# The Clinical Relevance and Potential Mechanism of Biomarkers in Elderly Heart Failure Patients

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“Men love to wonder, and that is the seed to science.”

*Ralph Waldo Emerson*

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## ABSTRACT

**Aim:** To study the clinical relevance and potential mechanism of biomarkers in elderly heart failure (HF) patients.

**Methods:** A retrospective study was conducted by access to Swedish Heart Failure Registry with focus on HF patients aged ≥85 years. A secondary study was conducted in our hospital cohort due to acute decompensated HF in elderly patients. A prospective study was conducted in elderly patients referred for echocardiography because of suspected HF.

**Results:** The ≥85 year group from Swedish Heart Failure Registry was characterized by higher incidence of cardiovascular and non-cardiovascular comorbidities compared with the ≤65 year group. Compared with the 85-90 year subgroup, the >90 year subgroup had a decline in cardiovascular and non-cardiovascular comorbidities. In the secondary study in elderly patients during hospital admission due to acute decompensated HF, multivariate analysis showed that N-terminal pro-B-type natriuretic peptide (NT-proBNP) was not prognostic predictor for all-cause mortality. However, a subgroup analysis demonstrated that in patients with NT-proBNP >8000 (ng/L), NT-proBNP was the only prognostic predictor for all-cause mortality. In the prospective hospital cohort referred for echocardiography because of suspected HF, red blood cell distribution width (RDW) was higher among patients with HF. In three multivariate analyses, biomarkers that were prognostic predictors of all-cause mortality were NT-proBNP, cystatin C, RDW, midregional pro-atrial natriuretic peptide (MR-proANP). Finally, when all the variables that were significant in above three multivariate analyses were analyzed in one multivariate analysis the only biomarker that

was prognostic predictor of all-cause mortality in elderly HF patients was NT-proBNP. Furthermore, the sensitivity and specificity of the two different multiple marker modalities are higher than NT-proBNP alone.

**Conclusion:** Elderly HF patients had increased cardiovascular and non-cardiovascular comorbidities that declined from >90 years. The prognostic value of NT-proBNP in elderly HF patients has to be interpreted with caution due to higher age and comorbidities. Two different multiple marker modalities incorporating biomarkers were able to improve prognostic prediction compared to NT-proBNP alone.

**Implication:** Our studies strongly suggest that the development of multiple marker models incorporating biomarkers reflecting different pathophysiological pathways might allow for better prognostic prediction in elderly HF patients.

**Keywords**: Heart failure, elderly, biomarkers, comorbidities, mortality
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# SAMMANFATTNING

Ungefär 250,000 patienter har hjärtsvikt i Sverige med en medelålder på 75 år och ungefär 30,000 nya patienter insjuknar varje år.

Hjärtsvikt är när hjärta inte orkar pumpa ut tillräckligt med blod till kroppen. Det kan antingen bero på att hjärtat är försvagat och har sämre förmåga att dra ihop sig och denna typ av hjärtsvikt heter systolisk hjärtsvikt. Eller att hjärtat inte fylls tillräckligt med blod innan det pumpas ut till kroppen och denna typ av hjärtsvikt heter diastolisk hjärtsvikt.

Hjärtsviktpatienter kan variera mycket, till exempel i sjukdomsgrad, bakomliggande sjukdomar, samsjukligheter och ålder. Dessa faktorer kan försvåra diagnosen och prognostisk prediktion av hjärtsvikt hos patienten.

Efter diagnos är det endast hälften av de äldre hjärtsviktspatienterna som lever mer än sex år. För att kunna öka detta behövs dagens prognos förbättras och behandlingen individanpassas. De flesta prognosmodeller har utvecklas på unga hjärtsviktspatienter och för de äldre patienterna är prognosmodellen inte lika utvecklad. Detta beror bland annat på att de äldre hjärtsviktspatienterna har oftast mer komplicerade sjukdomsbild med flera andra sjukdomar ihop med hjärtsvikten.

I denna avhandling har vi fokuserat på att hitta prognosmodeller som passar äldre patienter med hjärtsvikt och mycket andra sjukdomar. Detta görs genom att kombinera olika markörer som analyseras i blodprovet ihop med klinisk information.

Resultatet från denna avhandling är att äldre hjärtsviktspatienter har mera hjärtrelaterade och andra sjukdomar jämfört med unga hjärtsviktspatienter. Markören som rutinmässigt analyseras i blodprovet för hjärtsvikt, NT-proBNP, bör användas med försiktigheter pga att NT-proBNP kan variera kraftigt hos de äldre hjärtsviktspatienterna till följd av högre ålder och andra sjukdomar. Sedan visar denna avhandling också att en kombination av markörer ger bättre prognostisk information än bara NT-proBNP ensam.

# LIST OF PAPERS

I. Holmström A, Sigurjonsdottir R, Edner M, Jonsson A, Dahlström U, Fu M. **Increased comorbidities in heart failure patients ≥85 years but declined from >90 years: Data from the Swedish Heart Failure Registry.** *Int J Cardiol. 2012, In press*

II. Holmström A, Fu M. **Re-evaluation of prognostic significance of NT-proBNP in a 5-year follow-up study assessing all-cause mortality in elderly patients (≥75 years) admitted to hospital due to suspect heart failure.** *Eur Geriatr Med. 2012, In press*

III. Holmström A, Sigurjonsdottir R, Hammarsten O, Gustafsson D, Petzold M, Fu M. **Red blood cell distribution width and its relation to cardiac function and biomarkers in a prospective hospital cohort referred for echocardiography.** *Eur J Intern Med*. 2012, 23(7):604-9.

IV. Holmström A, Sigurjonsdottir R, Hammarsten O, Petzold M, Gustafsson D, Fu M. **An integrated multiple marker modality is superior to NT-proBNP alone in prognostic prediction in all-cause mortality in a prospective cohort of elderly heart failure patients.** *Manuscript, submitted*

# CONTENT

# ABBREVIATIONS

| | |
|---|---|
| HF | Heart failure |
| NT-proBNP | N-terminal pro-B-type natriuretic peptide |
| RDW | Red blood cell distribution width |
| MR-proANP | Midregional pro-atrial natriuretic peptide |
| LVEF | Left ventricular ejection fraction |
| SHF | Systolic heart failure |
| HFNEF | Heart failure with normal ejection fraction |
| BNP | Brain natriuretic peptide |
| Hs CRP | Highly sensitive C-reactive protein |
| Hs TNT | Highly sensitive troponin T |
| eGFR | Estimating glomerular filtration rate |
| PA | Pulmonary artery pressure |

# 1 INTRODUCTION

Heart failure (HF) is one of the leading causes of death, and it increases with age in line with increased comorbidity and mortality [1-12].

Hence, the aim of the work complied in this thesis has been to get a better understanding of elderly patients with HF from phenotype characterization to prognostic prediction. A multiple marker modality incorporating biomarkers is regarded as one of promising ways for a better prognostic prediction in this heterogeneous HF population in the elderly and therefore enable individually based tailored care.

## 1.1 Heart Failure

Approximately 250,000 patients have HF in Sweden, and the average age is 75 years.

HF is when the heart can't pump out enough blood to the body. It can either be because the heart has impaired contractile or pump function and this type of HF called systolic heart failure (SHF). Or that the heart has impaired relaxation, compliance or filling blood before it is pumped out to the body and this type of HF called heart failure with preserved or normal ejection fraction (HFNEF). It is common in advanced SHF to have diastolic dysfunction as assessed by changes in ventricular filling features. However, in general in HFNEF left ventricular systolic performance, contractility and function are normal. Most common risk factors for HF are high age, diabetes mellitus, hypertension, coronary artery disease and obesity. For SHF ischemic heart disease is the most common underlying cause whereas for HFNEF it is hypertension. In SHF there are increases in left ventricular cavity size, wall stress, wall mass, end-diastolic and end-systolic volumes. In the mean time the left ventricular ejection fraction (LVEF) is reduced and the thickness of the walls and mass/cavity ratio are either unchanged or decreased. In contrast, in HFNEF the cavity size is decreased or unchanged and the volumes of the end-diastolic and end-systolic remain normal or decreased. However, the wall thickness and mass are usually increased and mass/cavity ratio is increased substantially. Systolic wall stress and ejection fraction remain normal and end-diastolic wall stress is increased. The major mechanism for reduced ejection fraction in SHF is impaired contractile function. In HFNEF the functional derangement is impaired left ventricular relaxation and increased passive stiffness [13].

HF patients can vary greatly, for example, in disease severity, age, underlying diseases and associated diseases. These factors may complicate the diagnosis of HF in the patient.

## 1.2 Heart Failure in elderly patients

The number of people with HF aged ≥85 years is increasing rapidly as the aged population increases worldwide and this leads to that a more in-depth characterization and understanding of HF in this group are needed. HF is one of the leading causes of death, and it increases with age in line with comorbidity and mortality [1-12]. Comorbidities, for example cerebral dysfunction, renal dysfunction, anaemia, diabetes mellitus, liver dysfunction and pulmonary diseases frequently accompany HF and further aggravate clinical outcome and quality of life. Patients with HF have much higher prevalence of comorbidities compared to patients without HF. The unresolved issues are the pathophysiological processes that are underlying the interactions between comorbidities and HF. Factors associated with HF might cause neurohumoral activation, hemodynamic changes and inflammation that in turn make HF worse [14]. Braunstein et al. reported that five or more non-cardiac comorbidities were present in 39% of HF patients. Of all HF patients these patients with multiple comorbidities account for 81% of all hospital days [15].

Previous landmark randomized clinical trials have mostly been conducted in younger SHF patients. HF patients ≥85 years are rarely studied. Among available publications, the so-called “elderly” are mostly aged around 65 years [1, 5, 9-12, 16] and moreover with limited comorbidities. This leads to that HF in patients aged ≥85 years remains poorly understood. Available few studies in HF patients aged ≥85 reported inconsistent findings, particularly with regard to both cardiovascular and non-cardiovascular comorbidities [6-9, 17-21]. For instance, the incidence of hypertension has been reported to be both lower [18, 20-21] and higher [6-8], and the incidence of ischaemic heart disease has been shown to be similar [20], higher [8] and lower in elderly patients [7, 18, 21]. These differences may be due to small sample sizes in previous studies, a severe limitation because of the highly heterogeneous nature of the elderly HF patient population.

## 1.3 Biomarkers

Patients with HF have a poor prognosis, despite advances in treatment. The risk of death for patients with HF could only be partly explained by

established mortality risk factors such as the New York Heart Association functional class, LVEF and N-terminal pro-B-type natriuretic peptide (NT-proBNP). This is particularly true for elderly individuals, where HF is often coexisted with other diseases [15]. In this context we hypothesized that NT-proBNP alone is not sufficiently enough as prognostic indicator in elderly HF patients and additional biomarkers might have added value for more accurately predicting the prognosis of HF in elderly populations. Therefore a modality of multiple markers might be a novel approach for a better prognostic prediction.

### 1.3.1 N-terminal pro-B-type natriuretic peptide (NT-proBNP)

In healthy individuals NT-proBNP is normally very low in circulation. In cardiomyocytes brain natriuretic peptide (BNP) is activated in response to increased myocardial wall stress due to pressure- or volume-overload. This leads to that intracellular precursor propeptide is produced. This propeptide is further processed and then NT-proBNP and active BNP are released. In HF patients NT-proBNP is increased and correlates with severity of HF and ventricular wall stress. However, there are other diseases (renal dysfunction) and other cardiac diagnoses (valvular heart disease and atrial fibrillation) that could influence the NT-proBNP levels as well. SHF and HFNEF cause usually elevated NT-proBNP. However, in general HFNEF has lower levels of NT-proBNP than that in SHF. There are patients who have NT-proBNP level in the gray zone implying that NT-proBNP is slightly elevated above cut-off for exclusion for HF and too low to identify HF [23]. Nevertheless NT-proBNP is one of few cardiac biomarkers that have been well studied and currently recommended by international guidelines for diagnosis and prognosis for HF [22].

### 1.3.2 Red blood cell distribution width (RDW)

Red blood cell distribution width (RDW) is determined during standard complete blood count and is a numerical measure of the variability in the size of circulating erythrocytes. Normally the erythrocytes are in a standard size. However, higher RDW and greater heterogeneity in size of the erythrocytes can be caused by ineffective erythropoiesis or increased destruction [24].

In several cardiovascular conditions such as HF and coronary artery disease [24-32], RDW has been shown to be a strong prognostic predictor. There are increasing evidences uniformly supporting the prognostic importance of RDW in SHF. Oh et al. reported that in 100 patients admitted to hospital because of acute HF there was an independent correlation between RDW and

signs on echocardiography of diastolic dysfunction after adjustment for other risk factors [33]. Another study by Al-Naijar et al. showed association between increasing RDW and worsening heart function in the SHF population [26].

### 1.3.3 Multiple marker modality incorporating biomarkers

In HF patients almost half of their deaths are due to non-cardiac origins [15, 34] and therefore for better prognostic prediction a modality of multiple marker incorporating biomarkers has been proposed. There were few studies available in approach to multiple marker paradigm. Horwich et al found that all-cause mortality and urgent need for cardiac transplantation could be improved through combination of troponin-I and BNP in patients with advanced HF referred for cardiac transplantation evaluation [35]. Ishino et al reported that cardiac death or hospitalization due to worsening of HF were reduced in HF patients through combination of BNP, heart-type fatty acid-binding protein and pentraxin 3 [36].

HF has often heterogeneous aetiology and underlying mechanisms covering from cardiac overload or injury to a complex interplay among inflammatory, genetics, biochemical changes and neurohormonal acting on cardiac myocytes, interstitium or both [37]. Because of this a modality of multiple biomarkers is needed to represent diverse pathophysiological pathways. These pathways include hemodynamic stress (midregional pro-atrial natriuretic peptide (MR-proANP) and NT-proBNP); plasma volume and osmolarity (copeptin); damage to heart cells (highly sensitive troponin T (hs TNT)); inflammation (highly sensitive C-reactive protein (hs CRP)); renal function (cystatin C) and RDW [38].

Despite above biomarkers have been shown to be predictors for all-cause mortality [2, 28-29, 39-42], there are up to now no convincing evidence that these novel biomarkers are better than NT-proBNP. Therefore it remains unknown whether these new biomarkers are better than NT-proBNP and a multiple marker modality incorporating biomarkers is better than a single biomarker in elderly HF.

# 2 AIM

To study the clinical relevance and potential mechanisms of biomarkers in elderly HF patients.

# 3 METHODOLOGICAL CONSIDERATIONS

Methods that are used in this thesis are briefly presented here. In each paper a more detailed descriptions of methods are provided.

## 3.1 Swedish Heart Failure Registry

The Swedish Heart Failure Registry is an internet-based registry created in 2003 and allows participating units to register their HF patients online after diagnosis by a clinician (www.rikssvikt.se). More than 70 variables, including demographics, concomitant diseases, diagnostic procedures, hemodynamics, laboratory data and medication are recorded at discharge from hospital (within 1 month) or after visits to outpatient clinics [43].

Patients ≤65 years and ≥85 years were included in this study. Patients aged 66–84 years were excluded in this study in order to show the greatest contrast as possible between the much younger and much elderly patients. The ≥85 year group was further divided into two subgroups: 85–90 year and >90 year.

HF was diagnosed individually by physicians from participating registration sites where the current guidelines for the diagnosis and treatment of acute and chronic HF is recommended [44-45]. Cut-off for LVEF is defined as <50% in SHF, and ≥50% for HFNEF.

## 3.2 Patients with acute decompensated heart failure

Patients included in this study were those admitted to Department of Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden, due to suspect HF during 2005–2007. The current study was based on secondary analysis of baseline data from a part of this database with following inclusion criteria: age ≥75 years and NT-proBNP immediately taken after hospital admission. Outcome data were collected during January–March 2012. HF was initially diagnosed individually by physicians on duty in the emergency room based mainly on clinical symptoms and signs of HF. HF diagnose was confirmed during hospital stay. In current study, HF diagnosis was newly adjudicated by two independent cardiologists based on guidelines for the diagnosis and treatment of acute and chronic HF [44, 46].

## 3.3 Patients referred for echocardiography

Patients referred for echocardiography due to suspected HF at the Department of Clinical Physiology, Sahlgrenska University Hospital, Gothenburg, Sweden, during 2010 were recruited for present studies. Following patients were excluded: patients less than 60 years old in the non-HF and uncertain HFNEF groups (paper III).

The diagnosis of SHF was based on HF symptoms in combination with LVEF <50% [44]. HFNEF was defined as a combination of four criteria: 1) HF symptoms, 2) normal or mild abnormal LVEF ≥50%, 3) non-dilated left ventricle and 4) echocardiographic signs of diastolic dysfunction, left atrial enlargement, left ventricular hypertrophy and/or NT-proBNP ≥1500 ng/L [45]. The non-HF group met the following four criteria: 1) symptoms which might be explained by non-cardiac causes, 2) LVEF ≥50%, 3) NT-proBNP ≤300 ng/L, and 4) absence of left ventricular hypertrophy, left atrial enlargement and diastolic dysfunction by echocardiography. As the definition of HFNEF remains ambiguous, one group was assigned as uncertain HFNEF based on four criteria: 1) HF symptoms, 2) normal or mild abnormal LVEF ≥50%, 3) echocardiographic signs that only partially fulfilled the diastolic dysfunction in absence of left ventricular hypertrophy or left atrial enlargement and 4) NT-proBNP <1500 ng/L.

## 3.4 Measurement of biomarkers

The RDW and other blood cell variables were determined using a Model S-PLUS JR Coulter Counter (Beckman Coulter, INC, USA). The serum levels of NT-proBNP and hs TNT were measured by the Elecsys proBNP/hs TNT assay (Roche Diagnostics, Indianapolis, INC, USA) except in paper II where NT-proBNP was measured using the Stratus proBNP assay (Stratus CS Acute Care system, Dade Behring Holdings, INC, USA). MR-proANP was analyzed by immunoluminometric assay MR-proANP (B.R.A.H.M.S., Germany). Copeptin was analyzed by immunoluminometric assay copeptin (B.R.A.H.M.S., Germany).

All other laboratory variables examined were part of the routine laboratory services provided by the Clinical Chemistry Laboratory at Sahlgrenska University Hospital.

## 3.5 Collection of clinical data

Clinical variables were collected either from the Swedish Heart Failure Registry or medical records. At the end of the follow-up period, for prospective studies, all-cause mortality was collected from medical records and the Swedish populations register.

## 3.6 Statistical analyses

Student's unpaired t-test, Mann-Whitney U-test or One-way ANOVA with Bonferroni, and for discrete variables, the chi-square test, were used to assess statistical significance. For correlation analysis Spearman and Pearson correlations (two-tailed) were used. Stepwise multiple linear regression was used for analyzing which factors were independently associated with RDW. Both Kaplan-Meier analysis and Cox proportional-hazard regression survival model were used for survival analyses. The hazard ratios (HR) with confidence intervals (CI) and p-values were presented. Risk score systems were established based on Wald $X^2$ values from the Cox proportional-hazard regression survival model of variables that were significant in the multivariate analyses.

# 4 RESULTS

## 4.1 Swedish Heart Failure Registry

Compared to the ≤65 year group, the 85-90 year group and the >90 year group had characteristics that differ. The ≥85 year group was characterized by more women, lower BMI, higher systolic blood pressure, lower diastolic blood pressure, more left bundle branch block, more than twice as many patients with HFNEF compared to the ≤65 year group. Moreover, the ≥85 year group has more cardiovascular comorbidities (atrial fibrillation, ischaemic heart disease and hypertension), more non-cardiovascular comorbidities (pulmonary disease, anaemia, renal dysfunction and stroke) compared to the ≤65 year group. However, diabetes mellitus decreased with ageing (Table 1).

When comparing the 85–90 year group, in the >90 year group anaemia and renal dysfunction increase with age whereas cardiovascular and non-cardiovascular comorbidities decline (Table 1). In 85-90 year and >90 year groups NT-proBNP levels were twice as high as that in ≤65 year group. NT-proBNP level was also higher in the >90 year group compared to the 85-90 year group. Moreover, NT-proBNP level was higher in SHF compared to HFNEF in the same age group.

*Table 1. Comorbidities in elderly and younger HF patients from the Swedish Heart Failure Registry*

| **Variables** | | **≤65y** | **85–90y** | **>90y** |
|---|---|---|---|---|
| | *N* | 8,348 | 11,412 | 4,477 |
| **Cardiovascular comorbidity** | Ischaemic heart diseases (%) | 34.8 | 56.1** | 53.5##,¶¶ |
| | Hypertension (%) | 37.7 | 49.5** | 45.6##,¶¶ |
| | Atrial fibrillation (%)[1] | 29.7 | 57.3** | 56.1##,¶ |
| **Non-cardiovascular comorbidity** | Diabetes mellitus (%) | 24.4 | 20.0** | 14.6##,¶¶ |
| | Pulmonary diseases (%) | 13.3 | 19.1** | 15.5##,¶¶ |
| | Stroke (%) | 7.9 | 15.7** | 11.3##,¶¶ |
| | eGFR <60 (ml/min)[2] (%) | 10.9 | 89.8** | 97.2##,¶¶ |
| | eGFR <30 (ml/min)[2] (%) | 1.8 | 28.0** | 53.1##,¶¶ |
| | Anaemia (%)[3] | 22.4 | 44.3** | 47.2##,¶¶ |

[1]Atrial fibrillation in medical history. [2]Estimating glomerular filtration rate (eGFR), Cockroft-Gaults formula, [3]Anaemia according to WHO <130 g/L (male) and <120 g/L (female).
*$p$ <0.05, **$p$<0.01 between ≤65 and 85–90 years groups.
#$p$ <0.05, ##$p$<0.01 between ≤65 and >90 years groups.
¶$p$ <0.05, ¶¶$p$<0.01 between 85–90 and >90 years groups.

## 4.2 Patients with acute decompensated heart failure

When using the multivariate analysis in the HF patients, only pulmonary artery pressure (PA) (mmHg), history of valvular surgery and use of aldosterone receptor antagonist were significant prognostic indicators for all-cause mortality.

HF patients were divided into two subgroups, one with NT-proBNP ≤8000 (ng/L) and another with NT-proBNP >8000 (ng/L). Multivariate analysis in the subgroup with NT-proBNP >8000 (ng/L) demonstrated that NT-proBNP was the only significant independent indicator for all-cause mortality. However, when the subgroup with NT-proBNP ≤8000 (ng/L) was analyzed, enlargement of left atrium and PA (mmHG) were significant prognostic indicators for all-cause mortality (Figure 1).

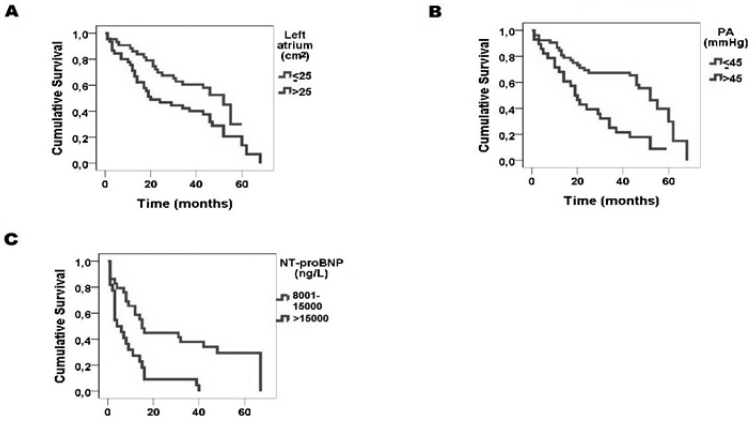


*Figure 1. Kaplan-Meier survival curve in patients with NT-proBNP ≤8000 (ng/L) (A-B) and NT-proBNP >8000 (ng/L) (C) in the HF cohort.*

The potential impact of NT-proBNP on prognostic prediction in HF patients was studied through different levels of NT-proBNP with Kaplan-Meier analysis. When NT-proBNP was between 2000-8000 (ng/L) the cumulative survival rate was almost the same. However, the cumulative survival rate in

group with NT-proBNP ≤8000 (ng/L) was significantly lower than group with NT-proBNP >8000 (ng/L).

## 4.3 Patients referred for echocardiography

Compared to the non-HF and with uncertain HFNEF (gray zone patients) the mean RDW levels were significantly higher among HF patients. However, the mean RDW levels did not differ between SHF and HFNEF.

A positive correlation between RDW and NT-proBNP and an inverse correlation between RDW and LVEF were shown when RDW was divided in quartiles. SHF was twice as often in the highest RDW quartile compared to the lowest RDW quartile. However, this trend was not significant for HFNEF.

Three multivariate analyses demonstrated that NT-proBNP, cystatin C, RDW, MR-proANP, PA (mm Hg), estimated glomerular filtration rate (eGFR), anaemia, sinus rhythm and diuretics were significant predictors of all-cause mortality. However, when all above variables that were significant was analyzed in one multivariate analysis, only NT-proBNP, eGFR <60 (ml/min), anaemia and diuretics remained significant predictors for all-cause mortality.

The potential impact of NT-proBNP on prognostic prediction in HF patients was studied through different levels of NT-proBNP with Kaplan-Meier analysis. When NT-proBNP was between 2000-8000 (ng/L) the cumulative survival rate was almost the same and the cumulative survival rate was significantly lower in group with NT-proBNP ≤8000 (ng/L) than group with NT-proBNP >8000 (ng/L).

According to Wald $X^2$ values from the univariate or the multivariate analyses, risk score systems were set up. In HF patients a score ≤2 indicates lower mortality risk, score 3-5 indicates intermediate risk and ≥6 indicates higher mortality (Figure 2).

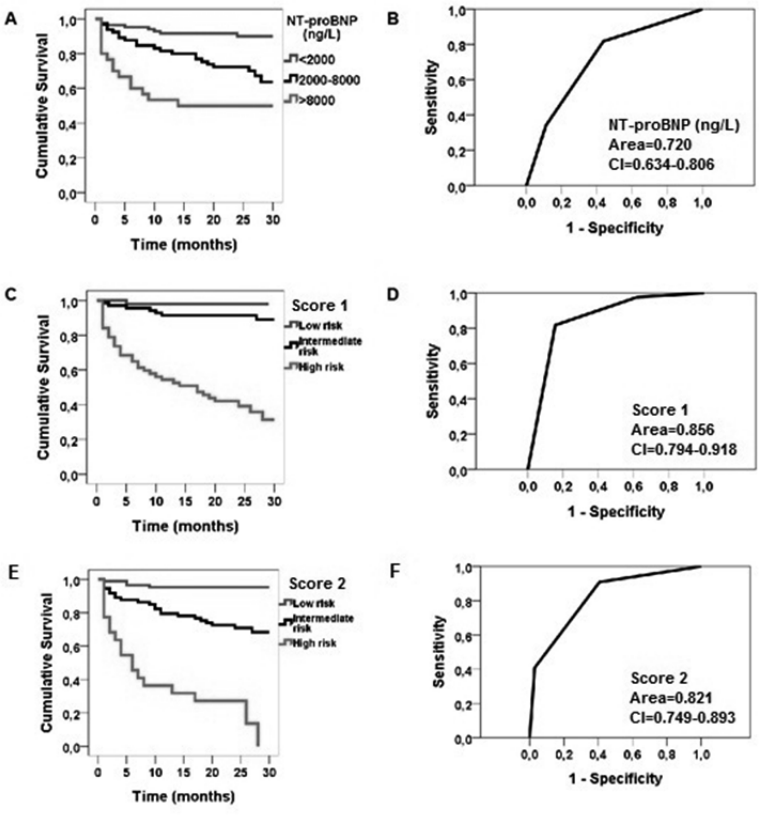


*Figure 2. Kaplan-Meier survival curve (A) and ROC curve (B) in patients with different levels of NT-proBNP (ng/L) in the HF cohort. Kaplan-Meier survival curve (C) and ROC curve (D) in patients with different levels of score in score system 1 in the HF cohort. Kaplan-Meier survival curve (E) and ROC curve (F) in patients with different levels of score in score system 2 in the HF cohort.*

# 5 DISCUSSION

## 5.1 Clinical phenotype of heart failure in the elderly vs study population

There were limited studies available in HF patients ≥85 years, most studies were in younger populations. The HF patients ≥85 years are estimated to constitute one-fourth of the HF population aged >65 years [9].

One study in HF patients ≥85 was Euro Heart Failure Survey II [6], however in this study there were not so many HF patients ≥85 years. In other studies there were not so many HF patients ≥85 years either [7, 21]. This might explain why previous findings about relations between ageing and incidences of comorbidities have not been consistent [6-9, 17-21]. Therefore our study is needed, since our sample size is almost 20 times larger in HF patients ≥85 years compared with previous studies [6, 7, 21]. According to our results, in HF patients ≥85 years there were increases in most of the cardiovascular and non-cardiovascular comorbidities compared with the ≤65 year group. Moreover most of the cardiovascular and non-cardiovascular comorbidities were declined in the >90 year group compared with the 85-90 year group.

However, there were comorbidities that continuously increase with age: renal dysfunction and anaemia. Therefore our study has extended previous findings about difference between elderly and younger through its large sample size.

In this thesis the population from prospective study should be regarded as representative as they are characterized by higher age, multiple comorbidities and a 2-year all-cause mortality in 25%, which was almost 8 times higher than those with non-HF. Regarding group with uncertain HF, it seems that many of them did not have HF since its all-cause mortality is less than 1/3 of HF group. This group with uncertain HF is quite large and constitutes about 17% of study population, indicating indeed an utmost challenge in HFNEF diagnostic in our daily clinical practice in particular in the elderly. In our retrospective studies, HF populations were decompensated hospitalized patients and were included without exclusions. Therefore they are also representative. As a complimentary to our retrospective studies, our prospective study are partly hospitalized and partly from Outpatient Clinic in one hand, and partly decompensated and partly stable in other hand. It is interesting to stress that it is important and clinically relevant to study such a heterogeneous population because HF in nature is extremely heterogeneous.

What is most important to point out is that in our prospective study all electrocardiogram, blood sampling and clinical examination were performed within approximately 24 hours from the time point when echocardiography was performed. To our knowledge most of previous clinical trials had echocardiography within 3-6 months prior to trials whereas in registry database or retrospective studies longer time in months or years could occur between echocardiography and biomarker analyses.

## 5.2 Prognostic value of NT-proBNP in elderly heart failure patients

In younger HF, NT-proBNP is a well-established prognostic biomarker [38, 41, 47-49]. However NT-proBNP is difficult for interpretation in the elderly partly because there are huge individual variations in particular when comorbidity is present, and partly because NT-proBNP increases dramatically with age. Our studies showed that only in a subgroup with NT-proBNP >8000 (ng/L), NT-proBNP was a significant prognostic indicator in those ≥75 years with acute decompensated HF. However, in the elderly HF patients referred for echocardiography NT-proBNP was significant in the multivariate analyses. This might be due to that patients with decompensated HF had more hemodynamic disturbance compared to the HF patients referred for echocardiography. Another factor could be that those elderly HF patients with decompensated HF had a mean follow-up by 5 years whereas HF patients referred for echocardiography had a mean follow-up only for 2 years.

Our results are in line with previous results. Several studies demonstrated that NT-proBNP was a prognostic indicator for mortality only in univariate but not in multivariate analyses in HF populations [41, 50]. Another study showed that NT-proBNP was significant in the multivariate analysis [42] in a similar HF population as elderly HF patients referred for echocardiography in our study.

Our results emphasized that in terms of prognostic value in the elderly, NT-proBNP level needs to be much higher than those in younger, in particular during acute decompensation. Therefore the prognostic value of NT-proBNP should be taken with caution when its level ≤8000 (ng/L) [2].

## 5.3 Prognostic value of a multiple marker modality incorporating biomarkers

It remains unknown whether new biomarkers are better than NT-proBNP in elderly HF patients. Therefore we analyzed a panel of biomarkers including both novel biomarkers and established NT-proBNP.

In this thesis we have demonstrated that all biomarkers in our study had significant associations with all-cause mortality in the univariate analyses in HF patients. However, in the three multivariate analyses only four biomarkers (NT-proBNP, cystatin C, RDW and MR-proANP) were significantly associated with all-cause mortality. Our results are in line with previous reports [28-29, 40, 42, 48-49, 51-54]. However when all above four biomarkers were analyzed in one multivariate analysis, the only biomarker that was significant for all-cause mortality was NT-proBNP.

Furthermore we were able to compare two different multiple marker modalities incorporating NT-proBNP and NT-proBNP alone in prognostic prediction. As shown by ROC curves in our study, the areas of ROC curves from two different score systems are higher than that using different levels of NT-proBNP. This is believed to be clinically relevant for those patients with NT-proBNP level between 2000-8000 (ng/L).

# 6 CONCLUSION

In HF patients most cardiovascular and non-cardiovascular comorbidities increase with age and decline in HF patients >90 years.

The prognostic value of NT-proBNP in elderly HF patients has to be interpreted with caution due to higher age and comorbidity.

In HF patients two different multiple marker modalities incorporating biomarkers were able to improve prognostic prediction compared to NT-proBNP alone.

Our studies strongly suggest that the development of multiple marker modality incorporating biomarkers reflecting different pathophysiological pathways might allow for better prognostic prediction in elderly HF patients.

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