Role of Comorbidities in Chronic Heart Failure

(RoC-HF) Study – Study Protocol

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Prinicipal Investigators:

KLEMENS ABLASSER, MD*

Department of Cardiology, Medical University Graz, Auenbruggerplatz 15, 8036

Graz, email: klemens.ablasser@medunigraz.at

NICOLAS VERHEYEN, MD

Department of Cardiology, Medical University of Graz, Auenbruggerplatz 15, 8036

Graz, email: nicolas.verheyen@medunigraz.at

*klinischer Prüfarzt

Heart Failure Outpatient Clinic, Department of Cardiology

FRITZ FRUHWALD, MD, PROF

Department of Cardiology, Medical University of Graz, Auenbruggerplatz 15, 8036

Graz, email: fritz.fruhwald@medunigraz.at

X-Ray, Department of Radiology

JOHANNES SCHMID, MD

Department of Radiology, Medical University of Graz, Auenbruggerplatz 9, 8036

Graz, email: Johannes.schmid@meduingraz.at

Osteoporosis Outpatient Clinic, Division of Endocrinology

ASTRID FAHRLEITNER-PAMMER, MD

Division of Endocrinology, Department of Internal Medicine, Medical University of Graz, Auenbruggerplatz 15, 8036 Graz, email: astrid.fahrleitner@medunigraz.at

Department of Cardiology

DIRK von LEWINSKI, MD, PROF

Department of Cardiology, Medical University of Graz, Auenbruggerplatz 15, 8036

Graz, email: dirk.von-lewinski@medunigraz.at

Contribution to Study Design

ANDREAS TOMASCHITZ, MD

Department of Cardiology, Medical University of Graz, Auenbruggerplatz 15, 8036

Graz, email: andreas.tomaschitz@medunigraz.at

STEFAN PILZ, MD, PhD, PROF

Division of Endocrinology, Department of Internal Medicine, Medical University of Graz, Auenbruggerplatz 15, 8036 Graz, email: stefan.pilz@medunigraz.at

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Abbreviations

ABPM, ambulatory blood pressure monitoring

CI, clinical investigator

DXA, dual energy X-ray absorptiometry

PWA, pulse wave analysis

PWV, pulse wave velocity

HFpEF, heart failure with preserved ejection fraction

HFrEF, heart failure with reduced ejection fraction

1. Protocol Synopsis

Studien ID: 01/2016/ROC-HF/KANV

Version: 6.0

Date: 07. Aug 2018

Study title:

Role of Comorbidities in Chronic Heart Failure (RoC-HF) Study

Study aims:

Aims:

- To quantify the prevalence of osteoporosis in HFrEF (primary aim)
- To quantify the prevalence of vertebral fractures in HFrEF (primary aim)
- To quantify the 24-hour central blood pressure in HFrEF (secondary aim)
- To quantify arterial stiffness in HFrEF (secondary aim)

Exploratory aims:

- To elucidate the role of the bone-vascular axis in HFrEF
    - Relationship between bone metabolism and cardiac systolic function in dependence of vascular status
    - Relationship between bone metabolism and cardiac diastolic function in dependence of vascular status
- To elucidate the role of central arterial pressures and cardiac function in HFrEF
- To elucidate the relationship between arterial stiffness and cardiac stiffness in HFrEF
- To find or apply novel blood- or urine-derived biomarkers that may improve our understanding of mechanisms linking heart and bone pathologies.

Study design:

Longitudinal, prospective, single-center, epidemiological cohort study.

Study procedures will be performed at the Medical University of Graz, Graz, Austria: Division of Endocrinology (DXA scans), Department of Radiology (X-ray) and Department of Cardiology (all other procedures).

Duration of active study participation: 2 x 4 hours on two separate days, follow-up visits planned as part of a separate protocol

Study population:

Male or female subjects with stable chronic heart failure with reduced ejection fraction

Number of participants: 205,

Inclusion criteria:

1. Age >= 18
2. NYHA class II-IV symptoms
3. Left ventricular ejection < 50% at Visit 1
4. Treatment according to current Heart Failure Guidelines of the European Society of Cardiology
5. Willingness and ability to provide signed informed consent form (ICF) prior to participation in any study-related procedures
6. Previous diagnosis of heart failure with reduced ejection fraction defined as symptomatic left ventricular ejection fraction < 40% requiring optimization of heart failure therapy

Exclusion criteria:

1. Unplanned hospitalization within 1 month prior to the Baseline Visit.
2. Discontinuation or initiation of a pharmacologic or device treatment for HFrEF within 1 month prior to the baseline visit.
3. Coronary or peripheral revascularization procedures, valvular procedures, OR any major surgical procedure within 3 months prior to the Screening Visit.
4. Acute coronary syndrome (ACS), stroke or transient ischemic attack (TIA) within 3 months prior to the Screening Visit.
5. Any acute illness
6. Disease reducing life expectancy to <1 year, except HFrEF
7. Recipient of any organ transplant
8. Primary significant valve disease (at least moderate to severe valve disease)

2. Introduction

Chronic Heart failure with reduced ejection fraction (HFrEF) is a major health problem, due to high and increasing prevalence with high morbidity and mortality (1). Currently, approximately 250.000 to 300.000 people in Austria have a diagnose of heart failure, a disease with a two-year mortality that is higher than in most patients with cancer (2). Around 50% of all patients hospitalized with heart failure have a serious event within 60 days of discharge (3). Patients with HFrEF are exposed to a death rate of up to 141 deaths per 1000 patient-years. After hospitalization for heart failure reported median survival range between 1.3 and 2.4 years (4); Ninety percent of heart failure patients die from cardiovascular causes; it is, however, important to acknowledge that co-morbidities crucially contribute to disease progression (5, 6) and the number of non-cardiac co-morbidities predicts all-cause hospitalizations and even short-term mortality (6, 7).

There are many etiologies leading to HFrEF and heart failure is more often considered to be a symptom with many co-morbidities influencing the prognosis, rather than an isolated cardiac dysfunction. The most common etiology leading to HFrEF is coronary artery disease (CAD). In randomized clinical heart failure trails 68% of patients presented with ischemic cardiomyopathy (8). In Europe the ischemic cardiomyopathy is believed to be the primary etiology in 41 % of chronic heart failure patients (9). Classic Risk factors for CAD include arterial hypertension, diabetes, smoking, obesity and hypercholesteremia. Central hemodynamic markers, such as central blood pressure, and measurements of arterial stiffness have shown independent and additive prognostic value for cardiovascular risk in the general population (10, 11), in patients with arterial hypertension (12, 13) and in patients with HFpEF (14). However this has not been shown in HFrEF patient.

The second most common reported HFrEF is dilative cardiomyopathy attributed to about 25% of chronic heart failure patients. Dilative cardiomyopathy is a descriptive term and has several underlying causes including inflammatory cardiomyopathy or toxic associated cardiomyopathy. CAD is often not present and does not significantly contribute to the progression of the disease. Arterial hypertension is thought to be risk factor for the development and progression of dilative cardiomyopathy. However little is known about the central blood pressures or arterial stiffness in this subset of patients.

HFrEF is associated with altered bone metabolism and novel evidence from our research group strongly suggests that bone metabolism interferes with cardiac function. However, the prevalence of altered bone metabolism and osteoporosis as well as the link with cardiac function has not been analyzed in patients with chronic heart failure. Given that bone metabolism in patients with HFrEF responds to hemodynamic changes (15), vascular function may play a mediating role in the bone-

heart axis. However, epidemiological studies totally lack to disentangle these complex relationships in HFrEF.

The role of bone disease in this context has attracted increasing attention. Experience of a hip fracture doubles the mortality rate in heart failure patients (16) Moreover, treatment of osteoporosis after hip fracture reduced mortality in the HORIZON trial (17). Bone strength is crucially determined by bone metabolism. The role of bone metabolism in HFrEF is, however, completely unknown.

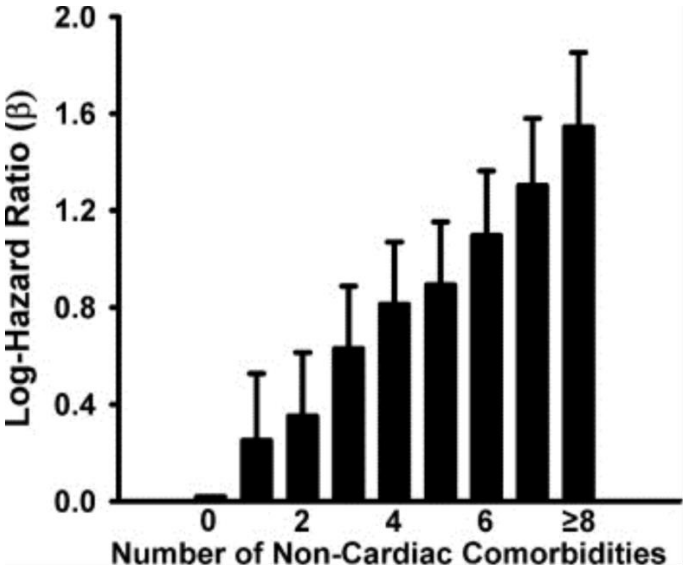

Figure 1: Risk for all-cause hospitalization as a function of number of non-cardiac co-morbidities in patients with heart failure (7).

Increasing evidence suggests that bone should be regarded as an endocrine organ. Of note, novel cross-sectional data from our research group and others point towards an independent link between bone metabolism and cardiac function. Proposed mechanistic links include cardiovascular effects of bone-derived peptides that are increasingly secreted in the course of HFrEF. These include effects on blood pressure, glucose metabolism or vascular calcification which may further promote cardiac remodeling and deterioration of cardiac function. The clinical relevance of these mechanistic links for cardiovascular outcomes in HFrEF remains elusive in large parts. This is due to the fact that available longitudinal analyses are limited by their retrospective approach and, importantly, the lack of profound profiling of study participants: studies on HFrEF patients lack important baseline information on bone characteristics such as bone mineral density or prevalence of vertebral fractures. Thus, current evidence is insufficient to fully enlighten the role of disturbed bone metabolism and bone status in HFrEF. The current “Role of Co-morbidities in Heart Failure”(RoC-HF study) is therefore designed to address the question whether disturbances in bone metabolism affect cardiac function and cardiovascular outcomes in a highly standardized cohort of HFrEF patients with comprehensive assessment of bone/ mineral metabolism, fracture status and bone mineral density at baseline.

Sample size calculation:

Based on previous epidemiological studies we expect a prevalence of osteoporosis in RoC-HF of 20 % (95% confidence interval: 14 -26). Calculating with a significance level of 5% and a two-sided P-value leads to a number of 171 participants to be analyzed. Calculating with 20% unavailable data due to invalid ABPM, missing laboratory values or other missing parameters, we will need to enroll 205 subjects in total.

3. Study aims

This study aims to create a large and previously unavailable database of patients with stable HFrEF with comprehensive assessment of bone, skeletal and vascular status. RoC-HF will facilitate cross-sectional and, eventually, longitudinal mechanistic epidemiological analyses to disentangle the role of the bone- vascular axis in HFrEF.

Aims:

- To quantify the prevalence of osteoporosis in HFrEF
- To quantify the 24-hour central blood pressure in HFrEF
- To quantify arterial stiffness in HFrEF

Exploratory aims:

- To elucidate the role of the bone-vascular axis in HFrEF
    - Relationship between bone metabolism and cardiac systolic function in dependence of vascular status
    - Relationship between bone metabolism and cardiac diastolic function in dependence of vascular status
- To elucidate the role of central arterial pressures and cardiac function in HFrEF
- To elucidate the relationship between arterial stiffness and cardiac stiffness in HFrEF
- To find or apply novel blood- or urine-derived biomarkers that may improve our understanding of mechanisms linking heart and bone pathologies.

4. Relevance

HFrEF is associated with altered bone metabolism and fractures increase mortality risk in patients with HFrEF (16). Yet. bone disease is not even listed among relevant co-morbidities in international HFrEF guidelines . Therefore, bone disease in HFrEF is underdiagnosed and undertreated.

Retrospective analyses indicate that osteoporosis as the manifestation of altered bone metabolism is present in approximately 1 of 5 patients with HFrEF (see **Table 5**). However, prospective studies lack to quantify the burden of osteoporosis in HFrEF. Given that some studies even indicated a mortality reduction by osteoporosis therapy (17) is it of critical importance to fill this gap in evidence. The RoC-HF study will prospectively determine the prevalence of osteoporosis, fractures and altered bone metabolism and the link with cardiac function in a well-standardized cohort of HFrEF patients. RoC-HF will thus provide novel and important evidence on the relevance of bone disease in HFrEF. It is the final aim of RoC-HF to establish bone disease as a relevant co-morbidity in international HFrEF guidelines and, thus, to improve medical treatment of these patients.

5. Study population

Male or female subjects with stable chronic heart failure with reduced ejection fraction.

Number of participants: 205

a. Recruitment

Patients will be recruited from the Department of Cardiology or the Department of Internal Medicine and will be contacted personally.

b. Inclusion criteria

1. Age >= 18
2. NYHA class II-IV symptoms
3. Left ventricular ejection < 50% at Visit 1
4. Treatment according to current Heart Failure Guidelines of the European Society of Cardiology
5. Willingness and ability to provide signed informed consent form (ICF) prior to participation in any study-related procedures
6. Previous diagnosis of heart failure with reduced ejection fraction defined as symptomatic left ventricular ejection fraction < 40% requiring optimization of heart failure therapy

c. Exclusion criteria

1. Unplanned hospitalization within 1 month prior to the Baseline Visit.

2. Discontinuation or initiation of a pharmacologic or device treatment for HFrEF within 1 month prior to the baseline visit.
3. Coronary or peripheral revascularization procedures, valvular procedures, OR any major surgical procedure within 3 months prior to the Screening Visit.
4. Acute coronary syndrome (ACS), stroke or transient ischemic attack (TIA) within 3 months prior to the Screening Visit.
5. Any acute illness
6. Disease reducing life expectancy to < 1 year, except HFrEF
7. Recipient of any organ transplant
8. Primary significant valve disease (at least moderate to severe valve disease)

d. Premature discontinuation of study participation

Subjects may be discontinued for the following reasons:

- CI decision for any reason
- Subject Decision: The subject or the subject's designee, (e.g., parents or legal guardian), requests to be withdrawn from the study.
- The subject is lost to follow-up

6. Investigational Plan

a. Study design

Longitudinal, prospective, single-center, epidemiological cohort study.

Study procedures will be performed at the Medical University of Graz, Graz, Austria:

- Division of Endocrinology (DXA scan)
- Department of Radiology (X-ray)
- Department of Cardiology (all other procedures).

Duration of active study participation: 3 (+2) days, follow-up visit planned

Study duration: 2 years for cross-sectional part, follow-up planned

b. Study schedule

Patients hospitalized at the Department of Internal Medicine/Department of Cardiology or who are consulting the Outpatient Clinic of the Department of Cardiology, will be screened for coherence with in/exclusion criteria either by a clinical investigator (CI) or by a study nurse. If a patient fulfills in/exclusion criteria, he or she will be informed by a CI about the possibility to participate at the RoC-HF study. If the patient is found to be eligible for study participation by a CI and agrees to participate, Visit 1 will be scheduled. All study-related procedures will only be initiated when the patient has signed the written informed consent form. Every study participant will be assigned a unique ID number. The first study participant will be assigned the study ID 001 and every further ID will increase by 1 with every new participant. All study IDs will be documented in the RoC-HF study book which will be kept locked safely at a place only accessible by the CIs. Study procedures include blood sampling (65 ml), spot urine sampling (12 mL), physical examination, assessment of vital signs, assessment of medication and medical history, echocardiography, electrocardiography, pulse wave analysis (PWA), pulse wave velocity (PWV), DXA-scan, 24-hours ambulatory blood pressure monitoring and X-ray of the spine.

NV will routinely assess the results of X-ray examinations and DXA scans following European Guidelines for diagnosis and management of osteoporosis (18). If found pathologies require initiation of medical treatment, the participant will be informed and an appointment at the Outpatient Clinic of the Division of Endocrinology will be scheduled.

c. Study visits

Visit 1

Visit 1 will be scheduled on the day of Screening or at a separate day. Study procedures will include the following:

1. Signing of informed consent forms (possible already during screening): General form, form of the Biobank Graz, separate form for data generation from stored samples.
2. Assessment of medical history
3. Assessment of medication history
4. Physical examination
5. ECG
6. Echocardiography
7. Spot urine pregnancy test for women of child-bearing age
8. 24-hours ambulatory blood pressure monitoring (ABPM).

9. Scheduling of visit 2, at least 2 days after visit 1 to facilitate performance of 24-hours ABPM. The patient will be advised to remove and switch off the ABPM device 24 hours after attaching. The patient will be advised to attend visit 2 as fasting and non-smoking over the preceding night.

Visit 2

Visit 2 will take place at least 2 days after visit 1 to facilitate performance of 24-hours ABPM. Visit 2 will take place in the Clinical Studies Outpatient Clinic of the Department of Cardiology. The participant should be fasting and should not have smoked over the previous night. Following study procedures will be performed:

1. X-ray of the thoracic and lumbar spine. Women of child-bearing age will not perform X-ray in case of a positive pregnancy test during visit 1.
2. Re-obtainment of ABPM device. If ABPM was unsuccessful it can be repeated once, if the patient agrees.
3. Vital signs (office blood pressure and heart rate)
4. Hand Grip
5. 4m Gait Speed
6. Sampling of blood (65 mL) and spot urine (12 mL)
7. PWA
8. PWV
9. Questionnaires
10. DXA-scan. Women of child-bearing age will not perform DXA in case of a positive pregnancy test during visit 1.

d. Study procedures

Blood/urine sampling – immediate determination

32mL of blood and 8mL-of spot urine will be processed directly after collection for the purpose of immediate determination of parameters, at the Clinical Institute of Medical and Chemical Laboratory Diagnostics (18mL) and at the Laboratory platform of Division of Endocrinology (14mL). An oversight is provided in **Table 1** and **Table 2**.

Table 1: Endo lab parameters from blood (14 mL).	
3mL EDTA-plasma (on ice)	**8mL Serum (room temperature)**
Parathyroid Hormone	TSH
Osteocalcin	fT3
FGF-23	fT4
CTX	25-hydroxy-vitamin D
	bALP
3mL EDTA-plasma (room temperature)	P1NP
Aldosterone (plasma aldosterone concentration)	P3NP
Renin (plasma renin concentration)	TRAP
	IGF-1
FGF-23, fibroblast growth factor-23; TSH, thyroid stimulating hormone; fT3, triiodothyronine; fT4, tetraiodothyronine; bALP. bone-specific alkaline phosphatase; P1NP, intact N-terminal propeptide of procollagen type 1; P3NP, amino-terminal propeptide of type III procollagen; TRAP, active isoform 5b of the tartrate-resistant acid phosphatase; IGF-1, Insulin-like growth factor 1.	

Table 2: KIMCL parameters from blood (18mL) and urine (8 mL)	
Hematology (3mL EDTA plasma):	**Clinical Chemistry (8mL Li-Hep plasma):**
Hemoglobin	Sodium (Na^+)
Hematocrit	Potassium (K^+)
Erythrocyte count (RBC)	Chloride (Cl^-)
Leukocytes (WBC)	Total calcium (Ca^{2+})
Neutrophils, segmented	Phosphate
Lymphocytes	Magnesium (Mg^{2+})
Monocytes	Total bilirubin
Eosinophils	Alkaline phosphatase (AP)
Basophils	Alanine aminotransferase (ALT)
Platelets	Aspartate aminotransferase (AST)
$HbaA1_c$	γ-glutamyl-transferase (GGT)
Coagulation (3mL sodium citrate)	Urea
International normalized ratio (INR)	Creatinine
Prothrombin time (PZ)	Calcium
Activated partial thromboplastin time (aPTT)	Glucose
Urinalysis (8mL spot urine):	Albumin
Sodium (Na^+)	Total protein
Potassium (K^+)	Total cholesterol
Chloride	HDL/LDL (Friedewald formula)
Total calcium (Ca^{2+})	Trigylcerides
Creatinine	eGFR (CKD EPI equation)
Magnesium	Iron (Fe^{2+})
Phosphate	Transferrin
Protein	Ferritin
Albumin	C-reactive protein
Venous blood (1mL syringe)	Creatine-kinase (CK)
Blood gas analysis	Creatine kinase myoglobin isoform (CK-MB)
Sodium (Na^+)	Lactate dehydrogenase (LDH)
Potassium (K^+)	hsTroponin T
Chloride (Cl^-)	NT-proBNP
Ionized calcium (Ca^{2+}_{ion})	Interleukin-6
Glucose	**Coagulation (3mL sodium citrate)**

Lactate	
	Von Willebrand factor (vWF)
$HbA1_c$, glycated hemoglobin A1c; NT-proBNP, n-terminal pro-brain B-type natriuretic peptide, hs, high sensitive.	

Blood/urine sampling – biobanking

Immediately after collection, 36 mL of blood and 4 mL of spot urine will be transferred to the biobanking facility of the Biobank Graz, located at the Department of the Clinical Institute of Medical and Chemical Laboratory Diagnostics (Auenbruggerplatz 15, first floor). A list of stored sampled is provided in **Table 3**. Subjects will be asked to sign the informed consent of the Biobank Graz.

Table 3: Blood and urine samples for biobanking (36mL + 4mL)		
Collected volumes	Aliquoted volumes	Total aliquoted volume (sum)
3x8mL serum	14*235 µL/aliquot 14* 580µL/aliquot	11.410 µL serum
2x6mL EDTA plasma	10*580µL/aliquot 1* 235µl buffy coat/aliquot	5.800 µL EDTA plasma 235µL buffy coat
1x4mL spot urine	4*580 µL/aliquot	2.320 µL spot urine

Analyses of gene mutations that may predispose for the development of heart failure or for cardiovascular risk factors will be analyzed. These analyses will be conducted from buffy coat that will be frozen to -80°C as part of the blood plasma sampling **(see Attachment 3)**. An overview on genes to be analyzed is provided in **Table 4**.

Table 4: Genes to be analyzed in genetic testing			
Gene name	**Location**	**Expressed protein**	**Function of protein**
MYBPC3	11.p11.2	Cardiac myosin binding protein C (cardiac MyBP-C)	Cardiac myocyte contractility
TTN	2q31.2	Titin	Myocyte contractility
MYH7	14q11.2	Myosin heavy chain beta (MHC-β)	
LMNA	1q22	Lamin A and C	Cytoskeleton stability
DES	2q35	Desmin	
CYP11B2	8q22	Aldosterone synthase	Regulates aldosterone concentration
CaSR	3q21	Calcium sensing receptor	Regulates parathyroid hormone concentration
Analyses of gene mutations that are not currently known to increase the risk of heart failure but may be identified in the future.			

Echocardiography

Echocardiographic examination will be performed with a Vivid 7 or Vivid 9 (GE Healthcare, Chalfont St Giles, UK). For acquisition of images in the parasternal view the participant will be placed in the steep left-lateral decubitus position. The participant's left arm will be raised. Images will be acquired during quiet respiration. Image rate will be set at least at 70 frames per second. Echocardiographic examination will be outlined in a separate SOP.

ECG

A 12-lead ECG will be obtained in supine position.

ECG intervals (PR, QRS, QT, QT_c), heart rate and pathologies (rhythm abnormalities, AV block, pathological Q, branch block, repolarization abnormalities) will be recorded for each subject.

Assessment of medical history

Detailed medical history (including dates):

- Any surgery
- Any cardiovascular interventions
- Smoking history (pack years)

- Cardiovascular events: stroke, myocardial infarction (STEMI, NSTEMI), other embolic or thrombotic event, cardiac decompensation, hospitalization for heart failure, peripheral vascular disease
- Cardiovascular disease: arterial hypertension, atrial fibrillation, heart failure signs (ankle edema, elevated jugular venous pulse) and symptoms (NYHA classification, angina pectoris, dizziness, syncope, palpitations)
- Chronic renal failure
- Pulmonary disease (COPD, asthma)
- Any other relevant illness
- Previous fractures?- traumatic/minor injuries/non traumatic
- Previous vertebral trauma
- Previous nephrolithiasis?
- Child-bearing potential? If yes, spot urine pregnancy test will be performed.

Assessment of medication history

Devices or drugs: names (INNs), dosing scheme (if applicable), date and indication of implantation/first prescription of regularly or intermittently taken drugs. Particular care will be taken to ask for treatment of:

1. Heart failure (medical): levosimendane, loop diuretics, ACE-inhibitors, angiotensin II receptor I blockers, mineralocorticoid receptor antagonists, neprilysin inhibitors, beta-blockers, ivabradine, digitalis or digoxin, amiodarone, other
2. Heart failure (devices): ICD, CRT
3. Coronary artery disease: aspirin, PY12 inhibitors, anti-anginal drugs (nitrates or similar)
4. Bone therapy: treatment for osteoporosis including PTH analogues, bisphosphonates, RANKL-antagonists, strontium-ranelate, estrogen receptor antagonists, vitamin D or analogues
5. Others: Pacemaker, oral anticoagulation, heparin, thiazide diuretics, other antihypertensive medication, drugs for psychiatric disorders

Pregnancy test

Women of child-bearing age will be asked to perform a routine spot urine pregnancy test. If the test result is positive, the participant will not perform X-ray and DXA during visit 2.

Physical examination

Lung auscultation, heart auscultation, assessment of peripheral edema, assessment of jugular venous pulse height, weight, height, height at 20 years of age. Further examinations will be symptom-oriented.

Questionnaires

- Minnesota Living with Heart Failure (MLWHF) questionnaires
- SF-36 questionnaire
- Physical Activity Questionnaire (IPAQ)
- Calcium questionnaire (CaQ, 19)
- Pedigree questionnaire

24-hours ambulatory blood pressure monitoring (ABPM)

The ABPM device will be attached by a study nurse in the Outpatient Clinic of the Department of Cardiology at Visit 1, before X-ray and after all other procedures of Visit 1 have been performed.

ABPM will be performed using the brachial, oscillometric, automated self-measurement mobil-O-Graph device (I.E.M. GmbH, Stolberg, Germany) with integrated ARCSolver®-Software (Austrian Institute of Technology Wien). The device was validated according to the criteria of the British Hypertension Society (20). The range of measurement is 70–260mmHg for systolic blood pressure, 45–180mmHg for diastolic blood pressure, and 40–240/min for heart rate. Before measurement arm circumferences will be measured and recorded to allow correct choice of cuff size. Measurements of blood pressure and heart rate will be performed every 30 minutes between 22:00h and 6:00h and every 20 minutes between 6:00h and 22:00h. Daytime will be defined as from 09:00 to 21:00, nighttime will be defined as from 01:00 to 06:00 and evening will be defined as from 21:00 to 01:00 according to the ESC position paper 2013 on ambulatory blood pressure monitoring (21). If the ambulatory recordings will be longer than 24 hours, only the first 24 hours will be used for analysis starting the 24 hours timeframe from the first valid recorded measurement. The ARCSolver®-method allows the calculation of PWA values and PWV with each blood pressure measurement.

The ABPM device will be placed on the non-dominant upper arm, except if the office blood pressure measurements reveals more than 20mmHg systolic or more than 10mmHg diastolic difference between the two arms. In that case the arm with the higher blood pressure will be used. The patient

will be instructed on proper handling of the ABPM including resting the arm at the level of the heart during the measurements. Patients will be informed that they should conduct normal daily activities, however they should restrain from strenuous sports.

If less than 70% of the measurements over 24 hours are invalid the ABPM will be considered as unsuccessful and will be redone.

X-ray of the spine

Women of child-bearing age will not perform X-ray in case of a positive pregnancy test during visit 1. X-ray examination, performed at the Department of Radiology, will be the first study procedure performed at visit 2. Afterwards, the participant will be transported to the Cardiology Outpatient Clinic

X-rays of thoracic and lumbar spine will be performed in anterior-posterior and lateral projections. The procedure will take approximately 5 minutes in total. All images will be analyzed by one radiologist (JS) who will be blinded to all patient-specific data. Unclear findings will be reassessed by a second radiologist and a consensus result will be reported. Fractures will be graded according to Genant Score (GS). Parameters will be collected according to **Table 5**.

Table 5: Parameters to be determined from X-ray of the vertebral spine.

		Thoracic						Lumbar		
Genant Score: 1: 20-25% height reduction 2: 25-40% height reduction 3: >40% height reduction 0: no fracture according to GS NA: not assessible	deformity: w: wedge b: biconcave c: crush n: no deformity	1.			7.			1.		
		2.			8.			2.		
		3.			9.			3.		
		4.			10.			4.		
		5.			11.			5.		
		6.			12.			(6.)		
Sum of fractures										
Sum of fractures > GS 1										
Severe scoliosis (0/1)										
Degeneration (mild/moderate/severe)										
Other pathologies										

If found pathologies require initiation of medical treatment according to (18), the participant will be informed and an appointment at the Outpatient Clinic of the Division of Endocrinology will be scheduled.

Vital signs (office blood pressure and heart rate)

The office blood pressure and heart rate will be measured according to the current ESH/ESC guidelines (22). A validated semi-automatic blood pressure monitor will be used to measure the blood pressure on both upper arms. The arm with the higher measured value will be used for the further measurements. In one-minute intervals the blood pressure will be recorded two more times. The first measurement will be ignored and the average of the second and third blood pressure measurement will be recorded in the CRF as office blood pressure and the average of the two heart rates will be recorded as office heart rate.

Hand Grip Strength

The hand grip strength will be determined as a measure of "weakness" to calculate the "Cardiovascular Health Study Frailty Screening Scale" (23) a recommended tool to assess frailty (24). The JAMAR Hydraulic Hand Dynamometer (Model J00105) (Lafayette Instrument Company, USA) will be used, a current gold standard to measure hand grip strength (25). The recording will be done according to the Southampton protocol, a well-established protocol for measurement of grip strength in epidemiological studies of older people, which is based on the ASHT protocol (25). The patients are seated comfortably in a standard chair with legs, back support and armrests. They will rest their forearms on the arms of the chair with their wrist just over the end of the arm of the chair in a neutral position with thumb facing upwards. The handgrip dynamometer will be demonstrated to the patient. The instrument is positioned so that the thumb is round one side of the handle and the four fingers are around the other side. The handgrip dynamometer has to sit comfortably in the hand for the patient, otherwise the position will be slightly altered. The base of the hand grip instrument will have to rest on the palm of the investigator, to support the weight, but care has to be taken to not restrict the movement. The patient will be encouraged to squeeze the handgrip dynamometer as long and tightly as possible until the needle of the instruments stops rising. The result will be documented to the nearest 1kg. First the right hand strength then the left hand strength will be examined and this will be repeated twice to get six overall measurements. The highest hand grip strength recording out of all six measurements will be documented and used for the study.

4-meter Gait Speed test

As a measurement of "slowness" to calculate the "Cardiovascular Health Study Frailty Screening Scale" (23) the 4 meters gait speed test will be conducted. Gait speed will be measured over a 4 meters. Subjects will begin the test from a standing position and are instructed to walk at their usual

pace a distance of 4 meters. The time will be recorded. A slow gait speed is considered with a walking speed <0.8 m/s (26, 27).

PWA

The pulse wave analysis (PWA) will be performed using the SphygmoCor® (AtCor Medical, Sydney, Australia), which allows a noninvasive central blood pressure assessment. The patient will have to lie in a supine position for 15 minutes before the start of the measurements and care will be taken to assure a quite environment. Initially the brachial blood pressure will be recorded in supine position and the SphygmoCor® will be calibrated using this measurement. Using the technique of the radial applanation tonometry the pulse wave analysis will be performed with the SphygmoCor® to measure at least two PWA recordings. Only recordings with a quality index above 80% will be accepted and otherwise the measurements will have to be repeated. If there are major differences between the parameters, such as an augmentation index difference of more than 4%, a third measurement will be done. The PWA results in which the measured values are closer to each other will be used.

PWV

The carotid-femoral pulse wave velocity (PWV) will be determined using the SphygmoCor® (ATCOR Medical, Sydney, Australia) immediately after performing the PWA in supine position. It is considered the noninvasive gold standard to determine the PWV and is approved by the Federal Drug Administration (FDA) (28). The recordings will be performed at the carotid artery and at the femoral artery. The distances from the palpable carotid pulse to the suprasternal notch (fossa jugularis sternalis) as well as the distances from the palpable femoral pulse to the suprasternal notch will be determined by measuring tape (29). To calculate the PWV the “Intersecting Tangents” algorithm will be chosen. The recording will be done twice. If the two results differ by more than 1,5 m/sec, a third measurement will be taken. The two PWV measurements, which are closer to each other, will be used and the other one discarded.

DXA-scan

Women of child-bearing age will not perform DXA in case of a positive pregnancy test during visit 1. The participant will be transported/accompanied by the study nurse to the Outpatient Clinic of the Division of Endocrinology. DXA scan will be performed and will include scan of lumbar spine, femoral neck, distal radius and full size for the purpose of estimating percentage body fat according to the departmental Standard Operating Procedure. Body regions are defined using standard anatomical partitions. Scan areas are analysed to determine lean mass, fat mass, bone mineral content, and total body composition. If found pathologies require initiation of medical treatment according to European Guidelines for diagnosis and management of osteoporosis (18), the participant will be

informed and an appointment at the Outpatient Clinic of the Division of Endocrinology will be scheduled.

If repetition of any study procedures is not necessary, DXA-scan will be the last procedure of Visit 2.

Pacemaker, ICD or CRT interrogation

Most chronic heart failure patients have, according to current guidelines, an ICD (implantable cardioverter-defibrillator) or CRT (cardiac resynchronization therapy) implanted. Those Devices can record arrhythmias and deliver therapeutic actions if necessary. Standard interrogation of the devices will be done during the study to record device parameters and recorded arrhythmic events.

6. Study medication

None.

7. Randomization

None.

8. Ethical considerations/safety

RoC-HF is designed as a cross-sectional, non-interventional study, with an option for longitudinal follow-up. Therefore, the only safety risk imposed to participants may refer from study procedures. None of the planned procedures will be invasive and only blood sampling will be semi-invasive. Particular care was taken to reduce the volume of blood samples to a minimum: many biomarkers of interest will be determined immediately which will save volume. Therefore, the volume of blood samples to be stored at the Biobank Graz will be relatively low when compared to other studies. Nevertheless, it will be crucially important to perform biobanking because this will facilitate achievement of one the major goals of the RoC-HF study: to biochemically disentangle the complex relationships between these co-morbidities (bone disease, vascular disease) with each other and with cardiac function in HFrEF. Radiation exposure should be mentioned as another safety issue:

Patients will undergo X-Ray examinations of the spine and DXA scans (femur, distal forearm, vertebral spine), which pose a certain radiation exposure. However the total radiation dose (depending on body constitution a maximum of 1.5 mSv for X-ray and approx. 0.3 mSv for DXA; in total <0.01% increased cancer mortality risk per patient) seems acceptable. Given that osteoporosis is largely underdiagnosed in HFrEF populations, participation in the RoC-HF study may turn out

beneficial for a significant proportion of participants as the comprehensive assessment of the patients' bone and mineral status may lead to consecutive initiation of preventive treatment by an experienced osteologist if indicated (AF, KA, SP). Of note, osteoporosis treatment reduces mortality and thus, performance of X-ray/DXA scan may, in fact, turn out to be beneficial for the participant. Women of child-bearing age will not perform X-ray and DXA in case of a positive pregnancy test during visit 1.

To conclude, the safety risk of participation in the RoC-HF is minimal; on the other hand, diagnosis and treatment of previously undiagnosed osteoporosis will be beneficial for many participants. Therefore, we consider the conduct of the RoC-HF study to be ethically justifiable.

9. Sample size calculation

The sample size calculation of RoC-HF is based on data from previous analyses that reported prevalence data of osteoporosis among patients with HFrEF leading to an expected prevalence of osteoporosis in RoC-HF of 20%. Osteoporosis is defined as a T-Score < -2.5 (measured by DXA), and severe osteoporosis is defined as a T-Score < -2.5 and presence of a fragility fracture, e.g., a vertebral fracture. In most of available studies on patients with HFrEF, data from DXA scans were not available. Therefore, presence of a vertebral fracture was commonly used as a criterion of osteoporosis. Thus, these studies likely underestimated the real prevalence of osteoporosis, because presence of a fracture may constitute only the "tip of the iceberg". Taken together, previous studies reported on prevalences of osteoporosis of 12 – 16% (see **Table 6**).

Table 6: Prevalence of osteoporosis as reported in previous heart failure cohorts

Study	Patient number	Heart failure definition	LVEF in %	Data
Lyons et al (30)	623	Listed for transplant	32 +/- 16	12% with vertebral fractures
Mazziotti et al (31)	1031 (430 vs 601 healthy controls)	ICD + revision of clinical records	27 (15-45)	16.1% with vertebral fracture
Youn et al (32)	65	Acute decompensated heart failure	28 +/- 12 (males) 36 +/- 16 (females)	15% with T-Score < -2.5

Given that the RoC-HF study procedures comprise DXA scan as well as X-ray of both the thoracic and lumbar spine, we expect a prevalence of osteoporosis in RoC-HF of 20 % (95% confidence interval: 14 -26). Calculating with a significance level of 5% and a two-sided P-value leads to a number of 171

participants to be analyzed. Calculating with 20% unavailable data due to invalid ABPM, missing laboratory values or other missing parameters, we will need to enroll 205 subjects in total.

Support in sample size calculation was provided by the Institute for Medical Informatics, Statistics and Documentation of the Medical University of Graz (Regina Riedl, MSc).

10. Statistical analyses

Data will be illustrated by descriptive statistics using numbers and percentages for categorical variables and means and standard deviation or medians and interquartile range for continuous variables, respectively, as appropriate.

To test our primary hypotheses, the correlation between a bone formation marker or central arterial systolic pressure (independent variable, respectively) with LV systolic function (dependent variable) will be analyzed using multivariate linear regression analyses, with adjustment for age, sex, blood lipids, blood glucose, kidney function and other potentially confounding variables.

Differences of echocardiographic and other variables between patients with and without osteoporosis or other categorical variables will be compared using student's t-test, Mann-Whitney U test or analysis of variance (ANOVA), as appropriate. These differences will also be analyzed using multivariate analyses of covariance (ANCOVA), with osteoporosis (yes/no) as fixed factor, echocardiographic or other continuous variables dependent variable and age, sex, blood pressure, blood lipids, blood glucose, kidney function and other potentially confounding variables as covariates. Also other categorical variables of interest and their association with continuous outcome variables will be analyzed in the same manner.

Statistical analyses of the RoC-HF study will be the responsibility of the PIs or persons who received the permission by the PIs to perform statistical analyses. The PIs have published more than 15 original manuscripts in the field of epidemiological research, partly as first authors, and have thus proven their expertise to conduct statistical analyses. Support in statistical analyses will also be provided by Prof. Stefan Pilz or PD Andreas Tomaschitz who have authored more than 100 peer-reviewed original manuscripts in the field of epidemiological research.

11. Data processing

All generated data will be recorded in case report forms (CRF) and will be transcribed into Excel or SPSS datasets. In electronic datasets participating subjects can only be identified by their unique study ID, name and surname will not be recorded.

12. Data access

All CIs will have unlimited access to the dataset.

13. Responsibilities and competences

Only the principal investigators (Klemens Ablasser, Nicolas Verheyen) will have unlimited access to study data and will have the right to nominate co-authorships and the position of co-authors, on publications that originate from data of the RoC-HF study. Forwarding of anonymized RoC-HF data may only occur with permission of both the PIs.

14. Literature

1. Bui AL et al. Epidemiology and risk profile of heart failure. Nat Rev Cardiol 2010; 8(1):30–41.

2. Stewart S et al. More 'malignant' than cancer? Five-year survival following a first admission for heart failure. European journal of heart failure 2001; 3(3):315–22.

3. Felker GM et al. Diuretic strategies in patients with acute decompensated heart failure. The New England journal of medicine 2011; 364(9):797–805.

4. Jhund PS et al. Long-term trends in first hospitalization for heart failure and subsequent survival between 1986 and 2003: a population study of 5.1 million people. Circulation 2009; 119(4):515–23.

5. Braunstein JB et al. Noncardiac comorbidity increases preventable hospitalizations and mortality among medicare beneficiaries with chronic heart failure. Journal of the American College of Cardiology 2003; 42(7):1226–33.

6. Lee DS et al. Predicting mortality among patients hospitalized for heart failure: derivation and validation of a clinical model. JAMA 2003; 290(19):2581–7.

7. Ather S et al. Impact of noncardiac comorbidities on morbidity and mortality in a predominantly male population with heart failure and preserved versus reduced ejection fraction. Journal of the American College of Cardiology 2012; 59(11):998–1005.

8. Gheorghiade M et al. Chronic heart failure in the United States: a manifestation of coronary artery disease. Circulation 1998; 97(3):282–9.

9. Maggioni AP et al. Are hospitalized or ambulatory patients with heart failure treated in accordance with European Society of Cardiology guidelines? Evidence from 12,440 patients of the ESC Heart Failure Long-Term Registry. European journal of heart failure 2013; 15(10):1173–84.

10. Wang K-L et al. Wave reflection and arterial stiffness in the prediction of 15-year all-cause and cardiovascular mortalities: a community-based study. Hypertension 2010; 55(3):799–805.

11. Chirinos JA et al. Arterial wave reflections and incident cardiovascular events and heart failure: MESA (Multiethnic Study of Atherosclerosis). Journal of the American College of Cardiology 2012; 60(21):2170–7.

12. Sung S-H et al. White coat hypertension is more risky than prehypertension: important role of arterial wave reflections. Hypertension 2013; 61(6):1346–53.

13. Weber T et al. Wave reflections, assessed with a novel method for pulse wave separation, are associated with end-organ damage and clinical outcomes. Hypertension 2012; 60(2):534–41.

14. Weber T et al. Pulsatile hemodynamics in patients with exertional dyspnea: potentially of value in the diagnostic evaluation of suspected heart failure with preserved ejection fraction. Journal of the American College of Cardiology 2013; 61(18):1874–83.

15. Wu C et al. Dynamics of bone turnover markers in patients with heart failure and following haemodynamic improvement through ventricular assist device implantation. European journal of heart failure 2012; 14(12):1356–65.

16. Carbone L et al. Hip fractures and heart failure: findings from the Cardiovascular Health Study. European heart journal 2010; 31(1):77–84.

17. Lyles KW et al. Zoledronic Acid in Reducing Clinical Fracture and Mortality after Hip Fracture. The New England journal of medicine 2007; 357:nihpa40967.

18. Kanis JA et al. Guidelines for diagnosis and management of osteoporosis. The European Foundation for Osteoporosis and Bone Disease. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 1997; 7(4):390–406.

19. Macdonald HM et al. Validation of a short questionnaire for estimating dietary calcium intakes. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2014; 25(6):1765–73.

20. Wei W et al. Validation of the mobil-O-Graph: 24 h-blood pressure measurement device. Blood pressure monitoring 2010; 15(4):225–8.

21. O'Brien E et al. European Society of Hypertension position paper on ambulatory blood pressure monitoring. Journal of hypertension 2013; 31(9):1731–68.

22. Mancia G et al. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Journal of hypertension 2007; 25(6):1105–87.

23. Fried LP et al. Frailty in older adults: evidence for a phenotype. The journals of gerontology. Series A, Biological sciences and medical sciences 2001; 56(3):M146-56.

24. Morley JE et al. Frailty consensus: a call to action. Journal of the American Medical Directors Association 2013; 14(6):392–7.

25. Roberts HC et al. A review of the measurement of grip strength in clinical and epidemiological studies: towards a standardised approach. Age and ageing 2011; 40(4):423–9.

26. Muscaritoli M et al. Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia-anorexia in chronic wasting diseases" and "nutrition in geriatrics". Clinical nutrition (Edinburgh, Scotland) 2010; 29(2):154–9.

27. van Abellan Kan G et al. Gait speed at usual pace as a predictor of adverse outcomes in community-dwelling older people an International Academy on Nutrition and Aging (IANA) Task Force. The journal of nutrition, health & aging 2009; 13(10):881–9.

28. Pauca AL et al. Prospective evaluation of a method for estimating ascending aortic pressure from the radial artery pressure waveform. Hypertension 2001; 38(4):932–7.

29. Weber T et al. Noninvasive determination of carotid-femoral pulse wave velocity depends critically on assessment of travel distance: a comparison with invasive measurement. Journal of hypertension 2009; 27(8):1624–30.

30. Lyons KJ et al. The unrecognized burden of osteoporosis-related vertebral fractures in patients with heart failure. Circulation. Heart Failure. 2011 Jul;4(4):419-24

31. Mazziotti G et al. Prevalence of thoracic vertebral fractures in hospitalized elderly patients with heart failure. European journal of endocrinology. 2012 Dec;167(6):865-72.

32. Youn JC et al. Exercise capacity independently predicts bone mineral density and proximal femoral geometry in patients with acute decompensated heart failure. Osteoporos Int. 2015 Aug;26(8):2121-9.