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Novel Biomarkers in Heart Failure with Reduced and Preserved Ejection Fraction in the General Population

Dissertation

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1 Introduction

1.1 Heart Failure

1.1.1 Epidemiology of Heart Failure

Cardiovascular diseases are among the leading causes of mortality worldwide, with heart failure (HF) contributing to a total amount of 5 % of all deaths (1). According to WHO statistics, there is no major difference in HF prevalence over the world (2), so the global burden of HF is massive. Worldwide, approximately 26 million people are living with HF (3).

HF is defined as a clinical syndrome, which is “caused by a structural and/or functional cardiac abnormality, resulting in reduced cardiac output and/or elevated intracardiac pressures at rest or during stress” (4).

Structural cardiac abnormality can be caused by a diseased myocardium (e.g. as a consequence of ischemic heart disease), abnormal loading conditions by arterial hypertension, valve diseases or arrhythmias (4).

While the overall mortality of HF has decreased over the last years, the morbidity of HF is growing continually (1).

Since 2006, HF has been the leading cause of hospital admissions in Germany, with over 455,000 admissions, approximately 1/50 of all hospitalizations (5).

HF becomes more common with increasing age, more than 80 % of all HF patients are older than 65 years (6). In the aging population, the prevalence of HF will increase further within the next decades (7).

HF also leads to significant costs in health care systems. In Germany, health expenditure costs due to HF have more than doubled within 10 years. They were around €5.3 billion in 2015 (8) compared with €2.5 billion in 2004 (9).

In the United States, health expenditure costs due to HF were around $31 billion, equivalent to more than 10 % of the total health expenditure for cardiovascular diseases (10). Total costs are expected to increase further by 127 % until 2030 (10), thereby leading not only to an increased disease burden but also to an economic burden.

1.1.2 Diagnosis of Heart Failure

At present, many cases of HF are not diagnosed correctly (11). Health professionals, especially non-cardiologists, are not confident in diagnosing HF and often fail to initiate the right medication according to guidelines (12).

Therefore, it is important to develop better diagnostic as well as therapeutic opportunities and clear management of HF.

A first step to diagnose HF can be the clinical diagnostics because patients with HF show typical signs and symptoms (Table 1).

Table 1 – Symptoms and signs typical of HF (4)

Typical symptoms	Specific signs
Breathlessness	Elevated jugular venous pressure
Orthopnea	Hepatojugular reflux
Paroxysmal nocturnal dyspnea	Third heart sound
Reduced exercise tolerance	Laterally displaced apical impulse
Fatigue, tiredness	
Ankle swelling	

The clinical degree of suffering can be classified by the New York Heart Association (NYHA) functional classification system (Table 2). It is well known that higher NYHA classes (III-IV) are connected to poor outcome and higher mortality (13,14).

Table 2 – NYHA functional classification system (15)

Functional Capacity	Objective Assessment
Class I. Patients with cardiac disease but without resulting limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	**A**. No objective evidence of cardiovascular disease.
Class II. Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	**B**. Objective evidence of minimal cardiovascular disease.
Class III. Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	**C**. Objective evidence of moderately severe cardiovascular disease.
Class IV. Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	**D**. Objective evidence of severe cardiovascular disease.

Clinical diagnostics can provide valuable information, but they are not reliable. Symptoms are unspecific and can be even present in subjects without HF, for example in the elderly or in patients with lung diseases. Signs are more specific but more difficult to diagnose (4).

Due to the latest guidelines of the European Society of Cardiology (ESC) in 2016, the diagnosis of HF is currently based on three essential investigations: electrocardiogram (ECG), echocardiography and the determination of blood levels of natriuretic peptides, which include the brain natriuretic peptide (BNP) and the N-terminal prohormone of brain natriuretic peptide (NT-proBNP).

Although the ECG has low specificity, this method can yield first information. HF is very unlikely in patients with a normal ECG (4).

In systematic echocardiography, several parameters are measured. The left-ventricular ejection fraction (LVEF or EF) is one of the most important parameters because it gives a measurable figure for the cardiac output. It is used to classify HF in three different types: HF with reduced EF (HFrEF), HF with preserved EF (HFpEF) and, since the newest ESC guidelines, HF with mid-range EF (HFmrEF).

HFrEF is present when the EF is < 40 %. HFmrEF is present when the EF is between 40–49 % and HFpEF when the EF is > 50 % plus evidence of diastolic dysfunction. The authors of the guidelines describe the term HFmrEF more as a new term for better classification in research. There are no target-oriented studies for the therapy of HFmrEF and the clinical relevance remains unclear.

HFrEF and HFpEF are seen as two different phenotypes with different etiology. Patients with HFpEF are older, more often female and typically suffering from hypertension and atrial fibrillation. As opposed to this, patients with HFrEF more often have a history of myocardial infarction (MI) (4).

These differences require different therapy approaches. At the moment, there is a lack of clear evidence for therapy methods for HFpEF, so further research is needed to define appropriate treatment (16).

Natriuretic peptides (BNP/NT-proBNP) are the current gold standard biomarker for HF, for both HFrEF and HFpEF (17). Currently, they are the only biomarker, which fit all criteria of a suitable biomarker (see 1.2 Biomarkers of Heart Failure):

1) the method is well-tested and established,
2) the measurement is easy, cheap and accurate,

3) important pathophysiological processes are reflected,
4) additional information to clinical examination is obtained (18).

Natriuretic peptides are synthesized in the myocardium due to mechanical and neurohumoral stimulation. For instance, ventricular wall stretch (e.g. because of fluid overload) activates the synthesis of natriuretic peptides, as well as the presence of cytokines, vasopressin or angiotensin-II, does (19). Cardiomyocytes release the prohormone proBNP, which is divided into the biological inactive peptide NT-proBNP and the active BNP in equal proportion. BNP decreases pre- and afterload, activates diuresis and acts vasodilating. Although it is biologically inactive, the measurement of NT-proBNP is preferred because of its longer half-life of 90-120 minutes compared to 20 minutes for BNP (20).
NT-proBNP has a high negative predictive value, thus HF is very unlikely in patients with NT-proBNP-levels below the cut-off (< 125pg/mL) (4). The disadvantage of NT-proBNP is, that the biomarker-level is influenced by other factors (age, obesity, atrial fibrillation, renal function), which makes it an unsafe parameter in populations e.g. of the elderly (21–23). Alternative biomarkers are therefore needed to improve the diagnosis of HF.

1.2 Biomarkers of Heart Failure

The term "biomarker" was defined by the Biomarkers Definitions Working Group: “A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” (24)
Following Morrow & De Lemos biomarkers should meet three criteria:
1) they should be measurable in a timely manner at a reasonable cost
2) they should add new information and
3) they should aid in the management of the disease (25).

For biomarkers in HF, the National Academy of Clinical Biochemistry set three important goals in their Practice Guidelines:
1) to identify causes of HF,
2) to confirm the HF syndrome and
3) to evaluate the risk stratification of the disease (26).

Furthermore, because of the occurrence of many novel biomarkers, Kimmenade & Januzzi declare four standards:
1) the method should be well tested and established,
2) the measurement should be easy, cheap and accurate,
3) the biomarker should reflect important pathophysiological processes. Biomarkers originating outside the myocardium are acceptable as long as they are providing independent useful information,
4) the biomarker should give additional information to clinical examination and other biomarkers (18).

Currently, the only biomarkers who fit these standards are natriuretic peptides (BNP, ANP) (18). They are the only established biomarkers in clinical care, but there are a few more novel biomarkers, which are promising candidates. Recent ESC guidelines do not address this topic, but the American Heart Association (AHA) states that novel biomarkers may be useful for providing additional risk stratification and the prognosis of chronic HF (27).
Among the currently discussed novel biomarkers are suppression of tumorigenicity 2 (ST2), growth differentiation factor-15 (GDF-15), and c-reactive protein (CRP).

1.2.1 Suppression of Tumorigenicity 2

The protein suppression of tumorigenicity 2 (ST2) is a member of the interleukin-1 receptor family. It was first described in 1989 when Shinichi Tominaga discovered that ST2 was similar to the extracellular portion of the interleukin 1-receptor. Without being sure about its function, he presumed that the “protein is possibly secreted as a signal molecule and has something to do with the growth signal transduction” (28). In further research, a transmembrane (ST2L) and soluble (sST2) isoform were identified as well as the ligand: interleukin-33 (IL-33) (29). The IL-33/ST2 system is upregulated in cardiomyocytes and fibroblasts as a response to mechanical stimulation or injury (30). Weinberg et al. also described that sST2 is inversely correlated with EF and raised the hypothesis that sST2 is increased chronically in patients with HF (31).
In an experimental mouse model, the interaction between IL33 and ST2L reduced cardiac hypertrophy and fibrosis. The cardioprotective effect of the pathway seems to be attenuated by sST2, which acts as a decoy receptor (32).

Elevated sST2-levels are discussed to be of importance to the diagnosis of HF and provide risk stratification in acute and chronic HF (33–35).
Because serial measurements of sST2 added value to established methods, it may also be a candidate for therapy guidance (36–38).
Many authors describe a multiple biomarker panel approach, in which sST2 was able to improve risk stratification (39–41).
In patients with HFrEF, increased sST2 was a stronger predictor of cardiovascular-mortality compared to natriuretic peptides (42). However, some authors could not confirm that sST2 is a useful predictor of cardiovascular events (43).
sST2 is less influenced by age, other traditional cardiovascular risk factors and renal function (33,44,45), which would make it superior to natriuretic peptides in this issue.

1.2.2 Growth Differentiation Factor 15

Growth differentiation factor 15 (GDF-15) was discovered as a member of the transforming growth factor-superfamily (46). It is weakly expressed in all tissues under physiological circumstances and shows increased plasma levels in response to pulmonary, cardiac, renal or cancer disease (47,48). In addition to this, the cardiac expression of GDF-15 is very low (49). Therefore GDF-15 is not viewed as a cardiac-specific biomarker. It is believed that GDF-15 reflects extracardiac disease manifestations in HF (50).
Although the exact biological function remains unclear, experiments in mice revealed that GDF-15 shows anti-hypertrophic (51), anti-inflammatory and anti-apoptotic properties (52). It is elevated as a response to inflammation and oxidative stress (53).
Previous studies showed that GDF-15 is an independent predictor of mortality and hospitalization in chronic HF (54). Lok et. al described that it was even stronger than NT-proBNP in predicting all-cause mortality (55).
GDF-15 is equally elevated and has comparable predictive value in patients with HFrEF and HFpEF (56). In discriminating HFpEF from a control group GDF-15 performed at least as well as NT-proBNP, and the ratio of NT-proBNP to GDF-15 was best to distinguish HFpEF from HFrEF (57).

1.2.3 C-Reactive Protein

C-reactive protein (CRP) belongs to the family of pentraxins and is an acute phase protein. It is synthesized in the liver and upregulated by interleukin-6 (IL-6), which is produced by stimulated monocytes (58). CRP mediates the complement system and activates macrophages. CRP has been established for years as an unspecific marker of systemic inflammation (59). Furthermore, studies have demonstrated the connection between CRP and cardiovascular diseases such as atherosclerosis (60) and hypertension (61). Increased levels of CRP in patients with chronic HF were firstly described in 1956 (62). CRP levels show the severity of HF (63). They are directly connected to the NYHA class and EF (64). Different authors demonstrated that CRP levels were an independent predictor of prognosis (65,66).

Because CRP tends to decrease with successful treatment, it may be useful for therapy guidance (67). It is not clear if CRP is just reflecting the inflammation, which is present in HF, or if it is directly involved in the pathogenesis of HF and therefore a potential target for the development of therapy (68,69).

1.3 Hypothesis and Aims of the Thesis

The prevalence of HF is increasing and the morbidity of HF, and especially HFpEF, is still high, while sufficient therapy is missing. The available data from other studies suggest that novel biomarkers may be useful in discriminating HF subtypes and risk stratification of HF.

Currently, only a few population-based studies, which analyzed the potential of novel biomarkers in larger cohorts and characterized the subtypes HFrEF and HFpEF are available so far.

Therefore, the aims of this doctoral thesis were:

1. to characterize circulating blood-levels of GDF-15, sST2, CRP, and NT-proBNP in a large sample of the general population,
2. to investigate the ability of these biomarkers for detecting prevalent HF and for predicting the outcome of HF and
3. to improve the discrimination of HFrEF from HFpEF by using a multi biomarker index based on the suggested biomarkers.

In order to achieve these aims, the data of the first 5,000 study participants of the population-based Gutenberg Health Study, with available biomarker levels, were analyzed.

2 Materials & Methods

2.1 Gutenberg Health Study

2.1.1 Study Design

The Gutenberg Health Study (GHS) was a population-based cohort study in the Rhine-Main region in Germany and was described in detail by Wild et. al (70). It was initiated at the Johannes Gutenberg-University Mainz in 2007 to improve the understanding of the pathogenesis and epidemiology of cardiovascular diseases. The focus of the study was to evaluate the prevalence of cardiovascular diseases and to identify risk factors. The study analyzed the association between influencing factors and disease outcomes and aims at deriving an improved individual cardiovascular risk stratification in detail. Additional goals were the scientific research of eye diseases, the immunologic and metabolic system, and cancer.

A random sample of 35,000 men and women was drawn from the governmental local registry offices in the city of Mainz and the district of Mainz-Bingen. The sample was stratified 1:1 by age and gender. Subjects of the higher age groups were overrepresented to count on enough cardiovascular events for statistical analysis.

Inclusion criteria were:

- Inhabitant of the city of Mainz or the district Mainz-Bingen at the time of inclusion into the study (inclusion is the time of written consent),
- age 35 to 74 years,
- personally signed informed consent.

Exclusion criteria were:

- Insufficient knowledge of the German language, in order to understand study documents and computer-assisted interviews without translation,
- physical or psychological incapability to travel to the study center and to cooperate in the investigations.

Therefore, subjects with prevalent heart disease were also included in the study. Finally, 15,010 subjects participated in the GHS and gave written informed consent. The subjects were examined in the study center at the Johannes Gutenberg-University and received a follow-up examination after 2.5 years (FU 1) and another after five years (FU 2). FU 1 was performed as a computer-assisted telephone interview with the following items:

- Endpoints
- Medical history and medication

- Sociodemographic data
- Lifestyle factors

Subjects were followed up after five years, and the primary endpoints of the study were:

- Incident myocardial infarction
- Cardiac death

The secondary endpoints of the study were:

- Death of all causes
- New onset of stroke
- New onset of diabetes mellitus
- New onset of heart failure

The tertiary endpoints of the study were:

- Cancer
- Eye diseases
- Diseases of the immune system
- Metabolic disorders

Various other tertiary endpoints (e.g. onset of hypertension, progression of cardiovascular disease) were analyzed in the project.

The study protocol was approved by the local review board of the University Medicine Mainz and the ethics committee of the State Chamber of Physicians of Rhineland-Palatinate, with reference number 837.020.07.
The analyses in this doctoral thesis were performed with the data of the first 5,000 study participants with available biomarker levels. The median follow-up time was seven years. During follow-up, 213 study participants of this subset died of any causes.

2.1.2 Examination and Biobanking

For comparable examination conditions, every subject was invited to the study center at the University Medical Center Mainz and received the same examination with the same preparation and examination-order. The whole examination took five hours and contained a computer-assisted interview with a standardized

questionnaire, several medical-technical examinations, and biobanking (see Appendix A).

Because of the relevance for this paper, the focus will be on the detailed procedure of biobanking and echocardiography here:

Every subject was asked to fast for several hours (at least 11 when the appointment was before 12.00 a.m. and at least five hours when the appointment was after 12.00 a.m.). The subjects could drink pure water and take their usual medication except for vitamin-containing medication. They were asked not to do any sports or consume alcohol within eight hours and not to eat rich food within twelve hours prior to the investigation.

Overall 114.5ml blood was collected by trained personnel, following a detailed standardized procedure. The biomaterial was processed according to standardized procedures into serum/EDTA plasma and nucleic acids and the different biomaterials were stored at -80°C.

2.1.3 Echocardiography

Every subject underwent systemic echocardiography with a Philips iE 33 ultrasound system. Trained medical technical assistants performed two- and three-dimensional echocardiography and measured basic parameters (see Appendix B).

Subjects were identified as having HF if symptoms of HF (according to NYHA class II-IV) were reported during a structured medical interview or typical medication for the treatment of HF was present. They were classified as HFrEF when LVEF measured by echocardiography was < 50 %. HFpEF was defined with the same criteria but an EF of ≥ 50 % and evidence of diastolic dysfunction, which was shown with echocardiographic parameters, either E/e'-ratio ≥ 12 or [(8 ≤ E/e′-ratio < 12) and (E/A ≤ 0.5)]. All other participants were defined as "No HF". The ESC-guidelines for the diagnosis and treatment of acute and chronic heart failure were updated in 2016. The authors created the new term "HF with mid-range EF (HFmrEF)" for patients with HF and an LVEF that ranges from 40 to 49 % (4). The cohort of patients with HFmrEF was examined additionally (n = 21). Nevertheless, subjects with HFmrEF were included in the HFrEF cohort for the analyses because characteristics were comparable (see 3.1 Characteristics of Study Participants). Therefore, the sample size of the HFrEF cohort increased (n = 38).

2.2 sST2 Measurements

The measurement of sST2 levels in serum samples was performed using the „Presage® ST2 Assay" by Critical Diagnostics, which is a quantitative sandwich monoclonal enzyme-linked immunosorbent assay (ELISA).

The principle of a sandwich ELISA is the use of two antibodies. The assay plate is coated with an antibody, which captures the antigen. After the antigen has been captured, the second antibody is added to bind on the surface of the antigen. Then the antigen is bound between two antibodies just as in a "sandwich". An enzyme-linked antibody is added, which binds the Fc region of the detection antibody. Any unspecific binding to the assay surface or antibody is prevented by washing the plate after every single step. A chemical substrate that is converted in a detectable form by the enzyme is also added. The detection can occur via photometric measurement of a color change or fluorescence.

Serum samples of GHS subjects (n = 5,000) were stored at -80°C in 96-well-plates. Samples were thawed overnight at 4°C prior to the measurements. The reagent assays were stored at 4°C. Prior to use the serum samples and the reagent assays were equilibrated to room temperature for one hour. Preparation and measurements of sST2 were performed according to the Presage® ST2 Assays manual.

Serum samples were diluted 1:50 with sST2 sample diluent (MOPS buffered). A serial dilution of the standard calibrator (recombinant human ST2) was performed to create a standard calibration curve. 100 µL diluted patient samples and standard calibration were filled in the assay microtiter plate (coated with mouse monoclonal anti-human sST2 monoclonal antibodies) and incubated at 600 revolutions per minute (rpm) for 60 minutes at room temperature.

After the incubation, the plates were cleaned by a mechanical plate washer (Tecan HydroFlex) with three cycles of 350 µL wash buffer (potassium phosphate buffer with NaCl and Tween 20) per well. Then 100 µL of anti sST2 biotinylated antibodies were added and the plate incubated at 600 rpm for 60 minutes at room temperature. The same washing procedure as previously described was followed by dispensing 100 µL streptavidin-HPR conjugate into each well and incubating for 30 minutes at 600 rpm.

After washing, 100 µL tetramethylbenzidine reagent was added to the wells to start the enzymatic reaction, followed by shaking at 600 rpm for 20 minutes, light-protected by covering the plate with aluminum foil.

100 µL stop solution of diluted HCl stopped the reaction and caused a color-change from blue to yellow.

The absorbance was measured with a microplate absorbance reader (Tecan Sunrise) at 450 nm, for the standards as well as for the samples. The software for data analysis (Tecan Magellan) generated a standard curve (Figure 1) via linear plot. This standard curve was used for calculating the sST2-levels for the corresponding absorbance for every sample. The standard curve was also used for evaluating the precision of the assays. Because of a significant deviation of the respective standard curve in comparison to the other standard curves, two assays were repeated at the end of the test series. The Presage® ST2 Assay has a limit of detection of 2 ng/mL, the intra-assay coefficient of variants (CV) was 5.6 %, the inter-assay CV 8.85 %.

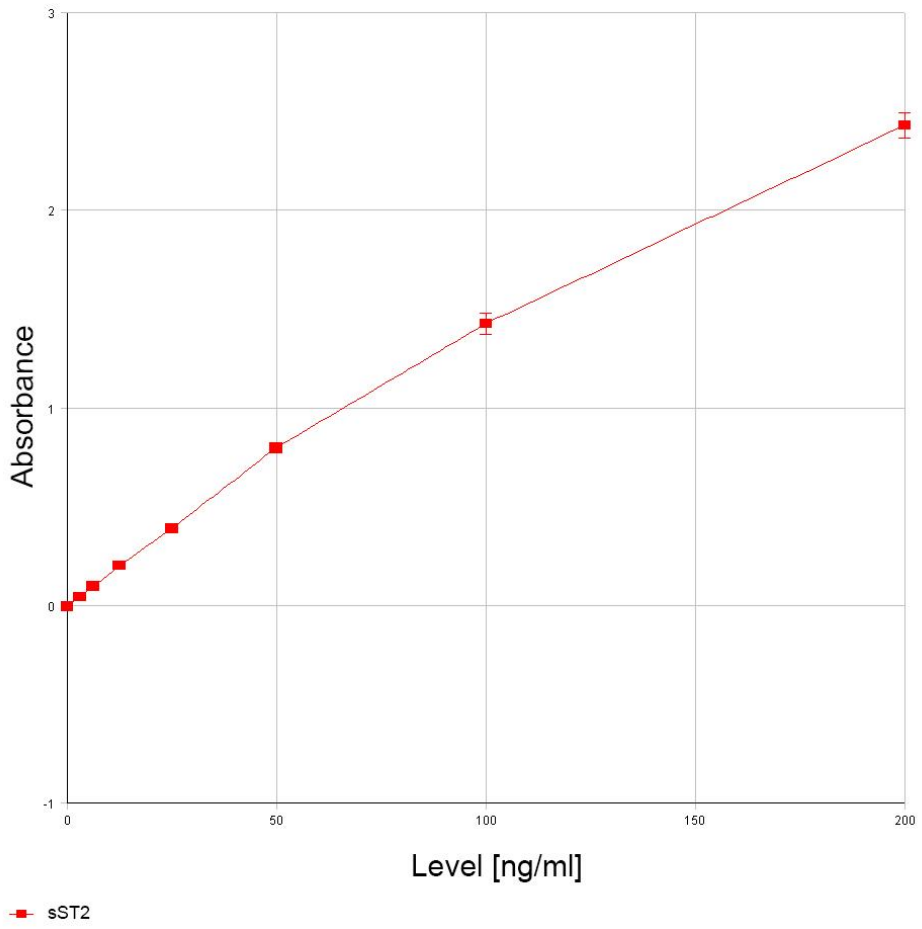


Figure 1 – An exemplary standard curve of sST2 measurements shown in Tecan Magellan

2.3 GDF-15 Measurements

The GDF-15 levels were measured as part of a collaborative project at the Department of Cardiology and Angiology at Hannover Medical School. Consent to the use of these results in this thesis was given. Measurements were performed with an immunoluminometric assay (ILMA), which was similar to the previously described immunoradiometric assay (IRMA) from Kempf et. al (71) except that the GDF-15 detection antibody was labeled with acridinium ester instead of Iodine-125 and assay results were quantified in a luminometer (Berthold) instead of a gamma counter. The assay has a limit of detection of 24 ng/L and a linear range from 200 to 50,000 ng/L. The intra-assay CV was below 5.9 % and the inter-assay CV below 10 %.

2.4 CRP and NT-proBNP-Measurements

The levels of CRP were measured by routine assays during the time of examination at the study center. CRP was measured on an Abbott Architect c8000 System with the CRP Vario assay, which is a quantitative immunoturbidimetric kit. The assay range was 0.2 to 320 mg/L.

NT-proBNP was measured at the biomarker laboratory of the GHS on a Roche Elecsys 2010 with an electrochemiluminescence immunoassay. The analytical range was 5 to 35,000 ng/L. The intra-assay CV was 0.8–3.0 % and the inter-assay CV 2.2–5.8 %.

2.5 Statistical Analysis

Continuous variables were described by its quartiles and binary variables by frequencies. All biomarkers were log-transformed before analyses. For each biomarker, histograms were produced as well as Spearman correlations were computed.

For each biomarker, receiver operating characteristic (ROC) curves were generated for the outcomes a) HF vs. no HF, b) HFrEF vs. no HF, c) HFpEF vs. no HF and d) HFrEF vs. HFpEF. The area under the curve (AUC) and 95 % confidence intervals were computed. ROC curves were also calculated for each of the following biomarker quotients GDF-15 / NT-proBNP, (GDF-15 + sST2)/NT-proBNP and (CRP + GDF-15 + sST2)/NT-proBNP.

For every biomarker, the optimal cut-off to distinguish HF was computed. Optimal cut-off means the cut-off that maximizes the Youden index (sensitivity + specificity − 1). An individual would be classified as healthy if his/her biomarker concentration is below the cut-off, and as diseased, if his/her biomarker concentration is equal to or greater than the cut-off. For the biomarker quotients and CRP (only in the case of HFrEF vs. HFpEF), an individual is classified as diseased if the biomarker value is less or equal than the described cut-off.

Logistic regressions were performed for the dependent variables a) HF vs. no HF, b) HFpEF vs. no HF, c) HFrEF vs. no HF and d) HFrEF vs. HFpEF, the independent variables of interest in these models are the analyzed novel biomarkers. A model adjusted for age, body mass index (BMI), estimated glomerular filtration rate (eGFR), sex, diabetes, hypertension, dyslipidemia, smoking and NT-proBNP and a model including additionally CRP, GDF-15, and sST2, each in turn, were computed. CRP values below the limit of detection (LoD) were substituted by the method described by Richardson & Ciampi (72). Additional logistic regressions were performed with the previously introduced biomarker ratios, GDF-15/NT-proBNP, (GDF-15 + sST2)/NT-proBNP, and (CRP + GDF-15 + sST2)/NT-proBNP, as covariates of interest. For each quotient, a separate model was computed.

Cox regressions for all-cause mortality, adjusted for age, sex, BMI, eGFR, diabetes, hypertension, dyslipidemia, and smoking, were performed including each marker as the predictor of interest.

The biomarkers were categorized into thirds using the respective tertiles. Unadjusted associations to mortality were examined using the cut-off for prevalent HF derived in the ROC analyses and computing Kaplan-Meier survival curves using thirds and performing the log-rank test.

All analyses were performed using R version 3.2.1 (R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org).

2.6 Materials & Reagents

Materials

Pipettes and pipette tips in different sizes	Eppendorf AG, Hamburg, Germany
Reagent reservoirs	VWR International GmbH, Radnor, PA, USA
HydroFlex microplate washer	Tecan Trading AG, Männedorf, Switzerland
Microtest Plate 96 Well	Sarstedt AG & Co. KG, Nümbrecht, Germany
Titramax 1000	Heidolph Instruments GmbH & Co. KG, Schwabach, Germany
Sunrise microplate absorbance reader	Tecan Trading AG, Männedorf, Switzerland

Presage® ST2 Assay (Critical Diagnostics, San Diego, CA, USA)

Anti ST2 Antibody Coated Wells (1 plate, 96 wells)
Lyophilized sST2 Calibrator (400 ng/vial)
ST2 Standard Diluent (13 mL/vial)
ST2 Sample Diluent (30 mL/bottle)
Anti ST2 Biotinylated Antibody Reagent (13 mL/vial)
Streptavidin-HRP Conjugate Concentrate 100X (0.2 mL/vial)
Streptavidin-HRP Conjugate Diluent (13 mL/vial)
20X Wash Buffer (50 mL/bottle)
TMB Reagent (11 mL/vial)
Stop Solution (11 mL/vial)

Software

Magellan	Tecan Trading AG, Männedorf, Switzerland
R version 3.2.1	R Development Core Team, Vienna, Austria

3 Results

3.1 Characteristics of Study Participants

The sample for the analyses in this thesis included 5,000 subjects. The cohort was stratified by age and gender, including 2,460 women (median age 55) and 2,540 men (median age 56). Measured values of all biomarkers of interest (NT-proBNP, sST2, GDF-15, and CRP) were available for 4,821 subjects, so analyses were done with this subset. Subjects with HFmrEF were included in the HFrEF cohort for the analyses because their baseline characteristics were comparable (Table 3). The characteristics of the summarized HFrEF cohort are shown in Table 4.

Table 3 – Characteristics of study participants with HFmrEF subjects as a separate cohort

	All (N = 5000)	No HF (N = 4864)	HF (N = 108)	HFpEF (N = 70)	HFmrEF (N = 21)	HFrEF (N = 17)
Age (years)	56.0 (46.0, 65.0)	55.0 (46.0, 64.0)	67.0 (62.0, 71.0)	67.0 (62.0, 72.0)	63.0 (53.7, 67.3)	69.0 (59.7, 71.0)
BMI (kg/m²)	26.5 (23.9, 29.8)	26.4 (23.8, 29.6)	30.4 (27.7, 34.6)	31.2 (27.8, 36.4)	28.5 (26.5, 33.1)	29.9 (29.0, 34.0)
eGFR (mL/min for 1.73m²)	90.4 (80.8, 98.8)	90.6 (81.2, 99.0)	82.5 (66.7, 91.7)	82.8 (67.8, 91.8)	87.1 (78.3, 92.9)	72.8 (61.1, 90.2)
Male No. (%)	2540 (50.8)	2461 (50.6)	65 (60.2)	35 (50.0)	15 (71.4)	15 (88.2)
Diabetes No. (%)	374 (7.5)	335 (6.9)	35 (32.4)	24 (34.3)	5 (23.8)	6 (35.3)
Hypertension No. (%)	2564 (51.3)	2458 (50.5)	84 (77.8)	55 (78.6)	15 (71.4)	14 (82.4)
Dyslipidemia No. (%)	1483 (29.7)	1431 (29.5)	42 (38.9)	25 (35.7)	8 (38.1)	9 (52.9)
Current smoking No. (%)	959 (19.2)	937 (19.3)	20 (18.5)	13 (18.6)	6 (28.6)	1 (5.9)
EF (%)	64.1 (60.3, 67.9)	64.2 (60.4, 67.9)	58.3 (47.2, 66.4)	63.9 (58.9, 69.5)	47.3 (44.9, 48.5)	35.2 (22.7, 37.2)
CAD No. (%)	226 (4.6)	196 (4.1)	26 (25.2)	14 (20.6)	6 (30.0)	6 (40.0)
MI No. (%)	156 (3.1)	128 (2.6)	24 (22.4)	11 (15.9)	3 (14.3)	10 (58.8)
Stroke No. (%)	95 (1.9)	86 (1.8)	7 (6.5)	4 (5.8)	0 (0)	3 (17.6)
NT-proBNP (pg/mL)	61.9 (28.5, 124.4)	60.3 (27.7, 119.3)	224.8 (78.9, 909.8)	145.5 (75.5, 293.9)	389.5 (39.9, 1584.0)	1457.0 (919.0, 1897.0)
CRP (mg/L)	1.7 (0.9, 3.3)	1.6 (0.9, 3.2)	2.9 (1.5, 5.5)	3.0 (1.6, 5.5)	3.9 (1.2, 7.4)	2.6 (1.3, 5.0)
sST2 (ng/mL)	24.7 (20.2, 30.8)	24.5 (20.1, 30.7)	28.0 (23.0, 38.1)	26.5 (21.7, 36.0)	28.6 (23.3, 48.3)	30.4 (23.4, 42.1)
GDF-15 (ng/L)	885.0 (725.9, 1136.0)	876.0 (723.0, 1120.0)	1370.0 (1025.2, 1891.5)	1290.0 (989.3, 1868.8)	1416.0 (1060.2, 1729.3)	1630.0 (1183.1, 2864.2)
All-cause mortality No. (%)	213 (4.3)	183 (3.8)	27 (25.0)	16 (22.9)	5 (23.8)	6 (35.3)

Table 4 – Characteristics of study participants with HFmrEF subjects included in the HFrEF cohort

	All (N = 5000)	No HF (N = 4864)	HF (N = 108)	HFpEF (N = 70)	HFrEF (N = 38)
Age (years)	56.0 (46.0, 65.0)	55.0 (46.0, 64.0)	67.0 (62.0, 71.0)	67.0 (62.0, 72.0)	64.0 (57.8, 70.0)
BMI (kg/m²)	26.5 (23.9, 29.8)	26.4 (23.8, 29.6)	30.4 (27.7, 34.6)	31.2 (27.8, 36.4)	29.6 (27.2, 33.4)
eGFR (mL/min for 1.73m²)	90.4 (80.8, 98.8)	90.6 (81.2, 99.0)	82.5 (66.7, 91.7)	82.8 (67.8, 91.8)	81.9 (65.6, 91.7)
Male No. (%)	2540 (50.8)	2461 (50.6)	65 (60.2)	35 (50.0)	30 (78.9)
Diabetes No. (%)	374 (7.5)	335 (6.9)	35 (32.4)	24 (34.3)	11 (28.9)
Hypertension No. (%)	2564 (51.3)	2458 (50.5)	84 (77.8)	55 (78.6)	29 (76.3)
Dyslipidemia No. (%)	1483 (29.7)	1431 (29.5)	42 (38.9)	25 (35.7)	17 (44.7)
Current smoking No. (%)	959 (19.2)	937 (19.3)	20 (18.5)	13 (18.6)	7 (18.4)
EF (%)	64.1 (60.3, 67.9)	64.2 (60.4, 67.9)	58.3 (47.2, 66.4)	63.9 (58.9, 69.5)	43.1 (35.8, 47.5)
CAD No. (%)	226 (4.6)	196 (4.1)	26 (25.2)	14 (20.6)	12 (34.3)
MI No. (%)	156 (3.1)	128 (2.6)	24 (22.4)	11 (15.9)	13 (34.2)
Stroke No. (%)	95 (1.9)	86 (1.8)	7 (6.5)	4 (5.8)	3 (7.9)
NT-proBNP (pg/mL)	61.9 (28.5, 124.4)	60.3 (27.7, 119.3)	224.8 (78.9, 909.8)	145.5 (75.5, 293.9)	955.7 (243.6, 1876.7)
CRP (mg/L)	1.7 (0.9, 3.3)	1.6 (0.9, 3.2)	2.9 (1.5, 5.5)	3.0 (1.6, 5.5)	2.6 (1.3, 5.5)
sST2 (ng/mL)	24.7 (20.2, 30.8)	24.5 (20.1, 30.7)	28.0 (23.0, 38.1)	26.5 (21.7, 36.0)	29.6 (23.4, 43.3)
GDF-15 (ng/L)	885.0 (725.9, 1136.0)	876.0 (723.0, 1120.0)	1370.0 (1025.2, 1891.5)	1290.0 (989.3, 1868.8)	1513.5 (1092.2, 2126.0)
All-cause mortality No. (%)	213 (4.3)	183 (3.8)	27 (25.0)	16 (22.9)	11 (28.9)

The prevalence of HF was 2.2 %, divided in HFpEF with 1.4 % and HFrEF with 0.8 %. Cardiovascular risk factors (diabetes mellitus, hypertension, dyslipidemia, smoking) were more prevalent in subjects with HF, despite smoking, which was not increased. Diabetes mellitus and hypertension were more common in HFpEF subjects, while dyslipidemia had a higher prevalence in subjects with HFrEF.

Prevalent coronary artery disease (CAD) and prevalent myocardial infarction (MI) were elevated in the HF subjects, in particular in individuals with HFrEF. Subjects with HF had a higher BMI (30.4) compared to subjects without HF (26.4), probands suffering from HFpEF showed the highest BMI with 31.2. According to the previously described stratification, 50.8 % of the collective were male. With an amount of 60.2 % male subjects, the sex ratio was shifted in subjects with HF, notably in the subgroup of HFrEF, where 78.9 % were male.

All biomarker levels were elevated in subjects with HF. Levels of NT-proBNP were 60.3 pg/mL in subjects without HF vs. 224.8 pg/mL in subjects with HF. sST2 levels were 24.5 ng/mL (no HF) vs. 28.0 ng/mL (HF). Subjects with HF had higher levels of GDF-15 (876 ng/L vs. 1370 ng/L) and CRP (1.6 mg/L vs. 2.9 mg/L). All biomarker levels were elevated in subjects with HFrEF (vs. HFpEF), despite CRP, which was

lower in the HFrEF cohort (2.6 mg/L) than in the HFpEF cohort (3.0 mg/L). During a follow-up time of 7.3 years, 213 subjects died of any causes. All-cause mortality was increased in both HF subtypes with 22.9 % (n = 16) for HFpEF and 28.9 % (n = 11) for HFrEF compared to 3.8 % (n = 183) in subjects without HF.

3.2 Spearman Correlations

The analyses of Spearman correlations were performed to examine the association between the biomarkers. The analyses showed a moderate correlation for GDF-15 and NT-proBNP with 0.32, followed by GDF-15 and CRP with 0.2. GDF-15 and sST2 showed a correlation of 0.14, together with NT-proBNP and CRP, which had a correlation of 0.13. The other combinations of biomarkers showed no correlation (Table 5 and Figure 2).

Table 5 – Spearman correlations between biomarkers adjusted for age and sex

	sST2	CRP	NT-proBNP	GDF-15
sST2		0.02 p=0.091	-0.03 p=0.019	0.14 p < 0.001
CRP	0.02 p=0.091		0.13 p < 0.001	0.20 p < 0.001
NT-proBNP	-0.03 p=0.019	0.13 p < 0.001		0.32 p < 0.001
GDF-15	0.14 p < 0.001	0.20 p < 0.001	0.32 p < 0.001	

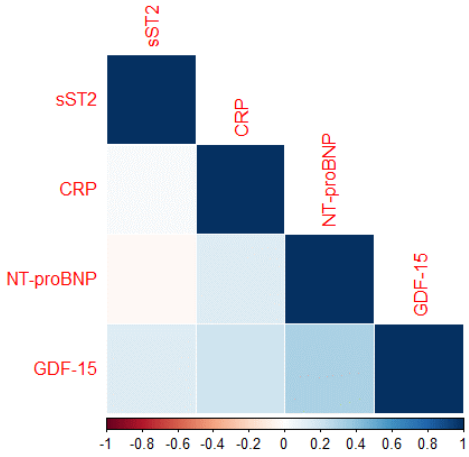


Figure 2 – Spearman correlations between biomarkers adjusted for age and sex

3.3 Receiver Operating Characteristic Analysis

Receiver operating characteristic (ROC) analysis was performed to assess the ability of the biomarkers to discriminate subjects with HF from subjects without HF. GDF-15 showed the highest area under the curve (AUC) with 0.79, compared to NT-proBNP with an AUC of 0.77. The AUC for CRP was 0.66, while sST2 showed the lowest AUC with 0.62 (Table 6 and Figure 3).

To discriminate HFrEF and HFpEF, NT-proBNP showed the highest AUC (0.74), followed by GDF-15 (0.60), which had a slightly higher AUC than sST2 (0.59). The AUC for CRP was 0.48 (Table 7 and Figure 4).

Table 6 – AUC analyses for biomarkers for the condition HF vs. no HF including biomarker combinations and ratios

	AUC (95 % CI)	N
NT-proBNP	0.77 (0.72, 0.82)	4957
CRP	0.66 (0.61, 0.71)	4969
sST2	0.62 (0.56, 0.67)	4867
GDF-15	0.79 (0.75, 0.83)	4664
GDF-15/NT-proBNP	0.29 (0.23, 0.34)	4662
(GDF-15 + sST2)/NT-proBNP	0.28 (0.22, 0.34)	4590
(CRP + GDF-15 + sST2)/NT-proBNP	0.28 (0.22, 0.34)	4590

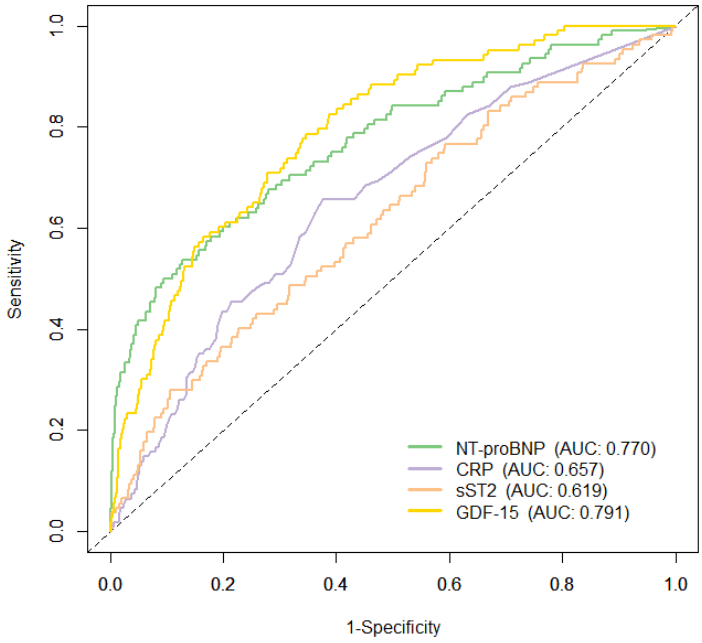


Figure 3 – ROC curve analyses for each biomarker for the condition HF vs. no HF

Table 7 – Biomarkers AUCs for the differentiation of HFrEF vs. HFpEF including biomarker combinations and ratios

	AUC (95 % CI)	N
NT-proBNP	0.74 (0.62, 0.86)	108
CRP	0.48 (0.36, 0.59)	108
sST2	0.59 (0.47, 0.70)	107
GDF-15	0.60 (0.48, 0.71)	103
GDF-15/NT-proBNP	0.27 (0.15, 0.38)	103
(GDF-15 + sST2)/NT-proBNP	0.27 (0.15, 0.38)	102
(CRP + GDF-15 + sST2)/NT-proBNP	0.27 (0.15, 0.38)	102

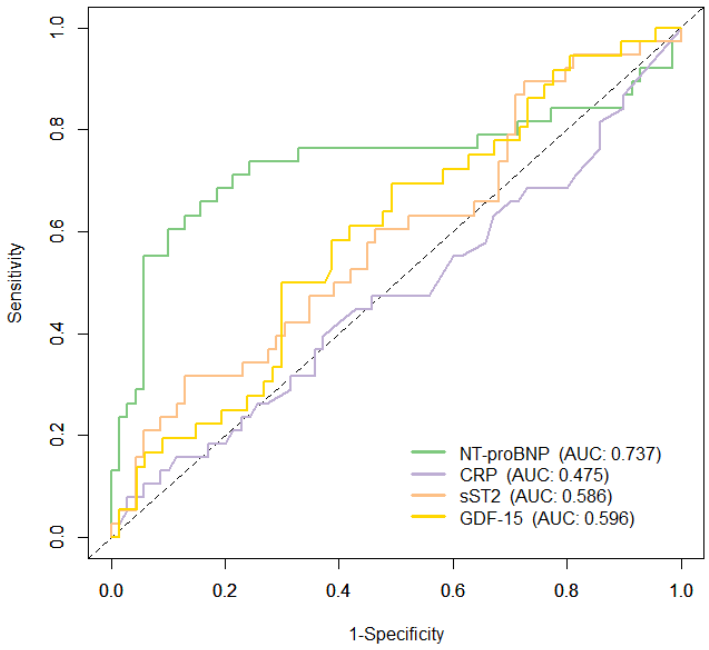


Figure 4 – ROC curve analyses for each biomarker for the differentiation of HFrEF vs. HFpEF

For every biomarker, the optimal cut-off to distinguish HF was computed. Optimal cut-off means the cut-off that maximizes the Youden index.

The tables 8 and 9 show the cut-offs for HF vs. no HF as well as for HFrEF vs. HFpEF.

Table 8 – Optimal cut-offs to distinguish HF vs. no HF for every single biomarker and ratios

Marker	Cut-off	Sensitivity	Specificity	PPV	NPV	N
NT-proBNP	199.1, pg/mL	50.8 (40.9, 60.4)	87.2 (86.3, 88.2)	8.2 (6.1, 10.5)	98.8 (98.4, 99.1)	4957
CRP	2.3, mg/L	64.3 (54.5, 73.1)	62.4 (61.0, 63.7)	3.6 (2.8, 4.6)	98.7 (98.3, 99.1)	4969
sST2	31.375, ng/mL	35.7 (26.4, 45.7)	77.2 (76.0, 78.4)	3.4 (2.4, 4.7)	98.1 (97.7, 98.5)	4867
GDF-15	1006, ng/L	75.4 (66.2, 82.9)	65.2 (63.8, 66.6)	4.6 (3.6, 5.7)	99.2 (98.8, 99.4)	4664
GDF-15/NT-proBNP	7.515	57.1 (47.0, 66.6)	75.7 (74.4, 76.9)	4.9 (3.7, 6.3)	98.7 (98.3, 99.1)	4662
(GDF-15 + sST2)/NT-proBNP	7.735	58.2 (48.0, 67.7)	75.9 (74.7, 77.2)	5.1 (3.9, 6.6)	98.8 (98.4, 99.1)	4590
(CRP + GDF-15 + sST2)/NT-proBNP	7.771	58.1 (48.0, 67.7)	75.9 (74.6, 77.1)	5.1 (3.8, 6.6)	98.8 (98.3, 99.1)	4590

Table 9 – Optimal cut-offs to distinguish HFrEF vs. HFpEF for every single biomarker and ratios

Marker	Cut-off	Sensitivity	Specificity	PPV	NPV	N
NT-proBNP	860.1, pg/mL	56.8 (39.6, 72.2)	88.1 (78.6, 94.0)	73.3 (54.4, 86.7)	78.8 (68.3, 86.9)	108
CRP	1.4, mg/L	26.3 (12.3, 43.4)	77.4 (66.1, 86.0)	40.6 (21.0, 61.1)	64.4 (53.2, 74.3)	108
sST2	42.119, ng/mL	26.0 (11.9, 43.0)	83.9 (73.6, 90.8)	51.3 (28.2, 72.4)	65.3 (54.5, 74.7)	107
GDF-15	1311, ng/L	64.3 (46.7, 78.5)	48.0 (35.5, 60.5)	38.9 (26.0, 52.6)	71.6 (56.5, 83.2)	103
GDF-15/NT-proBNP	2.669	57.9 (40.2, 73.6)	86.7 (76.5, 93.4)	71.0 (51.8, 85.4)	79.2 (68.3, 87.5)	103
(GDF-15 + sST2)/NT-proBNP	2.708	58.2 (40.6, 74.0)	86.4 (76.0, 93.1)	70.7 (51.5, 85.1)	79.1 (68.1, 87.5)	102
(CRP + GDF-15 + sST2)/NT-proBNP	2.719	58.2 (40.6, 73.9)	86.4 (76.0, 93.1)	70.6 (51.4, 85.0)	79.1 (68.1, 87.5)	102

3.4 Logistic Regressions

Logistic regressions were computed to evaluate the impact of serum levels of the different biomarkers on the presence of HF and HFrEF vs. HFpEF respectively.

The Odds Ratio (OR) increase per standard deviation (SD) for HF vs. no HF as a dependent variable was 1.4 for GDF-15 (p-value 0.0014), 1.2 for sST2 with a p-value of 0.089 and not significant for CRP (p-value 0.58) (Figure 5).

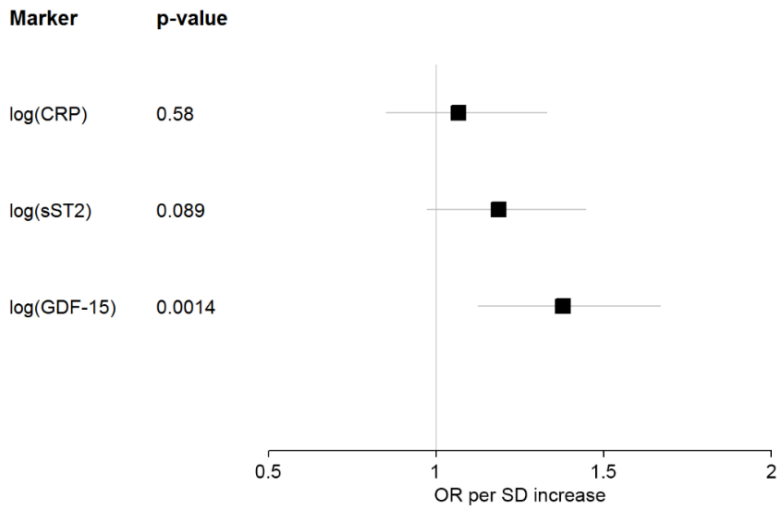


Figure 5 – Odds ratios per SD for the base model (adjusted for age, BMI, eGFR, sex, diabetes, hypertension, dyslipidemia, smoking, and NT-proBNP) including one biomarker

The analysis for HFrEF vs. HFpEF showed no significant increase for the single biomarkers ($p > 0.1$) (Figure 6). A calculated index of the sum of the biomarkers (CRP + GDF-15 + sST2)/NT-proBNP showed an OR of 3.7 ($p < 0.001$), as well as the ratio GDF-15/NT-proBNP ($p < 0.001$).

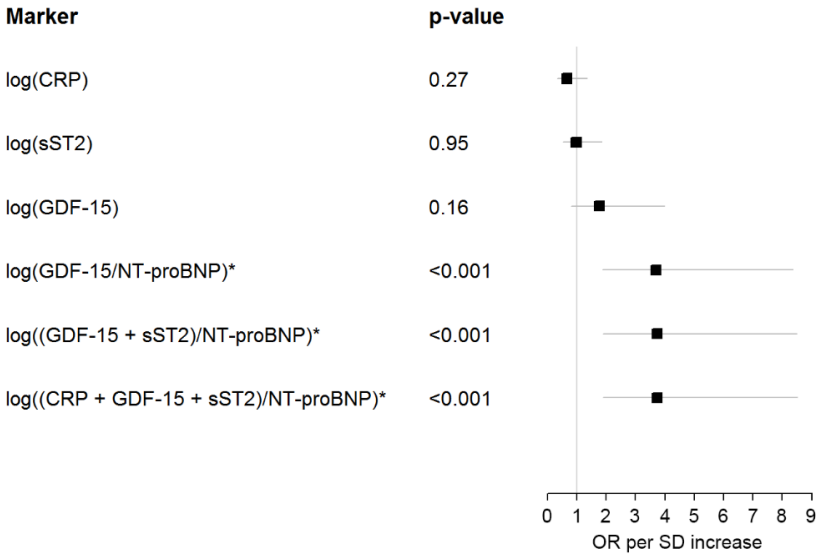


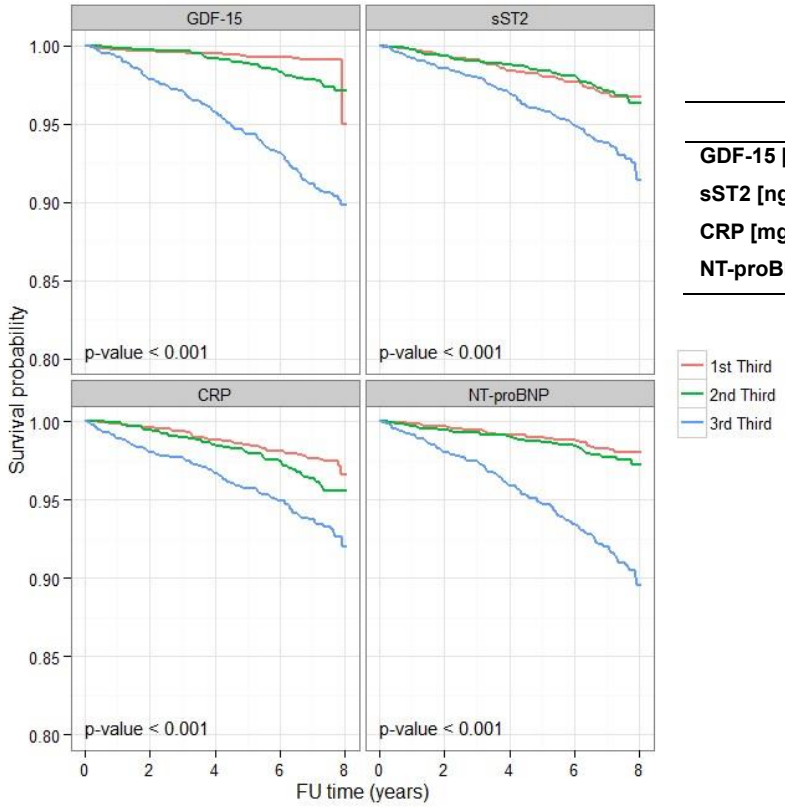
Figure 6 – Odds ratios per SD for the base model + marker for HFrEF vs. HFpEF (adjusted for age, BMI, eGFR, sex, diabetes, hypertension, dyslipidemia, smoking, and NT-proBNP). The model for the biomarker-indices is not adjusted for NT-proBNP.
*** OR presented is for minus the variable indicated in the graphic. This has the effect of showing 1/(original OR).**

3.5 Kaplan-Meier Survival Curves

The unadjusted survival of subjects regarding different biomarker levels was analyzed by means of Kaplan-Meier curves.

In the median follow-up time of 7.3 years (estimated by the reverse Kaplan-Meier estimator), 213 subjects died of any causes.

Higher biomarker values were connected to an increase of all-time mortality (Figure 7). The p-value showed in the figure was calculated by the log-rank test and shows the significance of every biomarker.



	1st Tertile	2nd Tertile
GDF-15 [ng/mL]	780.7	1037.3
sST2 [ng/mL]	21.8	28.4
CRP [mg/L]	1.2	2.6
NT-proBNP [pg/mL]	38.7	96.5

Figure 7 – Kaplan-Meier estimate of all-cause mortality according to biomarkers categorized using tertiles

3.6 Cox Proportional Hazards

Cox proportional hazards, adjusted for age, sex, BMI, eGFR, diabetes, hypertension, dyslipidemia, and smoking, were computed including each biomarker as an independent variable to investigate multivariable associations to mortality.

In Cox regression analyses for all-cause mortality, NT-proBNP had the highest hazard ratio (HR) per SD with 1.9, followed by GDF-15 with 1.7. CRP and sST2 showed lower HRs (1.5 and 1.4). The increase was significant for every biomarker ($p < 0.001$) (Table 10 and Figure 8).

Table 10 – Summary table for Cox regression analyses for all-cause mortality for every single biomarker (each line represents a different model)

	Beta (95 % CI)	HR (95 % CI)	HR per SD (95 % CI)	p-value	N	N events	EPV
log (NT-proBNP), log(pg/mL)	0.513 (0.394, 0.632)	1.7 (1.5, 1.9)	1.9 (1.6, 2.2)	<0.001	4965	210	21
log (CRP), log(mg/L)	0.374 (0.239, 0.509)	1.5 (1.3, 1.7)	1.5 (1.3, 1.7)	<0.001	4977	211	21.1
log(sST2), log(ng/mL)	1.003 (0.615, 1.390)	2.7 (1.9, 4.0)	1.4 (1.2, 1.6)	<0.001	4875	208	20.8
log (GDF-15), log(ng/L)	1.408 (1.151, 1.666)	4.1 (3.2, 5.3)	1.7 (1.6, 1.9)	<0.001	4675	197	19.7

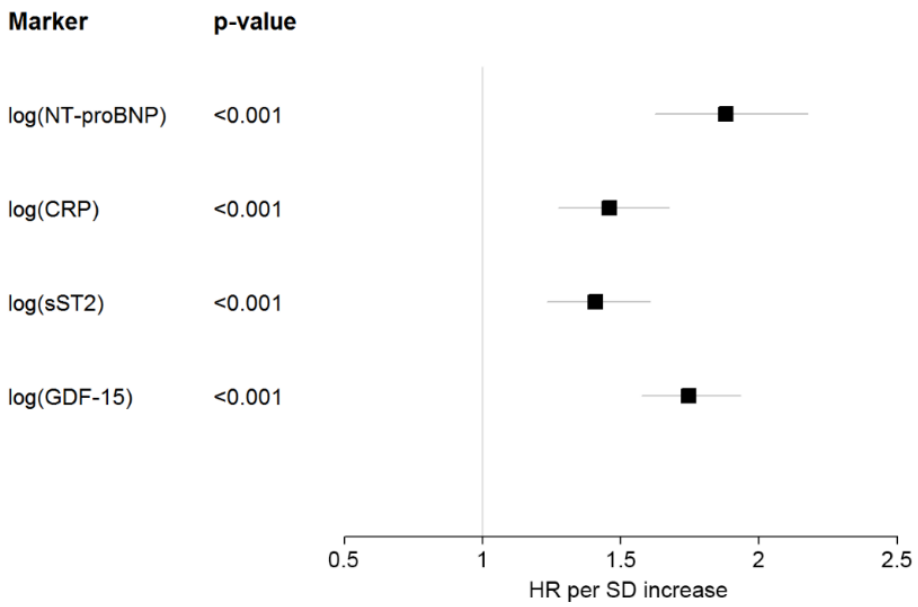


Figure 8 – Hazard ratios per SD for the base model (adjusted for age, sex, BMI, eGFR, diabetes, hypertension, dyslipidemia, and smoking) + marker for all-cause mortality. Each row represents a different model.

4 Discussion

The presented thesis aimed to investigate whether novel cardiovascular biomarkers GDF-15 and sST2, as well as the established marker CRP, are able to improve the current diagnosis and prognosis of HF.

In detail, this thesis characterized a population-based cohort, the presence of cardiovascular risk factors and the prevalence of HF. The mortality of subjects with HF was illustrated and the connection between biomarker serum levels and mortality was investigated.

The thesis evaluated whether novel biomarkers are able to ameliorate the detection of prevalent HF and the discrimination between HFrEF and HFpEF beyond the established biomarker NT-proBNP. This analysis was performed including the biomarkers of interest as single markers as well as in different multi-biomarker indices.

Our results showed that the presence of HF clearly increases mortality risk and that elevated biomarker levels are significantly connected to mortality. This thesis confirmed the value of NT-proBNP as a cornerstone in the diagnosis of HF and presented that GDF-15 was equally useful as a single marker. A biomarker index NT-proBNP/GDF-15 was best in differentiating HFrEF from HFpEF.

In our investigation, sST2 and CRP did not improve discrimination of HF as single markers, although both contributed value to a multi-biomarker index which included all biomarkers.

The necessity of improvements in current HF management

The global burden of HF is massive and it is expected that prevalence and health expenditure costs will keep growing in the next decades (7,10)**.**

Currently, the diagnosis of HF is based on a combination of ECG, echocardiography and the determination of NT-proBNP-levels (4).

However, the diagnosis of HF is challenging. Typical symptoms of HF are unspecific and some of them may be caused by another underlying disease (e.g. pulmonary disease, anemia, renal failure) (73). The ECG itself is also often unspecific and cannot provide a diagnosis on its own (74,75).

There is still little agreement on optimal NT-proBNP cutoffs, thus, it is unknown which one may be the best cutoff for diagnosing HF (73). Echocardiography requires the availability of a sonographic unit, which is not given at any place. Furthermore,

examination quality may vary due to patient-individual factors (obesity, availability of adequate acoustic windows) and clinician-individual experience in performing cardiac echo. The different phenotypes of HF (HFrEF and HFpEF) have a different appearance (as outlined in 1.1 Heart Failure) and are often not diagnosed correctly (12).

Therefore, it is important to improve current diagnostics and risk stratification using novel approaches such as data from circulating, blood-based biomarkers in epidemiological cohorts.

In this study, the value of blood-based biomarkers GDF-15, sST2 and CRP for HF, in particular HFrEF and HFpEF, in relation to all-cause mortality were investigated in the population-based Gutenberg Health Study.

4.1 Cardiovascular Risk Factors and Prevalence of Cardiovascular Disease

We could show that the presence of HFrEF and HFpEF is connected to different cardiovascular risk factors and comorbidities. HFpEF subjects had more often diabetes mellitus and hypertension. Wolsk et al. described an association between extra-cardiac disease burden and outcomes as hospitalization, stroke or death (76). Our findings underlined that extra-cardiac comorbidities are more prevalent in subjects with HFpEF. Furthermore, Wolsk et al. showed that the cardiac disease burden is higher in patients with lower LVEF (76). Similar, in this thesis, prevalent CAD, previous myocardial infarction and dyslipidemia had a higher prevalence in subjects with HFrEF.

Men were more common to have HF, especially HFrEF, with more than 75 % of the subjects being male. The demographic burden was higher in the HFpEF cohort, which was also described earlier (76).

4.2 Biomarker and Mortality

All-cause mortality was higher for subjects with HF than for subjects without HF.

It was shown before that elevated levels of biomarkers were able to predict the risk of death. In the Framingham Heart Study with more than 3,000 participants it was shown that sST2 and GDF-15 were able to predict the risk of death (35). Chen et al. published data from the Dallas Heart Study, which could underline the association of sST2 and mortality in another 3,300 subjects (44). A large meta-analysis with 84,000 participants from 14 studies showed the connection between CRP levels and

not only all-cause mortality, but also cardiovascular mortality (77).
This thesis confirmed that elevated serum levels of every analyzed biomarker were strongly connected to all-time mortality.
Triboulloy et al. pointed out that five-year survival rates in hospitalized HF patients were not significantly different between HFpEF and HFrEF (78). We also found no difference in the mortality of HFrEF or HFpEF, which underlines the poor outcome of HFpEF.
Because of the population-based study design, we could show that subjects suffering from HFpEF were already at risk of mortality, even if they are relatively young.

4.3 Prognostic Value of Biomarkers

We calculated Spearman correlations to analyze the association between the different biomarkers. None of the biomarker combinations showed a strong connection, all combinations had a weak positive or negligible correlation.
For diagnosing HF, the only biomarker which is established and recommended by the current ESC-guidelines is NT-proBNP (4).
In this thesis, NT-proBNP showed the highest ROC AUC for HF vs. no HF, confirming the leading role of NT-proBNP regarding the diagnosis of HF.
GDF-15 was equally useful as NT-proBNP in detecting prevalent subjects with HF in our study.
GDF-15 levels were not significantly different for HFrEF and HFpEF. This follows other studies which showed that inflammatory stress is present in both HFrEF and HFpEF (56).
In differentiating HFrEF from HFpEF NT-proBNP was best of all single biomarkers investigated in this thesis. Subjects with HFrEF had higher levels of NT-proBNP, which was shown before (79). This suggests that hemodynamic stress is higher in HFrEF.
Previous studies suggested that sST2 is a promising emerging biomarker in predicting mortality (33,34). We could confirm a connection between increased serum levels and mortality. Beyond that, the OR for discriminating HF from no HF just showed a weak association. The hazard ratio in Cox regression analyses for all-cause mortality was the lowest of all biomarkers. Therefore, we cannot confirm that sST2 is useful as a single marker in addition to NT-proBNP. This is in line with

another previous study, where sST2 was not even useful in predicting mortality (43). Furthermore, we could not confirm that CRP is able to detect subjects with HF.
CRP was the only biomarker that had lower serum levels in subjects with HFrEF (2.6 mg/L) than in subjects with HFpEF (3.0 mg/L). As a general marker of inflammation, it might be useful for differentiating HFrEF from HFpEF in a multi-biomarker-approach, because it is assumed that a systemic proinflammatory state is driving the cardiac remodeling in HFpEF (80). A recent study stated that CRP is associated with mortality reduction only in HFpEF but not in HFrEF (81).
Data suggest that the use of combined biomarkers can improve the predictive accuracy of single biomarkers (82). Various biomarker combinations are described (74,83–86).
We also analyzed different biomarker indices and their ability to discriminate HFrEF from HFpEF. Santhanakrishnan et al. introduced the ratio of NT-proBNP and GDF-15, which was best in differentiating HFrEF from HFpEF in their study (57). In our data, the ratio was most useful as well. Furthermore, we could prove that an index which included all biomarker of interest (CRP + GDF-15 + sST2)/NT-proBNP showed similar OR.
Additional research is needed to confirm the strength of the ratio NT-proBNP/GDF-15 or to identify another most suitable biomarker index.

4.4 Strengths and Limitations

The main strength of the thesis is that the GHS was a population-based cohort with a large sample size. Many subjects with measured circulating biomarkers were included in the analyses.
Because all subjects were examined according to harmonized protocols, the assessment of disease status and phenotypes was accurate.

The limitations of this thesis are the following:
As circulating biomarkers were evaluated in a population-based study, the size of subjects with HF was low and thus, only a small number of HF subjects were included in the analyses. Furthermore, we did not analyze the subjects with HFmrEF and HFrEF separately, as their respective numbers were too small. Therefore, we included the HFmrEF subgroup into the HFrEF cohort because the characteristics of both subgroups were comparable.

Median LVEF was comparatively high in the HFrEF cohort because only stable patients were included in the study. That may affect the validity of our study.

Measurements of biomarkers were performed in samples previously frozen at -80°C. Storage of samples may affect the blood serum levels. However, it is described in the literature that sST2 is stable for at least 1.5 years (87), NT-proBNP for at least two years (88) and CRP up to eleven years (89).

26 subjects showed NT-proBNP levels, which were below the proposed cut-off of 125 pg/mL (4), although they had the diagnosis of HF and received treatment of HF. The most probable reason is that all 26 subjects had a BMI of 30 or greater. It is known that NT-proBNP is lower in obese people, although the mechanism is not fully understood (90,91).

4.5 Conclusion

The value of circulating, blood-based biomarkers GDF15, sST2 and CRP for the prognosis of HF was investigated in a population-based cohort. Levels of all investigated biomarkers were connected to mortality, which represents the poor outcome of HF.

Our results confirmed the leading role of NT-proBNP as an established biomarker in HF clinical care.

Because of the heterogeneity of novel biomarkers, they need to be examined based on their own merits.

In our study, GDF-15 was equally useful as NT-proBNP for detecting prevalent HF, making GDF-15 the most promising novel biomarker.

In contradiction to previous studies, sST2 was not useful as a single biomarker. This is in line with one report from Hughes et al. (43). Based on these findings we cannot recommend focusing further research on sST2 for predicting HF events or mortality.

CRP was also not useful as a single marker. However, as it is described that CRP is associated with mortality only in HFpEF, it may be useful specifically in HFpEF or in a multi-biomarker approach.

In our research regarding the value of a multi-biomarker panel, the ratio of NT-proBNP and GDF-15 was best in differentiating HFrEF from HFpEF. A biomarker index that included all biomarkers of interest was also appropriate. Since none of

the novel biomarkers besides CRP is currently established in everyday clinical practice of HF, the calculation of a biomarker index with all novel biomarkers seems inconvenient for now.
Biomarker indices may play a more prominent role in the future, especially in the discrimination of HFrEF from HFpEF.

4.6 Outlook

Our findings from a large population-based study should be further validated in other studies, especially in treatment studies. It should be analyzed if biomarker levels can depict appropriate therapy of HF.
We could not analyze the HFmrEF subgroup separately as the sample size was too small. Additional research is needed for this relatively “young” category of HF.

Beyond our selection of biomarkers, there are other putative novel biomarkers such as galectin-3, mid-regional pro-adrenomedullin (MR-proADM) and microRNAs (miRNAs), which already showed their potential in prediction of HF and mortality in previous studies.
Galectin-3 is one out of 15 carbohydrate-binding proteins (lectins) and was first described in 1982 as a macrophage-specific marker (formerly known as Mac-2 Antigen) (92) and as an IgE-binding protein (93). The expression and secretion of galectin-3 are connected to progressive fibrosis and HF, and inhibition of galectin-3 reduces cardiac fibrosis (94). The association with outcome in chronic HF is less strong than for other biomarkers (27).

MR-proADM, the stable pro-hormone fragment of adrenomedullin (ADM), which is expressed in cardiac, renal, pulmonary and endocrine tissues (95), has shown a connection to mortality independently of cardiac risk factors or cardiac echo (96). Previous studies showed that MR-proADM was even superior to natriuretic peptides in predicting mortality and HF (97,98).

miRNAs are short non-coding RNAs, which regulate gene expression (99). Therefore many different pathways are regulated by miRNAs and their significance in various prominent diseases, e.g. diabetes and cancer, is currently investigated (100).

It is known that miRNAs are up- and down-regulated during cardiac remodeling (101). miRNAs are also involved in the regulation of pathways of calcium homeostasis (102), which can lead to the development of HF (103), or apoptosis (104). Thus, miRNAs do not serve only as a diagnostic tool, but also as a potential therapeutic target in HF.

Treatment studies are encouraged to determine whether these novel biomarkers can modify the current therapy of HF or change patient outcome.

The suggested multi-biomarker indices should be specified in other collectives and may be expanded through the aforementioned biomarkers to specify the ideal combination.

5 Summary

In this thesis, we tried to answer the question if the novel biomarkers growth differentiation factor 15 (GDF-15), soluble suppression of tumorigenicity 2 (sST2) and c-reactive protein (CRP) can improve the diagnostics and prognosis of heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF).

HF creates a massive disease burden to patients and an economic burden to healthcare systems worldwide, which is likely to increase in the next decades due to the aging population. The mortality of HFpEF is comparable to the mortality of HFrEF subjects, but until now current therapy concepts do not improve the outcome and adequate therapy guidance is missing.

Current German treatment guidelines of HF do not recommend the measurement of any other biomarker than NT-proBNP for clinical use.

In this thesis, data of 5,000 subjects of a population-based cohort study, the Gutenberg Health Study, were investigated. Blood serum levels of the biomarkers GDF-15, sST2, and CRP in comparison to NT-proBNP were measured and statistically analyzed in relation to cardiovascular risk factors, prevalent heart failure, discrimination of HFrEF and HFpEF and mortality for a follow-up time of seven years.

The main finding of our study is that GDF-15 was equally useful as NT-proBNP to detect prevalent HF. We also confirmed the status of NT-proBNP as a cornerstone of diagnosis. All analyzed biomarkers showed an association with mortality, but beyond that sST2 and CRP were not useful as single markers.

Additionally, we calculated biomarker indices, for which the ratio GDF-15/NT-proBNP was best in differentiating HFrEF from HFpEF.

In summary, it can be stated that the current status of NT-proBNP in the diagnostics of HF could be confirmed. Of the biomarkers examined, GDF-15 showed the most promising results, which, as well as the ratio GDF-15/NT-proBNP, have to be confirmed in further studies, especially treatment studies.

6 Zusammenfassung

In der vorliegenden Arbeit wurde untersucht, ob die zirkulierenden Biomarker growth differentiation factor 15 (GDF-15), lösliches suppression of tumorigenicity 2 (sST2) und C-reaktives Protein (CRP) die Diagnostik und Prognose der Herzinsuffizienz mit reduzierter Pumpfunktion (HFrEF) und der Herzinsuffizienz mit erhaltener Pumpfunktion (HFpEF) verbessern können.

Die Herzinsuffizienz belastet nicht nur betroffene Patienten, sondern führt auch zu einer enormen wirtschaftlichen Belastung, welche in den kommenden Jahrzehnten noch zunehmen wird. Obwohl die Mortalität von HFpEF-Patienten vergleichbar mit der Mortalität von HFrEF-Erkrankten ist, fehlen aktuell sowohl Therapiekonzepte für HFpEF, um das Überleben signifikant zu verbessern, als auch adäquate Mechanismen zur Therapiesteuerung.

In den deutschen Leitlinien wird für den klinischen Alltag aktuell NT-proBNP als einziger Biomarker für die Diagnose der Herzinsuffizienz empfohlen.

In der vorliegenden Arbeit wurden die Daten von 5000 Probanden der populationsbasierten Gutenberg Gesundheitsstudie untersucht. Im Blutserum wurden die Konzentrationen der Biomarker GDF-15, sST2 und CRP im Vergleich zu NT-proBNP vermessen und statistisch in Bezug auf kardiovaskuläre Risikofaktoren, eine vorbestehende Herzinsuffizienz, Diskriminierung von HFrEF und HFpEF und Mortalität während der siebenjährigen Follow-Up-Zeit analysiert.

Die Haupt-Erkenntnis dieser Arbeit ist, dass eine bestehende Herzinsuffizienz durch die Bestimmung von GDF-15 ebenso zuverlässig wie durch NT-proBNP nachgewiesen werden konnte. Zudem konnte der aktuelle Status von NT-proBNP als Grundpfeiler der Herzinsuffizienz-Diagnostik bestätigt werden. Alle analysierten Biomarker zeigten eine Assoziation zur Mortalität, darüber hinaus brachten sST2 und CRP jedoch als einzelne Biomarker keinen zusätzlichen Nutzen.

Weiterhin wurden Biomarker-Indizes berechnet, von denen der Quotient GDF-15/NT-proBNP die besten Ergebnisse für die Diskriminierung von HFrEF und HFpEF aufwies.

Zusammenfassend konnte der aktuelle Stellenwert von NT-proBNP für die Diagnostik von Herzinsuffizienz bestätigt werden. Von den untersuchten neuen Biomarkern zeigte GDF-15 die aussichtsreichsten Ergebnisse, die ebenso wie der Biomarker-Quotient GDF-15/NT-proBNP in zukünftigen Studien, insbesondere in Behandlungsstudien, bestätigt werden sollten.

7 List of Abbreviations

ADM	Adrenomedullin
AHA	American Heart Association
AUC	Area under the curve
BMI	Body mass index
BNP	Brain natriuretic peptide
CAD	Coronary artery disease
CRP	C-reactive protein
CV	Coefficient of variants
CVD	Cardiovascular disease
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EF	Ejection fraction
ELISA	Enzyme-linked immunosorbent assay
eGFR	Estimated glomerular filtration rate
ESC	European Society of Cardiology
FU	Follow-up examination
GDF-15	Growth differentiation factor 15
GHS	Gutenberg Health Study
HF	Heart Failure
HFmrEF	Heart Failure with mid-range ejection fraction
HFpEF	Heart Failure with preserved ejection fraction
HFrEF	Heart Failure with reduced ejection fraction
HR	Hazard ratio
ILMA	Immunoluminometric assay
IL-6	Interleukin-6
IL-33	Interleukin-33
IRMA	Immunoradiometric assay
LoD	Limit of detection
LVEF	Left-ventricular ejection fraction
MI	Myocardial infarction
mi-RNA	Micro ribonucleic acid
MR-proADM	Mid-regional proadrenomedullin

NT-proBNP	N-terminal prohormone of brain natriuretic peptide
NYHA	New York Heart Association
OR	Odds ratio
ROC	Receiver operating characteristic
rpm	Revolutions per minute
SD	Standard deviation
ST2	Suppression of tumorigenicity 2
sST2	Soluble suppression of tumorigenicity 2
WHO	World Health Organization

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9 Appendices

9.1 Appendix A – Baseline Examination of Every Subject in the Gutenberg Health Study

Computerassistiertes persönliches Interview

– Soziodemografie
– Inanspruchnahme medizinischer Versorgung
– Krebsvorsorge
– Geschlechtsspezifische Fragen
– Medizinische Anamnese – Gesundheit und Erkrankungen
– Klassische Risikofaktoren
– Erkrankungsspezifische Beschwerden und Pathologie
– Familienanamnese
– Kinder
– Gesundheitsverhalten
– Hobbys und Freizeitverhalten
– Rauchen, Passivrauchen, Alkoholkonsum
– Berufsanamnese
– Feinstaub- und Lärmbelastung
– Lebenszufriedenheit und Umweltfaktoren
– Häusliche Umgebung

Medizinisch-technische Untersuchungen

– Erfassung der Medikation
– Spirometrie
– Messung von Kohlenmonoxid in der Alveolarluft
– Ruheblutdruck und Ruhepuls
– Zahntaschenabstrich
– Simultane Bestimmung von flussmediierter Vasodilatation sowie arterieller Steifigkeit mittels Messung der Reaktivität der A. brachialis mittels Ultraschall, Volumenplethysmographie der Digitalarterie mittels Endo-PAT sowie digitaler photoplethysmographischer Pulskurvenanalyse
– Neurokardiale Regulation
– Verschlussdruckmessung der Beine mit Bestimmung des Ankle-Brachial-Index
– Erfassung der aktuellen Wetterdaten

– Anthropometrie
– Körpertemperatur
– Elektrokardiogramm, Rhythmusstreifen
– Venöse Blutentnahme mit Bestimmung laborchemischer Routineparameter
– Sonographie der Halsschlagadern
– Zwei- und dreidimensionale Echokardiographie
– Ophthalmologische Untersuchung mit Bestimmung von Visus und Refraktion, Perimetrie (FDT), Fundusfotografie, Pachymetrie, Tonometrie und Spaltlampenuntersuchung

Befragung mittels Fragebögen
– Körperliche Aktivität
– Persönlichkeit, psychische Erkrankung und seelische Belastung
– Alltägliche Belastungen
– Soziale Integration
– Psychosoziale Belastung am Arbeitsplatz
– Lebensereignisse
– Visuelle Lebensqualität
– Ernährung

Gewinnung von Biomaterialien für das Biobanking
– Blutplasma
– Blutserum
– DNA (isoliert)
– RNA (isoliert)
– Gewaschene Erythrozyten
– Urin
– Zahntaschenabstrich

9.2 Appendix B – Measured Parameters of Echocardiography

Two-dimensional echocardiography

M-Mode: RV, LVDD, LVDDI, LVSD, LVSDI, FS, IVST, PWT, Wall-Mass. 2D-Echo/B-Mode: EDV, EDVI, ESV, ESVI, EF, LAV, RAV, LAd (apico-basal), RAd (apico-basal). PW-Doppler: MV-VE, MV-VA, E/A-ratio, Dec-Time. Tissue-Doppler: Ma-VS´, Ma-VE´, Ma-VA´, E/E´-ratio, Dec-Time, IRT+ICT, ETAorta, Tei-Index, Ma-VS´ Stress, Ma-VE´ Stress, Ma-VA´ Stress, E/E´-ratio Stress. Evaluation of calcification: Calcification Score – aortic valve, Calcification Score – mitral valve.

Three-dimensional echocardiography

RV, LVDD, LVDDI, LVSD, LVSDI, FS, IVST, PWT, Wall-Mass; EF.

Device for measurements: iE 33, Philips Medical Systems, NL.

Analysis software: QLab, Philips Medical Systems, NL.

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11 Publication

Parts of this doctoral thesis were previously published in:

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12 Curriculum Vitae

Lebenslauf wurde aus datenschutzrechtlichen Gründen entfernt.

13 Eidesstattliche Versicherung

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.

Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: .....