UNIVERSITÄTSKLINIKUM HAMBURG-EPPENDORF

University Heart Center Hamburg
Department of General and Interventional Cardiology

Director: Univ.-Prof. Dr. S. Blankenberg

Obesity and Novel Biomarkers in Heart Failure – Results from the Gutenberg Health Study

Doctoral Thesis

To attain the degree of medical doctor at the Medical Faculty of Hamburg University

Presented by:

Sevenai Ohdah
(Hamburg, Germany)

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1. Introduction

1.1 Heart failure – definition and epidemiology

In the background of an aging population, the incidence of heart failure rises and was reported to be 5.8 million in the United States and 23 million worldwide in 2010 (1,2). The traditional assessment of heart failure relied on clinical assessment of patient history, physical examination and chest x-ray. However, these methods lack the needed accuracy in diagnosing heart failure (HF). In this setting, many patients in the population (up to 48%) are asymptomatic upon presentation to hospital, even with a severe systolic or diastolic dysfunction (3,4). These results show the need to further enhance diagnostic as well as prognostic assessment in heart failure to account for the low sensitivity and specificity in the general population relying only on the previous above mentioned methods.

Heart failure

The European Society of Cardiology (ESC) describes heart failure as a syndrome. Patients with heart failure have typical symptoms and signs resulting from an abnormality of cardiac structure or function (5). When the heart cannot provide the required cardiac output to the organism while maintaining normal end-diastolic ventricular pressure; heart failure (HF) is present. Heart failure is the disability of the heart to supply enough blood and oxygen to the tissue. This may manifest clinically by dyspnea, fatigue, dizziness and fluid retention.

There are many causes of heart failure for example diseases of the myocardium, endocardium, pericardium, heart valves, vasculature or metabolism and abnormalities of the heart rhythm or diastolic function (5). Therefore, therapy depends on the etiology of HF.

Patients with heart failure report symptoms during exercise or even at rest.

To assess the functional impairment by heart failure, the New York Heart Association (NYHA) – classification (**table 1**) is commonly used, dividing heart failure due to the clinical presentation.

Table 1: New York Heart Association functional classification based on severity of symptoms and physical activity

Class I	Patients with heart disease without resulting limitation of physical activity. Physical activity does not cause HF symptoms, like breathlessness, fatigue or palpitations.
Class II	Slight limitations of physical activity. Ordinary physical activity results in symptoms. No symptoms at rest.
Class III	Marked limitations of physical activity. Less than ordinary physical activity develop symptoms of HF.
Class IV	Patients cannot perform any physical activity without discomfort. Symptoms may occur even at rest.

The American Heart Association (AHA) stages the heart failure and this includes patients with a high risk for heart failure without a structural heart disease (6). The AHA classification emphasizes the structural heart disease (**table 2**).

Table 2: The classification of the American Heart Association

Stage A	Patients with a high risk for a HF, without structural heart disease or symptoms.
Stage B	Structural heart disease without symptoms of a HF, this corresponds to patients with NYHA Class I.
Stage C	Structural heart disease with prior or current symptoms of HF, this stage includes patients of NYHA class II and III.
Stage D	Structural heart disease, patients cannot perform any physical activity without discomfort. Symptoms may occur even at rest. This includes patients with NYHA class IV.

Because heart failure symptoms are often non-specific, it is difficult to determine the true cause and differentiate heart failure from a multitude of various other diseases. Further examinations often suggestive of heart failure including the patient history are the electrocardiogram, echocardiography and analysis of drawn blood samples. Furthermore, chest radiography, exercise testing, noninvasive stress testing, cardiac magnetic resonance (CMR), single photon emission computed tomography (SPECT), coronary angiography and endomyocardial biopsy may help to establish the correct diagnosis and the underlying diseases causing structural changes resulting in heart failure.

In the disease spectrum of heart failure it is important to distinguish between heart failure with reduced ejection fraction (HFrEF) also known as heart failure due to left ventricular systolic dysfunction (systolic heart failure) and the heart failure with preserved ejection fraction (HFpEF). Since clear definitions are lacking, HFrEF is most often described with a reduced left ventricular ejection fraction (LVEF) <55%. In contrast, diagnosis of HFpEF needs a LVEF of ≥55% besides different co-variables as described by additional parameters, including biomarkers (natriuretic peptides) and imaging parameters (tissue Doppler, left atrial size, strain) as this may improve the likelihood that HF symptoms are indeed of cardiac origin (7,8). Moreover, some patients with HFpEF may have non-diastolic abnormalities like chronotropic incompetence or changes in ventricular coupling. In general, heart failure is a highly prevalent disease associated with increased mortality, repeated and lengthy hospitalization, and disability. In this context, HFpEF still lacks proper management with a high morbidity and mortality (9).
Therefore, an important issue in the disease spectrum of heart failure is the rising incidence of risk factors like arterial hypertension and coronary artery disease and in the consequence of HFpEF in the population and the need to shift the focus from secondary to primary prevention. In this setting, uniformity regarding diagnosis, follow-up and patient characteristics is essential and can be achieved in population-based studies like the Gutenberg Health Study (10). Both, HFrEF and HFpEF, have different risk factors identifying these entities like ischemic heart disease as predominant risk factor for HFrEF, while hypertension, obesity, and

diabetes are risk factors for HFpEF (11). First study results in secondary prevention cohorts regarding additional biomarkers suggest that myocardial injury represented by high-sensitivity determined troponin T (hsTnT) and increased wall stress N-terminal pro B-type natriuretic peptide (NT-proBNP) are associated with HFrEF, and systemic inflammation as shown through growth differentiation factor 15 (GDF15) with HFpEF (12).

1.2 Obesity and the risk of heart failure

Obesity is a nutritional and metabolic disease. It is characterized by an above and beyond the normal degree increase in body fat with pathological effects. The body mass index (BMI) is defined as the weight in kilograms (kg) divided by the height in meters squared (m^2).

BMI= weight in kg/ height in m^2

According to the World Health Organization (WHO) definition of obesity is available from a body mass index (BMI) of ≥ 30 kg/m^2 (13) see **table 3**.

Table 3: WHO Classification for BMI

BMI kg/m^2	Classification
≤18,5	Underweight
≥18,5 to 24,9	normal weight
≥25 to 29,9	Overweight
≥30 to 34,9	class I obesity
≥35 to 39,9	class II obesity
≥40	class III obesity

However, Deurenberg et al. showed that the obesity classification also depends on the ethnic groups (14). People with South asian ancestry reach a higher body fat percentage even at a lower BMI, compared to Caucasian ethnicity. In contrast, African and afro-american ethnicity present with a higher body fat percentage with higher BMI (14,15).

Obesity is a chronic disease which is increasing worldwide and is associated with high morbidity and mortality. The National Health and Nutrition Examination Survey (NHANES) showed in the USA the prevalence of obesity in adults at 34.9 percent, the data was collected between 2011 and 2012 (16). The number of adults with a BMI≥ 25 kg/m² has increased worldwide between 1980 and 2013, from 28,8% to 36.9% in men and from 29,8 to 38% in women (17).

Obesity is a risk factor for diabetes mellitus, hypertension, hyperlipidemia and left ventricular hypertrophy, leading to an increased incidence of heart failure (18). It is often a challenge to diagnose heart failure in obese patients, since obesity may mask the symptoms of heart failure, because obese subjects also have typical heart failure symptoms such as dyspnea, orthopnea, ankle swelling and fatigue (5,18). Therefore a major task in the future is to relate diagnosis and outcome data to the important aspect of obesity and the connection to heart failure. Novel biomarkers represent a promising approach to individualize diagnosis, treatment and lastly prognosis. Concentrations of these modern, new biomarkers, each representing a different aspect in pathophysiology and the corresponding biologic reaction of the body have to be of future interest regarding concentration ranges in obese, as well as in the overall general population. With this information, future assessment of heart failure can be established, proposing cut-off values in obese subjects of the general population.

1.3 Biomarkers in heart failure – diagnosis and prognosis

In the emergency room we need to have variable diagnostic tools to distinguish the differential diagnosis and to establish heart failure. Therefore we use, among other investigations, the blood concentrations of the natriuretic peptides like B-type natriuretic peptide (BNP) and N-terminal pro B-type natriuretic peptide (NT-proBNP).

Diverse studies tried to determine the cut-off concentrations for natriuretic peptides. Maisel et al. showed 2002 in their prospective study with 1568 patients that a cut-off level of 100pg/ml for BNP would be optimal for diagnosing heart failure (20).

The B-type Natriuretic Peptide for Acute Shortness of Breath Evaluation (BASEL)

study was a prospective study which included 452 patients and used 2 cut-off values for the BNP (100 and 500pg/ml). If a patient had a BNP concentration of 100pg/ml to 500pg/ml the investigator had to decide whether further examinations were required or heart failure could be possible. For the patients with a BNP>500pg/ml the presence of heart failure was accepted and it was important to quickly initiate heart failure therapy. (84)

The ESC guidelines of 2012 for chronic and acute heart failure defined cut-off levels for BNP and NT-proBNP. The blood levels differ depending on the clinical status of the patient. In order to exclude heart failure for patients with acute symptoms the cut-off levels are 300pg/ml for NT-proBNP and 100pg/ml for BNP, in stable patients the levels are 125pg/ml for NT-proBNP and 35pg/ml for BNP. (5)

But the sensitivity and specificity in the stable patients is lower than in the patients with acute symptoms (85).

1.3.1 Criteria for a biomarker

What are the criteria for a candidate biomarker?

The following table (**table 4**) describes the main criteria that need to be fulfilled for a candidate biomarker to be established in clinical medicine. Currently management and treatment is mostly influenced by natriuretic peptides, representing the gold standard.

Table 4: Criteria for candidate biomarkers

Criteria
1. The marker should be evaluated across a wide range of patients using rigorous and contemporary statistical methods
2. Results should be easily obtained within a short period of time and provide acceptable level of accuracy-defined biological variation and low analytical imprecision
3. Results should reflect important pathophysiological processes in HF presence and progression
4. Results should provide clinically useful information beyond status quo

In comparison to the current gold standard, represented by natriuretic peptides, none of the presented candidate biomarkers in this manuscript meets all criteria from **table 4** but some come very close in doing so (21,22). Main reason for the complex process of evaluating such markers is that different pathophysiologic states like pressure or volume overload can finally manifest in heart failure. Biomarkers thus can indicate a variety of health and disease characteristics indicating a biological reaction in the human body to the exposure of different factors (83).

1.3.2 Current state-of-the-art for diagnosis and management of heart failure

Natriuretic Peptides

In healthy individuals, only low BNP levels are measured, whereas in states of increased myocardial stretch, like heart failure or myocardial infarction, BNP expression is increased. Recent studies already showed the value of BNP as well as NT-proBNP for improved diagnosis of heart failure (19,20). However, there are also limitations for the sole use of natriuretic peptides. The diagnosis of heart failure is not 100% specific when using natriuretic peptides as biomarker, as increased natriuretic peptide concentrations reflect structural heart disease and presence of cardiovascular risk factors. Apart from age and ventricular function, obesity, atrial arrhythmias, renal function and heart disease beyond heart failure influence natriuretic peptide concentrations.

It is still a continuous process when the heart function is impaired reaching from an at-risk but structurally normal organ to cardiac injury, ventricular dysfunction and finally symptomatic heart failure. In addition to the mentioned B-type natriuretic peptide, midregional pro atrial natriuretic peptide (MR-proANP) has emerged as a promising biomarker in patients with congestive heart failure (23). MR-proANP and the B-type natriuretic peptides exhibited similar associations to previous or prevalent cardiovascular disease and echocardiographic data. In subgroups with confounding conditions (female sex, obesity, renal dysfunction), MR-proANP did not exhibit stronger associations to echocardiographic data than the B-type natriuretic peptides and was not superior in diagnosing heart failure in patients with atrial fibrillation due to the different release pattern (23,24). In the

Gutenberg Health Study (GHS) there was a moderate to strong correlation of the biomarkers with age, diabetes, hypertension, smoking, renal function, prevalence of coronary artery disease and heart failure. Males showed lower MR-proANP concentrations than females (25). In general, MR-proANP was not inferior to BNP in diagnosing heart failure; however it offers additional information in patients within the grey zone of BNP concentrations between 100-500 pg/mL and in obese patients. In the PRIDE study MR-proANP was an independent predictor of heart failure diagnosis and provided information beyond BNP or NT-proBNP suggesting a superior accuracy in combining both natriuretic peptides (26).

Current studies could show that natriuretic peptide concentration is different in patients with HFpEF and HFrEF, with lower concentrations in patients with HFpEF. However, upon reaching a certain concentration regarding natriuretic peptides the prognosis is poor in both sub-types of heart failure (27).

For establishing diagnosis in HF patients natriuretic peptides are used reliably as HFrEF and HFpEF both influence load and filling of the left ventricle (21,22), however different biomarkers might be used as well to ascertain diagnosis of HF (28,29). In the acute setting, natriuretic peptides still remain the mainstay for the diagnosis but as HF is a result of multiple changes and pathophysiologic conditions, additional biomarkers allow to refine prognosis and to shed light on new treatment targets (21, 28-30).

1.3.3 High-sensitivity determined cardiac troponins (hs cTn)

According to the universal definition of myocardial infarction (31), cardiac troponin I and T are the biomarker of choice to diagnose acute myocardial necrosis. Evolution of troponin tests has recently led to the determination of troponin via high-sensitivity assays (32). Regarding the pathology represented by elevated high-sensitive troponin I (hsTnI) concentrations; the mechanisms are numerous like myocardial infarction type 1 and 2 (with and without coronary heart disease), inflammation, apoptosis and cytotoxicity as a result of cardiac remodeling and contribute to elevated troponin I concentrations (33,34). It is known from population-based studies that troponin T correlates with cardiovascular risk factors, age and impaired renal function (34, 35). Estimation of troponin in at-risk

subjects, like older community dwelling individuals showed the additive information regarding incident heart failure or cardiovascular death, mirroring the pathophysiology of different causes of heart failure and the resulting structural changes to the heart itself (34,36,37). These new high-sensitivity determined troponin assays allow for detection of troponin levels in the general population and therefore might add prognostic information in the general population as shown in diseased cohorts (38,39).

1.3.4 Midregional proadrenomedullin (MR-proADM)

Adrenomedullin was at first described in pheochromocytoma cells and increases myocardial contractility through a cyclic-AMP dependent mechanism, further it stimulates nitric oxide synthesis and thus causes vasodilatation (21,22). Although MR-proADM is not cardiac specific, the additional use to predict an adverse short term outcome in patients with acute heart failure or presenting with acute dyspnea was reported earlier from the BACH trial and the PRIDE study (26,40). Current data indicate that MR-proADM is associated with classical risk factors and cardiovascular diseases, especially heart failure and coronary artery disease (41,42).

The additional use of MR-proADM in patients with known stable coronary artery disease and as well the general population in comparison to natriuretic peptides was recently shown in two large population based studies (43,44).

1.3.5 Growth differentiation factor - 15 (GDF-15):

GDF-15 is a member of the transforming growth factor-β cytokine super family, and participates in mitigation of myocardial stress and remodeling; expression of GDF-15 is strongly induced in cardiomyocytes in response to metabolic stress such as cardiac ischemia or pressure overload state (45,46).

In diseased patients, suffering from heart failure, measurement of GDF-15 improved the prediction of mortality and an adverse outcome (47,48). Interestingly, GDF-15 levels seem to better correlate with diastolic dysfunction than NT-proBNP levels and thus add incremental information to NT-proBNP in a population at risk (48). The concentration of GDF-15 is low during lack of

cardiovascular events, inflammation or tumor genesis, however increases with apoptosis, cell death and chronic inflammation (22).

1.3.6 Soluble source of tumorogenicity 2 (sST2)/ receptor of IL-33

ST2 belongs to the interleukin-1 receptor family and consists of the transmembrane and soluble (sST2) isoform (49,50). The complex of interleukin-33 and its decoy receptor sST2 have an important role in pathogenesis of cardiovascular disease (50-52) as increased concentrations of sST2 lead to an impaired signaling by the cardioprotective interleukin-33 and subsequently to heart failure and an increased number of adverse events. The pathophysiology of increased concentrations of sST2 and thus impaired IL-33/ST2L signaling are cardiac hypertrophy, fibrosis, worsening left ventricular function and arterial hypertension (50,53-56).

In previous studies, the concentrations of sST2 were related to increased cardiovascular events and heart failure as singular biomarker and also in a combined approach of a biomarker panel (29) improved the prognostic information regarding cardiovascular events and heart failure. Risk factors influencing sST2 concentration in a reference population were male gender, increased age, arterial hypertension and diabetes (53,56). It was shown that increased concentrations of sST2 are connected to an adverse outcome in patients with non-ST-segment-elevation infarction and diagnosed chronic heart failure (57,58).

1.3.7 C-reactive protein (CRP)

The protein CRP is an acute- phase protein which is synthesized in the liver and regulated by cytokines. An increased concentration of interleukin 6, which is produced in the macrophages and adipocytes, leads to an increased production of CRP (59,60). CRP binds to phosphocholine, this activates the complement system and enhances phagocytosis of apoptotic cells by macrophages (61,62).

CRP is used as an inflammation marker and elevated CRP levels especially the high- sensitivity CRP (hs CRP) levels are associated with a higher cardiovascular risk (60,63,64).

The studies of Motie et al. and Visser et al. also showed us that a higher BMI is related with a higher CRP level (60,65).

1.4 Obesity and the diagnosis of heart failure with biomarkers

Obese individuals have an increased risk for heart failure. Although typical symptoms of heart failure maybe present in obesity as well and may mask coincidence of heart failure and obesity. The current gold standard represented by the natriuretic peptides have lower concentrations in obese subjects (66-69). In this background, the usefulness of the natriuretic peptides has been questioned because of the unequivocally observed inverse relationship with body mass index (BMI) (18,68). Even though obesity is related with cardiac pressure overload and volume expansion, which usually leads to an increased level of BNP, the BNP levels of obese people are in contrary lower (18,70-72). In some studies obesity is related to an elevated clearance of BNP (73,74). In contrast, the Suita Study disproved this theory, in which the multivariable regression analysis was adjusted for the serum creatinine but nevertheless showed the inverse relationship of natriuretic peptides with BMI (75). Another speculation related to the adipose tissue expansion in obesity. Since the adipocytes express their natriuretic peptide clearance receptor-C (NPR-C), this could lead to a low BNP level (18,76,77). But the Dallas Heart Study refuted this and also reported low BNP levels in obese subjects and that low levels were unrelated to NPR-C (66).
Therefore it is necessary to evaluate new cut off values for natriuretic peptides in obese subjects

Baessler et al. were able to demonstrate in their study that GDF- 15 levels are better associated with HFpEF than NT-proBNP levels in obese HF patients (48). An inadequate myocardial adaptation to chronic volume overload in obese patients with HFpEF is therefore better represented by GDF-15 than by natriuretic peptide levels (48).

The use of new biomarkers could be helpful to detect heart failure in obese subjects.

1.5 Aims and hypothesis of the doctoral thesis

The doctoral thesis targets to answer the following main questions by the analyses of the first 5000 subjects participating in the Gutenberg Health Study:

a.) Biomarkers can have a different concentration in obese subjects as already shown for natriuretic peptides in previous studies. Therefore we intend to investigate whether the NT-proBNP levels are dependent on BMI.

b.) If our hypothesis will be confirmed, we want to set new cut-off values for NT-proBNP for the different BMI categories BMI<30 or ≥ 30 kg/m² to detect a heart failure.

c.) In addition to the current gold standard, represented by natriuretic peptides, a panel consisting of novel biomarkers; sST2, GDF-15, hs TnI, CRP, MR-proADM and MR-proANP will be used to evaluate concentration ranges in obese subjects and define diagnostic concentration cut-off values in the overall first 5000 subjects of the GHS.

d.) Additional characterization is needed, if novel biomarkers might prove superior to natriuretic peptides for identification of overall heart failure in subjects with a BMI<30 or ≥ 30 kg/m² and to correlate the biomarkers in the different cohorts to clinical variables and risk factors.

e.) As prognostic data regarding overall mortality is present, the concentration of each novel biomarker is used to define patients at risk for fatal outcome in the different BMI categories BMI<30 or ≥ 30 kg/m².

2. Material and Methods

2.1 Patients and study cohort – the Gutenberg Health Study (GHS)

The GHS is designed as a prospective population-based, cohort study in the Rhine-Main region of Germany. The primary study aim is to evaluate and improve cardiovascular risk stratification. The study sample was drawn randomly from the governmental local registry offices in the city of Mainz. The sample was stratified 1:1 for gender and residence and in equal strata for decades of age. Briefly, study individuals aged 35 to 74 years and stratified according to gender and age were selected randomly by the registration office from the city of Mainz. Baseline recruitment was conducted between April 2007 and April 2012, finally including 15,010 individuals. All individuals were invited for a 5-hour baseline-examination at the study center. During this baseline examination the investigations and standardized blood draw were carried out patterned. The following medical examinations were performed for every person:

- Resting blood pressure and heart rate
- Spirometry
- Expired carbon monoxide – measurement
- Endothelial Function measurement
- Flow- mediated dilatation
- Arterial waveform collection
- Ankle-Brachial Index
- Anthropometric measures
- Electrocardiogram
- Sonography of the Carotid Arteries
- Echocardiography
- Ophthalmological Examination
- Laboratory routine parameters
- Optional assays, biomarker

In addition the participants were interviewed about medical history, medication, classical risk factors, socio demographic data, lifestyle factors and endpoints.

The Gutenberg Health Study was approved by the ethics committee of

Rhineland-Palatinate and the medical faculty of the Johannes Gutenberg-University, Mainz. Each study individual provided written informed consent before participating. The ethical application complied with the Declaration of Helsinki. A detailed study description has been published earlier (10). The first follow up (F1) examination started 2, 5 years after the baseline-examination in 2009 with a computer-assisted telephone interview (CATI), this needed 20- 30 minutes. Starting in 2012, study participants have been recruited for the 5 years follow up (F2) of the study including the same examinations and biobanking as during the baseline examination. Biomarker measurements (Nt-proBNP, MR-proANP, MR-proADM, GDF-15, sST2 and hs-TnI) were performed in the first 5000 study participants.

To address the specific question if obesity influences biomarker concentrations, we divided the cohort according to the calculated BMI into two sub-cohorts: 1. BMI <30 (N=3794, 1900 women and 1894 men), 2. BMI ≥30 with obesity (N=1204; 559 women and 645 men).

GHS Timeline:

Baseline-Investigation:	04/2007 – 04/2012
Follow-up examination 1:	10/2009 – 10/2014
Follow-up examination 2:	04/2012 – 04/2017

2.2 Study aims of the GHS

2.2.1 Primary study aim

The primary aim of the study is to achieve a new cardiovascular risk score, which takes into account additionally to the classical risk factors, psycho-social, environmental and lifestyle-risk factors, subclinical atherosclerotic disease, protein muster and genetic variability with respect to the primary endpoint (myocardial infarction and cardiovascular death).

2.2.2 Secondary study aims

Secondary study aims are

- To develop risk scores in term of the secondary endpoints (apoplex, overall deaths, development of heart failure, development of diabetes) takes into account additionally to the classical risk factors, psycho-social, environmental and lifestyle-risk factors, subclinical atherosclerotic disease, protein muster and genetic variability.
- To test the additional predictive value of measures of subclinical atherosclerosis (e.g. measurements of endothelial function or arterial stiffness) for cardiovascular risk prediction in comparison to risk models that are based on classical risk factor models only.
- To provide accurate, quantifiable measures of early cardiovascular disease;
- To characterize cardiovascular disease before it has become clinically manifest and therefore subject to interventions that disrupt natural history;
- To provide a sophisticated biobank including DNA, RNA, cells, and serum/plasma for comprehensive genetic, gene expression and proteomic studies; the biobank is installed to investigate risk factors and associations for cancer, eye diseases, diseases of the immune system and metabolic disorders;
- To explore the impact of cardiovascular candidate genes on cardiovascular risk stratification by using genome wide analyses and the biological system approach;
- To explore proteins relevant for cardiovascular disease;
- To allow identification of new therapeutic targets;
- To provide estimates for the incidence of myocardial infarction, cardiovascular death and stroke in the study region;
- To provide estimates for the prevalence of cardiovascular risk factors in the study region;
- To evaluate the impact of socio-economic, environmental and lifestyle factors on the cardiovascular risk.

2.2.3 Tertiary study aims

Tertiary study aims are

- To investigate risk factors and associations for cancer, eye diseases, diseases of the immune system and metabolic disorders+ using all data acquired and the collected biomaterial.
- To develop risk scores for the occurrence of cancer, eye diseases, diseases of the immune system and metabolic disorders.
- To characterize new genes and variants contributing to cancer, eye diseases, diseases of the immune system and metabolic disorders.
- To assess the overlap of cancer, eye diseases, diseases of the immune system and metabolic disorders with the prevalence of cardiovascular disease by use of the database and biobank characterising a variety of intermediate clinical phenotypes and establishing a sophisticated biobank in a prospective population based cohort study.
- To translate the genetic findings into protein identification and relate the candidate protein to cancer, eye diseases, diseases of the immune system and metabolic disorders using the established plasma and serum biobank.
- To evaluate a new risk model for cardiovascular risk stratification that amends the established risk scores (e.g. EURO HEART Score, Deutschland Score, PROCAM Score and Framingham Heart Score) by the results of an ocular fundus examination.

2.3 Biobanking and measurement of biomarkers

The blood withdrawal was performed in all participants; a total of 114,5ml blood was obtained.

Biomaterial was processed immediately after blood draw, and samples were stored at -80°C in a large biobank. Biomaterial includes serum, plasma, DNA, RNA, and blood cells.

Therefore the biomaterial will be long-term stored in the laboratories of the study center, but the longest warehousing will be 60 years.

To protect the storages an electronic temperature monitoring system is used, this will activate an alarm in case of an increasing temperature.

The primary goal of the GHS study with regard to biological analyses is to establish a biobank which enables researchers to investigate hypotheses in relation to different aspects of cardiovascular disease, cancer, the immune system and metabolic disorders and their risk factors by use of biological material which represents the different stages from DNA to protein. Material shall be used by contemporary, high throughput methods for genotyping, gene expression profiling and proteomic analyses.

2.4 Sampling of biomaterial

The following biomaterial will be sampled for biobanking:

- Blood
  - Serum
  - Plasma (EDTA, citrated, heparinized)
  - DNA
  - RNA
  - Washed erythrocytes
- Urine
- Tear fluid
- Tooth plaque sample

The samples will be collected under standardized conditions according to standardized operating procedures.

2.4.1 Blood sampling

The participants are asked to have an overnight fast of at least 11 hours when the appointment to the study center is before 12.00 a.m. and a prior fast of at least 5 hours for appointments after 12.a.m.. The participants are allowed to drink pure water during the fasting time. If they have long-term medication, they are taking it as usual except vitamin containing medication. They are asked to not do any sports and not consume alcohol within 8 hours and to eat no rich food within 12 hours prior to the investigation.

The blood withdrawal will be performed in lying position on the right or left forearm or in the elbow flexure. Altogether 114,5ml of blood will be collected. The detailed procedure is documented in the respective standard operating procedure.

The blood will be collected in the following sequence and tubes (purpose in brackets):

1. CPT Vacutainer BD à 8 ml (Biobanking)
2. 1 Citrat-Monovette à 9 ml (Biobanking)
3. 1 Citrat-Monovette à 2,7 ml (Routine laboratory)
4. 1 Heparin-Monovette à 2,6 ml (Routine laboratory)
5. 1 EDTA-Monovette à 2,7 ml (Routine laboratory)
6. 1 brown Serum-Monovette à 2,6 ml (Routine laboratory)
7. 4 white Serum-Monovette à 7,5 ml (Biobanking)
8. 6 EDTA-Monovette à 9 ml (Biobanking)
9. 1 EDTA-Monovette à 2,7 ml (Routine laboratory)
10. 1 Homocystein-Monovette à 2,9 ml (Routine laboratory)

Figure 1 shows the distribution of the subsets

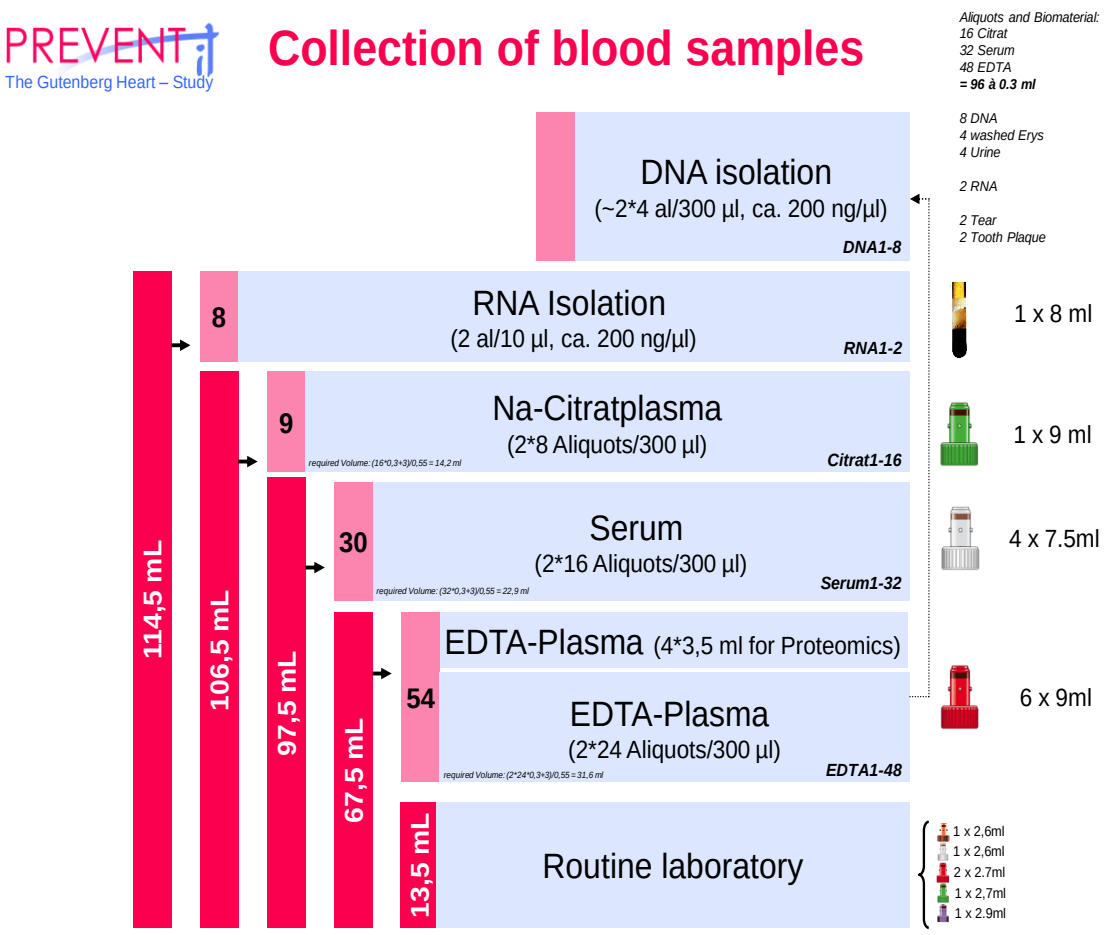

Figure 2 Collection of blood samples

2.5 Biomarker assays

Seven biomarkers reflecting hemodynamics and remodeling (Nt-proBNP, MR-proANP), inflammation (CRP, GDF-15), vascular function (MR-proADM), fibrosis (sST2) and cardiac damage (hsTnI) were measured by commercially available assay systems or antibodies. Serum creatinine was measured by the modified Jaffe routine method.

NT-proBNP: NT-proBNP concentration was measured on the ELECSYS 2010 using an electrochemiluminescence immunoassay (ECLIA, Roche Diagnostics). The analytical reporting range is 5–35.000 ng/L. The functional assay sensitivity

(defined as the lowest concentration detectable with an inter-assay CV of 20%) is <50 ng/L. Intra- and inter-assay imprecision for the luminescence immunoassay is 0.8%–3.0% and 2.2%–5.8%, respectively.

sST2: The concentration of sST2 was determined with a high-sensitivity, second-generation ELISA with a detection limit of 2 ng/mL (Presage ST2, Critical Diagnostics) (93). sST2 values >35 ng/mL have been linked to adverse outcomes in the setting of overt heart failure.

GDF-15: The measurement of GDF-15 was done with an immunoradiometric assay developed by Wollert and colleagues (89). The assay uses a polyclonal, affinity chromatography–purified goat antihuman GDF15 IgG antibody and had a detection limit of 20 ng/L, an intra-assay imprecision of ≤10.6%, and an inter-assay imprecision of ≤12.2% [2].

CRP: The concentration of CRP was measured with an Abbott Architect c8000 system. The CRP Vario kit is a latex in vitro diagnostic immunoassay for the quantitative determination of CRP in human serum and in heparinized and EDTA-plasma.

MR-proADM: Plasma MR-proADM was measured using a novel commercial assay in the chemiluminescence/coated tube format (MR-proADM LIA, BRAHMS AG) (94). The lower limit of detection of the assay is 0.08 nmol/L. The functional assay sensitivity (defined as the lowest concentration detectable with an interassay CV of 20%) is 0.11 nmol/L. The intra-assay CVs at 0.5 and 5 nmol/L are 3% and 3.5%, respectively; the inter-assay CVs at 0.5 and 5 nmol/L are 8.5% and 6.5%.

MR-proANP: MR-proANP was determined in EDTA plasma samples (stored at -80°C) with a commercially available automated immunfluorescence assay (BRAHMS MR-proANP KRYPTOR, BRAHMS GmbH, Hennigsdorf, Germany). The direct range of detection is 2.1-1000 pmol/L. The functional assay sensitivity (defined as the lowest concentration detectable with an inter-assay coefficient of variation of <20% is below 10 pmol/L. The intra-assay coefficient of variation was <2,5% for samples containing 20-1000 pmol/L MR-proANP with an inter-assay CV of <6.5% for the same concentration range (95).

Hs troponin I: Troponin I was assessed using a commercially available high-sensitivity cardiac troponin assay (ARCHITECT STAT highly sensitive Troponin I immunoassay, Abbott Diagnostics, USA, ARCHITECT i2000SR). The limit of detection (LoD) for the assay was 1.9pg/mL (assay range 0-50,000 pg/mL). The assay has a 10% coefficient of variation at a concentration of 5.2 pg/mL. Intra-assay and inter-assay coefficients of variation were 4.26 and 6.29%, respectively (96).

2.6 Assessment of cardiovascular risk factors and diseases

Risk factors were assessed as outlined in previous publications (10). Former history of stroke, coronary artery disease, myocardial infarction, heart failure and peripheral artery disease were assessed in a standardized interview. Arterial hypertension was defined as a systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg at rest obtained as the mean of the second and third measurement, or by taking any antihypertensive drugs within the last 2 weeks. Diabetes mellitus was defined as a fasting glucose ≥ 126 mg/dl, a spontaneous glucose concentration of ≥ 200 mg/dl, or as diagnosed by a physician. We defined dyslipidemia as a LDL/HDL-ratio of > 3.5 or as diagnosed by a physician. Smokers were classified into daily smokers (≥ 1 cigarette/day), occasional smokers (< 1 cigarette/day), former smokers, and non-smokers (never smoked). Any family history of myocardial infarction in first-degree relatives before the age of 65 years for females and before the age of 60 years for males was defined as positive family history. For glomerular filtration rate (GFR), as the best marker for renal function in health and disease, we used the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation (78). Since creatinine measurements according to the method described by Jaffe are not IDMS-traceable (being the “standard creatinine”) we multiply the creatinine value by 0.95 before using the CKD-EPI equation as described by Matsushita et al.(79).

2.7 Assessment of cardiac structure and function

All subjects underwent multimodal echocardiography with an iE33 echocardiography system with an S5–1 sector array transducer (Royal Philips Electronics, Amsterdam, The Netherlands), a phased array with 80 elements and a 5- to 1-MHz operating frequency range (80). The examinations were performed according to standard operating procedures by trained and certified medical technical assistants at a single center. Measurements were according to recommendations by the American Society of Echocardiography (81).

Echocardiographic measurements of systolic and diastolic function

The systolic function of each individual was assessed by use of the biplane simpson method in 2D echocardiography in the 4- and 2- chamber view, as recommended in the guidelines systolic function was defined normal ≥55%, mildly abnormal 45-54%, moderately abnormal 44-30% and severly abnormal <30% (81,82). Therefore, subjects with typical signs and symptoms of heart failure and an EF of <55% were defined as HFrEF and subjects with typical signs symptoms and an EF ≥55% as HFpEF.

2.8 Statistics

The data obtained a patient collective of N= 5000 in a period of 7 years in the Rhine Main Region of Germany.

To characterize the specific question if obesity influences biomarker concentrations, we divided the cohort according to the calculated BMI into two sub-cohorts: 1. BMI <30 (N=3766, 1879 women and 1887 men), 2. BMI ≥30 with obesity (N=1204; 559 women and 645 men).

Baseline table: Continuous variables will be described using quartiles and categorical ones using frequencies. This will computed with and without weights.

Distribution of biomarkers: Histograms and quartiles of the biomarkers (NTproBNP, MR-proANP, hs TnI, hsCRP, MR-proADM, GDF-15, sST2) will be computed for the following groups:

a) Overall sample
b) Stratified by BMI categories
c) Stratified by BMI categories and sex
d) Stratified by BMI categories and HF (vs no HF)
e) Stratified by BMI categories, HF (vs no HF) and sex

Correlations: Correlations and partial correlations adjusted for age and sex will be computed for the biomarkers: MR-proANP, hs TnI, hsCRP, MR-proADM, GDF-15, sST2 and NT-proBNP. This analyses will be performed in the overall sample.

Biomarker cut-offs for HF in the different BMI subgroups: For each biomarker (NTproBNP, MR-proANP, hs TnI, hsCRP, MR-proADM, GDF-15, sST2) and on each BMI category the following will be done:

a) Computation of the cut-off that maximizes the Youden index for the diagnosis of HF (vs no HF).

b) Sensitivity and specificity for this cut-off will be computed.

c) Bootstrap will be used to correct the over-optimism of the estimates in b).

Logistic regressions for each biomarker:

The following endpoints will be used:

a) HF vs no HF

The models will be adjusted for the following variables: age, sex, diabetes, hypertension, dyslipidemia, current smoking.

The explanatory variables of interest are:

i) Biomarker (only one per model of NT-proBNP, MR-proANP, hs TnI, hsCRP, MR-proADM, GDF-15, sST2).

ii) BMI categories.

iii) Interaction of i) and ii)

Logistic regressions for biomarker panel:

The endpoints and adjusting variables are the same as in the previous point. The explanatory variables of interests are:

i) Biomarker (NT-proBNP, MR-proANP, hs TnI, hsCRP, MR-proADM, GDF-15, sST2).

ii) BMI categories.

iii) Interaction of i) and ii)

Remark: On these models all biomarkers appear on the same model.

Association of biomarkers to all-cause mortality (Cox regressions):

The endpoint is all-cause mortality. The models will be adjusted for the following variables: age, sex, diabetes, hypertension, dyslipidemia, current smoking.

The explanatory variables of interest are:

i) Biomarker (NT-proBNP, MR-proANP, hs TnI, hsCRP, MR-proADM, GDF-15, sST2).

ii) BMI categories.

iii) Interaction of i) and ii)

As in the logistic models above a version with only one biomarker per model and a version where all biomarkers are used in one model will be computed.

3. Results

3.1 Baseline characteristics and concentrations of biomarkers

Analyses are done in A2 (N = 5000) excluding 28 individuals that are underweight, that is, with BMI < 18.5 kg/m², and 2 that have no BMI information. This leaves us with 4970 individuals for the analyses.

The GHS sample was drawn randomly with stratification by gender, residence (urban and rural) and decade of age. The stratification was designed to have the same proportion of men and women, the same proportion of individuals on each decade of age and the same proportion of individuals of urban and rural residence. Due to this stratification on some of the quantities estimated in what follows, weighting of the estimates is used according to the age and sex distribution of the underlying population. When weighting is used this is explicitly stated on the corresponding table or graphic.

For the baseline characteristics refer to **table 5**. The sample included 3766 individuals with a BMI<30 kg/m² (median age 49 years and 42/60 years, 48.8% male) and 1204 participants with obesity and a BMI≥30 kg/m² (median age 55 years and 45/64 years, 53.4% male).

According to symptoms and echocardiography examination, HF was diagnosed in 2.8% subjects with a BMI<30 kg/m² and in 9.8% in obese subjects.

Classical risk factors like hypertension, diabetes and dyslipidemia were more prevalent in obese subjects despite smoking, being more common in the cohort with a BMI <30. For biomarker concentrations GDF-15 showed higher concentrations in obese individuals with 908.0 ng/L vs. 818 ng/L in subjects with a BMI<30 kg/m². In the obese participants the CRP and the hsTnI level were higher and the MR-proANP level lower than in the subjects with a BMI<30kg/m². For the markers sST2, MR-proADM, NT-proBNP no weight related difference could be described. During follow-up with a median of 6.9 years, 192 subjects died from any-cause. Mortality within the cohort of subjects with BMI<30kg/m² was 133 and with a BMI≥30kg/m² 59.

Table 5: Baseline characteristics (weighted according to the distribution of age and gender in Mainz/Mainz-Bingen (N=210867).)

	BMI < 30 (N=3766)	BMI >= 30 (N=1204)	p-val
Age (years)	49.0 (42.0, 60.0)	55.0 (45.0, 64.0)	<0.001
BMI (kg/m²)	25.1 (22.9, 27.1)	32.6 (31.1, 35.3)	<0.001
eGFR (mL/min for 1.73m²)	93.0 (83.9, 102.0)	92.1 (82.1, 100.8)	0.016
Male %	48.8	53.4	0.0092
Diabetes %	3.5	14.5	<0.001
Hypertension %	38.8	67.7	<0.001
Dyslipidemia %	24.6	41.9	<0.001
Current smoking %	21.6	18.8	0.071
EF (%)	64.1 (60.4, 67.8)	63.6 (59.8, 67.3)	0.015
HF %	2.8	9.8	<0.001
NT-proBNP (pg/mL)	53.5 (24.0, 105.1)	54.2 (22.0, 120.9)	0.66
CRP (mg/L)	1.4 (0.8*, 2.6)	2.9 (1.7, 5.2)	<0.001
CRP above LOD %	67.1	91.4	<0.001
hsTnI (ng/L)	3.4 (2.1, 4.9)	4.1 (2.8, 5.8)	<0.001
hsTnI above LOD %	79.5	88.4	<0.001
MR-proADM (nmol/L)	0.4 (0.4, 0.5)	0.5 (0.4, 0.6)	<0.001
MR-proANP (pmol/L)	62.6 (46.7, 83.0)	59.1 (42.9, 84.6)	0.013
ST2 (ng/mL)	24.2 (19.9, 30.5)	24.9 (20.2, 31.0)	0.070
GDF 15 (ng/L)	818.0 (672.0, 1020.0)	908.0 (732.0, 1190.1)	<0.001
All-cause mortality %	2.63	3.85	0.017

Table 6: Quartiles of biomarkers according to BMI categories and No HF and HF in Both sexes (weighted according to the distribution of age and gender in Mainz/Mainz-Bingen (N=210867)).

	BMI < 30 and No HF (N=3526)	BMI >= 30 and No HF (N=1010)	p-val No HF BMI < 30 vs BMI >= 30	BMI < 30 and HF (N=131)	BMI >= 30 and HF (N=127)	p-val HF BMI < 30 vs BMI >= 30
NT-proBNP (pg/mL)	51.7 (23.2, 101.7)	47.8 (20.4, 106.7)	0.28	135.6 (51.9, 273.1)	86.1 (31.0, 222.9)	0.040
CRP (mg/L)	1.3 (0.8*, 2.5)	2.8 (1.6, 5.0)	<0.001	2.4 (1.3, 3.5)	4.2 (2.4, 7.3)	<0.001
CRP above LOD %	66.5	91	<0.001	85.4	95.2	0.0096
hsTnI (ng/L)	3.4 (2.0, 4.8)	4.0 (2.7, 5.6)	<0.001	4.3 (2.8, 7.3)	4.7 (3.3, 7.3)	0.35
hsTnI above LOD %	79.2	87.4	<0.001	84.6	95	0.012
MR-proADM (nmol/L)	0.4 (0.4, 0.5)	0.5 (0.4, 0.6)	<0.001	0.5 (0.5, 0.6)	0.6 (0.5, 0.7)	<0.001
MR-proANP (pmol/L)	61.8 (46.4, 81.5)	56.6 (42.1, 79.8)	<0.001	94.5 (61.9, 131.7)	71.5 (47.3, 121.6)	0.0088
ST2 (ng/mL)	24.2 (19.9, 30.4)	24.5 (20.0, 30.8)	0.39	25.4 (21.2, 32.8)	26.3 (22.6, 31.4)	0.50
GDF 15 (ng/L)	811.0 (669.0, 1001.0)	874.6 (715.0, 1129.4)	<0.001	1077.8 (869.8, 1459.0)	1143.2 (876.8, 1523.6)	0.42

The following Quartiles of biomarkers according to BMI categories and the existence of heart failure vs. no heart failure showed that 131 individuals in both sexes (**table 6**) with BMI<30 kg/m² had HF and 127 individuals with BMI≥30 kg/m². The NT-proBNP levels in the individuals with BMI≥30 kg/m² are lower compared to the levels with the BMI<30 kg/m². Especially the obese participants with a heart failure had lower NT-proBNP levels (86.1pg/mL) compared to the group with BMI<30 kg/m² and heart failure (135.6pg/mL). The same could be noticed in the MR-proANP levels in both groups, the obese individuals had lower MR-proANP levels compared to the groups with BMI<30 kg/m².

In contrast to that the GDF-15 levels showed higher levels in the obese subjects. Particularly in the obese participants with heart failure the GDF-15 levels were higher with 1143.2ng/L than in the cohort with BMI<30 kg/m² and heart failure with GDF-15 level of 1077.8ng/L.

After the separation of the quartiles for the gender, we noticed that the NT-

proBNP levels in women with obesity and heart failure (N=69, median NT-proBNP 95.4pg/mL) showed no difference compared to the women with heart failure and BMI<30kg/m² (N=64, median NT-proBNP 95.7pg/mL) (**table 7**). But the NT-proBNP levels in men with heart failure and obesity are lower (N=58, median NT-proBNP 72.1pg/mL) than in men with heart failure and BMI<30kg/m² (N=67, median NT-proBNP 196.1pg/mL) (**table 8**). The GDF-15 levels in obese individuals with heart failure were higher in men and women compared to none obese participants. In contrast to this observation, MR-proANP levels were lower in obese individuals independent of the gender. The sST2 and MR-proADM levels showed no relevant difference in obese vs. none obese participants.

Table 7: Quartiles of biomarkers according to BMI categories and No HF and HF in Women (weighted according to the distribution of age and gender in Mainz/Mainz-Bingen (N=210867)).

	BMI < 30 and No HF (N=1765)	**BMI >= 30 and No HF (N=456)**	**p-val No HF BMI < 30 vs BMI >= 30**	**BMI < 30 and HF (N=64)**	**BMI >= 30 and HF (N=69)**	**p-val HF BMI < 30 vs BMI >= 30**
NT-proBNP (pg/mL)	75.8 (41.9, 125.8)	72.2 (37.5, 146.3)	0.96	95.7 (52.0, 226.9)	95.4 (43.9, 224.7)	0.55
CRP (mg/L)	1.4 (0.8*, 2.6)	3.6 (2.1, 5.9)	<0.001	2.5 (1.1, 3.5)	4.8 (2.6, 7.3)	<0.001
CRP above LOD %	67.5	92.7	<0.001	83.9	95.7	0.025
hsTnI (ng/L)	2.8 (1.8*, 4.1)	3.5 (2.1, 4.8)	<0.001	3.5 (2.1, 5.4)	4.1 (2.9, 5.3)	0.31
hsTnI above LOD %	70.1	80.1	<0.001	79.4	92.5	0.040
MR-proADM (nmol/L)	0.4 (0.4, 0.5)	0.5 (0.5, 0.6)	<0.001	0.5 (0.4, 0.6)	0.6 (0.5, 0.7)	<0.001
MR-proANP (pmol/L)	66.8 (52.3, 86.1)	63.0 (48.5, 84.9)	0.077	85.0 (61.3, 118.3)	76.2 (46.9, 101.7)	0.14
ST2 (ng/mL)	21.9 (18.2, 26.4)	21.9 (18.2, 27.0)	0.64	22.8 (20.5, 28.5)	25.7 (20.2, 29.7)	0.15
GDF 15 (ng/L)	806.0 (669.0, 986.2)	852.0 (707.0, 1079.7)	<0.001	1018.5 (787.9, 1359.3)	1076.9 (811.8, 1383.4)	0.62

Table 8: Quartiles of biomarkers according to BMI categories and No HF and HF in Men (weighted according to the distribution of age and gender in Mainz/Mainz-Bingen (N=210867)).

	BMI < 30 and No HF (N=1761)	BMI >= 30 and No HF (N=554)	p-val No HF BMI < 30 vs BMI >= 30	BMI < 30 and HF (N=67)	BMI >= 30 and HF (N=58)	p-val HF BMI < 30 vs BMI >= 30
NT-proBNP (pg/mL)	32.0 (11.4, 67.1)	29.4 (9.4, 73.8)	1.00	196.1 (40.8, 400.1)	72.1 (24.3, 206.5)	0.033
CRP (mg/L)	1.3 (0.8*, 2.3)	2.4 (1.4, 3.9)	<0.001	2.3 (1.4, 3.5)	3.7 (2.1, 5.1)	0.016
CRP above LOD %	65.4	89.5	<0.001	87.1	94.6	0.17
hsTnI (ng/L)	4.0 (2.7, 5.6)	4.6 (3.3, 6.2)	<0.001	6.2 (3.5, 9.5)	6.2 (4.6, 8.3)	0.77
hsTnI above LOD %	88.5	93.4	0.0058	90.9	98.4	0.071
MR-proADM (nmol/L)	0.4 (0.4, 0.5)	0.5 (0.4, 0.6)	<0.001	0.5 (0.5, 0.6)	0.6 (0.5, 0.7)	0.0057
MR-proANP (pmol/L)	55.1 (42.2, 75.2)	50.5 (38.2, 72.7)	0.0057	105.4 (61.7, 140.2)	65.0 (48.1, 127.5)	0.031
ST2 (ng/mL)	27.6 (22.5, 34.1)	27.2 (22.1, 33.1)	0.28	29.2 (24.1, 35.8)	29.3 (23.5, 36.2)	0.64
GDF 15 (ng/L)	817.0 (668.6, 1031.0)	898.0 (717.0, 1175.2)	<0.001	1156.1 (965.1, 1539.4)	1312.5 (934.4, 1682.8)	0.44

3.2. Partial correlation

Correlations and partial correlations (adjusted for age and sex) between the biomarkers are computed in the different BMI categories. For the analyses on this section CRP and hsTnI values below the LoD were set to LoD/2.

Table 9: Spearman correlations between biomarkers for invididuals with BMI < 30

	NT-proBNP	CRP	hsTnI	MR-proADM	MR-pro-ANP	ST2	GDF 15	
NT-pro BNP		0.15 p<0.001	0.12 p<0.001	0.40 p<0.001	0.73 p<0.001	-0.04 p=0.0097	0.33 p<0.001	**NT -pro BNP**
CRP	0.15 p<0.001		0.09 p<0.001	0.27 p<0.001	0.07 p<0.001	0.02 p=0.25	0.19 p<0.001	**CRP**
hs TnI	0.12 p<0.001	0.09 p<0.001		0.17 p<0.001	0.13 p<0.001	0.17 p<0.001	0.07 p<0.001	**hsTnI**
MR-pro ADM	0.40 p<0.001	0.27 p<0.001	0.17 p<0.001		0.45 p<0.001	0.07 p<0.001	0.52 p<0.001	**MR-pro ADM**
MR-pro-ANP	0.73 p<0.001	0.07 p<0.001	0.13 p<0.001	0.45 p<0.001		0.03 p=0.047	0.34 p<0.001	**MR-pro-ANP**
ST2	-0.04 p=0.0097	0.02 p=0.25	0.17 p<0.001	0.07 p<0.001	0.03 p=0.047		0.12 p<0.001	**ST2**
GDF 15	0.33 p<0.001	0.19 p<0.001	0.07 p<0.001	0.52 p<0.001	0.34 p<0.001	0.12 p<0.001		**GDF 15**
	NT-proBNP	**CRP**	**hsTnI**	**MR-proADM**	**MR-pro-ANP**	**ST2**	**GDF 15**	

The spearman correlation analysis in **table 9** could describe a tight correlation for the natriuretic peptides NT-proBNP with MR-proANP with an r of 0.73 in individuals with BMI<30 kg/m², as well for MR-proADM with GDF-15 (r=0.52).
A moderate correlation could be described for NT-proBNP with MR-proADM (r=0.4), for MR-proANP with GDF-15 (r=0.34), for GDF-15 with NT-proBNP (r=0.33) and for CRP with MR-proADM (r=0.27).

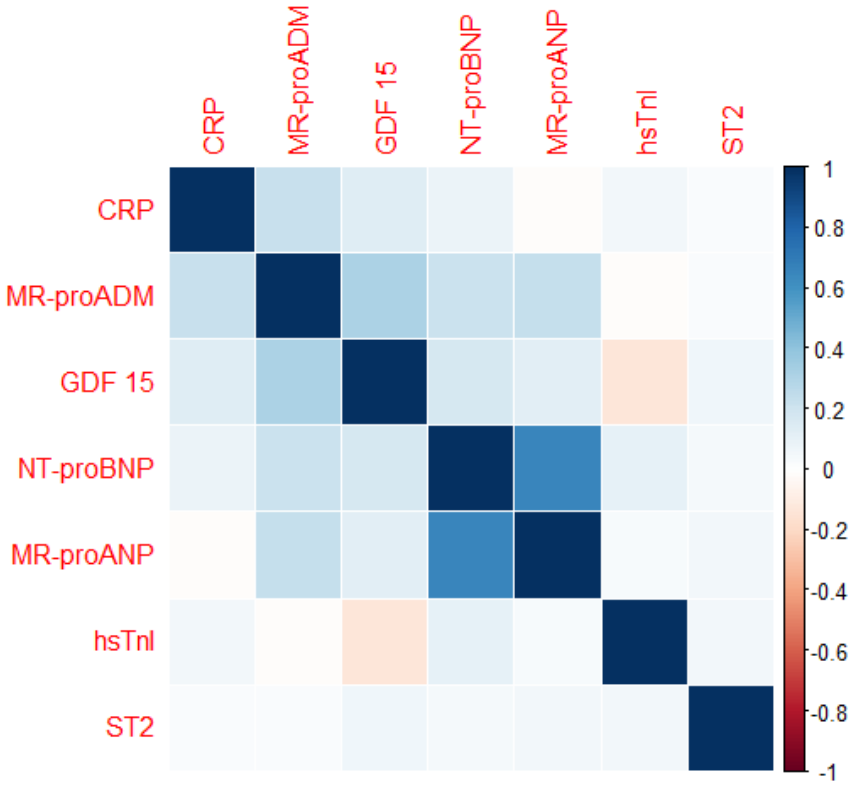

Figure 3: Partial Spearman correlations between biomarkers (adjusted for age and sex) for individuals with BMI< 30

To evaluate if the marker show a correlation between each other, partial correlation adjusted for age and sex was done.

The partial spearman correlation analysis in **table 10** and **figure 3** could describe a tight correlation for the natriuretic peptides NT-proBNP with MR-proANP with an r of 0.65 in individuals with BMI<30 kg/m².

A moderate correlation could be described for MR-proADM and GDF-15 (r=0.31).

Table 10: Partial Spearman correlations between biomarkers (adjusted for age and sex). BMI<30

	NT-proBNP	CRP	hsTnI	MR-pro ADM	MR-pro-ANP	ST2	GDF 15	
NT-pro BNP		0.08 p<0.001	0.10 p<0.001	0.22 p<0.001	0.65 p<0.001	0.04 p=0.013	0.17 p<0.001	**NT-pro BNP**
CRP	0.08 p<0.001		0.06 p<0.001	0.22 p<0.001	-0.02 p=0.34	0.02 p=0.13	0.13 p<0.001	**CRP**
hs TnI	0.10 p<0.001	0.06 p<0.001		-0.01 p=0.46	0.04 p=0.033	0.06 p=0.0017	-0.13 p<0.001	**hs TnI**
MR-pro ADM	0.22 p<0.001	0.22 p<0.001	-0.01 p=0.46		0.24 p<0.001	0.02 p=0.15	0.31 p<0.001	**MR-pro ADM**
MR-pro-ANP	0.65 p<0.001	-0.02 p=0.34	0.04 p=0.033	0.24 p<0.001		0.06 p<0.001	0.12 p<0.001	**MR-pro-ANP**
ST2	0.04 p=0.013	0.02 p=0.13	0.06 p=0.0017	0.02 p=0.15	0.06 p<0.001		0.06 p<0.001	**ST2**
GDF 15	0.17 p<0.001	0.13 p<0.001	-0.13 p<0.001	0.31 p<0.001	0.12 p<0.001	0.06 p<0.001		**GDF 15**
	NT-proBNP	**CRP**	**hsTnI**	**MR-pro ADM**	**MR-pro-ANP**	**ST2**	**GDF 15**	

Table 11:Spearman correlations between biomarkers for invididuals with BMI >= 30

	NT-proBNP	CRP	hsTnI	MR-pro ADM	MR-pro-ANP	ST2	GDF 15	
NT-pro BNP		0.08 p=0.0039	0.21 p<0.001	0.52 p<0.001	0.79 p<0.001	-0.01 p=0.64	0.28 p<0.001	**NT-pro BNP**
CRP	0.08 p=0.0039		0.05 p=0.13	0.29 p<0.001	0.00 p=0.96	0.02 p=0.57	0.09 p=0.0028	**CRP**
hs TnI	0.21 p<0.001	0.05 p=0.13		0.20 p<0.001	0.19 p<0.001	0.21 p<0.001	0.21 p<0.001	**hs TnI**
MR-pro ADM	0.52 p<0.001	0.29 p<0.001	0.20 p<0.001		0.51 p<0.001	0.07 p=0.023	0.52 p<0.001	**MR-pro ADM**
MR-pro-ANP	0.79 p<0.001	0.00 p=0.96	0.19 p<0.001	0.51 p<0.001		0.03 p=0.26	0.30 p<0.001	**MR-pro-ANP**
ST2	-0.01 p=0.64	0.02 p=0.57	0.21 p<0.001	0.07 p=0.023	0.03 p=0.26		0.18 p<0.001	**ST2**
GDF 15	0.28 p<0.001	0.09 p=0.0028	0.21 p<0.001	0.52 p<0.001	0.30 p<0.001	0.18 p<0.001		**GDF 15**
	NT-proBNP	**CRP**	**hsTnI**	**MR-pro ADM**	**MR-pro-ANP**	**ST2**	**GDF 15**	

In the individuals with obesity the spearman correlations showed also a tight correlation of NT-proBNP with MR-proANP (r=0.79), GDF-15 with MR-proADM (r=0.52) and also of NT-proBNP with MR-proADM (r=0.52). A moderate

correlation could be descried for MR-proANP with GDF-15 (r=0.30), MR-proADM with CRP (r=0.29) and NT-proBNP with GDF-15 (r=0.28). For further detail refer to **table 11**.

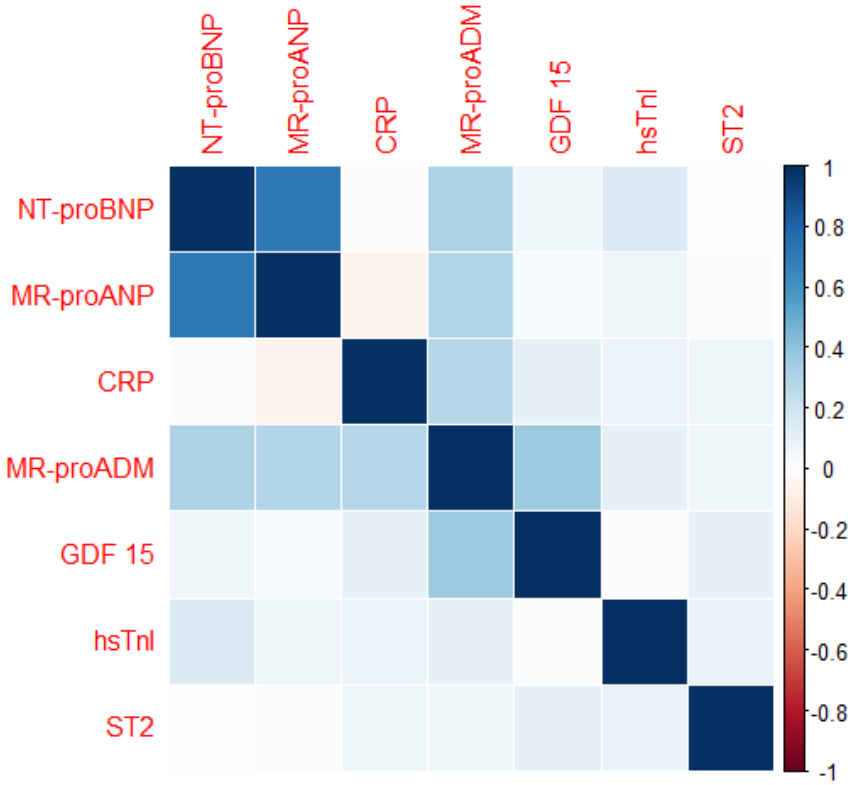

Figure 4: Partial Spearman correlations between biomarkers (adjusted for age and sex) for individuals with BMI >= 30

The partial spearman correlation analysis in obese subjects showed a tight correlation for the natriuretic peptides NT-proBNP with MR-proANP with an r of 0.71 (**table 12** and **figure 4**).

A moderate correlation could be noticed for NT-proBNP with MR-proADM (r=0.31) and for MR-proADM with GDF-15 (r=0.36).

Table 12: Partial Spearman correlations between biomarkers (adjusted for age and sex). BMI>=30

	NT-proBNP	CRP	hsTnI	MR-pro ADM	MR-pro-ANP	ST2	GDF 15	
NT-pro BNP		0.01 p=0.69	0.16 p<0.001	0.31 p<0.001	0.71 p<0.001	0.00 p=0.99	0.07 p=0.023	**NT-pro BNP**
CRP	0.01 p=0.69		0.09 p=0.005	0.28 p<0.001	-0.06 p=0.029	0.07 p=0.010	0.11 p<0.001	**CRP**
hs TnI	0.16 p<0.001	0.09 p=0.005		0.10 p=0.0014	0.07 p=0.021	0.09 p=0.0031	0.01 p=0.67	**hs TnI**
MR-pro ADM	0.31 p<0.001	0.28 p<0.001	0.10 p=0.0014		0.30 p<0.001	0.07 p=0.022	0.36 p<0.001	**MR-pro ADM**
MR-pro ANP	0.71 p<0.001	-0.06 p=0.029	0.07 p=0.021	0.30 p<0.001		0.01 p=0.70	0.04 p=0.21	**MR-pro ANP**
ST2	0.00 p=0.99	0.07 p=0.010	0.09 p=0.0031	0.07 p=0.022	0.01 p=0.70		0.11 p<0.001	**ST2**
GDF 15	0.07 p=0.023	0.11 p<0.001	0.01 p=0.67	0.36 p<0.001	0.04 p=0.21	0.11 p<0.001		**GDF 15**
	NT-proBNP	**CRP**	**hsTnI**	**MR-pro ADM**	**MR-pro ANP**	**ST2**	**GDF 15**	

3.3 Receiver operating characteristic (ROC) curve analysis

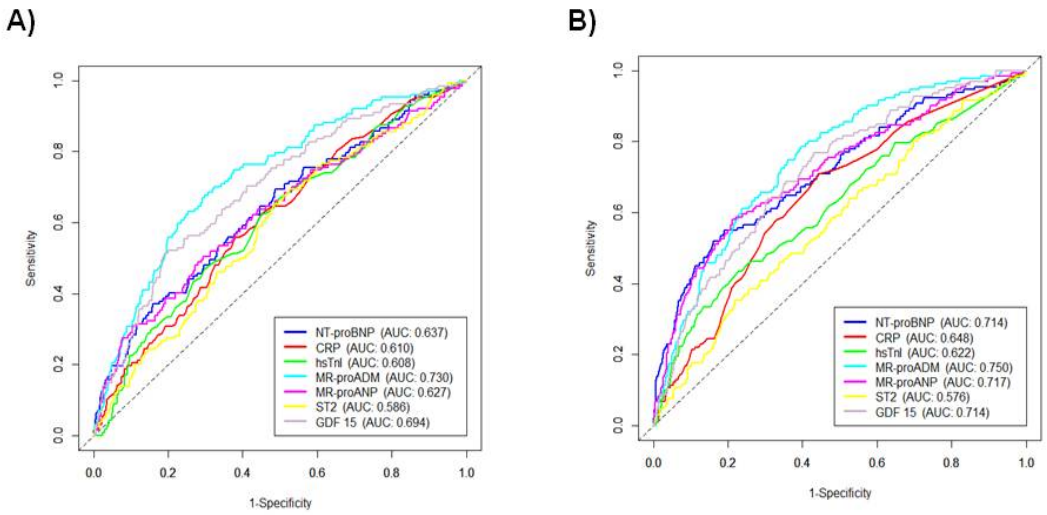

Figure 5 ROC curve BMI≥30 **A**) and ROC curve BMI<30 **B**)

The receiver operating characteristic (ROC) curve analysis was done to compare the different biomarkers in their ability to discriminate subjects with and without HF in the general population and according to the fact if obesity is present with BMI ≥30 or not BMI <30 (**table 13**).

For subjects with BMI <30 the highest area under the curve (AUC) was shown for MR-proADM with 0.75 followed by the natriuretic peptides with NT-proBNP 0.71 and MR-proANP 0.72 and GDF-15 with 0.72 (**Figure 5B**). The AUCs for the other markers were lower with CRP 0.65, hsTnI 0.62 and sST2 0.58. In subjects with BMI ≥30 still MR-proADM had the highest AUC with 0.73 and followed by GDF-15 with 0.69 (**Figure 5A**). In this cohort, NT-proBNP and MR-proANP had in contrast lower AUCs with 0.63 for both. The AUC for hsTnI and CRP was 0.61 and for sST2 0.59.

Table 13: AUCs for the diagnosis of HF using biomarkers

Marker	BMI category	AUC (95% CI)	N
NT-proBNP	BMI< 30	0.71 (0.66, 0.76)	3646
NT-proBNP	BMI >= 30	0.64 (0.58, 0.69)	1133
CRP	BMI< 30	0.65 (0.60, 0.69)	3654
CRP	BMI >= 30	0.61 (0.56, 0.66)	1137
hsTnI	BMI< 30	0.62 (0.56, 0.68)	3016
hsTnI	BMI >= 30	0.61 (0.55, 0.66)	956
MR-proADM	BMI< 30	0.75 (0.71, 0.79)	3643
MR-proADM	BMI >= 30	0.73 (0.68, 0.78)	1133
MR-proANP	BMI< 30	0.72 (0.67, 0.77)	3642
MR-proANP	BMI >= 30	0.63 (0.57, 0.68)	1134
ST2	BMI< 30	0.58 (0.53, 0.63)	3582
ST2	BMI >= 30	0.59 (0.53, 0.64)	1110
GDF 15	BMI< 30	0.71 (0.67, 0.76)	3433
GDF 15	BMI >= 30	0.69 (0.64, 0.74)	1069

3.4 Cut-off for the different biomarkers

The **table 14** with sensitivities and specificities for biomarker cut-off that maximizes the Youden index showed us that in participants with BMI<30 kg/m² NT-proBNP, GDF-15, MR-proADM, MR-proANP and CRP are more sensitive biomarkers than in obese participants. In contrast to that hsTnI and sST2 are more sensitive biomarkers in obese subjects.

The cut-off values for NT-proBNP, GDF-15, CRP and MR-proADM are higher and the cut-off values for hsTNI, sST2 and MR-proANP are lower in obese subjects compared to subjects with a BMI<30 kg/m².

The cut-off value for NTproBNP is 138pg/mL in individuals with BMI<30kg/m² and 175pg/mL in obese individuals. However, the sensitivity for NT-proBNP is higher in none obese. The sensitivity for MR-proADM and GDF-15 are higher than NT-proBNP but the specificity is lower.

Table 14: Sensitivities and specificities for biomarker cut-off that maximizes the Youden index. Sens. and spec. are corrected for over-optimism.

Marker	BMI category	Cut-off	Sens (95% CI)	Spec (95% CI)	N
NT-proBNP	BMI < 30	138.4 pg/mL	53.4 (44.4, 62.1)	81.0 (79.7, 82.3)	3646
NT-proBNP	BMI >= 30	175.2 pg/mL	34.1 (25.7, 43.1)	83.8 (81.4, 86.0)	1133
CRP	BMI < 30	1.6 mg/L	68.9 (60.3, 76.5)	55.5 (53.8, 57.1)	3654
CRP	BMI >= 30	3.7 mg/L	53.1 (44.0, 61.9)	61.6 (58.5, 64.6)	1137
hsTnI	BMI < 30	5.3 ng/L	40.6 (31.1, 50.5)	77.2 (75.6, 78.7)	3016
hsTnI	BMI >= 30	4.5 ng/L	58.6 (48.9, 67.6)	54.6 (51.1, 58.0)	956
MR-proADM	BMI < 30	0.463494 nmol/L	75.9 (67.9, 82.6)	60.4 (58.8, 62.0)	3643
MR-proADM	BMI >= 30	0.593892 nmol/L	65.4 (56.5, 73.4)	69.9 (67.0, 72.7)	1133
MR-proANP	BMI < 30	91.323778 pmol/L	55.9 (47.0, 64.5)	78.7 (77.3, 80.0)	3642
MR-proANP	BMI >= 30	80.807544 pmol/L	47.1 (38.1, 56.1)	70.2 (67.3, 73.0)	1134
ST2	BMI < 30	31.375 ng/mL	31.7 (23.5, 40.5)	77.9 (76.5, 79.3)	3582
ST2	BMI >= 30	25.034 ng/mL	62.8 (53.8, 71.2)	51.2 (48.0, 54.3)	1110
GDF 15	BMI < 30	899 ng/L	74.3 (65.9, 81.4)	56.9 (55.2, 58.6)	3433
GDF 15	BMI >= 30	1300 ng/L	49.0 (39.7, 58.2)	80.7 (78.0, 83.1)	1069

3.5 Logistic regression analysis

To evaluate the different biomarker level and presence of the disease condition HF logistic regression analyses were performed. In the model including all cardiovascular risk factors and each biomarker alone, there was a difference in subjects with BMI < and ≥30 especially for natriuretic peptides.

The odds ratio (OR) per standard deviation (SD) for heart failure from logistic regression in the fully adjusted model for the different BMI categories was significant ($p<0.01$) for all novel biomarkers except hsTNI in both BMI groups, MR-proANP ($p=0.09$) and NT-proBNP ($p=0.02$) in the individuals with obesity (**table 15**). The OD per SD in the subjects with BMI<30kg/m² was 1.8 for NT-proBNP, followed by MR-proADM with 1.7, MR-proANP with 1.6, CRP and GDF-15 with 1.4 respectively and sST2 with 1.3.

Regarding the obese subjects MR-proADM showed the highest OR per SD with 1.9, followed by CRP with 1.5, GDF-15 with 1.4 and sST2 with 1.3 (for all $p<0.01$). NT-proBNP showed an OR per SD of 1.3 and was not significant ($p=0.02$) in obese. See also **figure 6A**.

Table 15: One biomarker per model; Logistic regression models for HF including biomarker-BMI categories interaction. For each biomarker a separate model was computed.

Marker	p-val interac-tion	Category	OR (95% CI)	OR per SD (95% CI)	p-val	N	N Event
log(NT-proBNP)	0.028						
		BMI < 30	1.6 (1.4, 1.9)	1.8 (1.4, 2.2)	<0.001	3641	131
		BMI >= 30	1.2 (1.0, 1.5)	1.3 (1.0, 1.6)	0.018	1127	127
log(CRP)	0.73						
		BMI < 30	1.4 (1.2, 1.7)	1.4 (1.2, 1.7)	<0.001	3649	131
		BMI >= 30	1.5 (1.2, 1.9)	1.5 (1.2, 1.9)	<0.001	1131	127
log(hsTnI)	0.63						
		BMI < 30	1.3 (1.0, 1.7)	1.2 (1.0, 1.5)	0.059	3011	108
		BMI >= 30	1.2 (0.9, 1.6)	1.1 (0.9, 1.4)	0.30	951	111
log(MR-proADM)	0.51						
		BMI < 30	8.0 (3.7, 17.2)	1.7 (1.4, 2.1)	<0.001	3638	131
		BMI >= 30	11.3 (4.6, 28.7)	1.9 (1.5, 2.4)	<0.001	1127	127
log(MR-pro-ANP)	0.016						
		BMI < 30	2.7 (1.8, 4.0)	1.6 (1.3, 1.9)	<0.001	3637	131
		BMI >= 30	1.4 (0.9, 2.1)	1.2 (1.0, 1.4)	0.089	1128	127
log(ST2)	0.89						
		BMI < 30	2.1 (1.3, 3.5)	1.3 (1.1, 1.5)	0.0042	3577	130
		BMI >= 30	2.2 (1.2, 4.0)	1.3 (1.1, 1.6)	0.0071	1104	124
log(GDF 15)	0.78						
		BMI < 30	2.3 (1.5, 3.4)	1.4 (1.2, 1.6)	<0.001	3429	125
		BMI >= 30	2.5 (1.5, 4.2)	1.4 (1.2, 1.7)	<0.001	1063	121

Table 16: Multiple biomarkers per model; Logistic regression model for HF including biomarker-BMI categories interactions. This table describes a single model.

Marker	p-val interac-tion	Category	OR (95% CI)	OR per SD (95% CI)	p-val	N	N event
log(NT-proBNP)	0.98						
		BMI < 30	1.2 (0.9, 1.7)	1.3 (0.9, 1.8)	0.19	2749	101
		BMI >= 30	1.2 (0.9, 1.7)	1.3 (0.9, 1.9)	0.26	871	103
log(CRP)	0.56						
		BMI < 30	1.3 (1.1, 1.7)	1.3 (1.1, 1.7)	0.0089	2749	101
		BMI >= 30	1.2 (0.9, 1.6)	1.2 (0.9, 1.6)	0.21	871	103
log(hsTnI)	0.58						
		BMI < 30	1.1 (0.8, 1.5)	1.1 (0.9, 1.4)	0.41	2749	101
		BMI >= 30	1.0 (0.7, 1.4)	1.0 (0.8, 1.3)	1.00	871	103
log(MR-proADM)	0.084						
		BMI < 30	2.3 (0.8, 7.1)	1.2 (0.9, 1.7)	0.15	2749	101
		BMI >= 30	9.7 (2.6, 37.5)	1.8 (1.3, 2.7)	<0.001	871	103
log(MR-pro-ANP)	0.10						
		BMI < 30	1.4 (0.7, 2.9)	1.2 (0.8, 1.6)	0.37	2749	101
		BMI >= 30	0.6 (0.3, 1.3)	0.8 (0.5, 1.1)	0.17	871	103
log(ST2)	0.23						
		BMI < 30	1.1 (0.6, 2.1)	1.0 (0.8, 1.3)	0.70	2749	101
		BMI >= 30	2.0 (1.0, 3.9)	1.3 (1.0, 1.6)	0.053	871	103
log(GDF 15)	0.33						
		BMI < 30	1.4 (0.8, 2.4)	1.1 (0.9, 1.4)	0.25	2749	101
		BMI >= 30	2.1 (1.1, 4.1)	1.3 (1.0, 1.7)	0.029	871	103

In the model encompassing all risk factors and all biomarkers for the heart failure the highest OR per SD were shown for MR-proADM with 1.8 ($p<0.01$) in obese and for CRP with 1.3 in individuals with BMI<30kg/m² ($p<0.01$). The other biomarkers were not significant in both BMI groups (**table 16** and **figure 6B**).

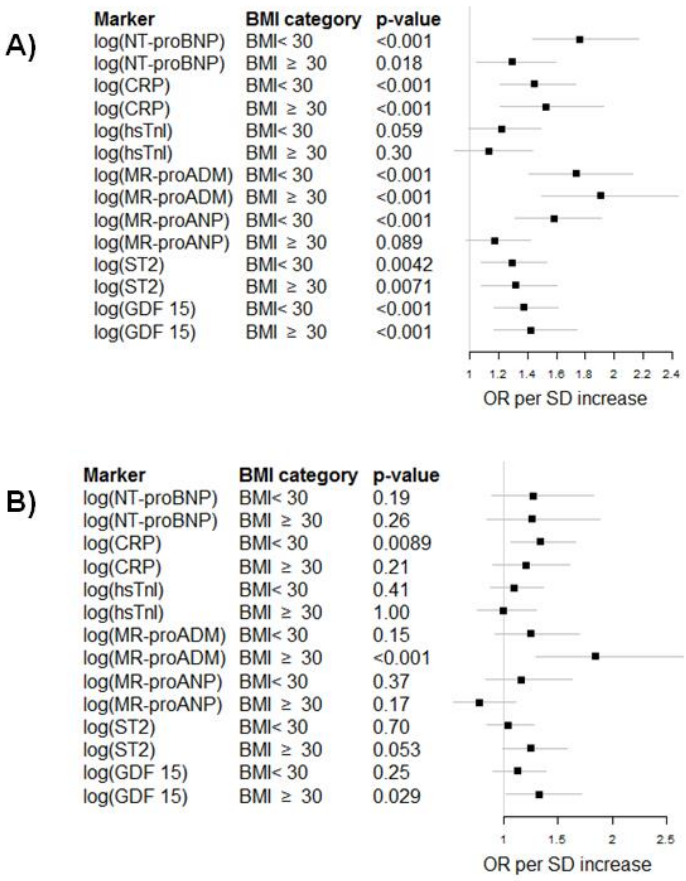

Figure 6 Forrest Plots showing the logistic regression for each biomarker alone **A**) and in the multiple marker model **B**)

3.6 Survival curves

The median follow-up time was 6.9 years (estimated by the reverse Kaplan-Meier estimator). There were 192 deaths.

Different Kaplan-Meier curves are shown below. The p-value given in the graphics is for the log-rank test.

Observe that tertiles and medians are computed only on the individuals used in the respective analyses (e.g. those with FU and biomarker information available).

According to BMI and the corresponding median of the biomarker concentration, for the upper half of the subjects mortality was higher in the BMI≥30 kg/m² cohort for both NT-proBNP and MR-proANP (**figure 7A and B**). For the most promising markers MR-proADM and GDF-15 there was no relevant difference for mortality between BMI<30 and ≥30 kg/m² (**figure 8A and E**).

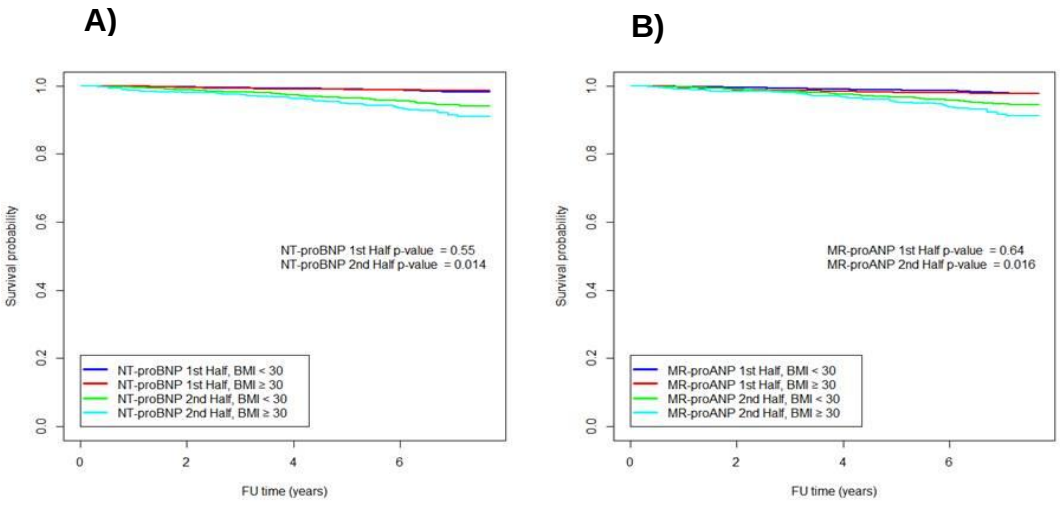

Figure 7 Kaplan-Meier survival curves showing the outcome according to median in the overall cohort and according to the median and BMI<30 and ≥30 kg/m². Figure **A)** for the natriuretic peptides and **B)** for MR-proANP.

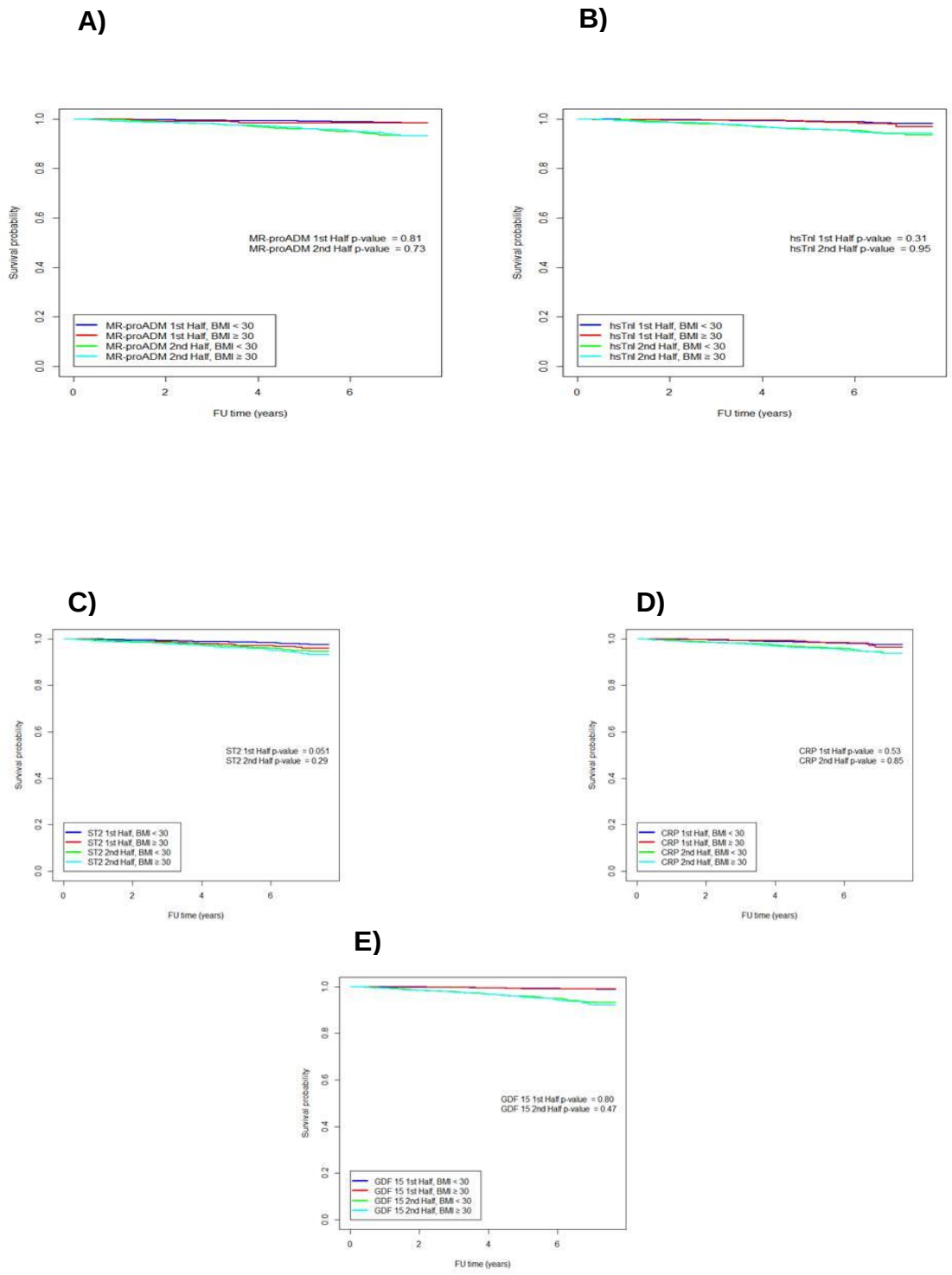

Figure 8 Kaplan-Meier survival curves showing the outcome according to median in the overall cohort and according to the median and BMI<30 and ≥30 kg/m². Figure **A)** for the MR-proADM and **B)** for hsTNI, **C)** for the sST2, **D)** for the CRP and **E)** for GDF-15.

3.7 Cox regression analysis

Table 17: One biomarker per model; Cox regression models for all-cause mortality including biomarker-BMI categories interaction. For each biomarker a separate model was computed.

Marker	p-val interaction	Category	OR (95% CI)	OR per SD (95% CI)	p-val	N	N Event
log(NT-proBNP)	0.80						
		BMI < 30	1.7 (1.4, 1.9)	1.9 (1.6, 2.2)	<0.001	3735	132
		BMI >= 30	1.7 (1.4, 2.2)	2.0 (1.5, 2.6)	<0.001	1192	58
log(CRP)	0.46						
		BMI < 30	1.5 (1.3, 1.8)	1.5 (1.3, 1.8)	<0.001	3743	132
		BMI >= 30	1.3 (1.0, 1.8)	1.4 (1.0, 1.8)	0.041	1196	59
log(hsTnI)	0.92						
		BMI < 30	1.7 (1.3, 2.1)	1.5 (1.3, 1.8)	<0.001	3091	105
		BMI >= 30	1.7 (1.2, 2.5)	1.5 (1.1, 2.1)	0.0041	1005	44
log(MR-proADM)	0.095						
		BMI < 30	18.8 (9.3, 37.9)	2.2 (1.8, 2.7)	<0.001	3732	132
		BMI >= 30	49.3 (16.4, 148.2)	2.9 (2.1, 3.9)	<0.001	1192	58
log(MR-proANP)	0.51						
		BMI < 30	2.6 (1.8, 3.8)	1.6 (1.3, 1.9)	<0.001	3731	132
		BMI >= 30	3.2 (1.9, 5.4)	1.7 (1.4, 2.2)	<0.001	1193	58
log(ST2)	0.92						
		BMI < 30	2.7 (1.7, 4.3)	1.4 (1.2, 1.6)	<0.001	3671	130
		BMI >= 30	2.8 (1.3, 5.8)	1.4 (1.1, 1.8)	0.0060	1167	58
log(GDF 15)	0.032						
		BMI < 30	3.9 (2.9, 5.2)	1.7 (1.5, 1.9)	<0.001	3515	126
		BMI >= 30	7.8 (4.3, 14.1)	2.3 (1.8, 2.8)	<0.001	1126	53

Cox proportional hazard analyses were performed to analyze the prognostic importance of the biomarker concentration during follow-up in the general population with the outcome of all-cause mortality. Biomarkers are related to disease progress and therefore prognosis as regarding prognosis all evaluated biomarkers influenced the outcome in the cohort with a BMI <30 and ≥30 kg/m².

The hazard ratio (HR) was evaluated in the same approach and the results for the model including all risk factors and each biomarker alone was reported here for both BMI categories. In this model the biomarkers GDF-15, sST2, NT-proBNP,

MR-proANP, MR-proADM and hsTnI were able to detect individuals with an adverse outcome in both BMI categories respectively with a significant result (p<0.01) (**table 17**). The HR per standard deviation (SD) in none obese subjects were 2.2 for MR-proADM, followed by NT-proBNP with 1.9, GDF-15 with 1.7, MR-proANP with 1.6, hsTnI and CRP each with 1.5 and sST2 with 1.4 respectively with a significant result (p<0.01).

In the obese individuals the HR per SD were 2.9 for MR-proADM, succeeded by GDF-15 with 2.3, NT-proBNP with 2.0, MR-proANP with 1.7, hsTnI with 1.5 and sST2 with 1.4. Only the CRP was not significant in obese participants (p=0.04) (see **figure 9A**).

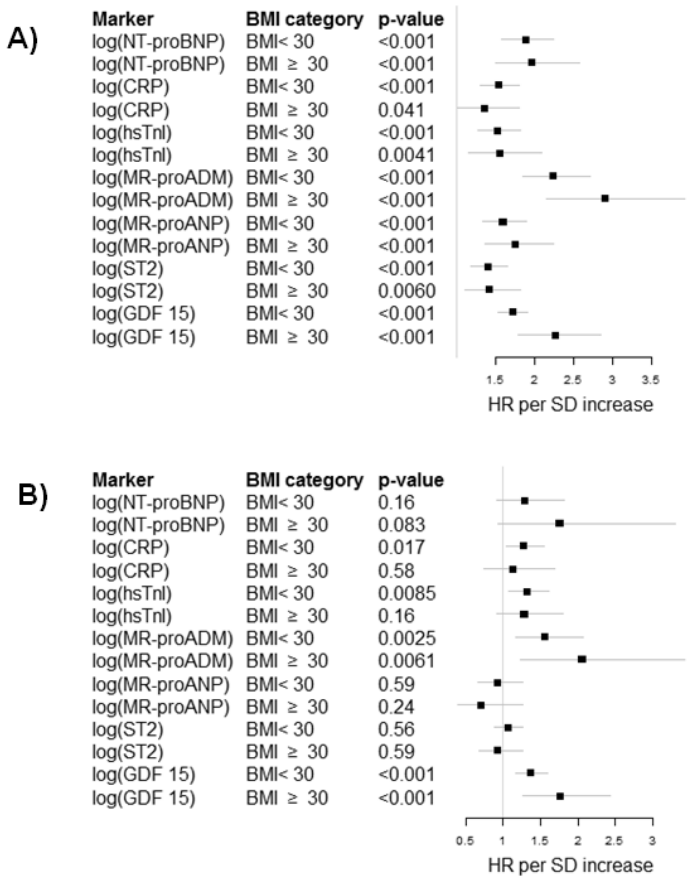

Figure 9 Forest plot showing the hazard ratio for each biomarker alone **A**) and in the multiple marker model **B**)

Regarding prognosis all evaluated biomarkers together with the cardiovascular risk factors influenced the outcome in the cohort with BMI <30 and ≥30 kg/m². After including all cardiovascular risk factors and all biomarkers using lasso selection:

In this model only the biomarkers GDF-15 and MR-proADM were able to detect individuals with an adverse outcome in both BMI categories respectively with a significant result ($p<0.01$) (**table 18**). The HR per standard deviation (SD) in none obese subjects were 1.6 for MR-proADM, followed by GDF-15 with 1.4 and hsTnI with 1.3 respectively with a significant result ($p<0.01$).

In the obese individuals the HR per SD were 2.1 for MR-proADM, succeeded by GDF-15 with 1.8, only these two biomarkers were significant to detect an adverse outcome (see **figure 9B**).

Table 18: Multiple biomarkers per model; Cox regression model for all cause-mortality including biomarker-BMI categories interactions. This table describes a single model.

Marker	p-val interaction	Category	HR (95% CI)	HR per SD (95% CI)	p-val	N	N event
log(NT-proBNP)	0.40						
		BMI < 30	1.2 (0.9, 1.6)	1.3 (0.9, 1.8)	0.16	2822	98
		BMI >= 30	1.6 (0.9, 2.6)	1.8 (0.9, 3.3)	0.083	922	40
log(CRP)	0.60						
		BMI < 30	1.3 (1.0, 1.5)	1.3 (1.0, 1.5)	0.017	2822	98
		BMI >= 30	1.1 (0.7, 1.7)	1.1 (0.7, 1.7)	0.58	922	40
log(hsTnI)	0.90						
		BMI < 30	1.4 (1.1, 1.8)	1.3 (1.1, 1.6)	0.0085	2822	98
		BMI >= 30	1.4 (0.9, 2.1)	1.3 (0.9, 1.8)	0.16	922	40
log(MR-proADM)	0.32						
		BMI < 30	4.9 (1.8, 13.9)	1.6 (1.2, 2.1)	0.0025	2822	98
		BMI >= 30	13.6 (2.1, 87.3)	2.1 (1.2, 3.4)	0.0061	922	40
log(MR-proANP)	0.44						
		BMI < 30	0.8 (0.4, 1.6)	0.9 (0.7, 1.3)	0.59	2822	98
		BMI >= 30	0.5 (0.1, 1.6)	0.7 (0.4, 1.3)	0.24	922	40
log(ST2)	0.44						
		BMI < 30	1.2 (0.7, 2.0)	1.1 (0.9, 1.3)	0.56	2822	98
		BMI >= 30	0.8 (0.3, 1.9)	0.9 (0.7, 1.3)	0.59	922	40
log(GDF 15)	0.17						
		BMI < 30	2.2 (1.5, 3.2)	1.4 (1.2, 1.6)	<0.001	2822	98
		BMI >= 30	4.2 (1.8, 9.6)	1.8 (1.3, 2.4)	<0.001	922	40

4. Discussion

The results of this study point out that beside the current standard of natriuretic peptides, novel biomarkers can ameliorate detection of heart failure in the general population and especially in obese people. In terms of prognosis all evaluated biomarkers were relevant, as well as the natriuretic peptides, being strong predictors of the outcome all-cause mortality in subjects with and without obesity. In this context it has to be stressed that for the first time an interaction between obesity and natriuretic peptide levels is reported which is interfering with the correct management of patients presenting with typical signs and symptoms of heart failure.

In individuals with BMI≥30 kg/m² the use of both NT-proBNP and MR-proANP is blunted to detect HF and to identify subjects with mortality during the follow-up period. On the other hand, the both markers MR-proADM and GDF-15 were especially useful in this subcohort of individuals to differentiate those with and without HF and to evaluate if subjects have an adverse outcome in the follow-up. On the contrary, in the cohort of subjects with BMI<30 kg/m² natriuretic peptides still outperformed the other biomarkers in terms of HF diagnosis and identification of subjects with mortality in the follow-up period.

4.1 Application of biomarkers in obese subjects regarding HF presence

Although the current HF guidelines recommend natriuretic peptides as biomarker in HF (5,6), establishing diagnosis and prognosis might be ameliorated by the additional application of candidate biomarkers being especially useful in cohorts were the use of the current standard is blunted. From our data non obese subjects with BMI<30 natriuretic peptides still remain the standard for establishing diagnosis and prognosis, being shown for NT-proBNP and MR-proANP. Of the novel biomarkers, midregional proadrenomedullin and growth differentiation factor-15 were linked to HF even after inclusion of NT-proBNP into the logistic regression analysis. Important in this context is the fact, that all models were already adjusted for NT-proBNP, thus indicating that different biologic reactions due to pathophysiological changes are related to HF.

This was especially pronounced in the obese cohort when GDF-15 and MR-proADM did perform even better than the natriuretic peptides.

From our results, the two markers MR-proADM and GDF-15 proved to be reliable to detect HF in subjects with BMI≥30 kg/m². However, this might be due to the fact that natriuretic peptides with NT-proBNP and MR-proANP had a lower sensitivity in this cohort. In comparison to the cohort with a BMI<30 kg/m² were NT-proBNP was the best biomarker to detect HF.

4.2 Cut-off variation in GHS regarding natriuretic peptides

Since the incidence of heart failure is rising and obesity can mask the symptoms of heart failure, therefore it is necessary to find new biomarkers to support the identification of heart failure preeminently in obese people and to describe new cut-off levels for the biomarkers.

In our study we were able to describe higher cut-off values for NT-proBNP to detect a HF compared to the ESC guidelines of 2012 (NT-proBNP level for stable patients 125pg/ml vs. 300pg/ml for patients with acute symptoms) (5). An explanation for this could be the general condition of the individuals in our study, because the sensitivity and specificity for NT-proBNP in stable participants are inferior to patients with acute symptoms (85). Our results determined for none obese the cut-off value of 138pg/mL vs. 175pg/mL for obese. Though the sensitivity for NT-proBNP is higher for people with a BMI<30kg/m². The sensitivity for MR-proADM and GDF-15 was higher in comparison to NT-proBNP and MR-proANP irrespective of the body weight, but the specificity is lower compared to NT-proBNP.

The GDF-15 cut-off levels in our study for none obese individuals are 899ng/L vs 1300ng/L for obese, though the sensitivity is higher in none obese and specificity is higher in obese. This could also be observed for the cut-off values of MR-proADM (0.46nmol/L none obese vs 0.59nmol/L obese) and CRP (1.6mg/L none obese vs 3.7mg/L obese). In contrast the sensitivity for sST2 (cut-off 31.38ng/mL none obese vs 25.03ng/mL obese) and hsTnI (cut-off 5.3ng/L none obese vs 4.5ng/L obese) is higher in obese and the specificity is higher in none obese. Only

MR-proANP has a higher sensitivity and specificity in none obese (cut-off 91.32 pmol/L none obese vs 80.81 pmol/L obese).
Due to the cause that every cut-off is determined by the sample size, variations cannot be recommended but need a larger sample size.

4.3 Pathophysiology of low natriuretic peptide levels and findings in GHS

This large study cohort showed the inverse relationship of natriuretic peptides with BMI like many studies in the past (18,66-69,70-77). Obese individuals had lower NT-proBNP levels than individuals with a BMI<30kg/m², particularly in obese individuals with heart failure we could describe significantly lower NT-proBNP levels compared to none obese individuals with heart failure. This could be especially noticed by the results of the male participants. The BASEL study examined especially the BNP levels in women and showed significantly differences between men and women (84). There are various theories that try to explain why the relationship of NT-proBNP with BMI is as described. Some studies discussed an increased clearance of NT-proBNP (73,74) but the Suita Study refuted this (75). Sugisawa et al. showed an inverse relationship of NT-proBNP with BMI after adjusting their multivariable regression analysis for the serum creatinine (75). Others debated about a higher expression of natriuretic peptide clearance receptor-C (NPR-C) on the adipocytes (18,76,77), which could be disproved by the Dallas Heart Study (66). Das et al. argued against this, because they could find low NT-proBNP levels in obese individuals and that these low levels were unrelated to the NPR-C levels (66).
However, the most common explanation is a response of the cardiac endocrine system to physiological and pathological interactions and that as well body fat distribution and the cardiac endocrine systems are regulated by the gonadal function (97). One of the theories is that the hypothalamic-pituitary- gonadal axis may lead to a decreased secretion of natriuretic peptides (66,68,98). The exact cause is not clarified. Currently it is known that obese patients often show a salt retention and as well an increased cardiac output which should result in rising natriuretic peptide levels (68,98). The conflictive reality therefore has to be attributed to a none hemodynamic factor which is present in obese subjects and

missing in individuals without obesity (68,98-100). A predominant role may be the metabolic syndrome present in subjects with obesity and also in our study the risk factors dyslipidemia, arterial hypertension and diabetes mellitus were more prevalent in obese subjects (98,101). In the favor of this hypothesis points the activation of the renin-angiotensin-aldosterone system present in metabolic syndrome resulting in insulin resistance and further, as natriuretic peptides have a role in blood pressure regulation, in an increased susceptibility to arterial hypertension in obese individuals as shown by our data (98,101). From our data there is no evidence that through the presence of a higher body fat mass enhanced clearance of natriuretic peptides, which is mediated through the NPR-C expressed in fat tissue, is related to low levels (66). The reason our data refute this hypothesis is, that we report low levels of NT-proBNP and MR-proANP as well, both not being target of the clearance receptor (66). Anyway, in the end all of these studies showed a lower NT-proBNP level in obese with HF.

4.4 Outcome and biomarkers in obese subjects

During the five year follow-up period, the biomarkers GDF-15, hsTnI, MR-proADM, MR-proANP, sST2 and NT-proBNP predicted an adverse outcome reflecting myocardial remodeling, damage and increased wall stress. Thus, these biomarkers each reflect an important pathway in pathophysiological progression of cardiovascular disease. Each of the biomarker was selected due to regression analysis using lasso analysis thus reducing estimation variance.

In this study we could also describe an association of CRP with HF in general but especially in individuals with BMI<30kg/m². The reason for this could be the underlying inflammation which is often suggested to be a main reason for development of heart failure, on the other hand CRP is a surrogate biomarker being as well increased at the presence of multitude risk factors, which are speculated as well to be causative for HF (92). If CRP has a role in managing heart failure is speculative, but reduction in presence of risk factors should be recommended in all patients, finally as well resulting in a CRP reduction.

The outcome of all-cause mortality during the first five years of follow-up from cox regression analysis including all risk factors for each biomarker alone in a model

was significantly related to the concentration of MR-proADM, MR-proANP, NT-proBNP, GDF-15, sST2 and hsTnI in both BMI categories, CRP was only significant in none obese individuals. Interestingly MR-proADM was the best biomarker for the outcome in obese and none obese individuals. Our data showed some parallels to data suggested from the population-based MORGAM project, which described NT-proBNP, CRP and sensitive Troponin I to be associated with cardiovascular events (86). This was confirmed in our study but particularly in none obese individuals.

Current results of previous studies suggest a role for GDF-15 for detection of precursors of cardiovascular diseases and the management of heart failure beside NT-proBNP (87-89). The results for MR-proADM in diseased subjects or general population are not as thorough as for GDF-15 but current results support the role of MR-proADM in cardiovascular disease and especially HF (42,44,90).

The results of our study could show the importance of the markers MR-proANP, MR-proADM and GDF-15 for the detection of a heart failure. These biomarkers are increased in concentration due to left atrial enlargement, left ventricular remodeling and other prequel of symptomatic HF (91).

Regarding the outcome of all-cause mortality, the biomarkers predominantly useful in the cohort with a BMI<30 kg/m² were the natriuretic peptides and as well GDF-15 and MR-proADM. Although the other candidate biomarkers hsTnI, CRP and sST2 were additionally useful, hazard ratios were lower than the first mentioned biomarkers. In the cohort with a BMI≥30 kg/m² GDF-15 and MR-proADM were the biomarkers with the strongest influence regarding mortality and GDF-15 data pointed out that it was predominantly useful in obese subjects to predict the outcome. Including all biomarkers and risk factors in one model, only MR-proADM and GDF-15 were useful to predict all-cause mortality in this sample of the GHS cohort.

Regarding the outcome, data underline the use of GDF-15 and MR-proADM, but the use especially in obese patients was not reported yet (44,48,87). The data from our study strengthen the use of natriuretic peptides in subjects presenting without obesity, but in obese subjects a different strategy might be warranted.

4.5 Limitations

There are limitations to this study which merit consideration. All biomarkers were measured from frozen samples, which may affect absolute risk estimates. Other novel biomarkers that we did not include in this study, might subsequently be found to improve the detection of HF or improve risk prediction. Furthermore, we cannot ascertain whether preventive treatment decisions based on the novel biomarkers would improve the outcome.

4.6 Conclusion

Our results could confirm the inverse relationship of NT-proBNP with BMI, obese individuals with heart failure had a lower NT-proBNP level than none obese participants with heart failure. The cut-off levels for NT-proBNP are 138pg/mL in none obese vs. 175 pg/mL in obese.

The novel biomarkers GDF-15 and MR-proADM have a higher sensitivity but a lower specificity compared to NT-proBNP for detecting heart failure in obese and none obese subjects. GDF-15 and MR-proADM provided information in characterizing subjects with HF. This was also present when the current standard of NT-proBNP was included into the model. Both biomarkers were also able to detect HF in obese participants and were better in doing so than NT-proBNP as well.

In terms of predicting the outcome of all-cause mortality MR-proADM was the most meaningful biomarker in obese and none obese subjects. Outcome during the first 5 years of follow-up for the participants of the GHS was significantly related to concentration of MR-proADM and GDF-15 in all individuals regardless of body weight, thus elucidating different pathophysiological pathways resulting in development of HF.

The results of our study strengthen the use of natriuretic peptides in none obese subjects of the general population to diagnose HF and to predict all-cause mortality. However, in obese subjects the use of NT-proBNP and MR-proANP is blunted and the use of additional biomarkers ameliorates establishing diagnosis and prognosis in the general population. The two biomarkers with a predominant role were MR-proADM and GDF-15 and especially GDF-15 was suited best to

predict prognosis in obese subjects. Although natriuretic peptides remain the standard biomarker in HF, application of biomarker candidates offer a new perspective in diagnosing and managing HF in obese individuals.

Future clinical studies have to elucidate the additional use of GDF-15 and MR-proADM on top of natriuretic peptides for HF management and for detecting HF in obese subjects.

5. Summary

The main finding of our study is that natriuretic peptide levels, represented by NT-proBNP and MR-proANP, were influenced by obesity and were not equally useful to identify prevalent HF in contrast to candidate biomarkers in HF currently investigated especially MR-proADM, GDF-15 and further sST2. In terms of prognosis all evaluated biomarkers were relevant, as well as the natriuretic peptides, being strong predictors of the outcome all-cause mortality in subjects with and without obesity. In this context it has to be stressed that for the first time an interaction between obesity and natriuretic peptide levels is reported which is interfering with the correct management of patients presenting with typical signs and symptoms of heart failure.

Regarding the interaction with obesity our results could point out the direct influence of obesity resulting in low natriuretic peptide levels, on the other hand GDF-15 was especially predictive of all-cause mortality in obese individuals. The pathophysiology causing this paradox in obese individuals could however not be clarified by our results, but underlines the importance of combining biomarkers to correctly identify subjects with HF and obesity and that there is need to individualize biomarker testing in subgroups of patients. Personalizing medicine in the future is a major step to take in the future and our results point out that candidate biomarkers might ameliorate this process.

6. Abbreviations

al/µl	Aliqouts per microlitre
a.m.	ante meridiem
CPT	Cell Preparation Tube
CV	Cardiovascular
°C	degree Celsius
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EF	Ejection Fraction
HDL	high-density lipoprotein
IgG	Immunoglobulin G
kg/m²	kilogram divided by meters squared
LDL	Low-density lipoprotein
LOD	Limit of detection
log	logarithm
m²	meters squared
mg/dl	milligram per decilitre
mg/L	milligram per litre
MHz	Megahertz
ml	millilitre
mL/min	millilitre per minute
mmHg	millimeter of mercury is a manometric unit of pressure
ng/L	nanogram per litre
ng/µl	nanogram per microlitre
ng/mL	nanogram per millilitre
nmol/L	nanomole per litre
pg/mL	picogram per millilitre
pmol/L	picomole per litre
r	correlation coefficient
RNA	Ribonucleic acid
2D	two dimensional
%	percent

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9. Affirmation

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.
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