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Cystatin C, a measure of renal function, as prognostic risk marker in acute heart failure

Studies on the cardiorenal syndrome

Johan Lassus

ACADEMIC DISSERTATION

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Abstract

Acute heart failure (AHF) is a complex syndrome associated with exceptionally high mortality. Still, characteristics and prognostic factors of contemporary AHF patients have been inadequately studied. Kidney function has emerged as a very powerful prognostic risk factor in cardiovascular disease. This is believed to be the consequence of an interaction between the heart and kidneys, also termed the cardiorenal syndrome, the mechanisms of which are not fully understood. Renal insufficiency is common in heart failure and of particular interest for predicting outcome in AHF. Cystatin C (CysC) is a marker of glomerular filtration rate with properties making it a prospective alternative to the currently used measure creatinine for assessment of renal function.

The aim of this thesis is to characterize a representative cohort of patients hospitalized for AHF and to identify risk factors for poor outcome in AHF. In particular, the role of CysC as a marker of renal function is evaluated, including examination of the value of CysC as a predictor of mortality in AHF.

The FINN-AKVA (Finnish Acute Heart Failure) study is a national prospective multicenter study conducted to investigate the clinical presentation, aetiology and treatment of, as well as concomitant diseases and outcome in, AHF. Patients hospitalized for AHF were enrolled in the FINN-AKVA study, and mortality was followed for 12 months. The mean age of patients with AHF is 75 years and they frequently have both cardiovascular and non-cardiovascular co-morbidities. The mortality after hospitalization for AHF is high, rising to 27% by 12 months.

The present study shows that renal dysfunction is very common in AHF. CysC detects impaired renal function in forty percent of patients. Renal function, measured by CysC, is one of the strongest predictors of mortality independently of other prognostic risk markers, such as age, gender, co-morbidities and systolic blood pressure on admission. Moreover, in patients with normal creatinine values, elevated CysC is associated with a marked increase in mortality. Acute kidney injury, defined as an increase in CysC within 48 hours of hospital admission, occurs in a significant proportion of patients and is associated with increased short- and mid-term mortality. The results suggest that CysC can be used for risk stratification in AHF.

Markers of inflammation are elevated both in heart failure and in chronic kidney disease, and inflammation is one of the mechanisms thought to mediate heart-kidney interactions in the cardiorenal syndrome. Inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) correlate very differently to markers of cardiac stress (i.e. NT-proBNP) and renal function. In particular, TNF-α showed a robust correlation to CysC, but was not associated with levels of NT-proBNP. Compared to CysC, the inflammatory markers were not strongly related to mortality in AHF.

In conclusion, patients with AHF are elderly with multiple co-morbidities, and renal dysfunction is very common. CysC demonstrates good diagnostic properties both in identifying impaired renal function and acute kidney injury in patients with AHF. CysC, as a measure of renal function, is also a powerful prognostic marker in AHF. CysC shows promise as a marker for assessment of kidney function and risk stratification in patients hospitalized for AHF.

Sammandrag

Akut hjärtsvikt är ett mångfasetterat tillstånd med exceptionellt hög dödlighet. Forskningen har dock inte i tillräckligt stor utsträckning undersökt kliniska karaktärsdrag och prognostiska faktorer hos patienter med akut hjärtsvikt. Njurfunktionen har visat sig spela en nyckelroll för prognosen hos patienter med hjärt-kärlsjukdomar. Mekanismerna bakom detta samband mellan hjärta och njurar, ofta kallat det kardiorenala syndromet, är otillräckligt klarlagda. Nedsatt njurfunktion är vanlig vid hjärtsvikt och därmed av speciellt intresse för bedömning av prognosen vid akut hjärtsvikt. Cystatin C (CysC) är en markör för den glomerulära filtrationen med egenskaper som gör den till ett intressant alternativ till kreatininet, som för närvarande används för att bedöma njurfunktionen.

Syftet med avhandlingen är att karaktärisera en representativ grupp patienter med akut hjärtsvikt och att identifiera riskfaktorer för dålig prognos vid akut hjärtsvikt. Särskild vikt läggs vid att utvärdera vilken roll CysC spelar som markör för njurfunktionen, samt vid att bedöma dess värde som prediktor av mortaliteten vid akut hjärtsvikt.

FINN-AKVA är en finländsk prospektiv multicenter-studie som utförs för att undersöka akut hjärtsvikt: klinisk bild, etiologi, komorbiditet, behandling och mortalitet. Patienter med akut hjärtsvikt som intagits på sjukhus rekryterades till FINN-AKVA-studien och följdes under 12 månader med avseende på mortaliteten. Medelåldern för personerna som ingår i studien är 75 år. De har ett flertal grundsjukdomar, både kardiovaskulära och icke-kardiovaskulära. Mortaliteten efter akut hjärtsvikt är hög, 27 % på 12 månader.

Resultaten i avhandlingen visar att försämrad njurfunktion är mycket vanligt vid akut hjärtsvikt. CysC upptäcker en nedsatt njurfunktion hos 40 % av patienterna och är dessutom en av de faktorer som är starkast relaterad till mortaliteten, även om man beaktar andra vanliga riskmarkörer så som ålder, kön, grundsjukdomar och systoliskt blodtryck. Studien påvisar att höga nivåer av CysC har samband med en ökad mortalitet även hos patienter med normala kreatininvärden. Akut njursvikt, i studien definierad som en ökning av CysC-nivåerna med 0.3mg/l under de första två dygnen av sjukhusvistelsen, konstateras hos en betydande andel av patienterna och medför också en ökad mortalitet, både omedelbar och på längre sikt under uppföljningstiden. Resultaten antyder att CysC är användbar för riskbedömning vid akut hjärtsvikt.

Förhöjda nivåer av olika inflammatoriska cytokiner, såsom interleukin-6 (IL-6) och tumörnekros faktor alfa (TNF-α) har påvisats både vid hjärtsvikt och vid kronisk njursjukdom. Inflammationen antas vara en av mekanismerna vid växelverkan mellan hjärta och njurar i det kardiorenala syndromet. IL-6 och TNF-α uppvisar rätt olika korrelation med markörer för njurfunktion och hjärtsvikt (NT-proBNP). Speciellt stark var korrelationen mellan CysC och TNF-α, som i sin tur inte korrelerade med NT-proBNP. CysC var dock en bättre riskmarkör för mortaliteten än de inflammatoriska markörerna.

Sammanfattningsvis kan konstateras att patienter med akut hjärtsvikt är äldre personer, har många grundsjukdomar, och att nedsatt njurfunktion är mycket vanlig. CysC visar sig ha goda diagnostiska egenskaper både för att upptäcka försämrad njurfunktion och akut njursvikt hos patienter med akut hjärtsvikt. CysC är också en stark prognostisk markör vid akut hjärtsvikt. CysC bör betraktas som en lovande markör för bedömningen av njurfunktionen och riskbedömningen hos patienter med akut hjärtsvikt.

Tiivistelmä

Akuutti sydämen vajaatoiminta on monimuotoinen oireyhtymä johon liittyy poikkeuksellisen suuri kuolleisuus. Siitä huolimatta näiden potilaiden kliinisiä piirteitä ja ennustetekijöitä on puutteellisesti tutkittu. Munuaistoiminta on noussut vahvaksi ennustetekijäksi sydän- ja verisuonisairauksissa. Tätä sydämen ja munuaisten välistä yhteyttä kuvataan nimellä kardiorenaalinen syndrooma, mutta sen mekanismit ovat huonosti tunnettuja. Munuaisten vajaatoiminta on tavallinen sydämen vajaatoimintapotilailla, ja siksi erityisen mielenkiinnon kohteena kuolleisuuden ennustajana akuutissa sydämen vajaatoiminnassa. Kystatiini C (CysC) on glomerulusfiltraation mittari jonka ominaisuudet tekevät siitä mielenkiintoisen vaihtoehdon nykyisin käytössä olevalle kreatiniinille munuaistoiminnan merkkiaineena.

Väitöskirjan tavoitteena on kuvata edustava aineisto akuuttia sydämen vajaatoimintaa sairastavia potilaita ja tunnistaa akuutin sydämen vajaatoiminnan huonon ennusteen tekijöitä. Erityisenä tavoitteena on tutkia CysC:tä munuaistoiminnan merkkiaineena akuutissa sydämen vajaatoiminnassa ja selvittää sen merkitystä kuolleisuuden ennustetekijänä.

FINN-AKVA on suomalainen prospektiivinen monikeskustutkimus akuutin sydämen vajaatoiminnan kliinisestä ilmentymästä, etiologiasta, liitännäissairauksista, sekä hoidosta ja kuolleisuudesta. Tutkimukseen otettiin akuutin sydämen vajaatoiminnan takia sairaalahoitoon joutuneita potilaita, ja kuolleisuutta seurattiin 12 kuukauden ajan. Potilaiden keski-ikä oli 75 vuotta, ja heillä esiintyi paljon sydän- ja verisuonisairauksia sekä useita muita liitännäissairauksia. Kuolleisuus sairaalahoitojakson jälkeisen vuoden aikana oli korkea (27 %).

Tutkimuksessa CysC:llä todetaan alentunutta munuaistoimintaa 40 %:lla akuutin sydämen vajaatoimintapotilaiden joukosta. Munuaistoiminta, CysC:llä mitattuna, on yksi vahvimmista itsenäisistä kuolleisuuden ennustajista senkin jälkeen, kun muut tavanomaiset riskitekijät on otettu huomioon. Niilläkin potilailla joilla kreatiniini on normaali, koholla olevaan CysC-arvoon liittyy huomattavasti korkeampi kuolleisuus. Akuutti munuaisvaurio, määritelmänä CysC-arvon nousu 0.3 mg/l kahden vuorokauden sisällä sairaalaan tulosta, havaitaan merkittävällä osalla potilaista, ja siihen liittyy lisääntynyt kuolleisuus sekä lyhyellä että keskipitkällä aikavälillä. Tulosten perusteella vaikuttaa siltä, että CysC on käyttökelpoinen merkkiaine riskinarvioon akuutissa sydämen vajaatoiminnassa.

Kohonneita pitoisuuksia tulehduksellisia sytokiineja, kuten interleukiini-6 (IL-6) ja tuumorinekroositekijä alfa (TNF-α), on todettu sekä sydämen että munuaisten vajaatoiminnassa. Tulehduksen ajatellaan olevan mukana välittäjänä sydämen ja munuaisten välisessä vuorovaikutuksessa, kardiorenaalisessa syndroomassa. IL-6 ja TNF-α korreloivat eri tavalla munuaistoimintaa ja sydämen kuormitusta (NT-proBNP) kuvaaviin merkkiaineisiin. Etenkin TNF-α:lla on vankka korrelaatio CysC:n kanssa, mutta ei taas assosioidu NT-proBNP-tasoihin. CysC:n verrattuna tulehdusmerkkiaineiden vaikutus ennusteeseen on heikompi.

Yhteenvetona voi todeta, että akuuttia sydämen vajaatoimintaa sairastavat potilaat ovat iäkkäitä, varsin monisairaita, ja etenkin munuaisten vajaatoiminta on tavallinen löydös. CysC osoittaa hyviä diagnostisia ominaisuuksia, sekä alentuneen munuaistoiminnan että akuutin munuaisvaurion havaitsemiseen akuuttia sydämen vajaatoimintaa sairastavilla potilailla. Munuaistoiminnan merkkiaineena CysC on myös vahva ennustetekijä. CysC vaikuttaa lupaavalta merkkiaineelta munuaistoiminnan arviointiin ja akuutin sydämen vajaatoimintapotilaiden riskinarvioon.

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List of original publications

This thesis is based on the following publications:

I Siirilä-Waris K, Lassus J, Melin J, Peuhkurinen K, Nieminen MS, Harjola VP. Characteristics, outcomes, and predictors of 1-year mortality in patients hospitalized for acute heart failure. *Eur Heart J 2006;27:3011–3017*

II Lassus J, Harjola VP, Sund R, Siirilä-Waris K, Melin J, Peuhkurinen K, Pulkki K, Nieminen MS, for the FINN-AKVA Study group. Prognostic value of cystatin C in acute heart failure in relation to other markers of renal function and NT-proBNP. *Eur Heart J 2007;28:1841-1847*

III Lassus JPE, Nieminen MS, Peuhkurinen K, Pulkki K, Sund R, Siirilä-Waris K, Harjola V-P. Markers of renal function in acute kidney injury in acute heart failure: definitions and impact on outcomes of the cardiorenal syndrome. *Eur Heart J 2010; 31(22): 2791-2798.*

IV Lassus J., Harjola V-P., Sund R., Peuhkurinen K., Mebazaa A., Siirilä-Waris K., Miettinen K., Punnonen K., Melin J., Pulkki K., Nieminen MS. Cystatin C, NT-proBNP and inflammatory markers in AHF. Insights into the cardiorenal syndrome. *Biomarkers; In press.*

The publications are referred to in the text by their roman numerals.

Abbreviations

ACS	acute coronary syndrome
ACEI	angiotensin converting enzyme inhibitor
ADCHF	acute decompensated chronic heart failure
AHF	acute heart failure
AKI	acute kidney injury
AKIN	acute kidney injury network
ARB	angiotensin 1 receptor blocker
AUC	area under the curve
BB	beta-blocker
BNP	B-type (brain) natriuretic peptide
CAD	coronary artery disease
CCr	24-hour urinary creatinine clearance
CI	confidence interval
CKD	chronic kidney disease
$CrCl_{C-G}$	creatinine clearance by Cockcroft-Gault equation
CRP	c-reactive protein
CRS	cardiorenal syndrome
CVP	central venous pressure
CVD	cardiovascular disease
CysC	cystatin C
DBP	diastolic blood pressure
ESC	European society of cardiology
FINN-AKVA	Finnish acute heart failure study
GFR	glomerular filtration rate
eGFR	estimated glomerular filtration rate
HFPEF	heart failure with preserved ejection fraction
HR	hazard ratio
ICU	intensive care unit
IL-6	interleukin 6
IL-10	interleukin 10
IQR	interquartile range
LOS	length of stay
LVEF	left ventricular ejection fraction
MDRD	modified diet in renal disease
NGAL	neutrofil gelatinase associated lipocalin
NKF/KDOQI	National Kidney Foundation/Kidney disease outcomes quality initiative
NT-proBNP	aminoterminal pro-brain natriuretic peptide
NYHA	New York heart association class
OR	odds ratio
RAAS	renin angiotensin aldosterone system
ROC	receiver operating characteristic
SBP	systolic blood pressure
SD	standard deviation
TNF-α	tumor necrosis factor alpha
TnT/TnI	troponin T/troponin I

1. Introduction

Acute heart failure (AHF) is characterized by rapid worsening of symptoms and signs of heart failure with the need for urgent therapy and usually requiring hospitalization. Heart failure arises from a disturbance of cardiac structure and function, either systolic or diastolic, and is characterized by inability to maintain sufficient cardiac output. Clinical findings in heart failure include symptoms like breathlessness at rest or on exertion, fatigue and signs of congestion and often volume overload. Historically, AHF was regarded as part of the natural history of chronic heart failure, and characteristics and outcomes of patients with AHF were not well known and poorly described. Numerous randomised trials in chronic heart failure have not been matched by similar studies in AHF. As a result, factors precipitating AHF have been inadequately understood and treatment largely empirical. Heart failure is generally associated with poor long term outcomes, and after hospitalization for AHF, mortality rates rise steeply. Identification of factors that contribute to this prognostic tragedy is one way of approaching the goal of better treatment and improved survival in AHF.

Renal function has emerged as a central feature affecting prognosis in heart failure. Since the first large publications describing the effect of renal function on prognosis in chronic heart failure a decade ago (Hillege et al. 2000, Dries et al. 2000), many papers have confirmed that renal insufficiency is a strong and independent risk factor for mortality and adverse outcomes in cardiovascular disease (CVD) in general, and in heart failure in particular (Go et al. 2004, Hillege et al. 2006, Anavekar et al. 2004, Smith et al. 2006). Concurrently, reports about the high cardiovascular event rate in patients on dialysis and with chronic kidney disease (CKD) have driven the interest in the link between heart and kidney disease.

The cardiorenal syndrome (CRS) is a general term to describe the bidirectional heart-kidney interaction. Specifically, primary dysfunction in one of these organs eventually impairs the function of the other organ, which amplifies the harmful effects affecting both organs in what could be depicted as a vicious circle. The interplay between heart and kidneys occurs at several levels through various pathways affecting hemodynamics, neurohumoral signalling as well as salt and water homeostasis. Although the normal physiology of the heart and kidney is rather well characterized, pathophysiological changes and disturbances associated with disease states and, in particular, mechanisms of organ dysfunction related to the CRS are poorly understood.

The CRS is typically manifested in AHF. Patients hospitalized for AHF have an acute cardiac dysfunction and frequently have evidence of impaired renal function on admission (Smith et al. 2006). Due to hemodynamic derangements, neurohumoral or inflammatory activation, volume overload, or as a result of treatment directed at relieving symptoms and congestion, renal function deteriorates in a significant proportion of patients with AHF (Smith et al. 2003, Gottlieb et al. 2002). Resistance to diuretics used as treatment for removal of excess fluid in AHF patients with impaired kidney function is also a phenomenon familiar to many physicians. Finally, both renal insufficiency on admission and worsening renal function during hospitalization for AHF are associated with poor

in-hospital outcomes and an increased risk of death shortly after discharge. Given these circumstances, assessing renal function is essential in patients with AHF.

Cystatin C (CysC) is a novel marker of renal function with many properties making it suitable for estimation of glomerular filtration rate (GFR). CysC has proven to be superior to the commonly used creatinine as a marker of GFR, especially in patients with mild to moderate impairment of renal function (Laterza et al. 2002, Kazama et al. 2002, Rule et al. 2006). CysC has also been a strong prognostic marker of adverse cardiovascular outcomes in different populations (Shlipak et al. 2005c, Sarnak et al. 2005, Jernberg et al. 2004). The aim of this thesis is to study the characteristics, prognosis and predictors of outcome in a contemporary population hospitalized for AHF. Renal function and aspects of the CRS are of particular interest with special focus on CysC as a marker of renal function in AHF.

2. Review of the literature

2.1 Heart failure

Heart failure is characterized by symptoms and signs of congestion and volume overload due to cardiac dysfunction, usually with decreased cardiac output. The most common symptoms are dyspnea, fatigue and exercise intolerance. Heart failure is usually not present in a structurally and functionally normal heart, but is caused by underlying disturbances in cardiac systolic or diastolic function, secondary to diseased myocardium, valvular dysfunction and pressure or volume overload in the heart.

In one cardiac cycle, blood is ejected into the arterial circulation from the left and right ventricles through contraction (systole) of the heart which is followed by the relaxation phase (diastole) during which venous blood fills the ventricles for the start of the next systole. The cardiac output is dependent on the volume ejected during each cycle (stroke volume) and the heart rate (beats/minute). Heart failure can arise both from an impaired ability to fill or to eject blood, secondary to structural or functional abnormalities of the myocardium and/or valves. Changes in systolic and diastolic function can occur suddenly (e.g. myocardial infarction) or develop during a prolonged period of time (myocardial disease, valve dysfunction).

2.1.1 Chronic heart failure

2.1.1.1 Definition and diagnosis

The definition of heart failure has continuously evolved during the past decades. Difficulties in finding a clear and uniform definition certainly reflect the complexity of the pathophysiology and clinical picture of heart failure, and have led to the use of the term heart failure syndromes. The definitions used in the current European society of cardiology (ESC) guidelines are presented in *Table 1* (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology). The guidelines of the American College of Cardiology/American Heart Association define heart failure as:

> "a clinical syndrome that is characterized by specific symptoms (dyspnea and fatigue) in the medical history and signs (oedema, rales) on the physical examination" (Hunt et al. 2005).

The clinical diagnosis of heart failure based on signs and symptoms is not always straightforward and echocardiographic evaluation of cardiac structure and left ventricular systolic and diastolic function is nowadays regarded mandatory (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology, Hunt et al. 2009).

Table 1. *Definition of heart failure according to current ESC guidelines (2008)*

Symptoms typical of heart failure
Dyspnea (at rest or on exertion), fatigue, ankle swelling
AND signs typical of heart failure
Tachycardia, tachypnea, rales, raised jugular venous pressure, oedema, hepatomegaly
AND objective evidence of structural or functional abnormality of the heart at rest
Chest radiograph, auscultation, echocardiography, elevated natriuretic peptide levels

The echocardiographic observations that not all patients with heart failure have abnormal left ventricular ejection fraction (LVEF) led to the distinction between systolic (having reduced LVEF) and diastolic heart failure, i.e. heart failure with preserved ejection fraction (HFPEF) (Aurigemma et al. 2004). The current guidelines find this distinction arbitrary and somewhat redundant (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology). Still, diagnostic criteria for HFPEF are presented, and include: 1) signs and symptoms of heart failure, 2) normal or only mildly abnormal systolic function (LVEF >40-45%) and 3) echocardiographic evidence of diastolic abnormalities (relaxation and filling properties).

The natriuretic peptides (e.g. B-type natriuretic peptide [BNP] and amino-terminal proBNP [NT-proBNP]) are neurohormones released in response to cardiac wall stress. Natriuretic peptide levels are elevated in heart failure and although no single definite cut-off for diagnosis has been identified, measurement of natriuretic peptide levels is useful in the evaluation of a patient with suspected heart failure (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology, Hunt et al. 2009, Maisel et al. 2008).

2.1.1.2 Epidemiology, aetiology and contemporary management

The prevalence of symptomatic heart failure in the adult population in Europe overall is about 2%, rising considerably in the elderly. In people aged over 75 years, the prevalence is as high as 10-20% (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology). Heart failure is never a solitary diagnosis. The presence of heart failure always requires identification of an underlying reason. Coronary artery disease (CAD) is the single most important cause of heart failure, being the aetiology in about two thirds of patients (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology). Hypertension is another major cause of heart failure, present in over half of patients with heart failure. Hypertension and CAD are often found concomitantly, and together they

account for 80-85% of heart failure cases. Valvular disease is the aetiology in a smaller proportion of patients with heart failure. Other causes like diseases of the myocardium in the absence of hypertension and CAD (i.e. cardiomyopathies) and infectious or inflammatory diseases each represent only a minority of cases (Krum et al. 2009). Half of the patients with heart failure have HFPEF, and preserved LVEF is more common in elderly, women and patients with a history of hypertension (Owan et al. 2006b, Bhatia et al. 2006, Fonarow et al. 2007b). Systolic dysfunction after myocardial infarction leading to heart failure is seen more often in men, and the age of this sub-population is on average lower. The incidence of heart failure is thought to be stable but, with the ageing population and more patients surviving after a myocardial infarction, the prevalence of heart failure is increasing, and there are over 15 million people in Europe who suffer from heart failure (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology).

Treatment of heart failure has progressed considerably in the last thirty years. Today, medication with angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blockers (ARB) and the use of beta blockers (BB) are the mainstay of heart failure therapy and are recommended to all patients unless not tolerated (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology). Many patients also need diuretics to control volume status, although no data exists on any mortality benefit with the use of loop diuretics (Krum et al. 2009). Aldosterone antagonists (spironolactone, eplerenone) have been shown to decrease morbidity and mortality in symptomatic (New York Heart Association [NYHA] functional class III-IV) heart failure with reduced LVEF and after myocardial infarction. In recent years, device therapy has had the strongest impact on the treatment of patients with heart failure. Both implantable cardioverter defibrillators and cardiac resynchronization therapy pacemakers have shown beneficial effects in well defined patients groups, and the use of these devices has been rapidly increasing. Most studies on heart failure therapy have had systolic dysfunction (LVEF <40%) as an inclusion criteria. Hence, patients with HFPEF have been excluded, the mean age of participants in these studies has been lower than in the general heart failure population and women have been underrepresented. Only a few studies in recent years have addressed the treatment of patients with HFPEF (Ghio et al. 2006, Flather et al. 2005, Cleland et al. 2006, Massie et al. 2008, Yusuf et al. 2003).

2.1.1.3 Prognosis in chronic heart failure

Patients with heart failure often have symptoms limiting activities of daily living. Reduced functional capacity and frequent hospitalizations affect quality of life. Despite the evidence that current treatment yield morbidity and mortality benefits in well defined cohorts of heart failure patients, the prognosis in heart failure is still poor. On average, the annual mortality rate still is around 10% in patients with chronic heart failure (Owan et al. 2006b, Bhatia et al. 2006). The need for hospitalization alters the survival curve dramatically, and one-year mortality increases 3- to 4-fold after hospitalization for AHF (Goldberg et al. 2007, Nieminen et al. 2006, Ahmed et al. 2008). Epidemiological studies

on temporal trends of survival in heart failure have found only modest improvement in the mortality of the heart failure population from the 1950:s to the 1990:s (Levy et al. 2002, Roger et al. 2004). The outcome is quite similar in systolic heart failure and HFPEF (Owan et al. 2006b, Bhatia et al. 2006, Tribouilloy et al. 2008). Most recent data show a slight decrease in hospitalizations for heart failure since mid- 1990:s and there is a trend towards improved survival after hospital discharge in patients with systolic heart failure (Owan et al. 2006b, Schaufelberger et al. 2004, Bueno et al. 2010, Teng et al. 2010). Epidemiological data may identify prognostic factors and show trends in survival for the overall heart failure population. Nevertheless, individual heart failure patients with poor predicted prognosis may stay alive for several years without hospitalization or disabling symptoms. The art of prognostication poses a real challenge for most clinicians.

2.1.1.4 Concomitant disease in heart failure

The abundance of underlying diseases and several co-morbidities are considered as the major reasons for the difficulty in improving outcomes in patients with heart failure syndromes. As already emphasized, heart failure is by definition not a single diagnosis.

The natural history and treatment success of any underlying cause is essential for the prognosis in heart failure. While the therapeutic results in hypertension and CAD have improved significantly, heart failure patients are elderly, and the list of concomitant diseases that affect morbidity and mortality is rather extensive (*Table 2*). Many of them have been found to be of prognostic importance in heart failure. Management of these co-morbidities plays a key role also for improving heart failure outcomes (Dahlström 2005).

2.1.2 Acute heart failure

2.1.2.1 Definition

Acute heart failure (AHF) is defined as an abrupt start or rapid worsening of heart failure symptoms requiring immediate care (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology, Nieminen et al. 2005a, Gheorghiade et al. 2005b). AHF usually requires urgent therapy, often with hospitalization, and some cases of AHF are medical emergencies. AHF may be the first presentation of heart failure (i.e. de novo heart failure) or it may be a deterioration of the clinical condition in a patient with known heart failure (acutely decompensated chronic heart failure [ADCHF]).

Table 2. *Important co-morbidities in heart failure*

Anemia
Arrhythmias
Atrial fibrillation
Ventricular arrhythmias
Atherosclerotic vascular disease
Cerebrovascular and peripheral vascular
Coronary artery disease
Depression
Diabetes
Gout
Hypertension
Malignancy
Pulmonary disease
Chronic obstructive pulmonary disease
Breathing abnormalities
Renal dysfunction
Valvular dysfunction

For a long time, no uniform definition on AHF existed. Compared to chronic heart failure, studies on characteristics, epidemiology and treatment of AHF have been surprisingly few. Only in recent years has more data on AHF patients become available. The clinical picture of AHF is highly variable, as is the pathophysiology which is not completely understood (Gheorghiade et al. 2005a, Nieminen et al. 2005b, Cotter et al. 2008). Treatment aims are relief of symptoms (dyspnea, anxiety, congestion and volume overload), myocardial protection to prevent further or progressive cardiac damage, and restoration of hemodynamic and neurohumoral balance. AHF is associated with high in-hospital mortality and poor long-term outcomes (Harjola et al. 2010, Adams et al. 2005, Gheorghiade et al. 2006a, Tavazzi et al. 2006, Huynh et al. 2006). Thus, improving the prognosis in AHF is also a primary therapeutic target.

2.1.2.2 Epidemiology

The epidemiology of AHF, including characteristics, in-hospital treatment and outcomes of patients has been described in more detail mostly during the last decade (Adams et al. 2005, Gheorghiade et al. 2006a, Tavazzi et al. 2006, Goldberg et al. 2005b, Cohen-Solal et al. 2000, Lee et al. 2003, Zannad et al. 2006). The incidence and prevalence of AHF has been estimated from national or hospital discharge registries, using hospitalizations with heart failure as the primary or secondary diagnosis. This method has some limitations

related to diagnostic criteria and coding of HF as discharge diagnosis. In addition, population data on incidence and prevalence may also be difficult to assess due to the frequent need of repeated hospitalization within a short time for individual patients with heart failure (Cowie et al. 2002). One study from Sweden examining only first hospitalization for heart failure found age-adjusted annual incidence rates of about 250 for men and 200 for women per 100 000 inhabitants (Schaufelberger et al. 2004). Others report slightly lower overall rates of first hospitalizations for AHF (Teng et al. 2010, Jhund et al. 2009). Nevertheless, because of higher prevalence of heart failure and the need for repeated admissions, heart failure related hospitalizations have risen steadily in the last thirty years, reaching almost epidemic proportions. It is estimated that there are over 1 million heart failure hospitalizations per year in Europe. Recent reports from Europe have found a decline in the hospitalization rates after the mid 1990:s, giving hope that the epidemic has reached its peak (Schaufelberger et al. 2004, Jhund et al. 2009, Stewart et al. 2001). Data from the U.S. showing a steady increase even in hospitalizations with heart failure as first diagnosis has not confirmed this finding (Fang et al. 2008). Nevertheless, HF hospitalizations cause a major burden on healthcare accounting for 5 % of all medical admissions and standing for over 70% of costs for management of heart failure patients (Nieminen et al. 2005a).

Characteristics and management of contemporary AHF patients have been described through prospective observational studies and registries (Nieminen et al. 2006, Gheorghiade et al. 2006a, Tavazzi et al. 2006, Cohen-Solal et al. 2000, Zannad et al. 2006, Fonarow et al. 2007a, Cleland et al. 2003). These have also been the base for identification of different patient groups and have put forward the complexity of the acute heart failure syndromes. It has been shown that 30-50% of patients hospitalized for AHF present to the emergency department without a previous history of heart failure (Nieminen et al. 2006, Tavazzi et al. 2006). These cases of de-novo acute heart failure differ in clinical profile and prognosis compared with patients hospitalized for acutely decompensated chronic heart failure. De-novo AHF is more often associated with an acute coronary syndrome (ACS) and the clinical picture on admission is often more severe (acute pulmonary oedema and cardiogenic shock). In-hospital mortality is higher in these patients, but after discharge the re-hospitalization and mortality rates are lower compared to patients with acutely decompensated chronic heart failure (Nieminen et al. 2006, Harjola et al. 2010, Tavazzi et al. 2006).

2.1.2.3 Clinical classification

The variability in the clinical picture and presentation of AHF has prompted the need for a practical classification in the management of these patients. Although the Forrester classification was introduced already over thirty years ago, it is still a simple and useful tool. The Forrester classification categorized patients as “warm-cold” based on peripheral perfusion and “wet-dry” depending on whether pulmonary congestion was present or not (Forrester et al. 1977). The first ever guidelines on the treatment and diagnosis of AHF were published by the ESC in 2005 (Nieminen et al. 2005a). These guidelines also

presented a clinical classification of AHF which incorporated clinical and hemodynamic findings on admission to categorize patients in six groups. In the recent revision of the guidelines, high-output AHF was left out, and five clinical classes remain, with a sixth entity being AHF in association with an ACS (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology). To some extent, there is overlapping between these classes *(Figure 1)*. The classes are presented in more detail below.

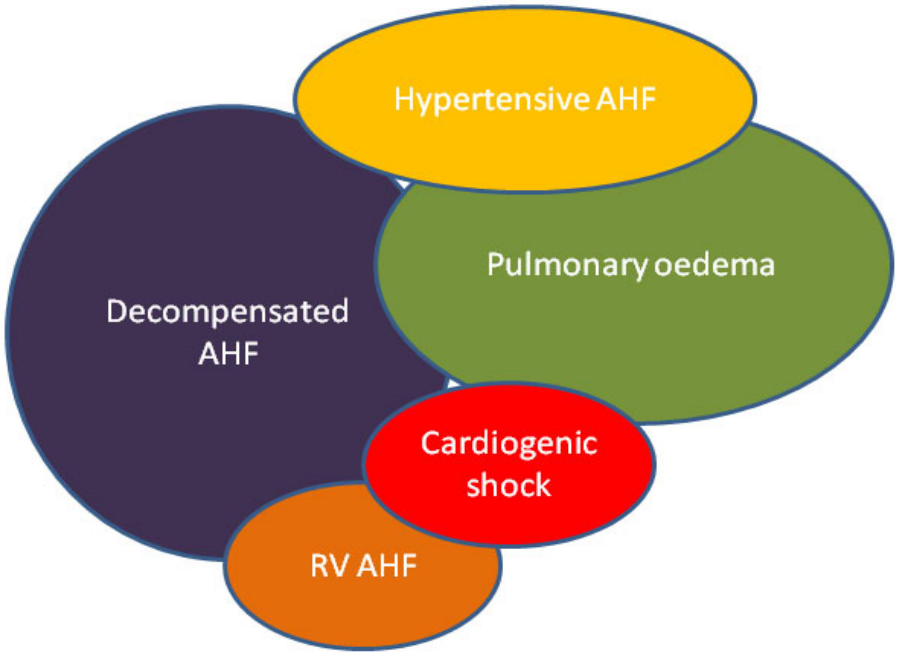

Figure 1 *ESC classes of acute heart failure (Modified after ESC guidelines 2005 and 2008) AHF=acute heart failure, RV=right ventricle*

Acute decompensated heart failure

Acute decompensated heart failure presents with signs and symptoms of AHF, which are mild to moderate and do not fulfill criteria for cardiogenic shock, pulmonary oedema or hypertensive AHF. Dyspnea at rest or on light exertion with signs of congestion (congestion on chest X-ray, elevated jugular venous pressure, and/or oedema of lower extremities) are the cardinal symptoms and findings.

Hypertensive heart failure

Hypertensive AHF is defined as signs and symptoms of heart failure accompanied by high blood pressure (systolic blood pressure [SBP] >180 mmHg) and relatively preserved left ventricular function with a chest radiograph compatible with acute pulmonary oedema.

Pulmonary oedema
Pulmonary oedema is described by severe respiratory distress, with O_2-saturation usually <90% on room air prior to treatment accompanied by orthopnea. Findings of pulmonary oedema should be present on chest X-ray and auscultation reveals crackles over the lung.

Cardiogenic shock
Cardiogenic shock is the most severe clinical picture of AHF, characterized by tissue hypoperfusion and usually with reduced blood pressure (SBP <90 mmHg) in the absence of hypovolemia. Low urine output (<0.5 ml/kg/h) and evidence of organ congestion may also be present.

Right heart failure
Right ventricle heart failure is characterized by low output syndrome with signs of increased jugular venous pressure, enlarged liver size and hypotension without concomitant pulmonary congestion and increase in left ventricular filling pressures.

2.1.2.4 Pathophysiology

The pathophysiology of AHF is complex and diverse. Although the clinical classification can serve as a tool for guiding management and a step towards better identification of different characteristics in AHF, no real breakthrough in our understanding of AHF has occurred during the last three decades. The traditional view of impaired systolic function, low cardiac output and fluid retention has been challenged in many ways (Gheorghiade et al. 2005a, Cotter et al. 2008, Gheorghiade et al. 2005b, Chen et al. 2006). Half of the patients have preserved systolic function, a substantial proportion have high, not low, blood pressure on admission (Gheorghiade et al. 2006a, Tavazzi et al. 2006, Milo-Cotter et al. 2007). Not all patients experience weight gain prior to hospitalization (Lewin et al. 2005, Zile et al. 2008, Chaudhry et al. 2007), and although symptoms resolve there is no or only modest weight loss during hospitalization in a proportion of patients (Gheorghiade et al. 2006b, Mehta et al. 2009). Nevertheless, some central mechanisms that contribute to the exacerbation of AHF can be identified.

First, cardiac output certainly plays a central role and disturbances in systolic or diastolic dysfunction makes patients susceptible to developing AHF. Factors that transiently or more permanently derange or damage cardiac function such as ischemia, ACS or myocarditis easily precipitate AHF. Impaired systolic and/or diastolic performance leads to activation of the sympathetic nervous system and renin angiotensin-aldosterone axis with sodium and water retention.

Second, the concept of AHF as a vascular disease has been put forward by some authors (Cotter et al. 2008, Milo-Cotter et al. 2007, Colombo et al. 2008). High-blood pressure on admission, together with preserved left ventricular ejection fraction seen in many patients with AHF, support the theory of increased vascular resistance and afterload mismatch as precipitating AHF. Activation of neurohumoral mechanisms plays a central

role in this model. The effect of inflammation in cardiac and vascular function is another complex and inadequately understood pathway in the pathophysiology of AHF.

Finally, renal dysfunction may be regarded as the third contributor to AHF. Impairment of renal function leads to: a) disturbances in sodium and water homeostasis and b) activation of neurohumoral pathways (e.g. the renin angiotensin-aldosterone [RAAS] and sympathetic nervous systems). These mechanisms promote fluid accumulation and increased vascular resistance and makes patients at risk for congestion and decompensation of heart failure.

2.1.2.5 Precipitating factors

Identifying factors that precipitate AHF may add information on the mechanisms leading to and help in the management of AHF. In patients with chronic heart failure, non-compliance to medical treatment or lack of adherence to dietary (salt and fluids) restrictions may lead to fluid accumulation, increased filling pressures and worsening symptoms requiring hospitalization. Ischemia is a frequent cause of cardiac dysfunction leading to AHF and an acute coronary event is one of the most common precipitating factors both in de-novo AHF and in patients with previous history of heart failure (Nieminen et al. 2006). Changes in hemodynamic status (high blood pressure, arrhythmias) or increased cardiovascular burden due to increased demand of oxygen delivery during infection are other major causes of AHF (Gheorghiade et al. 2009, Fonarow et al. 2008b). Finally, underlying diseases of the myocardium (myocarditis, cardiomyopathies) or valves (valvular stenosis or regurgitation) predispose for the development of AHF.

2.1.2.6 Treatment

The treatment of AHF aims at rapid symptom relief and stabilising hemodynamics if compromised. After these immediate goals have been achieved, intermediate and long-term management objectives include reducing myocardial damage and renal protection as well as improving morbidity and mortality (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology). Medical therapy shown to improve outcomes in chronic heart failure (i.e. ACEI/ARB:s and BB:s) should be initiated gradually as soon as possible when hemodynamically tolerated without compromising cardiac or renal function.

Therapies used for treatment of AHF (oxygen supplementation with or without ventilatory support, morphine, diuretics, vasodilators, and in selected cases inotropes) are largely empirical. Few studies have evaluated the efficacy of these therapies, and although they have a class I (beneficial) recommendation in the guidelines, the level of evidence is often limited (level of evidence B or C) (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology). The use of nitrates as

vasodilators is the only therapy which has been shown to improve outcome in AHF (Cotter et al. 1998, Mullens et al. 2008a).

2.1.2.7 Prognosis

The prognosis of AHF is disappointing. Characteristics and outcomes of selected populations of AHF are presented in *Table 3*. In-hospital mortality is around 5-10% (Nieminen et al. 2006, Adams et al. 2005, Tavazzi et al. 2006, Goldberg et al. 2005b, Goldberg et al. 2005a, Abraham et al. 2008). After discharge, mortality increases to around 20% by 90 days and reaches approximately 30% at 12 months after hospitalization for AHF (Harjola et al. 2010, Tavazzi et al. 2006, O'Connor et al. 2008). The mortality is highly variable between the clinical classes, being highest in cardiogenic shock (40-60% in-hospital mortality) and hypertensive AHF having the best prognosis (1.5% in-hospital mortality and high survival rates at 12 months) (Nieminen et al. 2006, Harjola et al. 2010, Zannad et al. 2006). Still, the overall survival one year after hospitalization for AHF is worse than many cancers. In addition, patients frequently require early re-hospitalization, 30-50% within 6-12 months depending on the population studied (Nieminen et al. 2005b, Tavazzi et al. 2006). Even though these patients are elderly with several co-morbidities that limit their life expectancy, four out of five deaths and early re-hospitalizations after AHF are due to cardiovascular causes (Tavazzi et al. 2006).

Table 3. *Characteristics and prognosis in AHF in various contemporary studies*

Study	n=	Mean age	Women	HF PEF	LOS	In-hosp. mortality	30-day	90-day	1-year
Cohen-Solal 2000	1086	76	45%	38%	11	8%	NA	NA	NA
Lee 2003	4031	76	51%	47%	NA	9%	11%	NA	32%
Adams 2005	105388	72.4	52%	44%	4.3	4.0%	NA	NA	NA
Goldberg 2005	2604	79	57%	NA	4.0	5.1%	NA	NA	NA
Gheorghiade 2006	48512	73	52%	51%	6	3.8%	NA	12	NA
Cleland 2003	11327	71	47%	55%	11	NA	NA	14	NA
Nieminen 2006	3580	70	62%	34%	9	7%	NA	15	28%
Zannad 2006	599	73	41%	27%	15	NA	27%	NA	47%
Tavazzi 2006	2807	73	40%	34%	9	7.3%	NA	NA	NA
Sato 2010	1110	73	41%	43%	21	7.7%	NA	NA	NA

LOS=length of hospital stay, HFPEF=heart failure with preserved ejection fraction

2.1.2.8 Underlying and concomitant disease

As for chronic heart failure, patients with AHF usually have several co-morbidities. These are mostly the same as in chronic heart failure (*Table 2*). In this elderly population, these concomitant illnesses increase morbidity and impact on quality of life. In addition, patients become frailer, with increased likelihood of treatment-related adverse events (bleeding, renal dysfunction, electrolyte disturbances and hypotension). Overall, this can make therapeutic decisions and interventions challenging.

2.2 Chronic kidney disease

If heart failure has reached epidemic proportions in the developed world, the same can be said about kidney disease. The number of patients with end-stage renal disease requiring dialysis is rapidly increasing. The incidence of initiation of dialysis rose by 23% during 1997-2007 in Finland up to approximately 90/million inhabitants/year (Finnish Registry for Kidney Diseases 2007). End-stage renal disease represents only the top of the iceberg of CKD, and for every patient with end-stage renal disease, there are numerous with moderate to mild renal insufficiency, which eventually may progress to end-stage renal disease and need of dialysis. An ageing population markedly contributes to the increase in the prevalence of CKD. Diabetes, hypertension and atherosclerotic vascular disease are the main causes for the increase of kidney disease (Finnish Registry for Kidney Diseases 2007). CKD represents a public health problem, not only through the considerable costs related to treatment of end-stage renal disease, but because renal insufficiency is also associated with significant morbidity and mortality, mainly of cardiovascular reasons.

2.2.1 Definition

Renal function is considered synonymous with glomerular filtration rate (GFR) (Brosius et al. 2006, Stevens et al. 2006, Lindeman et al. 1985). Normal glomerular filtration rates in young adults are 130 ml/min/1.73m^2 in men and 120 ml/min/1.73m^2 in women. GFR seems to decline ≤1 ml/min with normal ageing, but there is a high inter-individual variability (Lindeman et al. 1985, National Kidney Foundation 2002). By the age of 80 years, the mean GFR in both men and women would be approximately 60-70 ml/min/1.73m^2 (*Figure 2*).

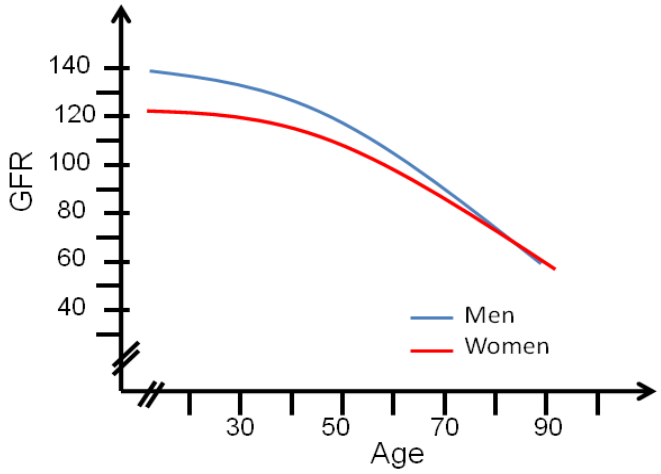

Figure 2 *Normal GFR and relation to age in men and women (adapted from Stevens 2006)*

The definition of CKD suggested by the National Kidney Foundation/Kidney disease outcomes quality initiative (NKF/KDOQI) has been widely accepted and used (National Kidney Foundation 2002, Sarnak et al. 2003). CKD is defined as evidence of structural or functional kidney damage, with or without decreased GFR *or* GFR <60 ml/min/1.73m^2 (with or without kidney damage) for ≥3 months. CKD is also divided into five stages as shown in *Table 4*. In general clinical practice and in the medical literature, a measured or estimated GFR <60 ml/min/1.73m^2 corresponding to the stages 3-5 of the NKF/KDOQI definition is commonly used as a cut-off for renal insufficiency. This is also supported by the guidelines which state that:

> "All individuals with GFR <60 mL/min/1.73 m2 for ≥3 months are classified as having chronic kidney disease, irrespective of the presence or absence of kidney damage. The rationale for including these individuals is that reduction in kidney function to this level or lower represents loss of half or more of the adult level of normal kidney function, which may be associated with a number of complications." (National Kidney Foundation 2002)

Table 4. *Stages of chronic kidney disease*

Stage	GFR		Description
I	>90ml/min	>1,5 ml/s	Signs of kidney damage (e.g proteinuria) with normal GFR
II	60-90 ml/min	1-1,5 ml/s	Mildly reduced GFR with kidney damage
III	30-60 ml/min	0,5-1 ml/s	Moderate decrease in GFR +/- damage
IV	15-30 ml/min	0,25-0,5 ml/s	Severe renal insufficiency
V	<15 ml/min	<0,25ml/s	End-stage renal disease +/- dialysis

2.2.2 Measurement of renal function

Assessment of renal function is a central element in the everyday practice of medicine. However, GFR by itself cannot be measured, but is determined as the filtration rate of a surrogate marker that is freely filtrated in the glomerulus. Many different methods are used for measurement or estimation of GFR, and every method has strengths and weaknesses (Stevens et al. 2009a).

The ideal marker of GFR should be easily measurable and accurately reflect actual GFR both in the steady state and with rapid changes of renal function. It should preferably be an endogenous substance with no binding to plasma proteins, produced at a constant rate and excreted by the kidney through free filtration at the glomerular level. No tubular (extra-glomerular) secretion or re-uptake and no extra-renal degradation should occur. The levels (production or elimination) of the marker should not be affected by age, sex, body composition, diet, disease states or medication, e.g. factors other than GFR. In the ideal scenario, the endogenous marker would be measured from a blood or urine sample, and any change in GFR (slow or rapid) would be detectable as a corresponding change in the circulating levels or urinary concentrations of that marker.

2.2.2.1 GFR measurement by urinary clearance of exogenous markers

Exogenous compounds such as Inulin, Iohexol or radiolabeled isotopes (^{51}Cr-EDTA, ^{99}Tm-DTPA, ^{125}I-Iothalamate) have most properties required of an ideal marker and renal clearance of these substances has become the golden standard for measurement of GFR. Direct measurement of GFR with an exogenous marker is time-consuming, requires repeated blood or monitored urine sampling and, because of the complex and relatively costly procedure, its use in larger scale clinical practice or studies is not usually possible. Moreover, research studies have reported an inter- and intra-procedural variation in measurements of 5 to 20% (Stevens et al. 2009a, Kwong et al. 2010).

2.2.2.2 Creatinine

Creatinine is an endogenous substance, and the most commonly used marker for estimating renal function. It is a product of the metabolism of phosphocreatine in muscle. As a small (113 D) amino acid derivative, creatinine is freely filtrated in the glomerulus. There is no tubular absorption. On the contrary, about 20% of creatinine is eliminated through active tubular secretion. Because most of the creatinine is produced by muscle, creatinine levels are strongly dependent on muscle mass and vary according to age, gender and race. Furthermore, ingestion of meat increases blood levels of creatinine, while disease states that affect muscle metabolism, including chronic illness with muscle wasting, usually lower creatinine levels (National Kidney Foundation 2002).

Creatinine can be measured from blood and/or urine. Since creatinine is cleared by glomerular filtration, measuring urinary excretion of creatinine (creatinine clearance [CCr]

from a 24-hour urine collection) and comparing it to plasma creatinine levels would give a rough estimate of glomerular filtration rate. An observed day to day variation in CCr values of up to 25% must be taken into account. However, even if creatinine production would be stable, CCr always overestimates GFR due to the tubular secretion of creatinine (National Kidney Foundation 2002). When kidney function declines, the tubular excretion of creatinine increases resulting in little or no change in creatinine plasma levels, concealing the decline in GFR until a substantial loss in renal function has occurred. On the other hand, tubular secretion can be inhibited by some medications (cimetidine, trimetoprime, dronedarone) which is reflected by a rise in plasma creatinine levels without actual change in GFR (Stevens et al. 2006, Tschuppert et al. 2007). Finally, extra-renal degradation of creatinine (both increased tubular secretion and bacterial degradation in the bowel) can account for up to over half of creatinine elimination in severe renal insufficiency (National Kidney Foundation 2002). Thus, plasma creatinine levels or urinary excretion of creatinine are not very accurate measures of GFR.

Still, plasma creatinine level (expressed as mg/dL or µmol/L; conversion coefficient 88.4 from mg/dL to µmol/L) is the most commonly used measure of renal function in clinical practice. Hereafter, creatinine levels will refer to serum/plasma concentrations unless otherwise stated. Overall, the association between creatinine levels and GFR is not linear, but inversely exponential. True GFR may decline to about half of normal before plasma levels of creatinine rise above the upper normal reference limit (*Figure 3*).

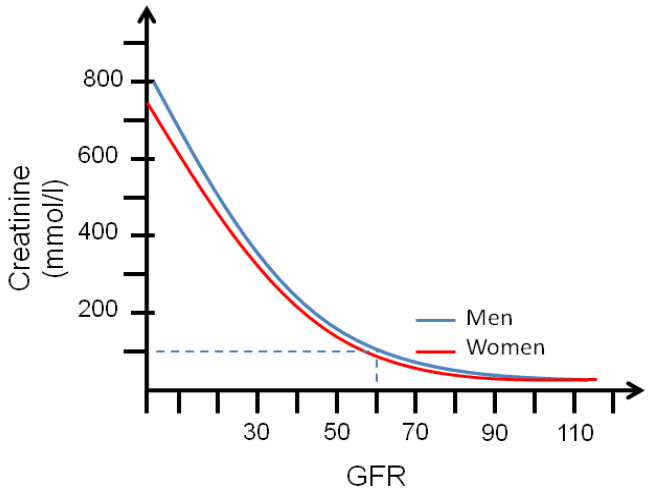

Figure 3 *Relationship between creatinine and GFR (Adapted from Levey et al AnnIntMed 1999)*

It has been estimated that up to 40% of patients with GFR below normal still have creatinine values within the normal range (National Kidney Foundation 2002). NKF/KDOQI guidelines state that "creatinine alone is not an accurate index of the level of GFR". In an attempt to overcome these problems with creatinine, and to convert creatinine values to better estimates of GFR, several equations have been developed. The equations incorporate information on age, gender, race and weight to correct for differences in

creatinine production associated with these features. The two most used in the adult patient population are presented below.

Urea, another product of protein metabolism, is >90% excreted by the kidneys. Urea is filtered in the glomerulus and apparently no tubular secretion occurs. Urea was one of the earliest markers used to estimate renal function. However, significant tubular absorption back into the circulation and large variations in serum urea generation with diet and different diseases (i.e. hepatic) makes it a less suitable alternative for monitoring GFR (Laterza et al. 2002).

2.2.2.3 Cockcroft-Gault formula

In 1976, Cockcroft and Gault published an equation for estimation of creatinine clearance ($CrCl_{C-G}$) from creatinine values adjusting for age and weight (as surrogate for muscle mass) (Cockcroft et al. 1976). The equation was developed based on measured 24-hour urinary CCr in 249 males. For females, a correction coefficient of 0.85 (assuming 15% less muscle mass/kg body weight in women) was adopted. The equation was found to correlate well with mean (two samples) measured CCr in 236 patients, but has been shown to consistently overestimate true renal function due to the tubular secretion of creatinine (Stevens et al. 2006). In heart failure patients, the $CrCl_{C-G}$ formula lacked both precision and accuracy in assessing GFR (Smilde et al. 2006). Increased body weight that does not relate to muscle mass and creatinine production (obesity, fluid overload with oedema) may decrease the accuracy of the estimate of renal function.

2.2.2.4 Modification of Diet in Renal Disease equation

More recently, the Modification of Diet in Renal Disease (MDRD) study cohort of patients with CKD was used to develop an equation for estimation of GFR (eGFR), with the result given as ml/min/1.73 m^2. Compared to the Cockcroft-Gault study, the MDRD cohort was larger (n=1070 for derivation cohort, n= 558 for validation cohort), included around 40% women and GFR was measured using a golden standard with Iothalamate-clearance. All patients in the study had CKD, with mean GFR values of 39.8 ml/min in the overall study population. From the MDRD study, a 4-variable (age, gender, race, creatinine) simplified equation was developed (Levey et al. 1999, Levey et al. 2006).

Validation of the 4-variable MDRD-equation in other cohorts has confirmed good accuracy of eGFR (91% of eGFR values being within 30% margin from measured GFR) and that the performance in estimating actual GFR is superior to CCr or C-G formula (Smilde et al. 2006, Lin et al. 2003, Stevens et al. 2007, Rule et al. 2004). However, the precision of MDRD is higher and bias lower in patients with eGFR values <60ml/min. In patients with eGFR above 60 ml/min and in patient populations not having CKD, the performance of the MDRD-equation, although still good, may be less precise than in patients with CKD (Stevens et al. 2006, Smilde et al. 2006, Stevens et al. 2007, Rule et al. 2004). In heart failure, the 4-variable MDRD-equation was found to be fairly accurate

(43% of estimations within 15% and 80% of values within 30% range of measured GFR) (Smilde et al. 2006).

Because age, gender and race are known or easily available demographic characteristics, and the simplified MDRD-equation does not require body weight, reporting eGFR from any measured creatinine value has become straightforward and has been widely adopted into clinical practice. Use of the MDRD-equation is also recommended by the guidelines (National Kidney Foundation 2002). Nowadays, many laboratories provide eGFR values calculated with the MDRD-equation directly from measured creatinine.

Because of the limitations of the MDRD-equation, an international collaboration collected a large database of cohorts with measured GFR, and patients from various clinical backgrounds. From this collaboration, a new equation for estimating GFR was developed and validated. This equation (CKD-EPI) has recently been published (Levey et al. 2009).

2.2.3 Cystatin C

CysC is a small 13 kDa protein with properties making it a candidate for a good marker of GFR. CysC was first discovered as an protein in an electrophoretic band in the 1960:s and subsequently became characterized as a member of the human cystatin superfamily (Seronie-Vivien et al. 2008, Newman 2002, Filler et al. 2005). The relation of CysC to glomerular filtration rate was described already 25 years ago (Simonsen et al. 1985). In the last decade, interest in the use of CysC as a replacement for creatinine as a marker of renal function has been rapidly growing. Furthermore, several studies have shown CysC to be a strong risk marker for adverse cardiovascular prognosis in various populations.

2.2.3.1 Metabolism of Cystatin C

CysC, a 122 amino acid cysteine protease inhibitor, is produced at a constant rate by all nucleated cells in the body. This is a consequence of CysC being a product of a "housekeeping" gene with stable and continuous expression (Laterza et al. 2002, Seronie-Vivien et al. 2008). As a small-sized protein, CysC is filtrated freely in the glomerulus, with no known extrarenal excretion or degradation. In the proximal tubule of the nephron, there is a re-uptake and complete degradation of CysC but without any reabsorption into the bloodstream. Normally, only small amounts of CysC are excreted into the urine.

In contrast to creatinine, age, gender, diet or body composition have no or little effect on levels of CysC (Laterza et al. 2002, Seronie-Vivien et al. 2008, Knight et al. 2004). Different medical conditions (inflammation or malignancy) do not appear to alter the levels of CysC with the exception of thyroid illness (Seronie-Vivien et al. 2008). Hypothyroidism has been associated with a decrease in GFR and higher creatinine levels, and several small studies report a decrease in creatinine and increase in eGFR upon restoration of euthyroidism (Jayagopal et al. 2003, Claus et al. 2005, Woodward et al.

2008). Paradoxically, it seems that CysC levels are lower in hypothyroid and higher in hyperthyroid patients and increase and decrease respectively, when patients are treated to the euthyroid state (Jayagopal et al. 2003, Fricker et al. 2003, den Hollander et al. 2003). The studies investigating the effects of thyroid function on levels of CysC have been small, used only estimated GFR or CCr values, and in other reports no association between thyroid hormones and CysC levels have been found (Kottgen et al. 2008, Kilic et al. 2009). Nevertheless, since the production of CysC is dependent on cellular metabolism, the finding that hypothyroidism is associated with lower and hyperthyreosis with increased levels of CysC would appear plausible. Data from one in-vitro study and one study in patients with renal transplants have suggested that glucocorticoids in high doses affect CysC levels (Risch et al. 2001, Bjarnadottir et al. 1995) but again not all studies have confirmed that steroids interfere with the relation between CysC and GFR (Kazama et al. 2002).

2.2.3.2 Cystatin C – a marker of renal function

CysC possesses most properties of an ideal marker of GFR (endogenous substance, constant production, free glomerular filtration, no extra-renal excretion, no tubular re-absorption, easily measured) (Laterza et al. 2002). Indeed, since the development of rapid automated assays for measurement of CysC, many studies have assessed the relationship of CysC with GFR and compared it with creatinine (Rule et al. 2006, Seronie-Vivien et al. 2008, Newman et al. 1995, Kyhse-Andersen et al. 1994).

Several authors have confirmed that CysC is a better marker of GFR than creatinine, CCr or eGFR, especially in patients with mild impairment of renal function (Laterza et al. 2002, Kazama et al. 2002, Rule et al. 2006, Hoek et al. 2003, Harmoinen et al. 2003, Coll et al. 2000, Pucci et al. 2007). Although not all studies have found advantages with CysC over creatinine or creatinine based equations (Macisaac et al. 2006, Spanaus et al. 2010, Oddoze et al. 2001), two meta-analyses consistently showed CysC to be superior to creatinine as a marker of GFR (Dharnidharka et al. 2002, Roos et al. 2007). Rule et al. (2006) used iothalamate clearance to establish the relationship between CysC and GFR in healthy adults (n=50), patients with CKD (n=204) and transplant recipients (n=206). CysC reflected GFR better than creatinine in all three groups. The eGFR prediction equation for CysC had higher correlation with measured GFR than equations for creatinine (including MDRD and $CrCl_{C\text{-}G}$) and was not improved by including age or gender as parameter (Rule et al. 2006).

Lately, reports of an association between CysC and factors not related to GFR have been published (Knight et al. 2004, Kottgen et al. 2008, Ognibene et al. 2006, Stevens et al. 2009b). A correlation between CysC and age is to be expected, as GFR declines with age (Odden et al. 2010). Therefore, different reference values of CysC have been developed for normal renal function in young adults and people aged over 50 years. Conflicting data exists about whether CysC levels vary with gender, but after the age of 60, no difference between men and women is seen (Rule et al. 2006, Seronie-Vivien et al. 2008). Dietary protein intake does not affect CysC levels (Tangri et al. 2010).

Attempts to convert CysC values (mg/L) to corresponding GFR (ml/min) have resulted in multiple equations in the literature (Macisaac et al. 2006, Herget-Rosenthal et al. 2004, Grubb et al. 2005, Stevens et al. 2008, Macdonald et al. 2006). Differences in studied population and size, CysC measurement as well as the reference GFR method makes validation difficult and gives poor generalizability (Seronie-Vivien et al. 2008, Herget-Rosenthal et al. 2004, Madero et al. 2006). One small study found CysC levels to be affected by lean body mass (as a surrogate for muscle volume) and that GFR prediction could be improved by incorporating lean mass or anthropometric measures in the equation (Macdonald et al. 2006). Some papers examining the association between CysC levels and other factors lacked direct GFR measurements and have compared CysC to creatinine or adjusted for CCr, eGFR or $CrCl_{C\text{-}G}$ (Knight et al. 2004, Ognibene et al. 2006, Singh et al. 2007, Young et al. 2008). These creatinine based estimations of renal function are not reliable measures of true GFR and diminish the strength of the observations.

The largest study so far comparing GFR estimates from CysC and creatinine was performed as pooled analysis of cohorts with CKD and measured GFR (Stevens et al. 2008). CysC was indeed less influenced than creatinine by non-renal factors, but a small variability (<10%) in CysC according to age, gender and race was still observed. GFR estimated from CysC alone performed nearly as well as the MDRD-based eGFR. Nevertheless, by incorporating age, gender and race or even creatinine with CysC, the bias in the GFR estimate was reduced. As acknowledged also by the authors (Stevens et al. 2008), this comparison was performed in CKD patients for whom the MDRD-equation was developed and therefore has optimal performance. Combining GFR estimates from creatinine and CysC seems to increase accuracy of the equations compared to measured GFR (Tidman et al. 2008). In a middle-aged population without heart or kidney disease and an average measured GFR of 91 ml/min, GFR estimated from CysC equations offered no advantage over eGFR from creatinine (Eriksen et al. 2010). Nevertheless, it is not excluded that CysC-based estimates of GFR can be better in other populations, especially in cases where the MDRD-equation may lack accuracy.

Based on these observations, CysC may not be an entirely specific marker of GFR to replace direct measurements using isotopes. Still, the influence of body composition or other non-renal factors on CysC levels is much smaller than for creatinine, and CysC has true potential for easy and reliable estimation of kidney function. The use of CysC for assessing kidney function could be advocated in populations where the disadvantages of creatinine might be particularly evident (elderly populations with many co-morbidities, hospitalized, malnourished or amputated subjects, heart failure patients) or where early detection of mild impairment of renal function (transplant recipients, diabetics, CVD) is of clinical importance (Filler et al. 2005, Pucci et al. 2007, Wasen et al. 2004, O'Riordan et al. 2003, Fliser et al. 2001, Perkins et al. 2005, Shlipak et al. 2006b). Compared to creatinine or creatinine based equations, CysC would allow better and more accurate assessment of renal function in clinical practice without affecting simplicity or availability.

2.3 Acute Kidney Injury

Acute kidney injury (AKI), formerly also known as acute renal failure, is a clinically significant decline in glomerular filtration rate secondary to kidney damage, and occurring within a limited time. This is reflected as oliguria and/or a rise in markers of renal function such as creatinine or CysC. Most clinicians are familiar with the phenomenon, and traditionally acute renal failure has been divided into pre-renal, renal and post-renal according to the supposed site of the problem. Pre-renal indicating abnormalities in the hemodynamic or volume status of the patient and suggesting that in the absence of such disturbances, the kidney would have normal function. Renal causes include intrinsic renal disease (infectious, immunologic and inflammatory) or effects of prolonged pre-renal ischemia, exogenous or iatrogenic toxins, while post-renal usually refers to hindrance of the flow of urine. This distinction offers little information about actual pathophysiological processes and is mainly useful in management: excluding the presence of post-renal obstruction by imaging and optimising volume status and hemodynamics to correct possible pre-renal causes. To date, specific treatment to prevent deterioration of established AKI or reverse kidney damage is not available (Waikar et al. 2008).

2.3.1 Definitions

Until recently, there was no uniform definition of acute renal failure. First presented by the Acute Dialysis Quality Initiative in 2004, the RIFLE-classification became a consensus definition and was well adopted (Bellomo et al. 2004). This classification defines acute renal failure by increases in creatinine and/or urine output, with growing severity from the R-criterion (*Risk*) through the I-criterion (Injury) to the F-criterion (*Failure*). *Loss* and *End-stage renal disease* constitute more of clinical endpoints with need of renal replacement therapy (dialysis) (Bellomo et al. 2004, Kellum et al. 2008). For entering the R-category, a rise in creatinine >50% from baseline is required.

The Acute Kidney Injury Network (AKIN) modified the RIFLE-criteria and introduced the expression Acute Kidney Injury (AKI) to illustrate the entire spectrum of acute renal failure (Mehta et al. 2007). AKI is now the preferred term to describe acute worsening of kidney function and what previously has been called acute renal failure. The AKIN classification of AKI was developed from the previous RIFLE-classification. An increase in creatinine or oliguria still forms the basis for detecting AKI (*Figure 4*).

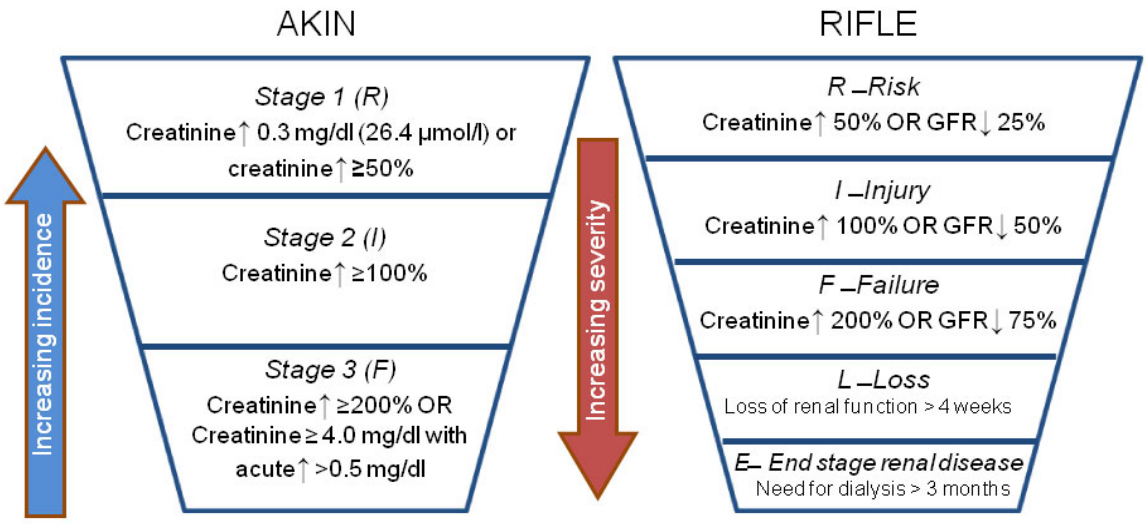

Figure 4 *The creatinine criteria of the RIFLE and AKIN classifications and stages. Modified from Bellomo et al 2004 and Mehta et al 2007*

In addition, a time frame of 48 hours for the rise in creatinine was suggested by AKIN (Mehta et al. 2007). This ensures that the change in renal function is acute and increases the probability of a clinically relevant event. Importantly, while the 50% increase in creatinine and oliguria criteria from the RIFLE-classification were retained, the definition also suggested that minor increments in creatinine of ≥0.3 mg/dL (≥26.5 µmol/L) would fulfill the definition of AKI. Detection of AKI relies on these modified criteria and diagnosis can be made using any of these measures. A separate staging system was proposed for assessing severity of AKI, which is not limited to the 48 hour time frame. Any patient meeting the AKIN-definition is classified as stage 1, if not meeting stage 2 or 3 criteria. A rise in creatinine of more than 2- to 3-fold (>200-300%) from baseline or prolonged oliguria (<0.5 ml/kg/h with duration >12 hours) is classified as stage 2, while an increase in creatinine exceeding 300% (rise to >3-fold) from baseline or reaching a creatinine value >4.0 mg/dL (with at least 0.5 mg/dL increment from baseline) was regarded the most severe stage 3 AKI. While the RIFLE-classification has proven to be useful in detection, classification and prognostication of acute renal failure, there is more limited experience with the use of the AKIN-criteria.

2.3.2 Incidence and clinical significance

The incidence of AKI is highly variable between different populations. The critically ill and patients undergoing major surgery are at very high risk of developing AKI, and the incidence is highest in these populations (Hoste et al. 2008, Liangos et al. 2006, Himmelfarb et al. 2007). Most of the studies on AKI have been performed in the intensive care unit (ICU) or surgical setting. Another population with high incidence of AKI is patients admitted with AHF (see chapter 2.4 on the CRS).

It needs to be emphasized that the incidence rate is highly dependent on the definition of an event. Death is an unambiguous event, and so is the initiation of renal replacement therapy. In contrast, the distinction between physiological adaptation of GFR and harmful kidney injury with decline in GFR is not clear cut. Moreover, actual GFR is not measured. The occurrence of AKI as an event is dependent on one hand of the magnitude of change of the marker required to define the event and on the other hand of the accuracy of the marker used to reflect true change in kidney function.

The incidence of acute renal failure in the ICU and after surgery in studies using the RIFLE-criteria has varied between 10-70% (Hoste et al. 2008). In a large retrospective analysis of 29 million hospitalizations in the United States, the incidence of AKI based on discharge diagnosis was 19.2/1000 hospitalizations (Liangos et al. 2006). Chertow et al analyzed in-hospital changes in creatinine from 9210 admissions with at least two creatinine measurements, and reported that 13% of patients had an increase of ≥0.5 mg/dL from baseline (Chertow et al. 2005). Using the RIFLE-criteria, 18% of patients admitted to a university hospital were found to have AKI: 9.1%, 5.2% and 3.7% were in the R-, I- and F-category respectively (Uchino et al. 2006).

The effect of AKI on outcomes has been investigated in numerous studies, and the results are summarized in recent meta-analyses (Coca et al. 2009, Ricci et al. 2008, Coca et al. 2007). The definition of AKI in the original publications has been variable, ranging from a rise in creatinine to the need for renal replacement therapy. Some studies have used the RIFLE-criteria after these were published (Ricci et al. 2008). In the majority of these studies, mortality has been the outcome of interest. Both meta-analyses reported that AKI was associated with a significant increase in mortality with a risk ratio for long term mortality above two. There was also a graded risk with higher mortality related to the severity of AKI (Coca et al. 2009, Ricci et al. 2008).

2.3.3 Detection of AKI

As for assessing renal function, the diagnosis of AKI has relied on creatinine for over half a century. Clinical signs such as oliguria can also be used but evidence of a reduction in GFR by a biomarker is often required. CysC might be an alternative to creatinine as marker of GFR in AKI. Obviously, the decline in renal *function* (i.e. GFR) as a consequence of an *injury* is a delayed event and prevention of further injury or salvage of kidney function may be difficult. New biomarkers thought to be specific for detection of kidney injury have become available only recently (see below). These are not functional markers but reflect damage to the kidney which eventually, if severe enough, results in a decline in GFR.

2.3.3.1 Creatinine as marker of AKI

As a marker of change in GFR, creatinine is easily available and a significant rise in creatinine has good specificity for a decline in renal function. However, as discussed

previously, creatinine is affected by many non-renal factors. Most importantly creatinine is a rather slow marker of changes in renal function. The drop in GFR required to be detected by creatinine is dependent on the baseline level of renal function and the time of observation. Using different mathematical models, Waikar et al (2009b) calculated the magnitude of change in GFR required and the time necessary before this change was detected as a rise in creatinine of 0.5 mg/dL (44 µmol/L). Assuming normal renal function at baseline, a >40% reduction in GFR will result in a rise in creatinine after approximately 24 hours (Waikar et al. 2009b). If renal function is already impaired (CKD 3-4) at the time of the injury, a numerically smaller (but clinically more important) 33% decrease in GFR may be detected by creatinine at 24 hours. To be detected as a rise in creatinine by 0.5 mg/dL within 12 hours from injury, GFR would need to drop over 50% from baseline. This severe reduction in kidney function is possible to observe as a rise in creatinine at 12 hours regardless of baseline kidney function. Overall, it is clear that creatinine is not a very sensitive marker of a rapid decline in renal function (Waikar et al. 2009b).

Equations estimating GFR from creatinine were developed in patients with stable CKD and require a stable creatinine level for accurate estimation of GFR. Besides having rather poor sensitivity for AKI, creatinine reaches a steady state slowly after a rapid change in GFR. After a 50% reduction in creatinine clearance, creatinine will be at a steady state only after 34 hours, if renal function was normal at baseline, and in stage 3 CKD, a steady state will be achieved only after several days (Waikar et al. 2009b). This severely compromises the utility of eGFR equations to assess renal function in AKI.

Thus, creatinine cannot be regarded as a very good marker of AKI, and the quest for novel and better markers for detection and quantification of AKI is of high priority (Waikar et al. 2009a, Parikh et al. 2009).

2.3.3.2 Cystatin C in AKI

As discussed in chapter 2.2, CysC is regarded to be a better marker of GFR than creatinine (Dharnidharka et al. 2002, Roos et al. 2007). CysC is not dependent on age, gender or muscle mass, and in brief, is less affected by non-renal factors. Only a few studies have investigated the use of CysC in AKI, mostly in the in the intensive care setting.

The first study assessed whether CysC could detect acute renal failure earlier than creatinine when using the RIFLE-criteria (Herget-Rosenthal et al. 2004). Of 85 patients in the ICU at risk, 52% developed AKI. While both renal markers were normal three days before the RIFLE-criteria were fulfilled, CysC rose significantly (mean increase 42%) already two days before the R-criterion was met. In patients developing AKI, a 50% increase in CysC was observed on average 1.5 days before a similar increase in creatinine was detected. The area under the curve (AUC) of the receiver operating characteristics (ROC) curve for CysC to predict the R-criterion was good (AUC 0.82) two days and excellent (AUC 0.97; 95% confidence interval [CI] 0.94-0.99) one day before the creatinine based R-criterion was fulfilled. At this time point, a significant rise in creatinine (21%) was detected for the first time, although creatinine values were still predominantly within the normal range (Herget-Rosenthal et al. 2004).

Most recently, serial measurements of creatinine and CysC performed in a larger population of patients admitted to ICU confirmed that CysC increased earlier than creatinine (Nejat et al. 2010). This was true both for a 25% and a 50% increase as cut-off. A rise in CysC was found to precede a rise in creatinine in two thirds of the patients with a significant increase in either or both markers. In patients where CysC rose first, mean creatinine remained astonishingly stable, and in many patients did not reach any of the defined thresholds (25% or 50% increase). A significant increase in CysC was seen without concomitant increase in creatinine. In the rest of the population either both markers rose above the cut-off or a smaller proportion of patients had a rise in creatinine before CysC, but in these CysC closely followed the rise in creatinine. In this study, a 20-25% decrease in creatinine values in patients not experiencing AKI was also detected during the 7 days of in-hospital follow-up. The authors suggested that fluid loading and progressive loss of muscle mass in the critically ill could explain this observation. In patients without AKI, the change in CysC was <10% (Nejat et al. 2010).

In other studies from the ICU, CysC had better correlation to urinary CCr, had superior sensitivity to detect renal dysfunction than creatinine and high CysC levels predicted mortality in patients with AKI (Villa et al. 2005, Bell et al. 2009). One study assessed the ability of a single CysC measurement to predict the requirement of dialysis or in-hospital death in AKI and compared its predictive performance to creatinine, serum urea nitrogen levels and urine output. All measures of renal function were independent predictors of the combined endpoint with similar odds ratios (OR). However, using ROC analysis adding renal function to a model with general clinical measures, markers of renal function did not improve the AUC for the prediction of the combined endpoint (Perianayagam et al. 2009).

Three studies have assessed CysC as a marker of AKI after cardiac surgery. Again, the performance of CysC for diagnosis of AKI was evaluated using the creatinine based RIFLE criteria as reference. In the first one, CysC and serum neutrophil gelatinase associated lipocalin (NGAL), a novel marker of kidney injury, were compared to creatinine and urea for prediction of AKI in 100 patients who underwent cardiac surgery (Haase-Fielitz et al. 2009b). In patients with AKI (23%), the relative increase in creatinine from preoperative values was 111% and peaked at 68 hours after surgery. As baseline renal insufficiency is a known risk factor for AKI, it is interesting to note that while preoperative values of the other markers did not differ, CysC levels were higher in the group who developed AKI postoperatively. On arrival in ICU, levels of NGAL ($p=0.005$), CysC ($p=0.001$) and creatinine ($p=0.03$) were already increased in patients with subsequent AKI compared to those without. All markers then continued to rise and were significantly higher in the AKI group at 24 hours after surgery compared to the non-AKI group where all markers remained stable. NGAL and CysC levels were independent predictors of AKI while creatinine was not. CysC and NGAL on arrival in ICU were found to be superior to creatinine for prediction of AKI. At 24 hours all markers showed similar performance statistics as assessed by the AUC. Wald et al (2010) also found that levels of CysC, but not creatinine, were higher in the group with subsequent AKI, but the ability of CysC measured as early as two hours after surgery to predict AKI was only modest. The third study performed on elderly patients undergoing cardiac surgery showed a slightly better performance of CysC measured on the first postoperative day (AUC 0.71)

compared to creatinine (AUC 0.66) to predict AKI but the difference was not statistically significant (p=0.11 for difference) (Ristikankare et al. 2010). In this study, the majority of patients had increases >50% from preoperative levels on day 2 for CysC (9/17) compared to day 3 for creatinine (15/30).

Outside the ICU or perioperative environment, Soto et al (2010) examined consecutive patients admitted to the emergency department, and measured both serum and urinary CysC. While urinary CysC was useful only in differentiating patients with AKI (21%) from those with normal renal function (51%), serum CysC had excellent early discriminative properties for AKI, both compared to normal renal function and pre-renal azotemia (26%). Creatinine was not able to differentiate between AKI and pre-renal azotemia, but performed well in prediction of AKI (Soto et al. 2010). All of the aforementioned studies assessed the predictive power of CysC measured at a single time-point, without assessing the performance of a change in CysC as marker of AKI.

A single, very recent, study has examined the discriminatory capacity of a change in CysC for prediction of contrast-induced nephropathy and assessed clinical outcomes up to 12 months. A 10% increase in CysC 24 hours after exposure to contrast media occurred in 21% of patients and showed good performance (AUC 0.92) for prediction of AKI (creatinine increase by 0.3 mg/dL at 48 hours) with excellent sensitivity (100%) and good specificity (86%). An increase in CysC at 24 hours was also associated with subsequent clinical events, i.e. mortality and need for dialysis, during one year of follow-up (Briguori et al. 2010).

2.3.3.3 New markers of tubular damage

Because of the rather poor ability of creatinine to detect or predict AKI, new biomarkers are needed. On the other hand, both plasma CysC and creatinine are markers of renal function (i.e. GFR). As mentioned above, detection of a functional decline will inevitably be a late event and the possibility of earlier detection of the actual injury opens new diagnostic and therapeutic frontiers. Urinary markers of tubular injury are of great potential and seem to have good specificity for kidney damage. In recent years, several biomarkers of kidney injury have been identified (*Table 5*) (Coca et al. 2008).

Of these, NGAL has been the most studied and methods for determining urinary or serum/plasma levels of NGAL are now commercially available (Mishra et al. 2005, Bolignano et al. 2008, Han et al. 2009). Most studies on NGAL and other markers have been performed in settings where the risk or incidence of AKI is high, such as intensive care populations or patients undergoing (cardiac) surgery or receiving radiocontrast agents (Haase et al. 2009). In a larger and unselected population of patients admitted to hospital, urinary NGAL was a good predictor of AKI and was able to differentiate AKI from patients with chronic renal diseases or acute infections (Nickolas et al. 2008). The use of urinary CysC for detection of tubular injury is not discussed here (Bagshaw et al. 2007, Bagshaw et al. 2010).

Table 5. *List of new putative urinary markers of tubular damage*

α-1-microglobulin	26kDa glycoprotein involved in the metabolism of heme. Normally filtrated and reabsorbed by tubular cells. Increased urinary secretion in kidney injury.
Cystatin C	Urinary concentration of CysC is normally undetectable or very low but increases in AKI due to renal tubular damage.
FABP Fatty Acid Binding Proteins	Members of the lipocalin superfamily. L- (liver) and H- (heart) type FABP found in proximal and distal tubule in the kidney. Measured in urine in AKI.
IL-18 Interleukin-18	Marker of tubular inflammation. Increases in urine after acute tubular necrosis.
KIM-1 Kidney Injury Molecule-1	Proximal tubular protein with increased concentrations in urine after ischemic kidney injury
MMP-9 Matrix MetalloProteinase-9	Normally degraded in the tubules, levels increase in urine after kidney injury
NAG N-Acetyl-β-D-Glucosaminidase	Lysosomal enzyme expressed in kidney tubular cells. Secreted in urine in kidney injury
NGAL Neutrofil gelatinase associated lipocalin	Measured in serum or in urine. Expressed and secreted in large amounts by kidney epithelia as a response to injury
NHE-3 Sodium-Hydrogen Exchanger isoform-3	Proximal tubular protein with increased concentrations measured in urine after kidney injury

Several questions concerning the use of new biomarkers of AKI are yet unanswered. These include identification of patients that benefit from the measurement of a new marker and the determination of useful cut-offs in different populations. Are these markers specific to kidney injury or how much are the levels influenced by other factors? Can some of these new markers give additional information about the aetiology of the injury (ischemic, toxic and inflammatory)? What interventions should be performed based on these markers? So far, the clinical use of markers of tubular damage and kidney injury has been limited.

2.4 The cardiorenal syndrome

The term cardiorenal syndrome is mentioned in the medical literature already 50 years ago (Odel et al. 1951, Ledoux 1951). The possible interaction between heart and kidneys in the pathophysiology of disease was first described almost a century earlier as an autopsy finding, but most of the publications about the CRS are from the 21st century. Still, a clear definition of the CRS has been lacking.

The excessive mortality from CVD in patients with end-stage renal disease is well known among nephrologists. Patients with end-stage renal disease have a 20- to 30- fold increase in cardiovascular risk (Sarnak et al. 2003, Schiffrin et al. 2007). In fact, the risk of death from a cardiovascular event in a young adult on dialysis equals that of an 80 years old person in the general population (Foley et al. 1998). Conversely, renal insufficiency is an independent predictor of outcomes in patients with different manifestations of CVD, but also in the general population (Go et al. 2004, Schiffrin et al. 2007, Fonarow et al. 2006, Muntner et al. 2002). Although the increase in cardiovascular risk is highest in patients with end-stage renal disease and most evident in patients with eGFR <60 ml/min (Go et al. 2004, Muntner et al. 2002), some reports also have found mild impairment in renal function to be associated with adverse cardiovascular prognosis (Anavekar et al. 2004, Tonelli et al. 2006, Van Biesen et al. 2007).

In addition to the effect of renal function on cardiovascular outcomes both in CKD and CVD, the heart-kidney interaction is particularly evident in heart failure patients. Renal insufficiency is highly prevalent in patients with heart failure (Hillege et al. 2006, Smith et al. 2006). Worsening renal function during hospitalization for heart failure is common and associated with prolonged length of hospital stay (LOS), higher re-hospitalization rates and increased mortality (Smith et al. 2003, Gottlieb et al. 2002, Damman et al. 2007). Finally, the clinical phenomenon of “diuretic resistance”, with poor response to diuretic treatment and decreased urine output in volume overloaded heart failure patients, is also recognised among cardiologists and has been attributed to the CRS (Tang et al. 2010).

2.4.1 Definition of the CRS

The CRS has recently been defined as a bidirectional disorder involving the heart and the kidneys where primary dysfunction in either one of these organs compromises the function of the other leading to a “vicious circle” with deleterious effects on prognosis (Ronco et al. 2010). The interplay between heart and kidney occurs at several levels: physical, chemical and biological (Ronco et al. 2008b, Ronco et al. 2008a). The clinical manifestations of the CRS briefly mentioned above are discussed more in detail below.

2.4.1.1 Classification and pathophysiology

The bidirectional nature of the CRS was emphasized by Ronco et al (2008a and 2008b). A new classification that incorporates both the primary initiator organ (cardio-renal vs. reno-cardiac) and nature of the dysfunction (acute or chronic) of the syndrome has been put forward by the Acute Dialysis Quality Initiative (Ronco et al. 2010). In addition to acute and chronic cardio-renal (CRS types 1 and 2) and reno-cardiac (CRS types 3 and 4) syndromes, CRS 5 is characterized by systemic disease damaging both organs simultaneously (*Table 6*).

Table 6. *Classification of the cardiorenal syndrome (according to Ronco et al 2010)*

Type	1	2	3	4	5
Definition	Acute cardio-renal syndrome	Chronic cardio-renal syndrome	Acute reno-cardiac syndrome	Chronic reno-cardiac syndrome	Secondary cardiorenal syndrome
Description	Acute cardiac event causing kidney dysfunction	Chronic heart disease causes CKD	Acute kidney disease causes disturbances in cardiac function	Cardiac disease related to CKD	Cardiac disease and renal failure caused by systemic illness
Clinical event	AKI: increase in creatinine or CysC, oliguria	Slowly (months-years) progressive kidney dysfunction	Volume overload, heart failure, electrolyte disturbances, arrhythmias	Ventricular hypertrophy, CAD, heart failure. High risk of cardiovascular events.	Heart failure, CAD, arrhythmias, CKD, proteinuria

AKI=acute kidney injury, CysC=cystatin C, CKD=chronic kidney disease, CAD=coronary artery disease.

The pathophysiologic processes behind CRS are not well understood, and most probably numerous and complex. The traditional view of low blood pressure and low cardiac output in heart failure leading to decreased renal perfusion is certainly an oversimplification. Firstly, most patients with heart failure have normal or elevated blood pressure, and approximately half of them preserved LVEF (Nieminen et al. 2006, Adams et al. 2005, Tavazzi et al. 2006). As will be discussed later, the CRS is not restricted to patients with heart failure. More importantly, kidney function, i.e. GFR, is regulated at multiple levels and dependent on perfusion pressure at the glomerular level, renal blood flow and filtration fraction. In the normal kidney there is a strong autoregulation keeping stable perfusion pressure despite large variations in arterial blood pressure. This autoregulation can be deranged in disease, like hypertension and CKD (Palmer 2002).

Renal blood flow and GFR are regulated through vasoconstriction or vasodilation of afferent end efferent arteriole. This regulation is controlled by the RAAS and additionally modulated by the tubulo-glomerular feedback mechanism mediated by adenosine (Persson 2002). Renal blood flow is also dependent on cardiac index. An early study in chronic heart failure patients found that renal blood flow was reduced by 40% when cardiac index

decreased 25% (Ljungman et al. 1990). This was however not accompanied by a decrease in GFR, which remained stable due to a concomitant increase in filtration fraction. In a more contemporary heart failure population on ACEI medication, which inhibits vasoconstriction of the efferent arteriole, a drop in renal blood flow was more linearly associated with a decrease in GFR (Smilde et al. 2009). Still, the main determinants of GFR in heart failure seem to be renal blood flow and central venous pressure (CVP), not cardiac index (Damman et al. 2007).

In a large cohort of patients with various cardiovascular diseases, CVP was a stronger determinant of GFR than cardiac index (Damman et al. 2009). In AHF, hemodynamic parameters like heart rate, SBP, pulmonary capillary wedge pressure and cardiac index were similar between patients with and without worsening renal function during hospitalization, and none of these were predictors of a decline in eGFR (Nohria et al. 2008, Mullens et al. 2009). In fact, in one study cardiac index was even higher in patients developing worsening renal function (Mullens et al. 2009). Nonetheless, factors other than blood pressure and cardiac index seem to be of importance in the CRS. The mechanisms thought to be centrally involved in the pathophysiology of CRS are schematically presented in *Figure 5*.

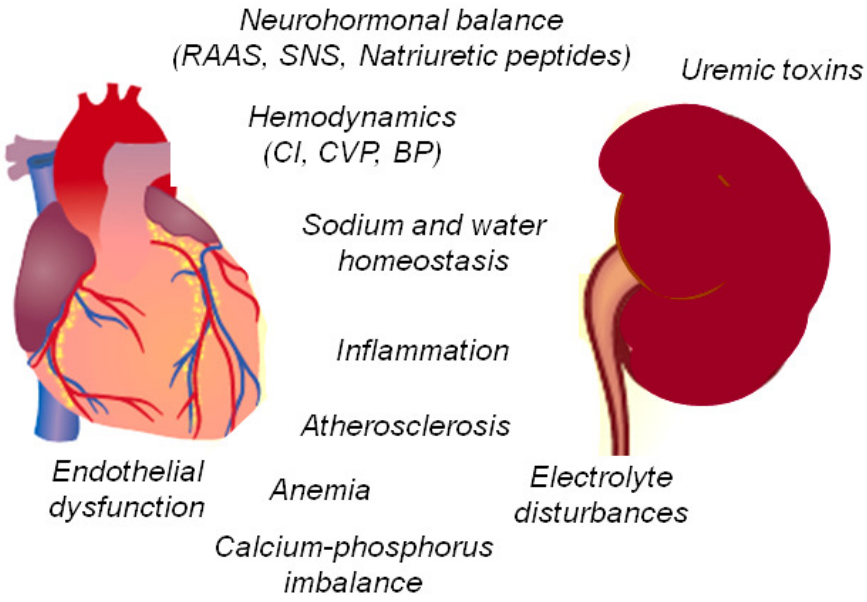

Figure 5 *Factors associated with the pathophysiology of the cardiorenal syndrome.*
RAAS= renin angiotensin-aldosterone system, SNS=sympathetic nervous system, CI=cardiac index, CVP= central venous pressure, BP= blood pressure

Although the exact pathways of the CRS are not known and may vary in different patient populations and between individuals, the new classification serves as a useful platform for identification of clinical scenarios and pathophysiological mechanisms. This forms the basis for the development of new therapeutic strategies.

2.4.2. Renal function and cardiovascular disease

The deleterious effect of renal insufficiency on cardiovascular outcomes was first noted in patients on dialysis. Subsequent studies found that CKD and reduced kidney function in patients not on dialysis was associated with increased mortality and higher rates of cardiovascular events (Tonelli et al. 2006). CKD stage 3 (eGFR <60 ml/min) has been considered a cut-off for identification of patients being at high-risk for cardiovascular morbidity and mortality, with higher prevalence of common cardiovascular risk factors. Renal insufficiency has also been associated with higher risk of death from non-cardiovascular causes (Fried et al. 2005). Go et al. published a large population study showing a stepwise increase in all-cause mortality and cardiovascular events with decreasing eGFR below 60 ml/min (Go et al. 2004). Increased cardiovascular risk has also been described in mild renal insufficiency (eGFR ≥60 ml/min) both in the general population and in patients with, or at high risk of, CVD (Anavekar et al. 2004, Tonelli et al. 2006, Mann et al. 2001, Fried et al. 2003).

Several studies have shown that worse renal function is an independent predictor of cardiovascular events both in the general population as well as in CVD and CKD. Moreover, CVD has been a risk factor for decline in renal function and development of CKD. In a population with mean eGFR about 90 ml/min at baseline, CVD was independently associated with the risk of kidney function decline and development of CKD (eGFR< 60 ml/min) (Elsayed et al. 2007). This highlights the bidirectional nature of the CRS.

2.4.3 Heart failure and CRS

Heart failure patients in many aspects represent the ultimate manifestation of CRS, with co-existence of renal insufficiency being highly prevalent and progressive worsening of both heart and kidney disease (CRS type 2). In addition, frequently occurring CRS type 1 (rapid decline in renal function in the setting of an acute cardiac event) during hospitalization for AHF and diuretic resistance are common problems in the care of heart failure patients.

Several studies have reported high prevalence of renal insufficiency in patients with heart failure, as measured by creatinine levels or estimations of $CrCl_{C\text{-}G}$ or eGFR (Hillege et al. 2000, Dries et al. 2000, Hillege et al. 2006, Ezekowitz et al. 2004, McAlister et al. 2004, de Silva et al. 2006). In a meta-analysis, approximately half of ambulatory heart failure patients had some degree of impairment of renal function and 10% had moderate to severe renal insufficiency (Smith et al. 2006). The individual studies examining renal function in chronic heart failure have consistently found renal insufficiency to be a strong and independent risk factor for mortality, both in patients with systolic and diastolic heart failure (Hillege et al. 2006, McAlister et al. 2004, Ahmed et al. 2007) and independent of the severity of heart failure (Dries et al. 2000). In the same meta-analysis, any impairment of renal function increased mortality risk by 50% and moderate to severe renal

insufficiency was associated with a 2-fold risk of death, even after adjusting for other prognostic factors (Smith et al. 2006).

2.4.4 CRS in AHF

AHF requiring hospitalization may be the first presentation of heart failure (de-novo AHF), or patients with previous heart failure may have deteriorating symptoms and need hospitalization for relief of congestion and stabilisation. On admission, 40-60% of AHF patients have abnormal kidney function, depending on the method used for assessment (Smith et al. 2006, Tavazzi et al. 2006, Zannad et al. 2006, Heywood et al. 2007). A study on over 6000 consecutive hospitalizations for AHF over 15 years reported a temporal trend towards worse renal function on admission (higher creatinine levels and lower eGFR values). Reasons for this observation probably include increasing mean age of AHF patients and increasing prevalence of hypertension and diabetes (Owan et al. 2006a). Impaired renal function in patients with AHF is extremely common and it is clear that this high prevalence of kidney dysfunction is not primarily due to intrinsic renal disease but a reflection of the CRS (type 2 CRS) in the heart failure population.

2.4.4.1 Renal function and effect on prognosis in AHF

The prognostic effect of renal insufficiency at admission on prognosis in patients with AHF has been examined in several different cohorts in recent years, and the message is clear (*Table 7*). After adjustment for age, gender, co-morbidities and often also clinical characteristics (blood pressure, NYHA class on admission), renal function emerges as a strong and independent risk factor for both in-hospital mortality and death during follow-up. In many studies, renal insufficiency has been the most powerful predictor of adverse outcome in AHF.

2.4.4.2 Worsening renal function in patients hospitalized for AHF

If renal insufficiency is common in patients with AHF on hospital admission, so is the occurrence of worsening renal function during hospital stay. This is a typical example of the acute (type 1) CRS. A decline in renal function has most often been measured by a rise in creatinine values, and assessed as the difference between admission creatinine and peak creatinine during hospitalization. The preferred term AKI will subsequently be used for in-hospital worsening renal function in AHF (Mehta et al. 2007).

Table 7. *Studies reporting effect of renal insufficiency on mortality in AHF*

Study	Year	N=	Mean SCr	Definition of kidney dysfunction	End-point	Follow-up	Risk increase[a]
Cowie	2002	332	1.3	/0.12 mg/dL ↑SCr	Mortality	19 month	7%*
Lee	2003	4031	1.5	/10mg/dL ↑Urea	Mortality	1 year	49%†
Akhter	2004	481	1.3	SCr>1.5 mg/dL	Mortality	6 months	172%†
Owan	2006	6440	1.6	/1 mg/dL ↑SCr	Mortality	3 months	12%†
Hyunh	2006	282	1.7	/10mg/dL ↑Urea	Mortality	14 years	20%†
Zannad	2006	599	2.3	History of CKD or elevated creatinine	Mortality	1 year	84%†
Tavazzi	2006	2807	1.7	/1 mg/dL ↑Urea	In-hosp. death	NA	0.7%†
Heywood	2007	118465	1.8	/10 ml/min ↓eGFR	In-hosp. death	NA	18%*
Nohria	2008	433	1.5	/10 ml/min ↓eGFR	Mortality	6 months	25%*
O'Connor	2008	4402	1.3	/1 mg/dL ↑SCr	Mortality	90 days	16%†
Abraham	2008	42612	1.8	/0.3 mg/dL ↑SCr	In-hosp. death	NA	18%†
Harjola	2010	2981	1.2	/0.57 mg/dL ↑SCr	Mortality	1 year	20%*

*SCr=serum creatinine in mg/dL (conversion factor 88.4 to µmol/L), NA=not available, ↑=increase, ↓=decrease, /=per unit change, *p<0.05, †p<0.01, [a]adjusted OR/HR*

The incidence of AKI in AHF populations is lower than in cohorts from the intensive care unit. AKI may also be less severe as only few patients have large (>50%) increases in creatinine or require renal replacement therapy. Nevertheless, increases in creatinine by 0.3 mg/dL (26.5 µmol/L) or 0.5 mg/dL (44 µmol/L) have been shown to increase LOS and are associated with higher in-hospital and short-term mortality. The incidence of AKI in AHF has been surprisingly consistent despite differences in patient characteristics and definitions between studies (*Table 8*). Using a rise in creatinine of >0.3 mg/dL during the index hospitalization as definition for AKI, 25-40% of AHF patients experience the event (Gottlieb et al. 2002, Forman et al. 2004, Cowie et al. 2006, Krumholz et al. 2000, Logeart et al. 2008, Metra et al. 2008). The incidence of AKI is naturally dependent of the definition employed. Smaller changes in creatinine are more common, while a larger increase of 0.5 mg/dL occurs in approximately 20% of patients (Smith et al. 2003, Chittineni et al. 2007).

Despite changes in patient characteristics and increasing prevalence of renal dysfunction the incidence of AKI seems to be stable in patients hospitalized for AHF (Owan et al. 2006a). Factors predicting the occurrence of AKI after hospital admission have included age, higher creatinine levels at baseline, diabetes and high admission blood

pressure (Forman et al. 2004, Cowie et al. 2006, Krumholz et al. 2000). One study has also suggested that baseline renal function is a stronger determinant of outcome than worsening renal function (Nohria et al. 2008). LVEF has been similar in patients with and without subsequent AKI (Cowie et al. 2006, Chittineni et al. 2007, Butler et al. 2004), and low cardiac index measured by pulmonary artery catheter was not a predictor of worsening renal function (Nohria et al. 2008, Mullens et al. 2009). Conflicting results exist about whether venous congestion is independently associated with in-hospital worsening renal function in AHF (Nohria et al. 2008, Mullens et al. 2009). The data about higher dose of diuretics as a risk factor for AKI has also been a matter of debate. Several studies have assessed the dose of furosemide on admission and in-hospital and investigated the association with AKI (Nohria et al. 2008, Mullens et al. 2009, Cowie et al. 2006, Logeart et al. 2008, Metra et al. 2008, Chittineni et al. 2007, Butler et al. 2004). The preliminary results from the only contemporary randomised trial examining effects and outcomes of diuretic treatment suggested that neither the mode of administration (bolus vs. continuous infusion) nor the dose (high vs. low dose) had any significant effect on renal function (Cleland et al. 2010).

Table 8. *Incidence of AKI in AHF studies*

Study	Year	N=	Baseline renal function[a]	Definition of AKI	Incidence	Time-frame
Krumholz	2000	1681	41% SCr>1.5	↑SCr >0.3	28%	During hosp.
Gottlieb	2002	1002	NA	↑SCr 0.1-0.5	72-20%	During hosp.
Smith	2003	412	SCr 1.8	↑SCr 0.1- 0.5	75%-24%	During hosp.
Forman	2004	1004	36% SCr>1.5	↑SCr >0.3	27%	During hosp.
Akhter	2004	481	44% SCr>1.5	↑SCr>0.5	25%	During hosp.
Cowie	2006	299	SCr 1.6	↑SCr >0.3	29%	In-hosp by day 15
Owan	2006	6440	SCr 1.6	↑SCr >0.3	24%	During hosp.
Chittinieni	2007	509	SCr 1.5	↑SCr>0.5	21%	Days 2-6 in-hosp.
Metra	2008	318	SCr 1.5	↑SCr >0.3 AND 25% ↑SCr	32%	During hosp.
Logeart	2008	416	eGFR 60	↑SCr>25 umol/L	37%	During hosp.
Nohria	2008	433	SCr 1.5	↑SCr >0.3	30%	During hosp.
Klein	2008	949	eGFR 51	↓eGFR >25%	12%	During hosp.
Mullens	2009	145	SCr 1.7	↑SCr >0.3	40%	During hosp.
Kociol	2010	20063	SCr 1.5	↑SCr >0.3	18%	Adm. vs. disch.
Rusinaru	2009	338	eGFR 59	↓eGFR >25%	12%	Adm. vs. disch.

SCr=serum creatinine expressed in mg/dL (conversion to µmol/L by multiplying with 88.4), eGFR=estimated glomerular filtration rate (ml/min), NA=not available, AKI=acute kidney injury, hosp=hospitalization, adm=admission, disch=discharge. ↑=increase, ↓=decrease. [a]Mean serum creatinine of eGFR or proportion of patients above specific value.

The effect of AKI on prognosis in AHF has also been investigated and most studies have observed a significant association between AKI and adverse outcomes with increased LOS, higher in-hospital mortality and poorer survival during follow-up (*Table 9*).

Table 9. *Effect of AKI on outcomes in AHF*

Study	N=	Baseline renal function[a]	Definition of AKI	LOS	Follow-up	Risk ratio for mortality with AKI
Krumholz	1681	41% SCr>1.5	↑SCr >0.3	↑ 2.3 d	In-hosp.	2.72
Gottlieb	1002	NA	↑SCr>0.3/ ↑SCr>0.5	↑LOS	In-hosp.	NA
Smith	412	SCr 1.8	↑SCr>0.3/ ↑SCr>0.5	NA	6 months	1.67 2.90
Forman	1004	36% SCr>1.5	↑SCr >0.3	↑LOS	In-hosp.	7.5
Akhter	481	45% SCr>1.5	↑SCr>0.5	↑ 2.1 d	6 months	1.61
Cowie	299	SCr 1.6	↑SCr >0.3	↑ 2 d	6 months	↑In-hosp.+30 days. NS at 6 months
Owan	6440	SCr 1.6	↑SCr >0.3	NA	3 months	1.39
Chittinieni	509	SCr 1.5	↑SCr>0.5	↑ 2.6 d	In-hosp.	9.3% vs. 4.3%
Metra	318	SCr 1.5	↑SCr>0.3 AND 25% ↑SCr	↑ 2.6 d	12 months	1.47
Logeart	416	eGFR 60	↑SCr>25 umol/L	↑ 3 d	6 months	NS
Nohria	433	SCr 1.5	↑SCr >0.3	NA	6 months	NS
Mullens	145	SCr 1.7	↑SCr >0.3	NS	NA	NA
Kociol	20063	SCr 1.5	↑SCr >0.3	↑ 1 d	1 year	1.12
Rusinaru	338	eGFR 59	↓eGFR >25%	NA	7 years	2.26 for 6 month NS for 7 year

SCr=serum creatninine expressed in mg/dL (conversion to µmol/L by multiplying with 88.4), eGFR=estimated glomerular filtration rate (ml/min), NS=not significant, NA=not available, NS=not significant, AKI=acute kidney injury, d=days, LOS=length of stay, hosp=hospitalization, ↑=increase, ↓=decrease. [a]*Mean serum creatinine of eGFR or proportion of patients above specific value.*

Worsening renal function (defined as an increase in creatinine or decrease in eGFR) was associated with a 60% increased mortality risk and 30% increased risk of hospitalization in a meta-analysis of studies in heart failure (Damman et al. 2007). The increase in risk of death was similar both in hospitalized as well as out-patient chronic heart failure populations. However, the effect on prognosis was also strongly dependent on the magnitude of increase in creatinine. Actually, only a rise in creatinine above 0.3 mg/dL was associated with higher risk of death and the larger the increase, the worse the prognosis. A second observation from the meta-analysis was that the effect on outcomes was greater early during follow-up, and weakened beyond 6 months (Damman et al. 2007). Still, one study has reported that in-hospital worsening of renal function is also associated with poor long-term outcome in patients with preserved ejection fraction (Rusinaru et al. 2009).

2.4.5 Role of inflammation in CRS

Inflammation is one of the putative mechanisms involved in the CRS (Ronco et al. 2008a). Elevated levels of inflammatory biomarkers have been found in heart failure as well as in renal insufficiency. In both cases, inflammation has also been associated with adverse outcomes (Stenvinkel et al. 2005, Anker et al. 2004). Studies investigating markers of inflammation in renal insufficiency and heart failure have not usually evaluated the effect of a possible heart-kidney interaction on the inflammatory response and levels of inflammatory markers.

Inflammatory cytokines are endogenous peptides that play a central role in the activation or modulation of the inflammatory response. The inflammatory cascade is complex, with numerous pathways and factors affecting the clinical response. Some of the essential markers and cytokines which are involved in the inflammatory process and which have been part of the current study are described below.

C-reactive protein (CRP) an acute phase reactant produced in the liver, is probably the most studied inflammatory biomarker in CVD. Plasma levels of CRP can increase 100- to 1000-fold in response to bacterial infection or tissue injury (e.g. myocardial infarction). The prognostic value of CRP as a marker of atherosclerotic disease and subsequent cardiovascular events has been investigated in stable populations with high-sensitivity assays (hsCRP) measuring also low (<3 mg/L) concentrations (Hansson 2005). Evaluating very low CRP levels may be difficult in unstable conditions and in emergency care (such as myocardial infarction and AHF), where the inflammatory cascade is often activated and CRP levels are above normal. One study using regular CRP reported worse outcome in AHF with higher levels of CRP (Mueller et al. 2006).

Tumor necrosis factor-α (TNF-α) is a potent pro-inflammatory cytokine produced mainly by activated macrophages but can be derived from a variety of tissues. TNF-α exerts its effects by binding to two cell-membrane receptors (TNFR1 and TNFR2). Degradation at the cellular level is one way of inactivating cytokines, but renal function seems to play a role in the clearance of circulating TNF-α levels (Stenvinkel et al. 2005).

Interleukin-6 (IL-6) is another pro-inflammatory cytokine the production of which is stimulated by inflammatory stress, but also induced by TNF-α. IL-6 is produced by various immune cells (leukocytes, macrophages) and endothelial cells. IL-6 further promotes the inflammatory response, and induces the production of acute phase reactants (e.g. CRP) from the liver (Hansson 2005). It is thought that IL-6 may also possess some anti-inflammatory properties (Stenvinkel et al. 2005). In addition to the acute inflammatory response, low-grade chronic inflammation by IL-6 has been associated with accelerated ageing, atherosclerosis and muscle wasting (Stenvinkel et al. 2005). IL-6 production can also be stimulated by adipose tissue (Hansson 2005) and may play a role in the metabolic syndrome and insulin resistance.

Interleukin-10 (IL-10) is regarded as one of the most important anti-inflammatory cytokines, counterbalancing the pro-inflammatory response at several levels. IL-10 downregulates the expression of pro-inflammatory cytokines such as TNF-α and IL-6 (Anker et al. 2004). Clearance of IL-10 is mainly by the kidneys, and increasing

circulating IL-10 levels are observed with decreasing renal function (Stenvinkel et al. 2005).

2.4.5.1 Inflammatory cytokines in heart failure

Several studies have found inflammatory markers to be elevated in heart failure (Bozkurt et al. 2010). Higher levels of especially TNF-α, but also IL-6, have been reported in both systolic and diastolic chronic heart failure (Wisniacki et al. 2005, Dunlay et al. 2008), and have also been associated with adverse outcomes (Dunlay et al. 2008, Levine et al. 1990, Rauchhaus et al. 2000, Deswal et al. 2001, Aukrust et al. 1999). TNF-α has been associated with negative inotropic effects on myocytes, left ventricular remodelling and dysfunction as well as negative effects on the vasculature and endothelium (Stenvinkel et al. 2005, Anker et al. 2004, Hilfiker-Kleiner et al 2006).

Levels of TNF-α have frequently but not invariably been higher in patients with more severe heart failure (Aukrust et al. 1999, Mann 2002, El-Menyar 2008). However, studies examining relations with LVEF or hemodynamic indices in heart failure, have not found an association between TNF-α and these parameters (Dunlay et al. 2008, Suzuki et al. 2005). A recent study in chronic heart failure showed a relationship between impaired renal function and higher levels of TNF-α (Dunlay et al. 2008).

IL-6 concentrations are elevated in patients with heart failure, particularly in the acute phase (Anker et al. 2004, Aukrust et al. 1999, Suzuki et al. 2005). In fact, IL-6 levels show a correlation with functional and hemodynamic indices (Aukrust et al. 1999, Suzuki et al. 2005, Tsutamoto et al. 1998, Lommi et al. 1997). IL-6 exhibits dynamic changes after myocardial infarction related to magnitude of injury, with TNF-α levels being more stable and not related to infarct size or LVEF (Puhakka et al. 2003). Elevated IL-6 levels seem related to worse prognosis in heart failure, although the association has been less strong than for TNF-α. Cardiodepressant effects on myocytes have been described for IL-6 as well, but also anti-apoptotic properties (Anker et al. 2004). Thus, the net effect of IL-6 in heart failure remains to be fully discovered.

Limited data exists on the anti-inflammatory cytokine IL-10 in heart failure (Aukrust et al. 1999, Miettinen et al. 2008, Kaur et al. 2009). Although elevated levels have been measured, IL-10 does not differ with severity of heart failure, as measured by NYHA functional class (Aukrust et al. 1999). Thus, levels of TNF-α are not matched by a concomitant increase in IL-10, leading to a higher TNF-α/IL-10 ratio in heart failure. As an important cytokine counterbalancing the effect of TNF-α, the growing TNF-α/IL-10 ratio with more severe heart failure has by some authors been attributed to a pro-/anti-inflammatory imbalance in these patients (Aukrust et al. 1999, Kaur et al. 2009).

It is commonly thought that inflammatory cytokines play a role in the pathogenesis and progression of heart failure (Mann 2002). As TNF-α was shown to have cardiodepressant effects in vitro and in vivo, it has even been the target for therapeutic trials, but administration of a monoclonal anti-TNF-α antibody infliximab or antibodies directed at TNF-α receptors did not improve survival in patients with systolic heart failure (El-Menyar 2008). These negative results are believed to be more a reflection of the

complexity of the cytokine network, and better knowledge about the different mechanisms and the role of inflammation in heart failure is still needed.

2.4.5.2 Inflammation in CKD

Inflammation is also activated in CKD. This is particularly evident in end-stage renal disease, where elevated levels of CRP, IL-6 and TNF-α have been found (Roberts et al. 2006, Bolton et al. 2001, Bologa et al. 1998). In patients on dialysis, inflammatory activation has also been associated with poor outcome (Stenvinkel et al. 2005, Roberts et al. 2006). Data in CKD patients not on dialysis is more limited, but existing evidence suggests higher levels of inflammatory markers and cytokines in moderate renal insufficiency as well (Bolton et al. 2001, Shlipak et al. 2003, Pecoits-Filho et al. 2003). In fact, rather strong correlations between creatinine and TNF-α have been reported (Bolton et al. 2001). Inflammation also seems to play a role in the prognosis of CKD patients (Roberts et al. 2006, Shlipak et al. 2005a, Barreto et al. 2010). Consequently, it is generally thought that inflammation could be a central link between renal insufficiency and increased cardiovascular risk (Stenvinkel et al. 2005, Roberts et al. 2006, Cachofeiro et al. 2008).

2.5 Cystatin C as a cardiovascular risk marker

The evidence of renal function as a strong prognostic factor in patients with CVD (see chapter 2.4), in combination with the search for new biomarkers in risk prediction, has been the driving force in assessing the utility and prognostic impact of CysC on cardiovascular outcomes. Numerous studies have been published in the last five years investigating the association between CysC and survival or cardiovascular events in different populations at risk. In a recent meta-analysis, higher levels of CysC were significantly predictive of mortality and adverse overall cardiovascular events, including independent associations with specific endpoints like heart failure, myocardial infarction and stroke (Lee et al. 2010).

2.5.1 General populations

The first paper to examine how levels of CysC influence cardiovascular outcomes in a broad population was published by Shlipak et al. in 2005. In the Cardiovascular Health Study, CysC was measured in over 4000 elderly ambulatory persons without previous history of cerebrovascular or coronary heart disease. During a median follow-up of 7.4 years, they found increased all-cause and cardiovascular mortality in subjects with higher CysC levels (Shlipak et al. 2005c). Moreover, higher rates of myocardial infarction and stroke were also observed in the top quintile of CysC. Similar associations between CysC and mortality, but without predicting incident myocardial infarction or stroke during

follow-up, were found in a slightly older cohort (Shlipak et al. 2006c, Deo et al. 2008). A secondary analysis of patients without renal insufficiency (eGFR ≥60 ml/min) at baseline in the Cardiovascular Health Study cohort showed that CysC levels were significantly related to all-cause, cardiovascular and non-cardiovascular mortality, incident myocardial infarction, stroke and heart failure, while creatinine was not associated with adverse outcomes (Shlipak et al. 2006a). CysC was a better predictor of death and cardiovascular events than creatinine in these studies.

More recently, data on subjects >40 years of age with eGFR >60 ml/min from a large population study in the United States also showed a clear association between high levels of CysC and mortality (all-cause, cardiovascular, non-cardiovascular)(Wu et al. 2010). CysC has been included in multimarker prediction models, and was found to improve cardiovascular and all-cause mortality risk stratification in elderly men (Zethelius et al. 2008). In a middle-aged cohort with lower cardiovascular risk profile, CysC was independently associated with cardiovascular and coronary events, but did not improve risk prediction in this population (Melander et al. 2009).

2.5.2 Coronary artery disease

CysC has been a consistent marker of poor prognosis in patients with CAD. In stable CAD patients, high CysC levels have been associated with a 2-3-fold risk of death, cardiovascular event or heart failure hospitalization during three years of follow-up (Ix et al. 2007, Koenig et al. 2005). Again this risk increase was present both in patients with and without renal insufficiency defined as eGFR <60ml/min at baseline.(Ix et al. 2007). In patients with CAD and eGFR >60 ml/min, CysC was a strong predictor of cardiovascular death (Keller et al. 2009). Compared to the first quartile, patients in the top CysC quartile had a five times higher adjusted risk of cardiovascular death. Data from a large population in the U.S. also showed that in patients without CKD (eGFR ≥60 ml/min and without albuminuria) higher CysC levels were associated with increased prevalence of myocardial infarction, angina pectoris symptoms and stroke (Muntner et al. 2008). CysC has also been associated with severity of CAD, both in subjects with eGFR below and above 60 ml/min.(Kiyosue et al. 2010) Preoperative GFR, estimated with CysC, also predicted outcome after heart surgery (Ledoux et al. 2007).

In patients with ACS, including both ST-elevation and non-ST-elevation myocardial infarction, CysC has been an independent predictor of death or death and myocardial infarction during follow up (Jernberg et al. 2004, Kilic et al. 2009, Windhausen et al. 2009).

2.5.3 Heart failure

Given the association with mortality and various cardiovascular events as described above, it is not surprising that higher levels of CysC also predict the incidence of heart failure. For each quartile of CysC, there was a stepwise increase in the risk of developing heart failure in elderly subjects, independently of other risk factors (Sarnak et al. 2005). This relation was not seen with creatinine. CysC has been a predictor of incident heart failure in CAD and even in patients without impairment in renal function (eGFR ≥60 ml/min) (Shlipak et al. 2006a, Ix et al. 2007).

One study found that there was a stepwise increase in the incidence of systolic heart failure with each quartile of CysC, while the increased risk of diastolic HF was evident only in the highest CysC quartile (Moran et al. 2008a). Still, in patients without a history of heart failure, elevated CysC has been primarily associated with markers of diastolic dysfunction such as left ventricular hypertrophy (increased wall thickness and higher left ventricular mass) and impaired diastolic tissue velocities, but not with left ventricular systolic function (left ventricular dimensions and LVEF) (Moran et al. 2008a, Ix et al. 2006). These findings were confirmed in another study evaluating cardiac function by magnetic resonance imaging (Patel et al. 2009). In patients with stable CAD, there was a relationship between higher levels of CysC and blood pressure. SBP and pulse pressure, but not diastolic blood pressure (DBP), increased linearly with CysC above normal. For CCr, the association between kidney function and SBP was present only for values below 60 ml/min (Peralta et al. 2006). In the Physicians Health Study, the association between CysC and heart failure was significant only in patients with hypertension (Djousse et al. 2008, Moran et al. 2008b), although mean measured blood pressure was similar between CysC categories.

There is a clear association between CysC and incident HF in different populations. Although CysC is not by itself related to LVEF, it can be regarded a risk marker both for systolic heart failure, left ventricular hypertrophy and diastolic dysfunction. These latter effects may be secondary to interactions between kidney function and higher blood pressure. The associations between CysC levels and outcome in studies of patients at risk of, or with heart failure, are summarized in *Table 10*.

CysC increases with severity of heart failure as measured by NYHA functional class and there is a relationship to echocardiographic measures of cardiac dysfunction (Arimoto et al. 2005, Tang et al. 2008, Alehagen et al. 2009). However, CysC levels do not correlate with LVEF, but to other indices of left ventricular function and to right ventricular systolic function (Tang et al. 2008). Patients in the Cardiovascular Health Study with heart failure at the baseline visit, and who subsequently died during follow up, had higher levels of CysC compared with patients who survived (Shlipak et al. 2005b). During a 10 years follow-up of heart failure patients, a graded, significant, increase in mortality was observed for each quartile of CysC (Alehagen et al. 2009). The same was not observed with creatinine or eGFR. CysC has been an independent predictor of mortality in heart failure even after adjustment for baseline creatinine or eGFR, alternatively after stratification by eGFR (Arimoto et al. 2005, Shlipak et al. 2005b).

Table 10. *Studies investigating the association between CysC levels outcomes in heart failure*

Author Pub year	Study population	Age M/F	CysC levels (mg/L)	Follow-up	Endpoint	Adj. RR (95% CI)
Risk of HF						
Sarnak 2005	4384 Elderly	75 41/59%	1.1	8.3 years	Incident HF	2.2 (1.6-2.9)[a]
Shlipak 2006	3659 eGFR>60	Elderly >65 years	1.0	9.3 years	Incident HF	1.3 (1.2-1.4)[b]
Djousse 2007	440 case-control	74 years 100/0%	0.41-3.9	N.R.	Incident HF	1.6 (0.9-2.9)[a] 4.2 (1.2-14.6)[a,c]
Ix 2007	990 CHD	67 years 82/18%	1.20	37 months	Incident HF	2.6 (1.0-6.9)[a]
					CV events	2.0 (1.0-3.8)
					All-cause death	3.6 (1.8-7.0)[a]
Moran 2008	4453 Elderly	39/61%	1.05 (0.92-1.23)	8 years	Systolic HF	3.2 (1.8-5.5)[a]
					Diastolic HF	1.8 (1.1-3.1)[a]
Chronic HF						
Arimoto2005	140	66 years 62/38%	1.14	480 days	All cause death or HF hosp.	1.9 (1.3-6.6)[b]
Shlipak 2005	279	76 years 49/51%	1.26	6.5 years	All-cause death	2.2 (1.3-3.5)[a]
Alehagen 2009	464	73 years 60/40%	1.43 (1.22-1.66)	10 years	CV mortality	2.9 (1.2-4.9)[a]
Tang 2008	139	57 years 77%	1.22	33 months	Death, transplantation. or HF hosp.	1.8 (1.3-2.7)[d]
AHF						
Campbell 2009	240	63 years 50/50%	1.39	12 months	Death	2.3 (N.R.)[a]
					Death or rehosp.	2.0 (N.R.)[a]
Naruse 2009	328 eGFR>30	73 years 62/38%	1.07 (0.91-1.31)	915 days	Cardiac death	20 (4-82)[a]
Manzano-Fernandez 2009	138	74 years 58/42%	1.2 (1.0-1.7)	261 days	Death or HF hosp	3.1 (1.5-6.1)[a]

CysC levels as mean, median (with IQR) or range. 95% CI = 95% confidence interval M= male, F=female, Adj.RR=adjusted risk ratio, CHD=coronary heart disease, HF=heart failure, CV=cardiovascular, hosp=hospitalization, N.R.=not reported. [a]Highest CysC tertile/quartile/quintile vs. lowest, [b]continuous variable (/SD increase),[c]in hypertensives, [d]CysC > 1.23 mg/L

2.5.4 Cystatin C in chronic kidney disease

For patients with established CKD, a retrospective analysis in the MDRD-cohort showed that higher CysC was a significant predictor of mortality (all-cause and cardiovascular) and progression to renal failure (need for dialysis or kidney transplant) during 10 years of follow-up (Menon et al. 2007). The increase in risk was similar for CysC and measured GFR, although CysC demonstrated slightly higher risk ratio for the mortality outcomes. In elderly subjects with eGFR ≥60 ml/min, a CysC concentration above normal was a strong predictor of developing renal insufficiency, with a four times increased risk of reaching stage 3 CKD (eGFR <60ml/min) during 4 years of follow-up (Shlipak et al. 2006a).

Shlipak et al (2009) elegantly compared rates of decline in kidney function assessed by creatinine and CysC in 4380 elderly persons followed for up to 7 years. CysC and creatinine values were converted to $eGFR_{CysC}$ and $eGFR_{MDRD}$, respectively. The authors showed that with $eGFR_{MDRD}$ the decline in renal function was on average 0.4 ml/min/year, and 16% of the population had a rapid decrease (>3 ml/min/y). Interestingly, 39% were found by $eGFR_{MDRD}$ to have an mean improvement in renal function during follow-up (Shlipak et al. 2009a). The overall change in $eGFR_{MDRD}$ was rather small in the study population, from a mean of 79 ml/min/1.73m^2 at baseline to 78 ml/min/1.73m^2 at study end. In addition, changes in renal function detected with $eGFR_{MDRD}$ differed significantly by gender and race (Shlipak et al. 2009a).

For CysC estimates, change in renal function was not different in males and females or depending on race. The mean annual decline for $eGFR_{CysC}$ was 1.8 ml/min and during the study, the average $eGFR_{CysC}$ in the population dropped from 79 ml/min/1.73m^2 at baseline to 70 ml/min/1.73m^2 at final follow-up. 25% of patients were categorized as having rapid kidney function decline and, compared to creatinine, fewer were found to have a positive trend in the annual $eGFR_{CysC}$ (Shlipak et al. 2009a). The study also pointed out age as a significant predictor of rapid kidney function decline and found that, compared to $eGFR_{MDRD}$, $eGFR_{CysC}$ identified twice as many patients reaching the endpoint of CKD (eGFR <60ml/min). Furthermore, separate analysis of the same cohort showed that rapid decline in kidney function (>3 ml/min/year), measured either with $eGFR_{CysC}$ or $eGFR_{MDRD}$ was associated with 50% increased risk of all-cause or cardiovascular death, irrespective of age, gender or baseline renal function (Rifkin et al. 2008).

In light of the findings above (Shlipak et al. 2009a), the combined analysis of the Multi-Ethnic Study on Atherosclerosis and Cardiovascular Health Study cohorts raise some new questions about the prognostic value of creatinine-based eGFR (Peralta et al. 2010). The study showed that CysC-based and creatinine-based GFR estimates give very divergent prevalence of CKD (eGFR <60 ml/min) in the population and that $eGFR_{CysC}$ and $eGFR_{Crea}$ have different effects on prognosis. Patients assessed as having CKD only by $eGFR_{Crea}$ (but not $eGFR_{CysC}$) did not have an increased rate of adverse outcome (mortality, cardiovascular event, heart failure, kidney failure), although these subjects would have been expected to be at high risk of events. Patients classified as having eGFR <60 ml/min by both CysC and creatinine had a nearly doubled risk of mortality, and approximately 50% increase in the risk of cardiovascular events and heart failure. This increase in risk was almost identical in patients with CKD by $eGFR_{CysC}$ alone, although $eGFR_{Crea}$ in these

patients was >60 ml/min. Finally, the risk of end-stage renal disease was increased in all patients with CKD at baseline, but the risk ratio was highest for the group with both estimates of GFR <60 ml/min (OR 23.8), followed by $eGFR_{CysC}$ (OR 6.1) and $eGFR_{Crea}$ (OR 2.6) (Peralta et al. 2010). In a different cohort, GFR estimated by CysC was also more strongly associated with all-cause and cardiovascular mortality than either $eGFR_{Crea}$ or a combination of $eGFR_{Crea}$ and $eGFR_{CysC}$ (Astor et al. 2009).

In conclusion, CysC is a strong predictor of outcomes. Consistently, higher CysC levels have been associated with increased risk of all-cause and cardiovascular death. CysC also predicts cardiovascular events and disease progression of heart failure and kidney failure in elderly patients at risk. The ability of CysC to predict adverse outcomes is better than creatinine and at least equals the effect on prognosis of renal function measured by GFR. The properties of CysC makes it an excellent candidate for assessing renal function and predicting cardiovascular outcomes in a variety of populations and disease states, both in renal insufficiency and in patients with more preserved renal function.

3. Aims of the study

The Finnish Acute Heart Failure Study (FINN-AKVA) is a national prospective multicenter study on AHF investigating the aetiology, concomitant diseases, treatment modalities, morbidity and mortality, as well as prognostic markers of AHF. The goal of the present thesis is to assess factors associated with poor prognosis in AHF and to investigate CysC as a marker of renal function in AHF. The specific aims are:

I- To characterize and identify prognostic risk markers in a contemporary, unselected population hospitalized for AHF.

II- To evaluate the prevalence of renal insufficiency in AHF and assess the value of CysC as a predictor of mortality and for risk stratification of AHF patients.

III- To examine the incidence of worsening renal function after hospital admission for AHF and analyze its effect on outcomes using CysC as marker of renal function.

IV- To explore the relationship between inflammatory cytokines and measures of cardiac stress and renal function in AHF for better understanding of the role of inflammation in heart-kidney interactions.

4. Materials and methods

4.1 Study population and data collection

The FINN-AKVA study enrolled consecutive patients hospitalized with AHF at 14 university, central, and regional hospitals in Finland during three months in the spring of 2004. Patients with new onset (de-novo) AHF as well as with exacerbation of chronic heart failure were included. Each subject was enrolled only once during the study period. Patients were characterized according to the ESC AHF guidelines criteria (Nieminen et al. 2005a) to five groups on the basis of their clinical presentation on admission: cardiogenic shock, pulmonary oedema, decompensated AHF, hypertensive AHF, and right heart failure. Cardiogenic shock was defined as SBP below 90 mmHg not responsive to fluid, or the need of vasopressor or inotrope therapy to maintain adequate blood pressure. Patients were classified as having pulmonary oedema if O_2-saturation on room air was below 90% and/or chest x-ray showed alveolar pulmonary oedema. Patients with SBP above 180 mmHg on admission were regarded as hypertensive AHF. Subjects with AHF not meeting any of the above mentioned criteria were classified as acute decompensated heart failure. Those presenting mainly with signs and symptoms of right heart failure without concomitant severe impairment of left ventricular function were categorized as right ventricle AHF. Patients with high output heart failure were not included.

The underlying diseases were systematically registered, including the presence or absence of a previous history of heart failure (for classification as de-novo AHF or ADCHF). Evidence of factors known to precipitate AHF (arrhythmia, infection, ACS, valvular causes) and clinical status (SBP, DBP, heart rate, NYHA class) on admission was assessed in detail. In-hospital management together with the most recent echocardiography findings and medication at discharge were also methodically recorded. In-hospital LOS and mortality were documented. At discharge the diagnosis of AHF had to be confirmed for acceptance in the final study database. Vital status and time of death was assessed for all patients through the national population register center (Väestörekisterikeskus) by the end of 2005. Survival follow-up was completed for all patients for at least one year after inclusion in the study and all-cause mortality rates determined. *Figure 6* shows a flowchart of the patients included in the different substudies.

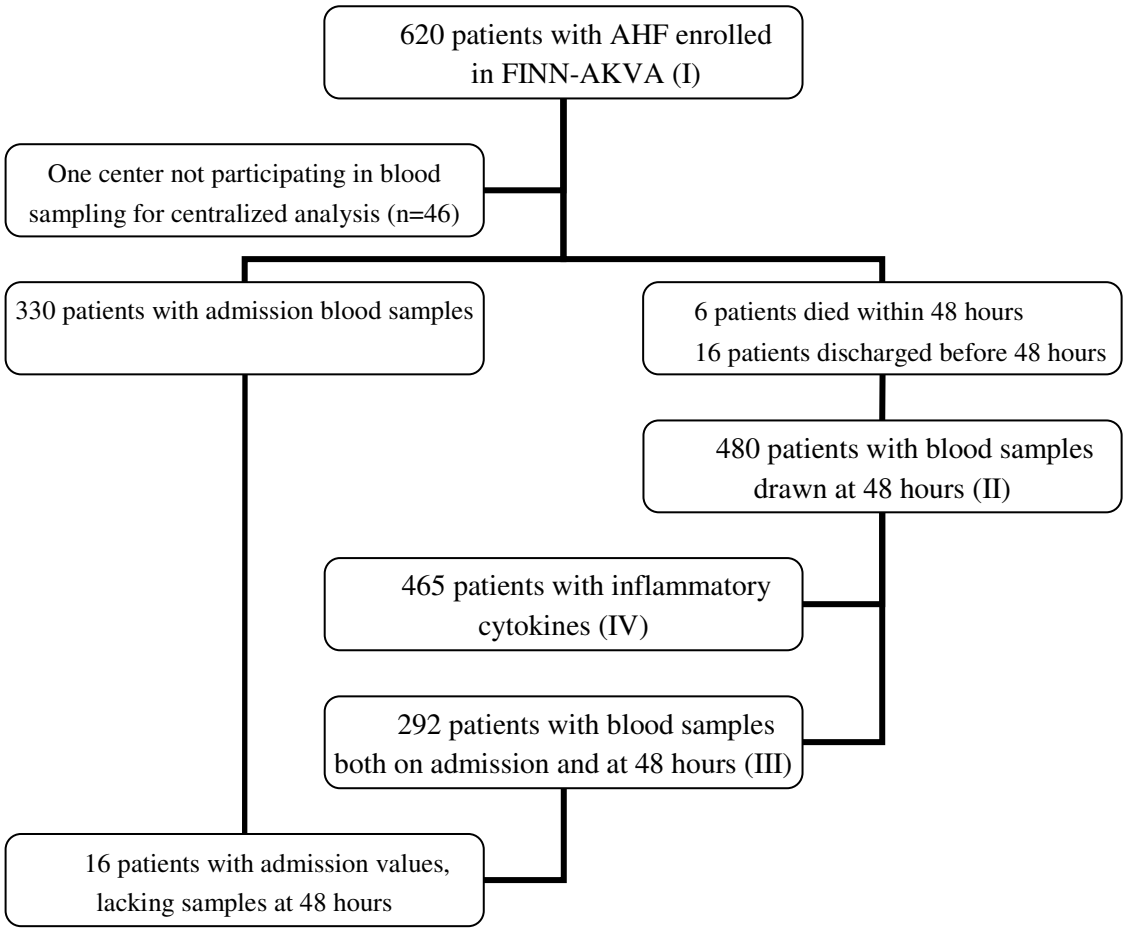

Figure 6 *Flowchart of the study population included in the different analyses*
AHF=acute heart failure, FINN-AKVA=Finnish acute heart failure study. The roman numerals refer to the different substudies

4.2 Analysis of biomarkers

After the patients had given informed consent, blood samples for analyses of cardiac, renal and inflammatory biomarkers were obtained on admission and again at 48 hours after admission. Samples were divided into plasma aliquots after centrifugation and frozen at -20°C (NT-proBNP and CysC samples) or -70°C (for plasma cytokines samples) until analysis in a centralized laboratory. Levels of CysC, creatinine, troponin T and I, natriuretic peptides (BNP, NT-proBNP) and inflammatory cytokines (TNF-α, IL-6, Il-10) were measured from these samples. In addition, data on locally analyzed biochemistry (including but not restricted to creatinine, CRP, hemoglobin, sodium) was recorded. Anemia was defined according to recommendations by the World Health Organisation (blood hemoglobin <130 g/L for men and <120 g/L for women). Hyponatremia was defined as plasma sodium <135 mmol/L.

CysC was analyzed using the Dako Cytomation immunoturbidimetric assay, with an intra-assay and inter-assay coefficient of variation of 2.0% and 4.1%, respectively, at 0.7

mg/L. The upper limit of the reference interval for healthy adults determined by the manufacturer was CysC <1.2 mg/L for young individuals and <1.4 mg/L for subjects aged over 50 years. Centralized analysis of plasma creatinine was performed using the CREA Plus Roche Diagnostics® enzymatic method (upper limit of reference interval 90 µmol/L for women and 100 µmol/L for men). From creatinine values, $CrCl_{C\text{-}G}$ was calculated using the Cockcroft-Gault equation (Cockcroft et al. 1976) and glomerular filtration rate estimated by the abbreviated four-variable MDRD formula (eGFR) (Levey et al. 1999, Levey et al. 2006). Renal insufficiency was defined as NKF/KDOQI CKD stages 3-5 ($CrCl_{C\text{-}G}$ or eGFR<60 ml/min).

BNP (Abbott Diagnostics) and NT-proBNP (Roche Diagnostics Elecsys) were analyzed using commercially available kits. Plasma concentrations of IL-6, TNF-α, and IL-10 were measured using commercial enzyme-linked immunosorbent assay systems (Quantikine kit, R&D Systems Inc). The lower limits of detection of the IL-6, TNF-α, and IL-10 assays were 0.70 ng/L, 0.12 ng/L, and 0.50 ng/L plasma, respectively.

4.3 Statistical methods and ethical considerations

Characteristics of the study population were assessed and patients categorized in different groups. Descriptive and comparative analyses were performed for demographic and clinical variables. Well established statistical methods were used in all analyses as presented in detail in each publication (I-IV). Numbers (n) and percentages (%), means and standard deviation (SD), median and interquartile range (IQR) were reported for descriptive purposes. Comparative analyses were done using chi-square, students *t*-, Kruskall-Wallis or Mann-Whitney U tests as appropriate. Correlations among continuous variables were assessed through correlation plots and by calculating Pearson's correlation coefficients. Linear regression models were used in study IV. Logarithmic transformation of biomarkers with a skewed distribution was performed as needed.

To assess differences in mortality between groups, Kaplan-Meier survival curves were plotted and statistical significance evaluated by log-rank test. In addition, univariate and multivariable logistic or Cox proportional hazard regression analyses were performed to assess predictors of mortality. Biomarkers were entered either as continuous variables (per SD increase) or as categorical variables after categorization in tertiles, quartiles or according to median value. Multivariable models were adjusted for predictors that were statistically significant on univariate analysis and variables of general clinical importance. Results were presented as odds ratios (OR) or hazard ratios (HR) with 95% confidence intervals (95% CI).

Receiver operating characteristic (ROC) curves were generated to assess the diagnostic performance of a change in CysC to detect AKI (III). Sensitivity and specificity of the chosen cut-off for the endpoint of interest was reported. ROC analysis was also used to evaluate the discriminative properties of biomarkers on mortality (IV). The area under the curve (AUC) with 95% CI was calculated.

Tests were two-sided with a statistical significance level of 0.05. Statistical analyses were performed using SPSS (SPSS Inc, versions 12.0.1 and 15.0.1), Survo MM and Stata 8.0 statistical software. The study was conducted according to the declaration of Helsinki.

All patients gave a written consent, and the study was approved by the local ethical committee. Collected variables were entered into the database using a center- and subject-specific identification number to allow anonymous handling of data.

5. Results

5.1 Characteristics, outcomes and predictors of mortality in AHF (I)

5.1.1 Demographic characteristics and medical history of the FINN-AKVA study population

The FINN-AKVA study enrolled 620 patients with AHF from February 2nd to April 30th 2004. Most of the patients (58%) were admitted at the five university hospitals, while five central (21%) and four regional (21%) hospitals contributed equally to recruitment. The mean age of the study population was 75.1 years. Half of the patients enrolled were women, and these were on average approximately 8 years older than the male participants. Heart failure had been previously diagnosed in 51% of the patients (acutely decompensated chronic heart failure), thus, almost half of the study population consisted of new-onset (de-novo) AHF. CAD (55%) and hypertension (55%), the two principal causes of heart failure, were also the most common co-morbidities in the study population. Significant valvular disease (13%) was found in a smaller proportion of patients. Diabetes (32%), chronic atrial fibrillation (27%), and clinical manifestations of atherosclerotic vascular disease (previous myocardial infarction [27%] and previous cerebrovascular accident [17%]) were frequently observed in this cohort of hospitalized AHF patients. Patients with de-novo AHF were on average slightly younger (77 vs. 74 years, p=0.0002), more often male and had fewer co-morbidities than patients with a previous history of heart failure. On the other hand, a presentation with concomitant ACS was more common in de-novo AHF (49% vs. 24%, p<0.0001).

5.1.2 Clinical presentation and classification

On admission, 75% of patients were in NYHA class III-IV. Blood pressure was on average 147/82 mmHg with heart rate at a mean of 91 beats/minute. Classification in the five clinical classes of AHF showed that acutely decompensated heart failure was the most common manifestation of AHF (64%), followed by pulmonary oedema (26%). Hypertensive AHF, cardiogenic shock and right heart failure accounted each only for a small proportion of patients (*Figure 7*). Hypertensive AHF and cardiogenic shock were more common in de-novo AHF. Cardiogenic shock was seen mainly in patients with concomitant ACS.

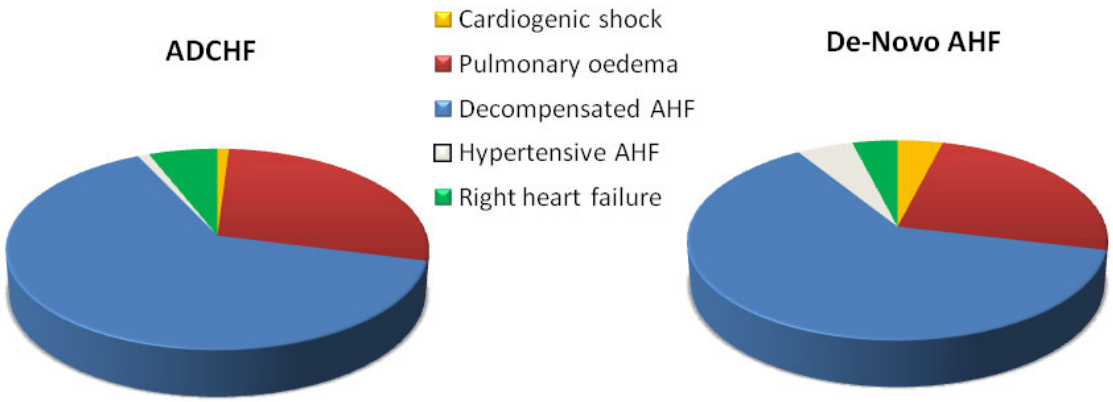

Figure 7 *Distribution of patients hospitalized for AHF in ESC clinical classes*

Factors known to precipitate AHF such as ischemia, arrhythmias, infection or valvular dysfunction, were also documented. Nearly one third of the patients presented with an ACS, and arrhythmia (mainly atrial fibrillation or flutter) was another principal precipitating factor. Infections and valvular causes accounted for 24% and 12% of the clinical deterioration of patients, respectively.

Echocardiographic data was also available in about two thirds of the FINN-AKVA study population and mean LVEF was 45%. More specifically, half of the patients had preserved left ventricular systolic function (LVEF >45%). One third had moderately depressed systolic function (LVEF 30-44%) while severe impairment of left ventricular function (LVEF <30%) was present in 16% of patients with echocardiographic data available. Mean LVEF and the proportion of patients with preserved, moderate or severe impairment of left ventricular function were similar in de-novo AHF and patients with ADCHF.

Of biomarkers measured on admission, mean hemoglobin was 127 g/L (SD 18) and sodium 138 mmol/L (SD 5). Creatinine was on average 113 µmol/L (SD 64), while the median CRP level was 10 mg/L (IQR 2-26) with a large variation (0-278 mg/L). BNP (median 174 ng/L; IQR [91-349]) and NT-proBNP (median 5627 ng/L; IQR [2605-11373]) levels were elevated as expected in a population with AHF (*Table 11*).

5.1.3 Medication on admission and at discharge

A large majority were taking some cardiovascular medication already on admission, with BB:s, ACEI/ARB:s and diuretics used in 63%, 51% and 48% of the study population, respectively. BB:s, ACEI/ARB medication and diuretics were all more common on admission in patients with a previous history of heart failure. During hospital stay, the prescription rates of heart failure medication increased, and at discharge 86% of the study population was treated with a BB, 76% were on ACEI/ARB therapy and 89% were prescribed furosemide. Although the use of furosemide, digitalis and spironolactone was

higher in discharged patients with previous heart failure, there were no differences between de-novo and ADCHF groups for the prescription of BB:s and ACEI/ARB medication at discharge.

Table 11. *Levels of biomarkers in FINN-AKVA*

Biomarker	Admission	At 48 hours
CysC (mg/L)	1.25 (1.00-1.63)	1.30 (1.04-1.70)
Creatinine (µmol/L)	98 (81-125)	88 (71-113)
BNP (pg/mL)	174 (91-349)	133 (56-264)
NT-proBNP (pg/mL)	5627 (2605-11373)	3895 (1950-8497)
TnT (µg/L)	0.01 (0.01-0.07)	0.01 (0.01-0.12)
TnI (µg/L	0.4 (0.1-0.21)	0.05 (0.02-0.34)
Hemoglobin (g/L)	127 (18)	-
Sodium (mmol/L)	138 (5)	-
Glucose (mmol/L)	7.4 (6.1-10.1)	-
CRP (mg/L)	10 (3-26)	-

Data shown as median (IQR) or means (SD)

5.1.4 In-hospital outcomes and mortality during follow-up

The in-hospital mortality in the FINN-AKVA study was 7.1% (44/620 patients). For patients discharged alive (n=576), median in-hospital LOS was 7 days (IQR 5-11). During follow up, mortality successively increased and at 1 year, 171 patients (27.4%) had died (*Figure 8).*

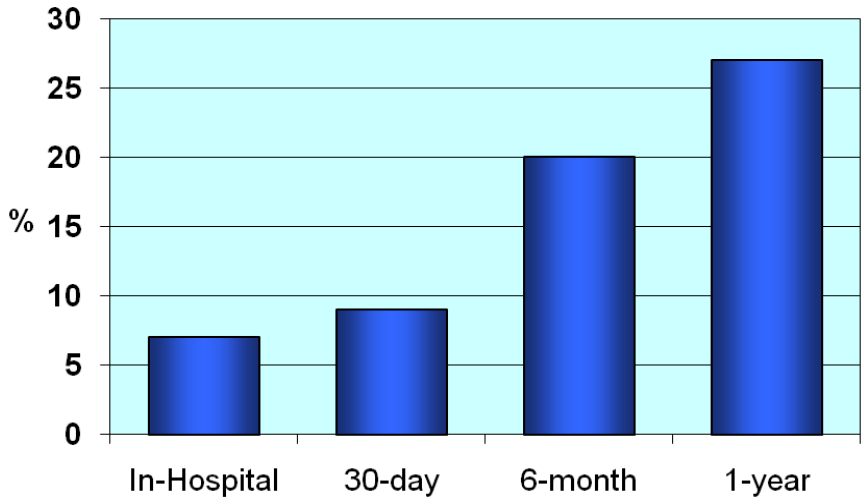

Figure 8 *Cumulative mortality in the FINN-AKVA study*

The crude mortality rate after one year of follow-up was higher in patients with previous history of heart failure compared to de-novo AHF (34 vs. 21%, p<0.001). Overall, patients who died during follow-up were older, had a higher prevalence of co-morbidities and more often had abnormal biochemistry (impairment of renal function [46 vs. 22%], hyponatremia [55 vs. 36%], higher BNP [263 vs. 152 ng/L], NT-proBNP [7363 vs. 4748 ng/L] and CRP [17 vs. 7 mg/L] on admission).

Mortality at 12 months also differed between the clinical classes: 16% in hypertensive AHF, 25% in decompensated AHF, 32% in pulmonary oedema and 36% in cardiogenic shock. While the in-hospital mortality for pulmonary oedema was similar to the overall population (9.8%) with a steady increase in mortalty during follow-up, 4/5 deaths in cardiogenic shock occurred in-hospital. All patients with right heart failure were initially discharged, but after 12 months 43% had died, resulting in the highest 1-year mortality rate and a prognosis even more dismal than in cardiogenic shock.

5.1.5 Predictors of mortality in AHF

The contemporary and unselected AHF population of FINN-AKVA enabled comprehensive analysis of demographic, clinical and biochemical variables as predictors of mortality. On univariate analysis, several co-morbidities were associated with poor prognosis. Previous heart failure (HR 1.7; 95% CI 1.2-2.3), CAD (HR 1.6; 95% CI 1.2-2.2), previous myocardial infarction (HR 1.5; 95% CI 1.1-2.0) and a history of kidney disease (HR 2.4; 95% CI 1.6-3.6) were all associated with worse outcome. Interestingly, a history of hypertension was associated with improved prognosis (HR 0.7; 95% CI 0.6-1.0). Diabetes did not predict 1-year mortality in this study. LVEF <40% was associated with increased mortality (HR 1.6; 95% CI 1.1-2.5) on univariate analysis, whereas higher blood pressure on admission, both systolic (HR 0.9; 95% CI 0.8-0.9 per 10 mmHg increase) and diastolic (HR 0.9; 95% CI 0.9-1.0 per 5 mmHg increase), was related to better prognosis.

Among biomarkers, anemia (HR 1.9; 95% CI 1.4-2.6), hyponatremia (HR 1.5; 95% CI 1.1-2.1 for Na< 135 mmol/L), renal insufficiency (HR 2.4; 95% CI 1.8-3.3 for creatinine >120 µmol/L on admission) as well as CRP (HR 2.2; 95% CI 1.6-3.0) and natriuretic peptide levels above median (HR 1.9; 95% CI 1.2-2.8 for BNP) were all associated with poor survival (*Table 12*).

Table 12. *Biomarker levels and association with mortality in the FINN-AKVA population*

Biomarker	Time of measurement	Univariate HR (95% CI)	Adjusted HR
CysC > median	At 48 hours	3.8 (2.5–5.8)	3.2 (2.0–5.3)
Creatinine >120 µmol/L	At 48 hours	3.1 (2.2–4.5)	2.3 (1.4–3.4)
$CrCl_{C\text{-}G}$ <60 ml/min	At 48 hours	2.2 (1.4–3.3)	N.S.
BNP > median	At admission	1.9 (1.2–2.8)	N.S.
NT-proBNP > median	At 48 hours	2.6 (1.8–3.8)	1.5 (1.0–2.3)
TnT >0.03 µg/L[a]	Admission/48hours	2.6 (1.5–4.4)	N.S.
TnI >0.03 µg/L[a]	Admission/48hours	2.0 (1.2–3.4)	N.S.
Anemia	At admission	1.9 (1.4–2.6)	N.S.
Hyponatremia	At admission	1.5 (1.1–2.1)	N.S.
CRP >median	At admission	2.2 (1.6–3.0)	1.9 (1.3–2.7)
IL-6 >median	At 48 hours	2.3 (1.6–3.4)	1.9 (1.2–2.9)

Adjusted for age, gender, co-morbidities, heart rate, SBP and DBP, and renal function. HR=hazard ratio
[a]*Data from Ilva et al (2008)*

To identify independent predictors of 1-year mortality in AHF, a multivariable model was built including and adjusting for the univariate predictors of mortality. This analysis retained age, male gender, impaired renal function and CRP levels above median as predictors of poor prognosis. Neither LVEF nor BNP (as dichotomous variables) were related to mortality on multivariable analysis in study I. Higher SBP on admission was independently associated with better survival (*Table 13).*

Table 13. *Independent predictors of one-year mortality in AHF (FINN-AKVA study)*

Variable
Older age
Male gender
Renal dysfunction
Systolic blood pressure
CRP above 10 mg/L
IL-6 above median
NT-proBNP above median

5.2 Prognostic value of cystatin C in AHF (II)

5.2.1 Cystatin C and prevalence of impaired renal function in AHF

In 480 patients from the FINN-AKVA study, CysC, creatinine and NT-proBNP levels were measured at 48 hours after admission for AHF (*Figure 6 and Table 11*).

Kidney disease was reported as a co-morbidity in 8% of the study population, but over twice as many (20%) had a creatinine value exceeding 120 µmol/L. Assessment of renal function using creatinine values to calculate CrCl with the Cockcroft-Gault equation or eGFR by the MDRD-equation showed that up to 40% of the study population had moderate to severe renal insufficiency ($CrCl_{C\text{-}G}$ or eGFR <60 ml/min). CysC identified a similar proportion of patients as having impaired renal function (*Figure 9*).

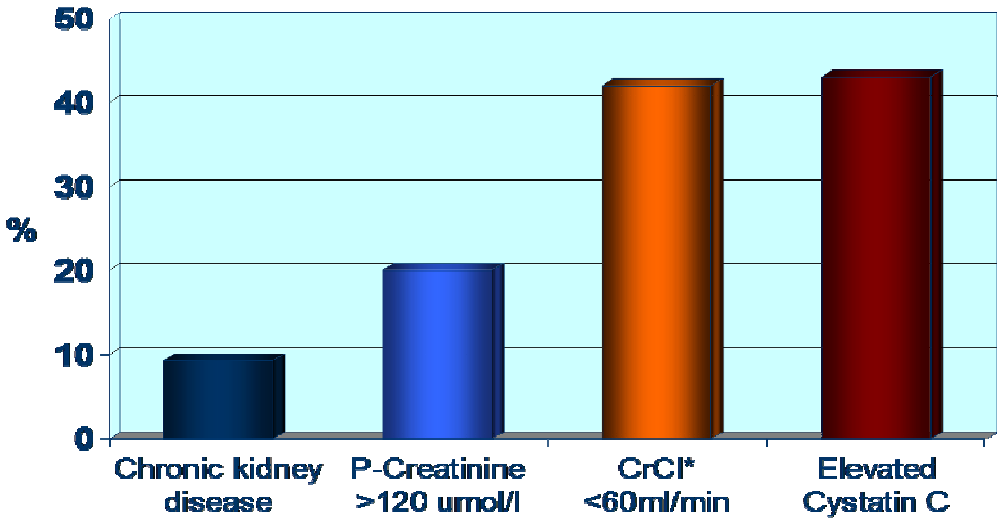

Figure 9 *Prevalence of impaired renal function as assessed by different measures*
** creatinine clearance calculated by the Cockcroft-Gault equation*

5.2.2 Levels of cystatin C and mortality

During the index hospitalization 24 patients died, and at 12 months the mortality was 25.4%. Death during follow-up was associated with higher age on admission, previous history of heart failure, CAD, CKD, lower blood pressure and hemoglobin on admission and higher biomarker levels of CysC, creatinine and NT-proBNP. Conversely, $CrCl_{C\text{-}G}$ was on average lower for deceased compared to patients alive at 12 months.

The median CysC level in the study population was 1.30 mg/L (IQR 1.04-1.70). CysC above median was associated with longer hospital stay (LOS 8 days [IQR 6-13] vs. 7 days [IQR 5-10]; p=0.005) at the index admission. Crude mortality rates at 12 months were 39% in patients with CysC above median compared with 12% if CysC was below the median (log rank p<0.0001). There was also a clear stepwise increase in mortality by

tertile of CysC: 10%, 25% and 42% mortality at 12 months for low, middle and high tertiles, respectively (log-rank $p<0.01$ for difference between each tertile) (II).

Using Cox proportional hazard modelling, CysC above median was associated with a univariate HR of 3.8 (95% CI 2.5-5.8, $p<0.0001$). For creatinine above 120 µmol/L and $CrCl_{C\text{-}G}<60$ml/min univariate HR were 3.1 (95% CI 2.2-4.5, $p<0.001$) and 2.2 (95% CI 1.4-3.3, $p<0.001$), respectively. Also NT-proBNP above median was a predictor of mortality on univariate analysis (HR 2.6; 95% CI 1.8-3.8, $p<0.001$)

Adjusting for demographic characteristics, medical history, clinical and biochemical parameters predictive of mortality on univariate analysis, renal function remained a strong and independent risk marker for 12 month mortality whether measured by creatinine or CysC. CysC was associated with a slightly higher HR (3.2; 95% CI 2.0-5.3, $p<0.0001$) than creatinine (HR 2.3; 95% CI 1.4-3.4, $p=0.0002$). The effect of $CrCl_{C\text{-}G}<60$ ml/min was attenuated by multivariable adjustment and did not reach statistical significance (HR 1.5; 95% CI 0.9-2.5, $p=0.1$).

Analysis of the biomarkers as continuous variables showed that CysC was a predictor of both in-hospital mortality and prognosis during follow-up with comparable OR. As a continuous variable, creatinine was also associated with both early and late mortality while $CrCl_{C\text{-}G}$ was a risk marker for prognosis at 12 months (II).

5.2.3 Cystatin C and risk stratification

As CysC was found to possess good prognostic properties and was an independent predictor of mortality, we explored the use of CysC for risk stratification of patients with AHF.

First, in the group of patients with normal creatinine (n=290) we found that a proportion (18%) still had an elevated CysC. In patients with normal renal function, as assessed by creatinine, CrCl or eGFR, an elevated CysC level was associated with significantly higher mortality. Mortality at 12 months was 13% in patients with normal CysC, while those with elevated values despite normal creatinine had a mortality rate of 40% (log-rank $p<0.001$) (II).

Second, combining CysC as marker of renal function and NT-proBNP, a marker of cardiac stress, resulted in comprehensive risk stratification in different subgroups. Patients stratified in the highest risk category had a ten-fold mortality compared to patients in the lowest risk category.

5.3 Cystatin C as a marker of acute kidney injury (III)

5.3.1 Incidence of AKI

A cohort of 292 patients from the FINN-AKVA study population had measurements of creatinine and CysC both on admission and at 48 hours. This allowed evaluation of a rise in CysC during early hospitalization, which was regarded equivalent to a decrease in GFR i.e. the occurrence of acute kidney injury (AKI). A decline in renal function after hospital admission for AHF is also characteristic of the acute (type 1) CRS. In addition the time frame of 48 hours conforms to the most recent definition of AKI by the AKIN (Mehta et al. 2007).

Patient characteristics were similar to the overall FINN-AKVA population. On admission, the median levels of CysC and creatinine were 1.25 mg/L (IQR 1.01-1.61mg/L) and 0.98 mg/dL (IQR 0.80-1.28 mg/dL), respectively.

The incidence of an increase in CysC levels by >0.1 mg/L, >0.2 mg/L, >0.3 mg/L, >0.4 mg/L and >0.5 mg/L was assessed. A minor rise in CysC was relatively common, with larger increases having smaller incidence. 44% of patients had an increase in CysC exceeding 0.1 mg/L while clinically more significant increases of 0.3 mg/L and 0.5 mg/L were seen in 16% and 8% of patients, respectively (*Figure 10*).

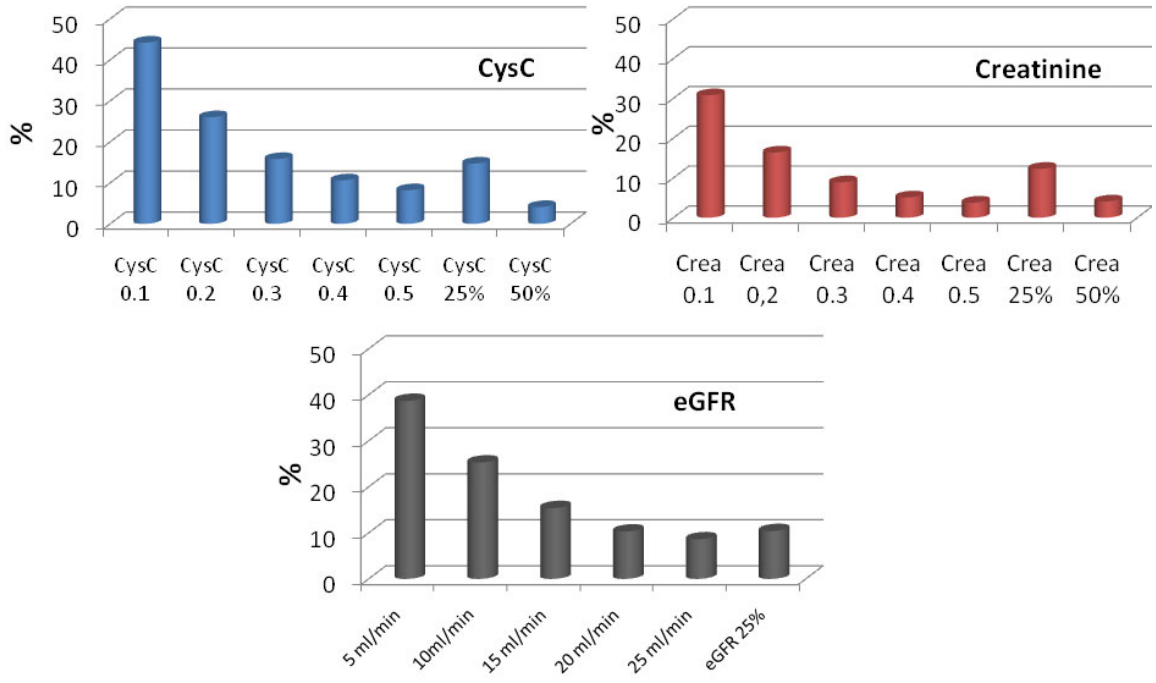

Figure 10 *Incidence of AKI depending on definition as assessed by increase in CysC and creatinine and decrease in eGFR. The x-axis represents different cut-offs of change in renal function biomarkers, while the y-axis depicts the incidence for AKI with that definition.*

The contemporary definition of AKI includes a rise in creatinine by 0.3 mg/dL or more. Using ROC analysis for a change in CysC with the creatinine-based definition of AKI as endpoint, we identified an increase in CysC >0.3 mg/L as a suitable cut-off to define AKI by CysC (AKI_{CysC}). Change in CysC had an AUC of 0.92 (95% CI 0.86-0.98, p<0.0001) and a rise in CysC >0.3 mg/L yielded a sensitivity if 90% and a specificity of 77% for detection of AKI (*Figure 11*).

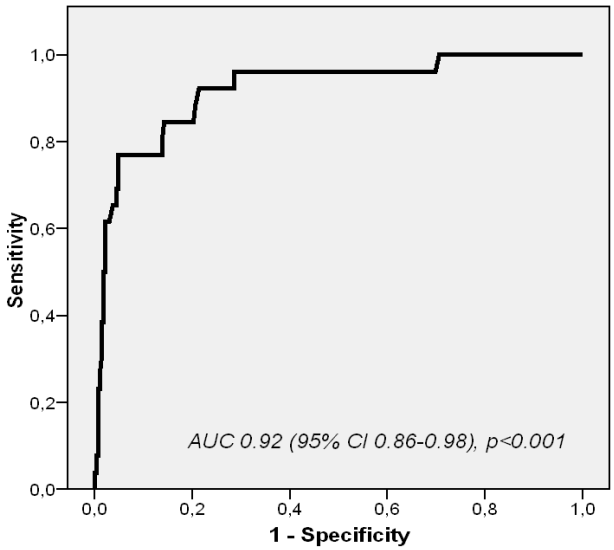

Figure 11 *ROC curve for change in CysC and diagnosis of AKI*

Elevated CysC levels on admission were measured in 38% of the study population (n=111), and patients with a subsequent AKI_{CysC} had higher CysC levels on admission. By comparison, creatinine levels did not differ between those with and without later rise in CysC. Some demographic and clinical characteristics were different in patients with and without AKI_{CysC}. Besides higher baseline levels of CysC, AKI_{CysC} was also associated with older age, previous history of heart failure and ACS on admission. NT-proBNP levels were also more elevated in patients with subsequent AKI_{CysC}. No difference was observed for LVEF, blood pressure or sodium levels on admission between patients with and without AKI_{CysC} (III).

5.3.2 Correlation between cystatin C and creatinine as markers of AKI

As markers of renal function (i.e. GFR), levels of CysC and creatinine were significantly correlated (R=0.76). CysC correlated to eGFR (R=-0.69) and creatinine and eGFR were strongly intercorrelated (R=0.82): p<0.0001 for all correlations. The magnitude of change between admission and 48 hours was also correlated between the markers of renal function (R=0.77 for CysC and creatinine and R=-0.61 for CysC and eGFR, R=-0.74 for creatinine and eGFR; p<0.0001 for all).

A rise in creatinine by 0.3 mg/dL or more, which is the consensus definition of AKI, was observed in 9% (n=26) of patients, which is comparable to the 8% increase in CysC >0.5 mg/L. Larger increases in creatinine were consequently rather uncommon. A rise in creatinine of 0.2 mg/dL or more had a similar incidence as AKI_{CysC} (16%) in this population. Assessing a change in renal function by eGFR showed that the RIFLE consensus definition of a decline in eGFR by 25% occurred in 10% of patients while a similar incidence as for AKI_{CysC} was observed for a decline in eGFR by 15 ml/min (15%).

However, the populations identified as having AKI by the different markers were not the same, as illustrated in *Figure 12*. In patients with AKI_{CysC} (n=46) only 29 patients (63%) had a concomitant increase in creatinine exceeding 0.2 mg/dL and in 22 patients (48%) a decline in eGFR >15mL/min was observed. In 20 of the 46 AKI_{CysC} patients (44%) creatinine increased >0.3 mg/dL (III).

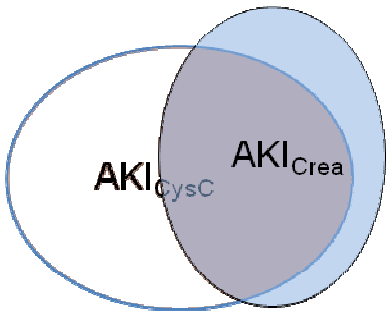

Figure 12 *Divergence of populations identified as having AKI by CysC and creatinine. 20/26 patients with a rise in creatinine >0.3 mg/dL (AKI_{Crea}) also had a rise in CysC >0.3 mg/L (AKI_{CysC}). AKI_{CysC} occurred in 26 additional patients, not identified as having AKI_{Crea}*

5.3.3 The effect of AKI_{CysC} on prognosis

The in-hospital mortality in study III was 5% with 85 patients (29%) dead at 12 months. Mean LOS in the overall population was 7 days (IQR 5-12), with AKI_{CysC} being associated with significantly longer LOS (10 days; IQR 6-16) compared to patients without AKI_{CysC} (7 days; IQR 5-10, p=0.01 for difference). Crude in-hospital, 30 day, 90 day, 6 month and 12 month mortality rates were evaluated for each change in CysC, and univariate OR calculated at all time points for each increase in CysC. Any rise in CysC from admission to 48 hours exceeding 0.2 mg/L was associated with higher odds of death. The larger the rise, the greater was the effect on mortality. The effect on mortality was more pronounced early during follow-up, and seemed to diminish with time (F*igure 13).* Greater increases were associated with poor prognosis even beyond 6 months.

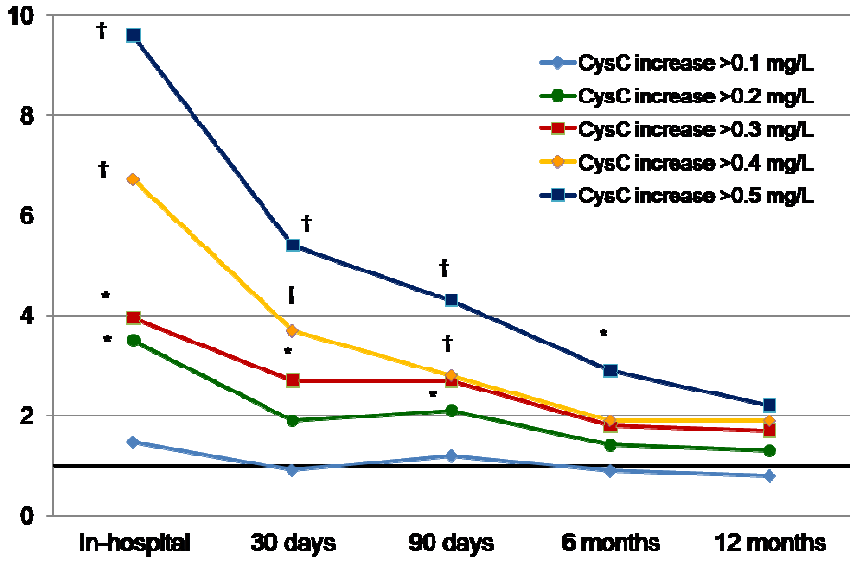

Figure 13 *Odds ratios for mortality during follow-up associated with different increases in CysC. *p<0.05, †p<0.01compared to increase <0.1 mg/L*

A rise in CysC >0.3 mg/L within 48 hours from admission (AKI_{CysC}) was a significant predictor of mortality both in-hospital and during follow-up. Crude mortality rates during hospitalization were 13% for patients with AKI_{CysC} and 3.7% in patients without AKI (OR of 4.0; 95% CI 1.3-11.7; p=0.01). During 12 months of follow-up, 39% of patients died in the group with compared to 27% in patients without AKI_{CysC} (p=0.06 for difference by log-rank test). However, the difference in mortality was significant at 90 days (*Figure 14*).

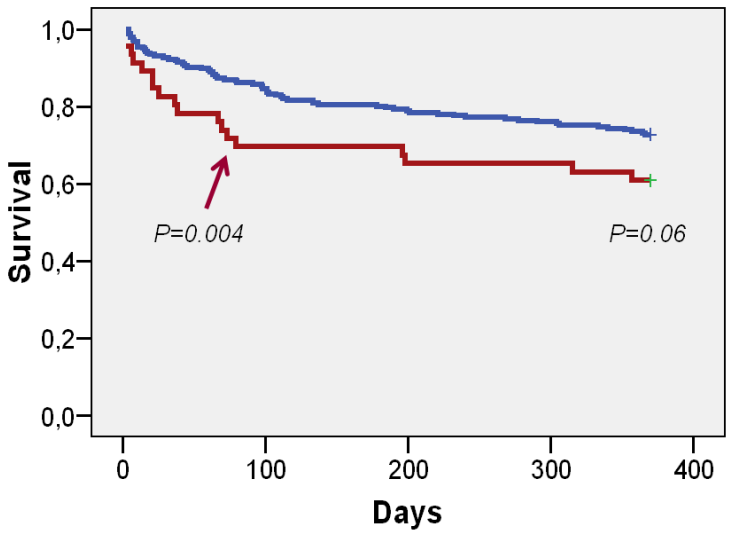

Figure 14 *Kaplan Meier survival curves for patients with (lower line) and without (upper line) AKI_{CysC}. Arrow indicates difference in survival at 90 days.*

Actually, AKI_{CysC} was found to be an independent predictor of mortality at 90 days. After adjustment for confounders, AKI_{CysC} was associated with an OR of 2.8 (95% CI 1.2-6.7, p=0.02) for mortality at 90 days. A rise in CysC >50% occurred only in 12 patients but was a strong risk marker for poor long-term prognosis (OR 7.5; 95% CI 1.6-34.7; p=0.01 for 12 month mortality). The effect on prognosis of a decline in renal function measured by creatinine and eGFR was also evaluated using cut-offs giving similar incidence of worsening renal function as AKI_{CysC}. All markers were found to be predictors of mortality with adjusted OR for AKI_{CysC}, creatinine increase >0.2 mg/dL and eGFR decline 15 ml/min of approximately similar magnitude.

In the multivariable analysis, NT-proBNP above median was also a marker of worse prognosis both at 90 days (OR 2.8; 95% CI 1.3-6.0, p=0.01) and at 12 months (OR 2.4; 95% CI 1.3-4.5, p=0.004). Using the combination of AKI_{CysC} and NT-proBNP above/below median for risk stratification showed that AKI_{CysC} increased both short-term as well as long-term mortality risk in patients with AHF and NT-proBNP below median. In patients with NT-proBNP above median, AKI_{CysC} increased the risk of death at 90-days, but was not associated with higher OR at 12 months. Finally, the observation that creatinine and CysC identified different populations with AKI also affected the prognostic properties. A rise in creatinine by 0.2 mg/dL or more was associated with higher mortality only in patients with a concomitant increase in CysC. Patients with a solitary rise in creatinine had a mortality rate similar to patients without AKI.

5.4 Inflammatory markers in the cardiorenal syndrome (IV)

5.4.1 Cytokine levels and correlations to cardiac and renal markers

Plasma concentrations of inflammatory cytokines (TNF-α, IL-6, IL-10), CysC and NT-proBNP were measured from samples taken at 48 hours after admission. Measurements of all five biomarkers were available in 465 subjects which formed the cohort for study IV (*Figure 6*). In these patients with AHF, median levels of TNF-α, IL-6 and IL-10 were 1.5 ng/L, 15.2 ng/L and 1.8 ng/L, respectively. Correlations between the cytokines and biomarkers of renal function and cardiac stress were calculated after logarithmic transformation, because the distribution of the plasma levels of the cytokines was heavily skewed to the right. Levels of CysC, NT-proBNP and TNF-α all exhibited a stepwise increase by quartile of eGFR (p<0.001 for all) (*Figure 15*). In contrast, IL-6 levels were markedly higher only in patients with worse renal function (median IL-6 14.4, 13.2, 13.2 and 20.2 ng/L in highest to lowest eGFR quartile, respectively).

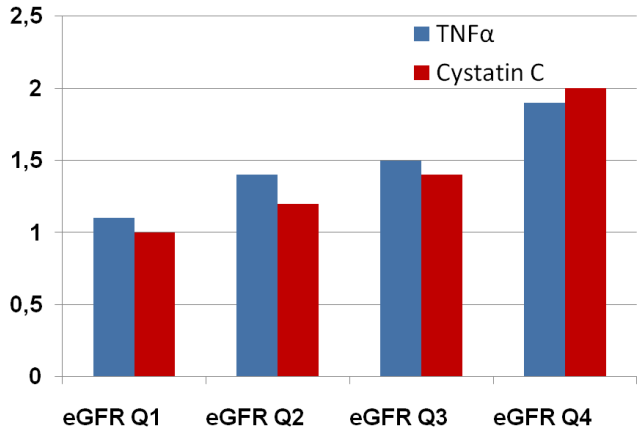

Figure 15 *Stepwise increase in median levels of CysC and TNF-α by quartile of eGFR. Q1=eGFR>81 ml/min, Q2=eGFR 64-81 ml/min, Q3=eGFR 48-64 ml/min and Q4=eGFR<48 ml/min. Units are ng/L for TNF-α and mg/L for CysC.*

CysC was found to correlate most strongly with TNF-α (R =0.47, p<0.001) whereas the correlation with IL-6 was weaker (R=0.17, p<0.001), and further attenuated by NT-proBNP. On univariate analysis, NT-proBNP correlated with IL-6 (R=0.26, p<0.001) and weakly with TNF-α (R=0.16, p<0.001). Using linear regression analysis, adjusting for confounding factors such as age, gender, history of previous heart failure, CAD, cerebrovascular disease, hypertension, diabetes, smoking status, medication at baseline (BB, ACEI/ARB, lipid lowering therapy), NYHA class, presence of ACS on admission and clinical infection during hospitalization did not significantly change the association to IL-6. However, after adjustment, no association remained between NT-proBNP and TNF-α (p>0.05). In fact, merely adjusting for CysC levels eliminated the relationship between NT-proBNP and TNF-α. By contrast, CysC was related both TNF-α (standardized β-coefficient=0.37, p<0.001) and IL-6 (β=0.18, p<0.001) even after considering confounders. NT-proBNP levels did not attenuate the association between CysC and TNF-α (β=0.42, p<0.001). The correlation of IL-10 with CysC and NT-proBNP was overall rather weak, but after adjustment a significant association with NT-proBNP was still detectable (β=0.15, p<0.01). NT-proBNP and CysC (β=0.26, p<0.0001) were associated after adjustment. The studied inflammatory cytokines were all significantly intercorrelated as well.

5.4.2 Stratification by cystatin C, NT-proBNP and relation to outcome

Levels of IL-6 and TNF-α differed in patient groups stratified by CysC and NT-proBNP (above/below median), whereas levels of the anti-inflammatory cytokine IL-10 showed no significant change between strata (*Table 15*). TNF-α levels were significantly higher in groups with CysC above median while NT-proBNP stratum had little effect on levels of TNF-α. IL-6 levels were more affected by NT-proBNP levels. In particular,

patients with both CysC and NT-proBNP above median had average IL-6 levels twice as high compared to patients with both markers below median.

Table 15. *Levels of inflammatory cytokines in groups stratified by CysC and NT-proBNP median*

	(A) $CysC_{low}$/ $NT\text{-}proBNP_{low}$ N=143	(B) $CysC_{low}$/ $NT\text{-}proBNP_{high}$ N=83	(C) $CysC_{high}$/ $NT\text{-}proBNP_{low}$ N=87	(D) $CysC_{high}$/ $NT\text{-}proBNP_{high}$ N= 147
IL-6	11.3 (5.9-21.9)	16.2 (7.6-29.9)*	12.4 (6.7-24.6)	23.6 (10.1-47.4)**‡†
TNF-α	1.1 (0.9-1.6)	1.1 (0.8-1.5)	1.7 (1.4-2.3)** ††	2.0 (1.5-2.5) **††
IL-10	3.3 (0.5-1.65)	2.1 (0.5-4.1)	1.6 (0.5-3.5)	2.1 (0.9-4.3)

*$CysC_{low/high}$=CysC below/above median of 1.30 mg/L, $NT\text{-}proBNP_{low/high}$=NT-proBNP below/above median of 3951 pg/mL, *p<0.05 vs A), ** p<0.01 vs A), †p<0.05 vs B), ††p<0.01 vs B) ‡p<0.01 vs C)*

Plasma levels of cytokines had only moderate discriminative capacity for mortality at 12 months. In the ROC-analysis, IL-6 and TNF-α had comparable AUC values of 0.64 and 0.66, respectively (p<0.001 for both), while the properties of IL-10 for risk stratification were weaker (AUC 0.56) and not significant. Multivariable Cox regression analysis still revealed IL-6 above median to be an independent predictor of mortality, even when CysC and NT-proBNP were included in the model. Kaplan-Meier analysis of IL-6 and TNF-α in CysC and NT-proBNP strata revealed that the effect on mortality of TNF-α above median was seen in the subgroup with $CysC_{low}$/$NT\text{-}proBNP_{low}$. For IL-6, separation of the curves occurred mostly in the subgroups with intermediate overall mortality, having either elevated CysC or elevated NT-proBNP. In the latter, the difference did not quite reach significance.

6. Discussion

6.1 Characteristics and outcomes in AHF

Epidemiological studies on the characteristics, clinical presentation and outcomes in AHF were surprisingly scarce until the last decade. In fact, FINN-AKVA is among the first studies to describe an unselected and well characterized population hospitalized for AHF and with mortality follow-up duration of 12 months. Important observations from this study and others can lead to a better understanding of AHF and help improve treatment strategies and risk stratification for patients.

The finding that half of the patients hospitalized for AHF are de-novo cases has several implications. First, initial management of AHF is usually necessary to perform without the knowledge of previous heart failure. Natriuretic peptide testing is useful in dyspneic patients without previous diagnosis of heart failure, and can help early diagnosis (Mueller et al. 2004, Baggish et al. 2006). For patients with de-novo heart failure, further diagnostic work-up, institution of heart failure medication and organisation of follow-up should be carried out during the index hospitalization. Together with natriuretic peptide testing, echocardiography with assessment of left ventricular function is the mainstay of diagnosis in patients presenting with signs and symptoms of AHF. Still, as shown also in the FINN-AKVA study, almost half of the patients have rather well preserved LVEF. Therefore, other signs of cardiac dysfunction need to be evaluated carefully. Second, de-novo AHF patients differ from patients with an acute decompensation of chronic heart failure both in matters of co-morbidities and clinical presentation, which also impacts prognosis (I) (Nieminen et al. 2006, Harjola et al. 2010).

Co-morbidities are abundant in the elderly AHF population. Although diseases known to cause heart failure (hypertension, CAD and valvular disease) are most common, CKD, pulmonary disease, diabetes, atrial fibrillation and manifestations of atherosclerotic disease are found in many patients with AHF. Although most of these co-morbidities are previously known, this study clearly shows that the prevalence of renal insufficiency is severely underestimated unless accurate and reliable methods are used to assess kidney function (II). Even when using creatinine-based measures of renal function, other studies have also found extremely high prevalence of renal dysfunction in patients hospitalized for AHF (Smith et al. 2003, Heywood et al. 2007).

The FINN-AKVA study used the ESC clinical classes to categorize the patients. Although this classification has not been widely used in other studies, it forms the basis for identifying clinically different patient categories within the very heterogeneous AHF cohort. As in EuroHeart Failure Survey II (Nieminen et al. 2006), the majority of AHF patients in FINN-AKVA presented with the clinical picture of decompensated AHF or pulmonary oedema which can be regarded as different grades of symptom severity, usually without circulatory compromise. Cardiogenic shock, hypertensive AHF and right heart failure each represent a smaller part of the clinical presentation of AHF, but are more distinct entities with more severe hemodynamic disturbances. The distribution in clinical classes between de-novo AHF and ADCHF is not the same. The former more often

presents with hypertensive AHF, a consequence of acute change in cardiac stress and/or vascular tone. Cardiogenic shock complicating ACS was also more common in de-novo AHF. These findings may point to a circulation less adapted to a rise in filling pressures and decrease in cardiac output. ADCHF most often presents with decompensated AHF suggesting better adapted hemodynamics and mild to moderate symptoms of worsening of heart failure.

Arrhythmias, especially atrial fibrillation, are frequently present as potential precipitators of AHF. Ischemia aggravating cardiac or valvular dysfunction and infection increasing oxygen consumption and thereby the demand on cardiovascular performance are the other main causes thought to precipitate AHF. Differences in precipitating factors can result in different clinical presentation as well. The distribution in clinical classes in FINN-AKVA and Euro Heart Failure Survey II were not completely analogous, but may be attributable also to geographical differences in the aetiology and presentation of AHF. In both studies, hypertensive AHF had the best prognosis. The high mortality observed in cardiogenic shock was mainly due to extremely poor outcome already during the index hospitalization.

The mortality early after hospitalization for AHF is very high. In-hospital mortality rates of 5-10% have consistently been reported both in Europe and in the United States (for details please refer to *Table 3)*. After discharge, the prognosis is still very poor for the first year after hospitalization. Mortality reaches 25-30% at 12 months, being even higher in patients with more severe clinical presentation. The ESC clinical classes are also associated with different mortality rates, reflecting severity of AHF at presentation, which can be used for early risk stratification.

6.2 Predictors of mortality in AHF

One of the central goals of this study was to investigate predictors of mortality in patients with AHF. For most diseases, risk stratification is a real clinical challenge. Still, improving outcome requires recognition of factors that affect prognosis and especially identification of those who can be modified by a preventive or therapeutic intervention.

6.2.1 Clinical predictors

In these elderly patients with AHF, older age is by itself associated with increasing mortality. This independent effect of age was very clear in the FINN-AKVA population (50% increase in annual mortality risk for each 10 years increase in age) and is confirmative of findings in other cohorts of AHF, with very similar risk ratios (3-5% risk increase per year) (Harjola et al. 2010, Tavazzi et al. 2006, Cowie et al. 2002, Blackledge et al. 2003, Gustafsson et al. 2004b). Some large studies do not report gender as being a predictor of outcome (Harjola et al. 2010, Lee et al. 2003), nor do the guidelines mention differences in prognosis associated to gender. Still some studies, including the present, suggest that male gender is independently associated with adverse outcome at least in

some subgroups (Blackledge et al. 2003, Mullens et al. 2008b, Gustafsson et al. 2004a). In an unselected population with AHF, female subjects are on average older than the male (I), (Cohen-Solal et al. 2000). This age-difference needs to be considered when evaluating the association of gender with mortality.

The AHF population has many concomitant diseases, which may affect survival. The co-morbidities recorded and assessed as predictors of outcome differ between studies, as do the results about their impact on short- or long-term mortality. In patients with heart failure, co-morbidities like CAD, previous myocardial infarction, hypertension, diabetes, pulmonary disease, kidney or even liver dysfunction have been suggested to be risk markers of poor prognosis in AHF (Harjola et al. 2010). In addition, some studies have assessed whether dementia, cancer or living in a dependent accommodation affects mortality in AHF (Lee et al. 2003). Although low LVEF is mentioned in the European and American guidelines as a strong predictor of poor prognosis in chronic heart failure (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology, Hunt et al. 2005), it seems that the effect on prognosis is seen mostly with severe impairment of LVEF (Hillege et al. 2000, Harjola et al. 2010, Pocock et al. 2005).

In the FINN-AKVA study, a previous history of heart failure, CAD, myocardial infarction, hypertension and CKD were all associated with prognosis. Still, using multivariable Cox proportional hazard modelling, no variable related to medical history was independently predictive of mortality at one year. The variables identified as independent risk factors for mortality were age, gender, SBP on admission, creatinine (as a marker for renal function), NT-proBNP and CRP. The earliest observations on the inverse relationship between blood pressure and mortality in AHF date back to the mid 1980:ies (Goldberger et al. 1986). Since then, several observational studies have confirmed this relationship (Harjola et al. 2010, Gheorghiade et al. 2006a, Tavazzi et al. 2006, Lee et al. 2003, Fonarow et al. 2005). Low blood pressure on admission, essentially systolic, is a marker of poor prognosis, not only in patients with cardiogenic shock (Zannad et al. 2006). Higher blood pressure on admission is associated with better survival in AHF (I, II).

6.2.2 Renal function

A creatinine value above 120 µmol/L was twice as common in patients who died during follow-up compared to survivors at one year (I). Creatinine was a strong predictor of mortality in univariate analysis and also one of five independent risk markers for mortality. Creatinine >120 µmol/L nearly doubled the risk of death even after adjustment for other risk factors. This is well in line with the meta-analysis of studies reporting the effect of renal function on prognosis in heart failure, in which moderate to severe renal insufficiency is associated with an adjusted HR for adverse outcome of around two (Smith et al. 2006). The observation in FINN-AKVA that renal function is one of the main predictors of outcome in AHF has been confirmed in many other cohorts and studies (Tavazzi et al. 2006, O'Connor et al. 2008, Fonarow et al. 2005). The current ESC

guidelines list renal dysfunction as associated with poor prognosis in chronic heart failure with a special mention in the section of AHF about high creatinine or urea levels as being adverse prognostic factors (Task Force for Diagnosis and Treatment of Acute and Chronic Heart Failure 2008 of European Society of Cardiology).

6.2.3 Other biomarkers as risk factors for adverse outcome

In addition to being useful in the diagnosis of heart failure, the natriuretic peptides, mainly BNP and NT-proBNP, have been shown to possess prognostic properties in heart failure, including AHF (Bettencourt et al. 2004, Logeart et al. 2004, Januzzi et al. 2006, Sakhuja et al. 2007). In study I, admission BNP above median was not independently associated with mortality while higher NT-proBNP levels measured at 48 hours from admission were predictive of worse prognosis in study II. Explanations for this discrepancy may be found in the different effects on outcome of admission and discharge values of natriuretic peptides (Logeart et al. 2004, O'Brien et al. 2003, Metra et al. 2007). One study with serial measurements of NT-proBNP found that after a peak level reached at 12 hours after admission, NT-proBNP levels decline by 48 hours after admission, but discharge levels were on average the same as at 48 hours (Metra et al. 2007). In addition, because median BNP levels in FINN-AKVA were lower than expected in AHF, analytical issues confounding the BNP results cannot be excluded.

Hyponatremia and anemia are two fairly common laboratory findings in heart failure, which also have been reported to be associated with increased mortality in several reports from AHF populations (Harjola et al. 2010, McClellan et al. 2002, Gheorghiade et al. 2007). In the FINN-AKVA cohort, mean sodium levels were not different between survivors and non-survivors (II). In contrast, patients who died during follow-up had both a higher prevalence of anemia and lower hemoglobin levels already on admission (I, II). One reason could be more severe renal dysfunction as a cause of anemia or it may be a reflection of the cardiorenal-anemia syndrome described in chronic heart failure (Harjola et al. 2010, Silverberg et al. 2006, Wexler et al. 2005). Hyponatremia was still more prevalent in non-survivors and associated with higher mortality on univariate analysis (I, II). Similarly, anemia was a predictor of death on univariate analysis, but neither hyponatremia nor anemia were independent risk markers of poor prognosis in FINN-AKVA. Both these parameters have been found to be significant in larger AHF cohorts, albeit with a modest increase in mortality risk (Harjola et al. 2010, Gheorghiade et al. 2007). Inflammation and CRP as prognostic markers are discussed in section 6.5. The next chapters will focus on the role of CysC as a marker of AKI and a predictor of poor prognosis in AHF.

6.3 Cystatin C and prognosis in AHF

CysC has been studied intensively as a risk marker for poor prognosis and cardiovascular events in various populations. This was the first study to show the relationship with mortality in patients hospitalized for AHF (II). Since, other publications have confirmed that higher levels of CysC are associated with worse prognosis in AHF, including cohorts with non-Caucasian ethnicities (Campbell et al. 2009, Manzano-Fernandez et al. 2009, Naruse et al. 2009). The effect of CysC on mortality was robust even after adjustment for possible confounders and other factors associated with increased mortality. The increase in mortality was present both in different CysC strata and as a continuous marker, affecting both short- and long-term survival in AHF (I).

The finding that CysC was a predictor of outcome after adjustment for creatinine or $CrCl_{C\text{-}G}$ as well may raise the question about effects not related to renal function (Koenig et al. 2005, Luc et al. 2006). Since comparison with directly measured GFR is not available, no definite answer can be given. Based on the available data it is impossible to fully adjust for true kidney function because, even at their best, $CrCl_{C\text{-}G}$ or eGFR are imperfect measures of GFR. Unfortunately, large studies with direct measurement of GFR are not likely to be performed with current methods. Given the documented strong effect of renal dysfunction on outcomes in AHF and the perception that CysC is a better marker of GFR than creatinine, it seems plausible that the superiority of CysC for predicting outcome is directly related to its properties as a marker of kidney function. As shown in study II, the effect of an elevated CysC on mortality in patients with normal creatinine was remarkable. Studies on other populations with normal or near normal kidney function assessed by creatinine have also documented an increased incidence of CVD and adverse events related to higher CysC levels (Shlipak et al. 2006a, Keller et al. 2009, Muntner et al. 2008). Interestingly, this increase in risk is seen mostly in the abnormal range of CysC. In all the studies in ACS, median CysC levels were $\leq$1.0 mg/L and the negative effect on cardiovascular outcomes was mostly visible in the highest quartile of CysC (Jernberg et al. 2004, Kilic et al. 2009, Windhausen et al. 2009). Thus, it is most probable that elevated CysC reflects a minor to moderate decline in renal function not detected by creatinine and consequently has a significant effect on prognosis.

6.4 Cystatin C as a marker of AKI

Diagnosis of AKI, i.e. a rapid decline in GFR, has been challenging. For half a century, creatinine and urea have been the only markers available to assess renal function. The dilemma of creatinine being a slow and insensitive marker for change in GFR has been highlighted in section 2.3. Therefore, detection of AKI with creatinine will inevitably be late and any interventions to prevent decline in kidney function usually futile. Signs of decrease in urine output, also used to detect AKI, are not invariably due to kidney damage but may be secondary to transient hypovolemia (pre-renal azotemia) or a post-renal obstruction of urine flow. In addition, low urinary output in hypovolemia could be considered more a physiological response (through effects of vasopressin and

concentration of urine) secondary to decrease in renal blood flow and GFR. The terms pre-renal and post-renal acute renal failure refer mainly to hemodynamic and anatomical factors, and are not descriptive of kidney damage or pathophysiology. On the other hand, tubular damage may cause inability to concentrate urine, and urine output can be normal despite injury to the kidney. Therefore, urinary output cannot be exclusively diagnostic of AKI.

The novel markers of tubular injury have the potential of early detection of kidney damage. Assuming that tubular injury precedes a decrease in GFR and that therapeutic interventions can be instituted to prevent or alleviate this decline, the tubular markers have a prospective role in clinical practice. Nevertheless, an accurate and rapid marker to detect decline in GFR should also be available. CysC has the potential to fulfil these requirements and has been studied as an alternative to creatinine as a marker of GFR in AKI.

6.4.1 Cystatin C and diagnosis of AKI

When assessing a new marker for diagnosis of AKI, one faces several methodological dilemmas. First, direct measurement of the variable of interest, i.e. GFR, is not practically feasible. Second, it is not known when and which magnitude of a change in GFR should be considered abnormal. The current definitions (RIFLE and AKIN) rely on changes in a surrogate marker, mostly because these changes have been shown to be associated with adverse events (need of renal replacement therapy, morbidity and mortality). Third, the actual change in GFR associated with an absolute or relative rise in creatinine will be dependent on baseline renal function. An absolute increase in creatinine will be of relatively greater magnitude if baseline renal function is normal (i.e. 0.3 mg/dL increase from 1 mg/dL compared to the same increase from 2.5 mg/dL). On the other hand, a 25% increase from 2.5 mg/dL will result in an absolute change in creatinine over 0.5 mg/dL compared to a rise of 0.25 mg/dL from a baseline value of 1 mg/dL. Fourth, in the absence of a true golden standard method for reference, showing that any new marker is superior to creatinine for diagnosis of AKI will be difficult. The occurrence of AKI is defined by creatinine, and the ability of the new biomarker to detect AKI (whether accurately identified by creatinine or not) will be compared to this definition. Hence, if the new biomarker detects AKI in subjects not classified as AKI by the creatinine definition, it will appear non-specific. If creatinine misclassifies patients as having AKI while the novel biomarker does not, it will be regarded as a lack of sensitivity of the new marker (Waikar et al. 2009a). Finally, the definition of AKI with creatinine will affect the performance (sensitivity and specificity) of the new marker (Haase-Fielitz et al. 2009a). All these issues are relevant when interpreting the results of study III as well.

Studies assessing CysC as a marker of AKI have mainly focused on two aspects: whether CysC rises earlier than creatinine or whether CysC measured at a specific time-point is able to predict a secondary outcome (need for renal replacement therapy, LOS or in-hospital mortality) better than creatinine. Of four studies using serial sampling of renal markers, three found CysC to be an earlier marker of AKI (Herget-Rosenthal et al. 2004,

Nejat et al. 2010, Haase-Fielitz et al. 2009b). The fourth did not find any statistically significant difference between CysC and creatinine, although the AUC for prediction of AKI (RIFLE-criteria) for CysC was higher in particular on the first post-operative day (Ristikankare et al. 2010).

The present study (III) was the first to examine the incidence of AKI by a change in CysC in a clinical population outside the cardiac surgery and intensive care setting. Although absolute increases in CysC and creatinine are not comparable with regard to changes in GFR, the results give the impression that a rise in CysC is detected more often than a rise in creatinine. The adopted threshold for AKI_{CysC} showed excellent performance for detection of AKI. The cut-off identified in this study is different from the threshold in a population at risk for contrast-induced nephropathy (Briguori et al. 2010).

As in other studies, the definition of AKI was by creatinine. Yet, the populations identified as having AKI by CysC and creatinine are not identical. A similar observation was made in another study, in which creatinine and CysC rose above a predefined cut-off simultaneously in only 20-25% of patients experiencing AKI. In more than half of the subjects with AKI, CysC not only preceded creatinine in time, but although a clear rise in CysC was observed creatinine levels remained below the diagnostic threshold in many patients (Nejat et al. 2010). Peralta et al (2010) also observed that the populations identified by $eGFR_{CysC}$ and $eGFR_{Crea}$ as having a change in kidney function differ. An effect on prognosis was seen only with a change in $eGFR_{CysC}$. In a small diabetic cohort with subjects of specific ethnicity and more stable renal function, changes in levels of CysC correlated well with the decline in measured GFR, and CysC was a better marker of decline in renal function than creatinine based measures (Perkins et al. 2005).

There are important differences between the population in this study and the cohorts experiencing AKI in ICU (Herget-Rosenthal et al. 2004, Nejat et al. 2010) or after cardiac surgery (Haase-Fielitz et al. 2009b, Wald et al. 2010, Ristikankare et al. 2010). The incidence of AKI is much higher in the ICU setting. Timing the injury in patients with cardiac surgery is more straightforward, and the disturbances associated with the surgical trauma can be regarded as a "one-hit" injury. While AKI after cardiac surgery resulted in a significant rise in creatinine, the peak creatinine value was observed only on the third day after surgery (Haase-Fielitz et al. 2009b). Still, many patients will have relatively severe kidney injury with substantial decline in GFR after cardiac surgery, and thus detected by creatinine with less delay than in milder forms of AKI (Waikar et al. 2009b). This is reflected also as a higher incidence of early AKI, with over half occurring within 48 hours and 76% within three days from surgery (Haase-Fielitz et al. 2009b, Ristikankare et al. 2010). Therefore, the advantage with early markers is to detect AKI mostly in the first post-operative hours. Markers of tubular damage may be most useful in detecting kidney injury very early after cardiac surgery (Haase et al. 2009, Haase-Fielitz et al. 2009a). CysC has properties enabling detection of a decline in GFR already in the first hours after cardiac surgery, but with less difference compared to creatinine beyond 24 hours post-operatively (Haase-Fielitz et al. 2009b, Ristikankare et al. 2010). If AKI ensues more gradually, with a minor and subsequently progressive decline in GFR, it is likely CysC will be able to: a) detect minor changes in GFR and b) at an earlier time-point compared to creatinine. CysC is at least equal, if not superior, to creatinine as a marker of GFR. The

independence from height, gender, age and muscle mass is advantageous (Filler et al. 2005). Recent evidence suggests that changes in creatinine and eGFR, whether acute or slow, in some cases do not mirror actual changes in renal function. In particular elderly hospitalized patients, with or without alterations in muscle mass, will benefit from estimating renal function with CysC. Therefore, CysC may yield even greater advantage compared to creatinine for detection of AKI in AHF patients, other elderly hospitalized or even general ICU populations.

6.4.2 Cystatin C and prognosis in acute CRS

AKI after hospital admission for AHF is the classical scenario that defines the acute (type 1) CRS. Numerous studies have assessed the effect of a rise in creatinine on prognosis in AHF (see also chapter 2.4.4) and it is clear that the acute CRS is associated with increased morbidity and mortality (Smith et al. 2006, Gottlieb et al. 2002, Damman et al. 2007, Forman et al. 2004).

Study III is the first to assess the effect of AKI detected by CysC on prognosis in the acute CRS. Moreover, only one other study has assessed the relationship between the change in CysC (i.e. AKI_{CysC}) and mortality during follow-up.(Briguori et al. 2010) Some of the publications that assessed the diagnostic performance of CysC in AKI discussed above also reported short-term outcome, but absolute number of events was very small. Furthermore, as already emphasized, the prediction was based on a single measurement rather than a change in CysC. In the prediction of in-hospital mortality or the need for renal replacement therapy, admission values of CysC and NGAL were independently associated with outcome, while creatinine was not (Haase-Fielitz et al. 2009b). CysC levels at a cut-off of 1.4 mg/L were reported to have excellent AUC for outcome prediction. In another study, levels of CysC and creatinine were similarly predictive of renal replacement therapy or 30 day mortality but with moderate performance on ROC-analysis (AUC 0.63 [95% CI 0.56–0.71] for CysC and 0.62 [95% CI 0.54–0.70] for creatinine) (Nejat et al. 2010). Finally, one study reported that although markers of renal function marginally increased the AUC compared to a comprehensive model incorporating clinical risk factors, the increase was not statistically significant (Perianayagam et al. 2009). It has to be noted that recent literature on how to assess the incremental value of novel biomarkers for risk prediction and stratification highlights the limitations both in the sensitivity and utility of the ROC-curve for these purposes. Measures like the Net Reclassification Improvement may provide additional information when assessing the incremental prognostic value of new biomarkers against validated clinical risk prediction models (Cook 2007, Pencina et al. 2008).

The results in study III suggest that changes in CysC do have prognostic implications. A rise in CysC of 0.3mg/dL or more (AKI_{CysC}) is sensitive enough to detect AKI in a significant proportion of AHF patients, with good diagnostic performance compared to the contemporary definition of AKI. In addition, AKI_{CysC} is associated with longer hospital stay and is an independent risk factor for short-term mortality. Larger increases in CysC conferred even greater increase in mortality risk, as expected with more severe decline in

GFR. Nevertheless, in patients with CKD and at risk of contrast-induced nephropathy, even a small 10% increase in CysC actually predicted clinical events.

No formal comparison was performed between AKI_{CysC} and a rise in creatinine on their effects on outcome, in part because of the observed discordance in the populations identified by each marker and then again because of methodological challenges with such a comparison. For changes in CysC, creatinine and eGFR having the same incidence in the studied population, the observed adjusted OR for mortality during follow-up were of comparable size. Still, an increase in creatinine was not associated with higher mortality unless accompanied by a simultaneous rise in CysC. An interesting interaction with NT-proBNP was also found, since AKI_{CysC} had greater impact on prognosis in patients with lower NT-proBNP levels.

Overall, the study showed that CysC can be used to detect a decline in renal function in patients with AHF. A rise in CysC within 48 hours from admission (type I acute CRS) was observed in a significant proportion of patients and was associated with adverse outcomes, most notably an increase in mortality. The present study yielded encouraging results about the properties of CysC as a marker of AKI and open the way for the further assessments of its clinical utility. The definition of AKI_{CysC} identified and used in this study needs to be tested and confirmed in other studies including populations other than AHF patients.

6.5 The cardiorenal syndrome

The increasing interest in the CRS is mainly driven by the very strong impact concomitant cardiac and kidney dysfunction has on patient prognosis. The current Acute Dialysis Quality Initiative consensus classification of the CRS (Ronco et al. 2010) broadens the picture, from just a state of diuretic resistance and renal insufficiency in severe heart failure to any situation, acute or chronic, where functional impairment of either heart or kidney negatively affects the other resulting in concomitant dual organ dysfunction. There is a vicious cycle of disease progression ultimately leading to death, through mechanisms thus far poorly understood.

6.5.1 Diagnostic and prognostic aspects

The results in studies I-III all point to the strong effect of the CRS on prognosis. Kidney function is one of the most powerful predictors of death in AHF, both in-hospital and after discharge during follow-up. The prevalence of impaired renal function on admission for AHF is very high, and far higher than would be expected from epidemiologic studies in chronic heart failure. There are two very different potential explanations to this finding. First, one could argue that renal insufficiency causes, or at least predisposes to the development of AHF or acute decompensation of chronic heart failure. Could this actually be regarded as a manifestation of CRS 4? The second scenario is that decompensation and worsening heart failure negatively affect kidney function already before hospitalization, which fits the definition of CRS 1. A rise in markers of renal function after admission

could be a simple continuation of AKI occurring even before presentation, which in turn could serve as a possible cause for the high prevalence of renal dysfunction on admission. Without knowledge of a recently measured pre-hospital value for comparison, it is very difficult to assess whether an elevated level on admission is a chronic state or caused by a rapid change in renal function in the days preceding hospitalization. Markers of tubular injury would be expected to be elevated in the latter case, but elevated levels of tubular markers have also been found in stable chronic heart failure patients without signs of AKI, i.e. rapid change in GFR (Damman et al. 2008, Damman et al. 2010).

Diagnosis of the CRS in patients with cardiac disease is very much depending on accurate assessment of renal function. In the outpatient setting, creatinine-based estimation of GFR is useful, but as different equations yield slightly different results, use of the same estimation method should be advocated (Smilde et al. 2006, Levey et al. 2006). A decline in kidney function over time is associated with increased mortality in the elderly, and heart failure is an independent predictor of rapid decline in kidney function (Rifkin et al. 2008, Shlipak et al. 2009b). Even so, creatinine-based estimations may underestimate or not be able to detect decline in kidney function, and the prevalence of CKD may still be underestimated in populations with CVD (Tang et al. 2008, Shlipak et al. 2009a). In acute situations with rapid change in renal function, estimation equations are not valid, and creatinine is a rather slow marker. CysC is a marker that seems more suitable for monitoring of GFR both in the acute and chronic setting.

6.5.2 Inflammation in the CRS

Inflammation has been studied in both heart failure and CKD. Despite high prevalence of concomitant disease of both organs in these patients, a potential effect of the heart-kidney interaction on the inflammatory markers has been mostly neglected by investigators. Studies in heart failure have been mainly in the chronic phase, and levels of several inflammatory markers have been found to be elevated (Dunlay et al. 2008, Rauchhaus et al. 2000, Deswal et al. 2001, Aukrust et al. 1999). Only a few small studies have tried to explore inflammatory biomarkers in AHF (Suzuki et al. 2005, Parissis et al. 1999, Milo et al. 2003, Parissis et al. 2009, Peschel et al. 2003, Vanderheyden et al. 1998, Milani et al. 1996).

The study on inflammation (IV) focused on two central pro-inflammatory cytokines (IL-6 and TNF-α) and an anti-inflammatory cytokine (IL-10). Suzuki et al (2005) had previously showed that IL-6 correlated with pulmonary capillary occlusion pressures in AHF, and IL-6 levels changed dynamically during hospitalization and correlated with changes in occlusion pressure. In the same study, TNF-α was elevated in AHF compared to controls, but levels remained stable throughout the AHF phase, and did not correlate with any hemodynamic or echocardiographic parameter (Suzuki et al. 2005). This confirmed previous observations that TNF-α levels remain mainly stable during an episode of AHF.(Parissis et al. 1999, Vanderheyden et al. 1998, Milani et al. 1996)

Investigators within the field of nephrology have repeatedly found elevated levels of inflammatory markers, including TNF-α, in CKD (Stenvinkel et al. 2005, Bolton et al.

2001, Shlipak et al. 2003, Cachofeiro et al. 2008, Landray et al. 2004). Again, most of these studies have been small and often performed in patients with advanced kidney disease. Nevertheless, there is consistent data showing that TNF-α levels increase with severity of renal failure and there is a significant correlation between kidney function and TNF-α (Bolton et al. 2001, Cachofeiro et al. 2008, Descamps-Latscha et al. 1995). Considering the high prevalence of renal dysfunction in cardiac patients, it is therefore surprising that its possible influence on TNF-α has been disregarded in studies investigating inflammatory cytokines in heart failure. Only one more recent study in chronic heart failure reported that levels of TNF-α increased with declining $CrCl_{C\text{-}G}$ (Dunlay et al. 2008).

IL-6 has mainly been studied in end-stage renal disease and dialysis patients, where elevated levels have been associated with increased mortality, notably cardiovascular death (Bologa et al. 1998, Pecoits-Filho et al. 2002). The association of IL-6 with kidney function and cardiac stress seems to be more complex, and it is probable that both renal insufficiency and heart failure contribute separately to elevated IL-6 levels. Whether markers of inflammation predict development of renal insufficiency was evaluated in a cohort with 4128 subjects, mean age 72 years, and average estimated GFR was 79 ml/min. Among several inflammatory markers measured at baseline, albumin, but not IL-6, was associated with a rapid decline in kidney function (Keller et al. 2010). However, these were ambulatory elderly, mainly without prevalent CVD. Whether IL-6 can predict CKD in patients with manifestation of CVD, e.g. heart failure patients, is not known.

The results from study IV indicate that levels of TNF-α in AHF are significantly associated with kidney function, and actually no relationship was found between NT-proBNP and TNF-α. Putting the available data from previous studies in CKD and heart failure together, this seems to be a reasonable finding. Similar correlations between CysC, IL-6 and TNF-α described in the current study have been reported in a cohort with a very low prevalence of heart failure (Keller et al. 2007).

Even though inflammation is thought to be part of the pathogenesis of heart failure, not much is known about the effect of inflammatory markers on outcome in AHF.(Chen et al. 2008) In the FINN-AKVA study, CRP above median was an independent risk factor for one year mortality (I). This finding is confirmatory to one other cohort in AHF (Mueller et al. 2006). CRP levels in AHF are usually elevated with an average or median above 10 mg/L (Mueller et al. 2006, Milo et al. 2003). The inflammatory cytokines were only moderate discriminators of survival and not very strong predictors of one year mortality (IV). Multivariable regression analysis suggested that IL-6 was independently associated with mortality while the effect of TNF-α on prognosis was weaker. Inflammatory markers could play a role in risk stratification in specific subpopulations. Better understanding of the interplay between inflammation and the CRS may help to identify areas of clinical utility for inflammatory cytokines in management or prognostication.

6.6 Future directions

The area of biomarkers has been rapidly expanding and will most certainly continue to do so. The use of new methods enables screening of a large number of biological factors in various populations in the search for disease specific markers. The evaluation of the clinical utility and benefit of these biomarkers in diagnosis, management and prognostication will pose a serious challenge for both the scientific and clinical community. In particular, assessing any incremental value of a putative diagnostic or prognostic marker compared to current standards will be essential. This will require representative study populations of adequate size, correct methods for defining useful cut-offs of biomarker levels and choice of clinically important endpoints. Finally, it is mandatory to use appropriate statistical instruments which incorporate the existing clinical knowledge and assess the incremental benefit of any specific or perhaps even multiple biomarkers.

Concerning renal function, accurate tools for assessing both kidney function (GFR) and injury, with the possibility to monitor patients at risk of AKI, are needed. Focus has been turning towards markers of tubular injury, and the emergence of clinically useful new biomarkers seems imminent. Diagnosis of injury will however necessitate reliable monitoring of kidney function as well. Adopting novel and better renal markers, both injury and functional, into clinical practice is to be expected and long-waited for. CysC has been regarded a promising novel marker of renal function for long, but only recently has the data from different clinical populations and settings been accumulating. Although not all studies find clear benefit with the use of CysC, there is evidence for a potential improvement in diagnostic and prognostic performance.

Adopting CysC into clinical practice has been rather slow due to different concerns about costs and differences in laboratory methods. CysC is not more expensive than troponin (TnT), the use of which is highly established. Moreover, the cost of CysC is far less than NT-proBNP, a biomarker recommended for diagnostic purposes by the guidelines, but for which the use in prognostication or guiding treatment is less clear. Equations for transforming CysC values to GFR estimates have been developed, but not widely validated. Different assays giving slightly different reference values, makes comparison of studies and use of different equations very difficult (Hossain et al. 2009). The International Federation for Clinical Chemistry and Laboratory Medicine published the first certified reference material for standardization of CysC laboratory platforms in the second half of 2010 (Grubb et al. 2010). Hopefully, this will help to calibrate different CysC assays, validate the GFR equations and subsequently improve the availability and interpretation of CysC values.

In the area of AHF, improved risk stratification of patients is required. Data from the Framingham study enabled the development of a risk prediction model for CAD and is used to make treatment decisions. Risk scoring systems for AHF have been suggested for in-hospital and short term mortality based on some larger studies and registries (Lee et al. 2003, Abraham et al. 2008, O'Connor et al. 2008, O'Connor et al. 2010, Fonarow 2008a). Still, in AHF the risk score is not used primarily to select treatment, but may be used to identify patients at high risk of adverse outcome and patients needing more intense

monitoring and follow-up after hospital discharge. Whether these more intense efforts are transformed into improved prognosis remains to be proven.

Since many factors of poor prognosis in AHF are constitutive, prevention will be a primary target for improving prognosis. Hospitalization for AHF causes a dramatic shift downward in the survival curve and consequently efforts to prevent deterioration to AHF could improve outcomes. New devices for hemodynamic surveillance with the possibility of early intervention have been tested in HF populations, but in small cohorts with well defined inclusion criteria (Vanderheyden et al. 2010, Bourge et al. 2008). Similarly, the use of biomarkers for monitoring patients and preventing hospitalization is only in the beginning. More research to identify modifiable factors is needed. Whether novel biomarkers of cardiac or hemodynamic stress, renal, endothelial or vascular damage and inflammatory markers can provide insight in the pathophysiology of the different clinical scenarios and even become therapeutic targets in AHF is yet to be seen.

Finally, the interaction between different organ systems in the complex syndrome of AHF needs to be better understood. The CRS is one example of such an interaction. Interest towards the influence of the liver and endocrine systems is also emerging (van Deursen et al. 2010, Jankowska et al. 2006, Jankowska et al. 2010, Anker et al. 2009). With the high prevalence of renal dysfunction in AHF, it seems reasonable to consider whether impairment of kidney function is a risk *marker* or a risk *factor* for HF hospitalization.

7. Conclusions

The AHF population consists of mainly elderly subjects with multiple cardiovascular and non-cardiovascular co-morbidities. The clinical picture on admission is heterogeneous, ranging from AHF with pulmonary oedema or precipitated by a hypertensive burst, through milder decompensated AHF, to severe cardiogenic shock in a minority of cases. Overall, the mortality is high in AHF, but varies according to clinical presentation. The diversity in clinical picture and the fact that half of the patients hospitalized for AHF have HFPEF has brought about the need for more individualized assessment and therapeutic strategies.

Risk stratification and prediction of prognosis is part of the management approach of patients with AHF. The FINN-AKVA study identified some easily available factors as risk markers of mortality in AHF. As previously reported and subsequently confirmed in many other cohorts as well, renal function and SBP were among these important predictors of prognosis.

CysC is a novel marker that can be used as a measure of renal function. CysC identifies forty percent of patients hospitalized for AHF as having impaired kidney function. CysC was found to be a strong and independent predictor of mortality in AHF. As a sensitive marker of a decrease in kidney function, CysC detects also patients at high risk of mortality not identified by creatinine. The results in this study imply that CysC is useful for risk stratification of patients with AHF.

A decline in renal function after hospital admission has repeatedly been reported in many patients with AHF. In the FINN-AKVA population, a rise in CysC within 48 hours from admission is observed in a considerable proportion of the patients, with at least a similar incidence as a rise in creatinine. AKI_{CysC}, with a rise by 0.3 mg/L as the cut-off identified in this study, is associated with prolonged hospital stay and increased mortality. The observation suggests that CysC can be used as an alternative to creatinine for detection of early AKI, and that even small increases in CysC have unfavourable effects on, mainly short-term, prognosis.

Inflammatory cytokines like IL-6 and TNF-α showed dissimilar patterns of association to levels of CysC and NT-proBNP. Inflammation is clearly activated in the CRS, but inflammatory cytokines seem to relate differently to cardiac stress and kidney function, the two components of the CRS. In particular, the correlation between TNF-α and renal function in patients with heart failure needs further consideration. Nevertheless, patients with severe dual organ dysfunction also presented with the highest levels of inflammatory markers.

CysC possesses several interesting properties making it a prospective marker of renal function in clinical practice. CysC is useful for diagnosis of both kidney dysfunction and AKI in patients with AHF. As a marker of renal function, CysC has shown to be of value for prognostication in AHF.

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