Kidney function and congestive heart failure

The Norwegian perspective

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Abbreviations

ACEi	= Angiotensin converting enzyme inhibitor
ARB	= Angiotensin receptor blocker
BNP	= B-type natriuretic peptide
CKD	= Chronic kidney disease
CRS	= Cardio renal syndrome
DM	= Diabetes mellitus
ESA	= Erythropoiesis stimulating agent
ESRD	= End stage renal disease
eGFR	= Estimated glomerular filtration rate
GFR	= Glomerular filtration rate
HF	= Heart failure
IHD	= Ischemic heart disease
K/DOQI	= Kidney Disease Outcome Quality Initiative
LVEF	= Left ventricular ejection fraction
LVH	= Left ventricular hypertrophy
NEP	= Neutral endopeptidase
NPR-C	= Natriuretic peptide receptor type C
NT-ProBNP	= Amino terminal fragment of Pro B-type natriuretic peptide
NYHA	= New York Heart Association
RAAS	= Renin angiotensin aldosterone system
SNS	= Sympathetic nervous system

List of Papers

Paper I: Waldum B, Westheim AS, Sandvik L, Flonaes B, Grundtvig M, Gullestad L, Hole T, Os I. **Renal function in outpatients with chronic heart failure.** J Card Fail 2010 May;16(5):374-80.

Paper II: Waldum B, Westheim AS, Sandvik L, Flønæs B, Grundtvig M, Gullestad L, Hole T, Os I. **Baseline anemia is not a predictor of all-cause mortality in outpatients with advanced heart failure or severe renal dysfunction. Results from the Norwegian Heart Failure Registry.** J Am Coll Cardiol. 2012 Jan 24;59(4):371-8.

Paper III: Waldum B, Stubnova V, Westheim AS, Omland T, Grundtvig M, Os I. **Prognostic utility of B-type natriuretic peptides in patients with heart failure and renal dysfunction.** Submitted

1. Background

1.1 Chronic heart failure

Heart failure (HF) is a clinical condition where the heart is not able to meet the tissue need for oxygen delivery. Many definitions have been utilized the last decades. Current definition from European Society of Cardiology states that HF is a syndrome in which the patients should have symptoms of HF, typically shortness of breath and/or fatigue, signs of fluid retention and objective evidence of an abnormality of structure or function of the heart at rest (1). The term chronic HF is applied for patients with persistent abnormalities of heart structure or function. Coronary heart disease is the most common cause of HF and accounts for the majority of patients (2). Other important causes include valvular disease, cardiomyopathy and hypertension.

Chronic HF is a major public health issue with a prevalence of more than 23 million worldwide and is associated with significant morbidity and mortality (3;4). The incidence increases with age. Prevalence in the developed world is found to be about 1 % in the general population in persons aged around 50, rising to about 10 % in persons aged above 80 (5;6). At 55 years of age the lifetime risk of heart failure is estimated to 33 % for men and 29 % for women (7). Despite improvement in treatment options the last decades, the prognosis remains poor with 5-year mortality rates reported as high as 65 % (7).

Studies and clinical trials have documented survival benefits from modern heart failure treatment including medication and various device therapies, in HF patients with reduced left ventricular ejection fraction (LVEF). The recent years, awareness on the HF syndrome with preserved LVEF has increased. These patients account for half the patients with HF. To date no effective therapies have proven to change the natural history of these patients.

1.2 Chronic kidney disease

Epidemiological research in kidney diseases was long hampered by lack of a common definition of chronic kidney disease (CKD). In 2002 the Kidney Disease Outcome Quality Initiative (K/DOQI) proposed a classification of CKD for diagnosis and risk stratification (8). CKD stages 1 and 2 were defined as signs of kidney damage in patients with a normal or slightly reduced glomerular filtration rate, while CKD stage 3-5 were defined solely based on

glomerular filtration rate (GFR), and these patients with GFR < 60 ml/min/1.73 m^2 were all classified to have renal dysfunction (Table 1).

Table 1: Definition of chronic kidney disease (CKD) stages according to the K/DOQI guidelines (8).

CKD stage	
1	eGFR ≥ 90 ml/min/1.73 m^2 + proteinuria or structural kidney abnormality
2	eGFR 60-89.9 ml/min/1.73 m^2 + proteinuria or structural kidney abnormality
3	eGFR 30-59.9 ml/min/1.73 m^2
4	eGFR 15-29.9 ml/min/1.73 m^2
5	eGFR < 15 ml/min/1.73 m^2

Based on this classification increasing knowledge of the epidemiology and significance of chronic kidney disease have evolved. The prevalence of CKD has been estimated to be 11 % in the United States (9), and similar prevalence is found in the Norwegian population (10). Furthermore, the prevalence in the United States increased by 30% from 1990 to 2000 (11). More prevalent hypertension, diabetes mellitus and obesity partly explained the increase. In addition to increasing age, these are all important risk factors for development and progression of CKD. This raises concern about future increased incidence of end stage renal disease (ESRD) and other complications of CKD including increased cardiovascular disease (11;12).

1.3 Cardiorenal associations

Patients with ESRD are well known to suffer a high risk of cardiovascular morbidity and mortality, having more than 10-fold increased risk of cardiac death than age-/gender-matched controls in the general population (13). Cardiovascular risk is shown to increase even in early stages of CKD (14-17). Death due to cardiovascular disease is much more common in patients within all stages of CKD than reaching ESRD needing renal replacement therapy (18). Multiple explanations may exist for the high cardiovascular risk in CKD patients. Traditional risk factors for cardiovascular disease, such as hypertension, diabetes mellitus, smoking and dyslipidemia are risk factors for CKD as well, and highly prevalent in this population (19). In the course of declining renal function, kidney specific risk factors for cardiovascular disease like accumulation of uremic toxins, inflammation, anemia, disturbances in calcium and phosphorous balance, lack of active vitamin D and sodium and

water retention among others, will contribute to left ventricular hypertrophy (LVH), chronic inflammation, accelerated atherosclerosis, extravascular calcifications and endothelial dysfunction (20;21). CKD patients are also less likely to receive risk-modifying medication and interventions compared to non-CKD patients (22;23).

Just like cardiovascular disease is prevalent in patients with renal disease, primary heart conditions are commonly associated with renal dysfunction (24-28). Reduced renal perfusion, often predisposed by microvascular and macrovascular disease in the context of the same vascular risk factors associated with cardiovascular disease, may hemodynamically affect kidney function in HF patients (21). Furthermore, neurohormonal activation, renal venous congestion and adverse effects of pharmacotherapies used in the management of HF, have been suggested as causes for the high prevalence of renal dysfunction in HF patients (29;30). Regardless the cause, impaired and worsening, renal function in patients with acute and chronic HF is associated with adverse outcomes and prolonged hospitalization (25;26;28;31). Even slight decrease in GFR is found to significantly increase mortality risk in patients with chronic HF (25;27).

Combined heart and kidney dysfunction is common. Primary disorders of either kidney or heart often result in secondary dysfunction or injury to the other organ (32). A vicious circle with worsening renal and cardiac disease occurs, a condition with serious prognosis and high burden of symptoms.

The cardiorenal syndrome (CRS) was earlier used to describe a relatively normal kidney that is dysfunctional because of a diseased heart, with the assumption that in the presence of a healthy heart, the same kidney would perform normally (33). The more recent recognition of the numerous negative effects of heart disease on kidney function and kidney disease on heart function led to a new definition of the CRS (29), dividing into 5 different subtypes of cardiorenal syndrome. (Table 2)

Table 2: Definition of cardiorenal syndrome (CRS) (21)

Cardiorenal syndrome (CRS) general definition	A complex pathophysiological disorder of the heart and the kidneys whereby acute or chronic dysfunction in one organ may induce acute or chronic dysfunction in the other organ.
CRS type 1 (acute cardiorenal syndrome)	Abrupt worsening of cardiac function (e.g. acute cardiogenic shock or acute decompensation of chronic heart failure) leading to kidney injury.
CRS type 2 (chronic cardiorenal syndrome)	Chronic abnormalities in cardiac function (e.g. chronic heart failure) causing progressive chronic kidney disease.
CRS type 3 (acute renocardiac syndrome)	Abrupt worsening of renal function (e.g. acute kidney failure or glomerulonephritis) causing acute cardiac disorder (e.g. heart failure, arrhythmia, pulmonary edema).
CRS type 4 (chronic renocardiac syndrome)	Chronic kidney disease (e.g. chronic glomerular disease) contributing to decreased cardiac function, cardiac hypertrophy and/or increased risk of adverse cardiovascular events.
CRS type 5 (secondary cardiorenal syndrome)	Systemic condition (e.g. diabetes mellitus, sepsis) causing both cardiac and renal dysfunction.

1.3.1 Cardiorenal syndrome type 2.

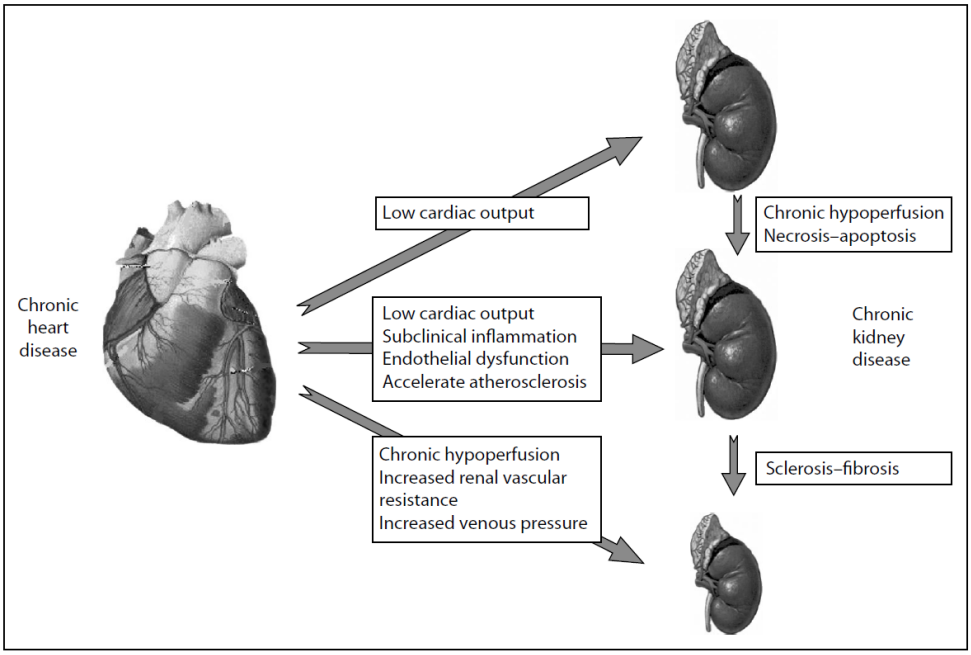

Figure 1: Cardio Renal Syndrome type 2. Ronco et al. Contrib.Nephrol. 2010 (21)

The present study included patients with chronic HF and thereby investigates topics related to cardiorenal syndrome type 2 (Figure 1). Impaired kidney function in patients with chronic HF is associated with adverse prognosis and even slight decreases in estimated GFR (eGFR) significantly increase mortality risk (27). Much of our knowledge on the relationship between HF and renal function originate from selected patient populations in clinical trials or hospitalized patients. There is limited understanding of the pathophysiology of renal dysfunction even in advanced heart failure. Hemodynamic issues as venous congestion and reduced renal perfusion due to powerful vasoconstriction are considered important (34;35). However, neurohormonal activation including the RAAS and SNS, oxidative stress, comorbidities and pharmacological heart failure treatment have a role as cardiorenal connectors affecting renal function (35). The explanation for the impaired prognosis observed in patients with chronic HF and CKD is also incompletely understood. A higher burden of comorbidities, more severe vascular disease and worsened cardiac function may explain part of the alteration. Furthermore, large randomized trials that have shaped the treatment of chronic HF in the two last decades, have consistently excluded patients with significant renal disease. Lack of evidence based clinical treatment may cause that patients with chronic HF and renal dysfunction are less likely to receive potential life-saving treatment (26), thereby altering prognosis.

1.4 Anemia in heart failure

Chronic heart failure is a syndrome with various pathophysiological consequences. Neuroendocrine, metabolic and immunological changes are identified as HF develops. Anemia has been recognized by clinicians in HF patients for decades, but has only recent years received attention with systematic research. CKD is a common comorbidity in HF patients with anemia. The kidneys are essential in the endogenous production of erythropoietin, and renal dysfunction may induce anemia in HF patients creating a vicious circle, termed the “cardio-renal-anemia syndrome” (36). In addition to renal dysfunction, anemia of chronic disease, iron-deficiency and hemodilution appears to be important etiologies of anemia in HF patients (37;38). Treatment with ACEi and ARB may also contribute to anemia as these agents may inhibit erythropoiesis (39). Depending on the definition used and population studied, the prevalence of anemia in HF patients varies from

9 to 69 % (40). Anemia is identified as an independent prognostic factor of mortality in HF patients (41) and hemoglobin levels are related to symptoms and quality of life (42). Increased myocardial workload due to hemodynamic and neurohormonal changes is a consequence of anemia, thereby inducing cardiac remodeling and left ventricular hypertrophy (LVH). This may partly explain the impaired prognosis of anemia in HF patients. Clinical trials investigating specific anemia treatment with erythropoiesis stimulating agents (ESA) and intravenous iron in HF patients with anemia have been promising in regard to symptoms and quality of life (43-45). Convincing data on mortality are missing and we do not yet know when to start anemia treatment, treatment goals and who will benefit from treatment. Actually, we don't know if anemia is a mediator or just a marker of poor prognosis in patients with chronic heart failure.

1.5 B-type natriuretic peptide in heart failure

B-type natriuretic peptide (BNP) is secreted from the cardiac myocytes predominantly as a response to wall distension and stretching. In addition to mechanical stimuli, neurohormonal activation including the renin-angiotensin-aldosterone system (RAAS) and the sympathetic nervous system (SNS) can act on cardiac myocytes to modulate BNP synthesis and secretion (46;47). BNP leads to vasodilatation and natriuresis, and acts as an antagonist on the RAAS and SNS (47;48). Patient BNP secretion may be measured as serum levels of the bioactive hormone BNP or its inactive amino terminal fragment (NT-proBNP). BNP and NT-proBNP are highly correlated and may be assumed to provide the same information about cardiac production of natriuretic peptides (49;50). Both are established as sensitive diagnostic markers of HF, and elevated levels are strong independent predictors of mortality in HF patients (51).

Renal dysfunction has been considered a confounding factor when evaluating the level of natriuretic peptides in HF patients. The kidneys are thought to be important in the clearance of both BNP and NT-proBNP, and levels are often elevated in patients with renal dysfunction even in the absence of clinical overt heart failure (52;53). Elevated natriuretic peptides in patients with renal dysfunction may therefore be a result of accumulation due to reduced renal clearance rather than a marker of cardiac disease.

The contribution of the kidneys in the removal of the natriuretic peptides is, however, controversial (54;55). CKD is associated with cardiovascular disease (29;56) and BNP elevation in asymptomatic patients with CKD has been shown to independently reflect ischemic heart disease and left ventricular hypertrophy (57;58). Thus, elevated BNP levels in heart failure patients with renal dysfunction might reflect increased cardiac production and not exclusively decreased renal clearance. As renal dysfunction is common in HF patients (59), the uncertainty of the interpretation of the natriuretic peptides in patients with renal dysfunction limits the use in clinical practice.

2. Aims of the thesis

1. Current knowledge on the prevalence and prognostic impact of renal dysfunction in HF patients is derived predominantly from hospitalized patients and highly selected study groups, and less is known about outpatients. We wanted to:
   - Describe the prevalence of chronic kidney disease in a Norwegian outpatient population with heart failure.
   - Evaluate the prognostic impact of renal dysfunction and identify associated factors to renal dysfunction in this population.

2. Convincing data on benefit of specific anemia treatment and who eventually will benefit from such treatment is still lacking. We wanted to:
   - Describe the prevalence and prognostic impact of anemia in Norwegian outpatients with heart failure and its relation to renal function.
   - Investigate if anemia is an independent prognostic variable also in patients with the worst prognosis.

3. The natriuretic peptides are proven to be an important prognostic variable in chronic HF, but the use in patients with renal dysfunction is questionable as levels of the natriuretic peptides are increased in renal dysfunction. Our aim was to:
   - Analyze the prognostic impact of BNP and NT-proBNP in patients with renal dysfunction to investigate if the natriuretic peptides are reliable prognostic markers also in these patients.

3. Material and Methods

3.1 Study Population

The Norwegian Heart Failure Registry was initiated in October 2000 with the intent to collect data on outpatients attending office visits in heart failure clinics (60). By March 2011, 6,482 patients were included and the number of participating clinics counted 24, situated in all regions in Norway. Cardiologists in cooperation with specially trained nurses run the heart failure outpatient clinics. Patients with heart failure of any etiology, New York Heart Association (NYHA) function class I-IV, diagnosed clinically according to guidelines from the European Society of Cardiology (1), were enrolled consecutively in the heart failure clinics. At the first visit (baseline) medical history, physical examination, echocardiography, laboratory results, and the medical management of heart failure were registered. After adjustment of medical therapy and undergoing an educational program, visit 2 was recorded. If required, additional clinical visits could be scheduled to ensure optimization of therapy before visit 2. Finally, visit 3 was planned to occur 6 months after visit 2. The chronology of visits and the number of patients that have attended the different visits by March 2011 are outlined in figure 2. Selected baseline characteristics of the population by March 2011 are presented in table 3.

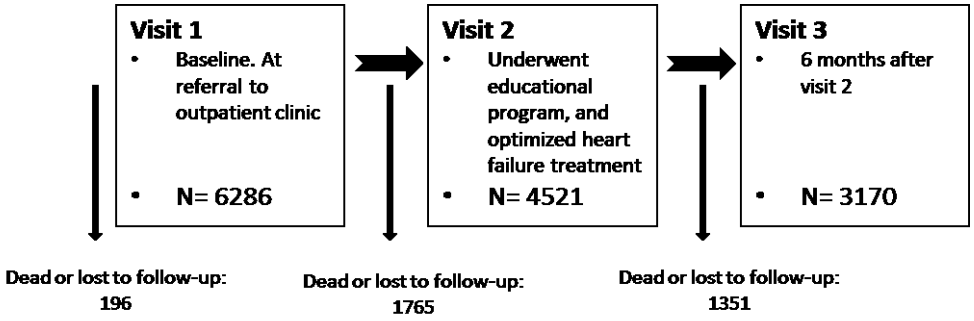

Figure 2: Flowchart of patients attending the registered visits and patients lost to follow-up between the visits, in the 6482 patients included in the Norwegian Heart Failure registry by March 2011.

Mortality data were retrieved from the Norwegian death registry kept by Statistics Norway. All participants provided written informed consent prior to inclusion in the database. Only

unidentifiable data were entered in the database. Permission for this analysis was granted from the National Data Inspectorate and the Regional Committee of Medical and Health Research Ethics. The clinical, echocardiographic and laboratory analyzes were performed locally at the different outpatient clinics.

Table 3: Selected baseline characteristics of 6286 patients included in the Norwegian Heart Failure Registry stratified by estimated glomerular filtration rate (eGFR).

	Valid data %	Total N=6286	eGFR<60 ml/min/1.73 m^2 N=2453	eGFR≥60 ml/min/1.73 m^2 N=3753	P-value
Age (years)	100	69.5 (12.1)	75.2 (9.3)	65.9 (12.2)	<0.001
Male gender	100	71.2 %	64.2 %	75.8 %	<0.001
BMI (kg/m^2)	88.1	26.3 (5.1)	25.8 (4.8)	26.6 (5.2)	<0.001
EF (%)	89.0	32.4 (11.3)	32.9 (11.9)	32.0 (10.9)	0.010
Coronary heart disease	96.0	54.9 %	64.4 %	52.5 %	<0.001
Vascular disease	94.1	15.1 %	19.5 %	12.2 %	<0.001
Diabetes mellitus	94.2	18.7 %	22.9 %	17.8 %	<0.001
NYHA class	98.5				<0.001
I		4.7 %	2.5 %	6.2 %	
II		43.2 %	35.8 %	49.2 %	
III		48.9 %	59.2 %	43.4 %	
IV		1.7 %	2.5 %	1.2 %	
eGFR (ml/min/1.73m^2)	99.5	67.3 (24.1)	44.8 (11.2)	82.0 (18.1)	<0.001
Hemoglobin (g/dl)	90.1	13.8 (1.7)	13.3 (1.7)	14.1 (1.6)	<0.001
Use of RAAS blocking drugs	99.8	88.2 %	83.7 %	91.1 %	<0.001
Loop diuretic dose (mg/day)	95.9	56.9 (58.2)	76.8 (70.7)	43.8 (43.8)	<0.001
Use of spironolactone	99.9	23.9 %	29.2 %	20.3 %	<0.001
Use of β-blocking drugs	99.9	83.2 %	82.8 %	83.6 %	0.436

BMI: body mass index, *EF*: left ventricular ejection fraction, *vascular disease*: earlier stroke or peripheral vascular disease, *eGFR*: estimated glomerular filtration rate, *RAAS blocking drugs*: use of ACE inhibitors or angiotensin receptor blocker, *loop diuretic dose*: daily furosemide equivalent dose.
Data presented as mean (SD) for continuous variables and percentage for categorical variables.

3.2 Definitions

3.2.1 Renal function

Renal function assessed as GFR, was estimated based on the simplified Modification of Diet in Renal Disease (sMDRD) prediction equation (61) and expressed as eGFR as follows:

$$eGFR\ (ml/min\ per\ 1.73\ m2) = 186.3*(serum\ creatinine\ (mmol/l))^{-1.154} *(age)^{-0.203} *0.742(Female)*1.212(Black)$$

Renal dysfunction was defined as eGFR < 60 ml/min/1.73 m2. Renal function was stratified according to GFR limits in the K/DOQI guidelines (8) (Table 1). Patients with eGFR greater than 60 ml/min per 1.73 m^2 were stratified into CKD stage 1 and 2, despite lack of data on renal damage. Severe renal dysfunction was defined arbitrarily as eGFR ≤ 45 ml/min per 1.73 m^2 as the number of patients with CKD stage 4 and 5 were low. This was done prospectively to add statistical power to the severe renal failure group.

3.2.2 Heart failure severity

Heart failure was categorized as New York Heart Association (NYHA) functional class I-IV based on symptoms during varying activities (Table 4). NYHA functional class III was divided into class IIIa and IIIb; patients in NYHA class IIIb were not able to cover 325 meters during the 6-minute walk test performed at the local heart failure clinics. Advanced heart failure was defined prospectively as NYHA class IIIb or IV.

Table 4: Definition of NYHA functional class

NYHA functional class	
I	Patient with cardiac disease but without resulting limitations of physical activity.
II	Patient with cardiac disease resulting in slight limitations in physical activity.
IIIa	Patient with cardiac disease resulting in marked limitation in physical activity. 6 minutes flat walking distance more than 325 meters.
IIIb	Patient with cardiac disease resulting in marked limitation in physical activity. 6 minutes flat walking distance less than 325 meters.
IV	Patient with cardiac disease resulting in symptoms during rest and discomfort during any physical activity.

3.2.3 Anemia

Anemia was defined according to the World Health Organization (WHO) guidelines as blood hemoglobin below 13.0 g/dl in men and below 12 g/dl in women (62). Hemoglobin levels were analyzed at the local heart failure clinics. Baseline anemia was defined as anemia at visit 1. Sustained anemia was defined as anemia at both visit 1 and visit 3. Transient anemia

was defined as anemia at baseline but not at the last visit, while patients with new onset anemia were not anemic at baseline, but anemic at the last visit.

3.2.4 B-type natriuretic peptide

The analyses on BNP and NT-proBNP were performed at the various hospitals using different assays. Natriuretic peptides analyzed with various methods are not necessarily comparable even when analyzing the same peptide (63). In order to take account of different reference values and methods, patients were allocated to BNP groups center-wise to be able to analyze the prognostic information of higher and lower levels of natriuretic peptides across centers. The BNP groups were defined by quartile limits in patients without renal dysfunction i.e. estimated glomerular filtration rate (eGFR) > 60 ml/min per 1.73 m^2. Patients with eGFR ≤ 60 ml/min per 1.73 m^2 were center-wise then assigned to BNP group 1 to 4 according to their level of natriuretic peptides. Centers included had to have sufficient number of patients, arbitrarily set to 40 or more, analyzed with the same assay. Patients from 13 outpatient clinics were included in the analyses. The patients were stratified according to their BNP or NT-proBNP level measured at the last attended visit. The average NT-proBNP levels which were used to define the BNP groups at the different centers were for group 1 < 495 pg/ml, group 2 495-1006 pg/ml, group 3 1006-2180 pg/ml and group 4 > 2180 pg/ml, while for BNP < 91 pg/ml, 91-204 pg/ml, 204-504 pg/ml and > 504 pg/ml in the 4 groups respectively.

3.2.5 Other definitions

A high proportion of the population was already treated with diuretics, RAAS blocking drugs and β-blockers at baseline. To differentiate between treatment intensity daily dose equivalents were calculated. Daily doses of loop diuretics were expressed in furosemide equivalents (bumetanide 1 mg = furosemide 40 mg). Doses of angiotensin converting enzyme inhibitors (ACEi) were expressed as enalapril equivalents per day (captopril 5 mg = ramipril 0.5 mg = lisinopril 1 mg = enalapril 1 mg). ACEi were more frequently used than angiotensin receptor blockers (ARB), and patients using ARB were excluded when analyzing ACEi daily dose in the baseline characteristics. Daily dose of β-blocker was expressed as metoprolol equivalents (bisoprolol 1 mg = carvedilol 5 mg = atenolol 10 mg = metoprolol 20 mg).

Vascular disease was defined as previous stroke and/or peripheral arterial disease. The diagnosis of ischemic heart disease (IHD) as the cause of heart failure was based on clinical evaluation at the time of inclusion.

3.3 Statistical analyses

3.3.1 General statistics

Analyses were performed using SPSS statistical software (SPSS inc. Chicago, IL, v.14.0-19.0). Level of significance was set to 0.05.

Continuous variables were presented as mean ± standard deviation. Selected non-normally distributed continuous data were presented as median and interquartile range. Categorical data were presented as percentages. Student T-test and analysis of variance (ANOVA) were used when comparing continuous data across categories of patients. For the same purpose Chi Square test was utilized on categorical data. Cases with missing data were deleted from the multivariate analyses.

Multivariate linear regression was used to identify associated independent variables of renal function at baseline and predictors of change in GFR during optimizing heart failure treatment. The assumption of linearity of continuous variables were checked and verified and residual analyses were used to check for normal distribution and equal variance. Spearman's test was used to check for multicollinarity, i.e. if pairs of independent variables were found to correlate to such a degree that they could not be simultaneously entered together in the regression analyses.

Multivariate logistic regression was used to identify associated independent variables when the outcome variable was dichotomous. The assumption of linearity of the logit of the outcome for continuous variables entered into the logistic models, were checked and found to be adequately met (64).

Kaplan-Meier plots were used to describe survival during the observation period in different patient groups. Log rank statistics identified differences between groups.

Multiple Cox regression analyses were performed to determine the association of different variables with all-cause mortality. For each Cox model the proportional hazard assumption was checked and found to be adequately met.

Analyses on selected interactions were performed by entering product terms into the different regression analyses.

3.3.2 Paper specific statistics

3.3.2.1 Paper I

The population was randomized into two equal sized samples: a model building sample where independent variables were identified, and a second sample to validate the independent variables identified in the model building sample. In the validation model only significant independent variables from the model building samples were included. Our study population was large enough to allow such analyses that served to improve the final model stability of the regression models.

3.3.2.2 Paper III

Nelson-Aalen plots were constructed to describe the change in hazard by length of follow-up time in patients with renal dysfunction within BNP group 1-3. This demonstrated that the assumption of proportional hazards were not met and led to separate Cox analyses on 2-year all-cause mortality and all-cause mortality beyond 2 years of follow-up. Figures were computed in STATA version 11.0 as the appearance and presentation of the figures were considered nicer using this software.

4. Summary of results

4.1 Paper I

The relationship between eGFR and all-cause mortality in outpatients with HF was investigated. Furthermore, factors associated with and predictive of impaired renal function were assessed. Renal dysfunction, defined as eGFR<60 ml/min/1.73 m^2, was highly prevalent (44.8 %) at baseline. Estimated GFR was an independent predictor of all-cause mortality together with age and NYHA class. Lower eGFR was independently associated with higher age, female gender, lower hemoglobin level, more severe heart failure (NYHA class), comorbidities (hypertension and vascular disease) and medications at baseline (spironolactone and higher diuretic dose). Higher systolic blood pressure, lower hemoglobin levels and the use of spironolactone predicted worsening renal function during follow-up, while higher dose of diuretics tended to predict worsening renal function.

4.2 Paper II

The impact on all-cause mortality of anemia in outpatients with HF, and specifically its prognostic utility in patients with severe renal dysfunction or advanced heart failure, was investigated. Baseline anemia was present in 24 % and was a strong independent predictor of all-cause mortality. However baseline anemia did not predict mortality in patients with severe renal dysfunction or advanced HF. In the selected population with valid follow-up data after optimizing heart failure treatment, sustained anemia implied worse prognosis irrespective of renal function and NYHA class, whereas transient and new-onset anemia did not.

4.3 Paper III

The prognostic utility BNP and NT-ProBNP levels in HF outpatients with renal dysfunction was investigated and compared with HF patients with preserved renal function. The majority of patients with renal dysfunction had BNP levels in the highest BNP group (59.1%). Patients with renal dysfunction and BNP levels in the lower three groups had similar 2-year survival as patients without renal dysfunction and comparable BNP levels. Beyond two years of follow-up renal dysfunction predicted worse outcome irrespective of levels of natriuretic peptides.

5. Discussion

5.1 Methodological considerations

5.1.1 Study population and study design

During the last decades an increasing number of medical patient registries have been established in Norway and the rest of the world. The ambition has been to continuously evaluate the treatment and outcome of defined patient groups in different treatment centers. The registries are organized as longitudinal observation of individual patients by recording course of treatment and outcome, thereby creating a continuously growing dynamic cohort reflecting real life management of a defined patient group. The Norwegian Heart Failure Registry was initiated in October 2000. Starting with 10 outpatient HF clinics, the number of participating clinics has continuously grown. The registry is now about to be given the status of a national quality registry, which will impose participation from all heart failure clinics in Norway.

A crucial term in epidemiology is causality. Observational studies are not able to conclude on causality as associations found may be an effect of confounding variables and other systematic errors rather than being a cause of the outcome. Furthermore, selection of the subjects is a common problem in cohort studies. In the present study, patients attending outpatient heart failure clinics were included in the cohort. This is probably a selected heart failure population as institutionalized patients were not admitted and patients with advanced comorbidities, including advanced renal dysfunction, were treated in other specialist clinics. Patients with less severe heart failure may not have got the heart failure diagnosis or were not admitted to the outpatient clinics. Loss to follow-up was another concern in the present study as a considerable amount of patients did not attend visit 2 and 3. The population that attended all visits differed significantly from patients who did not complete all visits, thereby weakening the reliability of the results from the follow-up analyses. Still another weakness concerning the registry cohort design was lack of clinical and laboratory records beyond visit 3. Change in important risk factors and habits were not detected as only all-cause mortality was registered beyond visit 3. As a consequence, the results in this study should be considered hypothesis generating rather than conclusive considering causality.

Experimental studies are considered the method of choice to investigate for causality. The randomized controlled trial (RCT) is designed to correct for all types of systematic errors, leaving the difference between the investigated groups to rely only on real effect of intervention or variation by chance (given by the p-value). However, experimental studies are highly consuming and not all questions are suitable to be answered by this study design. Furthermore, the studies are often carried out in highly selected patient populations, questioning the reliability of the results in the unselected population. In paper II this concern was illustrated when baseline anemia was found to be an independent marker of all-cause mortality, but not in patients with advanced heart failure or severe renal dysfunction. This interaction was not present in the selected population that attended subsequent visits where sustained anemia was an independent prognostic marker irrespective of NYHA class and level of eGFR. Thus, conclusions from experimental studies in highly selective populations are not necessarily transferable to the unselected population.

Well performed observational studies are necessary to generate good hypotheses prior to planning and performing good experimental studies. Well-organized patient registries will most likely, be increasingly important in the future, as the number of registries and the number of patients enrolled continuously rises.

5.1.1.1 Ethics

The study was observational. The patients were not exposed to any extra treatment or additional examinations as a consequence of participating in the study. All participants provided written informed consent prior to inclusion in the database. Only unidentifiable data were entered in the database. Permission for this analysis was granted from the National Data Inspectorate and the Regional Committee of Medical and Health Research Ethics.

The large set of data increases the possibility to find false statistically significant correlations and associations. The hypotheses and aims were defined prior to analyses to prevent publication of false positive results.

All authors have taken sufficiently part in the work to be listed as authors. The research and methods have been continuously discussed in the research group.

5.1.2 Estimation of GFR by creatinine based formulas

The definition and staging of CKD depends on the assessment of GFR, proteinuria and other markers of kidney disease. GFR cannot be measured directly. However, if a substance is present in stable concentrations in the plasma, is freely filtered in the glomerulus and neither secreted, reabsorbed, synthetized nor metabolized by the kidney the amount of that substance in the urine will equal the amount filtered in the glomerulus. Therefore, inulin clearance is considered an ideal method and gold standard of GFR measurement. Inulin clearance is however, demanding and difficult to use in daily practice. Alternative measures for estimating GFR have emerged including radionuclide technics of clearance of exogenous markers, 24-hour creatinine clearance or serum creatinine and cystatin C concentration, all having significant limitations in accuracy or availability.

Equations to predict GFR from serum creatinine have been tested in a large number of studies. Relevant equations have been shown to give more valid estimates of GFR than serum creatinine alone (65). Both American (66) and European (67) guidelines state that GFR estimation formulas are superior to serum creatinine alone, recommending the use of the Modification of Diet in Renal Disease (MDRD) formula (65) or the Cockcroft-Gault (CG) equation (68). More than 90% of the estimates were within 30% of the measured GFR when using MDRD formula (65), and this formula is found to perform better than the CG equation (66). Still, both equations are inaccurate in individuals, and loose accuracy in patients with near normal range GFR (GFR> 60 ml/min/1.73 m^2).

All creatinine based formulas are dependent on calibration of the serum creatinine assay. The analysis of serum creatinine was standardized in Norway between 2002 and 2005 to adjust to common Nordic reference intervals (69).

In patients with heart failure the MDRD formula was considered the most precise of the traditional creatinine based equations, especially in the lower ranges of GFR (70). The simplified MDRD (sMDRD) formula was slightly less accurate (70), but the easy accessibility in clinical practice by taking only serum creatinine, age, gender and ethnicity into account, makes it attractive to use. The sMDRD formula was used in our study to estimate GFR. The sMDRD equation tend to underestimate GFR in the near normal range of renal function (GFR > 60 ml/min/1.73 m^2), and overestimate GFR in the lower ranges, thus, the observed

associations between GFR and covariates and outcome in our study could be underestimated. Our study population was large, thereby correcting for the inaccuracy of the equation in individuals.

In paper 2, severe renal dysfunction was defined as eGFR < 45 ml/min/1.73 m^2. This arbitrary stratification was done prospectively to increase statistical power to the group with the worst renal function. Few patients in the registry had CKD class 4 and 5, perhaps because these patients were treated in nephrology clinics.

The chronic kidney disease epidemiology collaboration (CKD-EPI) equation was published in 2009 in an effort to be more generalizable across various clinical settings than the MDRD formula (71). The new equation appear to be more accurate than MDRD and result in lower prevalence of CKD (72). However, this equation is still not validated in patients with heart failure, and was not available when the current study was planned.

5.1.3 NYHA classification

Severity of HF is commonly classified due to symptoms and exercise capacity using the New York Heart Association functional classification (NYHA class). The classification was developed in 1928, and the latest revision was presented in 1994 (73). Despite that NYHA classification is a rough and subjective classification system with large inter- and intra-observer variability (74), it is proved to provide important prognostic information (75) and is applied routinely in clinical research.

The user manual in the Norwegian Heart Failure registry defines the criteria of classification into NYHA class (Table 4). According to the user manual NYHA class III was subdivided into IIIa and IIIb based upon the patient's ability to walk more or less than 325 meters during a 6-minute walk test. NYHA class IIIb has been increasingly used to categorize patients with advanced heart failure as the original classification system is not refined enough to characterize the broad spectrum of patients with advanced heart failure. NYHA IIIb is meant to classify patients who are more ill than the overall group of class III patients, but not as ill as those who are NYHA class IV. However, NYHA class IIIb has no consensus definition in the medical literature, and its use in clinical practice and research has been criticized (76). In the Norwegian Heart Failure Registry the stratification into NYHA class was done locally at the outpatient clinics, and no data on distance achieved during the 6-minute walk test was

available. In paper II, NYHA class IIIb and IV defined patients with advanced heart failure. This arbitrary classification was done prospectively to ad statistical power to the subgroup of patients with the most symptomatic heart failure. As the number of patients presenting NYHA class IV was low (1.6%) and the majority of patients were classified to NYHA class III (50.1%) a subclassification was required. A stepwise increase in risk of mortality with increasing NYHA class in our study supported the classification used (Figure 3).

Despite the subjective nature of the NYHA classification system, our study confirms its strength as a prognostic variable in the heart failure population.

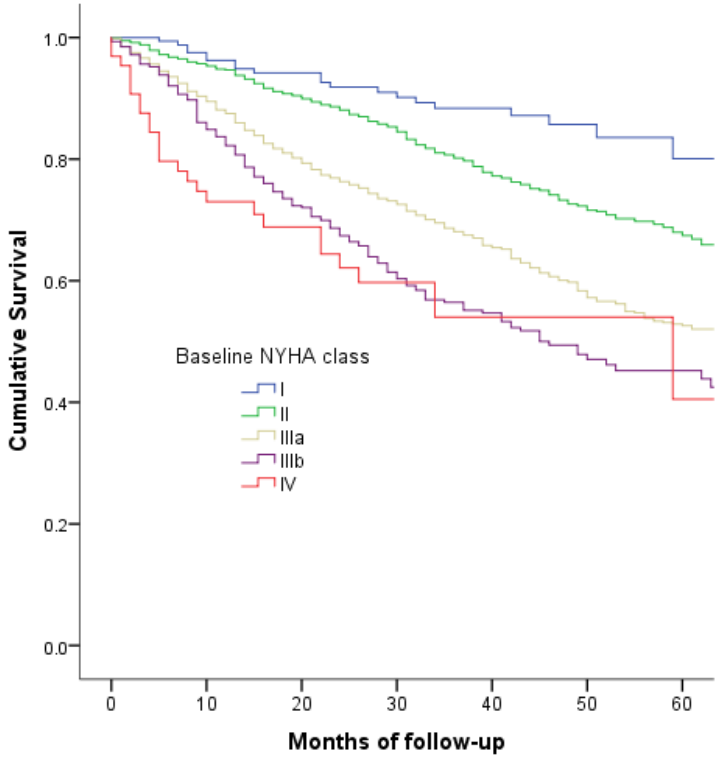

Figure 3: Kaplan-Meier survival plot in 4069 outpatients with heart failure stratified by New York Heart Association (NYHA) functional class at baseline. Log –rank statistics = 158.03 (p<0.001)

5.1.4 Definition of anemia

Anemia was defined according to the World Health Organization (WHO) guidelines as blood hemoglobin below 13.0 g/dl in men and below 12.0 g/dl in women (62). The definition was proposed by a WHO expert committee over 40 years ago, based on very few data using methods that were inadequate (77). Knowledge from more recent population studies suggest that the hemoglobin level for defining anemia should be adjusted for age, gender and ethnicity. Furthermore, the WHO definition generally underestimates the prevalence of anemia if the reference value should describe a hemoglobin level above which 95% or 97.5% of the normal subjects are included (77). In older patients and in blacks the WHO definition may overestimate the prevalence of anemia (77). Still the WHO definition is the most frequent definition of anemia used, also in the heart failure population (41).

5.1.5 Measurement of the natriuretic peptides

The natriuretic peptides were analyzed at the different participating centers using different assays. Patient BNP level may be measured either as the bioactive hormone BNP or the inactive amino terminal fragment (NT-ProBNP). BNP and NT-ProBNP are different biomarkers with different kinetics. While secretion is similar in response to appropriate stimuli (78), BNP is cleared from the circulation through proteolytic cleavage by neutral endopeptidase 24.11 (NEP) and binding to the clearance receptor natriuretic peptide receptor-C (NPR-C) (47). NT-ProBNP is not cleared by NPR-C and has a longer half-life than BNP (78). The majority of centers in the Norwegian Heart Failure Registry measured NT-ProBNP. Even within analyzes of the same peptide, different assays exists using different epitopes and with different epitope specificity. No applicable conversion factors exist between the different analyzes and assays. Hence, the levels of natriuretic peptides between the centers were not directly comparable. To overcome this methodological problem, BNP groups were created center-wise defined by quartile limits in patients without renal dysfunction i.e. eGFR > 60 ml/min/1.73 m^2. Thereby, it was assumed that the populations at each center were comparable and that this method classified the patients into 4 groups with increasing levels of natriuretic peptides irrespective of assay used and peptide measured. To check if these assumptions were fulfilled treatment center was entered into the multivariate

models in Paper III and this did not affect our results. Neither was treatment center an independent predictor of all-cause mortality. Furthermore, interaction analyses did not detect any differences between BNP and NT-ProBNP as the measured peptide. Therefore, we assumed that center-wise allocation into BNP groups would provide similar information irrespective of whether BNP or NT-ProBNP were used at the different centers. However, we cannot exclude the possibility that difference may exist between peptide analyzed and assay used.

Both BNP and NT-ProBNP are inversely related to GFR (79;80). Since both NEP and NPR-C are highly expressed in renal tissue and renal filtration and passive excretion might be responsible for some BNP clearance as well (78), higher levels in patients with renal dysfunction could be explained as an effect of accumulation (79;81). NT-proBNP level have been reported to be more affected by GFR (58;79). This could have affected our results if more patients with renal dysfunction were classified to higher BNP groups when NT-ProBNP was measured compared to BNP. However, Van Kimmenade et al. (54) have found BNP and NT-ProBNP to be equally dependent on renal function for their clearance, and both peptides are found to be similarly useful prognostic tools in renal patients (82;83). Again, no significant interaction between BNP and NT-ProBNP in the Cox-analyzes supported that patients with renal dysfunction were categorized into BNP groups independently of peptide analyzed.

Last visit BNP/NT-ProBNP levels were used to categorize patients into BNP groups. At visit 2 in the heart failure registry the treatment should have been optimized, and all patients did not complete all visits in the registry. Optimizing heart failure treatment is known to affect BNP levels (84-87). Therefore, the last registered visit was thought to represent the most standardized point zero for analyses of natriuretic peptides and length of follow-up.

Only 2076 patients, accounting for about 1/3 of the registry population, were allocated to a BNP group and included in the analyses in Paper III. Measurement of the natriuretic peptides has been increasingly available in Norwegian heart failure outpatient clinics since the start of the heart failure registry in the year 2000. Most patients included the first years had no registrations on natriuretic peptides, while most patients included the later years had multiple registrations. Furthermore, in order to categorize patients into BNP groups based

on quartile limits in patients without renal dysfunction, each center had to have a sufficient number of patients analyzed with the same method to be included in the analyses. Patients from 13 of the participating centers were included, excluding small centers, centers that changed their method of analysis during the period or introduced methods for analysis or registration of natriuretic peptides more recent years. The population with valid BNP registrations differed in baseline characteristics significantly from the rest of the registry population by having shorter follow-up time, younger age, higher systolic blood pressure, higher se-sodium level, lower ejection fraction and less IHD. Thus, selection bias, meaning that registrations of last visit BNP/NT-ProBNP were more likely in certain patients, cannot be excluded.

5.1.6 Statistical considerations

5.1.6.1 Assumptions of the regression analyses

Statistical models are approximation of data. In linear regression, the linear assumption does not mean that all observations fit a linear model; it means that a line is a good representation of the association between variables. Likewise, logistic and proportional hazard regression analyses are theoretical models with assumptions which have to be fulfilled if the analyses should be valid.

For every regression model presented in the papers the assumptions of each model were checked. As a consequence some variables were transformed to fit the assumptions. In paper III, the assumption of proportionality was not met in the Cox analysis that analyzed the prognostic effect of lower BNP levels in patients with renal dysfunction. The analyses were then split into the first 2 years of follow-up and beyond 2-years of follow-up to fulfill the assumption of proportionality.

5.1.6.2 Variable selection techniques

The database included a large number of variables and a decision which variables to enter into the different regression models had to be made. The sample size in the current study was large and did not restrict the number of variables entered into the models. However, a manageable number of independent variables necessary to estimate outcome was desired.

Automatic variable selection techniques available in the statistical software use statistical criteria to decide which and in what order, the variable should enter the model. The forward selection technique is desirable when sample size is small to minimize the number of variables entered into the model. However, forward selection does not deal well with suppresser effects. Likewise, a backward selection technique has a potential of not entering important confounders into the model. The variable selection in this study was mainly carried out by a combination of theoretical understanding and the univariate statistical relation with the outcome. In addition selected variables were not entered into regression models because of low numbers of valid data. Modest confounding variables may therefore have been missed in our analyses. Furthermore, analyses were restricted to existing variables in the heart failure registry.

5.1.6.3 Interactions

Still another assumption of multivariate regression analyses is that the independent variables should have additive effect on the outcome. Interactions are present when the variables are not additive, but rather something greater or less than additive. The regression models calculate the best coefficient for each variable for the sample as a whole, this may not be best for every subgroup of subjects.

In paper II, checking for interactions were predefined as one of the aims of the study. Knowing that anemia was an independent marker of all-cause mortality, the aim was to investigate if this was valid also in patients with severe renal dysfunction or advanced heart failure. The conclusion was that there was a significant interaction and that anemia was not an independent prognostic marker in these patients. This may indicate that anemia should be interpreted different in different subjects.

In the current study a large number of variables were analyzed, and thus a huge amount of potential interactions may exist in the dataset. It would have been almost impossible to identify all significant interactions in the present analyses, and some statistically interactions are difficult to interpret clinically when recognized. Furthermore, with increasing number of interaction analyses it is likely that at least one of them will be statistically significant, thereby increasing the risk for type I errors, i.e. rejecting the null hypothesis of no interactions when the null hypothesis is really true. As a consequence, interaction analyses

by product terms were performed when they were thought to be theoretically important. All interaction analyses performed where described in the methods section of each paper.

5.1.6.4 Validation sample

In paper I the regression analyses were performed in two steps to strengthen the final model stability. This type of analysis is rarely done in medical research. We observed that the effects of the independent variables in the regression models were, on average, about 30% lower in the validation sample than in the initial model building sample. This observation is not surprising to statisticians (88) and indicates that regression models without validation may overestimate the effect of individual variables and thus, could lead to faulty conclusions.

5.2 Discussion of results

5.2.1 Paper I

5.2.1.1 Prevalence and consequence of impaired renal function in outpatients with HF

Nearly half of outpatients with HF attending Norwegian heart failure clinics had significant reduction in renal function i.e. eGFR < 60 ml/min/1.73 m^2. In accordance with other studies (25-27;31), this confirmed that renal dysfunction is highly prevalent in the HF population. Furthermore, GFR was verified as a strong independent predictor of all-cause mortality (24;25;27;89). The proportion of patients in CKD stage 4 and 5 (i.e. eGFR< 30 ml/min/1.73 m^2) was lower than expected from comparable studies (25;26;31). This might indicate that the prevalence of renal dysfunction was actually underestimated as those with the most advanced renal failure would be more likely to attend a renal clinic rather than a HF clinic in Norway. Nevertheless, cardiorenal associations are common and awareness of its consequences and how to preserve renal function ought to be important for every physician dealing with heart failure patients.

5.2.1.2 Medications associated to GFR in HF patients

The relationship between CKD and HF is not fully understood (29). There is limited understanding of the pathophysiology of renal dysfunction even in advanced heart failure. Hemodynamic issues like venous congestion and reduced renal perfusion are important

(34;35). A 25 % reduction in cardiac output is shown to at least halve the renal perfusion (90). The positive effect on GFR by cardiac resynchronization therapy (91) underlines the importance of hemodynamic issues in cardiorenal syndrome type 2 and should motivate optimized heart failure treatment in these patients. However, medications used in heart failure therapy may potentially influence renal function. Lack of evidence in patients with renal dysfunction and fear of further decline in GFR may contribute to that HF patients with impaired kidney function are less likely to be described efficacious therapies. Evolving data though, indicate that patients with renal dysfunction have better outcomes if they receive these medications (25;26). In our study, close to 90% of patients received RAAS-blocking agents, and nearly 80% used β-blocking drugs at baseline. This is higher than in comparable studies (25;26;31), indicating that our study population was reasonably well treated at inclusion. Still the treatment was intensified during the follow-up period to the last visit in the registry (60).

At baseline a wide range of variables were found to be associated with GFR. Increasing age, female gender, history of hypertension and previous stroke or vascular disease were all independently related to lower GFR. More interestingly, the potentially modifiable factors: higher NYHA class, lower hemoglobin level, higher loop-diuretic dose and the use of spironolactone were independently associated with worse renal function at enrollment in the study. Use of β-blocking drugs and treatment with RAAS blocking drugs were not independently associated with GFR. Associated factors at enrollment are not easy to evaluate as they could be a consequence as well as a cause of renal dysfunction. However, the results may be read as that Norwegian HF patients are likely to be prescribed RAAS- and β-blocking drugs irrespective of renal function. Diuretics, however, were prescribed at a higher degree among patients with impaired kidney function at baseline. Higher doses of loop diuretics are required in patients with renal insufficiency than in those with normal renal function to achieve the same diuretic response (92). This may explain the association between loop diuretic dose and GFR found at baseline. More surprisingly we found that the use of spironolactone was independently related to declining GFR. Despite convincing data regarding prognostic effect in patients with advanced systolic HF (93), concern has been raised about worsening renal function and hyperkalemia with this treatment (94). Treatment

with spironolactone is actually considered contraindicated in patients with renal dysfunction.

During a median follow-up time of 9 months whereas the heart failure medication was optimized and the patients underwent an educational program, changes in GFR were analyzed in a subpopulation of the registry. While mean change in eGFR was modest, 1/3 of the patients experienced a clinical significant drop in GFR of more than 10 %. These patients would be expected to carry a worse prognosis (28;95). Increased blood pressure, lower hemoglobin levels and the use of spironolactone predicted deterioration of renal function, while a trend was found for intensified dosage of loop-diuretics.

HF patients with renal dysfunction have largely been excluded from the large clinical trials that have established modern HF treatment. RAAS blocking drugs have been a concern as they interfere with the kidney's regulation of glomerular filtration pressure and are a major risk factor for acute kidney injury in situations where kidney perfusion is affected. However, the same drugs have important renoprotective properties by slowing the decline in GFR, preventing diabetic nephropathy, reducing proteinuria and reducing risk of dialysis (96;97). Actually, growing evidence exists that patients with HF and renal dysfunction may benefit more from ACEi and ARB than patients with HF alone (96). In our study change in intensity of RAAS blocking drugs, together with β-blockers, did not predict changes in GFR during a median 9-month of follow-up. This may indicate that fear of worsening renal function by introducing or intensifying these treatments is exaggerated.

Treatment with spironolactone was an independent predictor of declining renal function during follow-up. It is a concern that the group of patients proven to benefit from the treatment (the most advanced HF), is the group of patients with the lowest GFR and at highest risk of the feared adverse effects of hyperkalemia and worsening renal function (94). Careful selection of patients and close monitoring seems warranted for HF patients treated with spironolactone.

Loop-diuretics are widely used in HF, but their effect on outcomes has not been fully evaluated in large clinical trials. No doubt, diuretics are efficacious in relieving clinical symptoms of shortness of breath and signs of peripheral edema. Decongestion may also improve cardiac performance by reducing left ventricular preload. However, diuretic

activation of neurohormonal systems like RAAS and SNS may be associated with increased morbidity and mortality (98-102). Influence on GFR may explain part of this association as neurohormonal activation is important in the pathophysiology of both HF and progression of CKD. Higher dose of loop diuretics was strongly associated with low GFR in our study. This is known from other studies (103). Furthermore, higher doses of loop diuretics tended to predict further decrease in GFR, though it did not reach statistical significance in the validation sample. Neurohormonal activation in combination with risk of volume depletion with excessive use of diuretic, may affect renal perfusion and GFR in HF patients. Conversely, diuretic therapy may favorably affect renal and tubular function by decreasing congestion (104). The definitive role of loop diuretics remains unclear and they appear as a two-edged sword with negative consequences on heart and renal function by both too low and too high doses. A large randomized trial is longed for, but probably will never occur. Hopefully, new therapies will emerge that demonstrate effective decongestion with less neurohormonal activation (105).

5.2.2 Paper II

5.2.2.1 Epidemiology of anemia in HF patients

Baseline anemia was common in our registry patients. Renal dysfunction, activation of neurohormonal and inflammatory responses, malnutrition, iron deficiency, drug effects and bone marrow hyporesponsiveness seem to contribute to the development of anemia in HF patients (38;39). Estimates of anemia prevalence in HF patients have varied widely (40;106;107), much due to different definitions of anemia used and differences in study population. Hospitalized patients with the highest degree of accompanying diseases and congestion tend to have the highest prevalence, whereas highly selected clinical trial cohorts have the lowest prevalence as patients with comorbidities are largely excluded from clinical trials. Compared to other outpatient HF populations, our baseline prevalence of 24% was coincident.

5.2.2.2 Prognostic impact of anemia in patients with HF and renal dysfunction

Baseline anemia was strongly associated with all-cause mortality in our population. Median survival was more than 3 years shorter in anemic, compared to non-anemic patients. Thus, anemia - independent of cause - identified patients with a poor prognosis and should alert

the clinician to the need for particular attention. After adjustment for a large range of confounders, anemia remained a strong independent risk factor for all-cause mortality. The mechanisms behind the increased mortality risk in anemic HF patients are not completely understood. Increased cardiac work, neurohormonal activation, reduced renal perfusion and salt and water retention could be consequences of anemia which might affect prognosis (107).

Both lower GFR and higher NYHA functional class were independently associated with anemia at baseline. The simultaneous presence of anemia, HF and CKD is referred to as the cardio-renal-anemia syndrome (36;108). Anemia can cause or worsen CKD and HF, at the same time it may be a consequence of either of the 2 conditions as well (108). As epidemiological data have suggested an inverse relationship between serum creatinine level and the prognostic information of anemia in HF patients (41), we wanted to evaluate the prognostic impact of baseline anemia on all-cause mortality in the subgroups of HF patients with the most advanced NYHA class and most severe renal dysfunction. Strikingly, we found that anemia was not an independent predictor of mortality in these patients. This might indicate that the ill prognosis in these patients accounts for a greater proportion of the mortality risk than anemia, but also may indicate that lower hemoglobin is somewhat compensatory in patients with the worst prognosis by lowering blood viscosity and thereby potentially reduce the risk of thromboembolic events (109) and improve tissue perfusion.

Hemoglobin correction trials in CKD patients undergoing dialysis were initially promising and proved to significantly improve symptoms and quality of life. However, concerns about increased risk of cardiovascular events and mortality have evolved following treatment with ESA in the CKD population (110-112). As a consequence current CKD guidelines recommend hemoglobin levels not to exceed 13 g/dl when treated with ESAs, to prevent excess cardiovascular events (113;114). Likewise hemoglobin correction with iron supplement and ESA in heart failure patients is promising with proved improved exercise capacity and improved hemodynamics (43-45;109). However, no evidence on gain in life expectance exists and target hemoglobin level is uncertain. Our data may be important in selecting patients that would probably not profit from anemia treatment, thereby guiding a design of future clinical trials to investigate the prognostic effect of anemia treatment.

Anemia was not necessarily persistent; a high proportion of patients who were anemic at baseline had normal hemoglobin levels at follow-up. Likewise, new-onset anemia was equally prevalent. The patients with transient anemia did not have significantly higher mortality rates compared to patients without anemia at any visit, a finding that is supported by Tang et al. (115). New onset anemia is, however, shown to predict outcome in HF patients (115;116). After multivariate correction, we could not confirm this, but low number of patients in this group gave small statistical power to this analysis. Sustained anemia however, strongly predicted mortality and among these patients no interactions with NYHA class and GFR were found.

The ideal hemoglobin level for patients with heart failure is not known and the definition of anemia in HF patients is arbitrary as outlined in section 5.1.4. Lower hemoglobin levels in our patients with sustained anemia were associated with increased mortality, thus the degree of anemia would probably identify patients at especially high risk of mortality. Hemoglobin level was not associated with all-cause mortality in patients with no or transient anemia. Increased risk of mortality and hospitalization and reduced exercise capacity are found in HF patients with hemoglobin levels below 13 g/dl (117;118). In concert with these studies, our data did not imply better prognosis with hemoglobin levels exceeding 13 g/dl.

An interesting observation in our material was the different conclusions drawn when we analyzed baseline anemia in the entire population compared to sustained anemia in the selected subgroup of the patients completing all visits in the study. Completing all visits was independently associated with better survival from the date of the last visit. This might reflect that being able to attend subsequent visits is an independent marker of better health. Randomized clinical trials are considered the gold standard of clinical evidence. However, if being able to participate in clinical trials is an independent variable of better health, it is uncertain whether the conclusions drawn from such studies would be applicable for an unselected outpatient cohort.

5.2.3 Paper III

5.2.3.1 Prognostic utility of BNP/NT-ProBNP in patients with HF and renal dysfunction

Renal dysfunction is considered a confounding factor when evaluating the level of natriuretic peptides in HF patients. The association between BNP/Pro-BNP and renal function is complex. Patient with CKD tend to have higher atrial pressure, systemic blood pressure, ventricular mass and more vascular disease than the general population, all of which could lead to higher "true" physiological levels of natriuretic peptides (78). On the other hand, decreased renal filtration and renal clearance by NPR-C and NEP within renal tissue might increase the levels. Furthermore decreased renal responsiveness to BNP in the kidneys may contribute to the higher levels observed in CKD patients (78).

Our study confirmed the strong association between renal dysfunction and level of BNP/NT-ProBNP. The majority of patients with eGFR ≤ 60 ml/min/1.73 m^2 had BNP/NT-ProBNP levels in the highest BNP group (59 %). Earlier studies have demonstrated the impaired prognosis in HF patients with renal dysfunction and elevated natriuretic peptides (119;120). In contrast to these studies, the main focus in paper III was on the patients with renal dysfunction and still not levels of BNP/NT-proBNP in the highest BNP group, accounting for 41 % of the patients with renal dysfunction. Interestingly, these patients presented equal 2-year all-cause mortality as patients without renal dysfunction and with comparable BNP/NT-ProBNP levels. Thus, BNP/NT-ProBNP predicted 2-year mortality irrespective of renal function in these patients. This has to our knowledge not previously been highlighted. In the discussion whether impaired renal function is a confounding variable when evaluating BNP/NT-ProBNP in HF patients, this could be an important observation. Our study was not designed to decide if the strong association between renal dysfunction and levels of natriuretic peptides is a consequence of increased production, decreased clearance or both. However, our data stated that BNP/NT-ProBNP was an important prognostic marker also in patients with impaired renal function. Furthermore, our data could propose that BNP/NT-ProBNP should be interpreted as the current burden of heart and vascular disease at the time of investigation also in CKD patients, without considering the actual mechanism behind.

We observed an exponentially increasing hazard of all-cause mortality by time in the patients with impaired renal function and within BNP group 1-3. Beyond two years of follow-up the initial BNP/ProBNP level did not predict mortality in patients with renal dysfunction. As BNP/NT-ProBNP are short acting hormones, reflecting primarily actual myocardial wall stress (78;121), it would not be surprising that they primarily predicted shorter term prognosis. The exponentially increasing hazard of all-cause mortality by time in CKD patients may provide insight in the increased susceptibility to progressive heart and cardiovascular disease in patients with CKD (29;122;123). Consecutive measurements of natriuretic peptides would have been interesting. Then, it would be possible to evaluate progression of HF and changes in BNP/NT-ProBNP levels in patients with renal dysfunction compared to patients with preserved GFR, and thereby investigate whether changes in BNP/NT-ProBNP during follow-up would yield prognostic information in patients with renal dysfunction. This question is to our knowledge not addressed yet and should be investigated in a future prospective trial.

6. Summary

Renal dysfunction is common in outpatients with heart failure as nearly half the patients admitted to Norwegian outpatient HF clinics had eGFR<60 ml/min/1.73 m^2. GFR was a strong independent predictor of all-cause mortality and preserving renal function during treatment and follow-up, ought to be important for every physician dealing with heart failure patients. The relationship between GFR and HF is not fully understood, but hemodynamic issues and neuroendocrine activation seem important. Spironolactone and higher doses of loop diuretics were associated with worse renal function at baseline and deterioration of renal function during follow-up, while RAAS- and β-blocking drugs were neutral concerning kidney function. While our registry data could not determine causal effect, they indicate that use of loop diuretics and spironolactone should be carefully evaluated as they are associated with negative effect on renal function, and thereby may worsen prognosis. Prospective randomized trials would possibly have answered this question, but such studies will probably never be undertaken.

Anemia was prevalent, 24% of the patients presented with anemia at baseline. Anemia was independently associated with impaired GFR and increasing NYHA class. Baseline anemia was a strong independent marker of prognosis, but was not a predictor of all-cause mortality among the patients with the worst prognosis i.e. patients with severe renal dysfunction and advanced heart failure. Anemia was not necessarily persistent, transient and new-onset anemia did not predict worse prognosis in our study in contrast to sustained anemia. Clinical trials are needed to investigate the effect of anemia treatment on all-cause mortality. Our data suggest that the patients with preserved GFR and lower NYHA class may benefit the most from anemia treatment.

Renal dysfunction has been considered a confounding factor in the interpretation of natriuretic peptides in HF patients, thereby limiting their use in daily practice. Our study confirmed the strong association between renal dysfunction and level of BNP/NT-ProBNP. However, as BNP/NT-ProBNP predicted 2-year mortality irrespective of renal function in patients within the lower three BNP groups, our data might indicate that the natriuretic peptides should be interpreted the same way in patients with renal dysfunction as in patients with preserved renal function. BNP is released from cardiac myocytes predominantly as a result of myocyte stretch and cardiovascular diseases associated with

renal dysfunction are prone to increase cardiac work load. Therefore, BNP/NT-ProBNP levels might reflect current burden of cardiovascular disease in patients with renal dysfunction. Evaluation of both BNP/NT-ProBNP and GFR in patients with HF might prove valuable in the evaluation of both short- and long-term prognosis.

7. Future perspectives

The prevalence of HF is high and may continue to rise in the following years as the number of elderly is rising. Traditional variables used to identify HF patients with poor prognosis have been higher age, low LVEF, ischemic etiology of HF, higher NYHA class and hyponatremia among many others (1). In our study; GFR, anemia and the natriuretic peptides together with increasing age and NYHA class were the most important independent prognostic factors concerning all-cause mortality. LVEF was not an independent risk factor of all-cause mortality in our analyses, neither was ischemic heart disease. Traditional prognostic markers were established in an era where heart failure patients may have been different from today's HF population. Treatment of coronary heart disease, the main cause of HF, has improved during the last decades, while the prevalence of hypertension and chronic diseases, like CKD, that predispose to HF is increasing. Thus, the etiology of HF might be changing. Furthermore, treatment possibilities in patients having or being at risk of HF have evolved. Traditional prognostic markers may therefore fail to alert the clinicians about which patients are at high risk of impaired prognosis. Predictive factors for the prognosis in HF patients ought to be updated and reconsidered in the future guidelines.

Furthermore, current knowledge of HF treatment is founded on studies of HF patients with reduced LVEF and relative lack of comorbidities. A suspected change in etiology of HF, growing number of elderly with HF and increasing number of HF patients living with comorbid chronic diseases would make these studies less representative for the real life HF population. No therapies have proven to alter the natural history of HF patients with preserved LVEF, and likewise there is lack of evidence of effective therapies in HF patients with comorbidities like renal dysfunction. Randomized controlled trials in these patients would be of great interest. Such studies are however, demanding and it is not likely that trials of currently established therapies will ever occur in additional groups of HF patients. Well-designed observational registry studies might therefore be increasingly important, and lead to improved treatment and outcome of HF patients in the future. Establishing, and continuously improving, well-functioning national and international HF registries should be given priority and be a part of the planning of healthcare.

8. Further research

As demonstrated, the cardiorenal associations in patients with heart failure are complex and not fully understood. Diabetes mellitus (DM) is a major risk factor of both cardiac and renal disease and the prevalence of DM is rising in the overall population. As a consequence increasing numbers of HF patients will have DM in the future. DM is considered an important factor of poor outcome in patients with HF, but there are no robust data in patients with HF with preserved LVEF and non-ischemic heart disease. Furthermore, treatment of patients with combined HF, renal dysfunction and DM needs to be evaluated. The Norwegian Heart Failure Registry may provide insight into the prevalence and prognostic importance of DM in HF patientsand current HF treatment options may be assessed in relationship to the presence of DM.

Uric acid is associated with poor prognosis in patients with HF and is associated with renal function, use of diuretics and DM. Whether uric acid is a mediator or marker of disease is debated, but a growing number of reports gives theoretical rationale that uric acid might contribute to disease progression in HF patients. Data from our registry might give further insight into this intricate question.

Health related quality of life is given increasingly attention as validated forms are developed to measure this dimension of health. Self-perceived quality of life is important for the patients and quality of life should most likely be routinely assessed regulary in HF patients. Our registry can provide data of quality of life using a validated tool, and this might expand the horizon of cardiorenal associations.

9. Errata list

Paper I, Heading Table 3: "…dependent variable Δ New York Heart Association…" was a misprint and should be "…dependent variable Δ estimated glomerular filtration rate…."

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