement.

6.5.3 Metabolic acidosis, ketoacidosis and diabetic ketoacidosis (DKA)

In case of metabolic acidosis, ketoacidosis and DKA further investigations should be done according to the medical judgment and the clinical course until a diagnosis is made and/or the patient is recovered.

DKA is defined by the diagnostic criteria in the table below, and as defined by the American Diabetes Association (ADA).

Investigators should note that not all criteria in the table below need to apply for the diagnosis of DKA, and clinical judgment should also be taken into consideration. Due to its mechanism of action, Empagliflozin may potentially

modify the clinical presentation of DKA which may occur at lower plasma glucose levels than stated in the table below.

	DKA		
	Mild	Moderate	Severe
Plasma glucose (mg/dL)	>250	>250	>250
Arterial pH	7.25-7.30	7.00-7.24	<7.00
Serum bicarbonate (mEq/L)	15-18	10 to <15	<10
Urine ketones*	Positive	Positive	Positive
Serum ketones*	Positive	Positive	Positive
Effective serum osmolality (mOsm/kg)**	Variable	Variable	Variable
Anion gap***	>10	>12	>12
Alteration in sensoria or mental obtundation	Alert	Alert/drowsy	Stupor/coma

Table 3 Diagnostic criteria for DKA

* Nitroprusside reaction method
** Calculation: 2[measured Na (mEq/L) + glucose (mg/dL)/18
*** Calculation: (Na+) – (Cl- + HCO3-) (mEq/L)

6.5.4 Events involving lower limb amputation

This definition includes amputation (i.e. resection of a limb through a bone), disarticulation (i.e. resection of a limb through a joint) and auto-amputations (i.e. spontaneous separation of non-viable portion of the lower limb).

Not included in this definition are debridement (removal of callus or dead tissue), procedures on a stump (like stump revision, drainage of an abscess, wound revision etc.) and other procedures (e.g., nail resection or removal) without a concomitant resection of a limb (amputation or disarticulation).

Each lower limb amputation, disarticulation, or auto-amputation should be reported separately. The SAE report should include the date of the procedure, the level of amputation or disarticulation, the medical condition(s) leading to the procedure and if the patient had some of the known risk factor(s) for lower limb amputation.

6.6 Serious Adverse Events (SAE)

An SAE is defined as any event that

- Results in death;
- Is immediately life-threatening*;
- Requires hospitalisation or prolongation of existing inpatient's hospitalisation**
- Results in persistent or significant disability or incapacity;
- Is a congenital abnormality or birth defect;

* "Life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

** "Hospitalisation" means any unexpected admission to a hospital department. It does not usually apply to scheduled admissions that were planned before study inclusion or visits to casualty (without admission).

Medical judgement should be exercised in deciding whether an adverse event/reaction is serious in other situations. Important adverse events/reactions that are not immediately life-threatening, or do not result in death or hospitalisation but may jeopardise a subject, or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

Patients may be hospitalised for administrative or social reasons during the study (e.g. days on which infusion takes place, long distance from home to site,). These and other hospitalisations planned at the beginning of the study do not need to be reported as a SAE in case they have been reported at screening visit in the source data and have been performed as planned.

Worsening of the underlying disease or other pre-existing conditions

Worsening of the underlying disease or of other pre-existing conditions will be recorded as an (S)AE in the (e)CRF.

Changes in vital signs, ECG, physical examination, and laboratory test results

Changes in vital signs, ECG, physical examination and laboratory test results will be recorded as an (S)AE in the (e)CRF , if they are judged clinically relevant by the investigator.

6.6.1 Reporting of SAEs

The Sponsor shall report (i.e., from signing the informed consent onwards through the trial defined follow-up period <please define>) all SAEs and non-serious AEs which are relevant for a reported SAE and Adverse Events of Special Interest (AESI) by fax or other secure method using BI IIS SAE form to the BI Unique Entry Point immediately (within twenty four (24) hours or next business day whichever is shorter).

BI Unique Entry Point:
Boehringer Ingelheim RCV GmbH & Co KG,
Pharmacovigilance Austria
Dr. Boehringer-Gasse 5-11, 1121 Vienna, Austria
Phone: +43 (0) 1 80 105 2099
Fax: +43 (0) 1 80 105 6384
E-mail: PV_local_Austria@boehringer-ingelheim.com

For each adverse event, the investigator will provide the onset date, end date, intensity, treatment required, outcome, seriousness, and action taken with the investigational drug. The investigator will determine the expectedness of the investigational drug to the AEs as defined in the Listed Adverse Events section of the Boehringer Ingelheim's (BI's) Investigator Brochure for the Productor BI Drug Information e.g. Summary of Product Characteristics (SmPC) or Product Information (PI) for the authorised Study Drug provided by BI,

The investigator does not need to actively monitor patients for adverse events once the clinical trial has ended. However, if the investigator becomes aware of an SAE(s) that occurred after the patient has completed the clinical trial (including any protocol specified follow-up period), it should be reported to BI if investigator considers it as relevant to the BI study drug.

The sponsor will report the SAE to the ethics committee and the local authorities as well as Boehringer Ingelheim Pharmacovigilance in accordance with the aforementioned SAE reporting instructions.

A suspected unexpected serious adverse reaction (SUSAR) is designated as such according to Guideline 2001/20/EG. A serious adverse reaction is deemed unexpected when it is not listed in the corresponding basic document (Summary of Product Characteristics, IB, IMPD).

7. STATISTICAL ANALYSES

7.1 Sample size and power considerations

Previous data showed that nt-proBNP levels decrease by about 50% within 6 months after acute myocardial infarction[11]. To detect a relative 40% larger reduction in nt-proBNP levels in the Empagliflozin group as compared to the placebo group with a power of 80% and an alpha-level of 0.05%, and assuming a correlation for nt-proBNP levels of 0.85, a sample size of 216 subjects in each group is necessary. To account for a dropout rate of about 10% each group will consist of 238 patients.

7.2 Data analysis

Patient characteristics will be summarized. Summaries of continuous variables will be presented as means and standard deviations, if normally distributed, and as medians and inter-quartile ranges for skewed data, whilst categorical variables will be presented as frequencies and percentages. The analysis will be performed according to the intention to treat principle. All statistical tests will be two-tailed with a 5% significance level.

Analysis will be based on the intention to treat cohort, while a sensitivity analysis for the per-protocol group will be done in addition. A detailed statistical analysis plan including pre-specified subgroup analyses will be finalized ahead of database lock.

8. DATA MANAGEMENT

Data Management is the responsibility of the Medical University of Graz, Department of Endocrinology and Diabetology and the study Monitor. The subject and the biological material obtained from the subject will be identified by subject number, trial site and trial identification number. Appropriate measures such as encryption or deletion will be enforced to protect the identity of human subjects in all presentations and publications as required by local/ regional and national requirements.

This study will capture and process data using an electronic Case Report Form (Clincase) which is a fully validated high quality electronic data capture system, which has a full audit trail and controlled level of access. Data and reports will be extracted from the database throughout the study to monitor progress and training will be provided to all study staff on use of the database.

9. TREATMENT

9.1 Study medication

Empagliflozin is a sodium-glucose cotransporter 2 (SGLT2)-inhibitor. It works by increasing the amount of sugar that leaves the body in the urine. The study dose is 10mg once daily in the morning, taken with or without food. Each tablet contains 10mg Empagliflozin or Placebo. The pharmaceutical form is a round, pale yellow, biconvex, bevel-edged film-coated tablet debossed with "S10" on one side and the Boehringer Ingelheim logo on the other (tablet diameter: 9.1 mm). Empagliflozin has minor influence on the ability to drive and use machines. Patients should be advised to take precautions to avoid hypoglycemia while driving and using machines, in particular when Empagliflozin is used in combination with a sulfonylurea and/or insulin. Pharmacy (Anstaltsapotheke) at the University Hospital of Graz, Austria will pack the medication as 26 weeks supplies for study participants and label study medication according to current regulatory requirements.

9.2 Method of administration

The tablets can be taken with or without food, swallowed whole with water. If a dose is missed, it should be taken as soon as the patient remembers. A double dose should not be taken on the same day.

9.3 Special precautions for storage

The study drug must be kept locked in a locked secure area. For this study drug no particular storage conditions are required.

9.4 Dispensing of Study Drug

The study team shall dispense the study drug only to subjects entered into the study, under the direction of the PI or sub-investigators authorized to receive or dispense it. The study drugs will not be dispensed or supplied to any person not authorized to receive it. Each time a study drug is dispensed; there must be documentation in the sponsor provided log (drug accountability log) as to the amount dispensed, to whom it is dispensed, and the date and signature or initials of the person dispensing the drug. Subjects should be advised to follow the study protocol and as appropriate to protocol return all used and unused containers to the site at study visit 4 (week 26). Any discrepancies between the amounts used by the subjects and the amount returned should be documented.

9.5 Return/Destruction of Study Drug

Assure that unused supplies of the study drug are returned to the sponsor in accordance with sponsor requirements. Obtain authorization from the sponsor if unused supplies of the study drug will be disposed of at the end of the study. Maintain records of the return or disposition of the unused study drug, including the unused study drug amount, lot numbers and quantity, method of return or disposition, and any documentation related to shipment or destruction. At the end of the study ensure that all documentation regarding receipt, storage, dispensing, return of used containers, and accountability is complete and accurate.

10. REGULATORY, ETHICAL AND LEGAL ISSUES

10.1 Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformity with the 1964 Declaration of Helsinki and all subsequent revisions.

10.2 Good Clinical Practice

The study will be conducted in accordance with the guidelines laid down by the International Conference on Harmonisation for Good Clinical Practice (ICH GCP E6 guidelines).

10.3 Independent Ethics Committee/ competent authority

10.3.1 Initial Approval

Prior to the enrolment of subjects, the Ethics Committee at the Medical University of Graz must provide written approval of the conduct of the study at named sites, the protocol and any amendments, the Patient Information Sheet and Consent Form, any other written information that will be provided to the subjects, any advertisements that will be used and details of any subject compensation.

10.3.2 Approval of Amendments

Proposed amendments to the protocol and aforementioned documents must be submitted to the Ethics Committee for approval as instructed by the Sponsor. Amendments requiring approval may be implemented only after a copy of the approval letter has been obtained.

Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving Sponsor or the Ethics Committee approval. However, in this case, approval must be obtained as soon as possible after implementation.

10.3.3 Competent Authority

Clinical Trial Applications (EudraCT application and the documents required for the assessment) should be addressed to the Austrian Competent Authority, the Federal Office for Safety in Health Care (Bundesamt für Sicherheit im Gesundheitswesen, BASG) under the following address:

Austrian Federal Office for Safety in Health Care (BASG)
Austrian Agency for Health and Food Safety (AGES)
Institute Surveillance (INS), Department for Clinical Trials (CLTR)
Traisengasse 5
A-1200 Vienna
Austria

10.4 Insurance

Participant insurance according to legal requirements will be contracted.

10.5 Informed Consent

The participation of a subject in this clinical trial is voluntary. The investigator or a member of the research team will approach the patient to obtain informed consent. The background of the proposed study, the procedure, the follow-

up schedule and all potential benefits and risks will be carefully explained to each subject. The person obtaining the informed consent shall:

- Avoid any coercion or undue influence of subjects to participate
- Not waive or appear to waive subject's legal rights
- Use language that is non-technical and understandable to the subject
- Clarify the subject that his/her data are confidential and are encoded with a subject ID number during the investigation
- Provide plenty of time for the subject to consider his/her participation
- Include dated signatures of the subject and of the clinical investigator
- Ask whether the subject has any questions about the study

After a subject has received and read the patient information sheet and agrees to participate in the study, the informed consent form approved by the Ethics Committee must be signed by the subject prior to any study specific tests being performed. It will also be signed by the person responsible for collecting the informed consent. The original will be kept in the subjects study research notes (source documents), a copy will be given to the subject and a copy kept in their hospital notes.

10.6 Subject Confidentiality

The investigator must ensure that the subject's privacy is maintained. On the CRF or other documents submitted to the Sponsors, subjects will be identified by a subject ID number only. Documents that are not submitted to the Sponsor (e.g., signed informed consent form) should be kept in a strictly confidential file by the investigator. The investigator shall permit direct access to subjects' records and source document for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, Regulatory Authorities and Ethic Committees.

10.7 End of Trial

The trial will end after the last subject has completed the follow-up telephone assessment (Study visit 5). All patients will be reviewed by a clinician at their last study visit in order to arrange return to appropriate routine clinical care pathways.

10.8 Study Documentation and Data Storage

The investigator must retain essential documents until notified by the Sponsor, and at least for 15 years after study completion, as per the Sponsor's requirements. Subject files and other source data (including copies of protocols, CRFs, original reports of test results, correspondence, records of informed consent, and other documents pertaining to the conduct of the study) must be kept for the maximum period of time permitted by the institution. Documents should be stored in such a way that they can be accessed/data retrieved at a later date, either in paper or electronically. Consideration should be given to security and environmental risks.

No study document will be destroyed without prior written agreement between the Sponsor and the investigator.

11. ADMINISTRATIVE MATTERS

11.1 Source Data

Source documents comprise the CRF and hospital records, laboratory records and correspondence. All documents will be stored safely in a confidential manner at the performing site. The subject will be referred to by a unique study subject number/code, their initials and date of birth on all study-specific documentations. The only exceptions will be the signed Consent Forms, Subject Identification log and subject clinical file, all of which will be stored securely by the clinical site. Source data will be made available for internal and external audits or inspections by regulatory authorities to authorised personnel only.

As a minimum requirement the following data must be source data verifiable in source documentation other than the eCRF:

- Existence of subject (subject identifier, subject number and date of birth)
- Confirmation of participation in the trial (subject identification number (ID), trial ID and signed and dated informed consent forms)
- Diagnosis/ indication under investigation
- Visit dates
- Data from AEs, safety information form and pregnancy forms
- Relevant medical history, concomitant illness
- Reason for exclusion or withdrawal

11.2 Language

CRFs will be in German. Generic names for concomitant medications should be recorded in the CRF wherever possible. All written material to be used by subjects must use vocabulary that is clearly understood, and be in the language appropriate for the study site.

11.3 Data Collection

All data collected will be documented in the source documents and eCRF (Clincase).

11.4 Electronic Recording of data

This study will capture and process data using Clincase (electronic data capture) which will be built by Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz. Clincase is a fully validated high quality electronic data capture system, which has a full audit trail and controlled level of access. Data and reports will be extracted from the database throughout the study to monitor progress and training will be provided to all study staff on use of the database. The Investigator must ensure that the data is recorded in the eCRFs as soon as possible after the visit preferably within 7 working days.

11.5 Study Management Structure

11.5.1 Trial Steering Committee (TSC)

A Trial Steering committee (TSC) comprised by all principal investigators and chaired be the Co-Chief investigators will be responsible for overseeing the progress of the trial. A TSC Charter will be devised to list the roles and responsibilities of the TSC members. TSC will be convened at least biannually either in person or by teleconference.

11.6 Monitoring

A monitoring plan will be devised based on risk analysis and described in detail in the monitoring manual. During the course of the trial the Monitor will visit the trial sites to ensure that the protocol is adhered to, that all issues have been recorded and to perform source data verification. The study will be monitored periodically by a Clinical Trial Monitor responsible for this study.

Initiation visits will be completed at all trial centres prior to the recruitment of participants, and will consist of review of protocol and trial documents, training with respect to trial procedures (informed consent, SAE reporting, inclusion and exclusion criteria), review of recruitment strategy, review of site facilities and equipment, review of GCP principles, essential document receipt, collection and filing, and archiving and inspection. Copies of the trial specific procedure manuals and related documents will be given to the investigators. The approved version of the protocol should be followed at all times, and any significant protocol deviations will be documented in a Protocol Deviation Form and any significant deviations will be recorded on a Protocol Violation Form submitted to the study coordination centre and Sponsor as soon as possible. The investigators will allow the monitors to:

- inspect the site, the facilities, device management and materials used for the trial
- meet all members of the team involved in the trial, and ensure all staff working on the trial are experienced and appropriately trained, and have access to review all of the documents relevant to the trial
- have access to the electronic case record forms and source data
- discuss with the investigator and site staff trial progress and any issues on a regular basis

The monitor will ensure that:

- A percentage of records will be inspected for confirmation of existence, eligibility based on the results of the Risk Assessment
- 100 % of consent forms will be reviewed along with all SAE's.
- there is adherence to the protocol, including consistency with inclusion/exclusion criteria
- there is GCP and regulatory compliance
- trial documentation is complete and up to date (e.g. correct versions of documents being used, source data captured) and relevant documents are collected for the Trial Master File (TMF)
- the monitored eCRFs have been completed correctly and accurately, and all entries correspond to data captured in source documents

The Monitor must be given direct access to the source documents (original documents, data and records). Direct access includes permission to examine, analyse, verify and reproduce and record reports that are important to evaluation of the clinical trial.

All information dealt with during such visits will be treated as strictly confidential. At the end of the trial, close out visits will be performed by the monitor after the final participant visit has been completed and prior to database lock. During this visit the monitor will verify that all trial close out activities are completed – all queries resolved, missing data completed, monitoring completed, archiving arrangements in place, ISF completed and TMF documents collected, and end of trial notification. Each investigator will also be notified that an audit or inspection may be carried out - by the sponsor, sponsor's representatives or the host institution, or regulatory authorities - at any time, before, during or after the end of the trial. The investigator must allow the representatives of the audit or inspection team:

- to inspect the site, facilities and material used for the trial,
- to meet all members of his/her team involved in the trial,
- to have direct access to trial data and source documents, to consult all of the documents relevant to the trial. If an Investigator is informed of an impending audit or inspection, the trial coordination centre should be notified immediately.

11.7 Disclosure of data and publication

Information concerning the study, patent applications, processes, scientific data or other pertinent information is confidential and remains the property of the Sponsor. The investigator may use this information for the purposes of the study only. Verbal or written discussion of results prior to study completion and full reporting should only be undertaken with written consent from the Sponsor. Therefore all information obtained as a result of the study will be regarded as CONFIDENTIAL, at least until appropriate analysis and review by the investigators is completed. Permission from the Trial Steering Committee is necessary prior to disclosing any information relative to this study outside of the Steering Committee.

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Appendix A Power and sample size calculation

Power and sample size calculation for the project: Impact of Empagliflozin on cardiac function and biomarkers of heart failure in patients with acute myocardial infarction with or without type 2 diabetes

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Contents

1 Introduction

The objective here is to show the steps followed to derive the sample size when using the change, from baseline to 6 months, in the levels of NT-proBNP as the primary endpoint.

Let $X_0^{n_1} = (X_{01}, X_{02}, ..., X_{0n_1})$ and $X_1^{n_1} = (X_{11}, X_{12}, ..., X_{1n_1})$ be the samples at baseline and at follow-up (6 months) in the placebo group and $Y_0^{n_2} = (Y_{01}, Y_{02}, ..., Y_{0n_2})$ and $Y_1^{n_2} = (Y_{11}, Y_{12}, ..., Y_{1n_2})$ be the samples at baseline and at follow-up (6 months) in the treatment group. We assume the following distributional assumptions:

$$X_{0i} \sim N(\theta_0, \sigma_{0x}^2)\ i = 1, 2, ..., n_1 \tag{1.1}$$

$$X_{1i} \sim N(\theta_1, \sigma_{1x}^2)\ i = 1, 2, ..., n_1 \tag{1.2}$$

$$Y_{0i} \sim N(\gamma_0, \sigma_{0y}^2)\ i = 1, 2, ..., n_2 \tag{1.3}$$

$$Y_{1i} \sim N(\gamma_1, \sigma_{1y}^2)\ i = 1, 2, ..., n_2 \tag{1.4}$$

Let us define $X_i = X_{0i} - X_{1i}\ i = 1, 2, ..., n_1$ and $Y_i = Y_{0i} - Y_{1i}\ i = 1, 2, ..., n_2$ as the change from baseline to follow-up in the placebo and treatment group respectively. Then from equations (1.1), (1.2), (1.3) and (1.4) we have that:

$$X_i \sim N(\theta = (\theta_0 - \theta_1), \sigma_x^2)\ i = 1, 2, ..., n_1$$

$$Y_i \sim N(\gamma = (\gamma_0 - \gamma_1), \sigma_y^2)\ i = 1, 2, ..., n_2$$

where $\sigma_x^2 = \text{var}(X_i)$ and $\sigma_y^2 = \text{var}(Y_i)$ are the variance of the change over time in the placebo and treatment group respectively. In this framework, we assume that these two variances are equal. More specifically, we assume that:

$$\sigma_x^2 = \sigma_y^2 = \sigma_c^2 \tag{1.5}$$

This assumption of a common variance of the change over time can be easily satisfied by assuming that: 1- the correlation between baseline and follow-up measurements is the same in the placebo and treatment groups and 2- the ratio of the follow-up and baseline variances is the same in the placebo and treatment groups. To show this, let's first note that:

$$\begin{aligned} \sigma_x^2 = \text{var}(X_i) &= \text{var}(X_{0i} - X_{1i}) \\ &= \text{var}(X_{0i}) + \text{var}(X_{1i}) - 2cov(X_{0i}, X_{1i}) \\ &= (1 + m_x - 2r_x\sqrt{m_x})\sigma_{0x}^2 \end{aligned} \tag{1.6}$$

and similarly that $\sigma_y^2 = \text{var}(Y_i) = (1 + m_y - 2r_y\sqrt{m_y})\sigma_{0y}^2$ where m_x (respectively m_y) is the ratio of the baseline and follow-up variances in the placebo (respectively. treated) group and r_x (respectively r_y) is the correlation coefficient between baseline and follow-up measurements in the placebo (respectively. treated) group. The assumption of equal variances for the change in (1.5) is satisfied if:

$$m_x = m_y = m, r_x = r_y = r \text{ and } \sigma_{0x}^2 = \sigma_{0y}^2 = \sigma_0^2 \tag{1.7}$$

2 Sample size calculation

In this study we want to test the null hypothesis that the average change in the level of the biomarker (NT-proBNP) is the same in the treated compared to the placebo group. More specifically, let $\Delta = \theta - \gamma = (\theta_0 - \theta_1) - (\gamma_0 - \gamma_1)$. We want to carry out the following test:

$$\begin{aligned} H_0 &: \ \Delta = 0 \Leftrightarrow (\theta_0 - \theta_1) = (\gamma_0 - \gamma_1) \\ \text{against} & \\ H_1 &: \ \Delta \neq 0 \Leftrightarrow (\theta_0 - \theta_1) \neq (\gamma_0 - \gamma_1) \end{aligned} \tag{2.8}$$

2.1 Formula for the sample size calculation

The null hypothesis in (2.8) can be tested by using the standard t-test for comparing two averages. The formula for the required sample size can be analytically derived as follows:

$$n_1 = kn_2 \quad \text{with} \quad n_2 = \frac{(z_{\frac{\alpha}{2}} + z_\beta)^2 \sigma_c^2}{\Delta^2}(1 + \frac{1}{k}) \tag{2.9}$$

where k is the sample size ratio (assumed here equal to 1), α and $1 - \beta$ are the significance level and the power of the test and σ_c^2 is the common variance for the change over time as defined in (1.5).

To derive the required sample size in (2.9), one need to specify a minimal clinically relevant value for Δ and obtain an estimate of the common variance of the change σ_c^2. In what follows we show how one can derive estimates of these parameters.

2.2 Derivation of σ_c^2 and Δ

Using equation (1.6) and assumption (1.7), one can easily derive the common variance as follows:

$$\sigma_c^2 = (1 + m_x - 2r_x\sqrt{m_x})\sigma_{0x}^2 \tag{2.10}$$

where σ_{0x}^2 is the variance at baseline in the placebo group, $m_x = \frac{\sigma_{1x}^2}{\sigma_{0x}^2}$ is the variance ratio between follow-up and baseline in placebo group and r_x is the correlation coefficient between follow-up and baseline measurements in the placebo group. Also, one can express Δ as a function of the desired relative reduction RR by $\Delta = (\theta_0 - \theta_1)RR$ where $RR = \frac{(\theta_0 - \theta_1) - (\gamma_0 - \gamma_1)}{\theta_0 - \theta_1}$ is the desired relative reduction associated to treatment.

Now, one can estimate the parameters needed for the sample size calculation *i.e.* Δ and the common variance of the change σ_c^2 by finding estimates of the means θ_0 and θ_1, variances σ_{0x}^2 and σ_{1x}^2 and correlation r_x from previous studies. In practice unfortunately, not all studies report summary results in terms of means, standard deviations and correlations. This is for instance the case of our study where previous similar studies reported only quartiles and sample size at baseline and follow-up. The next question is then how can we approximate means and variances using quartiles and sample size. To do this, we used the approximation

results in [1]. According to [1], the mean μ and standard deviation σ can in general be approximated from the quartiles and sample size as follows:

$$\hat{\mu} \approx \frac{q_1 + q_2 + q_3}{3} \tag{2.11}$$

$$\hat{\sigma} \approx \frac{q_3 - q_1}{2\Phi^{-1}\left(\frac{0.75n-0.125}{n+0.25}\right)} \tag{2.12}$$

where q_1, q_2 and q_3 are respectively the first, second (median) and the third quartiles, n is the sample size and $\Phi^{-1}(.)$ is the cdf inverse of a unit Gaussian distribution.

3 Application to our project

According to a similar study [2] that reported the quartiles of NT-proBNP at baseline and at 6 months follow-up and using the approximations in (2.11) and (2.12), the estimates of the parameters needed for the sample size calculation are as follows:

$$\theta_0 \approx \frac{201 + 518 + 1242}{3} = 653.66$$

$$\theta_1 \approx \frac{113 + 232 + 503}{3} = 282.66$$

$$\sigma_{0x} = \frac{1242 - 201}{2\Phi^{-1}\left(\frac{0.75 \cdot 877 - 0.125}{877+0.25}\right)} = 772.98$$

$$\sigma_{1x} = \frac{503 - 113}{2\Phi^{-1}\left(\frac{0.75 \cdot 856 - 0.125}{856+0.25}\right)} = 289.60$$

$$m_x = \frac{\sigma_{1x}^2}{\sigma_{0x}^2} = 0.14$$

Assuming $\alpha = 5\%$ and a power $1 - \beta = 80\%$, the required sample size as a function of the correlation between baseline and follow-up measurements is given in tables 1, 2 and 3 according to different levels of the desired relative reduction $RR = 0.3, 0.35$ and 0.4.

Table 1: Sample size as a function of the correlation between baseline and follow-up measurements using a relative reduction RR = 0.3

res	Correlation coefficient	Sample size in each arm	Total sample size
1	0.00	865	1730
2	0.05	837	1674
3	0.10	808	1616
4	0.15	780	1560
5	0.20	751	1502
6	0.25	723	1446
7	0.30	695	1390
8	0.35	666	1332
9	0.40	638	1276
10	0.45	610	1220
11	0.50	581	1162
12	0.55	553	1106
13	0.60	524	1048
14	0.65	496	992
15	0.70	468	936
16	0.75	439	878
17	0.80	411	822
18	0.85	383	766
19	0.90	354	708
20	0.95	326	652
21	1.00	298	596

Table 2: Sample size as a function of the correlation between baseline and follow-up measurements using a relative reduction RR = 0.35

res	Correlation coefficient	Sample size in each arm	Total sample size
1	0.00	636	1272
2	0.05	615	1230
3	0.10	594	1188
4	0.15	573	1146
5	0.20	552	1104
6	0.25	532	1064
7	0.30	511	1022
8	0.35	490	980
9	0.40	469	938
10	0.45	448	896
11	0.50	427	854
12	0.55	407	814
13	0.60	386	772
14	0.65	365	730
15	0.70	344	688
16	0.75	323	646
17	0.80	302	604
18	0.85	282	564
19	0.90	261	522
20	0.95	240	480
21	1.00	219	438

Table 3: Sample size as a function of the correlation between baseline and follow-up measurements using a relative reduction RR = 0.4

res	Correlation coefficient	Sample size in each arm	Total sample size
1	0.00	487	974
2	0.05	471	942
3	0.10	455	910
4	0.15	439	878
5	0.20	423	846
6	0.25	407	814
7	0.30	391	782
8	0.35	375	750
9	0.40	359	718
10	0.45	344	688
11	0.50	328	656
12	0.55	312	624
13	0.60	296	592
14	0.65	280	560
15	0.70	264	528
16	0.75	248	496
17	0.80	232	464
18	0.85	216	432
19	0.90	200	400
20	0.95	184	368
21	1.00	168	336

References

[1] W.L.J.T.T. Wan Xiang Wang, *Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range.*, BMC medical research methodology 14 (2014), pp. 135–155.

[2] B.P.L.B. Eggers Kai M.Lagerqvist, *Prognostic Value of Biomarkers During and After Non-ST-Segment Elevation Acute Coronary Syndrome*, Journal of the American College of Cardiology 54 (2009), pp. 357–364.

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Appendix C Echo Manual

1 Preamble

This manual is a summary of all echocardiographic images and loops that should be assessed as part of the EMMY trial echocardiography sub-study. All information given here is based upon the publications "Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging", published by Lang et al in 2015 and "Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging", published by Nagueh et al in 2016.

1.1 Equipment

All study centres will use their own equipment. The echo machines should be maintained adequately and of best available quality. The framing rate should be set to the highest possible value (preferable at least > 50 fps).

1.2 Saving images and loops

For the best quality in the echocardiographic evaluation of the heart function we need either full loops, here preferably **4 cardiac cycles** independent of sinus rhythm or atrial fibrillation (please adjust the saving options of your ultrasound system), or images as indicated. **Please note that the loops and images should not include any measurements**. Record these raw images and loops in an anonymized file. We recommend at this point to create two different files for each patient: one for your clinical usage and one with anonymized raw data. The anonymized file should be named as "last name = study ID" and "first name = baseline (0) or follow up (1)". Birth date and date of exam as well as gender should be included in the file. You may save it on USB-sticks or DICOM-DVDs. The raw data will be transferred to Graz and measured by an ESC certified investigator (*KA*).

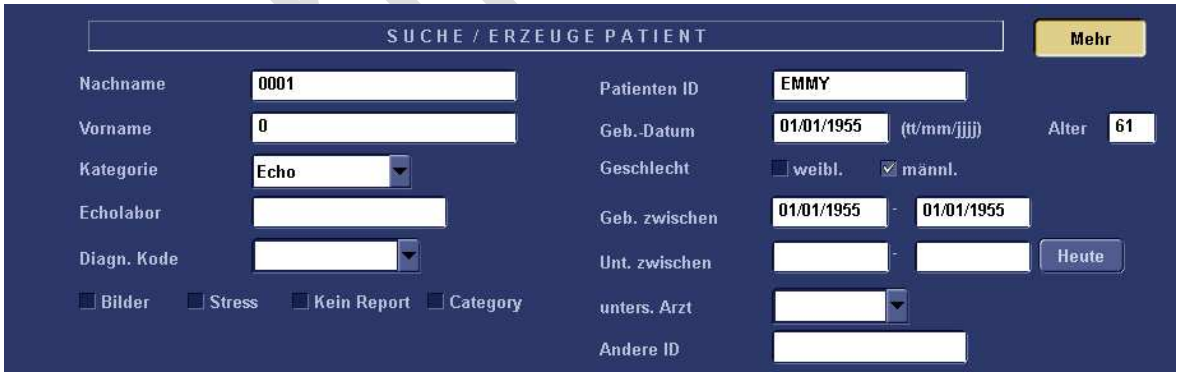


Figure 3 Example of an anonymised patient data input

1.3 What do we need for analysis?

Every file must include – if the quality is sufficient – loops and images of the (*1-3*) parasternal long axis including M-mode of the dimensions of the left ventricle and M-mode of the dimensions of the aorta/left atrium; (*4, 5*) parasternal short axis on the level of the papillary muscle and on the level of the apex; (*6-12*) apical 4-chamber view with and without colour Doppler including one image with pulsed wave Doppler at the leaflets of the opened mitral valve and tissue Doppler on the medial mitral annulus and continuous wave Doppler at the origin of a tricuspid regurgitation (gradient over tricuspid valve); (*13, 14*) native apical 2-chamber view; (*15*) native apical 3-chamber view; and (*16, 17*) one loop of the apical 5-chamber view with colour Doppler and one image with the continuous wave Doppler in the aortic outflow tract. ECG leads should be connected to the echocardiography machine and ecg tracings should be recorded on each loop or image.

2 Echocardiographic measurements

2.1 The parasternal long axis (*1-3*)

In this axis one loop is required (*1*). Additionally, saved images of the linear measurements of the left ventricle (*2*) and the aorta/left atrium (*3*) each measured in M-mode.

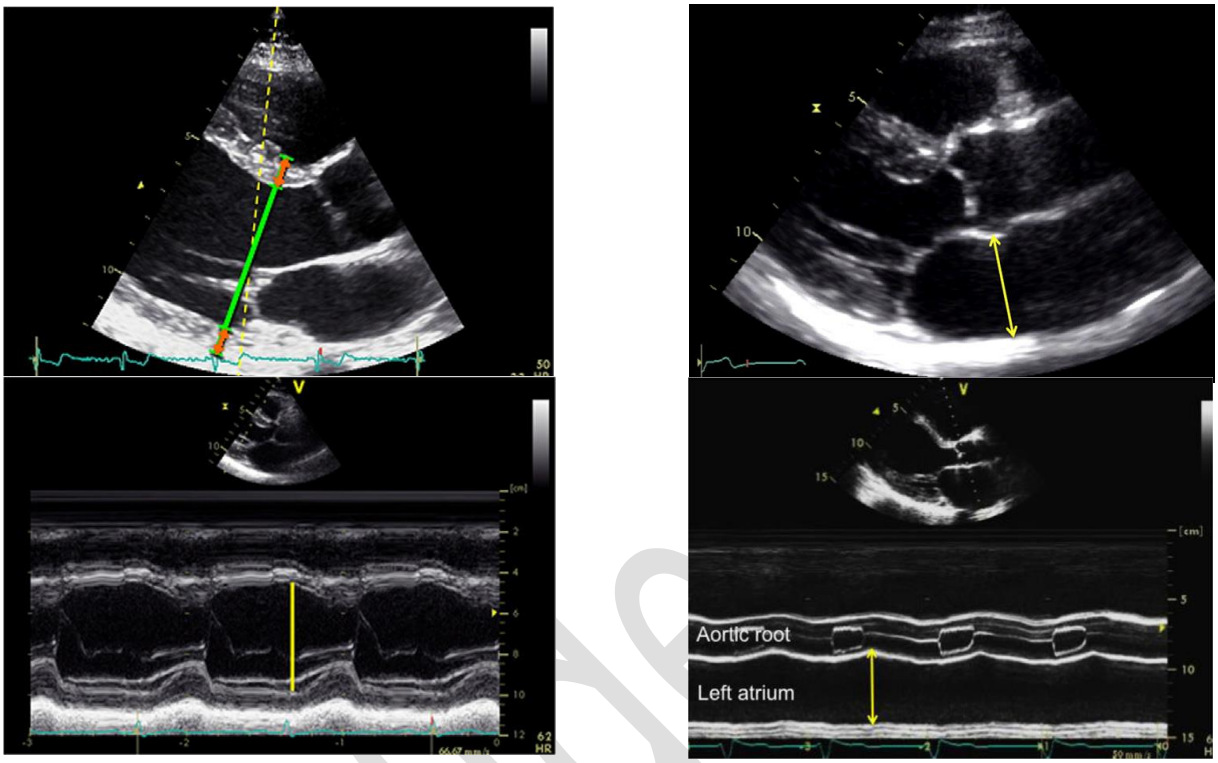


Figure 4 Example of an M-mode in the parasternal long axis (left: left ventricle; right: aorta/left atrium)

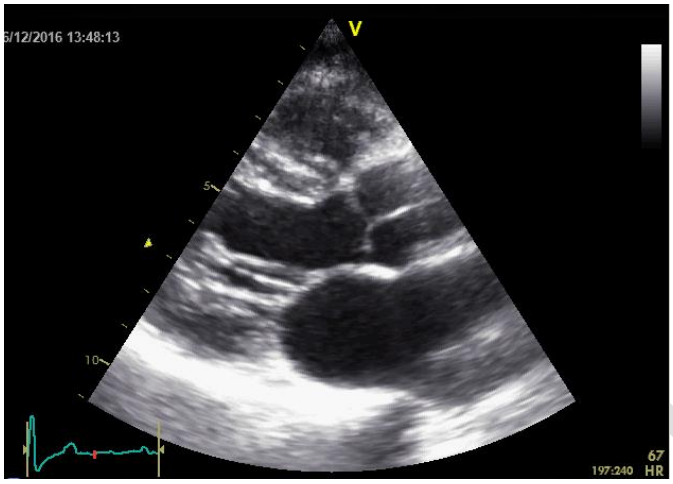


Figure 3 Example of a parasternal long axis (loop should be recorded)

2.2 The parasternal short axis (*4, 5*)

In the parasternal short axis, 2 loops should be obtained and saved: at the level of the papillary muscles (*4*) and at the level of the apex (*5*)

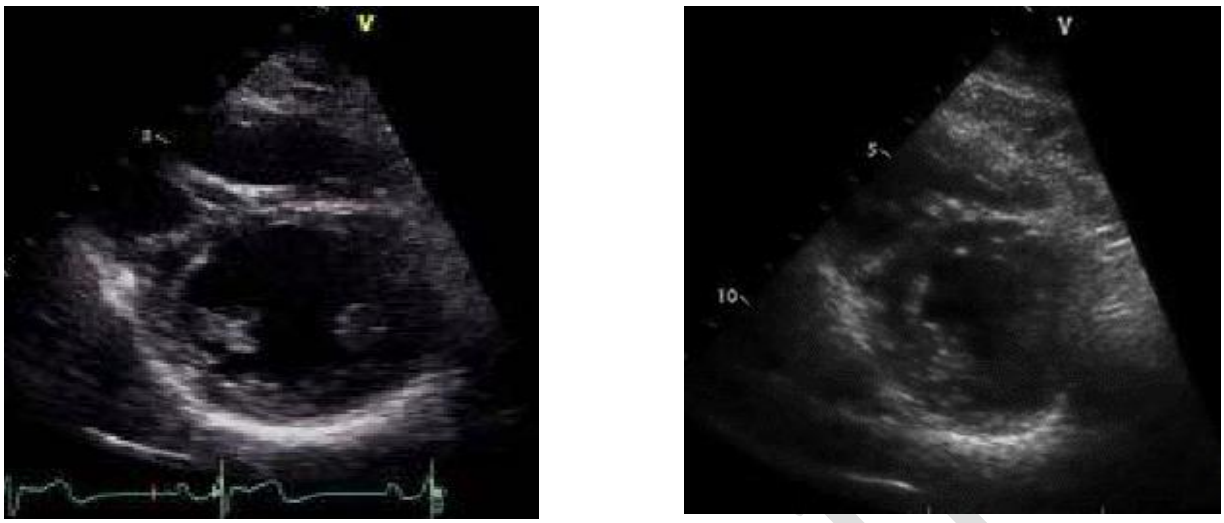


Figure 4 Examples of the parasternal short axis (left: level of the papillary muscles; right: level of the apex)

2.3 The apical 4-chamber view (*6-12*)

In the apical 4-chamber view one native loop is required (*6*). Make sure to record the left and right ventricles and the left atrium in the full length. If the left atrium can't be recorded at full length together with the left ventricle, you **should** record an **extra loop** with focus on the left atrium (*7*).

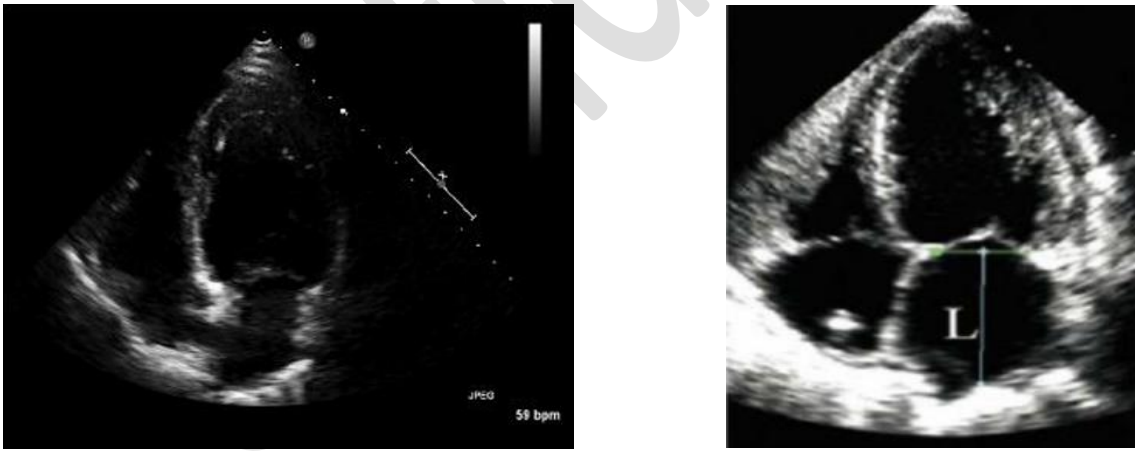


Figure 5 Example of an apical 4-chamber view (left: focus on the left ventricle; right: focus on the left atrium)

Record a loop with colour Doppler placed on the left atrium (*8*; assessment of mitral regurgitation) and a loop with colour Doppler placed on the right atrium (*9*; assessment of tricuspid regurgitation). After recording loop *#9* (tricuspid regurgitation), place the continuous wave Doppler directly on the origin of the jet, if present (*10*). The measured signal can then be used to gain information about the pulmonary artery systolic pressure.

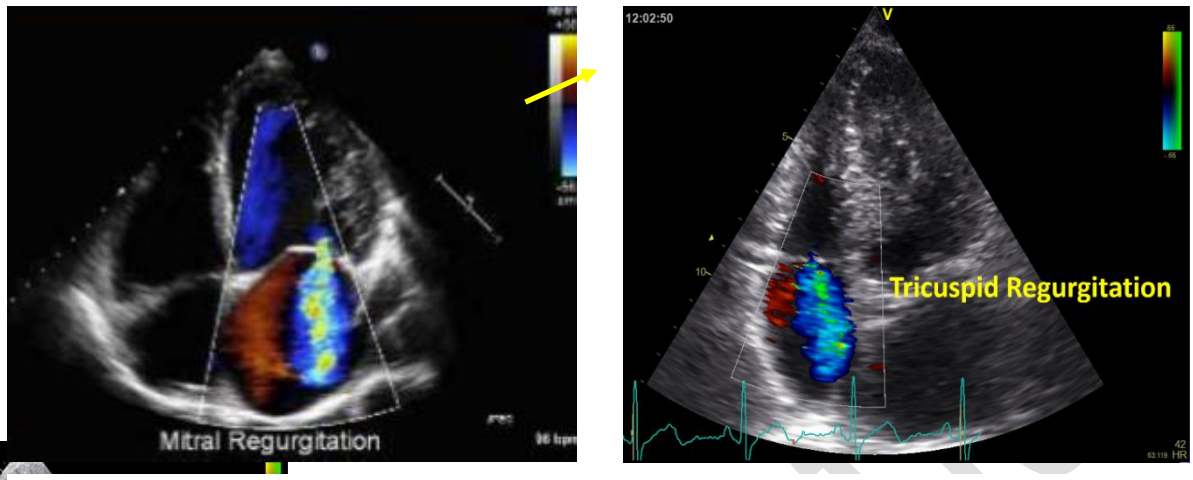


Figure 6 Left: Example of a mitral regurgitation (colour Doppler placed on the left atrium); right: Example of a tricuspid regurgitation (colour Doppler placed on the right atrium)

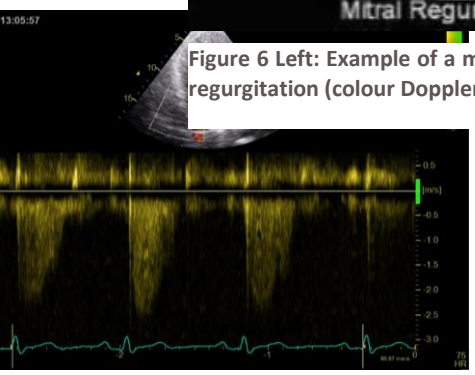


Figure 7 Example of a continuous wave Doppler placed on the origin of a tricuspid regurgitation, the signal can be used to gain information on pulmonary systolic pressure

To finish the recordings in the apical 4-chamber view, two more images need to be recorded: one image with the pulsed wave Doppler placed on the leaflets of the opened mitral valve (*11*; assessment of E and A wave) and the pulsed wave Doppler with tissue Doppler placed on the medial mitral annulus (*12*; assessment of é). Both, E and é are main parameters in the evaluation of the diastolic function (Nagueh et al. 2016).

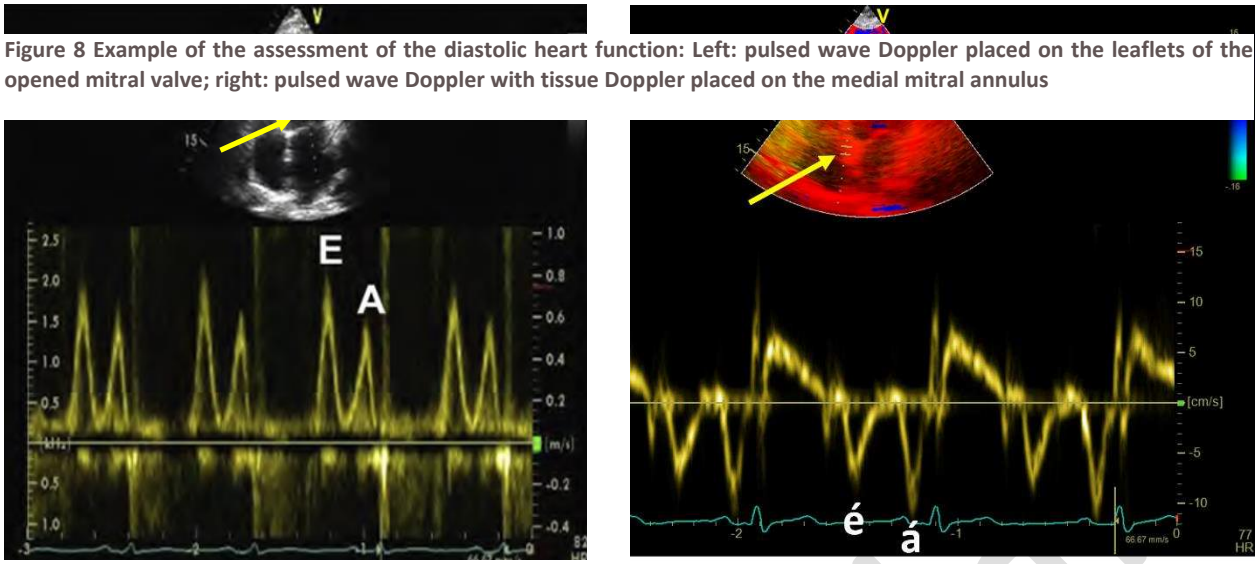


Figure 8 Example of the assessment of the diastolic heart function: Left: pulsed wave Doppler placed on the leaflets of the opened mitral valve; right: pulsed wave Doppler with tissue Doppler placed on the medial mitral annulus

2.4 The apical 2 chamber view (*13,* [*14*])

Like the apical 4 chamber view, left ventricle and left atrium should be recorded in full length (*13*). If the left atrium can't be recorded in full length together with the left ventricle, an additional loop with focus on the left atrium **should be** recorded (*14*; assessment of the left atrial volume with the Biplane Area-Length Method). No further loops or images are required.

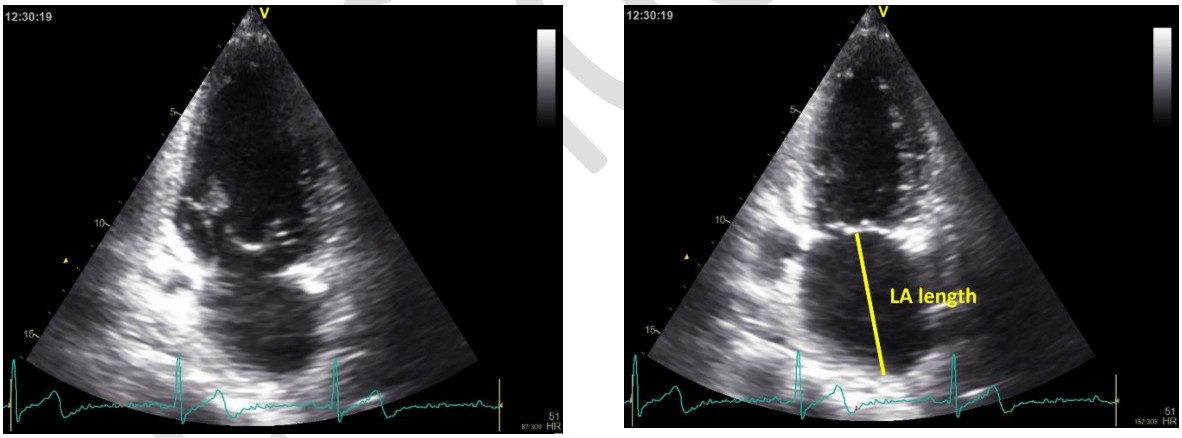


Figure 9 Example of an apical 2-chamber view (left: focus on the left ventricle; right: focus on the left atrium)

2.5

The apical 3 chamber view (*15*)

In the apical 3 chamber view the left ventricle at full length, left atrium and aortic root must be visible (*15*). No further loops or images are required.

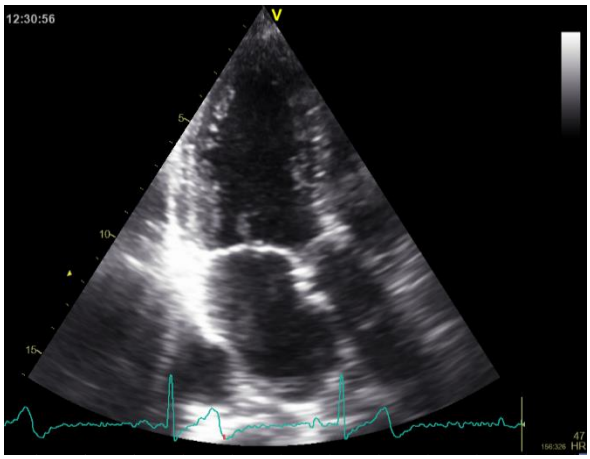
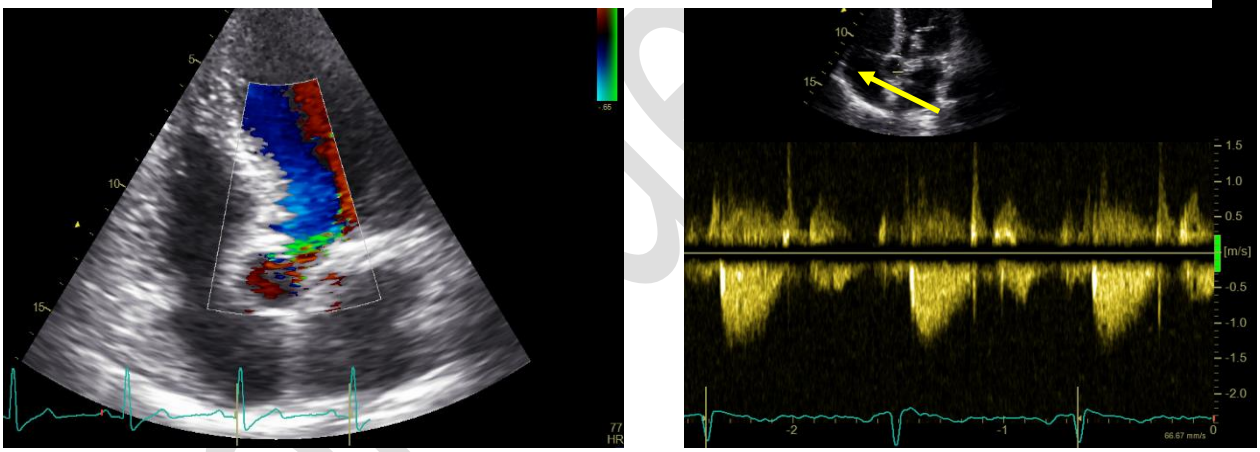


Figure 10 Example of an apical 3-chamber view

2.6 The apical 5 chamber view (*16 17*)

In the apical 5 chamber view, one loop with colour Doppler over the aortic valve should be recorded (*16*). Finally, one image with the continuous wave Doppler placed directly on the aortic valve should be recorded (*17*).

Figure 11 Example of an apical 5-chamber view (Left: colour Doppler placed over the aorta to gain information of an aortic regurgitation; right: continuous wave Doppler placed on the position of the aortic valve (measurement of gradients)



3 Summary

- ECG recordings on each loop or Image

PLAX

1 **Loop** of the PLAX

2 Image with M-mode of the left ventricular dimensions

3 Image with M-mode of the aortic root and the left atrium

PSAX

4 **Loop** of the PSAX at the level of the papillary muscles

5 **Loop** of the PSAX at the level of the apex

Apical 4 chamber view

6 **Native loop** of the 4-chamber view with focus on the left ventricle

7 Native loop of the 4-chamber view with focus on the left atrium

8 **Loop** of the 4-chamber view with **colour Doppler** placed on the **left atrium**

9 **Loop** of the 4-chamber view with **colour Doppler** placed on the **right atrium**

10 Image of the tricuspid regurgitation signal (CW Doppler on origin of jet into right atrium)

11 Image of E and A wave (PW Doppler placed on the leaflets of the opened mitral valve)

12 Image of septal é (TVI and PW Doppler placed on the medial mitral annulus)

Apical 2 chamber view

13 **Native loop** of the 2-chamber view with focus on the left ventricle

14 **Native loop** of the 2-chamber view with focus in the left atrium

Apical 3 chamber view

15 **Native loop** of the 3-chamber view with focus on the left ventricle (left atrium and aortic root must be visible)

Apical 5 chamber view

16 **Loop** of the 5-chamber view with **colour Doppler** placed on the aortic valve/left ventricle

17 Image of aortic blood outflow velocity (CW Doppler placed directly on the aortic valve)