RESEARCH PROTOCOL

A dynamic, multiple-biomarker approach aiming for individualized treatment of heart failure with preserved ejection fraction

(ADAPT-HFpEF)

(Een dynamische, multi-biomarker aanpak gericht op geïndividualiseerde behandeling van hartfalen met behouden ejectie fractie)

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CHF	**Chronic heart failure**
CRF	**Case report forms**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EMC	**Erasmus Medical Centre**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
HF	**Heart failure**
HFmrEF	**heart failure with mildly reduced ejection fraction**
HFpEF	**Heart failure with preserved ejection fraction**
HFrEF	**Heart failure with reduced ejection fraction**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
LVEF	**Left ventricular ejection fraction**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical**

	company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Heart failure with preserved ejection fraction (HFpEF) is a dynamic, heterogeneous clinical syndrome. Its prognosis is just as ominous as that of heart failure with reduced ejection fraction (HFrEF), with approx. 50% mortality in the first five years after diagnosis. There is much to gain in the assessment of individual prognosis of HFpEF patients, in order to contribute to better timing of additional treatment and therefore to a more favourable disease course. Repeated blood biomarker measurements may detect unfavourable changes before they become clinically apparent, and could herewith contribute to improved risk assesment. Moreover, blood biomarkers could be used to derive HFpEF sub-phenotypes with differences in clinical characteristics and prognosis. Such protein-based sub-phenotyping could help us gain further insights into the underlying pathophysiological mechanisms of HFpEF.

Objective: The primary objective is to investigate the association between temporal evolutions of blood biomarkers and clinical adverse events, in order to produce a dynamic, individual, and accurate prediction model for patients with HFpEF

Study design: This is a prospective, observational multi-center cohort study which will be conducted at the cardiology department of the Erasmus Medical Centre (EMC) as well as five peripheral hospitals. A total of 200 HFpEF patients will be included in the study via the outpatient clinics during 1.5 years. The follow-up period will constitute a minimum of 2 years and a maximum of 3.5 years. Clinical data will be collected at baseline and blood samples will be collected at baseline and subsequently every 6 months.

Study population: This study will include adults capable of understanding and signing informed consent with a diagnosis of HFpEF according to the ESC HFA-PEFF algorithm or a high (90%) probability of HFpEF according to the H2FPEF score. The exclusion criteria are: scheduled for surgery or intervention, severe kidney disease, liver disease, COPD, congenital heart disease, life expectancy of <1 year, unlikely adherence to follow-up visits, linguistic barrier.

Intervention (if applicable): not applicable.

Main study parameters/endpoints: The primary endpoint is a combined endpoint of urgent visit resulting in intravenous therapy for HF, hospital readmission for acute or worsened HF, and cardiovascular death.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The regular treatment of the patients will not be interfered with as this is an observational study. The main burden of this study consists of 5 to 8 visits to the outpatient clinic during 2 to 3.5 years of follow-up. However, by combining the study visits with planned outpatient clinic visits as much as possible, extra visits will be kept to a

minimum. Procedures during the extra study visits include venipuncture and also baseline echocardiography. Risks associated with these procedures are generally considered negligible. There are no individual benefits of participation, besides the contribution to obtaining scientific knowledge for the future.

1. INTRODUCTION AND RATIONALE

Burden of heart failure

Heart failure (HF) is caused by a structural or functional cardiac abnormality, resulting in reduced cardiac output and/or elevated intra-cardiac pressures.[1] It is the final common pathway of most heart diseases. HF is a clinical syndrome that includes signs and symptoms of dyspnea, fatigue and peripheral oedema.[1] The societal burden of HF is substantial. The prevalence is >10% in people aged 70 years or over, increases with age and is estimated to increase by 46% from 2012 to 2030.[2,3] The lifetime risk of HF at the age of 55 is 33% in men and 29% in women.[4] HF is a progressive disease; patients suffer severely diminished quality of life and episodes of hemodynamic decompensation, resulting in recurrent hospital admissions. On average, around 50% of HF patients die within 5 years after diagnosis, making HF outcome equivalent to that of some common types of cancer.[5]

Heart failure with preserved ejection fraction (HFpEF)

HF is categorised according to left ventricular ejection fraction (LVEF), commonly measured with echocardiography. Patients with symptoms and signs of HF and LVEF ≤40% or LVEF 41-49% are categorized as having heart failure with reduced ejection fraction (HFrEF) and heart failure with mildly reduced ejection fraction (HFmrEF), respectively, with the most common cause being myocardial infarction.[1] Patients with clinical signs of HF, structural and/or functional cardiac abnormalities and LVEF ≥50% are categorized as having heart failure with preserved ejection fraction (HFpEF). Approximately 50% of HF patients have HFpEF.[6] HFpEF typically evolves from a combination of risk factors and comorbidities, including among others advanced age, female sex, obesity, systemic arterial hypertension, diabetes mellitus, and renal dysfunction.[7] Although HFpEF patients have a preserved ejection fraction, they experience hemodynamic problems due to abnormal cardiac filling pressures.[1,8] More than half of all HF hospital admissions are accounted for by HFpEF, and its mortality rate is similar to that of HFrEF.[3,9]

For pharmacological treatment of patients with HFrEF, general recommendations have been formulated by the European Society of Cardiology (ESC).[1] However the treatment strategy of HFpEF is not as straightforward, mostly due to its heterogenic nature and remaining gaps in the understanding of the underlying pathophysiology, as well as lack of tools for adequate risk assessment. This is also illustrated by the fact that recently, sodium–glucose cotransporter (SGLT) 2 inhibitors, which are compounds that were developed to lower elevated blood glucose levels, have been shown to hold great promise for the treatment of patients with HFpEF. More detailed pathophysiological insights, and a more accurate and individual assessment of prognosis, could contribute to improved tailoring and timing of additional therapeutic interventions, and are thus urgently warranted.

Blood biomarkers

HFpEF is a heterogeneous syndrome, which is reflected by its risk factors and comorbidities. Circulating proteins are a promising tool for gaining early and personalized insights into prognosis as well as additional insights into underlying disease mechanisms. Circulating

proteins represent underlying biological processes such as inflammation, remodeling and neuro-hormonal pathways, as well as biological systems related to HF, such as kidney and liver function.[10] In the past years the ability has emerged of such circulating proteins to monitor subtle changes in HF patients that reflect and predict adverse changes before they become clinically apparent. This ability gives them potential to detect progression and increased risk in an earlier stage than traditional physiological markers such as symptoms, blood pressure and weight, which appear late in the time course of decompensation, even when evaluated via home-monitoring.[11] Moreover, their non-invasive nature and limited costs make circulating proteins interesting alternatives for other upcoming early indicators of progression, such as pulmonary artery pressure.[12] Circulating proteins carry potential to outperform other blood biomarkers as well; compared to e.g. the genome, they provide measures in closer proximity to actual disease status. Modern laboratory techniques provide the possibility to determine thousands of proteins from a single drop of blood.

Limitations of previous research

Several observational studies have examined prognostic value of blood biomarkers in HFpEF; recent overviews are provided by Carnes et al and Cypen et al.[13,14] However these studies carry important limitations. The first limitation is that previous studies have traditionally performed protein measurements at study baseline and related them to adverse events occurring over many years thereafter; or have left several years between (usually at most two) repeated measurements. Thus, these studies are not able to properly reconstruct individual temporal protein patterns, nor to examine their prognostic value. Consequently, the necessary frequency of protein assessment for risk stratification has never been scientifically substantiated, nor tailored to individual patients. Since HFpEF is a dynamic condition, not capturing these dynamic protein patterns is a missed opportunity (Figure 1).

The second limitation is that although it is known that HFpEF is multifactorial, and that plasma contains thousands of proteins, the interplay between the proteins and their relative importance has hardly been examined in epidemiological studies. Studies on circulating proteins and HFpEF prognostication traditionally investigate only single (or at best a few) proteins.[13,14] This has resulted in fragmented information and lack of a comprehensive prognostic strategy.

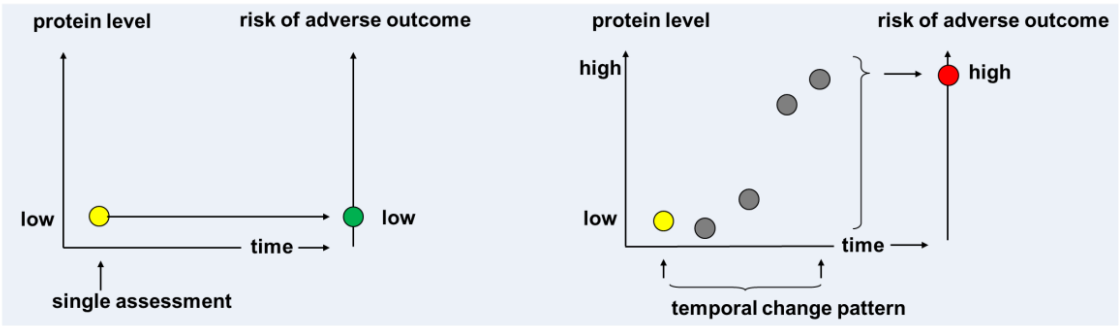


Figure 1. Single protein measurement (left), and serial protein measurement (right) for risk assessment. The right model uses additional information (missed by the left model), leading to a different risk estimate.

With regard to pathophysiology, the increasing prevalence of HF and its poor prognosis emphasize the importance of understanding the factors contributing to its heterogeneity and its underlying mechanisms. Several phenomapping (comprehensive description of phenotype

components) studies have been performed in HFpEF until now. This phenomapping usually entailed cluster analyses to discern pathophysiologically relevant subgroups of patients. The lack of effective treatments for HFpEF represents a large and growing unmet need in cardiology and has spurted these studies, because such deep phenotyping could contribute to better tailoring of treatment regimens. The studies attempted to identify HFpEF sub-phenotypes based on clinical, echocardiographic and hemodynamic characteristics, and suggested several HFpEF phenogroups with different comorbidities, pathophysiologies and outcomes.[15-19] Also, studies have investigated limited sets of circulating proteins to underscore that HFpEF has different properties than HFrEF.[20-23] Only one study has yet examined a wider protein panel (415 proteins), and demonstrated 2 HFpEF clusters with different cardiovascular phenotypes and outcomes.[24] Additional phenomapping studies examining larger sets of proteins, as well as temporal patterns of these proteins, are warranted.

Current research project

In this project, a cohort of HFpEF outpatients will be built. Repeated blood sampling will be performed and the disease-course will be monitored. When follow-up is complete, an elaborate set of proteins will be determined in the blood samples.

Based on these measurements, the association between temporal protein patterns and prognosis will be investigated. We hypothesize that temporal evolutions of circulating proteins are associated with clinical adverse events.

Moreover, HFpEF sub-phenotypes will be constructed based on the protein patterns in order to gain further insights into pathophysiology. We hypothesize that multiple proteomic-based sub-phenotypes will be present and that these sub-phenotypes are associated with differing clinical characteristics and disease outcomes.

2. OBJECTIVES

Primary Objective:

- To investigate the association between temporal evolutions of blood biomarkers and clinical adverse events, in order to produce a dynamic, individual, and accurate prediction model for patients with HFpEF

Secondary Objective(s):

- To describe temporal evolution of biomarkers in patients that experience the primary endpoint and in patients that do not
- To uncover HFpEF sub-phenotypes based on baseline levels and temporal evolutions of blood biomarkers
- To investigate differences in clinical characteristics and prognosis of these HFpEF sub-phenotypes.
- To obtain insights into underlying pathophysiological mechanisms of HFpEF, based on these sub-phenotypes.

3. STUDY DESIGN

This is a prospective, observational multi-center cohort study. It will be conducted at the Cardiology departments of the Erasmus Medical Center (EMC) as well as five peripheral hospitals. A total of 200 HFpEF patients will be included in the study through the outpatient clinics. The follow-up period is a minimum of 2 years and a maximum of 3.5 years. Clinical data will be collected at baseline, and blood samples will be repeatedly collected, at baseline followed by 6-month intervals. Protein measurements will be performed at the end of follow-up.

Start	**Up until**	**Research activities**
April 2021	October2022	Preparations, including completion of protocol and standard operation procedures (SOPs) and implementation of protocol/SOPs
November 2022	August 2024	Inclusion of 200 patients
May 2022	August 2026	Patient follow-up (minimum of 2 years and maximum of 3.5 years per patient)
September 2026 onwards		Protein measurements and reporting

4. STUDY POPULATION

4.1 Population (base)

Patients with a (suspected) diagnosis of HFpEF will be recruited through the Cardiology outpatient clinics of the EMC and five peripheral hospitals. Based on a recent Dutch multi-center registry (CHECK-HF registry), HFpEF patients constitute up to ~20% of all HF patients followed at Dutch centers.[25] In the EMC, since this is a tertiary center, this is expected to be somewhat lower, i.e. 10-20%. In the EMC, in total approx. 600 HF patients are being treated at the outpatient clinic; for the peripheral hospitals, this ranges from 400 to 800, depending on center size. Altogether this would imply there are 80 to 160 HFpEF patients available per peripheral center (and at least 60 in the EMC). With an expected participation rate of ~50%, 40-80 patients could potentially be included per center. With a total of 6 centers, inclusion of 200 patients during 1.5 years is therefore deemed feasible, even if HFpEF frequency and participation rates turn out lower than expected.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet the following criteria:

I. Age of 18 years or older

AND

II. Capable of understanding and signing informed consent

AND

III. A diagnosis of HFpEF according to the HFA-PEFF diagnostic algorithm of the ESC[7],

OR

IV. a high (90%) probability of HFpEF according to the H2FPEF score[26], i.e. a score of 6 or higher

AD. III: A diagnosis of HFpEF according to the HFA-PEFF diagnostic algorithm of the ESC[7] includes the following:

a. One or more symptoms and signs of HF
b. LVEF ≥50%

AND

c. objective evidence of cardiac structural and/or functional abnormalities consistent with the presence of LV diastolic dysfunction/raised LV filling pressures (based on echocardiography and raised natriuretic peptides (NPs))[1]; or specifically, a (noninvasive) HFA-PEFF score of ≥5 points[7]

OR

d. Previous HFpEF diagnosis based on echo stress test or based on invasive haemodynamic measurements, according to the HFA-PEFF criteria[7]

Further to above-described criteria c) and d), subjects will be included if: 1) diagnosis of HFpEF has been set, or can be set at the time of inclusion, according to the HFA-PEFF scoring system without additional invasive hemodynamic testing, or 2) invasive hemodynamic testing has previously been performed in the context of usual care and was positive according to the HFA-PEFF diagnostic criteria.
If additional invasive hemodynamic testing is necessary to confirm diagnosis at the time of screening, such invasive testing will not be performed for study purposes and the patient will not be included at that moment.

AD IV: A high (90%) probability of HFpEF according to the H2FPEF score[26] of the AHA entails a score of 6 to 9 according to the following criteria:

- Heavy: 2 points (BMI > 30 kg/m2)
- Hypertensive (using ≥2 antihypertensives): 1 point
- Atrial Fibrillation (any form): 3 points
- Pulmonary hypertension (sPAP > 35 mmHg): 1 point
- Elder (age > 60): 1 point
- Filling pressure (E/e' septal > 9): 1 point

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- History of LVEF ≤40%
- Scheduled for surgery or intervention for both coronary and non-coronary indication within 6 months of inclusion
- Impaired renal function, defined as eGFR < 20 mL/min/1.73 m2 (CKD-EPI) or requiring dialysis at the time of screening
- Acute or chronic liver disease, defined by serum levels of transaminases or alkaline phosphatase more than three times the upper limit of normal at screening
- COPD Gold stage IV
- Congenital heart disease
- Pregnancy
- Coexistent condition with life expectancy of <1 year
- Unlikely to appear at all scheduled follow-up visits
- Linguistic barrier

4.4 Sample size calculation

The sample size needed is estimated at 200 patients (180 patients plus 20 patients to account for patient attrition).

The annual incidence of the primary endpoint is estimated at ~18% based on a recent, large, multicenter trial in ambulatory HFpEF patients.[27] Assuming similar prognosis in HFpEF and HFrEF, this incidence also in line with our Bio-SHiFT study, which was performed in HF patients in the EMC and NWZ, and which showed an annual incidence of the PE of ~15%, without including urgent visits in the composite endpoint (manuscript submitted). The average follow-up will, by design, be 2.75 years (minimum of 2 years and maximum of 3.5 years). Therefore, the primary endpoint is expected to occur in at least 90 (180*0.18*2.75) patients and up to 100 (200*0.18*2.75) patients. Using the rule of thumb of 10 events per determinant, this provides sufficient statistical power to examine the association of 9 to 10 biomarkers (simultaneously) with occurrence of the primary endpoint.

When larger numbers of biomarkers will be studied simultaneously, suitable statistical approaches will be used such as elastic net regression, that provide variable reduction and are thus suitable for situations where the number of independent variables is much larger than the number of events.[28]

Moreover, since in the secondary objectives, temporal biomarker patterns will be compared between those that do and do not experience adverse clinical events, we have calculated the difference in change over time that can be demonstrated in circulating protein level between patients that experience the primary endpoint and those that do not. We have assumed a linear association between time and protein level and a continuous autoregressive correlation matrix. We have used available data on repeated NT-proBNP measurements from another patient cohort to derive the measurement error standard deviation (sigma) and the input parameter (rho) for the autoregressive correlation matrix. Using 500 simulations, the above numbers showed to be sufficient to demonstrate a difference in the slope (‘difference in the change over time’) of 10 pmol/L per month, between patients with and without the primary endpoint (with 80% power and a Bonferroni corrected α-error of 0.05/5000 = 0.00001). This difference is small in clinical terms, further demonstrating that the study has sufficient statistical power.

5. TREATMENT OF SUBJECTS

< This chapter is only applicable for intervention studies>

Not applicable

5.1 Investigational product/treatment

Not applicable

5.2 Use of co-intervention (if applicable)

Not applicable

5.3 Escape medication (if applicable)

Not applicable

6. INVESTIGATIONAL PRODUCT

Not applicable

6.1 Name and description of investigational product(s)

Not applicable

6.2 Summary of findings from non-clinical studies

Not applicable

6.3 Summary of findings from clinical studies

Not applicable

6.4 Summary of known and potential risks and benefits

Not applicable

6.5 Description and justification of route of administration and dosage

Not applicable

6.6 Dosages, dosage modifications and method of administration

Not applicable

6.7 Preparation and labelling of Investigational Medicinal Product

Not applicable

6.8 Drug accountability

Not applicable

7. NON-INVESTIGATIONAL PRODUCT

Not applicable

7.1 Name and description of non-investigational product(s)

Not applicable

7.2 Summary of findings from non-clinical studies

Not applicable

7.3 Summary of findings from clinical studies

Not applicable

7.4 Summary of known and potential risks and benefits

Not applicable

7.5 Description and justification of route of administration and dosage

Not applicable

7.6 Dosages, dosage modifications and method of administration

Not applicable

7.7 Preparation and labelling of Non Investigational Medicinal Product

Not applicable

7.8 Drug accountability

Not applicable

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The primary endpoint is a combined endpoint of urgent visit resulting in intravenous therapy for HF, hospital readmission for acute or worsened HF, and cardiovascular death.

The main study parameters will include the associations of blood- and urine protein biomarkers, with the primary endpoint.
On the one hand, biomarkers whose measurements have already shown associations with adverse prognosis in previous studies will be examined. These include measures of myocyte stress (NT-proBNP, ST2), myocyte injury (troponins T and I), inflammation (CRP,GDF-15), and remodeling (galectin-3, matrix metalloproteinases). On the other hand, “novel” biomarkers that are currently under investigation will be examined, as will biomarkers well known in other pathological states that may be helpful in heart failure. Biomarker measurements will be performed after all follow-up data have been collected. Thus, we will be able to select the most relevant biomarkers from the literature at that moment for measurement. Of note is that current multiplex technologies enable assessment of hundreds or even thousands of biomarkers simultaneously. Examples that are currently gaining interest include the so-called Olink Proximity Extension Assay and the SomaScan Assay. We will choose the platform with optimal characteristics at the moment of measurement.

8.1.2 Secondary study parameters/endpoints (if applicable)

The secondary endpoints are:

- the separate components of the combined primary endpoint; urgent visit resulting in intravenous therapy for heart failure, hospital readmission for acute or worsened HF, and cardiovascular death.
- The combined endpoint urgent visit resulting in intravenous therapy for HF, hospital readmission for acute or worsened HF, and all-cause death.
- All-cause death
- Myocardial infarction (fatal and non-fatal), stroke (fatal and non-fatal), percutaneous coronary intervention (PCI) and coronary artery bypass grafting (CABG)
- Cardiovascular disease (includes all of the above, except all-cause death)

Associations of blood- and urine protein biomarkers as described under 8,1,1 with the secondary endpoints will be examined.

8.1.3 Other study parameters (if applicable)

The baseline data will include demographics, medical history, medication use, anamnesis, physical examination, ECG and echocardiography measurements.

Follow-up data will include occurrence of clinical endpoints and changes in medication use. Blood samples will be collected as described in the study procedures below. Biomarker measurements will not be performed real-time but batch-wise after completion of follow-up or during interim assessments.

8.2 Randomisation, blinding and treatment allocation

Not applicable

8.3 Study procedures

Clinical data collection

PhD students will be employed by Erasmus MC and will be provided hospitality agreements by the participating centers. The PhD students will screen for enrollment and perform the baseline and follow-up assessments.
Clinical data will be collected at baseline and at each 6-month follow-up visit. Baseline data include demographics, medical history, medication use, anamnesis, results of physical examination, resting ECG and echocardiography. During the follow-up visits, changes in medication use and information on the occurrence of outcomes will be assessed. Clinical data will be collected in the form of electronic Case Report Forms (eCRF), according to the rules and regulations concerning the safety and confidentiality of patient data. The database will enable electronic checks for internal consistency of data-elements. Data sets for each subject will be identified by number. Study data will only be accessible to authorized personnel. In all cases, caution will be exercised to assure the patient's confidentiality.

Echocardiography

Echocardiography including tissue Doppler imaging and speckle tracking will be performed at baseline. A detailed protocol will be developed for echocardiography performance and reporting. The echocardiography procedure will be identical to that of a routine echocardiogram and will be conducted by experienced echocardiographists at the hospital where the patient is included. Echocardiographic parameters will also be entered into the eCRF at these hospitals.
Planning the baseline echocardiography may pose logistic challenges due to lack of available machines or echocardiographists. Therefore, if a recent (≤6 months) echocardiogram is available that meets the above protocol standards, this may alternatively be used for assessment of the baseline echocardiographic parameters.

The protocol does not dictate echocardiography at the time of the follow-up visits. However, in centers/patients in whom echocardiography is performed in the context of usual care during follow up and coincides with the study visits, echocardiographic parameters from these echocardiograms will be registered in the eCRF in the follow-up sections, so that repeated echocardiographic measurements will be available in a subset of the patients..

Blood sampling and urine collection

Blood and urine samples will be collected from all participants at baseline and then repeatedly every six months until the end of follow-up or death. The follow-up period is a minimum of 2 years and a maximum of 3.5 years, resulting in 5 to 8 blood and urine sampling moments per patient. Blood samples will be collected by venipuncture and a total of approximately 30ml of blood will be drawn per visit (serum, EDTA plasma, citrate plasma). Each visit, a single urine specimen will be collected. Blood and urine samples will be collected by a research nurse, a heart failure nurse or by personnel of the clinical laboratory. Thereafter they will be transferred to the laboratory where they will be processed. Subsequently the blood and urine samples will be frozen at -80°C and stored at the Central Biobank of the EMC until biomarker measurement takes place. Samples from peripheral hospitals will be frozen at -80°C and temporarily stored at those hospitals, and transported to the EMC batch-wise and under controlled conditions. A detailed protocol will be developed for sample collection, sample handling and (long-term) storage. The use of a standardized protocol for sample collection, preparation, and storage will provide the proper safeguards and assurances relating to sample quality and consistency for the assessment of clinical usefulness of the biomarkers. Study data will be accessible to authorized personnel only.

Biomarker measurements

Biomarker measurements will be performed after all follow-up data have been collected. Thus, we will be able to select the most relevant biomarkers from the literature at that moment for measurement. Of note is that current multiplex technologies enable assessment of hundreds or even thousands of biomarkers simultaneously. Examples that are currently gaining interest include the so-called Olink Proximity Extension Assay and the SomaScan Assay. We will choose the platform with optimal characteristics at the moment of measurement.

Overall, the following strategy will be applied. First, the value of serial measurements will be investigated for biomarkers whose baseline measurements have already shown associations with adverse prognosis in previous studies. These include measures of myocyte stress (NT-proBNP, ST2), myocyte injury (troponins T and I), inflammation (CRP,GDF-15), and remodeling (galectin-3, matrix metalloproteinases). Second, “novel” biomarkers that are currently under investigation will be examined, as will biomarkers well known in other pathological states that may be helpful in heart failure. A detailed protocol for biomarker analysis will be developed in the course of the study.

In addition to analyses of biomarkers in the form of plasma proteins, the collected blood samples may also be used for genetic research. The aim of these genetic analyses is to investigate the possibility of a potentially increased risk of occurrence of adverse cardiac events or divergent biomarker patterns in case of certain DNA-polymorphisms, single nucleotide polymorphisms (SNPs) or genetic variants. For this purpose, genetic data from this study may be pooled with those from other studies, in

order to enhance statistical power for genetic analyses. On the basis of current academic knowledge in cardiovascular genetic research, the results of the above-mentioned genetic research will not have any effect on diagnosis, prognosis, future health, required treatment or inheritance of cardiovascular disease by the offspring of study patients. Therefore, informed consent will be asked, but study patients will not be informed of these results.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal (if applicable)

Not applicable

8.5 Replacement of individual subjects after withdrawal

Since we have accounted for patient attrition in the sample size estimation, we will not replace individual subjects after withdrawal.

8.6 Follow-up of subjects withdrawn from treatment

All efforts will be made to complete and report observations as thoroughly as possible at the time of withdrawal. If a patient withdraws from the study, the reason for withdrawal will be recorded. Patient data collected up until withdrawal will be used in the final analysis, unless the participant objects to this.

8.7 Premature termination of the study

Due to the observational nature of the study, premature termination of the study is not to be expected and interim analysis is therefore not applicable. Nevertheless, in case the study is ended prematurely, the investigator will notify the accredited METC, including the reasons for the premature termination.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

AEs are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to trial procedure. All AEs reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

This study is an observational, investigator-initiated study that does not interfere with regular treatment. Patients are not subjected to any invasive procedures or therapeutic procedures other than standard medical care.

SAEs will be recorded. Given the observational nature of this study and since none of the SAEs are expected to occur as an effect of the study, we propose to report SAEs by providing line-listings to the Institutional Review Board once a year. We also propose to limit reporting to those SAEs that are cardiovascular in nature. These will include the following:

- Cardiovascular death
- Cardiac transplantation
- Left ventricular assist device implantation
- Re-hospitalization for acute or worsened heart failure
- Emergency room visit resulting in intravenous therapy for HF
- Myocardial infarction
- Stroke
- Percutaneous coronary intervention (PCI)
- Coronary artery bypass grafting (CABG)
- In addition, all-cause mortality

We will report study identification number, gender, and date of birth of the patients, as well as the date of occurrence of the SAE and consequences for the study.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable

9.3 Annual safety report

Not applicable

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated in the context of usual care, and/or referral to the general physician or a medical specialist.

SAEs will reported till the end of the study.

9.5 [Data Safety Monitoring Board (DSMB) / Safety Committee]

Not applicable

10. STATISTICAL ANALYSIS

Categorical variables will be described as numbers and percentages. Continuous variables will be described as means and standard deviations for normally distributed variables and as medians and interquartile ranges for not normally distributed variables. All study subjects will be included into the statistical analysis. In multivariable models, missing values of covariates will be handled by (multiple) imputations.

10.1 Primary study parameter(s)

The association between the temporal biomarker evolutions and the primary endpoint will be investigated using joint modelling. Joint models combine linear mixed modelling (for temporal biomarker evolutions) with survival analysis (for clinical adverse events).[29] Since joint models take into account all repeated measurements, they result in less bias compared to 'simpler' approaches such as deltas between two time points. In addition to joint modeling of repeatedly measured biomarker levels, instantaneous slopes (rate of biomarker change) and area under the temporal evolution (long term biomarker elevation) will also be examined in the joint models. Adjustment for potential confounders will be performed using baseline clinical characteristics. Covariates will be chosen based on existing literature and will include commonly known cardiovascular confounding variables such as sex, age, blood pressure, diabetes mellitus, body mass index and New York Heart Association Functional Classification.

Apart from examining associations between individual biomarkers and outcome, we will also construct a multivariable model by entering a wide range of biomarkers. In case of a large number of biomarkers, as obtained from multiplex biomarker assays, we will apply penalized regression such as elastic net to obtain a multivariable model that contains the most informative subset of biomarkers. Penalized regression methods provide variable reduction and are thus suitable for situations where the number of independent variables is much larger than the number of events.[28] In the penalized model, clinical covariates, summarized in a clinical score (such as the MAGGIC score,[30] will be fixed, and biomarkers will be added on top of the score. To accommodate the repeated measurements, we will combine the penalization with time-dependent Cox modelling. After obtaining the optimal selection of biomarkers in this way, we will enter the obtained selection into a multivariable joint model. The time horizon of the models will equal the follow-up time of the study.

Assessment of model performance will include c-statistics. Internal validation will be performed by means of bootstrapping and/or cross-validation.

For the purpose of external validation, during the project, collaborations will be initiated and consolidated with other HFpEF cohorts that have biomarker measurements available.

We will perform several statistical tests to obtain significance levels for relations between individual biomarkers and the primary endpoint. Some biomarkers will be selected based on pre-specified hypotheses. For these hypothesis-driven tests, a two-tailed significance level of 0.05 will be used. Some biomarkers will be part of large multiplex panels. For such hypothesis-free tests, corrections for inflation of the type I error due to multiple testing will be applied (Benjamini-Hochberg correction).

10.2 Secondary study parameter(s)

Secondary endpoints (clinical adverse events mentioned in 8.1.2) will be analyzed in a similar way as the primary endpoints.

Differences in temporal biomarker evolutions between patients with and without the primary endpoint will be illustrated by making effect plots based on linear mixed models. Time will be used as the independent variable and as a random effect, the primary endpoint and its interaction with time as independent variables, and biomarkers as the dependent variable.

Sub-phenotypes of HFpEF will be constructed by using a full set of repeated multiplex biomarker measurements and applying a cluster analysis. First, linear mixed models will be fitted to model the temporal evolutions of the biomarkers. The individual biomarker trajectories with be used as input for the cluster analysis. The number of clusters will be determined using multiple indices and varying all combinations of number of clusters, distance measures, and clustering methods.[31] Then patients will be assigned to clusters using the K-means algorithm. Cluster stability will be assessed using several schemes (bootstrapping, subsetting, jittering).[32,33]

After the sub-phenotypes are constructed, differences in baseline clinical characteristics between the sub-phenotypes will be examined using one-way ANOVA, Kruskal-Wallis, Chi2 and Fisher's exact tests when appropriate. Additionally, biomarker levels will be visualized with the use of heatmaps (after applying z-standardization for comparability). Moreover, the association between cluster membership and the primary outcome will be investigated using Cox regression models. Subsequently, these models will be corrected for potential confounders in a multivariable analysis.

Finally, Ingenuity Pathway Analysis (IPA) will be used to characterize pathways underlying differences in circulating proteins between the phenotypes. IPA enables visualization and analysis/interpretation of complex omics data by placing the results in the context of biological systems.

10.3 Other study parameters

The other study parameters as described in chapter 8 will serve as input for the above-described analyses when needed (e.g., adjustment for confounders).

10.4 Interim analysis (if applicable)

Not applicable

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted in compliance with Good Clinical Practice (GCP), with the principles of the Declaration of Helsinki (version of 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other guidelines, regulations and Acts.

11.2 Recruitment and consent

All potential subjects will be screened for eligibility for the study. If patients are eligible for participation, they will be asked by the treating physician whether the (sub)investigator may contact them. They will be informed on the background and goal of the study by a patient information letter (document E1/E2). They will be given one week to consider participation. Patients have to give their written informed consent to participate in the study. Written informed consent will be obtained for this study by the (sub-)investigator /PhD student from all patients before the performance of any protocol specific procedures. Templates are provided and attached to the protocol documents. As stated before, this study is based on voluntary participation and refraining from participation will not result in any change in therapy.

11.3 Objection by minors or incapacitated subjects (if applicable)

Not applicable.

11.4 Benefits and risks assessment, group relatedness

This is an observational study that does not interfere with regular treatment. Although the participants will not benefit personally from the research, there is a reasonable likelihood that the population in which the research is carried out stands to benefit from the results of the research. The additional prognostic value of multiple, different biomarkers repeatedly-measured during longer-term follow-up in HFpEF patients, has not been evaluated before, and may result in a better prediction of adverse events of individual HFpEF patients.

The burden of participation will be low. The time needed to perform the baseline investigations, including taking the clinical data, blood sample, and echocardiography, is estimated at approximately 60 minutes. Follow-up visits are estimated at approx. 20 minutes. The main burden of this study consists of extra visits to the outpatient clinic. However, by combining the study visits with planned outpatient clinic visits, this will be kept to a minimum. During standard outpatient visits, blood sampling is common to monitor electrolytes and renal function. Procedures during the extra study visits include

venipuncture and also baseline echocardiography. Risks associated with these procedures are generally considered negligible. There are no individual benefits of participation, besides the contribution to obtaining scientific knowledge for the future.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives (if applicable)

Patients will receive compensation for travel costs when their study visit cannot be combined with their outpatient visit.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Research data will be recorded into a central, digital eCRF. The handling of personal data complies with the General Data Protection Regulation (GDPR) (in Dutch: de Algemene Verordening Gegevensbescherming, AVG). Data will be handled confidentially and anonymously. Data will be coded, and consecutive patients will be assigned consecutive numbers (e.g. 001, 002 etc.). The key to the code will be safeguarded by the investigators. The source data will only be accessible to the investigators, the members of the committee that approved the study and authorised personnel of the health authorities. Data and blood will be kept for 15 years at least and there is a possibility of 15 years additional storage, only after patients' consent (this is an option for the patient in the informed consent form). No personalized data will be used in scientific reports or presentations. Blood samples may also need to be transferred to countries outside the European Union, for example for specific laboratory tests. Only coded data and blood samples will be transferred, if necessary for study purposes.
After the research project has been completed, participants may receive an overview of the results defined at group level if desired.

12.2 Monitoring and Quality Assurance

The investigational sites will be monitored to ensure accuracy of study data. Monitoring will also be used to verify that patients consented with participating in this study; submitted study data are complete and accurate with respect to the patient records. Monitoring will be performed in accordance to the Erasmus MC monitoring plan as described in document K6.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.
Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the

first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.
The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.
In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

All data will be collected in accordance with the privacy rules of the Erasmus MC, WMO, GDPR and rules of conduct for health research. Only investigators involved in the study will have access to the data. No personalized data will be used in scientific reports or presentations.

13. STRUCTURED RISK ANALYSIS

Not applicable

13.1 Potential issues of concern

Not applicable

13.2 Synthesis

Not applicable

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