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Biomarkers for eligibility and surrogate endpoints in heart failure trials

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To my Parents

“Science knows no country, because knowledge belongs to humanity, and is the torch which illuminates the world. Science is the highest personification of the nation because that nation will remain the first which carries the furthest the works of thought and intelligence”

Louis Pasteur

The Karolinska Institutet, Department of Medicine
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ABSTRACT

Background

In heart failure (HF) with reduced ejection fraction (HFrEF), randomized controlled trials have provided effective treatments, but prognosis still remains poor. HF with mid-range EF (HFmrEF) has no evidence-based therapy and represents a newly characterized and relevant population for future trials. Trials in HF with preserved EF (HFpEF) have failed to provide any effective treatment, with several concerns about their design.

Aims

Overall aim is to provide evidence to improve trial design in HF, investigating the use of natriuretic peptides (NPs) as surrogate endpoint, and as eligibility criterion to foster the enrichment of trials for cardiovascular (CV) vs. non-CV events. Specific aims were:

- to assess the associations between changes in NP [B-type NP (BNP) and N-Terminal pro-BNP (NT-proBNP)] levels over time and prognosis in chronic HFpEF and HFmrEF (**Study I**) and in acute decompensated HFpEF (**Study II**);
- to compare levels, the independent determinants of levels and the prognostic role of NT-proBNP across EF categories (**Study III**);
- to evaluate the associations between NT-proBNP and CV and non-CV outcomes across EF categories and in specific subgroups, and the associations between HF therapies and outcomes according to NT-proBNP levels (**Study IV**).

Changes in NT-proBNP and prognosis in chronic HFpEF and HFmrEF

We studied 650 HFpEF/HFmrEF outpatients enrolled in the Swedish Heart Failure registry (SwedeHF) between 2000 and 2012, reporting serial NT-proBNP assessments. A reduction in NT-proBNP at the median time of 7 months from the first measurement was associated with a reduction of mortality/HF hospitalization risk by 54% in the overall population, by 51% in HFpEF and by 61% in HFmrEF.

Changes in BNP/NTproBNP levels and prognosis in acute decompensated HFpEF

From the Karolinska-Rennes (KaRen) study, 361 patients with acute decompensated HFpEF and BNP/NT-proBNP measurements at the baseline and at the 4-8 weeks follow-up visit were analyzed. Changes in NPs from baseline to follow-up visit were not significantly associated with the risk of mortality/HF hospitalization although a trend toward a reduction in risk following the reduction in levels was observed.

Levels, predictors of levels and prognostic/discriminatory role of NT-proBNP across EF categories

We analyzed 9,847 outpatients with HFpEF (18%), HFmrEF (22%) or HFrEF (60%) with at least one NT-proBNP assessment, enrolled in the SwedeHF between 2000 and 2012. NT-proBNP levels were significantly higher in HFrEF (2,288 pg/ml) vs. HFpEF (1.428 pg/ml) and HFmrEF (1,540 pg/ml). Across EF categories, there were several different independent determinants for NT-proBNP levels, with atrial fibrillation more important in HFmrEF and HFpEF, diabetes and hypertension in HFmrEF, and age and body mass in HFrEF and HFmrEF, whereas there were no differences for renal function, New York Heart Association class, heart rate and anemia. NT-proBNP >vs. ≤median was associated with increased risk of mortality and mortality/hospitalization with hazard ratios significantly higher in HFmrEF and HFpEF vs. HFrEF. NT-proBNP had greater area under the curve for death/HF hospitalization in HFmrEF vs. HFpEF and HFrEF.

NT-proBNP levels and risk of CV/non-CV events across EF categories

We studied 15,849 patients with HFpEF (23%), HFmrEF (21%) and HFrEF (56%) and at least one NT-proBNP assessment, enrolled in SwedeHF between 2000 and 2012. Increasing NT-proBNP levels were associated with a steeper increase in CV vs. non-CV event rates in HFpEF vs. HFmrEF vs. HFrEF. CV to non-CV event ratio increased together with the increase in NT-proBNP in HFpEF and HFrEF, but only in the lower range in HFmrEF. The association between HF treatments (angiotensin converting enzyme inhibitors or angiotensin receptor blockers and beta-blockers) and CV/non-CV events risk was consistent in NT-proBNP ≤ and >median.

Conclusions

The association between NT-proBNP levels and prognosis across the EF spectrum, together with the association between reduction in NT-proBNP levels and improvement in prognosis in HFpEF, HFmrEF and HFrEF supports the use of NT-proBNP as surrogate endpoint in phase II trials in chronic HF. We did not observe any significant association between changes in BNP/NT-proBNP and prognosis in acute decompensated HFpEF. The observed relationship between NT-proBNP levels and CV and non-CV events supports the use of NT-proBNP for eligibility and enrichment for CV events in HF trials, but the cut-off levels should consider the differences in comorbidities across the EF spectrum. Potential treatment response according to NT-proBNP levels deserves further investigation.

LIST OF ORIGINAL PAPERS

Study I

Savarese G, Hage C, Orsini N, Dahlström U, Perrone-Filardi P, Rosano GM, Lund LH. Reductions in N-Terminal Pro-Brain Natriuretic Peptide Levels Are Associated With Lower Mortality and Heart Failure Hospitalization Rates in Patients With Heart Failure With Mid-Range and Preserved Ejection Fraction.

Circ Heart Fail. 2016;9(11). pii: e003105

Study II

Savarese G, Donal E, Hage C, Oger E, Persson H, Daubert JC, Linde C, Lund LH; KaRen investigators.

Changes in natriuretic peptides after acute hospital presentation for heart failure with preserved ejection fraction: A feasible surrogate trial endpoint? A report from the prospective Karen study.

Int J Cardiol. 2017; 226:65-70

Study III

Savarese G, Orsini N, Hage C, Dahlström U, Vedin O, Rosano GMC, Lund LH

Associations With and Prognostic and Discriminatory Role of N-terminal pro-B-type Natriuretic Peptide in Heart Failure with Preserved vs. Mid-Range vs. Reduced Ejection Fraction

In manuscript

Study IV

Savarese G, Orsini N, Hage C, Vedin O, Cosentino F, Rosano GMC, Dahlström U, Lund LH

Using NT-proBNP for Eligibility and Enrichment in Trials in HFpEF, HFmrEF, and HFrEF

JACC Heart Fail 2018; in press

LIST OF ABBREVIATIONS

ACE-I: angiotensin converting enzyme inhibitor
ADHF: acute decompensated heart failure
ANOVA: analysis of variance
ANP: A-type natriuretic peptide
ARB: angiotensin receptor blocker
BNP: B-type natriuretic peptide
CI: confidence interval
CNP: C-type natriuretic peptide
CRT: cardiac resynchronization therapy
CV: cardiovascular
EF: ejection fraction
ESC: European Society of Cardiology
HF: heart failure
HFmrEF: heart failure with mid-range ejection fraction
HFpEF: heart failure with preserved ejection fraction
HFrEF: heart failure with reduced ejection fraction
HR: hazard ratio
ICD: implantable cardioverter defibrillator
IQR: interquartile range
Karolinska: Karolinska-Rennes Study
LVAD: left ventricular assist device
MRA: mineralocorticoid receptor antagonist
NYHA: New York Heart Association
NP: natriuretic peptide
NT-proBNP: N-terminal pro-B-type natriuretic peptide
OR: odds ratio
ROC: receiver operating characteristic
SwedeHF: Swedish Heart Failure Registry
US: United States

INTRODUCTION

Global burden

Heart failure (HF) represents a global pandemic. Worldwide, around 26 million people are affected by HF. The prevalence of the disease reports geographical differences, ranging 1-2% in Western countries and Australia, and reaching 1.3-6.7% in Asia, but is even expected to rise following the global aging of population. Incidence ranges 0.1-0.4% in Western Countries, approximating 1% in China [1]. HF is the most common cause of hospitalization among >65 years adults in the United States (US) [2] and even in the overall population [3]. In 2012 health expenditure for HF approximated $31 billion and projections show that by 2030 the total cost of HF will increase by 127% to around $70 billion, corresponding to $244 for every US adult [4]. Prognosis is still poor, with <50% 4-year survival, similar to the most common cancers, and low quality of life [5, 6].

Definition

From a physiological perspective, HF can be defined as a clinical syndrome characterized by the reduced ability of the heart to pump (systolic dysfunction) or fill (diastolic dysfunction) with blood, that leads to an inadequate cardiac output to meet metabolic needs, or to a preserved cardiac output due to compensatory mechanisms (manifest as increased left ventricular filling pressures) [7]. Indeed, neurohormonal activation sustains cardiac output at the early stages of HF, but causes progressive maladaptive cardiac remodeling leading to full-blown HF in the long term [8].

From a more clinical perspective, HF has been well defined in the current European Society of Cardiology (ESC) guidelines on HF as a clinical syndrome characterized by typical symptoms (e.g. breathlessness, ankle swelling and fatigue) and signs (e.g. elevated jugular venous pressure, pulmonary crackles and peripheral edema), caused by structural and/or functional cardiac abnormalities, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress [9].

Notably, left ventricular ejection fraction (EF), which is a particularly important parameter in clinical practice and has been used as inclusion criterion for randomized controlled trials in the HF setting, does not contribute to the overall definition of HF but is key for characterizing the HF population. Indeed, according to EF and other additional criteria, HF can be classified as 1) HF with preserved EF (HFpEF) and 2) HF with mid-range EF (HFmrEF), characterized by EF≥50% and EF=40-49%, respectively, together with elevated levels of natriuretic peptides (NPs) and the presence of relevant structural heart disease (left ventricular hypertrophy and/or left atrial enlargement) or diastolic dysfunction; 3) HF with reduced EF (HFrEF), characterized by EF<40% [9]. The old HF classification into systolic and diastolic HF has been abandoned since both systolic and diastolic dysfunction coexist, even though at different extent, throughout the EF spectrum [10, 11].

Another classification of HF considers the different onset of symptoms. Thus, patients may suffer of chronic or acute decompensated HF (ADHF), where ADHF refers to a rapid onset of or a progressive worsening of HF symptoms/signs in a patient with chronic HF [9].

Etiology, risk factors and clinical characteristics

There are geographical differences for HF etiologies [1]. Overall, predominant cause of HF is hypertension in HFpEF and ischemic heart disease in HFmrEF and HFrEF [9]. Other causes may be cardiomyopathies, toxic damage (e.g. drug abuse, medications, radiation), abnormal loading conditions (e.g. valvular and pericardial diseases, severe anemia, sepsis, renal failure), arrhythmias, infiltrative diseases (e.g. amyloidosis, sarcoidosis, hemochromatosis) and metabolic derangements (e.g. thyroid disease, growth hormone deficiency) [9]. Important risk factors for HF are age, male sex, hypertension, left ventricular hypertrophy, ischemic heart disease, diabetes mellitus, valvular disease, overweight/obesity, smoking and atrial fibrillation [12]. Around 60% of the HF population has HFrEF, 20% has HFmrEF and 20% has HFpEF [13]. In HFpEF vs. HFmrEF vs. HFrEF patients are older, more likely females and overweight/obese, no smokers, with higher arterial blood pressure and more likely to have history of hypertension, atrial fibrillation, lung disease, renal disease and anemia. Those with HFrEF and HFmrEF vs. HFpEF are less likely to be diabetic and have valvular disease but more likely to report history of coronary artery disease [13, 14].

Pathophysiology

HFrEF

In HFrEF, the key is a direct cardiomyocyte injury [15, 16]. This triggers compensatory neurohormonal mechanisms, such as:

- the activation of the sympathetic nervous system that initially contributes to maintain cardiac output increasing heart rate and myocardial contractility;
- the activation of renin-angiotensin-aldosterone system that fosters an increase in systemic vascular resistance and fluid reabsorption in the kidneys (by the antidiuretic hormone), supporting arterial blood pressure.

All these mechanisms are compensatory at the early stages of HF, but later become maladaptive leading to adverse cardiac remodeling (left ventricular dilatation and eccentric hypertrophy) and further worsening of cardiac function (Figure 1,2).

HFpEF

In HFpEF, the key is endothelial dysfunction [15, 17]. Comorbidities (e.g. hypertension, diabetes, chronic kidney disease, anemia, chronic obstructive pulmonary disease, obesity) induce microvascular inflammation and thus, endothelial activation [17]. Low availability of nitric oxide and cyclic guanosine monophosphate adversely affects the adjacent cardiomyocytes leading to increased myocardial stiffness, and induces the endothelial-mesenchymal transition leading to enhanced fibrosis [17]. All these mechanisms foster concentric left ventricular remodeling [17] (Figure 2). Neurohormonal activation is also involved in HFpEF but at a smaller extent as compared with HFrEF [18].

Figure 2 reports the paradigms for pathophysiology in HFpEF vs. HFrEF.

Natriuretic peptides

Secretion of NPs represents one of the compensatory mechanisms in HF. The family of NPs consists of three peptides: A-type (ANP), B-type (BNP) and C-type NP (CNP). ANP is secreted by atrial myocardium secondary to its dilatation. Similarly, BNP is secreted by

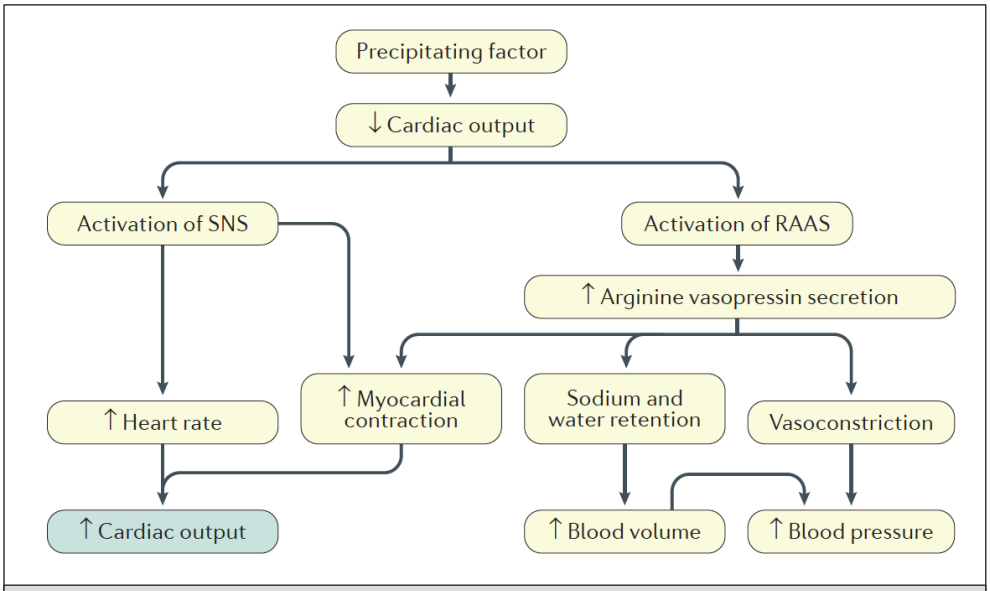

Figure 1. Compensatory mechanisms in early heart failure.
Reproduced with permission from [16]. Copyright Springer Nature. SNS: sympathetic nervous system; RAAS: renin-angiotensin-aldosterone system.

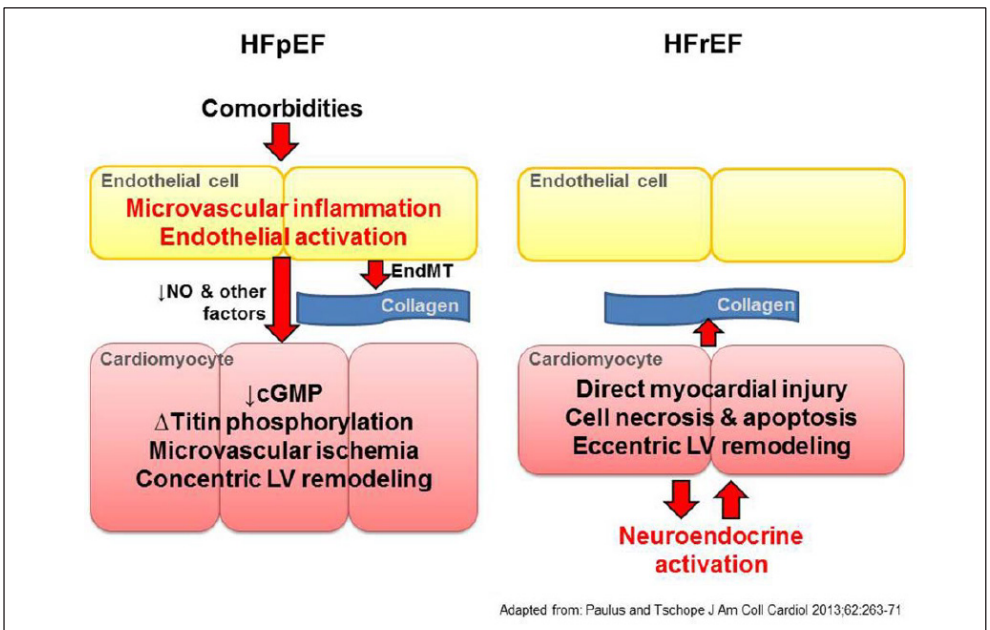

Figure 2. Paradigm for pathophysiology in HFpEF vs. HFrEF.
Reproduced with permission from [15]. Copyright BMJ Publishing Group Ltd. NO: nitric oxide; cGMP: cyclic guanosine monophosphate; HFpEF: heart failure with preserved ejection fraction; HFrEF: heart failure with reduced ejection fraction.

the ventricular myocardium in response to elevated end-diastolic pressure/volume. CNP is secreted by endothelial cells exposed to shear stress. The amount of NP secreted is directly correlated with the magnitude of the stress [19]. BNP has been particularly investigated in HF. Following the increase in ventricular end-diastolic pressure/volume that characterizes HF, the gene encoding BNP is transcribed and the derived mRNA translated into a 134 amino acid length pre-pro-hormone, which is cleaved by a neutral endopeptidase into a 108 amino acid pro-hormone, the proBNP, and a 26 amino acid peptide. Then, when secreted, proBNP is further cleaved by the convertase corin into a biologically active 32 amino acid C-terminal fragment (BNP) and a biologically inactive 76 amino acid N-terminal fragment (NT-proBNP) [19, 20]. BNP reduces preload promoting the shifting of intravascular fluid into the extravascular compartment, increasing venous capacitance, fostering natriuresis that reduces extracellular fluid retention, and diuresis. Additionally, it reduces sympathetic tone in the peripheral vasculature and suppresses renin-angiotensin-aldosterone system (Figure 3) [19, 21].

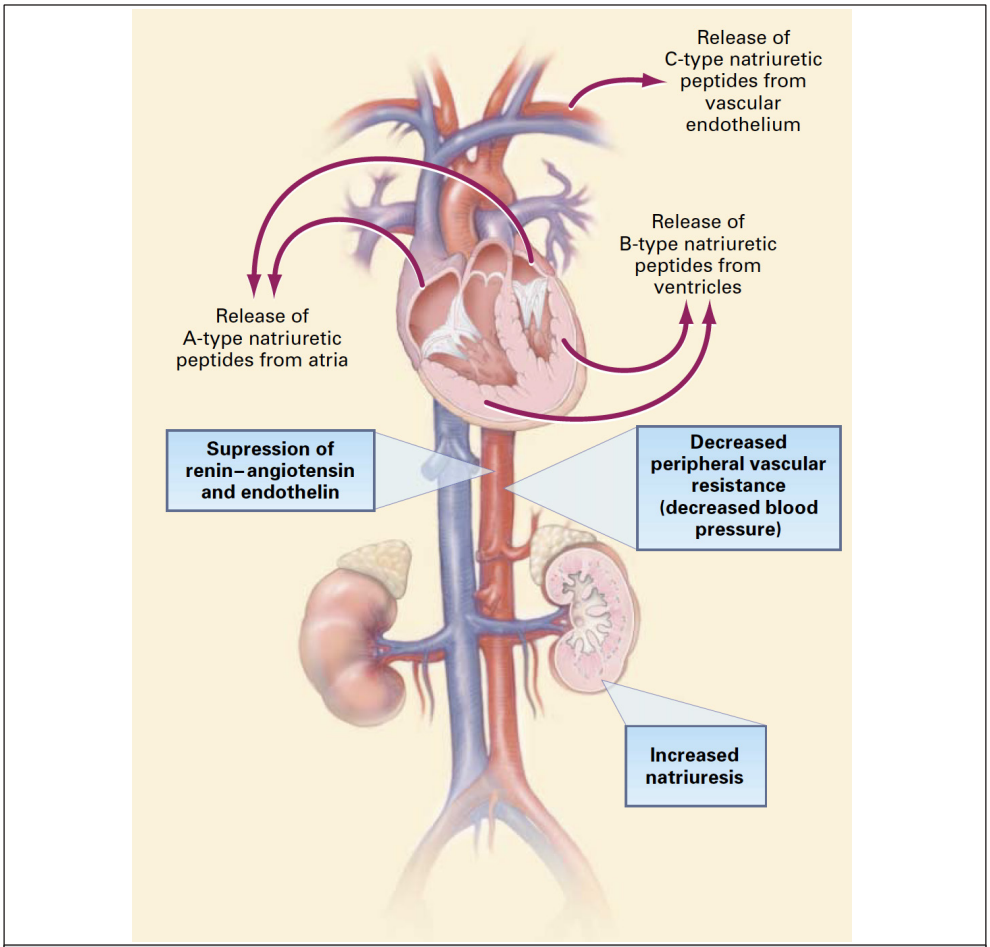

Figure 3. The ABC of natriuretic peptides.
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BNP and NT-proBNP are useful tools for the management of HF patients, with NT-proBNP more used than BNP over the last years because of its longer half-time (120 vs. 20 mins respectively) [22]. Indeed, because of their high negative predictive value, current HF ESC guidelines suggest to measure BNP or NT-proBNP in order to potentially exclude the diagnosis of HF in a patient with clinical history/symptoms/signs/ECG suggesting HF [9]. Additionally, BNP and NT-proBNP blood levels correlate with New York Heart Association (NYHA) class, EF, left ventricular end-diastolic and pulmonary artery wedge pressure, and there is data supporting their use for hospital stay/discharge decision making [23-26]. Finally, in HFrEF, but also to a less extent in HFpEF, there is evidence supporting the role of BNP/NT-proBNP as predictors of clinical events [27-32].

1-year mortality and prognosticators

One-year mortality shows geographical differences, ranging 22-37% in North America and 8-17% in Europe [1, 13]. Differences in prognosis across HFpEF, HFmrEF and HFrEF are difficult to investigate, in particular because of the inconsistent definitions of HFpEF and HFmrEF used in different studies, but the general impression is that crude 1-year mortality rates are higher in HFrEF vs. HFmrEF vs. HFpEF, whereas in some studies, but not in others, differences disappear after adjustments for confounders [13, 33, 34].

Independent predictors of 1-year mortality regardless of EF are older age, NYHA class and chronic kidney disease. Body mass index is associated with mortality risk in HFrEF and HFpEF, low systolic blood pressure and high heart rate in HFmrEF and HFrEF and atrial fibrillation in HFpEF [35].

Treatments in HF

HFrEF (Figure 4)

Over the last years several treatments have been demonstrated to be effective in reducing mortality/morbidity in HFrEF. Inhibition of the renin-angiotensin-aldosterone system and the blockade of sympathetic nervous system represent the foundation of HF therapy. In the CONSENSUS and SOLVD trials angiotensin converting enzyme inhibitor (ACE-I) therapy vs. placebo reduced mortality by 27% in NYHA class IV and by 16% in NYHA class II-III, respectively [36, 37].

Later in 1990s, the MERIT-HF, COPERNICUS and CIBIS II trials reported a 34-35% mortality reduction in patients randomized to beta-blockers vs. placebo [38-40].

At the beginning of 2000s, in the Val-HeFT and in the CHARM-Alternative trials, valsartan and candesartan, two angiotensin receptor blocker (ARB), significantly reduced mortality/morbidity vs. placebo in NYHA class II-IV and in patients intolerant to ACE-I, respectively [41, 42]. Spironolactone first and later eplerenone, two mineralocorticoid receptor antagonist (MRA), have been shown to reduce mortality by 30% in NYHA III-IV and by 24% in NYHA II vs. placebo on top of other HF treatments in the RALES and EMPHASIS-HF trials, respectively [43, 44]. Recently, in the PARADIGM-HF trial, the angiotensin receptor–neprilysin inhibitor LCZ696 has been tested vs. enalapril [45]. This new drug, consisting of the combination of the neprilysin inhibitor sacubitril and the ARB valsartan, blocks at the same time the renin-angiotensin system and inhibits neprilysin, a neutral endopeptidase that degrades several endogenous vasoactive peptides, including NPs. As result, BNP levels increase leading to the beneficial effects already discussed. In PARADIGM-HF, LCZ696 vs. enalapril significantly reduced mortality by 16% on top of all the other current HF treatments [45].

Beyond pharmacological treatments, device therapies have been demonstrated to be beneficial in HFrEF. HFrEF patients are at risk of arrhythmia-related sudden death. Implantable cardioverter defibrillators (ICDs), able to identify and treat life threatening arrhythmias, have been shown to further reduce mortality by 31% in the MADIT-II trial enrolling patients with prior myocardial infarction and EF≤30%, and by 23% in SCD-HeFT enrolling patients with NYHA class II-III and EF≤35% [46, 47]. Cardiac resynchronization therapy (CRT) has been demonstrated to reduce electric dyssynchrony that is often observed in HFrEF, fostering reverse remodeling and thus, decreasing left ventricular volumes by a synchronous pacing of left and right ventricles. In randomized controlled trials, CRT has been shown to improve quality of life, NYHA class, hospitalization by 37% and mortality by 22% [48]. The benefit of combining ICD and CRT is still debated. Indeed, MADIT-CRT trial reported a significant reduction of mortality/HF events in NYHA class I-II HFrEF patients randomized to CRT-ICD vs. ICD alone [49], whereas in the DANISH trial ICD significantly reduced the risk of sudden death but not the primary outcome (all-cause death) in a non-ischemic HFrEF population including patients with CRT (53%) [50].

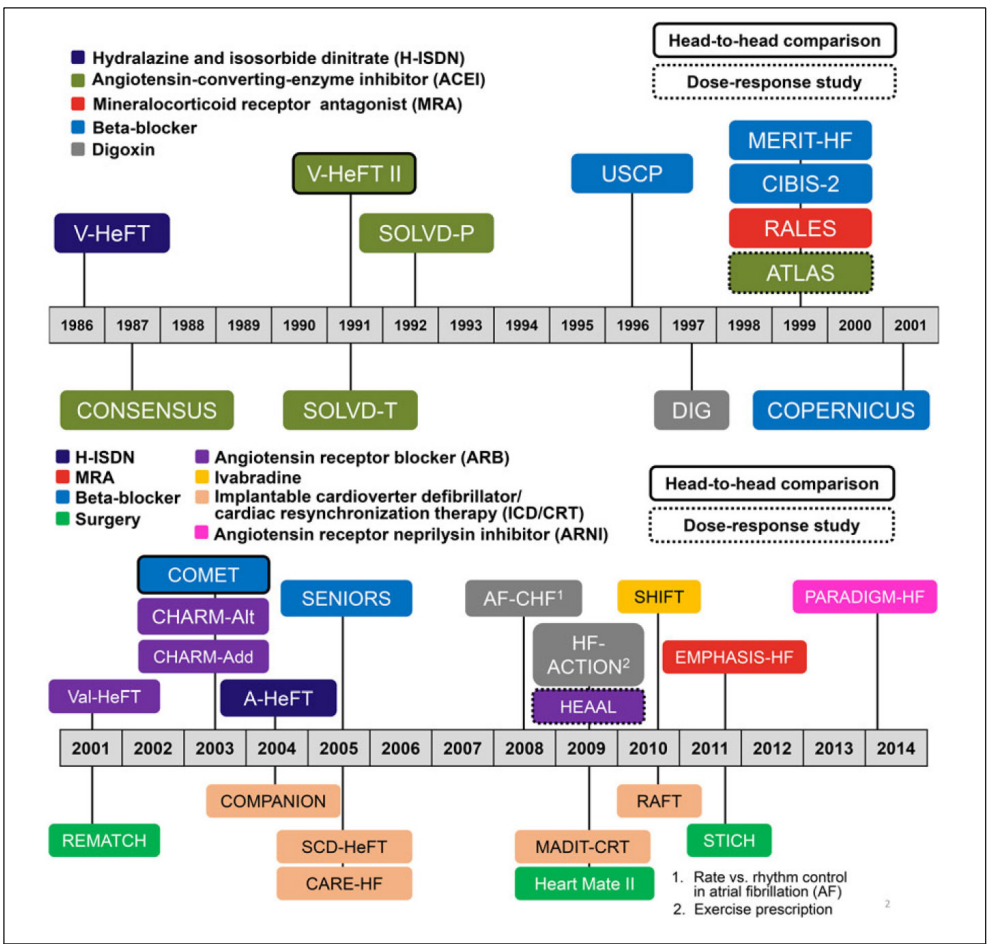

Figure 4. Trials in heart failure with reduced ejection fraction.
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In patients with HFrEF refractory to pharmacological and device treatments, heart transplantation still represents the gold standard. However, over the last years, due to shortage of organs, left ventricular assist devices (LVADs) have been used as bridge to the transplantation, or as bridge to candidacy, or as bridge to recovery or as destination therapy [51]. The use of LVAD is supported by the REMATCH trial that reported a 48% reduction in risk of mortality vs. medical therapy in NYHA class IV patients [52]. Advances in technology have led to further improvements in prognosis in terms of survival free of adverse events in patients with LVAD. Continuous flow pumps (HeartMate II) have been shown to be superior to pulsatile flow pumps (HeartMate XVE) in patients ineligible for transplantation [53], fully magnetically levitated centrifugal-flow pumps (HeartMate III) superior to axial-flow pumps (HeartMate II) in patients receiving LVAD as bridge to transplantation or destination therapy [54], and no differences between the axial-flow pump (HeartMate II) and the centrifugal-flow pump (Heartware) have been shown in patients receiving LVAD as destination therapy [55].

HFpEF

Randomized trials in HFpEF have not been as successful as in HFrEF. Thus, currently there is no established treatment for HFpEF patients. Indeed, in the CHARM-Preserved trial enrolling patients with NYHA class II-IV and EF≥40%, candesartan vs. placebo failed to reduce the primary outcome consisting of the composite of cardiovascular (CV) death or HF hospitalization, but fewer patients in the candesartan than in the placebo group were admitted to hospital for HF [57]. Similarly, in I-PRESERVE, enrolling patients with NYHA class II-IV, EF≥45% and age≥60 years, irbesartan failed to reduce the primary outcome (death or CV hospitalization) or any secondary outcome [58]. Also perindopril vs. placebo in the PEP-CHF trial did not reduce the risk of mortality/HF hospitalization in patients aged ≥70 years, with diastolic dysfunction and treated with diuretics [59]. Finally, in the TOPCAT trial enrolling patients with symptomatic HF and EF≥45%, spironolactone vs. placebo did not reduce the primary outcome of the study (CV death, aborted cardiac arrest, or HF hospitalization), but reduced the risk of HF hospitalization by 17% [60].

HFmrEF

HFmrEF has emerged only recently as an independent entity [9], and thus, currently it has no evidence-based therapy. The CHARM program evaluated the efficacy of candesartan in symptomatic HF across the whole EF spectrum. Recently, a post-hoc analysis analyzing CHARM data reported higher risk of CV death/HF hospitalization in HFmrEF and HFrEF vs. HFpEF with candesartan significantly reducing the risk of events in HFrEF and HFmrEF but not in HFpEF (in absence of any statistical interaction between EF category and candesartan treatment effect) [61]. Additionally, in PARADIGM-HF, enrolling patients with EF≤40% sacubitril/valsartan was effective to reduce CV death/HF hospitalization throughout the EF spectrum [62], whereas in TOPCAT (EF≥45%) there were signals for potential efficacy of spironolactone at lower EF [63]. These evidences might suggest an effect for these drugs in HFmrEF.

ADHF

As in HFpEF, there are no treatments improving outcomes in ADHF, thus current HF ESC guidelines recommend inotropic agents (only in patients symptomatically hypotensive or hypoperfused), vasodilators, vasopressors and diuretics only for symptom relief with class/level of evidence I-II/B-C [9].

Serelaxin and ularitide have been recently tested in ADHF. Serelaxin, a recombinant human relaxin-2, is a naturally occurring peptide contributing to the maternal adaptations to pregnancy. It has been shown to increase arterial compliance, cardiac output and renal blood flow that are beneficial effects for ADHF patients [64]. Ulartide is a chemically synthesized analogue of the naturally occurring vasodilator urodilatin, with hemodynamic effects that may be relevant in ADHF [65]. Although encouraging signals for improved outcome had been observed in the phase 2 trial RELAX-AHF, the phase 3 trial, RELAX-AHF-2, enrolling ADHF patients within 16 hours from presentation to 48-hour intravenous infusions of serelaxin or placebo, failed to demonstrate any effect of the treatment on the primary outcomes of the study (180-day CV death and worsening HF through day five) [66]. Similarly, in the TRUE-AHF trial, randomizing ADHF patients to receive ularitide vs. placebo for 48 hours starting within 12 hours from the hospital admission, ularitide failed to reduce the coprimary outcomes (CV death; a hierarchical composite end-point evaluating the initial 48-hour clinical course) [65].

Failure of trials in HF

Trials in HFrEF have provided several drugs and devices that significantly improve survival/morbidity. HFmrEF has currently no evidence-based therapy yet, since it has emerged very recently as an independent HF phenotype and its characterization is ongoing. Previously, HFmrEF patients have been enrolled inconsistently in HFpEF or HFrEF trials. Potential treatments have been unsuccessfully tested in HFpEF and ADHF that still lack treatments able to significantly improve clinical outcomes.

What are the reasons for failure of trials in HFpEF and ADHF? Some of the explanations could be:

- wrong treatments/doses: neurohormonal antagonists may not work in HFpEF; short-term infusion of a pharmacological compound may not be able to reduce long-term outcome in ADHF; treatments for chronic HF may be not effective in ADHF
- wrong patient selection: in HFpEF trials patients may not have had HFpEF or may have had HFpEF but poorly enriched (meaning low risk of CV events or high risk of non-CV events, that make testing new HF therapies ineffective or requiring excessive sample size); ADHF encompasses multiple syndromes, thus it is very unlikely that the same drug will be effective in all patients with ADHF ("one size fits all" approach)
- wrong outcomes: including extra components in the primary outcome, although increasing the number of events, may merely contribute to generate random noise, diluting a potential effect of the treatment; wrong surrogate endpoints may lead to positive phase II trials but to the failure of the following phase III trial
- wrong trial conduct: e.g. in TOPCAT spironolactone significantly reduced the primary outcome in Americas, but not in Georgia/Russia where canrenone concentration, a metabolite of spironolactone, was undetectable in 30% of the patients investigated, leading to hypothesize misconduct in the trial or compliance issues [67, 68]; in TRUE-AHF, 17% of the patients did not meet entry criteria and 63% of the sites in the Czech Republic, Estonia, Poland, and Serbia had 3 or more ineligible patients.

AIMS

Against this background, the overall aim is to provide evidence to improve trial design in HF, investigating the potential use of NPs as surrogate endpoint, and as eligibility criterion to foster the enrichment of trials for CV vs. non-CV events.

Specific aims are:

1. to evaluate whether a reduction of NT-proBNP levels over time is associated with improved prognosis in chronic HFpEF and HFmrEF (**Study I**)

2. to evaluate whether a reduction of NP levels (BNP or NT-proBNP) over time is associated with improved prognosis in acute decompensated HFpEF (**Study II**)

3. to compare NT-proBNP levels, to assess the independent determinants of high NT-proBNP levels, and to compare the prognostic role and discriminatory power of NT-proBNP levels in HFpEF vs. HFmrEF vs. HFrEF (**Study III**)

4. to evaluate in HFpEF, HFmrEF and HFrEF, and in relevant subgroups 1) the association between NT-proBNP and CV and non-CV outcomes, 2) the association between HF treatments and CV and non-CV outcomes according to NT-proBNP levels (**Study IV**)

PATIENTS AND METHODS

A summary of data and statistical methods used in the four studies is summarized in Table 1.

Table 1. Overview of data used in the thesis.
SwedeHF: Swedish heart failure registry; EF: ejection fraction; BNP: B-type natriuretic peptide; N-terminal B-type natriuretic peptide; HF: Heart failure; ROC: Receiver operating curves; KaRen: Karolinska-Rennes.

Study	I	II	III	IV
Data source	SwedeHF	KaRen	SwedeHF	SwedeHF
Time of data collection	2000-2012	2007-2011	2000-2012	2000-2012
Study population	EF≥40%, outpatient, 2 consecutive NT-proBNP measurements, follow-up ≧1 day	2 consecutive BNP/NT-proBNP measurements	Known EF, at least 1 NT-proBNP measurement, outpatient, follow-up ≥1 day	Known EF, at least 1 NT-proBNP measurement, follow-up ≥1 day
Design	Registry based	Prospective cohort	Registry based	Registry based
Number of patients	650	361	9,847	15,849
Outcomes	All-cause mortality, HF hospitalization, their composite	All-cause mortality, composite of all-cause mortality and HF hospitalization	All-cause mortality, composite of all-cause mortality and HF hospitalization	CV events, non-CV events
Adjustments	17 variables significantly associated with at least 1 outcome at the univariate analysis	10 variables significantly associated with at least 1 outcome at the univariate analysis	32 variables	39 variables
Main statistical analysis	Kaplan Meier, Cox regression	Kaplan Meier, Cox regression	Logistic regression, Kaplan Meier, Cox regression, ROC curves	Poisson regression, Kaplan Meier, Cox regression

Data Source

Studies I, III and IV – the Swedish Heart Failure Registry (SwedeHF)

For Studies I, III and IV, data from the SwedeHF have been analyzed.
SwedeHF (www.SwedeHF.se) is a nationwide continuous health quality and research registry created in 2000, with widespread use in Sweden since 2003. The only inclusion criterion is clinician-judged HF. The EF variable is not required but it is available in ~90% of the registrations. Pediatric patients are excluded. Approximately 80 variables are entered at hospital discharge or after out-patient clinic visit into a web-based case report form. The Uppsala Clinical Research Center, Uppsala, Sweden (www.UCR.UU.se) manages the

database. The coverage of SwedeHF (calculated as all the unique patients with an echo assessment available registered from 2014, divided by all the patients hospitalized in Sweden in 2014 with a primary discharge diagnosis of HF) according to the last annual report published in 2015 was 54%. Active centers, defined as hospitals with more than 10 registrations/year, are considered for the calculation. The coverage in primary care is lower, only 12%, but only few patients are followed-up exclusively in primary care and are, therefore, caught and registered in cardiology and internal medicine departments. By the end of 2015, more than 70,000 unique patients were registered in SwedeHF.
We matched data from SwedeHF with the Population Registry, the Patient Registry and Statistics Sweden by the personal identification number that all permanent residents in Sweden have regardless of citizenship.
The Swedish Board of Health and Welfare (www.socialstyrelsen.se) administers the Population Registry that provided the date of death, and the Patient Registry that supplied baseline comorbidities beyond those available in SwedeHF, hospitalizations and their causes, defined according to ICD-10 codes in the first position, and causes of death (where we used underlying cause rather than immediate mode of death).
Socioeconomic data were obtained by Statistics Sweden (www.scb.se).

Study II – the Karolinska-Rennes (KaRen) study

KaRen was a prospective, multicenter study including 11 centers in France and 3 centers in Sweden. Patients presenting with acute signs and symptoms of HFpEF were enrolled. The main purpose of KaRen was to test the prognostic value of electrical and/or mechanical dyssynchrony in HFpEF after a follow-up of 18 months. No investigational intervention was tested and all patients were treated according to the standard of care. Inclusion criteria, to be established within 72 h of hospital presentation, were: 1) acute presentation to the hospital with clinical signs and symptoms of HF, according to the Framingham criteria; 2) BNP >100 pg/ml or NT-proBNP >300 pg/ml; 3) EF≥45% by echocardiography within the first 72 h. Key exclusion criteria were: evidence of primary restrictive or obstructive cardiomyopathy or pericardial constriction, known cause of right HF not related to left ventricular dysfunction, renal disease requiring dialysis, pulmonary disease requiring chronic supplemental oxygen, existing cardiac resynchronization therapy, any CV disorder with indication for surgical or percutaneous intervention. Extensive baseline variables including symptoms, signs, laboratory and echocardiographic parameters and information about medications were assessed by local investigators at the time of the acute hospital presentation and when the patient returned in stable condition 4-8 weeks after enrollment for the follow-up visit.

Study I

Aim

To evaluate whether a reduction of NT-proBNP levels over time is associated with improved prognosis in chronic HFpEF and HFmrEF.

Patients

In SwedeHF, between May 11th 2000 and December 31th 2012, 80,772 registrations were recorded from 51,060 unique patients. A number of 650 were outpatients with HFmrEF (EF = 40-49%, n=380, 58%) or HFpEF (EF ≥50%, n=270, 42%), who reported at least two NT-

proBNP measurements, and thus, were included in the study. If a patient reported more than one NT-proBNP measurement at the follow-up, the value recorded at the closest visit to 6 months of follow-up from the first registration was used.

Endpoints

Endpoints were:

- Time to all-cause death
- Time to HF hospitalization
- Time to all-cause death or HF hospitalization (composite outcome)

The index date was defined as the outpatient clinic visit for HF, occurring between 2000 and December 31, 2012, at which the second NT-proBNP measurement was performed. End of follow-up was December 31, 2012.

Study II

Aim

To evaluate whether a reduction of NP (BNP/NT-proBNP) levels over time is associated with improved prognosis in acute decompensated HFpEF.

Patients

KaRen study recruited 584 patients between 2007 and 2011. After the exclusion of those who had violation of eligibility criteria (29 patients), withdrew the consent (16), did not report baseline or follow-up BNP/NT-proBNP or died or declined follow-up at 4-8 weeks (178), 361 patients were considered for the current analysis.

Endpoints

Endpoints were:

- Time to all-cause mortality or HF hospitalization (composite outcome)
- Time to all-cause mortality

Outcomes were adjudicated and defined according to the clinical judgment by the local investigators. End of follow-up was November 15th, 2012. The index date was defined as the 4-8 weeks follow-up visit at which the second NP measurement was performed.

Statistics in Studies I-II

Change in NP levels was calculated as the percent variation between the two measurements (%ΔNT-proBNP or BNP =[final NTproBNP or BNP − baseline NT-proBNP or BNP]/ baseline NT-proBNP or BNP*100).
Baseline characteristics of patients at the time of the first NP measurement were compared by t or Kruskal-Wallis tests (continuous variables) or $\chi 2$ test (categorical variables) in those who reported an increase versus a reduction in NP levels.
The association between change in NP levels and outcomes was assessed as follows:

- change in NP was considered as a continuous variable, thus restricted cubic splines were fitted to flexibly model potential non-linearity

- change in NP was considered as a categorical variable (increase/decrease, with increase as reference), thus Kaplan-Meier curves were fitted and adjusted proportional hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated by Cox regression models
- the study cohort was divided in four groups according to the median NP value at baseline and at follow-up: low levels at baseline and at follow-up (stable low levels), low at baseline and high at follow-up (increase in NP levels), high at baseline and low at follow-up (decrease in NP levels), and high at baseline and high at follow-up (stable high levels; reference group). Kaplan-Meier curves were fitted and adjusted proportional HR with 95% CI were calculated by Cox regression models.

Multivariable logistic regression models, using a decrease in NP as dependent variable, were performed to identify the independent predictors of a reduction in NP levels.
All the multivariable Cox regression/logistic regression models reported in the current analyses were adjusted for the variables that correlated with at least one outcome/dependent variable at the univariate analysis with a p-value <0.05 (marked with * in the tables reporting baseline characteristics of **Studies I and II**).
To address the presence of missing data in multivariable models, multiple imputation using chained equations method (n=10) was performed in **Study I**, whereas in **Study II** the mode was used to replace missing values for categorical and the mean for continuous variables, since the amount of missing data was limited.
A p-value<0.05 was considered statistically significant.
Statistical analyses were performed by Stata 14.2 (StataCorp LLC, College Station, Texas, USA) or IBM SPSS Statistics 24.0 (IBM Corp, Armonk, NY, USA).

Study III

Aim

To compare in HFpEF vs. HRmrEF vs. HFrEF

- NT-proBNP levels and assess the independent determinants of high NT-proBNP
- the prognostic role and discriminatory power of NT-proBNP levels

Patients

In SwedeHF, between May 11th 2000 and December 31th 2012, 80,772 registrations were recorded from 51,060 unique patients. A number of 9,847 outpatients with NT-proBNP assessment available, no missing value for EF and follow-up ≥1 day were included in the study. When a patient reported more than one registration, the first including a NT-proBNP assessment was selected.

Endpoints

Endpoints were:

- Time to all-cause mortality
- Time to all-cause mortality or HF hospitalization

End of follow-up was December 31, 2012.

Statistics

High vs. low NT-proBNP levels were defined according to the different median values of NT-proBNP in HFpEF, HFmrEF and HFrEF. Baseline characteristics were compared in patients with high (> median value) vs. low (≤ median value) NTproBNP in HFpEF vs. HFmrEF vs. HFrEF by t-test or analysis of variance (ANOVA) or Wilcoxon-Mann-Whitney or Kruskal-Wallis tests for continuous variables and by chi-squared for categorical variables.
In order to assess the different determinants of high NT-proBNP levels in HFpEF vs. HFmrEF vs. HFrEF, multivariable logistic regressions using high NT-proBNP levels as dependent variable and 32 variables as covariates were run. As consistency analysis, we investigated the potentially different impact of atrial fibrillation type on NT-proBNP levels across EF categories. Thus, multivariable models were performed including atrial fibrillation categorized as no vs. paroxysmal vs. permanent atrial fibrillation [8,751 patients (89% of the overall cohort) with known atrial fibrillation status and ECG collected were considered (those with pacemaker rhythm were excluded)].
The relationship between NT-proBNP and time-to-outcomes was assessed within each EF group using > vs. ≤ median NT-proBNP or modelling NT-proBNP as a quantitative predictor of events using restricted cubic splines (3 knots at fixed percentile of the distribution) to flexibly model potential non-linearity.
In both logistic and survival models, statistical interactions with EF were tested using a Wald-type test since the aim of all the analyses was to perform a comparison across EF categories for predictors of high NT-proBNP levels and prognosis.
To address the presence of missing data in multivariable models, multiple imputation using chained equations method (n=10) was performed.
In order to assess the discriminatory power of NT-proBNP in HFpEF vs. HFmrEF vs. HFrEF, receiver operating characteristic (ROC) curves were fitted and areas under the curves calculated.
A p-value<0.05 was considered statistically significant.
Statistical analyses were performed by Stata 14.2 (StataCorp LLC, College Station, Texas, USA) or IBM SPSS Statistics 24.0 (IBM Corp, Armonk, NY, USA).

Study IV

Aim

To evaluate in HFpEF, HFmrEF and HFrEF, and in relevant subgroups:

- the association between NT-proBNP levels and CV and non-CV outcomes
- the association between HF treatments and CV and non-CV outcomes according to NT-proBNP levels

Patients

In SwedeHF, between May 11th 2000 and December 31th 2012, 80,772 registrations were recorded from 51,060 unique patients. Consequently, 15,849 patients with no missing values for EF, NT-proBNP concentration and with a follow-up ≥1 day were enrolled. When a patient reported more than one registration, the first including a NT-proBNP assessment was selected.

Endpoints

Endpoints were:

- Time to first CV event
- Time to first non-CV event

End of follow-up was December 31, 2012.

Statistics

Baseline characteristics of patients included were reported according to EF category and compared by ANOVA or Wilcoxon-Mann-Whitney for continuous variables, and by chi-squared for categorical variables.
Kaplan Meier curves for outcomes were fitted in HFpEF, HFmrEF and HFrEF. Unadjusted and adjusted HRs with 95% CIs were calculated by Cox proportional hazard models. Univariate Poisson regression models were fitted to calculate the crude rates of CV and non-CV events according to the continuous levels of NT-proBNP (modelled using restricted cubic splines with 4 knots at fixed percentiles of distribution). In this analysis, adjustments were not performed since the primary aim was to estimate event rates by EF and NT-proBNP "as is" when selected for trials where there is no adjustment in patient selection.
Adjusted Cox regression models were performed to assess the associations between HF therapies (ACE-Is or ARBs and beta-blockers) and outcomes according NT-proBNP levels ($\leq$ or >median value). Variables used for adjustments are marked with * in the table reporting baseline characteristics for this study. In all the multivariate models, missing data were managed by multiple imputation using chained equations method (n=10).
A p-value<0.05 was considered statistically significant.
Statistical analyses were performed by Stata 14.2 (StataCorp LLC, College Station, Texas, USA).

Ethical considerations

All studies were performed in accordance with good clinical practice guidelines (ICH-GCP) and followed the recommendations of the Helsinki Declaration. In the health quality and research registry SwedeHF, individual patient consent is not required, but patients are informed of entry and allowed to opt out. Establishment of SwedeHF, its linking with the mentioned registries, and all the analyses reported in this thesis using SwedeHF data were approved by a multisite ethics committee.
The KaRen study and the related substudies on characterization of and prognosis in acute decompensated HFpEF were approved by Regional Ethical Review Boards. All patients provided oral and written informed consent prior to study participation.

RESULTS

Study I

Of 650 patients enrolled, 380 (58%) had HFmrEF (EF 40-49%) and 270 (42%) had HFpEF (EF≥50%). In the overall population, mean age was 73±12 years, 40% were women, the median time between first and second NT-proBNP measurement was 7 months [Interquartile Range (IQR): 4-13].

361 (55%) patients reported a decrease, whereas 289 (45%) an increase in NT-proBNP levels [137 (51%) vs. 133 (49%) in HFpEF, and 224 (59%) vs. 156 (41%) in HFmrEF, respectively]. Baseline characteristics according to NT-proBNP increase/decrease are reported in Table 2.

Table 2. Baseline characteristics in Study I. Variables labeled with * were significantly associated with the risk of overall mortality or of HF hospitalization or of the composite outcome and were included in the Cox regression models together with percent changes in NT-proBNP levels.
NYHA: New York Heart Association; EF: ejection fraction; NT-proBNP: N-terminal pro-B-type natriuretic peptide; SD: Standard Deviation; IQR: interquartile range; ACE-I: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist.

Variables	NT-proBNP Decreased 361 pts (55%)	NT-proBNP Increased 289 pts (45%)	p	Missing values
Demographics				
1. Gender				0 (0)
Male	213 (59)	175 (61)	0.748	-
Female	148 (41)	114 (39)		
2. Age, mean (SD), y*	72 (12)	74 (10)	0.052	0 (0)
3. Location				
Outpatient physician	66 (18)	66 (23)	0.170	-
Outpatient nurse-based HF clinic	295 (82)	223 (77)		
4. Specialty				130 (21)
Cardiology	114 (39)	98 (43)	0.370	-
Internal medicine or Geriatrics	178 (61)	130 (57)		
5. Follow-up referral specialty*				16 (2%)
Primary care or Other care	84 (24)	86 (31)	0.058	-
Cardiology or Internal medicine	269 (76)	195 (69)		
6. Follow up referral to outpatient HF nurse clinic	256 (72)	186 (66)	0.118	16(2%)
7. Follow up median (IQR), years	1.81 (0.74-2.93)	1.38 (0.65-2.56)	0.026	-
Clinical				
8. Duration of heart failure, months*				2 (0)
<6	190 (53)	119 (41)	0.004	-
≥6	170 (47)	169 (60)		
9. NYHA*				58 (9)
I	47 (14)	31 (12)	0.255	-
II	169 (51)	116 (45)		
III	117 (35)	109 (42)		
IV	1 (0)	2 (1)		

Table 2 continuing.				
Variables	**NT-proBNP Decreased** 361 pts (55%)	**NT-proBNP Increased** 289 pts (45%)	**p**	**Missing values**
Demographics				
10. EF, %*				
≥ 50	137 (38)	133 (46)	0.045	-
40 - 49	224 (62)	156 (54)		
11. Blood pressure, mean (SD), mmHg				-
Systolic	131 (21)	130 (20)	0.271	8 (1)
Diastolic	74 (11)	74 (11)	0.370	7 (1)
12. Mean arterial blood pressure, mean (SD), mmHg*	93 (13)	93 (12)	0.984	8 (1)
13. Heart Rate, mean (SD), beats/min	71 (15)	72 (14)	0.312	10 (1)
Laboratory Values				
14. Creatinine clearance, mean (SD), ml/min*^	71 (31)	69 (32)	0.303	61 (9%)
15. Hemoglobin, mean (SD), g/L*	135 (16)	134 (16)	0.738	0 (0)
16. NT-proBNP, median (IQR), pg/mL*	1,837 (964-4,069)	1,372 (630-2,627)	<0.001	0 (0)
Concomitant Medications				
17. ACE-I*	228 (63)	179 (62)	0.807	0 (0)
18. ARB*	122 (34)	78 (27)	0.072	0 (0)
19. MRA	112 (31)	73 (25)	0.096	1 (0)
20. Digoxin	48 (13)	44 (15)	0.499	0 (0)
21. Diuretic*	274 (76)	225 (78)	0.574	2 (0)
22. Nitrate*	46 (13)	39 (13)	0.815	0 (0)
23. Platelet inhibitor	158 (44)	114 (39)	0.263	1 (0)
24. Oral anticoagulant	159 (44)	145 (50)	0.133	0 (0)
25. Statin	183 (51)	137 (47)	0.430	0 (0)
26. Beta-Blocker*	311 (86)	239 (83)	0.227	1 (0)
History and Comorbidity				
27. Hypertension	229 (63)	165 (57)	0.107	0 (0)
28. Diabetes Mellitus*	70 (19)	68 (23)	0.211	0 (0)
29. Myocardial Infarction	124 (34)	77 (26)	0.040	0 (0)
30. Peripheral artery disease	29 (8)	16 (6)	0.276	0 (0)
31. Atrial fibrillation/flutter*	187 (51)	178 (62)	0.014	0 (0)
32. Stroke or transient ischemic attack incl intracranial bleed	57 (16)	53 (18)	0.401	0 (0)
33. Aortic stenosis*	27 (8)	26 (9)	0.564	0 (0)
34. Lung disease*	85 (24)	86 (30)	0.088	0 (0)

Briefly, patients reporting a decrease vs. an increase in NT-proBNP levels had a shorter duration of HF, higher baseline NT-proBNP levels, were more likely to have history of myocardial infarction but less likely to suffer of atrial fibrillation. A larger proportion of patients who reported a decrease in NT-proBNP levels had HFmrEF vs. HFpEF, as compared with patients who showed an increase.

The rate of change in risk of clinical outcomes depended on the actual values of change in NT-proBNP, with an inverse association for those with a decrease and a positive association for those with an increase in NT-proBNP levels (Figure 5)

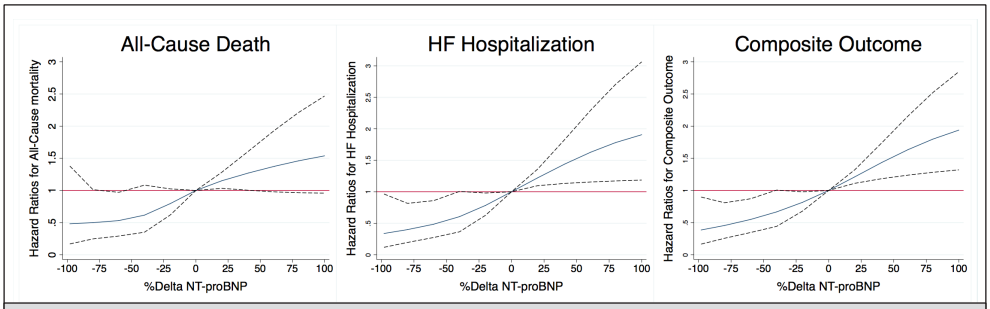

Figure 5. Association between continuous percent changes in NT-proBNP from baseline to follow-up evaluation and risk of all-cause death, HF hospitalization and composite outcome.
NT-proBNP: N-terminal pro-B-type natriuretic peptide; HF: heart failure.

Table 3 reports number of events and HRs (95% CIs) for the analyses assessing the associations between a decrease / increase in NT-proBNP levels and outcomes in the overall population and separately in HFpEF and HFmrEF. We reported that, after adjustments, a reduction in NT-proBNP levels was associated with significantly reduced risk of all-cause death, HF hospitalization and of the composite of all-cause mortality and HF hospitalization. Similar results were reported in both HFpEF and HFmrEF (Figure 6).

Table 3. Cox regression models fitted for all-cause death, HF hospitalization and the composite outcome according to decreasing vs. increasing NT-proBNP levels in the overall cohort and in patients with HFpEF and HFmrEF, separately.
HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; NT-proBNP: N-terminal pro-B-type natriuretic peptide; HR: hazard ratio; CI: confidence interval.

		All-cause Death		HF Hospitalization		Composite Outcome	
		No. (%)	HR (95% CI) P-value	No. (%)	HR (95% CI) P-value	No. (%)	HR (95% CI) P-value
Overall cohort	%Delta NT-proBNP<0 (361 pts, 55%)	57 (16%)	0.53 (0.36-0.77) 0.001	61 (17%)	0.41 (0.29-0.60) <0.001	96 (27%)	0.46 (0.34-0.62) <0.001
	%Delta NT-proBNP≥ 0 (289 pts, 45%)	78 (27%)	1.00 ref	86 (30%)	1.00 ref	125 (43%)	1.00 ref
HFpEF (EF≥50%)	%Delta NT-proBNP<0 (137 pts, 51%)	25 (18%)	0.43 (0.25-0.75) 0.003	22 (16%)	0.46 (0.26-0.83) 0.010	39 (28%)	0.49 (0.31-0.77) 0.002
	%Delta NT-proBNP≥ 0 (133 pts, 49%)	43 (32%)	1.00 ref	37 (63%)	1.00 ref	58 (44%)	1.00 ref
HFmrEF (EF40-49%)	%Delta NT-proBNP<0 (224 pts, 59%)	32 (14%)	0.53 (0.30-0.92) 0.024	39 (17%)	0.36 (0.22-0.59) <0.001	57 (25%)	0.39 (0.26-0.59) <0.001
	%Delta NT-proBNP≥ 0 (156 pts, 41%)	35 (22%)	1.00 ref	49 (31%)	1.00 ref	67 (43%)	1.00 ref

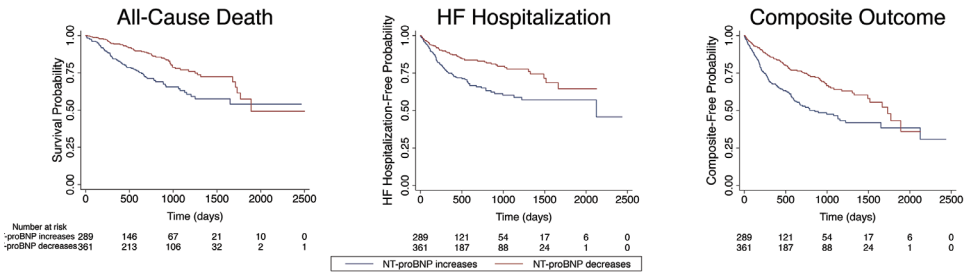

Figure 6. Kaplan Meier curves fitted for all-cause death, HF hospitalization and the composite outcome according to decreasing vs. increasing in NT-proBNP levels.
NT-proBNP: N-terminal pro-B-type natriuretic peptide; HF: heart failure.

Table 4 reports number of events and HRs (95% CIs) for the analyses exploring the associations between the different combinations of NT-proBNP at the first and at the second assessment and outcomes. As compared with stable high NT-proBNP levels (i.e. above median at time 1 and at time 2), stable low (i.e. below/equal to median at time 1 and at time 2) and a decrease in NT-proBNP levels (i.e. above median at time 1 and below/equal to median at time 2) were associated with improved all-cause death, HF hospitalization and composite outcome risk, whereas an increase in NT-proBNP levels (i.e. below/equal to median at time 1 and above median at time 2) was associated with similar risk of outcomes.

Table 4. Cox Regression model fitted for all-cause death, HF hospitalization and the composite outcome according to categorical changes in NT-proBNP.
HR: hazard ratio; CI: confidence interval; HF: heart failure.

	All-cause Death		HF Hospitalization		Composite Outcome	
	No. (%)	**HR (95% CI) P**	**No. (%)**	**HR (95% CI) P**	**No. (%)**	**HR (95% CI) P**
Low-Low (258 pts, 40%)	27 (10%)	0.47 (0.27-0.79) 0.005	33 (13%)	0.38 (0.23-0.63) <0.001	52 (20%)	0.44 (0.29-0.65) <0.001
Low-High (67 pts, 10%)	21 (31%)	1.49 (0.87-2.57) 0.15	18 (27%)	0.97 (0.56-1.68) 0.91	29 (43%)	1.11 (0.71-1.73) 0.66
High-Low (67 pts, 10%)	9 (13%)	0.45 (0.22-0.94) 0.033	10 (15%)	0.38 (0.19-0.76) 0.006	16 (24%)	0.39 (0.22-0.68) 0.001
High-High (258 pts, 40%)	78 (30%)	1.00 ref	86 (33%)	1.00 ref	124 (48%)	1.00 ref

Of all the variables reported in Table 2, those independently associated with a decrease in NT-proBNP levels were shorter HF duration (Odds Ratio [OR]: 1.63; 95% CI: 1.15 to 2.31; p=0.006), use of ARBs (OR: 1.66; 95% CI: 1.14 to 2.40; p=0.007), MRAs (OR: 1.59; 95% CI: 1.10 to 2.31; p=0.014), no history of atrial fibrillation (OR: 0.65; 95% CI: 0.46 to 0.92; p=0.016) and above median NT-proBNP baseline values (OR: 2.03; 95% CI: 1.42 to 2.89; p<0.001). There was a strong trend toward a statistically significant association between therapy with ACE-Is or ARBs and decrease of NT-proBNP levels (OR: 1.74; 95% CI: 0.99 to 3.07; p=0.055).

Study II

The overall population (361 patients) had at the time of the hospital presentation a median age of 78 years (IQR: 72-83) and 56% were females. A proportion of 90% were in NYHA class III-IV. Median EF was 55% (IQR: 50-60), median BNP was 555 ng/l (IQR: 298 – 1,266; collected in 45 patients) and median NT-proBNP was 2,331 (IQR: 1,217 – 4,465; collected in 316 patients).

At the follow-up visit at 4-8 weeks, 267 (74%) patients reported an improvement in BNP/NT-proBNP levels whereas 94 (26%) showed a worsening. Median change in NP levels was -57% (IQR: -76%, -29%) in those who showed a decrease and +55% (IQR: +23%, +96%) in those who showed an increase in NPs. Baseline characteristics of patients reporting an increase and a decrease in NP levels were similar except for heart rate that was higher in those showing worsening BNP/NT-proBNP levels (Table 5).

The endpoint death or death/HF hospitalization occurred in 59 (22%) and 123 (46%) patients, respectively, who reported a decrease and in 26 (27%) and 50 (53%) patients who showed an increase in NP levels. Thus, an improvement vs. a worsening in NP levels was not significantly associated with the risk of all-cause mortality (HR: 0.73; 95% CI: 0.46 to 1.17) or all-cause mortality/HF hospitalization (HR: 0.81; 95% CI: 0.58 to 1.14) (Figure 7), although the HRs were < 1.0.
Similar trend toward reduced risk of outcomes in patients reporting a reduction vs. an increase in NP levels was reported when change in BNP/NT-proBNP was considered as a continuous variable (Figure 8).
When the associations between the different combinations of BNP/NT-proBNP at the first and at the second assessment and outcomes were analyzed, no significant differences in prognosis were observed among patients with stable high, stable low, increasing (low-high) and decreasing (high-low) BNP/NT-proBNP levels (Table 6).
The only predictor of improvement in NP levels was higher heart rate at the baseline (OR: 1.014; 95% CI: 1.003 to 1.025).

Table 5. Baseline characteristics in Study II. Variables labeled with * were significantly associated with the risk of overall mortality or of the composite of mortality and HF hospitalization; thus were included in Cox regression models together with changes in NP levels.
NP: natriuretic peptide; NYHA: New York heart association; EF: ejection fraction; BNP: B-type natriuretic peptide; NT-proBNP: N-terminal pro-B-type natriuretic peptide; ACE-I: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; SD: standard deviation.

	NP Decreased 267 pts (74%)	**NP Increased** 94 pts (26%)	**p**
Demographics			
1. Gender			
Male	114 (42.7)	46 (48.9)	0.334
Female	153 (57.3)	48 (51.1)	
2. Age, mean (SD), y*	76 (9)	77 (9)	0.266
3. Follow up median (IQR), days	598 (283 – 998)	565 (161 – 998)	0.002
Clinical			
4. NYHA*			
I	2 (1)	0 (0)	0.180
II	23 (9)	9 (9)	
III	97 (36)	45 (48)	
IV	144 (54)	40 (43)	
5. EF, %*	55 (7)	57 (8)	0.091
6. Mean arterial blood pressure, mean (SD), mmHg*	102 (21)	101 (17)	0.507
7. Heart Rate, mean (SD), beats/min	86 (26)	79 (20)	0.005
8. Tachycardia (>100 bpm)	89 (33)	24 (25)	0.196
9. Body Mass Index (kg/m^2)*	29 (6)	30 (6)	0.241
Laboratory Values			
10. Creatinine clearance, mean (SD), ml/min	67 (29)	62 (27)	0.196
11. Hemoglobin, mean (SD), g/L*	117 (33)	119 (29)	0.574
12. BNP, median (IQR), ng/L	744 (367 – 1307)	272 (168 – 441)	<0.001
13. NT-proBNP, median (IQR), pg/mL	2740 (1430 – 5311)	1419 (791 – 2520)	<0.001
Concomitant Medications			
14. ACE-I	135 (49)	39 (42)	0.149
15. ARB*	66 (25)	28 (30)	0.340
16. MRA	64 (24)	26 (28)	0.489
17. Thiazide diuretic	26 (10)	8 (9)	0.839
18. Loop-acting diuretic*	219 (73)	82 (27)	0.250
19. Calcium channel blocker	71 (27)	24 (26)	0.892
20. Beta blocker*	193 (73)	73 (78)	0.335
21. Nitrate	28 (11)	11 (12)	0.703
History and Comorbidity			
22. Hypertension	209 (79)	74 (79)	1.000
23. Diabetes mellitus	71 (27)	24 (25)	0.892
24. Coronary artery disease	83 (31)	28 (30)	0.897
25. Coronary revascularization	46 (18)	14 (15)	0.746
26. Cardiomyopathies*	55 (21)	26 (29)	0.144
27. Atrial fibrillation/flutter	166 (62)	63 (67)	0.456
28. Pacemaker	39 (15)	9 (10)	0.289
29. Stroke	25 (9)	14 (15)	0.175
30. Moderate or severe valvular disease	51 (19)	15 (16)	0.640
31. Renal disease	73 (27)	28 (30)	0.689
32. Chronic obstructive pulmonary disease	35 (13)	11 (12)	0.858

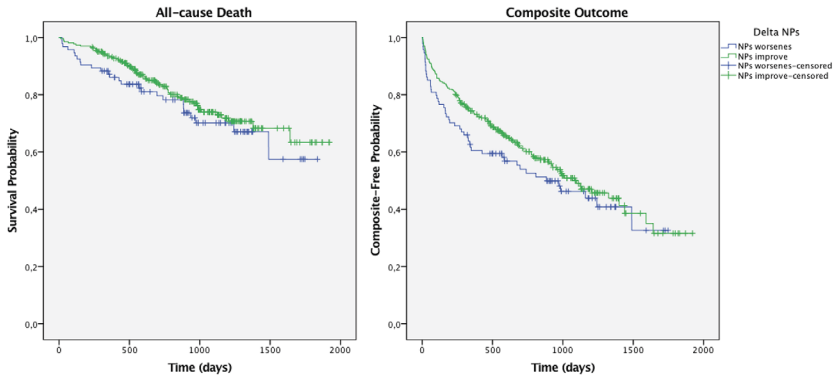

Figure 7. Kaplan Meier curves fitted for all-cause death and the composite outcome according to decreasing vs. increasing NP levels.
NPs: natriuretic peptides.

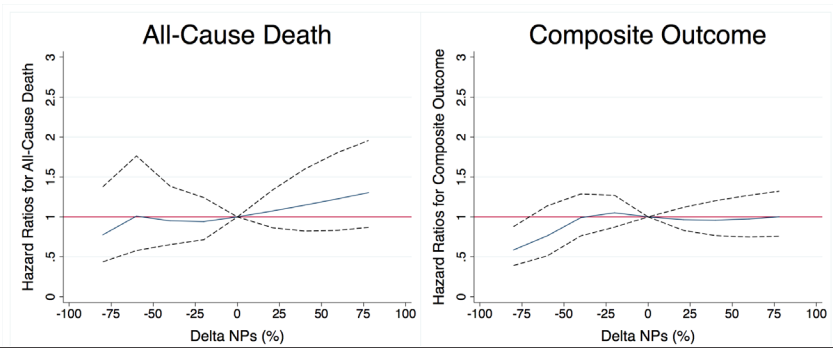

Figure 8. Association between continuous percent changes in NPs from baseline to follow-up evaluation and risk of all-cause death and of the composite outcome.
NPs: natriuretic peptides.

Table 6. Cox Regression models fitted for all-cause death and the composite outcome according to categorical changes in NPs.
HR: hazard ratio; CI: confidence interval; NPs: natriuretic peptides.

	All-cause Death		Composite Outcome	
	No. (%)	**HR (95% CI) P**	**No. (%)**	**HR (95% CI) P**
Low-Low (124 pts, 34%)	21 (17%)	0.69 (0.37-1.27) 0.26	51 (41%)	0.84 (0.56-1.26) 0.42
Low-High (57 pts, 16%)	16 (28%)	1.26 (0.68-2.33) 0.47	31 (54%)	1.20 (0.77-1.85) 0.42
High-Low (57 pts, 16%)	15 (26%)	1.06 (0.55-2.04) 0.87	28 (16%)	0.95 (0.59-1.52) 0.95
High-High (123 pts, 34%)	33 (27%)	1.00 ref	63 (36)	1.00 ref

Study III

Of 9,847 patients, 1,811 (18%) had HFpEF (EF≥50%), 2,122 (22%) had HFmrEF (EF 40-49%) and 5,914 (60%) had HFrEF (EF<40%). Mean age was 70±12 years, 32% were women and median NT-proBNP was 1,940 pg/ml (IQR: 829-4,191).

Median NT-proBNP in HFmrEF (1,540 pg/ml, IQR: 652-3,317) was minimally and non-significantly higher than in HFpEF (1,428 pg/ml, IQR: 623-3,000), but considerably lower than in HFrEF (2,288 pg/ml, IQR: 1,022-4,835; p<0.001).

Table 7 reports baseline characteristics of the population according to high (>median value) vs. low (≤median value) NT-proBNP. Briefly, except for diabetes and ARBs, patients with high vs. low NT-proBNP were different for all baseline variables collected across the EF categories. In particular, patients with high NT-proBNP levels were more likely to be female, older, with NYHA class III-IV, lower body mass index, creatinine clearance and hemoglobin, but higher heart rate, more history of atrial fibrillation and more diuretic use.

Differences in baseline characteristics in Table 7 are unadjusted. In Figure 9 we reported adjusted ORs (95% CIs) for the associations between several baseline characteristics and NT-proBNP levels (high NT-proBNP included in the models as dependent variable) in HFpEF vs. HFmrEF vs. HFrEF. We also included a p-value for interaction between each variable and EF categories.
Atrial fibrillation was associated with increased risk of having high NT-proBNP regardless of EF, but the OR was significantly higher in HFpEF and HFmrEF vs. HFrEF. When the type of atrial fibrillation (paroxysmal, permanent) was compared to no atrial fibrillation, permanent and paroxysmal atrial fibrillation independently predicted high NT-proBNP in HFpEF (OR: 4.67, 95% CI: 3.59 – 6.09; OR: 1.65, 95% CI: 1.15 – 2.36, respectively) and in HFmrEF (OR: 3.30, 95% CI: 2.57 – 4.23; OR: 1.94, 95% CI: 1.36 – 2.75), but with higher OR for permanent vs. paroxysmal atrial fibrillation. On the other hand, in HFrEF permanent but not paroxysmal vs. no atrial fibrillation independently predicted high NT-proBNP levels (OR: 1.52, 95% CI: 1.29 – 1.80; OR: 1.03, 95% CI: 0.83 – 1.27)(p for interaction for HFpEF vs. HFmrEF vs. HFrEF <0.001). Other variables that differently predicted high NT-proBNP levels were hypertension and diabetes, with ORs significantly higher in HFmrEF vs. HFpEF and HFrEF. Additionally, although without any interaction with EF categories, older age (>75 years), body mass index ≤30 and heart rate ≥70 bpm were significantly associated with the likelihood of having high NT-proBNP in HFmrEF and HFrEF but not in HFpEF where their role was neutral. NYHA class III-IV, shorter HF duration, lower creatinine clearance, anemia and use of diuretics were similarly associated with the risk of high NT-proBNP across the EF categories.

Table 8 reports the HRs (95% CIs) and number of events for the outcomes all-cause death and the composite of all-cause death and HF hospitalization in high vs. low NT-proBNP levels stratified by EF category. High vs. low NT-proBNP was associated with higher risk of all-cause death and of the composite outcome regardless of EF. In particular, for the composite outcome high NT-proBNP predicted higher risk of events in HFmrEF and HFpEF vs. HFrEF (Figure 10). This was not the case for all-cause mortality where there was no interaction between NT-proBNP levels and EF categories. Similarly, when the relationship between continuous levels of NT-proBNP and outcomes was analyzed, a strong positive dose-response association between NT-proBNP values and risk of outcomes was reported regardless of EF, but high levels of NT-proBNP were associated with a greater increase in risk of the composite outcome, but not of mortality alone, in HFpEF and HFmrEF vs. HFrEF (Figure 10).

Table 7. Baseline characteristics in Study III. High/low NT-proBNP levels were defined as > / ≤ median NT-proBNP values in HFpEF (1,428 pg/ml), HFmrEF (1,540 pg/ml) and HFrEF (2,288 pg/ml). NYHA: New York heart association; NT-proBNP: N-terminal pro-B-type natriuretic peptide; BMI: body mass index; IQR: interquartile range; ICD: implantable cardioverter defibrillator; CRT: cardiac resynchronization therapy; SD: standard deviation; HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction.

Variables	HFpEF 1,811 pts (18%)			HFmrEF 2,122 pts (22%)			HFrEF 5,914 pts (60%)			p for overall comparison	Missing (%)
	Low NT-proBNP 906 pts (50%)	High NT-proBNP 905 pts (50%)	p	Low NT-proBNP 1,061 pts (50%)	High NT-proBNP 1,061 pts (50%)	p	Low NT-proBNP 2,958 pts (50%)	High NT-proBNP 2,956 pts (50%)	p		
Demographics											
1. Gender*											0
Male	500 (55%)	429 (47%)	0.001	710 (67%)	664 (63%)	0.04	2,267 (76%)	2,163 (73%)	0.002	<0.001	
Female	406 (45%)	476 (53)		351 (33%)	397 (37%)		691 (23%)	793 (27%)			
2. Age, mean (SD), y*	72 (12)	77 (9)	<0.001	67 (13)	76 (10)	<0.001	66 (12)	72 (11)	<0.001	<0.001	0
3. Year of registration*											0
2001-2007	153 (17%)	103 (11%)	<0.001	150 (14%)	123 (12%)	0.08	439 (15%)	377 (13)	0.02	0.001	
2008-2012	753 (83%)	802 (89%)		911 (86%)	938 (88%)		2,519 (85%)	2,579 (87%)			
4. Specialty*											14
Cardiology	353 (60%)	336 (52%)	0.002	537 (60%)	486 (56%)	0.07	1,792 (65%)	1,677 (61%)	<0.001	<0.001	
Internal medicine or Geriatrics	231 (40%)	314 (48%)		359 (40%)	386 (44%)		946 (35%)	1,092 (39%)			
5. Follow-up referral specialty*											2
Primary care or Other care	404 (45%)	380 (43%)	0.37	267 (26%)	332 (32%)	0.001	2,548 (83%)	2,382 (81%)	0.04	<0.001	
Cardiology or Internal medicine	486 (55%)	503 (57%)		764 (74%)	695 (68%)		436 (15%)	500 (17%)			
6. Follow up referral to outpatient HF nurse clinic*	344 (39%)	454 (52%)	<0.001	548 (53%)	603 (59%)	0.02	1,771 (60%)	1,895 (64%)	0.001	<0.001	3
Clinical											
8. Duration of heart failure, months*											0.2
<6	381 (42%)	380 (42%)	0.98	513 (48%)	473 (45%)	0.08	1,528 (52%)	1,488 (50%)	0.34	<0.001	
≥6	523 (58%)	523 (58%)		545 (52%)	585 (55%)		1,427 (48%)	1,460 (50%)			
9. NYHA*											10
I-II	490 (68%)	351 (49%)	<0.001	736 (77%)	512 (55%)	<0.001	1,873 (67%)	1,330 (48%)	<0.001	<0.001	
III-IV	226 (32%)	361 (51%)		216 (23%)	412 (45%)		917 (33%)	1,414 (52%)			
10. BMI*	29 (6)	28 (6)	<0.001	29 (6)	27 (5)	<0.001	28 (6)	26 (5)	<0.001	<0.001	50

11. Blood pressure, mean (SD), mmHg											1
Systolic	132 (21)	130 (21)	0.009	129 (20)	129 (21)	0.81	126 (20)	121 (21)	<0.001	<0.001	
Diastolic	74 (12)	72.3 (11)	0.004	74 (11)	73 (12)	0.009	74 (11)	72 (12)	<0.001		
12. Mean arterial blood pressure, mean (SD), mmHg*	93 (13)	91 (13)	0.001	93 (13)	92 (13)	0.10	91 (13)	88 (13)	<0.001	<0.001	1
13. Heart Rate, mean (SD), beats/min*	70 (13)	73 (15)	<0.001	69 (13)	73 (15)	<0.001	70 (14)	73 (15)	<0.001	<0.001	2
Laboratory Values											
14. Creatinine clearance, mean (SD), ml/min^*	76 (34)	57 (25)	<0.001	86 (34)	60 (58)	<0.001	86 (35)	63 (30)	<0.001	<0.001	9
15. Hemoglobin, mean (SD), g/L*	134 (15)	129 (17)	<0.001	138 (15)	131 (17)	<0.001	140 (15)	135 (16)	<0.001	<0.001	0
16. NT-proBNP, median (IQR), pg/mL*	1,428 (623-3,000)			1,540 (652-3,317)			2,288 (1,022-4,835)			<0.001	0
Concomitant Medications											
17. Angiotensin converting enzyme inhibitor*	508 (56%)	510 (56%)	0.92	734 (69%)	679 (64%)	0.01	2,124 (72%)	2,082 (70%)	0.25	<0.001	0.1
18. Angiotensin II receptor blocker*	261 (29%)	258 (28%)	0.85	268 (25%)	306 (29%)	0.06	848 (29%)	771 (26%)	0.02	0.07	0.4
19. Mineralocorticoid receptor antagonist*	219 (24%)	264 (29%)	0.02	256 (24%)	292 (28%)	0.07	1,093 (37%)	1.117 (38%)	0.50	<0.001	0.2
20. Digoxin*	99 (11%)	184 (20%)	<0.001	112 (11%)	206 (19%)	<0.001	414 (14%)	501 (17%)	0.002	<0.001	0.2
21. Diuretic*	644 (71%)	776 (86%)	<0.001	604 (57%)	867 (82%)	<0.001	1,960 (67%)	2,531 (86%)	<0.001	<0.001	0.3
22. Nitrate*	108 (12%)	144 (16%)	0.015	100 (9%)	164 (16%)	<0.001	294 (10%)	331 (11%)	0.12	<0.001	0.3
23. Platelet inhibitor*	396 (44%)	322 (36%)	<0.001	505 (48%)	455 (43%)	0.03	1,425 (48%)	1,320 (45%)	0.006	<0.001	0.3
24. Oral anticoagulant*	315 (35%)	485 (54%)	<0.001	373 (35%)	551 (52%)	<0.001	1,201 (41%)	1,385 (47%)	<0.001	<0.001	0.2
25. Statin*	414 (46%)	355 (39%)	0.005	568 (54%)	514 (49%)	0.019	1.539 (52%)	1,410 (48%)	0.001	<0.001	0.2
26. Beta-Blocker*	685 (76%)	758 (84%)	<0.001	900 (85%)	942 (89%)	0.005	2,697 (91%)	2,735 (93%)	0.07	<0.001	0.1
27. ICD/CRT*	82 (9%)	96 (11%)	0.28	149 (14%)	146 (14%)	0.84	437 (15%)	538 (18%)	<0.001	<0.001	0.3
History and Comorbidity											
28. Smoking*											14
Never	365 (49%)	398 (54%)	0.109	385 (42%)	445 (50%)	<0.001	1,014 (38%)	1,037 (40%)	0.43	<0.001	
Previous	311 (42%)	282 (39%)		400 (44%)	366 (41%)		1,237 (47%)	1,180 (46%)			
Current	66 (9%)	52 (7%)		122 (14%)	75 (9%)		387 (15%)	364 (14%)			
29. Hypertension*	525 (59%)	548 (61%)	0.40	482 (46%)	596 (57%)	<0.001	1,255 (44%)	1,288 (45%)	0.40	<0.001	2
30. Diabetes mellitus*	208 (23%)	187 (21%)	0.24	208 (20%)	257 (24%)	0.01	682 (23%)	678 (23%)	0.90	0.08	0.3
31. Ischemic heart disease*	309 (35%)	317 (36%)	0.74	444 (43%)	497 (48%)	0.016	1,249 (45%)	1,390 (50%)	0.001	<0.001	5
32. Coronary revascularization*	176 (20%)	156 (17%)	0.24	320 (31%)	316 (30%)	0.79	834 (28%)	861 (29%)	0.47	<0.001	1
33. Atrial fibrillation/flutter*	361 (40%)	640 (71%)	<0.001	392 (37%)	690 (65%)	<0.001	1,081 (37%)	1,413 (48%)	<0.001	<0.001	0.3
34. Atrial fibrillation type¶											1§
Paroxysmal	119 (14%)	89 (11%)	<0.001	95 (10%)	105 (11%)	<0.001	283 (11%)	274 (11%)	<0.001	<0.001	
Permanent	211 (25%)	506 (61%)		246 (26%)	521 (55%)		697 (26%)	977 (38%)			
No atrial fibrittlation	503 (60%)	229 (28%)		601 (64%)	313 (33%)		1,671 (63%)	1,311 (51%)			
35. Valve disease*	202 (23%)	302 (34%)	<0.001	157 (15%)	258 (25%)	<0.001	404 (14%)	574 (20%)	<0.001	<0.001	2
36. Lung disease*	222 (24%)	232 (26%)	0.58	250 (24%)	290 (27%)	0.046	639 (22%)	720 (24%)	0.01	0.004	2

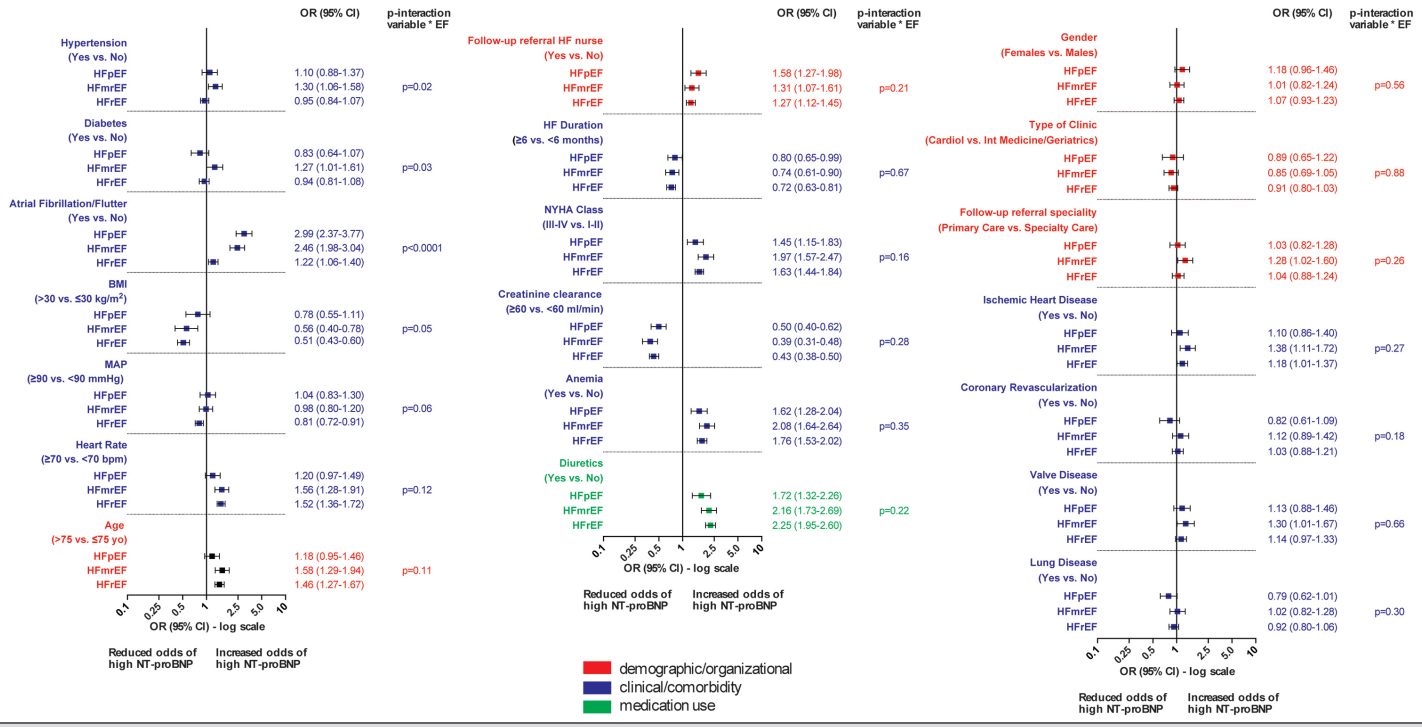

Figure 9. Forest plot reporting the predictors of high NT-proBNP levels according to the EF category

High/low NT-proBNP levels were defined as > / ≤ median NT-proBNP values in HFpEF (1,428 pg/ml), HFmrEF (1,540 pg/ml) and HFrEF (2,288 pg/ml). Variables included in multivariable models are marked with * in Table 7.

BMI: body mass index; HF: heart failure; NYHA: New York heart association; NT-proBNP: N-terminal pro-B-type natriuretic peptide; HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction, MAP: mean arterial pressure.

Table 8. Cox Regression models fitted for all-cause death and the composite of all-cause death and HF hospitalization according to high vs. low NT-proBNP levels in HFpEF vs. HFmrEF vs. HFrEF.

High/low NT-proBNP levels were defined as > / ≤ median NT-proBNP values in HFpEF (1,428 pg/ml), HFmrEF (1,540 pg/ml) and HFrEF (2,288 pg/ml). For all-cause mortality: unadjusted p for interaction = 0.22, adjusted p for interaction = 0.68; for the composite outcome: unadjusted p for interaction = 0.0001, adjusted p for interaction: 0.0005.

NT-proBNP: N-terminal pro-B-type natriuretic peptide; HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction; HR: hazard ratio; CI: confidence interval.

		All-cause Death			Composite Outcome		
		No. (%)	**Unadj. HR (95% CI) P-value**	**Adj. HR (95% CI) P-value**	**No. (%)**	**Unadj. HR (95% CI) P-value**	**Adj. HR (95% CI) P-value**
HFpEF	**High NT-proBNP levels (905 pts, 50%)**	299 (33%)	2.70 (2.23-3.28) <0.001	1.90 (1.55-2.32) <0.001	407 (45%)	2.29 (1.95-2.68) <0.001	1.86 (1.58-2.18) <0.001
	Low NT-proBNP levels (906 pts, 50%)	156 (17%)	1.00 ref	1.00 ref	247 (27%)	1.00 ref	1.00 ref
HFmrEF	**High NT-proBNP levels (1,061 pts, 50%)**	331 (31%)	3.27 (2.67-4.00) <0.001	1.87 (1.52-2.31) <0.001	512 (48%)	2.75 (2.36-3.20) <0.001	2.00 (1.71-2.34) <0.001
	Low NT-proBNP levels (1,061 pts, 50%)	132 (12%)	1.00 ref	1.00 ref	246 (23%)	1.00 ref	1.00 ref
HFrEF	**High NT-proBNP levels (2,956 pts, 50%)**	860 (29%)	2.67 (2.37-3.01) <0.001	1.73 (1.53-1.97) <0.001	1,496 (51%)	1.89 (1.75-2.05) <0.001	1.48 (1.36-1.61) <0.001
	Low NT-proBNP levels (2,958 pts, 50%)	393 (13%)	1.00 ref	1.00 ref	996 (34%)	1.00 ref	1.00 ref

NT-proBNP as a continuous variable had good prognostic discrimination for both outcomes. Overall, the area under the curve for all-cause mortality and all-cause mortality/HF hospitalization was largest in HFmrEF (Figure 11) in the overall population, whereas HFmrEF and HFpEF but not HFrEF reported lower area under the curve in atrial fibrillation vs. sinus rhythm (Figure 12).

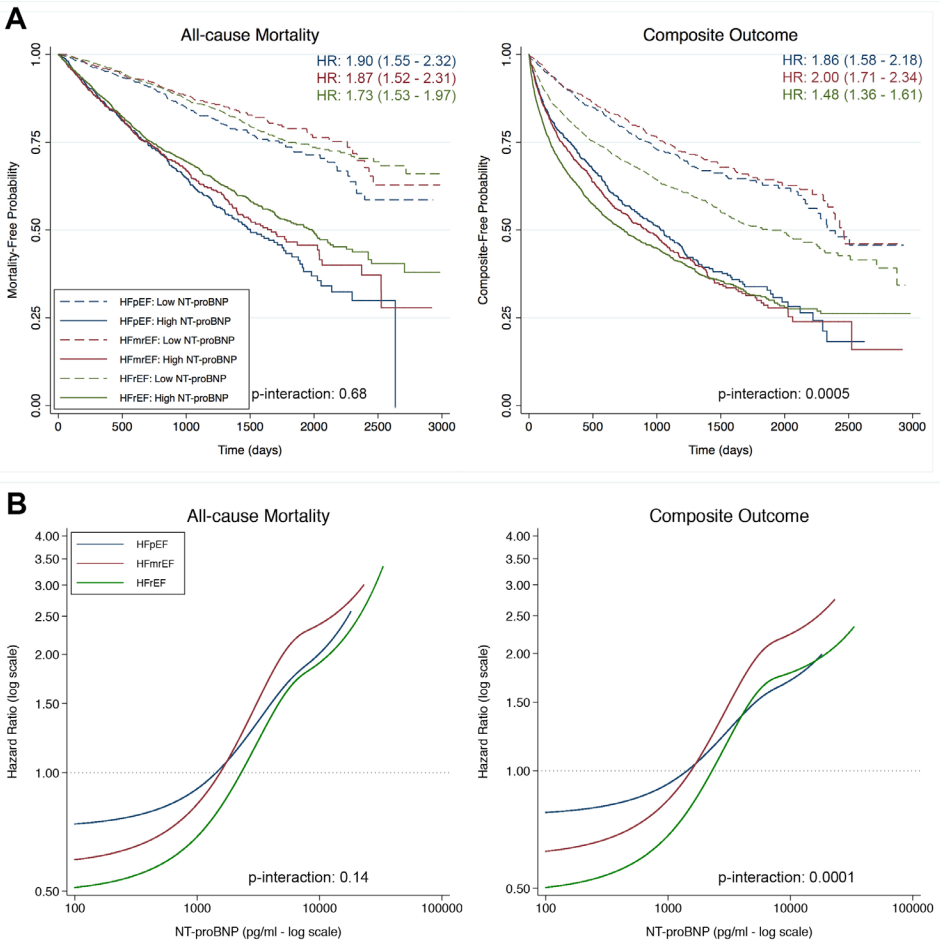

Figure 10. Outcome analysis.
Panel A. Kaplan–Meier curves fitted for all-cause death and the composite outcome (all-cause death / HF hospitalization) in patients with high vs. low NT-proBNP levels and different EF categories.
Panel B. Association between continuous NT-proBNP levels and risk of all-cause death and of the composite outcome in the different EF categories.
High/low NT-proBNP levels were defined as > / ≤ median NT-proBNP values in HFpEF (1,428 pg/ml), HFmrEF (1,540 pg/ml) and HFrEF (2,288 pg/ml).
NT-proBNP: N-terminal pro-B-type natriuretic peptide; HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction; HR: hazard ratio.

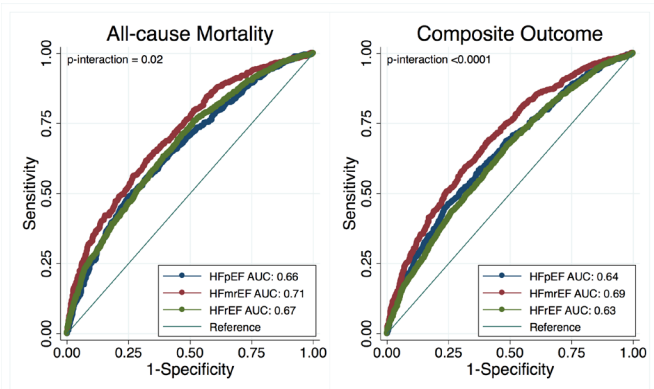

Figure 11. ROC curves and areas under the curves for NT-proBNP levels using all-cause death and the composite of all-cause death and HF hospitalization as outcomes in the different EF categories.
NT-proBNP: N-terminal pro-B-type natriuretic peptide; ROC: receiver operating characteristics; AUC: area under the curve; HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction.

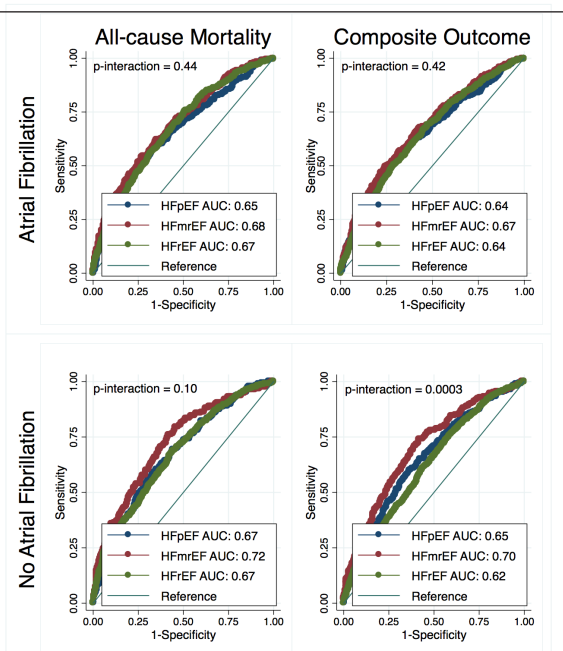

Figure 12. ROC curves and areas under the curves for NT-proBNP levels using all-cause death the composite of all-cause death and HF hospitalization as outcomes in the different EF categories according to the presence/absence of concomitant atrial fibrillation.
NT-proBNP: N-terminal pro-B-type natriuretic peptide; ROC: receiver operating characteristics; AUC: area under the curve; HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction.

Study IV

Out of 15,849 patients included, 23% had HFpEF, 21% HFmrEF and 56% HFrEF.
In the overall population, mean age was 73±12 years, 36% were female, median NT-proBNP was 2,640 [IQR :1,140-5,914] pg/ml. Table 9 reports patients' characteristics by EF category. HFmrEF was intermediate between HFpEF and HFrEF for age, gender, renal disease, hypertension, anemia, atrial fibrillation, valvular disease, lung disease, use of ACE-Is or ARBs, of beta-blockers and nitrates; more similar to HFpEF for body mass index, arterial blood pressure, prevalence of cancer, HF duration and use of MRAs; more similar to HFrEF for ischemic heart disease, use of platelet inhibitors, diuretics and statins. There were no differences across EF categories in prevalence of diabetes, use of digoxin and oral anticoagulants (Table 9).

Median NT-proBNP values in HFpEF (2,037 pg/ml, IQR:912-4,420) and HFmrEF (2,192, IQR:930-4,899) were similar and much lower than in HFrEF (3,141, IQR:1,370-7,080) (Figure 13).

Figure 14 reports the risk of CV and non-CV events across the EF categories. In particular, risk of CV events was higher in HFrEF vs. HFpEF and HFmrEF, whereas the risk of non-CV events was highest in HFpEF, intermediate in HFmrEF and lowest in HFrEF.

Figure 15 reports the associations between NT-proBNP and CV/non-CV outcomes in HFpEF vs. HFmrEF vs. HFrEF. Crude rates for CV and non-CV events ranged 20-160 and 30-100 per 100 patient-years in HFpEF, 20-130 and 20-100 in HFmrEF, 20-110 and 20-50 in HFrEF, respectively.
Overall, event rates for both CV and non-CV events increased together with increasing NT-proBNP in all EF categories, but with some differences: 1) rates for non-CV events were higher in HFpEF and HFmrEF vs. HFrEF regardless of NT-proBNP levels; 2) CV event rates increased with increasing NT-proBNP more steeply than non-CV event rates in all EF groups, but the increase occurred at lower NT-proBNP levels in HFpEF vs. HFmrEF vs. HFrEF and was steeper at lower NT-proBNP and flatter at higher NT-proBNP; 3) CV to non-CV event ratio increased with increasing NT-proBNP levels in HFpEF and HFrEF, but in HFmrEF the ratio increased at lower NT-proBNP levels and then remained almost stable at higher NT-proBNP levels.

Therapy with ACE-I/ARB was significantly associated with reduced risk of CV and also non-CV events in HFmrEF and HFrEF, but only of CV events in HFpEF. Beta-blockers were significantly associated with reduced risk of CV and also non-CV events in HFmrEF, but only of CV events in HFrEF. In HFpEF, therapy with beta-blocker was associated with no change in risk of neither CV or non-CV events (Figure 16).

The associations between therapy with ACE-Is/ARBs or with beta-blockers and outcomes (both CV and non-CV) reported in the overall population were consistent in patients with NT-proBNP ≤ and > median regardless of EF category (p for interaction non-significant) (Figure 17 and 18).

Table 9. Baseline characteristics in Study IV.
NYHA: New York heart association; NT-proBNP: N-terminal pro-B-type natriuretic peptide; ACE-I: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; MRA: mineralocorticoid receptor antagonist; ICD: implantable cardioverter defibrillator; CRT: cardiac resynchronization therapy; hb: hemoglobin; TIA: transient ischemic attack; SD: standard deviation; HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction; IQR: interquartile range.

Variables	HFpEF (n=3,623; 23%)	HFmrEF (n=3,322; 21%)	HFrEF (n=8,904; 56%)	p-value
Investigated subgroups				
Location*				
Inpatient	1,960 (54%)	1,383 (42%)	3,645 (41%)	<0.001
Outpatient	1,663 (46%)	1,939 (58%)	5,259 (59%)	
Atrial fibrillation*	2,360 (65%)	2,011 (60%)	4,566 (51%)	<0.001
Renal function*				
Creatinine clearance <60 ml/min	1,931 (58%)	1,510 (50%)	3,785 (45%)	<0.001
Creatinine clearance ≥60 ml/min	1,385 (42%)	1,521 (50%)	4,632 (55%)	
Age*				
≥75 years	2,890 (80%)	2,310 (70%)	5,163 (58%)	<0.001
<75 years	733 (20%)	1,012 (30%)	3,741 (42%)	
Gender*				
Male	1,690 (47%)	2,041 (61%)	6,476 (73%)	<0.001
Female	1,933 (53%)	1,281 (39%)	2,428 (27%)	
Ischemic heart disease*	1,564 (44%)	1,725 (53%)	4,627 (54%)	<0.001
Diabetes*	994 (27%)	925 (28%)	2,449 (27%)	0.92
Duration of HF, months*				
<6	1,640 (45.5%)	1,521 (46.0%)	4,366 (49.2%)	<0.001
≥6	1,961 (54.5%)	1,789 (54.0%)	4,504 (50.8%)	
Demographics				
Age, mean (SD), y	77 (11)	74 (12)	71 (12)	<0.001
Specialty*				
Internal medicine or Geriatrics	1,333 (44%)	1,222 (41%)	3,072 (36%)	<0.001
Cardiology	1,730 (56%)	1,761 (59%)	5,474 (64%)	
Follow-up referral specialty*				
Cardiology or Internal medicine	1,700 (49%)	2,015 (64%)	6,638 (77%)	<0.001
Primary care or Other care	1,756 (51%)	1,152 (36%)	1,941 (23%)	
Follow-up referral to outpatient HF nurse clinic*	1,187 (34%)	1,493 (47%)	4,931 (57%)	<0.001
Year of registration*				
2001-2009	1,669 (46%)	1,557 (47%)	4,197 (47%)	0.55
2010-2012	1,954 (54%)	1,765 (53%)	4,707 (53%)	
Clinical				
NYHA class*				
I	375 (15.5%)	341 (13.1%)	600 (8.1%)	<0.001
II	1,119 (46.2%)	1,372 (52.8%)	3,330 (45.2%)	
III	869 (35.9%)	827 (31.8%)	3,154 (42.8%)	
IV	60 (2.5%)	59 (2.3%)	280 (3.8%)	
Body mass index, mean (SD), kg/m2*	27.9 (6.2)	27.6 (5.8)	26.7 (5.3)	<0.001

Table 9 continuing.

Variables	HFpEF (n=3,623; 23%)	HFmrEF (n=3,322; 21%)	HFrEF (n=8,904; 56%)	p-value
Investigated subgroups				
Location*				
Blood pressure, mean (SD), mmHg				
Systolic	132 (21)	130 (21)	123 (20)	<0.001
Diastolic	73 (12)	74 (12)	73 (12)	0.008
Mean arterial blood pressure, mean (SD), mmHg*	93 (13)	92 (13)	90 (13)	<0.001
Heart rate, mean (SD), beats/min*	73 (15)	73 (15)	74 (15)	<0.001
Laboratory values				
NT-proBNP, median (IQR)*, pg/ml	2,037 (912, 4,420)	2,192 (930, 4,899)	3,141 (1,370, 7,080)	<0.001
Creatinine clearance, mean (SD), ml/min	61 (30)	67 (33)	70 (34)	<0.001
Hb, mean (SD), g/l	128 (17)	132 (17)	135 (17)	<0.001
Treatments				
ACE-I or ARB*	2,766 (76%)	2,868 (86%)	8,262 (93%)	<0.001
Digoxin*	656 (18%)	545 (16%)	1,480 (17%)	0.088
Diuretic*	3,113 (86%)	2,553 (77%)	7,219 (81%)	<0.001
Nitrate*	596 (16%)	510 (15%)	1,207 (14%)	<0.001
Platelet inhibitor*	1,550 (43%)	1,558 (47%)	4,278 (48%)	<0.001
Oral anticoagulant*	1,500 (42%)	1,396 (42%)	3,786 (43%)	0.53
Statin*	1,443 (40%)	1,567 (47%)	4,313 (49%)	<0.001
Beta-blocker*	2,879 (80%)	2,850 (86%)	8,118 (91%)	<0.001
MRA*	1,078 (30%)	932 (28%)	3,408 (38%)	<0.001
Device therapy*				
No	3,562 (99%)	3,208 (97%)	8,040 (91%)	<0.001
CRT-P	13 (0.4%)	33 (1.0%)	250 (2.8%)	
CRT-D	8 (0.2%)	21 (0.6%)	283 (3.2%)	
ICD	26 (0.7%)	45 (1.4%)	283 (3.2%)	
Comorbidities				
Smoking*				
Never	1,461 (51%)	1,232 (45%)	3,027 (40%)	<0.001
Previous	1,132 (40%)	1,177 (43%)	3,396 (45%)	
Current	243 (9%)	300 (11%)	1,145 (15%)	
Hypertension*	2,646 (73%)	2,176 (65%)	5,053 (57%)	<0.001
Coronary revascularization*	787 (22%)	1,073 (32%)	2,908 (33%)	<0.001
Peripheral artery disease*	366 (10%)	368 (11%)	843 (9%)	0.028
Stroke/TIA*	708 (19%)	552 (17%)	1,368 (15%)	<0.001
Anemia*	1,412 (39%)	1,157 (35%)	2,635 (30%)	<0.001
Valvular disease*	1,166 (33%)	804 (25%)	1,851 (21%)	<0.001
Lung disease*	1,108 (31%)	940 (28%)	2,229 (25%)	<0.001
Cancer within 3 years*	551 (15%)	482 (15%)	1,051 (12%)	<0.001
Socio-economics				
Family type*				
Living alone	2,022 (56%)	1,671 (50%)	4,272 (48%)	<0.001
Married/cohabitating	1,596 (44%)	1,649 (50%)	4,601 (52%)	
Education*				
Compulsory school	1,831 (51%)	1,552 (47%)	3,890 (44%)	<0.001
Secondary school	1,247 (35%)	1,227 (37%)	3,503 (40%)	
University	503 (14%)	520 (16%)	1,418 (16%)	
Income below median*	2,120 (59%)	1,697 (51%)	4,057 (46%)	<0.001
Number of children, mean (SD)*	2.0 (1.4)	2.0 (1.4)	2.0 (1.4)	0.001

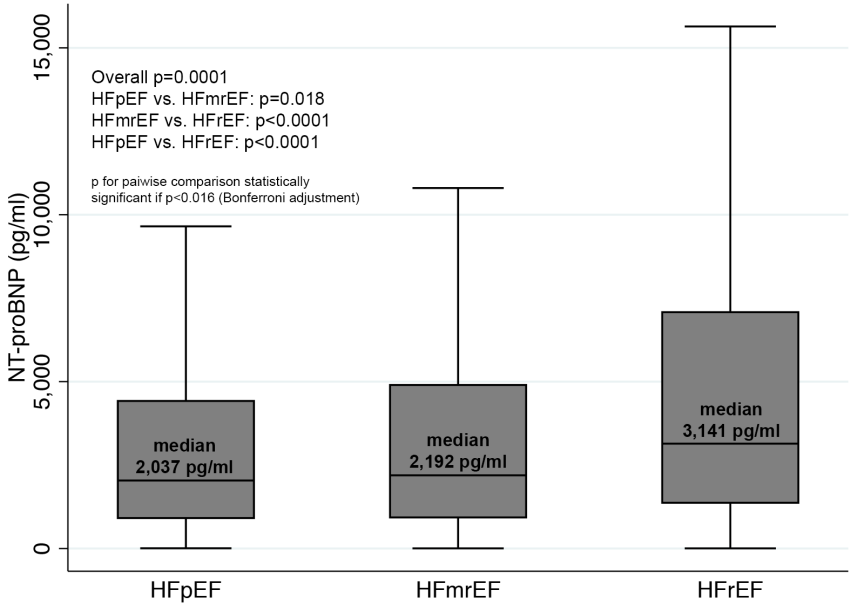

Figure 13. NT-proBNP levels according to the EF category.
NT-proBNP: N-terminal pro-B-type natriuretic peptide; HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction.

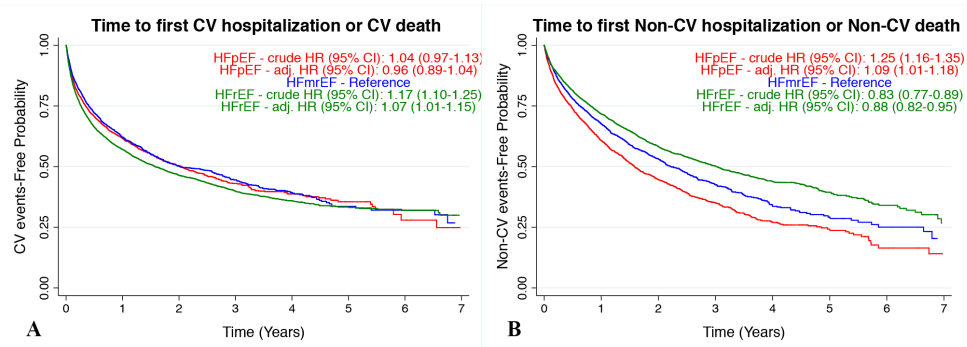

Figure 14. Kaplan Meier curves fitted for first CV (A) and non-CV (B) event according to the EF category.
CV: cardiovascular; HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction; HR: hazard ratio; CI: confidence interval.

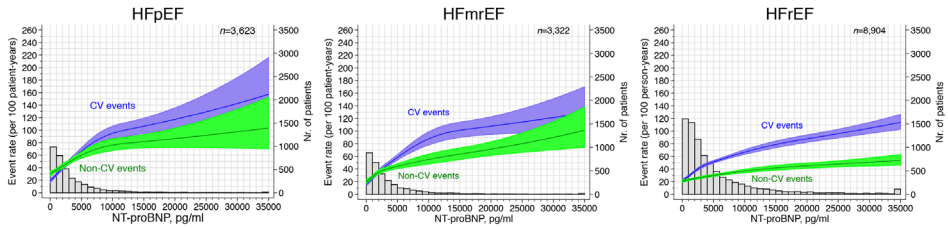

Figure 15. Association between continuous NT-proBNP levels and risk of outcomes in the different EF categories. The histogram reports the distribution of patients according to the NT-proBNP levels.
HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

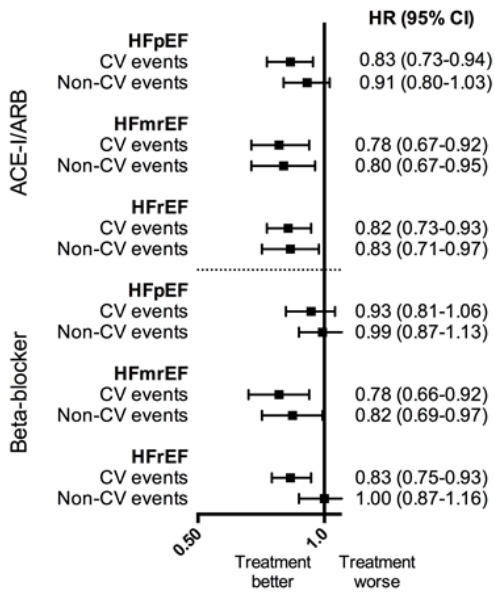

Figure 16. Associations between HF therapies and outcomes by EF category. HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction; HR: hazard ratio; CI: confidence interval; CV: cardiovascular; ACE-I: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker.

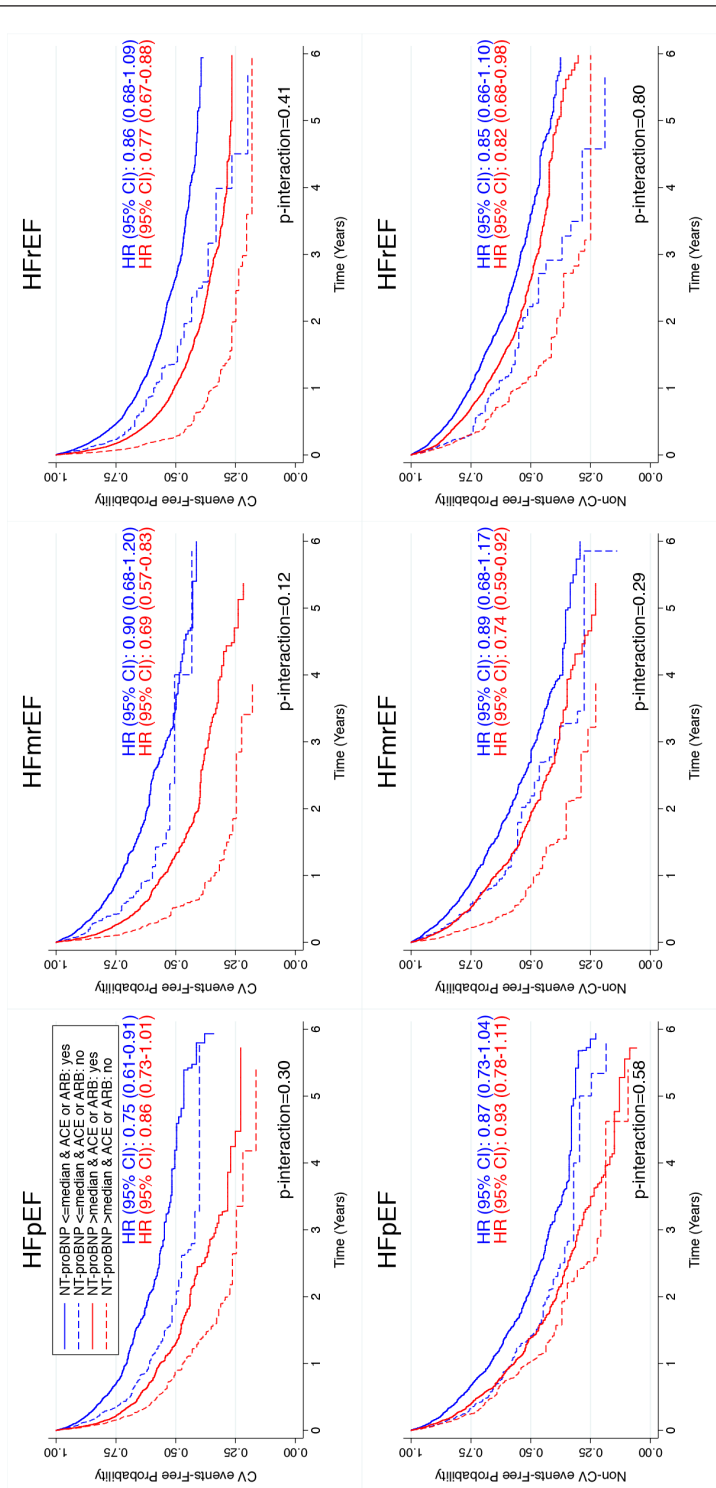

Figure 17. Kaplan Meier curves fitted for first CV (top panel) and non-CV event (bottom panel) according to the EF category, the use of ACE-I or ARB and NT-proBNP ≤ and > median value. HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction; HR: hazard ratio; CI: confidence interval; CV: cardiovascular; ACE-I: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

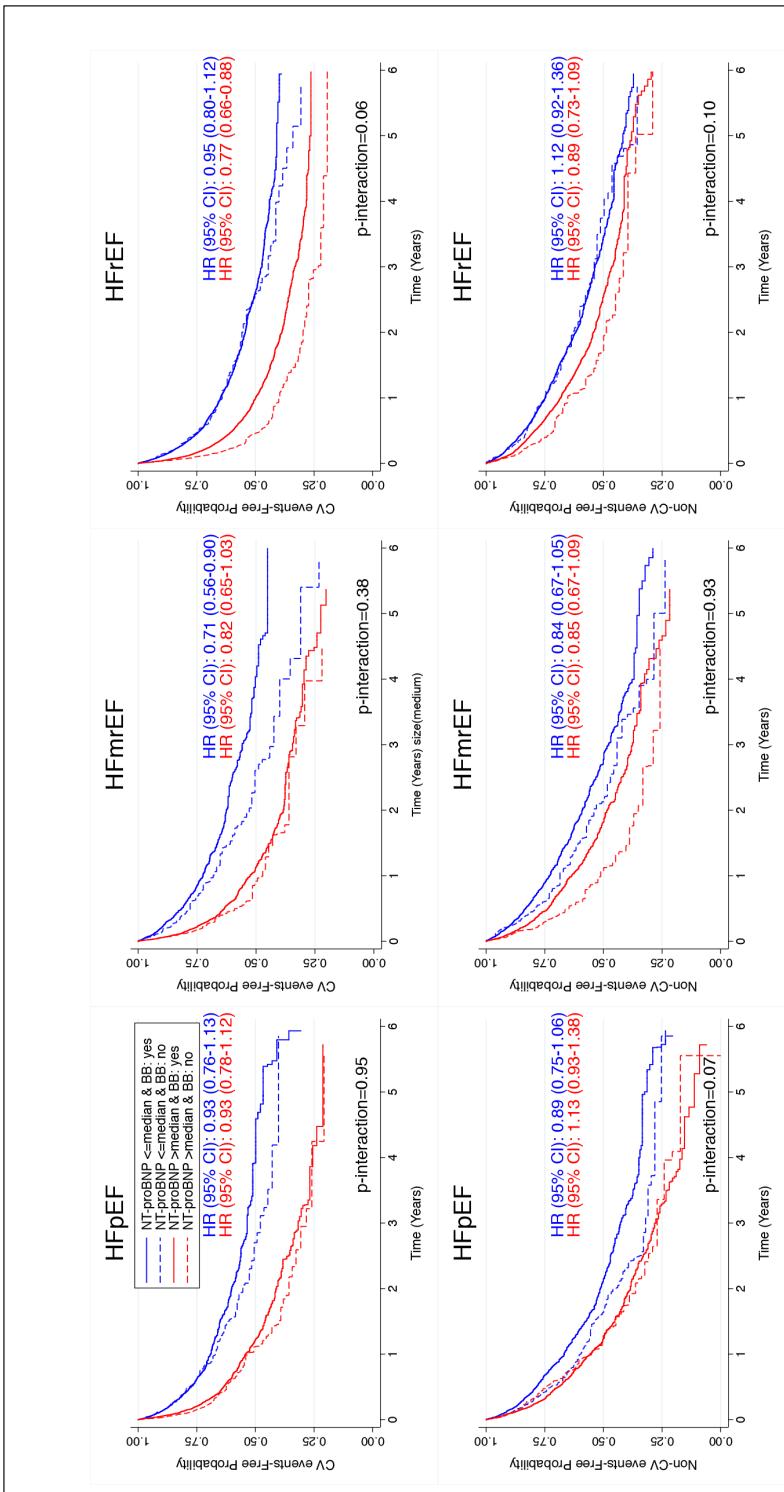

Figure 18. Kaplan Meier curves fitted for first CV (top panel) and non-CV event (bottom panel) according to the EF category, the use of beta-blockers and NT-proBNP ≤ and > median value. HFpEF: heart failure with preserved ejection fraction; HFmrEF: heart failure with mid-range ejection fraction; HFrEF: heart failure with reduced ejection fraction; HR: hazard ratio; CI: confidence interval; CV: cardiovascular; BB: beta-blocker; NT-proBNP: N-terminal pro-B-type natriuretic peptide.

We explored the following subgroups: inpatients/outpatients, atrial fibrillation, creatinine clearance <60/≥60 ml/min, age ≥75/<75 years, gender, ischemic heart disease, diabetes, HF duration ≥6/<6 months.

The risk of CV events was increased regardless of EF in patients with vs. without atrial fibrillation, ischemic heart disease, renal disease and diabetes. Age ≥ vs. <75 years and female vs. male sex were associated with lower risk of CV events in HFrEF but not in HFpEF and HFmrEF, even though there was no significant interaction between these subgroups and EF category. In all EF categories, inpatients had higher risk of CV and non-CV events as compared to outpatients, but with higher risk of CV events in HFpEF vs. HFmrEF vs. HFrEF and higher risk of non-CV events in HFpEF and HFmrEF vs. HFrEF. Overall, the risk of non-CV events was higher in HFrEF with vs. without atrial fibrillation and age ≥75 vs. <75 years, creatinine clearance <60 vs. ≥60 ml/min (even though the association was still significant after adjustments only in HFrEF), in HFmrEF and HFrEF women vs. men and diabetes vs. no diabetes. However, although these subgroups were significantly associated with prognosis in some EF categories but not all, there was no significant interaction with EF. Longer HF duration (≥ vs. <6 months) predicted higher risk of CV events in HFmrEF and HFrEF and of non-CV events only in HFrEF.

In all subgroups, regardless of EF, crude CV event-rates were higher than non-CV event rates throughout the NT-proBNP range. Consistent with the main analysis, CV and non-CV event rates increased mostly at lower NT-proBNP levels in HFpEF vs. HFmrEF vs. HFrEF, but the splines for CV and non-CV event rates diverged at lower NT-proBNP in HFrEF vs. HFpEF and HFmrEF. In high risk profile patients (i.e. patients with atrial fibrillation, ischemic heart disease and diabetes, age ≥ years, males, inpatients, creatinine clearance <60 ml/min and in HF duration ≥6 months), increasing NT-proBNP was associated with a more rapid increase in both CV and non-CV risk. Similarly, splines *diverged* at lower NT-proBNP levels in patients with higher risk (except for younger patients and those with creatinine clearance<60 ml/min who reported the same finding although at lower risk). Additionally, in higher risk patients, the increase in CV events risk was steeper than in non-CV events risk.

DISCUSSION

Randomized controlled trials have provided evidence for effective pharmacological and device treatments that have significantly improved mortality/morbidity in HFrEF, but prognosis still remains poor [1]. HFmrEF has no evidence-based therapy and constitutes a newly characterized and relevant population for future trials. Trials in HFpEF have failed to provide any effective treatment, and several concerns about their design have been raised.
The four studies included in the current thesis report evidences that may be of interest for and support future trial design in HF. In **Study I-IV** we provide data supporting the use of NPs as surrogate endpoints in HFpEF, HFmrEF and HFrEF phase II randomized trials, whereas in **Study III-IV** we explore the role of NT-proBNP as an eligibility criterion to enrich phase III trials for CV vs. non-CV events.

NPs as surrogate endpoints

Previous studies, post-hoc analyses from trial databases and registry-based and cohort studies, showed NT-proBNP levels higher in HFrEF vs. HFpEF [28-31, 69, 70], but no direct comparison among EF categories for NT-proBNP concentrations was performed. In **Study III-IV**, we directly compared EF categories for NT-proBNP levels in the SwedeHF, confirming NT-proBNP levels significantly higher in HFrEF vs. HFpEF, with HFmrEF more similar to HFpEF. This could be potentially explained by higher end-diastolic wall stress in HFrEF vs. HFpEF and HFmrEF and is also consistent with higher CV risk in HFrEF compared to HFmrEF and HFpEF [61]. We also observed higher levels of NT-proBNP in HFpEF patients enrolled in SwedeHF as compared with those in trials [28-30], that may be potentially addressed by 1) the less selective nature of SwedeHF and/or 2) the inclusion in HFpEF trials of patients with less severe HF, i.e. at less risk of events, that may have contributed to the lack of a significant difference in outcome between the experimental drugs and placebo.
A biomarker, in order to be used as surrogate endpoint, needs to have the following characteristics: 1) its levels have to correlate with risk of hard events (e.g. mortality/morbidity); 2) an improvement of its levels, induced by a treatment, is associated with a reduction in risk of hard outcomes.

Relationship between NT-proBNP levels and prognosis

Previous studies, mainly post-hoc analyses of randomized trials, have shown NT-proBNP levels to be associated with risk of mortality/moribidity (e.g. HF hospitalization) in HFpEF and HFrEF [28-31, 69, 70]. We confirmed these findings in a large unselected cohort of HF patients, the SwedeHF, reporting adjusted HRs for all-cause mortality/HF hospitalization ranging 1.48-2.00 in above vs. below/equal to median NT-proBNP levels, that is roughly comparable to those observed in trials such as COPERNICUS [31], Val-HeFT [69], I-PRESERVE [28], PEP-CHF [29], and CHARM-Preserved [30] (**Study III**). On top of this finding, we observed that although NT-proBNP levels were lower in HFpEF and HFmrEF vs. HFrEF, 1) the concentration above vs. below/equal to median but also continuously higher levels predicted significantly higher risk of mortality/HF hospitalization in HFpEF and HFmrEF vs. HFrEF and 2) the discriminatory power of NT-proBNP was higher in HFmrEF vs. HFpEF and HFrEF. These results are surprising and may suggest that the same NT-proBNP concentration is associated with different prognosis according to the EF category (also shown in **Study IV)**, thus different

cut-offs of NT-proBNP may be considered for eligibility in HFpEF vs. HFmrEF vs. HFrEF trials. Conversely, these findings may be also explained by HFpEF and HFmrEF patients with low NT-proBNP levels having mild HF or even symptoms driven by comorbidities and thus, being at lower risk of CV events, compared to those with higher NT-proBNP, whereas all HFrEF patients, regardless of NT-proBNP levels, had "true" HF. **Study IV** also reports a clear strong association between continuous NT-proBNP levels and risk of CV and non-CV events in HFpEF, HFmrEF and HFrEF.

Relationship between changes in NT-proBNP levels and prognosis

A previous meta-analysis of randomized trials in HFrEF reported NT-proBNP changes from baseline to end of follow-up to be significantly associated with the risk of HF hospitalization [27]. In the I-PRESERVE trial, enrolling patients with HFpEF (EF≥45%) and NYHA class II-IV, a decrease and increase in NT-proBNP levels <1000 pg/mL from baseline to 6-month follow-up predicted a 27% reduction and 2-fold increase in CV death or HF hospitalization risk, respectively, whereas beyond a 1000 pg/mL rise or fall, there was only little additional change in risk [32]. This analysis was performed on randomized trial data, that are highly selective and may not consider the heterogeneity, the competing risk and the comorbidities that characterize HFpEF real-world patients. Additionally, HFpEF and HFmrEF were not considered as distinct entities with EF=40-44% excluded from the analysis. Therefore, we investigated the association between changes in NT-proBNP and outcomes in 650 outpatients with HFpEF or HFmrEF enrolled in the SwedeHF (**Study I**). Notably, we reported that a decrease vs. an increase in NT-proBNP levels at a median time of 7 months between the two measurements was associated with a 51% and 61% reduction in risk of mortality/hospitalization in HFpEF and HFmrEF, respectively, and the risk of outcomes increased together with the continuous increase in NT-proBNP levels. Conversely, in **Study II** enrolling patients with acute decompensated HFpEF, we could show only a non-statistically significant 19% reduction in risk of mortality/HF hospitalization in those who reported a decrease vs. an increase in BNP/NT-proBNP levels (4-8 weeks between the first and the second NP measurement). Changes in NPs have been demonstrated to be prognostic in acute decompensated HFrEF [71]. The difference between acute decompensated HFpEF and HFrEF may be explained by lower NP levels and thus, less remarkable changes in NPs that may lead to fail to find an association with outcomes. Furthermore, the difference between acute decompensated and chronic HFpEF may be addressed by the more complex and heterogeneous population with acute decompensated HFpEF, together with the more important confounding effect of tachycardia, hypertension and acute ischemia that affects both the presentation, the levels of NPs and the association between these variables and the outcomes.

NT-proBNP for eligibility and enrichment in HF trials

Higher NT-proBNP levels are associated with higher risk of outcomes (**Study III-IV**), thus enrolling patients with high NT-proBNP levels in HF trials should ensure the presence of HF and enrich for CV events, leading to higher chances of success for the experimental treatment, if the pathophysiological target is correct. HFpEF and HFmrEF vs. HFrEF exhibit different NT-proBNP concentration, that may be explained by higher end-diastolic wall stress in HFrEF vs. HFpEF and HFmrEF. Thus, a different value of NT-proBNP may be chosen for eligibility in trials according to the EF category (**Study III-IV**). Additionally, previous studies reported high NT-proBNP levels associated with several comorbidities, e.g. atrial fibrillation and chronic

kidney disease, and lower body mass [72-74]. Thereby, in some cases, higher NT-proBNP could reflect the presence of comorbidities rather than the severity of HF, and thus different (i.e. higher) NT-proBNP values may be chosen as inclusion criterion in trials. In **Study III** we assessed the independent predictors of high NT-proBNP levels across the EF spectrum. We reported that regardless of EF category, higher age, NYHA class and heart rate, lower body mass index, presence of atrial fibrillation, chronic kidney disease and anemia, and diuretic use were independently associated with higher NT-proBNP levels. Notably, atrial fibrillation was associated with significantly higher NT-proBNP levels in HFpEF and HFmrEF vs. HFrEF independently of heart rate and use of rate control medication, that may be explained by atrial fibrillation, and in particular paroxysmal atrial fibrillation, contributing relatively less to filling pressure and wall stress in HFrEF vs. HFpEF and HFmrEF. We also observed obesity to be a weak predictor of NT-proBNP in HFpEF but to be strongly associated to NP levels in HFpEF. This may support the role for obesity as a driver for HFpEF rather than a maker of disease, whereas in HFmrEF and HFrEF low body mass may be linked to cachexia and thus, be tied to the severity of HF. All together, these findings suggest that optimal NT-proBNP cut-offs for eligibility in randomized trials should be carefully tailored, especially according to the EF category, since HFpEF, HFmrEF and HFrEF report different NT-proBNP levels and several differences in predictors of high NT-proBNP levels, and in specific subgroups such as in presence/absence of atrial fibrillation and renal disease.
Consequently, in **Study IV**, we explored the relationship between continuous NT-proBNP levels, CV and non-CV events, according to the different EF categories and in relevant subgroups, in order to provide to trialists and sponsors reference data facilitating the choice of appropriate values for NT-proBNP for eligibility in randomized trials. Importantly, we evaluated the ratio CV to non-CV events ratio, since the optimal cut-off for NT-proBNP for eligibility and enrichment for CV events is supposed to increase the likelihood of CV but not of non-CV events (i.e. increase CV to non-CV event ratio) where the latter may not be affected by HF therapies. We reported 1) higher non-CV event rates with higher EF across the whole NT-proBNP spectrum; 2) that higher NT-proBNP levels as inclusion criteria enrich for CV events but also increase the CV to non-CV event ratio; 3) higher CV to non-CV event ratio at lower NT-proBNP levels in HFrEF vs. HFmrEF vs. HFpEF, suggesting a potential optimal NT-proBNP cut-off lower in HFrEF than in HFpEF and HFmrEF; 4) that the trade-off with increasing NT-proBNP levels is the reduction of eligible patients and thus, less feasible recruitment rates and adequacy of enrollment; 5) patients at higher risk (with atrial fibrillation, ischemic heart disease, diabetes, renal disease, men, inpatients, longer HF duration) present an increase in CV and non-CV event rates and in the CV to non-CV event ratio (except for renal disease) at lower NT-proBNP levels. Additionally, we could not observe any difference in the association between ACE-I or ARB and beta-blocker therapies and outcomes according to NT-proBNP levels, and current literature on the topic reports inconsistent evidence [28, 31, 75-78]. However, this last result has to be interpreted considering that we used observational data that cannot demonstrate any potential treatment effect because of unmeasured confounders.

Strengths and Limitations

Strengths of **Study I, III and IV** is the use of data from SwedeHF, one of the largest HF registries worldwide, including a large and unselected cohort of patients with HFpEF, HFmrEF and HFrEF, with around 80 variables recorded, linkage with other registries

providing additional data (comorbidities, socioeconomics) and no missing data for outcomes. Additionally, the large sample of patients with a NT-proBNP value measured in SwedeHF allowed us to characterize NT-proBNP throughout the EF spectrum. Strengths of **Study II** is the availability of serial measurements of NT-proBNP/BNP in patients with acute decompensated HFpEF, who have been rarely investigated.

A limitation of all the studies included in this thesis is the use of observational data that are subject to selection bias and confounding. Even though the analyses were extensively adjusted for potential confounders when needed, we cannot rule out unmeasured confounders. Limitations of **Study I, III and IV** are linked with the use of SwedeHF, that has as only inclusion criterion clinician-judged HF, thus some patients, in particular those with preserved EF, may not have HF. There are missing data for some variables, but we used multiple imputation to reduce the bias due to data not missing at random. Generalizability of our findings to other countries may depend on similarities in population characteristics, health care and HF management. The limited amount of longitudinal data allowed us to include only 650 patients in **Study II** and did not permit us to investigate NT-proBNP levels in patients with increasing/decreasing EF in **Study III**. Furthermore, NT-proBNP is indicated in Sweden for diagnostic and prognostic purposes in HF but not collected for serial follow-up or guiding therapy. Thus, longer surviving patients may have had higher chances to get a second NT-proBNP assessment (survival bias) and, on the contrary, deteriorating patients may have had greater indication to get more than one measurement (bias by indication). In addition HFpEF, HFmrEF and HFrEF were defined only according to the EF. **Study II** included a relatively small cohort of patients, thus we cannot rule out that with larger sample size the association between NT-proBNP levels and prognosis would have become statistically significant.

CONCLUSIONS

NT-proBNP levels are associated with prognosis across the EF spectrum (**Study III-IV**). A reduction in NT-proBNP levels is associated with improved mortality/morbidity in HFpEF and HFmrEF (**Study I**), with previous studies reporting similar findings in HFrEF [27]. These data support the use of NT-proBNP as surrogate endpoint in phase II trials in chronic HF. We do not report any significant association between changes in NP and prognosis in acute decompensated HFpEF, but only a trend, thus further investigation in larger cohorts may be needed (**Study II**). The observed relationship between NT-proBNP levels and CV and non-CV events supports the use of NT-proBNP for eligibility and enrichment for CV events in HF trials, but cut-off levels should be carefully tailored to comorbidities (**Study III**). The role of NT-proBNP in predicting potential treatment response remains unclear (**Study IV**).

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