MARKERS OF INFLAMMATION AND CARDIOVASCULAR DISEASE IN DIALYSIS PATIENTS: VARIABILITY AND PROGNOSTIC VALUE

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Stockholm 2016
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THESIS FOR DOCTORAL DEGREE (Ph.D.)

which, by due permission of Karolinska Institutet, will be publicly defended in the English language at R64 lecture hall, Karolinska University Hospital, Huddinge on Friday 13th of May 2016 at 9.00 am

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ABSTRACT

Patients with end-stage renal disease are burdened with a high cardiovascular morbidity and mortality. Inflammation is a common feature in these patients and strongly associates with cardiovascular complications and outcome. Variability in markers of inflammation is known to a certain degree but associated factors and the difference between dialysis modalities have been scarcely studied. N-terminal pro-brain natriuretic peptide (NT-proBNP) is a predictor of cardiac events and death in the general population and in chronic kidney disease. It is known to be greatly elevated in dialysis patients but little is known about variation over time within individuals, the difference between individuals and the prognostic effect of NT-proBNP variation. Similarly, data on clinical factors relating to increases in NT-proBNP in end-stage renal disease is restricted. High-sensitivity cardiac troponin I (hs-cTnI) and T (hs-cTnT) are elevated in dialysis patients even without signs of cardiac ischemia. Cardiac troponins have been shown to predict prognosis but very little is known about variability of hs-cTnI and T in dialysis patients. Our aim was to characterize short-term variability of high-sensitivity CRP (hs-CRP), interleukin-6 (IL-6), NT-proBNP, hs-cTnI and hs-cTnT and to explore factors associated with variability of these markers in prevalent dialysis patients. Another aim was to evaluate the prognostic value of serial measurements of hs-CRP, NT-proBNP, hs-cTnI and hs-cTnT.

In paper I, weekly hs-CRP was measured in 228 prevalent hemodialysis (HD) patients during three months. Results showed large intra- and interindividual variation in hs-CRP and associations with 7 out of 12 clinical symptoms/events, comorbidity, male sex and age. hs-CRP at baseline correlated to the time averaged hs-CRP (Spearman's rho 0.76) and the individual median hs-CRP (Spearman's rho 0.78) both p<0.001. Hazards ratios for all-cause mortality were significant for age, comorbidity, dialysis vintage and serial hs-CRP values. Median hs-CRP associated with a higher hazard ratio for death than baseline hs-CRP, maximum hs-CRP, minimum hs-CRP and average hs-CRP. In paper II, repeated values of IL-6 (monthly) and hs-CRP (weekly) were measured in 228 prevalent HD (same as in paper I) and 80 prevalent peritoneal dialysis (PD) patients. IL-6 was higher in HD than PD patients; median IL-6 8.3 (IQR, 5.3-14.5) vs. 6.7 (IQR, 4.2-10.0) pg/ml; and median hs-CRP 6.1 (IQR, 2.5-14.0) vs. 5.4 (IQR, 1.6-9.0) mg/l; p<0.001 for both. Clinical events, age, male sex, protein-energy wasting (PEW) and HD predicted increased hs-CRP and IL-6 variability. Increased comorbidity predicted IL-6, but not hs-CRP, variability. In paper III, monthly measurements of NT-proBNP were obtained from 211 prevalent HD patients (same cohort as in paper I and II). Inflammation, age, PEW and comorbidity were predictors of NT-proBNP variability. Patients with constantly high NT-proBNP (above 18443) during three months had a significantly increased risk for death adjusting for age, sex, vintage and comorbidity but not when also adjusting for PEW. In paper IV, hs-cTnI and hs-cTnT monthly measurements were obtained from 198 prevalent HD and 78 PD patients (same cohorts as in paper II). The levels of troponins were similar in HD and PD patients; median (IQR) hs-cTnI; 25ng/L (14–43) vs. 21ng/L (11–37), hs-cTnT; 70ng/L (44–129) vs. 67ng/L (43–123). 42% of hs-cTnI and 98% of hs-cTnT measurements were above the decision level for myocardial infarction. Variability of troponins was associated with age, male sex, PEW and congestive heart failure but not ischemic heart disease or dialysis modality. Constantly high hs-cTnT levels (above 108 ng/L) predicted death (HR 2.09 95% CI 1.03-4.26) after adjusting for confounders, whereas hs-cTnI levels did not.

Levels of IL-6 and hs-CRP are increased and have a high variability in dialysis patients, more so in HD than PD. Comorbidity, PEW and acute clinical events are strongly related to inflammatory activity in dialysis patients. In spite of the variability, inflammation as assessed by hs-CRP is a strong predictor of mortality in HD patients. Serial measurements provide additional information compared to single measurements. Longitudinal changes of NT-proBNP are associated with inflammation, PEW, age and comorbidity. Repeatedly high NT-proBNP levels predict mortality. Dialysis patients without signs of ischemic cardiac events have elevated hs-cTnI and hs-cTnT. The large intra-individual differences in cardiac troponins support the use of reference change levels when assessing a patient with a possible acute cardiac event. Constantly high levels only of hs-cTnT and not hs-cTnI predicted risk of death.
LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers. They are referred to in the text by their Roman numerals:


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LIST OF ABBREVIATIONS

ACE: Angiotensin-converting enzyme
ADMA: Asymmetric dimethylarginine
ARB: Angiotensin II-receptor blockers
BMI: Body mass index
BNP: Brain natriuretic peptide
CHF: Congestive heart failure
CI: Confidence interval
CKD: Chronic kidney disease
CRP: C-reactive protein
CV: Coefficient of variation
CVD: Cardiovascular disease
DM: Diabetes mellitus
EF: Ejection fraction
eGFR: Estimated glomerular filtration rate
ESA: Erythropoiesis-stimulating agent
ESRD: End-stage renal disease
HD: Hemodialysis
HIV: Human immunodeficiency virus
HR: Hazard ratio
hs-CRP: High-sensitivity C-reactive protein
hs-cTnI: High-sensitivity cardiac Troponin I
hs-cTnT: High-sensitivity cardiac Troponin T
ICAM-1: Intercellular Adhesion Molecule 1
ICC: Intraclass correlation
IHD: Ischemic heart disease
IL: Interleukin
IQR: Interquartile range
ISRN: International Society of Renal Nutrition and Metabolism
IU: International units
LVD: Left ventricular dysfunction
LVMI: Left ventricular mass index
MI: Myocardial infarction
MIA: Malnutrition inflammation atherosclerosis
MIMICK: Mapping of Inflammatory Markers in Chronic Kidney disease
MRSA: Methicillin-resistant Staphylococcus aureus
NSTEMI: Non ST-segment elevation myocardial infarction
NT-proBNP: N-terminal pro-brain natriuretic peptide
PD: Peritoneal dialysis
PEW: Protein-energy wasting
PTX3: Pentraxin 3
PVD: Peripheral vascular disease
RCV: Reference change value
ROC: Receiver-operating characteristic
RRT: Renal replacement therapy
SD: Standard deviation
SE: Standard error
SGA: Subjective Global Assessment
TNF: Tumor necrosis factor
VCAM-1: Vascular cell adhesion molecule 1
1. INTRODUCTION

1.1 CHRONIC KIDNEY DISEASE

Chronic kidney disease (CKD) is a global health problem with a continually increasing prevalence.\(^1,2\) CKD is defined as an abnormality of kidney structure or function, present for more than three months, with implications for health. Based on estimated glomerular filtration rate (eGFR), CKD is divided into five stages. Stage 1 (eGFR ≥ 90 mL/min/1.73 m\(^2\), normal or high with signs of kidney damage), stage 2 (eGFR 60-89 mL/min/1.73 m\(^2\)), stage 3 (eGFR 30-59 mL/min/1.73 m\(^2\)), stage 4 (eGFR 15-29 mL/min/1.73 m\(^2\)), and CKD 5 also called end-stage renal disease (ESRD) (eGFR <15mL/min/1.73 m\(^2\) or need for dialysis).\(^3\)

1.2 EPIDEMIOLOGY

The current prevalence of ESRD patients on renal replacement therapy (RRT) in Sweden 2014 was 946 per million population (9,220 patients) and the corresponding incidence was 116 per million population. They divide into 3,859 (42%) patients on dialysis and 5,361 (58%) with a functioning kidney transplant. The most common diagnoses in prevalent ESRD patients are glomerulonephritis (and has been since the start of renal registries in Sweden in 1991) 25%, followed by diabetic nephropathy 18%, polycystic kidney disease 10%, nephrosclerosis 9%, and pyelonephritis 4%. Various or unknown causes constitute the remaining 34%.\(^2\) Many patients with CKD will never reach ESRD, either because of stable renal function or death from other causes prior to ESRD.\(^4\)

1.3 RENAL REPLACEMENT THERAPY

Patients with ESRD require RRT with either kidney transplantation or dialysis. For many patients the future entails both. This thesis focuses on patients with CKD 5 (ESRD) receiving life-sustaining treatment with dialysis. The two main methods of conducting dialysis, hemodialysis (HD) and peritoneal dialysis (PD), vary in patient availability for both medical and logistic reasons. In Sweden 79% of dialysis patients are on HD and 21% on PD.\(^2\)
### 1.4 THE UREMIC MILIEU

Uremia (“urine in the blood”) or the uremic syndrome develops with progressive loss of renal function and in the final stage manifestations from several body systems occur simultaneously. Reduced renal function leads to an increase in various detrimental agents as well as a decrease in protective elements, together leading to a toxic internal milieu that predisposes to complications such as vascular disease, protein-energy wasting, osteoporosis, premature aging, and frailty.

Vascular disease is mediated through among other things endothelial dysfunction, which in ESRD is promoted in part through increased asymmetric dimethylarginine (ADMA) (further detail in chapter 1.5.4). Endothelial dysfunction is also promoted by oxidative stress which is closely related to inflammation. Markers of oxidative stress are prominent in ESRD and dialysis is ineffective in correcting this. Hyperphosphatemia, inflammation, bone protein elevations, and low fetuin-A in ESRD all facilitate vascular calcification and vascular stiffness. Additionally, hypertension and ventricular hypertrophy are promoted in ESRD through salt and fluid retention. Inflammation, in addition to promoting vascular disease, is a risk factor for protein-energy wasting (PEW) in uremia (Figure 1). Other metabolic disturbances, such as insulin resistance (with impaired anabolic effect of insulin) and abdominal fat distribution relate to PEW in ESRD.

![Figure 1. Uremia and risk factors for cardiovascular disease and protein-energy wasting](image-url)
1.5  DISORDERS IN DIALYSIS

Dialysis itself has some detrimental side effects. The dialysis procedure adds to PEW, partly because it induces further loss of residual renal function. There is also loss of nutrients and proteins into dialysate. A dialysis related hypermetabolism and inflammation also contribute.\textsuperscript{13,17,18} Central dialysis catheters are a major cause of inflammation and infection.\textsuperscript{19} ESRD and dialysis are related to depression, fatigue and loss of appetite, which are contributing factors to PEW.\textsuperscript{20,21} Many other factors, such as intradialytic hypotension, affect the myocardium and outcome.\textsuperscript{22} Finally, recent data show that prolonged intradialytic hypoxemia associates with inflammation, higher erythropoietin requirements, and higher all-cause hospitalization and mortality.\textsuperscript{23}

1.6  MORBIDITY AND MORTALITY IN ESRD

Mortality risk increases inversely with decreasing renal function.\textsuperscript{24,25} In the 1970s Lindner et al. first described the excessive cardiovascular morbidity and mortality in patients on maintenance HD.\textsuperscript{26} Cardiovascular mortality is up to 30 times higher than in the general population.\textsuperscript{27} Patients on dialysis have an annual mortality rate of about 20\% compared to 2.4\% in patients with a kidney transplant.\textsuperscript{2} Mortality rates for dialysis patients differ between countries. This is in part explained by a difference in proportion of ESRD patients receiving kidney transplants. Cardiovascular diseases are the leading cause of mortality in European dialysis patients, explaining 40\% of all deaths. Infectious causes account for around 15\% of all deaths.\textsuperscript{28} The mortality rates are similar in HD and PD patients taking into account the difficulty in direct comparison between these groups.\textsuperscript{29} Data on causes of mortality in Sweden are shown in Figure 2. The two-year mortality for dialysis patients after myocardial infarction (MI) is roughly 70\% and has not changed much during the last decades.\textsuperscript{30}
1.6.1 Morbidity in ESRD

The risk of cardiovascular disease increases gradually with decreasing kidney function.\textsuperscript{31} According to the dialysis outcome and practice patterns study data, the prevalence of comorbid conditions in European dialysis patients was high and 29% had coronary artery disease, 25% congestive heart failure (CHF), 14% cerebrovascular disease, 23% peripheral vascular disease (PVD), and 20% diabetes mellitus (DM).\textsuperscript{32} Others have found a 40% prevalence of both ischemic heart disease (IHD) and CHF.\textsuperscript{33} The burden of comorbidity is strongly and quantitatively related to inferior outcome in dialysis patients.\textsuperscript{34,35}

1.6.2 Protein-energy wasting in ESRD

An important comorbidity in ESRD is PEW, defined as loss of body protein mass and fuel reserves. According to a proposal by the International Society of Renal Nutrition and Metabolism (ISRNM), the optimal diagnosing of PEW is based on four factors: biochemical criteria, low body weight/reduced total body fat/weight loss, decrease in muscle mass, and low protein or energy intake.\textsuperscript{36}

The prevalence of PEW in maintenance dialysis patients is 30-50%.\textsuperscript{37-39} Some effort has been made in recent years to engage a routine assessment of nutritional status into
dialysis practice. This is important since PEW is a prognostic factor for death.\textsuperscript{37,40,41} Increased awareness as to causes and possible ways of interfering with the development of PEW resulted in a consensus statement by the ISRNM regarding several possible means of prevention and treatment such as preservation of muscle mass, nutritional interventions and treatment of systemic inflammation.\textsuperscript{42}

![Figure 3. A conceptual model for etiology of protein-energy wasting in chronic kidney disease, and clinical implications\textsuperscript{13}](image)

Causes of PEW in CKD are diverse (Figure 3). Decreased protein and energy intake is common and caused by for example dietary restrictions, poor appetite, nausea, and depression. Other underlying factors in ESRD are decreased physical activity, comorbidities that lead to PEW, decreased anabolism, increased resting energy expenditure, increased glucocorticoid activity, and metabolic acidosis.\textsuperscript{13} Inflammation is in itself a participant in many pathways leading to PEW. It is associated with loss of appetite,\textsuperscript{43,44} depression,\textsuperscript{45} increased resting energy expenditure and oxidative stress,\textsuperscript{46,47} resistance of skeletal muscle to insulin, muscle atrophy,\textsuperscript{15,41} as well as reduced albumin concentration.\textsuperscript{44,48-51} Since treatment of muscle wasting is difficult and seldom successful in this commonly old, frail, sedentary and exercise-hesitant patient group, novel treatment strategies are needed.\textsuperscript{40}
1.6.3 **Inflammation in ESRD**

The presence of inflammation is associated with all-cause and cardiovascular death in the general population.\(^{52,53}\) The phenotype of CKD is often accompanied by systemic inflammation which promote progression of CKD, premature ageing\(^ {54}\) and CVD\(^ {55,56}\) In the late 1990s C-reactive protein (CRP) was acknowledged a powerful predictor of cardiovascular death and overall mortality in HD\(^ {12,57}\) and PD patients.\(^ {58}\) In 1998 interleukin-6 (IL-6) was also shown to be a strong predictor of mortality in HD patients.\(^ {59}\) IL-6 and CRP are measurable markers of inflammation. IL-6 is a cytokine produced by various cells; T-cells, mast cells, macrophages, endothelial cells and many more.\(^ {60}\) CRP, an acute-phase protein of the pentraxin family, is produced by hepatocytes largely by regulation of IL-6.\(^ {61}\)

The inflammatory process in general has various origins; in the vasculature, at sites of infection, in remodeling of organs after necrosis, in chronic wounds, and foreign material, many of these apply in dialysis. Various clinical events are common causes of inflammation, such as periodontal disease, bioincompatible dialysis membranes, unpure dialysate, and vascular access.\(^ {62-66}\) Bioincompatible dialysis membranes were more common with earlier membranes, especially cuprophan membranes, which induced inflammation.\(^ {67}\) Synthetic membranes, used today, are far more biocompatible.\(^ {68}\) In PD there is bioincompatibility of dialysate related to low pH and glucose degradation products.\(^ {69}\) The use of bicarbonate as a buffer, with a higher pH of dialysate, has been important in increasing biocompatibility in PD.\(^ {70}\) Intraperitoneal inflammation does not necessarily translate to systemic inflammation although data have shown a moderate relation between IL-6 in plasma and dialysis effluent.\(^ {71}\) In ESRD there is also retention of cytokines\(^ {72}\) and sympathetic overactivity, potentially leading to increased inflammation.\(^ {73}\) Inflammation (measured as high-sensitivity (hs)-CRP) has also been linked to overhydration in PD patients\(^ {74}\) and prolonged intradialytic hypoxemia.\(^ {23}\) Another possible contributor to inflammation in ESRD is the altered composition of the gut microbiome and disruption of the intestinal barrier seen in uremia.\(^ {75,76}\)

In addition to causal involvement in PEW, inflammation plays a central role in the development of CVD.\(^ {77}\) The formation of atherosclerosis is partly caused by the adhesion of leukocytes to the vascular endothelium which is mediated by cellular adhesion molecules (ICAM-1, VCAM-1, E-selectin) expressed on the surface of
endothelial cells in response to various pro-inflammatory cytokines. Adhesion molecules are known to be elevated in patients on dialysis. Fibrinogen and lipoprotein(a) may also contribute to atherosclerotic plaque growth, both of which are elevated and related to inflammation in CKD. It has been proposed that CRP in itself promotes the formation of atherosclerotic lesions through binding with modified LDL activating the complement system and causing foam cell formation.

The observation that elevated body mass index (BMI) confers a survival advantage to ESRD patients was first reported in 1999 and subsequently confirmed in most, but not all, studies based on European, North-American, and Asian dialysis cohorts. At the same time increased BMI and fat body mass are positively related to IL-6 and CRP in dialysis patients. The distribution of fat is an important factor to consider; abdominal fat deposition is positively linked to inflammation (IL-6) and PEW in HD patients resulting in an increased mortality risk. Recent findings show the advantageous effect of high BMI only in the presence of inflammation since it is mitigated in non-inflamed dialysis patients.

Inflammation is related to left ventricular systolic dysfunction and CHF in non-renal patients as well as left ventricular hypertrophy in PD patients. The pathophysiology of how inflammation affects the heart is not well understood. A study on animal models induced left ventricular dysfunction (LVD) by infusing tumor necrosis factor (TNF) and then reversed the condition with TNF receptor antagonists implicating a direct cardiotoxic effect of TNF. Inflammatory mediators have been proposed to affect cardiomyocytes and fibroblasts leading to hypertrophy and fibrosis and induce apoptosis and myocardial remodeling.

Of the large number of pro-inflammatory cytokines, IL-6 is one of the most studied. It has an important role as a pro-inflammatory cytokine activating B-cells, a mediator of the acute-phase response, and a possible role in the pathogenesis of atherosclerosis. Recent studies of haplotypes have shown associations of a functional IL-6 polymorphism with cardiovascular events in CKD patients but not with a CRP haplotype. Although it is still not settled whether CRP is simply a downstream product of ongoing inflammation or poses a role in the pathogenesis of atherosclerosis available evidence suggests that it is not a causal risk factor for CVD. IL-6 has been shown to be a more reliable predictor of mortality in ESRD than hs-CRP and even albumin. A recent study of 543 CKD stage 5 patients investigating 12
Biomarkers confirmed the robustness of IL-6 as a classifier of clinically overt CVD and a predictor of all-cause mortality.\textsuperscript{103} CRP assays are far more available than the more expensive IL-6 assay and given the strong correlation between hs-CRP and IL-6, analysis of hs-CRP may suffice in the clinical situation.\textsuperscript{104} Some mechanisms in which pro-and anti-inflammatory cytokines are involved in uremia are shown in Figure 4.

Pentraxin 3 (PTX3), as well as CRP, belongs to the pentraxin family of proteins and is an acute phase reactant involved in pathogen recognition, complement activation and regulation. PTX3 is elevated in dialysis patients compared to healthy controls and reflects endothelial dysfunction. It is associated with cardiovascular disease and mortality risk.\textsuperscript{105,106}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Potential mechanisms by which pro- and anti-inflammatory cytokines may promote accelerated atherosclerosis, other uremic complications, and protein-energy wasting.\textsuperscript{72}}
\end{figure}

1.6.4 \textbf{Cardiovascular disease in ESRD}

The cardiovascular risk in CKD cannot solely be explained by traditional risk factors such as hypertension, dyslipidemia, smoking, DM or obesity.\textsuperscript{107} The uremic phenotype entails a wide variety of metabolic disturbances known to increase cardiovascular risk.
such as oxidative stress and inflammation, endothelial dysfunction, and vascular calcification to name a few.\textsuperscript{108,109} Arteriosclerosis due to both intimal disease and medial calcification is a common finding in CKD affecting small and large arteries.\textsuperscript{110} Endothelial dysfunction is an important and early contributor to atherosclerosis.\textsuperscript{9} ADMA is linked to endothelial dysfunction through its inhibition of nitric oxide production and its effect amplified in the presence of inflammation.\textsuperscript{111,112} ADMA is cleared by the kidneys and metabolised by an enzyme partly generated in the kidneys. This explains the three-fold increase in ADMA seen in ESRD patients.\textsuperscript{5} DNA methylation, a mechanism for regulation of gene expression has a potential role in atherosclerosis\textsuperscript{113} and inflammation may cause aberrant DNA methylation.\textsuperscript{114} In addition, hypermethylation has been associated with inflammation and increased mortality in CKD.\textsuperscript{115}

The relation between inflammation and medial calcification is less clear than with atherosclerosis. Cardiovascular ossification is common in CKD patients.\textsuperscript{116} A possible mechanism is through fetuin-A, an inhibitor of extra-skeletal calcification, which like serum albumin acts as a negative acute phase reactant.\textsuperscript{117} Fetuin-A is often decreased in the inflamed uremic milieu and has a positive relation to survival in HD and PD patients,\textsuperscript{118,119} while osteoprotegerin, associated with progression of coronary artery calcification and mortality, is increased in ESRD.\textsuperscript{9} In medial calcification a differentiation from smooth muscle cells to osteoblast-like cells takes place.\textsuperscript{120,121} Consequently a bone-like matrix production and mineralization happens in the presence of enough calcium and phosphorus.\textsuperscript{122} Vascular calcification and inflammation are interrelated in CKD, perhaps in part mediated via phosphate retention and/or bone disease.\textsuperscript{123}

The interrelationship of inflammation, malnutrition, and atherosclerosis in CKD was first described in 1999\textsuperscript{55,124} based on the observation that elevated CRP (>20mg/L) was more common in malnourished HD patients and that inflamed and malnourished incident-dialysis patients more often had carotid plaques.\textsuperscript{55,125} The coexistence of malnutrition, inflammation, and atherosclerosis (MIA) in dialysis patients (Figure 5) has been related to vascular calcification, fluid overload, non-alcoholic fatty liver disease, high peritoneal transport, gut dysbiosis, depression, and outcome during dialysis or after renal transplantation.\textsuperscript{126-133} The fact that conventional cardiovascular risk factors such as overweight, hypercholesterolemia and hypertension associate with greater survival in dialysis patients is thought to be partly explained by the presence of the MIA
syndrome. This is sometimes referred to as reversed epidemiology in ESRD.\textsuperscript{134} Although evidence is still lacking for a causative role of inflammation in causing PEW in ESRD there is interest in the possible pathways in CKD leading to both PEW and CVD and hence possible interventions of these conditions.\textsuperscript{111,135-137}

\textbf{Figure 5}. The presence of protein-energy wasting, inflammation and cardiovascular diseases, and combinations, in a cohort of 815 end-stage renal disease patients at three months after the start of chronic dialysis treatment\textsuperscript{138}

1.6.5 \textbf{Left ventricular dysfunction in ESRD}

CHF is 10-30 times more prevalent in patients with CKD stages 4 and 5 compared to the non-renal population.\textsuperscript{1} LVD predicts CHF.\textsuperscript{139} The presence of CHF predicts an exceedingly high mortality rate in dialysis patients.\textsuperscript{140} Because of its high prevalence and severity, current guidelines propose an evaluation of cardiac function with echocardiography at dialysis commencement, after the patient has achieved dry weight, and at three-yearly intervals thereafter.\textsuperscript{141} The causes of LVD are manifold in uremia. High blood pressure may predispose to left ventricular hypertrophy, volume overload to diastolic dysfunction, arteriovenous fistulas may lead to LVD because of high blood flow and increased cardiac output.\textsuperscript{142} IHD and anemia also contribute.\textsuperscript{139}
1.6.6 Diabetes in ESRD

DM is a prominent and increasingly common cause of ESRD. The “behavior” of DM in dialysis patients is intriguing and a state of burnt-out DM with diminished insulin requirements is sometimes described to take place in type 2 DM patients on dialysis. Since insulin sensitivity usually improves with initiation of dialysis treatment uremic toxins are likely to contribute to insulin resistance. Renal gluconeogenesis is reduced with decreased renal function. Since the half-life of insulin is prolonged and PEW and gastroparesis are frequently part of the uremic phenotype, these factors may contribute to a higher risk for hypoglycemia.

1.7 Grading in ESRD

1.7.1 Comorbidity

There are several ways of measuring comorbidity and there is currently no consensus on which one to use in ESRD. A score for use in dialysis patients was presented by Davies et al. which included IHD, LVD, PVD, active malignancy, systemic inflammatory disease, DM and other significant diseases. It has been compared to the Khan index and the Charlson comorbidity index in dialysis patients, all three indexes were deemed equal in adjusting for the potential confounding effect of comorbidity on health status.

1.7.2 Protein-energy wasting

Several approaches have been used through the years to assess nutritional status and PEW. One of them is the Subjective Global Assessment scale (SGA) that has been validated for use in dialysis patients and repeated use of it has been recommended for assessment of nutritional status. SGA is based on six subjective assessments, three come from the patient’s history of weight loss, incidence of anorexia, and vomiting and the other three are based on a clinicians grading of muscle wasting, edema, and loss of subcutaneous fat. Based on this, an SGA score is given and divided to normal nutritional status (1 point), mild malnutrition (2 points), moderate malnutrition (3 points), and severe malnutrition (4 points).
1.7.3 Assessment of inflammation

In recent years CRP has been measured with a high-sensitive method improving the detection of lower levels and the insight that CRP may vary within an individual (both non-renal and renal) without obvious clinical reasons causing interest in what may underlie such variation.\textsuperscript{153,154} In 2003 the American Heart Association recommended hs-CRP measurements for individuals at intermediate risk of cardiovascular disease.\textsuperscript{155} Conventional CRP assays have a detection limit of 3mg/L while the high-sensitivity assays detect levels down to 0.1mg/L. CRP measured with both methods has similar associations with mortality in ESRD.\textsuperscript{156} CRP is measured routinely in dialysis patients in many countries and dialysis units. Regular CRP-measurements have been related to improved survival in HD patients\textsuperscript{157} and are by some seen as part of a good quality practice of dialysis patients.\textsuperscript{158} IL-6 is commonly used in research for assessment of chronic inflammation. A few small studies have investigated IL-6 variability in non-renal individuals and results are conflicting concerning the usefulness of a reference interval for IL-6.\textsuperscript{159,160} Repeated measurements of IL-6 in CKD patients have been found to better predict cardiovascular outcome than single measurements.\textsuperscript{161}

Measuring markers of inflammation on a regular basis captures the dynamic inflammatory response that is prominent in ESRD. Considering the extensive data on the correlation between inflammation and various conditions such as CVD and PEW, studying variability of inflammatory markers and relating it to clinical events in carefully phenotyped patients is a way to increase knowledge of the inflammatory process in ESRD.

1.7.4 Cardiac injury

In response to stretching of the myocardial wall, natriuretic peptides are released from the ventricles. Strain, ischemia, inflammation, and sympathetic overactivity may also promote release of natriuretic peptides\textsuperscript{162} such as pro-brain natriuretic peptide (proBNP) that cleaves into a biologically active BNP and an inactive N-terminal pro brain natriuretic peptide (NT-proBNP). An increase in BNP leads to inhibition of the renin-angiotensin system, natriuresis, and vasodilatation.\textsuperscript{163} NT-proBNP is more commonly used as a result of increased stability \textit{in vitro} and longer half-life compared to BNP.\textsuperscript{164} It is used today as a marker of CHF in non-renal patients, both to rule out an
acute episode of CHF and for treatment evaluation in chronic CHF.\textsuperscript{165,166} Due to increased secretion and decreased renal clearance, NT-proBNP increases when renal function declines, which results in that almost 100\% of ESRD patients have elevated NT-proBNP.\textsuperscript{167-169} Even after correcting for volume overload, NT-proBNP predicts the presence of LVD in HD patients.\textsuperscript{170}

NT-proBNP is a prognostic marker for death in heart failure patients\textsuperscript{171} and for LVD and death in ESRD.\textsuperscript{170,172-174} NT-proBNP may increase during HD with a low flux filter partly due to hemococoncentration but levels remain stable with high-flux dialyzers. NT-proBNP is also elevated with the use of catheters or grafts compared to native fistulas\textsuperscript{175} and is higher in HD than PD patients, probably because of larger fluctuations in volume overload.\textsuperscript{176} NT-proBNP release can be caused by other factors, such as atrial fibrillation in HD patients.\textsuperscript{177} Elevated NT-proBNP levels can predict future cardiovascular events in CKD.\textsuperscript{178}

For detection of cardiac ischemia, troponins are today the most used and sensitive markers, released from myocytes in response to myocardial injury.\textsuperscript{179,180} Troponin I and T together with troponin C form a complex involved in muscle contraction.\textsuperscript{181,182} Their elevation can be caused by factors other than IHD, such as sepsis, CHF, cerebrovascular incidences, pulmonary embolism, gastrointestinal bleeding, and even physical exertion.\textsuperscript{183,184} Troponins are, like natriuretic peptides, elevated in ESRD both due to increased release and reduced clearance.\textsuperscript{185,186} Although this renders the interpretation of troponin elevations difficult in dialysis patients, troponins have been shown to predict cardiovascular events in ESRD patients.\textsuperscript{187,188} Troponin levels may also predict mortality in ESRD (Figure 6) and some effort has been made to define a cut-off value above which patients should be evaluated for risk of cardiac death.\textsuperscript{186,189}

hs-cardiacTnT (hs-cTnT) predicts mortality better than hs-cardiacTnl (hs-cTnl) in heart failure patients\textsuperscript{190} and based on studies on earlier troponin assays TnT is also more predictive of death in ESRD.\textsuperscript{191,192} The HD treatment per se may affect NT-proBNP and troponin levels. Whereas a post-dialysis decrease in both Tnl and TnT has been described,\textsuperscript{193} others found no change in TnT and a decrease in Tnl caused by adherence of Tnl to the dialyzer membrane.\textsuperscript{194} Other factors characteristic for dialysis patients, such as reduced diuresis, fluid overload, systolic dysfunction, time on dialysis, and myocardial stunning may also induce NT-proBNP and troponin release.\textsuperscript{195-197}

With the introduction of a new generation of hs-cTnT assays almost 100\% of the levels in ESRD patients are above the “normal” level or 99\% percentile leading some research
groups to recommend regular measurements of hs-cTnT in dialysis patients\textsuperscript{198} and others to call for further research of variability and prognostic value of hs-cTnT in this population.\textsuperscript{199}

A variability of both NT-proBNP and troponins between and within dialysis patients has been reported. This has led to the conclusion that a relative change strategy is better suited to identify pathological changes of these cardiac markers.\textsuperscript{200,201}

Markers of inflammation (CRP) and cardiac disease (NT-proBNP and cTnT) have been found to be interrelated in dialysis patients pointing to a relation between overhydration, inflammation and cardiac biomarkers.\textsuperscript{202} Others have found the combination of elevated cTnT and CRP to be a strong predictor of mortality in PD patients.\textsuperscript{203}

\textbf{Figure 6.} Kaplan–Meier event-free survival curve according to baseline pre-dialysis serum cardiac Troponin T concentration (1 µg/l = 100 ng/L)\textsuperscript{186}
2. **AIMS**

**Overall aim**

To characterize the variability and prognostic value of indicators of cardiovascular disease and inflammation in ESRD.

To characterize the association of clinical events and comorbidity with the variability of markers of cardiovascular disease and inflammation in ESRD.

**Specific aims**

To study the short-term variability of hs-CRP and IL-6 and association to clinical events and comorbidity in patients on dialysis.

To estimate the short-term variability of NT-proBNP, hs-cTnI, and hs-cTnT and association with comorbidity in patients on dialysis without acute ischemic cardiac events.

To compare cardiovascular disease and inflammation markers in patients on PD versus HD.

To estimate the prognostic value of serial assessments of cardiovascular and inflammation markers in patients on dialysis.
3. METHODS

3.1 STUDY DESIGN AND POPULATION

The work presented in all four papers is part of a prospective, observational cohort study (MIMICK). MIMICK is the acronym for Mapping of Inflammatory Markers In Chronic Kidney disease. The study was implemented in two different time periods, in the first part (MIMICK 1) non-selected, prevalent HD patients from six HD units in the Stockholm-Uppsala region were included. A total of 254 HD patients were eligible to

Figure 7. Flow chart of the hemodialysis cohort
HD, hemodialysis; HIV, human immunodeficiency virus; hs-CRP, high-sensitivity C-reactive protein; hs-cTnl, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; IL-6, interleukin-6; NT-proBNP, N-terminal pro-brain natriuretic peptide
participate in the study (Figure 7). Patients were recruited from October 2003 to September 2004. The second part (MIMICK 2) includes prevalent PD patients from Stockholm recruited from March 2008 through April 2011. A total of 164 patients were invited to participate (Figure 8). Inclusion criteria for participation were more than three months on regular dialysis, age 18 years or older. Exclusion criteria were unwillingness to participate and infections such as human immunodeficiency virus (HIV) and methicillin-resistant Staphylococcus aureus (MRSA). In paper IV an MI during or three months prior to study start led to exclusion of three patients (HD cohort; non ST-segment elevation MI (NSTEMI) just under three months before study start n=1, NSTEMI six days after study start n=1, PD cohort; NSTEMI four days into study n=1).

The Ethics Committee of Karolinska Institutet, Stockholm, Sweden approved the study protocol and written informed consent was obtained from all participants.

The study for biomarker variability lasted for three months during which time the patients answered weekly questionnaires on symptoms that can be related to increased
systemic inflammation (fever, cold/sore throat, cough, otalgia, dyspnea, diarrhea, vomiting, dysuria, arthralgia, rashes, wounds, injuries, and/or antibiotic treatment).

3.2 CLINICAL FACTORS

Data on comorbidity at inclusion was gathered from patients’ medical charts. Comorbidity was defined according to Davies et al. The scoring of comorbidity included seven chronic and active conditions; 1) IHD (e.g. angina pectoris, previous MI) 2) DM (type I or II), 3) LVD (clinical pulmonary oedema, dysfunction according to echocardiography), 4) peripheral (e.g. claudication, amputation, or endovascular procedures) and cerebral vascular disease (e.g. hemorrhagic or ischemic stroke), 5) non-cutaneous, active malignancies, 6) systemic collagen vascular disease (e.g. systemic lupus erythematosus, rheumatic arthritis), and 7) other significant pathology severe enough to affect patient outcome, for example chronic obstructive pulmonary disease, heart valve disease, and chronic infections. Patients were graded as low risk (score 0, no comorbidity), medium risk (1-2 comorbidities), or high risk (3 or more comorbidities). The comorbidity grading was performed by a single clinician (S Snaedal). Forty-three patients (19%) were graded as low risk, 129 patients (56%) medium risk and 56 patients (25%) as high risk in MIMICK 1, whereas 21 patients (26%) as low risk, 46 patients (58%) medium risk and 13 patients (16%) high risk in MIMICK 2.

Nutritional status was evaluated at study start using SGA (described in Chapter 1.3). Patients were given a score from 1 to 4 (1=normal nutritional status, 2=mild malnutrition, 3=moderate malnutrition, and 4=severe malnutrition). PEW was defined as an SGA score of more than one. Research nurses performed SGA on all patients. Muscle strength was estimated by handgrip strength using the Harpenden Dynamometer. The test was performed with both arms on PD patients and the fistula free-arm in HD patients, and was repeated three times and the highest score recorded. Loss of subcutaneous fat was estimated with Harpenden Skinfold Caliper. Skinfold thickness was measured at four sites i.e. at the biceps, triceps, subscapularis, and suprailiaca. Oedema was estimated at the ankles and lower legs.
Table 1 shows clinical characteristics and comorbidities at baseline for both cohorts. The two cohorts of HD and PD dialysis patients did not differ with regard to comorbidity, age, sex or nutritional status. The HD patients had been on dialysis 18 months longer than the PD patients. Serum albumin, NT-proBNP, hs-CRP, and IL-6 were all significantly higher in HD than PD patients (p-value ranging from <0.001 to 0.01).

Table 1. Biomarkers and clinical characteristics at baseline in hemodialysis and peritoneal dialysis patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HD patients (n=228)</th>
<th>PD patients (n=80)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>66 (51–74)</td>
<td>65 (56–77)</td>
<td>0.54</td>
</tr>
<tr>
<td>Sex (male), %</td>
<td>56</td>
<td>68</td>
<td>0.09</td>
</tr>
<tr>
<td>Dialysis vintage, months</td>
<td>29 (14–57)</td>
<td>11 (6–29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoker no/yes, %</td>
<td>80/20</td>
<td>80/20</td>
<td>1.0</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24 (21–27)</td>
<td>25 (23–28)</td>
<td>0.02</td>
</tr>
<tr>
<td>PEW (SGA&gt;1), %</td>
<td>48</td>
<td>40</td>
<td>0.23</td>
</tr>
<tr>
<td>Comorbidity (low/medium/high), %</td>
<td>19/57/24</td>
<td>26/58/16</td>
<td>0.18</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>26</td>
<td>24</td>
<td>0.82</td>
</tr>
<tr>
<td>Peripheral/cerebral vascular disease, %</td>
<td>31</td>
<td>28</td>
<td>0.64</td>
</tr>
<tr>
<td>Ischemic heart disease, %</td>
<td>30</td>
<td>31</td>
<td>0.92</td>
</tr>
<tr>
<td>Left ventricular dysfunction, %</td>
<td>21</td>
<td>16</td>
<td>0.45</td>
</tr>
<tr>
<td>Albumin, g/l</td>
<td>35 (32–38)</td>
<td>32 (28–35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NT-proBNP, pg/ml</td>
<td>9,205 (2,954–26,876)</td>
<td>3,080 (1,188–8,845)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP, mg/l</td>
<td>6.7 (2.5–21.0)</td>
<td>4.6 (1.5–10.6)</td>
<td>0.01</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>8.7 (5.4–15.5)</td>
<td>6.6 (4.0–9.6)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Note: Data expressed as median values (IQR) or %. Abbreviations: BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; IQR, interquartile range; NT-proBNP, N-Terminal pro-brain natriuretic peptide; PEW, protein-energy wasting; SGA, subjective global assessment

All patients on HD were treated using bicarbonate dialysate and synthetic or semisynthetic membranes (polyamide 140 patients, polysulfone 74 patients, cellullosynthetic 12 patients, data missing on two patients). The use of low/high flux membranes was approximately 70/30%. Among PD patients, use of the manually performed continuous ambulatory PD versus the mechanical automated PD was
around 75/25%. Median time on HD treatment was 29 months (range 1 to 378 months) and on PD 11 months (range 2-169 months).

Most patients were on drugs routinely used in ESRD, such as phosphate and potassium binders, diuretics, vitamin B, C, and D supplementation. Some of the drugs used are listed in Table 2. The underlying causes of ESRD are listed in Table 3.

### Table 2. Medication use among hemodialysis and peritoneal dialysis patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HD patients (n=228)</th>
<th>PD patients (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-blockers, %</td>
<td>49</td>
<td>71</td>
</tr>
<tr>
<td>Calcium channel blockers, %</td>
<td>25</td>
<td>33</td>
</tr>
<tr>
<td>ACE/ARB, %</td>
<td>32</td>
<td>56</td>
</tr>
<tr>
<td>Statin, %</td>
<td>32</td>
<td>49</td>
</tr>
<tr>
<td>Acetylsalicylic acid, %</td>
<td>30</td>
<td>41</td>
</tr>
<tr>
<td>ESA, IU</td>
<td>10,000 (6,000-15,000)</td>
<td>5,000 (3,000-8,000)</td>
</tr>
</tbody>
</table>

**Abbreviations:** ACE, angiotensin-converting-enzyme inhibitors; ARB, angiotensin II receptor blockers; ESA, erythropoiesis-stimulating agent; HD, hemodialysis; IU, international units; PD, peritoneal dialysis

### 3.3 BIOCHEMICAL METHODS

Venous blood was collected weekly from study start and on for 12 weeks. Samples were drawn from dialysis accesses at the start of dialysis sessions (HD patients) and from peripheral veins (PD patients). Plasma was separated and samples kept frozen at −70°C if not analyzed immediately. hs-CRP was measured from the weekly samples. IL-6, IL-10, TNF, NT-proBNP, hs-cTnI, hs-cTnT, albumin, and hemoglobin were measured from monthly samples. Routine biochemistry was analyzed at the local laboratory of each dialysis unit. The measurements of inflammatory markers and NT-proBNP were centralized in one single laboratory (Renal Medicine Huddinge).

Troponins were analyzed at Aleris Medilab, Täby, Sweden. Hs-CRP concentration was measured by nephelometry, plasma IL-6, TNF, and IL-10 analyzed on an Immulite Automatic Immunoassay Analyzer (DPC, Los Angeles, CA, USA) and NT-proBNP by an immunometric assay on an Immulite 1000 Analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA, upper limit 35,000 pg/mL). hs-cTnT was analyzed using the Roche Diagnostics Cobas E 411 analyzer and hs-cTnI using Abbott Diagnostics Architect i4000SR analyzer.
Table 3. Renal diagnoses among hemodialysis and peritoneal dialysis patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HD patients (n=228)</th>
<th>PD patients (n=80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic glomerulonephritis, %</td>
<td>18</td>
<td>15</td>
</tr>
<tr>
<td>Diabetic nephropathy, %</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Adult polycystic kidney disease, %</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Interstitial nephritis including pyelonephritis, %</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>Miscellaneous and unknown diagnoses, %</td>
<td>23</td>
<td>45</td>
</tr>
<tr>
<td>Renal vascular disease including nephrosclerosis, %</td>
<td>16</td>
<td>13</td>
</tr>
</tbody>
</table>

*Abbreviations: HD, hemodialysis; PD, peritoneal dialysis*

3.4 STATISTICAL ANALYSES

3.4.1 Statistical comparisons

For comparison of clinical characteristics and biomarkers between groups standard nonparametric tests were used; Wilcoxon signed-rank test, Kruskal-Wallis, Fisher’s exact test or Pearson chi-square test as appropriate. Categorical data were reported as proportions (percentages) and continuous data presented as median values and the interquartile range (IQR) or means± standard deviation (SD) as appropriate. Spearman rho correlation was used to evaluate the association between CRP, IL-6, and albumin (Paper II) and Pearson’s test for correlation between high-sensitivity cardiac troponin I and T (Paper IV). Outliers were determined using a Box-Cox transformation in paper IV.

3.4.2 Measures of variability

In all papers multivariate analyses were done for estimating factors relating to the three-month variability of biomarkers under study. The multivariate, linear mixed-effect model was used that includes both fixed and random effects. From the model intraclass correlation (ICC) was acquired describing the portion of inflammation variability explained by inter- vs. intra-individual variation (Paper II-IV), i.e. an ICC of 70% means that the 70% of the overall variation is explained by differences between
individuals and 30% of differences within individuals. The model takes into account every biomarker measurement and every symptom for each patient separately. Correlations between symptoms and biomarker variability are independent of how many times symptoms are reported. The mixed model assumes a normal distribution and hence values for biomarkers were logarithmized before entered into the model. The output, or estimate, derived is hence not an absolute number but can be converted to absolute values (in different ways based on which logarithm is used). Furthermore the estimate describes an average change of the biomarker under study that patients with that particular condition, characteristic or symptom experience during the three months compared to reference patients.

In paper IV variability was also described using coefficients of variation (individual, $CV_i$ and grouped, $CV_g$). For estimation of troponin variation exceeding that of cardially stable patients the rise and fall of log-normal reference change values (RCV) were calculated. RCV is found using the median of each patient’s $CV_i$ and a Z-score of 1.96 (for the likelihood that 95% of changes fall within the RCV rise and fall limits found). $CV_i$ is based on the median of $CV_t$ and the analytical $CV$ (given $CV$ of the assays). The index of individuality is calculated from the $CV_i$, $CV_a$ and average $CV_g$. For further information on equations, see the supplement to Paper IV.

### 3.4.3 Survival analyses

Survival analyses were presented in papers I, III and IV and determined after a follow-up for 42-52 months. Kaplan–Meier survival curves and Cox proportional hazard models were used. Patients receiving a kidney transplant were censored from the analysis at the time of transplantation. In paper I a receiver-operating characteristic (ROC) predicted optimum biomarker cut-off levels for survival. Based on that, Kaplan–Meier curves were presented for baseline and median hs-CRP. In papers III and IV the survival analyses were based on biomarker tertiles at baseline, dividing patients into groups by how and if they varied between tertiles of NT-proBNP, hs-cTnI and hs-cTnT during the study period. Significance of data was presented with p-values and/or 95% confidence intervals. In all analyses a p-value of less than 0.05 was considered significant.
4. RESULTS AND DISCUSSION

4.1 HS-CRP AND IL-6 VARIABILITY (PAPER I AND II)

In paper I, data on hs-CRP variation was presented along with prognostic data of serial hs-CRP and baseline IL-6 values. This was based on one cohort of HD patients (Figure 7). In paper II data on hs-CRP and IL-6 variation was presented. This was based on two cohorts, the HD cohort from paper I and a PD cohort (Figures 7 and 8). In paper II information on PEW was added in the analyses.

A large variation in hs-CRP within and between patients based on weekly values was observed during three months in 228 prevalent HD patients. In fact, only 27% had all CRP-levels below 10 mg/L and few (13%) had all hs-CRP values below 5 mg/L. A total of 19% had all serial CRP values above 10 mg/L. IL-6 also showed variation within and between patients based on monthly measurements. Among HD patients, 11% had all IL-6 levels below 5 pg/mL (a normal IL-6 level) and 51% had all levels above. Each participant’s individual minimum, median, and maximum hs-CRP (Figure 9) and IL-6 values are shown (Figure 10). Median hs-CRP in HD was 6.1 mg/L (IQR 2.5–14.0), in PD 5.4 mg/L (IQR 1.6–9.0), median IL-6 in HD 8.3 pg/mL (IQR 5.3–14.5), and in PD 6.7 pg/mL (IQR 4.2–10.0). Range of hs-CRP was 0.2–389 mg/L in HD and 0.1–236 mg/L in PD, the range of IL-6 was 0.9–204 pg/mL and 0.5–91.5 in HD and PD, respectively.

![Figure 9. high-sensitivity CRP variation in hemodialysis and peritoneal dialysis patients. CRP, C-reactive protein](image-url)
Comparing the two cohorts, all biomarkers analyzed were higher in HD compared to PD (IL-6, hs-CRP, albumin and NT-proBNP, Table 1 chapter 3.2). The cohorts were in many ways similar with no significant difference in sex, age, smoking, presence of comorbidity, or wasting. The PD patients had a shorter dialysis vintage and higher BMI.

**Figure 10.** Interleukin-6 variation in hemodialysis and peritoneal dialysis patients.

IL-6, interleukin-6

Univariate analyses showed that HD patients with more comorbidity had higher IL-6 and hs-CRP while PD patients with more comorbidity only had higher IL-6. Specifically, HD patients with CHF and PVD had higher CRP and IL-6, whereas PD patients with these conditions only had higher IL-6. The same was seen for PEW. IHD was associated with increased CRP and IL-6 in HD but not in PD. No significant differences were observed in inflammatory markers in patients with and without DM and no differences were found in TNF or IL-10 by comorbidity in HD patients (data not shown).
Along with repeated hs-CRP and IL-6 values the patients’ weekly report on clinical events, data on comorbidity and clinical characteristics were analyzed with multivariate models with hs-CRP and IL-6 variability being the outcomes. PEW was adjusted for in Paper II but not in Paper I. Age, sex, and comorbidity was associated with hs-CRP (Paper I) and IL-6 (Paper II) variability. Comorbidity did not associate with hs-CRP variability in Paper II when the added effects of dialysis modality and PEW were incorporated (Table 4). HD led to more hs-CRP and IL-6 variation compared to patients on PD. The presence of PEW also related to increased hs-CRP and IL-6 variation. Based on the ICC, 70% of the overall hs-CRP variation was caused by between patient variation and 30% by within-patient variation. For IL-6 the corresponding numbers were 63% variation between patients and 37% variation within patients. IL-6 and hs-CRP at baseline had a significant correlation (Spearman’s rho=0.72; p<0.001 for both HD and PD cohorts).

The results of weekly measurements of CRP and monthly of IL-6 during three months demonstrate a high variability of inflammation markers in prevalent HD and PD patients. The majority of patients have a dynamic inflammatory activity depending on a

Table 4. Factors related to hs-CRP and IL-6 variation, a multivariate analysis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Estimate (SE)</th>
<th>p-value</th>
<th>Estimate (SE)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>hs-CRP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age ≤45 vs. 45-65, y</td>
<td>0.70 (0.22)</td>
<td>0.001</td>
<td>0.41 (0.12)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age ≤45 vs. &gt;65, y</td>
<td>0.92 (0.21)</td>
<td>&lt;0.001</td>
<td>0.63 (0.12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, women vs. men</td>
<td>0.28 (0.14)</td>
<td>0.04</td>
<td>0.18 (0.08)</td>
<td>0.02</td>
</tr>
<tr>
<td>Modality, PD vs. HD</td>
<td>0.47 (0.16)</td>
<td>0.003</td>
<td>0.28 (0.09)</td>
<td>0.002</td>
</tr>
<tr>
<td>Dialysis vintage, &lt;24 vs. ≥24 months</td>
<td>0.02 (0.14)</td>
<td>0.91</td>
<td>0.06 (0.08)</td>
<td>0.44</td>
</tr>
<tr>
<td><strong>Comorbidity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low vs. medium risk</td>
<td>0.31 (0.17)</td>
<td>0.06</td>
<td>0.38 (0.10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low vs. high risk</td>
<td>0.32 (0.21)</td>
<td>0.13</td>
<td>0.51 (0.12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fever (&gt;38ºC)</td>
<td>0.54 (0.09)</td>
<td>&lt;0.001</td>
<td>0.46 (0.10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cold</td>
<td>0.26 (0.05)</td>
<td>&lt;0.001</td>
<td>0.17 (0.05)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>0.21 (0.05)</td>
<td>&lt;0.001</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>0.32 (0.05)</td>
<td>&lt;0.001</td>
<td>0.12 (0.06)</td>
<td>0.04</td>
</tr>
<tr>
<td>Use of antibiotics</td>
<td>0.38 (0.05)</td>
<td>&lt;0.001</td>
<td>0.19 (0.05)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dysuria</td>
<td>0.23 (0.09)</td>
<td>0.01</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Injury</td>
<td>0.20 (0.06)</td>
<td>&lt;0.001</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>0.36 (0.36)</td>
<td>&lt;0.001</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>PEW (SGA &gt;1)</td>
<td>0.30 (0.09)</td>
<td>0.03</td>
<td>0.35 (0.08)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>IL-6</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| **Note:** Data log-transformed. Estimate, effect of each factor on hs-CRP variation. Abbreviations: HD, hemodialysis; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; NS, not significant; PD, peritoneal dialysis; PEW, protein-energy wasting; SE, standard error; SGA, subjective global assessment; y, years
variety of stimuli and few patients exhibit either stable high or stable low levels of inflammation. Earlier studies on serial CRP measurements show similar results.209,210 Less is known about IL-6 variability in ESRD. The study presented in paper I and II is the largest on variation in inflammatory markers in ESRD with a detailed phenotyping. Even in healthy subjects there is a certain variation in CRP, more prominent between than within individuals.211 Intraindividual measurement stability in hs-CRP is similar to total cholesterol.154 Smaller studies have shown increased levels and variability212 of inflammatory markers in dialysis patients (measured weekly and then monthly) indicating that the dialysis process itself may not play an important role in inflammation induction.209 Others have related clinical events to CRP-levels and found that in almost 100% where CRP is above 10mg/L a clinical or chronic comorbidity was present further supporting that raised inflammation in these patients should be evaluated and the cause of inflammation searched for.213 A study on CRP before and after HD sessions showed an increase in CRP in 25% of patients, CRP lowered back to pre-HD levels before the next dialysis, and furthermore an increase in CRP levels by 1mg/L resulted in 9% higher mortality.214 Even after excluding patients with apparent clinical inflammatory events, inflammation varies greatly in HD patients, mostly due to intraindividual variation of IL-6.153

Levels of IL-6 and hs-CRP were higher in HD compared to PD patients and the difference in inflammation based on comorbidity was more prominent in HD than PD patients. Data on inflammation in HD versus PD have been conflicting. Two studies concluded that there is no difference in IL-6 and CRP in HD compared to PD patients215,216 whereas Haubitz et al.217 showed an increase in CRP after starting HD but not PD; and the PD group had CRP levels similar to healthy controls. Even though the proportion of patients having PEW, PVD, CHF and IHD did not differ significantly between the cohorts the patients on PD most likely constitute a healthier patient population which may in part explain the difference in inflammation. The causes of inflammatory activity also differ between the two dialysis modalities. Lower GFR correlates with higher inflammation218,219 and it is likely that HD patients in our study had a lower residual function compared to the PD patients. Dialysis related factors are linked to inflammation (see also chapter 1.6.3) Dialyzer membranes and endotoxins in dialysate may provoke inflammation in HD.220,221 Infections at exit sites or in catheter tunnels can cause inflammation in PD. Since the PD cohort was smaller than the HD cohort this may explain the lack of effect of e.g. PEW, CHF and comorbidity on hs-
CRP, factors that showed highly significant differences in IL-6 in both cohorts as well as hs-CRP in HD.

The association between inflammation and overall comorbidity, CHF, PVD, IHD and PEW has been well established in both non-renal and renal cohorts. However, less is known about a causative correlation although much is speculated. For instance, in PVD associated wounds and gangrene are likely sources of inflammation as is the underlying vascular damage or atherosclerosis. hs-CRP is a prognostic factor for progression of PVD in vessels of varying sizes and has even been shown to be higher in patients with PVD than stable IHD. However a direct pathogenic role of CRP has not been proven. Earlier studies have shown a potential role of chronic infections as a link to carotid atherosclerosis via inflammation in ESRD. Others have found a link between IL-6 and left ventricular hypertrophy in PD patients. These are observational data that do not confirm a causal role for inflammation in cardiovascular disease or PEW. The results may however indicate sources of inflammation in dialysis patient when inflammation is present without a detectable clinical source. In the multivariate analyses presented in Paper I and II, acute events were accounted for as reported by patients. Only a part of the increase in inflammation was predicted by intercurrent events, as has been reported by others while unfavorable patient factors; high age, male sex, and a high burden of comorbidity predicted some. A protective effect of estrogen may explain the link between male sex and increased inflammation, as IL-6 increases after menopause. Testosterone deficiency has been inversely related to endothelial dysfunction and increased risk of cardiac events in CKD male patients.

In Paper II, PEW was added as a confounder to a multivariate model combining the HD and PD cohorts. PEW showed a strong association with monthly fluctuation of IL-6 and CRP. Comorbidity did not predict CRP variability after PEW was added as a confounder, perhaps explained by the strong association between PEW and comorbidity. Previous studies demonstrate significant correlations between inflammation and PEW. The interrelationship between inflammation, PEW and atherosclerosis is well-known in ESRD as described in chapter 1.6.4. The relationship between inflammation, various comorbidities, and PEW are complex and may be bidirectional, i.e. comorbidity and PEW drive inflammation or inflammation cause the disease, protein degradation, and PEW. A central role of IL-6 in vascular damage has
been proposed in earlier studies, in part by acting in the arterial wall and stimulating uptake of lipids into macrophages.\textsuperscript{235,236}

As expected, IL-6 correlated well with CRP and showed an even stronger association to comorbidity than CRP. IL-6 has been found to be a stronger predictor than CRP of CVD, cardiovascular mortality, and all-cause mortality in ESRD.\textsuperscript{102,237,238} All the same CRP has a potential causative role in CVD e.g. to attenuate endothelial progenitor cell survival and function.\textsuperscript{239} The more stable nature, no diurnal variation and longer half-life of CRP compared to IL-6 may explain why more of the clinical symptoms reported by patients correlated with hs-CRP variation.\textsuperscript{240-242}

Studies on IL-6 polymorphisms have led to a proposal of IL-6 involvement in pathogenesis of CVD in both non-renal\textsuperscript{99} and CKD patients.\textsuperscript{101,243} Studies on polymorphisms in the CRP gene have shown varying results. Zhang et al. proposed that CRP haplotypes did not predict CVD,\textsuperscript{100} while Lange et al. showed an increased risk for cardiovascular events in the elderly based on CRP polymorphism\textsuperscript{244} suggesting that CRP is not just an innocent bystander. However, Zacho et al. did not find an increased risk of IHD for four different CRP polymorphisms.\textsuperscript{245} A recent publication based on polygenic overlap suggested that CRP polymorphism might have a causative role in coronary artery disease.\textsuperscript{246} A polymorphism in the promoter region of IL-6 (-174 G—C, the high producer genotypes for IL-6 (G/G and G/C vs. low producer C/C) has been related to higher comorbidity, lower nutritional status, and cardiovascular events in non-renal, CKD 2-5, and dialysis patients.\textsuperscript{101,247,248} A variant in the IL-6 gene (-14C carriers in absence of 162 Val allele) was associated with higher IL-6 and higher CVD, supporting a causal role for IL-6 in CVD.\textsuperscript{243} Since vascular disease in CKD is in part a calcification problem the finding that IL-6 polymorphisms have been linked to increased bone resorption in postmenopausal women is interesting.\textsuperscript{249}
4.2 HS-CRP AND PROGNOSIS (PAPER I)

Repeated CRP-values as described with CRP\textsubscript{median} or CRP\textsubscript{mean} significantly associated with decreased survival while a baseline CRP value did not (Figure 11) after adjustments for confounders. There was a significant correlation between CRP\textsubscript{baseline} and CRP\textsubscript{median} ($r^2=0.61$, $p<0.001$).

![Comparison of Hazard Ratios for Death](image)

**Figure 11.** Comparison of hazard ratios for death in hemodialysis patients. Adjusted for age, sex, vintage, comorbidity, access type and albumin.
CRP, C-reactive protein; IL-6, interleukin-6

Cut-off values for best predicting survival were found with ROC analyses; CRP\textsubscript{baseline} 6.1 mg/L, CRP\textsubscript{median} 5.7 mg/L, CRP\textsubscript{mean} 10.0 mg/L and IL-6 baseline 10.1 pg/mL. Groups above and below the cut-off values for CRP\textsubscript{baseline} and CRP\textsubscript{median} were expressed in Kaplan-Meier survival curves unadjusted and adjusted (Figure 12). In the figure values are adjusted for age, sex, vintage and comorbidity (Figure 12 c) and d).
Figure 12. Survival by high-sensitivity CRP in hemodialysis patients. a) Survival by hs-CRP at baseline, b) adjusted survival by hs-CRP at baseline, c) survival by median hs-CRP, d) adjusted survival by median hs-CRP. p-value <0.001 for all hs-CRP, high-sensitivity C-reactive protein

Prognostic values with hazard ratios for death are presented in Table 5. An increase in CRP\textsubscript{median} by 1mg/L resulted in a 1% increase in mortality risk. Patients with high risk comorbidity had a roughly six-fold hazard ratio for death. One year in older age resulted in an average 6% increase in risk of mortality. Vintage, sex, and access type did not significantly predict survival. Higher albumin by 1g/L related to a lower hazard ratio (HR) for death (Table 5).
### Table 5. Cox proportional hazard ratios for death

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Hazards ratio</th>
<th>95% confidence interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP median (mg/L)</td>
<td>1.01</td>
<td>1.00 - 1.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Age, year</td>
<td>1.06</td>
<td>1.04 - 1.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, men</td>
<td>0.8</td>
<td>0.5 - 1.3</td>
<td>0.4</td>
</tr>
<tr>
<td>Comorbidity, low vs. medium risk</td>
<td>2.9</td>
<td>1.1 - 7.4</td>
<td>0.02</td>
</tr>
<tr>
<td>Comorbidity, low vs. high risk</td>
<td>6.2</td>
<td>2.4 - 16.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vintage &lt; 24 months vs. &gt;24 months</td>
<td>0.995</td>
<td>0.99 - 1.001</td>
<td>0.1</td>
</tr>
<tr>
<td>Access type, fistula vs. graft</td>
<td>0.8</td>
<td>0.5 - 1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Access type, fistula vs. catheter</td>
<td>0.9</td>
<td>0.5 - 1.7</td>
<td>0.8</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>0.93</td>
<td>0.88 - 0.98</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Abbreviations:** hs-CRP, high-sensitivity C-reactive protein

Studies have shown a single CRP value to be valuable in risk assessment of morbidity, cardiovascular mortality, and overall mortality in HD and PD patients.\textsuperscript{12,57,250-253} Similar risk estimates to our study have been shown before.\textsuperscript{254} There is limited information from prior studies on serial CRP measurements and prognosis. One study, where patients with inflammatory disease and infections were excluded, showed a significantly higher risk of death for patients with four CRP values >5.1 mg/L.\textsuperscript{210} Median levels of CRP were much lower compared to our study of unselected prevalent patients.

Repeated CRP values from our study (as presented by CRP\textsubscript{median}) were more strongly related to mortality than a single value. Thus repeated measurements (here weekly), give more information on prognosis in dialysis patients than an incidental value, supporting data from the NECOSAD study on incidental HD patients where CRP was measured at 3 and 6 months and high levels at both points gave a higher risk for both CV and non-CV mortality.\textsuperscript{255} The results from the HEMO study were similar with repeated IL-6 values more strongly predicting all-cause and cardiovascular mortality in HD patients.\textsuperscript{256}

Increase in age and comorbidity significantly associated with higher mortality, but sex, vintage, and access type (catheter vs. graft or AV-fistula) did not. In a large US study of incident HD patients (DOPPS) the use of dialysis catheters related to increased mortality risk.\textsuperscript{257} A total of 67% of the patients had catheters as opposed to 21% in our study, where patients were not included until at least 3 months in their HD treatment.
This may in part explain the discrepancy between the two studies. Albumin has for long been recognized as a strong predictor for mortality in ESRD, which was confirmed in our study.\textsuperscript{102,258} It is a rather unspecific marker though, associated with inflammation, overhydration, nephrotic range proteinuria, liver disease, loss in dialysate and to some extent poor nutrition.\textsuperscript{209,259-262} The association between albumin and nutrition has been debated, for example albumin did not correlate strongly with SGA in a large study on incident and prevalent HD patients, unless they also had some degree of inflammation. The correlation with handgrip strength and lean body mass was also poor.\textsuperscript{263} IL-6 and CRP have been found to be better markers of nutrition than albumin.\textsuperscript{44} IL-6 and hs-CRP were in the present study strongly and inversely related to plasma albumin levels in both cohorts, which is logical since inflammation directly affects the synthesis of albumin.\textsuperscript{264} Adding both CRP and albumin to the same model predicting for survival may thus diminish the weight of CRP on survival, although we did not see a difference in HR of albumin with or without CRP.

The prognostic value of IL-6 for survival was not significant after adjustments for confounders. Others have found a single IL-6 level to be a strong predictor of death in incident HD and PD patients\textsuperscript{265} and in prevalent HD patients.\textsuperscript{266} Later data from our group based on the same cohort showed a similar prognostic effect of CRP and IL-6 on mortality based on two values, three months apart.\textsuperscript{267} Other groups have found IL-6 to be a stronger predictor of total and cardiovascular mortality in HD patients compared to CRP.\textsuperscript{268,269} Perhaps by correcting for albumin in the model, the prognostic effect of IL-6 was lost.

Repeated measures add information about outcome over a single value.\textsuperscript{161,210} Possibly, fluctuation in inflammation represents an unstable condition leading to a long-term decline in health and increased risk for mortality.
4.3 NT-PRO-BNP VARIABILITY (PAPER III)

In paper III, NT-proBNP variability was estimated for 211 HD patients. Levels of NT-proBNP varied greatly within individuals and even more between individuals as seen in Figure 13. One single value fell within a normal reference interval (279 pg/mL). The median of all NT-proBNP levels was 30-fold higher compared to the normal reference. A total of 12% (n=25) had all values above the method’s upper limit 35,000 pg/mL and 1.4% (n=3) had all values below 1,000 pg/mL. Based on tertiles used for survival analyses (see section 4.4), 19% (n=41) had constantly low, 16% (n=34) constantly medium, and 24% (n=50) constantly high levels, thus 59% had a stable NT-proBNP pattern and the remaining 41% a varying pattern, between tertiles.

Figure 13. NT-proBNP variability in hemodialysis patients. Ordered by patients maximum value

Tertiles of NT-proBNP were: 279-4,389 pg/mL (low), 4,390-18,442 pg/mL (middle), 18,443- >35,000 pg/mL (high). There was a significant relation between older age, increased comorbidity and repeatedly high levels of NT-proBNP. Higher NT-proBNP levels also related to lower albumin and higher hs-CRP. Comparing NT-proBNP between patients with or without certain comorbid conditions showed that CHF and PEW related to higher levels while IHD and DM did not (Table 6).
Table 6. NT-proBNP levels in patients with and without congestive heart failure, ischemic heart disease, protein-energy wasting, and diabetes mellitus

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No (%)</th>
<th>NT-proBNP pg/mL (median IQR)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHF</td>
<td>44 (21)</td>
<td>24,614 (8,831-35,001)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No CHF</td>
<td></td>
<td>6,958 (2,667-20,006)</td>
<td></td>
</tr>
<tr>
<td>IHD</td>
<td>66 (31)</td>
<td>11,253 (4,093-27,069)</td>
<td>0.2</td>
</tr>
<tr>
<td>No IHD</td>
<td></td>
<td>8,044 (2,672-26,465)</td>
<td></td>
</tr>
<tr>
<td>PEW</td>
<td>97 (47)</td>
<td>12,932 (5,658-35,001)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No PEW</td>
<td></td>
<td>6,092 (2,248-17,670)</td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>55 (26)</td>
<td>10,677 (3,533-24,861)</td>
<td>0.8</td>
</tr>
<tr>
<td>No DM</td>
<td></td>
<td>8,831 (2,909-26,683)</td>
<td></td>
</tr>
</tbody>
</table>

Note: NT-proBNP values at baseline. Abbreviations: CHF, congestive heart failure; DM, diabetes mellitus; IHD, ischemic heart disease; IQR, interquartile range; NT-proBNP, N-terminal pro-brain natriuretic peptide; PEW, protein-energy wasting

Estimating which factors related to the short-term variability described above, we found that inflammation (hs-CRP) significantly associated with NT-proBNP variability, as did PEW, age, and comorbidity (Table 7). Sex and time on dialysis did on the other hand not. ICC showed that 86% of NT-proBNP variation was explained by variation between patients and 14% within patients.

Table 7. Factors related to NT-proBNP variation, a multivariate analysis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Estimate</th>
<th>Lower</th>
<th>Upper</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: &lt;45 vs. &gt;45-65 years</td>
<td>0.02</td>
<td>-0.06</td>
<td>0.11</td>
<td>0.6</td>
</tr>
<tr>
<td>Age &lt;45 vs. &gt;65 years</td>
<td>0.11</td>
<td>0.03</td>
<td>0.19</td>
<td>0.008</td>
</tr>
<tr>
<td>Sex, women vs. men</td>
<td>-0.01</td>
<td>-0.06</td>
<td>0.04</td>
<td>0.8</td>
</tr>
<tr>
<td>Dialysis vintage, &lt;24 vs. &gt;24 months</td>
<td>0.03</td>
<td>-0.02</td>
<td>0.08</td>
<td>0.2</td>
</tr>
<tr>
<td>Comorbidity:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>low vs. medium risk</td>
<td>0.08</td>
<td>0.02</td>
<td>0.15</td>
<td>0.01</td>
</tr>
<tr>
<td>low vs. high risk</td>
<td>0.12</td>
<td>0.05</td>
<td>0.20</td>
<td>0.002</td>
</tr>
<tr>
<td>Log2 hs-CRP</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PEW, SGA&gt;1</td>
<td>0.09</td>
<td>0.04</td>
<td>0.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Study week 4</td>
<td>0.01</td>
<td>-0.00</td>
<td>0.03</td>
<td>0.08</td>
</tr>
<tr>
<td>Study week 8</td>
<td>0.02</td>
<td>0.00</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Study week 12</td>
<td>0.02</td>
<td>0.00</td>
<td>0.03</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Note: Estimate, effect of each factor on NT-proBNP variation. Abbreviations: hs-CRP, high-sensitivity C-reactive protein; PEW, protein-energy wasting; SGA, subjective global assessment
Our finding of very high NT-proBNP in all HD patients supports previous data on increased NT-proBNP in ESRD.\textsuperscript{173,271} NT-proBNP, with a half-life of 120 minutes in non-renal subjects, accumulates in ESRD since its clearance is partly renal. Extra-renal clearance by endopeptidases which may accumulate in renal failure counteracts decreased renal clearance to some extent.\textsuperscript{175,176,272} Fluctuation in NT-proBNP levels was substantial within and between patients, the median range being 4,102 pg/mL. Fahim et al. presented NT-proBNP variability in HD and PD patients where between-person CV was higher than the within-person CV (148% vs. 35%). They proposed that reference change levels would be more appropriate than absolute values in estimating changes of NT-proBNP in ESRD.\textsuperscript{200}

Blood samples were collected at the start of dialysis when most patients have excessive fluid overload. High NT-proBNP in CHF patients compared to patients without known CHF may be explained by less tolerance of CHF-patients to overhydration. Patients with PEW also had higher NT-proBNP levels. A plausible explanation is overhydration, i.e. PEW leads to chronic fluid overload which in turn causes myocardial stretch. PEW is associated with loss of fat mass\textsuperscript{13} and adiposity has been negatively correlated with NT-proBNP\textsuperscript{273} with the possible explanation that adipocytes contribute to natriuretic peptide clearance.\textsuperscript{274} Lean body mass has also been inversely associated with NT-proBNP and BNP, stronger so than fat mass.\textsuperscript{275} In Paper I we established that inflammation, measured by hs-CRP, was strongly linked to CHF. NT-proBNP significantly correlates with LVD in HD patients in spite of generally elevated levels.\textsuperscript{170} In the multivariate mixed model, hs-CRP did associate with NT-proBNP variability, as did PEW that is known to have an important relation to inflammation.\textsuperscript{13}

The association between NT-proBNP variability and comorbidity increased with higher Davies score, an index that has emphasis on cardiovascular disease. There is certainly a complex interrelation between wasting, inflammation, fluid overload, and NT-proBNP although studies have been inconclusive.\textsuperscript{202} Some have shown the relation between NT-proBNP and markers of volume overload (measured by bio-impedance) to be stronger than between NT-proBNP and cardiac dysfunction in HD patients, with the conclusion that wasting and fluid overload are central in raising NT-proBNP.\textsuperscript{202,276} Others have shown no link between extra-cellular volume and NT-proBNP while observing a relation between NT-proBNP and left ventricular mass index (LVMI) and ejection fraction (EF) in PD patients. Others yet have not seen a link between NT-proBNP and
left ventricular hypertrophy or ultrafiltration volume.\textsuperscript{277-280} We had information on ultrafiltration volume at the baseline dialysis session for 173 patients which did not show an independent association to NT-proBNP variation when added to the multivariate analysis.

An everyday challenge in the treatment of dialysis patients is the estimation of “dry weight”. A patient losing weight on account of for example PEW, is likely to have fluid overload that is not treated during several dialysis sessions because of an overestimation of the dry weight. This may go unobserved until clinical symptoms such as refractory hypertension, oedema or dyspnea appear.\textsuperscript{281}
4.4 NT-PRO-BNP AND PROGNOSIS (PAPER III)

Based on baseline (Figure 14) and repeated data (Figure 15) survival was worse with increasing NT-proBNP. Patients with high tertile NT-proBNP had significantly worse survival while similar results were seen in the middle and low tertiles. When divided into six groups according to variation in monthly levels, patients with consistently high NT-proBNP had the poorest outcome with a hazard ratio for death of 3.75 in crude analyses (reference group: constant low levels) and 1.99 for patients with middle to high levels (Table 8). After adjustments for age, sex, vintage, comorbidity and PEW the significant prognostic value of NT-proBNP was lost.

Figure 14. Survival by tertiles of NT-proBNP at baseline
NT-proBNP, N-terminal pro-brain natriuretic peptide
Figure 15. Survival by constant levels within tertiles of NT-proBNP (Low, Medium, High) or varying levels between tertiles (Low medium, Medium high, Low medium high). Based on tertiles at baseline NT-proBNP, N-terminal pro-brain natriuretic peptide

Table 8. Multivariate Cox proportional hazard ratios (95% CI) for death by NT-proBNP variability groups

<table>
<thead>
<tr>
<th>NT-proBNP</th>
<th>Crude</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Low-middle</td>
<td>0.93 (0.42-2.05)</td>
<td>1.09 (0.49-2.41)</td>
<td>1.00 (0.45-2.22)</td>
<td>0.86 (0.38-1.93)</td>
<td>0.89 (0.39-2.04)</td>
</tr>
<tr>
<td>Middle</td>
<td>0.83 (0.37-1.87)</td>
<td>0.65 (0.29-1.48)</td>
<td>0.58 (0.25-1.33)</td>
<td>0.60 (0.26-1.37)</td>
<td>0.63 (0.27-1.45)</td>
</tr>
<tr>
<td>Middle-high</td>
<td>1.99 (1.01-3.92)</td>
<td>1.78 (0.89-3.57)</td>
<td>1.39 (0.69-2.81)</td>
<td>1.32 (0.65-2.67)</td>
<td>1.38 (0.67-2.86)</td>
</tr>
<tr>
<td>Low-middle-high</td>
<td>1.47 (0.53-4.08)</td>
<td>1.21 (0.39-3.72)</td>
<td>1.42 (0.46-4.42)</td>
<td>1.23 (0.39-3.89)</td>
<td>1.13 (0.35-3.64)</td>
</tr>
<tr>
<td>High</td>
<td>3.75 (2.02-6.95)</td>
<td>2.77 (1.45-5.29)</td>
<td>2.00 (1.02-3.89)</td>
<td>1.70 (0.86-3.36)</td>
<td>1.63 (0.80-3.33)</td>
</tr>
</tbody>
</table>

Note: Model 1, adjusted for age, sex, vintage; Model 2, Model 1 + comorbidity; Model 3, Model 2 + protein-energy wasting; Model 4, Model 3 + high-sensitivity C-reactive protein. Low, values within low NT-proBNP tertile; middle, values within middle tertile; high, values within high tertile; low-middle, values within low and middle tertiles; middle-high, values within middle and high tertiles; low-middle-high, values within low, middle and high tertiles. Abbreviations: CI, confidence interval; NT-proBNP, N-terminal pro-brain natriuretic peptide
The baseline survival analysis showed patients with high tertile NT-proBNP (above 18,442 pg/mL) had poorer survival compared to those with lower levels (Figure 14). Other studies have had similar findings.\textsuperscript{282} Few studies have reported repeated measurements, which in our study gives the added information that patients with constantly high NT-proBNP levels have the worst prognosis while those varying between medium and high levels come second. These are unadjusted data that show the prognostic value of NT-proBNP before taking into account other factors affecting survival. After adjusting for age, sex, time on dialysis, and comorbidity, levels that are constant within the high tertile still predict a two-fold risk for death after 50 months of follow-up. After adding the confounding effect of PEW to mortality analyses, constantly high NT-proBNP was not prognostic for death. Most studies that have found NT-proBNP to be a prognostic marker for survival did not account for nutritional status as a confounder.\textsuperscript{167,168,173,283,284} Gutierrez et al. made an attempt to take malnutrition into account, by adjusting for albumin in a study on HD patients based on two NT-proBNP measurements in three months.\textsuperscript{172} As discussed previously (chapter 4.2) albumin is a powerful marker of mortality\textsuperscript{102,285} but debated as a marker for nutrition or PEW in dialysis patients.\textsuperscript{263,286} Albumin did for instance not discriminate well between malnutrition and normal nutritional status in HD patients in the absence of inflammation.\textsuperscript{287}

Since wasting has been linked to mortality in ESRD it is an important factor to adjust for in survival analyses. Doing that, the prognostic value of NT-proBNP decreased, supporting the importance of assessing PEW and NT-proBNP together in the clinical setting. The association between PEW, fluid overload, CHF and even inflammation is complex and multifactorial, explaining why the prognostic value of NT-proBNP is different in ESRD than in non-renal patients. As such NT-proBNP should be used as a prognostic marker taking into account the interaction of comorbidity, inflammation, and nutritional status as well as high levels of the biomarker in general in ESRD and great inter-and intraindividual variation.
4.5 TROPONINS AND VARIABILITY (PAPER IV)

In paper IV, variability of hs-cTnI and hs-cTnT in HD and PD patients was assessed. Both hs-cTnI and hs-cTnT varied greatly within and between patients. A total of 42% of all hs-cTnI values were above the suggested diagnostic level for MI (>27 ng/L) and 98% of all hs-cTnT values (MI level >14 ng/L). Nearly 100% of all troponin measurements were above the assays’ limits of detection. The individual lowest, highest and median hs-cTnI and hs-cTnT are presented in Figure 16.

![Figure 16](image.png)

**Figure 16.** Troponin variation in peritoneal dialysis and hemodialysis patients  
HD, hemodialysis; PD, peritoneal dialysis; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T

Patients with CHF, IHD, and PVD had higher hs-cTnI and hs-cTnT compared to patients without these conditions in univariate analyses. Hs-cTnT, but not hs-cTnI, was increased in patients with PEW and DM. Among PD patents, not HD patients, a difference in both troponins was seen between the sexes (higher in men). hs-cTnI and hs-cTnT levels did not differ based on dialysis modality. Variation within- and between individuals (as expressed by CV_i and CV_g) was higher in hs-cTnI than hs-cTnT but similar between dialysis cohorts. Analyses excluding outliers which gave lower CV_i for hs-cTnI (16%) and hs-cTnT (8%) and lower CV_g for hs-cTnI (125%) and hs-cTnT (94%). A large gap between CV_i and CV_g results in a very low index of individuality (II)
The RCV was higher for hs-cTnl (Table 9). Excluding outliers RCVs decreased slightly for hs-cTnl (+62/-38%) but not for hs-cTnT (+29/-22%). RCV and total CV were similar between age-tertiles and the sexes (data not shown).

The two troponins correlated moderately with each other (Pearson’s correlation r=0.62 (95% confidence interval (CI) 0.58-0.65). Age showed positive correlations with hs-cTnl and hs-cTnT levels (Spearman’s p 0.38 vs. 0.39 both p<0.001)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HD patients (n=198)</th>
<th>PD patients (n=78)</th>
<th>All patients (n=276)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-cTnl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR), ng/L*</td>
<td>24 (14–41)</td>
<td>21 (11–45)</td>
<td>23 (13–41)</td>
</tr>
<tr>
<td>Range, ng/L</td>
<td>&lt;2–4,057</td>
<td>2–1,764</td>
<td>&lt;2–4,057</td>
</tr>
<tr>
<td>CVi, %</td>
<td>17</td>
<td>18</td>
<td>19</td>
</tr>
<tr>
<td>CVg, %</td>
<td>312</td>
<td>248</td>
<td>304</td>
</tr>
<tr>
<td>Reference change value, %</td>
<td>+67/-40</td>
<td>+70/-41</td>
<td>+68/-41</td>
</tr>
<tr>
<td>Index of individuality</td>
<td>0.06</td>
<td>0.08</td>
<td>0.07</td>
</tr>
<tr>
<td>hs-cTnT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (IQR), ng/L**</td>
<td>70 (45–130)</td>
<td>71 (42–138)</td>
<td>70 (45–132)</td>
</tr>
<tr>
<td>Range, ng/L</td>
<td>&lt;5–1,961</td>
<td>12–1,008</td>
<td>&lt;5–1,961</td>
</tr>
<tr>
<td>CVi, %</td>
<td>9</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>CVg, %</td>
<td>146</td>
<td>123</td>
<td>127</td>
</tr>
<tr>
<td>Reference change value, %</td>
<td>+30/-23</td>
<td>+23/-19</td>
<td>+29/-23</td>
</tr>
<tr>
<td>Index of individuality</td>
<td>0.06</td>
<td>0.06</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Note: *p=0.26 HD vs. PD, **p=0.66 HD vs. PD. Abbreviations; CVi, individual coefficient of variation; CVg, grouped coefficient of variation; HD, hemodialysis; hs-cTnl, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T, IQR, interquartile range; PD, peritoneal dialysis;

To determine the association of clinical characteristics and comorbidities with troponin variation multivariate analyses were conducted. Factors adjusted for were age, sex, time on dialysis (dialysis vintage), treatment modality, cardiovascular disease (IHD; CHF; PVD), DM, and PEW. Age, male sex, CHF, and PEW significantly related to hs-cTnl and hs-cTnT variation but IHD, dialysis modality, and vintage did not. The ICC showed a vast
part of troponin variability to be caused by differences between patients (hs-cTnI; 74% and hs-cTnT; 87%) (Table 10).

Table 10. Factors related to high-sensitivity troponin variation, a multivariate analysis

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>hs-cTnI</th>
<th></th>
<th></th>
<th></th>
<th>hs-cTnT</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model 1</td>
<td>Model 2</td>
<td>Model 1</td>
<td>Model 2</td>
<td>Model 1</td>
<td>Model 2</td>
<td>Model 1</td>
<td>Model 2</td>
</tr>
<tr>
<td>Age, ≤45/46-65/&gt;65 years</td>
<td>0.54 (0.17)</td>
<td>&lt;0.001</td>
<td>0.45 (0.17)</td>
<td>&lt;0.001</td>
<td>0.44 (0.15)</td>
<td>&lt;0.001</td>
<td>0.34 (0.14)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, women vs. men</td>
<td>0.27 (0.1)</td>
<td>0.02</td>
<td>0.31 (0.11)</td>
<td>0.005</td>
<td>0.3 (0.09)</td>
<td>0.002</td>
<td>0.38 (0.09)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Modality, PD vs. HD</td>
<td>0.15 (0.13)</td>
<td>0.23</td>
<td>0.14 (0.12)</td>
<td>0.25</td>
<td>0.11 (0.11)</td>
<td>0.29</td>
<td>0.09 (0.10)</td>
<td>0.39</td>
</tr>
<tr>
<td>Dialysis vintage, &lt;24 vs. ≥24 months</td>
<td>&lt;0.01 (0.01)</td>
<td>0.98</td>
<td>&lt;0.01 (&lt;0.01)</td>
<td>0.8</td>
<td>&lt;0.01 (&lt;0.01)</td>
<td>0.86</td>
<td>&lt;0.01 (&lt;0.01)</td>
<td>0.84</td>
</tr>
<tr>
<td>IHD</td>
<td>0.03 (0.13)</td>
<td>0.82</td>
<td>-0.05 (0.1)</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVD</td>
<td>-0.02 (0.12)</td>
<td>0.83</td>
<td>-0.05 (0.1)</td>
<td>0.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHF</td>
<td>0.45 (0.15)</td>
<td>0.002</td>
<td>0.46 (0.12)</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>0.1 (0.13)</td>
<td>0.42</td>
<td>0.14 (0.1)</td>
<td>0.17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEW, (SGA &gt;1)</td>
<td>0.25 (0.11)</td>
<td>0.02</td>
<td>0.28 (0.09)</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICC</td>
<td>0.73</td>
<td>0.74</td>
<td>0.88</td>
<td>0.87</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Estimate, effect of each factor on troponin variation. Abbreviations: CHF, congestive heart failure; DM, diabetes mellitus; HD, hemodialysis; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; ICC, intraclass correlation; IHD, ischemic heart disease; PD, peritoneal dialysis; PEW, protein-energy wasting; PVD, peripheral vascular disease; SE, standard error; SGA, subjective global assessment

The large proportion of high-sensitivity troponin levels above the 99th percentile (MI level) is consistent with previous findings as is the larger proportion of increased hs-cTnT compared to hs-cTnI. Baseline hs-cTnI and hs-cTnT levels did not differ based on dialysis modality which also supports previous findings. This study on prevalent patients showed more prominent differences between patients than within. Since elevated troponin levels are prognostic for adverse events in dialysis patients, regular measuring of troponins may identify patients at risk that are otherwise not recognized. A monthly troponin measurement can also serve as a patients reference level and be used together with dynamic changes in the acute setting to
determine the likelihood of acute coronary syndrome. hs-cTnT is more suitable for this based on the lower intra-individual variation found compared to hs-cTnI supporting previous findings that hs-cTnT is superior in ruling out MI in ESRD.\textsuperscript{192,292} The large differences in cTn levels between patients limits the use of absolute reference levels for dialysis patients.\textsuperscript{292-294} Instead, a RCV is of interest. In paper III, RCV was higher for hs-cTnI than hs-cTnT, confirming recent data from a small HD cohort and healthy subjects.\textsuperscript{292} There was some difference in RCV between modalities; larger changes of hs-cTnI were seen in PD patients and of hs-cTnT in HD patients. This difference could be real or explained by a relatively small PD-cohort. A RCV may be useful to detect changes that are outside of fluctuations normally seen. Based on the calculated RCV a portion of the participants had variations outside of it. This can be explained by clinical incidents causing troponin leakage since patients were only excluded from analyses if they had an MI: Alternatively, it could indicate that the RCV is too small to take into account the actual variation in dialysis patients, i.e. some false positive results are seen. Larger studies with detailed information on clinical events likely to cause troponin leakage are needed to more accurately determine a true RCV in ESRD.

The fluctuation in troponins observed in our studies does not present normal biologic variation since it may be caused by different underlying pathologic clinical conditions. These can be acute or subclinical e.g. anemia, paroxysmal arrhythmias, angina, intradialytic hypotension, myocardial stunning or fluid overload.\textsuperscript{196,295-297} Troponin elevation in dialysis patients is often considered to be caused by renal failure, the dialysis procedure, or clinical factors such as those mentioned above.\textsuperscript{176,195,298} Findings from the multivariate analyses showed that troponins fluctuate partly in relation to the presence of CHF and/or PEW, increasing age and male sex. A significant association exists between LVD and cTn increase as well as increased left ventricular mass and cTn in HD patients.\textsuperscript{288,299-301} Some studies have shown a stronger correlation of hs-cTnI with LVD than of hs-cTnT.\textsuperscript{302} We found that both troponins fluctuated with the presence of CHF. The Dallas Heart Study with over 3,500 participants showed that older age and male sex increased the likelihood of detectable hs-cTnT.\textsuperscript{301} They also showed (using the same hs-cTnT assay as done in paper IV), in a multivariate analysis, that increased left ventricular mass, wall thickness, and chamber dilation predicted detectable hs-cTnT, but coronary artery calcium score did not.\textsuperscript{301} Omland et al, had similar findings, and showed that increased hs-cTnT predicted heart failure and cardiovascular death but not incident MI in patients with stable coronary artery disease.\textsuperscript{303} Taken together, there
seems to be a difference to troponin release in an acute situation compared to a chronic setting. The stronger association with CHF than IHD implicates myocardial ischemia could be due to reduced perfusion within a hypertrophied myocardium.

Troponins are related to CHF and PEW as seen from our results and others.\textsuperscript{36} PEW, as discussed before (chapter 4.3) is a common condition in ESRD which may lead to overestimation of dry weight.\textsuperscript{281} The resulting chronic fluid overload could potentially cause myocardial stretch with troponin release and pose a risk for the patient to develop fulminant CHF. Malnutrition and troponins have been associated with fluid overload in dialysis patients.\textsuperscript{196,304} Clinically it is therefore important to assess, among other things, patients’ fluid and nutritional status when fluctuations in troponins are found without signs of acute cardiac events. This can preferably be done with SGA, a validated clinical tool which is easy to use.\textsuperscript{151}

### 4.6 TROPONINS AND PROGNOSIS (PAPER IV)

Based on tertiles at baseline survival was lowest for patients with hs-cTnl within the high tertile (Figure 17) while an increased risk was seen across higher tertiles of hs-cTnT (Figure 18).

![Figure 17. Survival by tertiles of high-sensitivity cardiac troponin I at baseline. terTNI0=0, low tertile, terTNI0=1, middle tertile; terTNI0=2, high tertile](image-url)
Patients were also grouped by troponin stability within one tertile (low, middle or high) or moving values between tertiles, in the same way as done for NT-proBNP in Paper III. The tertiles of hs-cTnI were (ng/L): <2-15 (low), 16-32 (middle), 33-2615 (high) and of hs-cTnT (ng/L): 12-51 (low), 52-107 (middle), 108-1175 (high). In hs-cTnI measurements, 23% of patients (n=64) had constantly low, 13% (n=36) middle, and 24% (n=67) high levels i.e. 61% patients had a stable troponin pattern and 39% a varying pattern. Similarly, for hs-cTnT 26% of patients (n=72) had constantly low, 18% (49) middle and 27% (n=75) high levels i.e. 71% of patients had a stable pattern and 29% had a varying pattern.

Crude analyses showed that constantly high hs-cTnI predicted a roughly three-fold increased risk for mortality and hs-cTnT a more than five-fold increased risk. After adjustments for age, sex, modality, vintage, cardiovascular disease and DM, hs-cTnI did not predict outcome while high hs-cTnT throughout the study predicted a roughly two-fold risk of death as compared to the reference group of constantly low hs-cTnT levels (Table 11).

Table 11. Multivariate Cox proportional hazard ratios (95% CI) for death by troponin variability groups
<table>
<thead>
<tr>
<th></th>
<th>Crude</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 3</th>
<th>Model 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low hs-cTnT</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Low-middle</td>
<td>0.80</td>
<td>0.74</td>
<td>0.75</td>
<td>0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>hs-cTnT</td>
<td>0.37 - 1.72</td>
<td>0.34 - 1.61</td>
<td>0.34 - 1.62</td>
<td>0.33 - 1.59</td>
<td>0.33 - 1.60</td>
</tr>
<tr>
<td>Middle hs-cTnT</td>
<td>1.20</td>
<td>1.08</td>
<td>0.86</td>
<td>0.80</td>
<td>0.85</td>
</tr>
<tr>
<td>Middle high</td>
<td>0.57 - 2.53</td>
<td>0.51 - 2.29</td>
<td>0.40 - 1.86</td>
<td>0.37 - 1.75</td>
<td>0.39 - 1.85</td>
</tr>
<tr>
<td>hs-cTnI</td>
<td>2.07</td>
<td>1.48</td>
<td>1.13</td>
<td>1.25</td>
<td>1.21</td>
</tr>
<tr>
<td>Low-middle</td>
<td>1.69</td>
<td>1.54</td>
<td>1.35</td>
<td>1.33</td>
<td>1.19</td>
</tr>
<tr>
<td>high hs-cTnI</td>
<td>0.62 - 4.62</td>
<td>0.56 - 4.23</td>
<td>0.48 - 3.80</td>
<td>0.45 - 3.93</td>
<td>0.40 - 3.52</td>
</tr>
<tr>
<td>High hs-cTnI</td>
<td>3.26</td>
<td>2.39</td>
<td>1.7</td>
<td>1.57</td>
<td>1.40</td>
</tr>
<tr>
<td>(1.84 - 5.80)</td>
<td>(1.31 - 4.37)</td>
<td>(0.89 - 3.26)</td>
<td>(0.81 - 3.05)</td>
<td>(0.72 - 2.72)</td>
<td></td>
</tr>
<tr>
<td>Low hs-cTnT</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
<td>1.0 (ref)</td>
</tr>
<tr>
<td>Low-middle</td>
<td>2.49</td>
<td>2.28</td>
<td>1.95</td>
<td>1.60</td>
<td>1.45</td>
</tr>
<tr>
<td>hs-cTnT</td>
<td>1.13 - 5.48</td>
<td>1.02 - 5.08</td>
<td>0.87 - 4.40</td>
<td>0.71 - 3.61</td>
<td>0.64 - 3.29</td>
</tr>
<tr>
<td>Middle hs-cTnT</td>
<td>2.66</td>
<td>2.64</td>
<td>2.35</td>
<td>2.01</td>
<td>2.07</td>
</tr>
<tr>
<td>Middle-high</td>
<td>1.26 - 5.59</td>
<td>1.22 - 5.71</td>
<td>1.08 - 5.08</td>
<td>0.93 - 4.33</td>
<td>0.96 - 4.46</td>
</tr>
<tr>
<td>hs-cTnT</td>
<td>4.33</td>
<td>3.53</td>
<td>2.84</td>
<td>2.22</td>
<td>1.84</td>
</tr>
<tr>
<td>(2.04 - 9.17)</td>
<td>(1.64 - 7.60)</td>
<td>(1.28 - 6.32)</td>
<td>(1.00 - 4.93)</td>
<td>(0.83 - 4.10)</td>
<td></td>
</tr>
<tr>
<td>Low-middle</td>
<td>2.26</td>
<td>2.78</td>
<td>1.77</td>
<td>1.16</td>
<td>1.08</td>
</tr>
<tr>
<td>high hs-cTnT</td>
<td>0.50 - 10.21</td>
<td>0.61 - 12.68</td>
<td>0.36 - 8.57</td>
<td>0.24 - 5.71</td>
<td>0.22 - 5.39</td>
</tr>
<tr>
<td>High hs-cTnT</td>
<td>5.65</td>
<td>4.19</td>
<td>3.02</td>
<td>2.26</td>
<td>2.09</td>
</tr>
<tr>
<td>(2.92 - 10.92)</td>
<td>(2.10 - 8.35)</td>
<td>(1.49 - 6.12)</td>
<td>(1.11 - 4.62)</td>
<td>(1.03 - 4.26)</td>
<td></td>
</tr>
</tbody>
</table>

Note: Model 1, adjusted for age, sex, vintage, modality; Model 2, Model 1 + ischemic heart disease, peripheral vascular disease, congestive heart failure, diabetes; Model 3, Model 2 + protein-energy wasting; Model 4, Model 3 + high-sensitivity C-reactive protein at baseline. Low, values within low tertile of troponin; middle, values within middle tertile; high, values within high tertile; low-middle, values within low and middle tertiles; middle-high, values within middle and high tertiles; low-middle-high, values within low, middle and high tertiles. Abbreviations: CI, confidence intervals; hs, high-sensitivity; cTnT, cardiac troponin T; cTnI, cardiac troponin I; ref, reference.

Our results support previous data on CHF patients comparing sensitive TnI and hs-cTnT where the latter had a better performance, predicting mortality. Mallamaci et al. showed an adjusted risk of 2.39 (CI 1.13-5.06) for a high cTnT tertile compared to a low in stable, prevalent dialysis patients without previous CHF, which is similar to our findings for patients constantly within the high tertile. Khans meta-analysis of 28 studies including 3,900 ESRD patients showed a cTnT cut-off point of >0.1 ug/L giving a relative risk of 2.64 for all-cause mortality. Data of cTnI from that study were weaker and more diverse. A study on 238 HD patients with a similar median and tertiles of hs-cTnT showed that hs-cTnT is a strong predictor for cardiovascular and all-cause death, although not adjusting for as many comorbidities. Most prognostic studies on troponins in ESRD have been cross-sectional. The serial data presented in our work together with detailed phenotyping is what differs it from many others.
The two troponins correlated moderately and, as mentioned before, may have some difference in pathophysiology and prognostic values. hs-cTnT has a stronger inverse correlation to eGFR in chronic kidney disease compared to hs-cTnI. \(^1\) \(^{91,307,308}\) Studies show differences in half-life, stability, and clearance (both renal and by dialysis). \(^1\) \(^{93,194,309-311}\) Since both are markers of myocardial injury but not perfectly correlated, there is a possibility of added information using both measures. A study on troponins in patients with atrial fibrillation showed an added prognostic value for cardiac death and cardiovascular events when hs-cTnI and hs-cTnT were both above median, separately they had similar prognostic values to each other. \(^3\) \(^\) \(^\) \(^1\) \(^\) \(^\) Additional data (not shown in Paper IV) from our study confirm this for patients with all levels in the high tertile (both hs-cTnI and hs-cTnT), HR 3.2 (95% CI 1.05-9.72) (Figure 19).

### 4.7 MARKERS OF INFLAMMATION AND CARDIOVASCULAR DISEASE

In the papers presented, the markers in focus have in common a vast variability in prevalent patients on dialysis. Hence, more information can be collected from repeatedly measuring levels of hs-CRP, IL-6, NT-proBNP, hs-cTnI, and hs-cTnT in the...
circulation of dialysis patients than evaluating a single value. Some problems are imposed when describing variability. The CV is good and intuitive for describing repeated values and variation over time. A problem is that CV is based on the mean and SD. With skewed variation, for example of CRP measurements, the mean will be higher than the median and extreme values affect the mean (and hence the CV) disproportionally.

The same multivariate method was used in all papers for estimating factors related to the outcome of variation in markers of CVD and inflammation. Two factors were associated with all the markers; higher age and PEW. The strength of the mixed model used is its ability to take into account every single value and describe the relation between various factors (both fixed and changing) and the outcome. The model allows for inclusion of subjects that may have some values missing and does not call for imputation. A limitation of the model is that it assumes the values to be independent of the time when they are measured, although not assuming serial values from the same individual to be independent of each other. Using logarithmic values in the mixed model means the extremes or outliers are lost. This has two sides, one is the true variability is not expressed in the model, the other is the extremes do not skew or dominate the results. The ICC received from a mixed model gives information on the proportion of overall variability being explained by within subject fluctuations as opposed to between subject fluctuations. In the presented data within-person changes caused 30% of hs-CRP (paper II), 37% of IL-6 (paper II), 14% of NT-proBNP (paper III), 26% of hs-cTnI, and 13% of hs-cTnT overall changes. The ICC does not express which associated factors adjusted in a multivariate model are more related to within-person compared to between-person variation. In general, analyzing variability is a way to increase insight of which possible conditions are related to markers such as the ones assessed here.

All markers presented (CRP, IL-6, NT-proBNP and troponins) have some prognostic value. After correcting for various factors this value diminishes which doesn’t mean they are clinically insignificant for prognosis. They may be an “alarm system” guiding the clinician to some underlying and important factors that may be causing the production or release of these markers leading to further investigations and possibly treatments. The value of repeated measurements to predict survival is showed in Paper I where median hs-CRP predicted survival better than a baseline (random) value and in
a different way in Paper III and IV where survival differs according to constantly low or middle values as compared to variable values.
5. CONCLUSIONS

• There is great short-term variability in hs-CRP and IL-6 within and between individuals on HD and PD. This is in relation to clinical events, but also comorbidity, PEW, age, sex and dialysis modality.

• Repeated measurements of hs-CRP are superior to single measurements in predicting survival of HD patients.

• The short-term variability of NT-proBNP is great within and between individuals on HD and is associated with comorbidity, PEW, inflammation and age.

• High levels of NT-proBNP during three months predict poor survival in HD patients, but not significantly when adjusted for clinical characteristics including PEW.

• In HD and PD patients that do not express signs of acute cardiac ischemia, there is short-term variability of both cardiac troponin I and T associated with age, sex, CHF, and PEW.

• hs-cTnT is a stronger predictor of survival than hs-cTnl in dialysis patients.
6. ACKNOWLEDGEMENTS

Writing this thesis is not a solo accomplishment. I am indebted to many people for helping me along the way. I want to thank you all.

My utmost respect and gratitude to all the individuals on dialysis participating in these studies. Without their engagement to improve the health of themselves and others by taking part in research there would be no study results.

My main supervisor Peter Bárány, for showing me that one can be a devoted clinician and scientist at the same time, each role strengthening the other. For getting me involved in research and encouraging me along the way. You have shown support at very crucial moments along the way, even in the face of large obstacles.

My co-supervisor Peter Stenvinkel for including me in this project from the start. For having such a positive attitude, I admire your use of exclamation points, it’s very encouraging!

My other co-supervisor Juan Jesús Carrero-Roig, for sharing your experience in manuscript writing, always quick to respond.

Tony, co-author and statistical wizard, for making me laugh while discussing statistics, that’s a gift! For reminding me from day one of the responsibilities of scientific work.

Olle Heimbürger, colleague, co-author and former boss. You hired me to Huddinge in the first place, otherwise I would not have had this opportunity at all. For a lot of great advice along the way and for pushing me to move forward in your very own supporting and candid way.

Karolina Szummer, co-author and friend, for a lot of friendly advice on science and manuscript writing and for great collaboration, it is such fun working with you.

Sigrún H. Lund, co-author and friend, for stepping in with invaluable statistical advice at a crucial moment and for all the great energy!

To friends and colleagues at Njurmedicin Huddinge whom I miss not working and laughing with anymore – Peter Hemmingsson, Annette, Maarit, Niklas, Hadi, Ulf, Marie H, and many more. To friends from other clinics at Karolinska, Huddinge who made it seem like a good idea to become a PhD and working clinically at the same time; Christian Unge, Karin Hellgren and especially Emilia Titelman, who convinced me that defending my thesis would be great fun 😊

My colleagues at the Nephrology department, Landspítali, for receiving me so well in 2012 and for stepping in when I needed time off for science, my boss Runólfur for giving
me the time off, and special thanks to Margrét Birna and Hrefna, my roommates at Meyjarskemman, for a lot of laughs and motivational talks on research and things.

Collaborators in this work both at the nephrology department and outside; especially Åsa Lindé and Björn Anderstam, always so helpful. Christian Löwbeer, co-author, for your great engagement in our work. Evianne Larsson for valuable help with all the administration necessary, so important in my long-distance situation.

Unnur, Arna and Dóra at Public Health Sciences, University of Iceland, for letting me take part in your research environment and for giving me the access to an office, (and lots of good coffee), all crucial things for finishing my work.

My ASA-sisters, my friends, so clever and so fun. You made me laugh through the whole process. Gunna, my partner in crime at Huddinge and in thesis-writing – you are an incredible motivator! Arna, who approaches every situation with logic, positive attitude and ways to move forward. Sigga and Guðrún for helping me focus on celebrating ones accomplishments.

To my dear friend Dísa for all the advice and you-go-girl attitude in science and in life. My friend Ásgeir, pushing me in thesis-writing as if pushing me to climb up a mountain.

To my good friends Ingibjörg, Andri and Kata for all the interest and enthusiasm in the progress of my work. To my Cecilia and my Tobba, for your friendship.

To Signý Vala for laughs and pep-talks at and outside the hospital. In the fall we will really have something to celebrate at our after-works! To the rest of doktorander anonymous, Steinunn, Silla and Lóa – girl power goes a long way in science! To Sigga Björns, my friend at Forskarskolan and outside, for good advice along the way.

My friends Gunnar and Þorvarður, for the interest you´ve shown in my work and for letting me gain from your knowledge in science, technology and stuff.

My friend and personal photographer Diddi, for shooting the front cover of this thesis, always ready to help. Hadda Fjóla, friend and amazing artist, for letting me use your beatiful work to complete mine.

The rest of the Icelandic gang in Stockholm, all part of this journey that were our 10 years there. Sara, Kolla, Helgi, Jóí, Einar, Alfred, Björk, Inga and many more.

My sister Drífa, for motivational talks on deadlines, imposter syndrome and so much more. For coming at me with solid counter-arguments when I´m convinced I can´t pull something off. Sometimes it seems you´re the big sister.
My mother Sigga for raising me to critical thinking and to think I could do it all, with a little help. Your faith in me is endless, I feel it.

My father Jón who encouraged me to seize the possibilities I could find in doing science in Sweden. You make it seem intriguing to combine clinical work, science and social activities.

My parents-in-law, Ásta and Kristinn, for believing in me and for being a part of our whole journey, always ready to help in every way you can.

My extended family, Linda for helping me keep an eye on the prize, Ragnheiður, Gunna, Berta, Ögmundur, Gunní, Ólöf Andra, Hrafnkell and Silja, what an amazing and supporting family I have.

My son Kristinn, for realising this work was important to me and helping out in many ways, I’m sure you can find a reference to football in here somewhere...! My daughter Katla, for all the help and for pushing me, you inspire me to inspire you © My daughter Vala, for all the hugs and positive attitude; “if this was a contest you would be winning mom!” Love you all to the moon and back.

My Sigurður Yngvi, you know how I feel, this thing can’t go wrong. This happened with you and because of you. Thank you. 8
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