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PROGNOSIS AND PROGRESSION IN CHRONIC KIDNEY DISEASE

EPIDEMIOLOGICAL STUDIES ON RISK FACTORS FOR DISEASE, DECLINE IN ESTIMATED RENAL FUNCTION AND MORTALITY

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ABSTRACT

Chronic kidney disease (CKD) is a life threatening condition with high risk of pre-term death and need for dialysis. The population prevalence of CKD has been estimated to 10-13% for both Europe and the USA. We investigated if analgesic use, occupational lead exposure and other patient characteristics were related to decline in kidney function in a population-based cohort of 920 patients with CKD. We also studied how survival related to timing of dialysis initiation.

The inclusion took place in Sweden between May 20 1996 and May 31 1998. All Swedish-born patients, 18-74 years old, who had been tested with a serum creatinine ≥300µmol/l for men and 250µmol/l for women for the first time were included and interviewed. At three occasions, during 1996-1998, age and sex-matched controls were included from the general population for comparison. Through linkages with the Swedish Renal Registry, the Swedish Population and Cause of Death Registry, and the patients' medical records we followed these patients until either death, start of renal replacement therapy (RRT [dialysis or transplantation]) or Dec 31 2005.

At the end of the follow-up 756 patients had initiated RRT while 46 were still alive and without RRT. After one and three years, the proportion of patients alive and without RRT was 64% and 29%, respectively. The mean unadjusted decline in estimated glomerular filtration rate (eGFR) was 9.0ml/min/1.73 m^2 per year, while the median decline was 5.1ml/min/1.73 m^2 per year. Younger patients, those with higher blood pressure, and more albuminuria had a faster decline in glomerular filtration rate. However, patients with regular acetaminophen or aspirin use progressed with -5.1/-4.4 ml/min/1.73m^2 compared to -5.3/-5.1 ml/min/1.73m^2 among non-regular users. There was no difference in progression rate among patients with a high lifetime cumulative analgesic use compared to non-exposed. We could not detect any significant risk of CKD with occupational lead-exposure (OR 0.97, 95% CI 0.7-1.4). Neither did patients who had been occupationally exposed to lead differ in progression rate or risk for RRT compared to those who had never been lead exposed.

Mortality was high both before and after dialysis, one and five-year survival was 97% and 61%. Compared to the general population the adjusted standardized mortality ratio was 8.3 (95% CI 7.5-9.2). Patients with diabetic nephropathy, low body mass index, and high co-morbidity score, blood pressure, and albuminuria had a significantly higher risk of death. Mortality increased by eGFR and progression rate. The HR (death) for eGFR <7.5 ml/min/1.73 m^2 was 4.65 (95% CI 1.28, 9.49) compared to non-RRT patients with eGFR 7.5-10ml/min/1.73 m^2. After dialysis start mortality increased further; the HR for patients who had started dialysis was 2.64 (95% CI 1.80, 3.89) relative to patients who had not yet started dialysis. However, timing of dialysis initiation was not associated with survival. The HR for patients initiating dialysis with an eGFR <7.5 ml/min/1.73 m^2 was 0.84 (95% CI 0.64-1.10) relative to those who started dialysis at higher eGFR. In summary, most patients with Stage 4 or 5 CKD progress to RRT, and the factors that most importantly affect the progression rate are age, blood pressure and proteinuria. Mortality is very high in this population, relates to eGFR and does not improve after initiation of dialysis.

LIST OF PUBLICATIONS

I. The natural history of chronic renal failure: Results from an unselected, population-based inception cohort in Sweden
Evans M, Fryzek JP, Elinder C-G, Cohen SS, McLaughlin JK, Nyrén O, Fored CM. *Am J Kidney Dis* (2005) 46:(5) 863-870

II. Acetaminophen, aspirin and progression of advanced chronic kidney disease
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III. Occupational lead exposure and severe CKD in a Swedish population-based investigation.
Evans M, Fored CM, Nise G, Bellocco R, Nyrén O, Elinder CG. *Am J Kidney Dis* (2010) 55:(3) 497-506

IV. No survival benefit from early start dialysis in a large, population-based inception cohort of CKD-patients in Sweden
Evans M, Tettamantti G, Bellocco R, Nyrén O, Fored CM, Elinder C-G. *Accepted for publication, J Intern Med 100624*

Every exit is an entrance somewhere else

-Tom Stoppard

CONTENTS

LIST OF ABBREVIATIONS

AAC	Abdominal aortic calcification
ACE	Angiotensin Converting Enzyme
ARB	Angiotensin II Receptor Blocker
BMI	Body Mass Index (kg/m^2)
CCI	Charlson co-morbidity index
CI	Confidence interval
CKD	Chronic kidney disease
ESRD	End-Stage Renal Disease. Defined as the condition/renal function when RRT is needed.
eGFR	Estimated Glomerular filtration rate ($ml/min/1.73m^2$)
HDL	High density lipoprotein
HR	Hazard ratio
IGF	Insulin-like growth factor
IL	Interleukin
KDIGO	Kidney Disease Improving Global Outcomes
KDOQI	Kidney Disease Outcomes Quality Initiative
LDL	Low-density lipoprotein
MDRD	Modification of Diet in Renal Disease (a study and an equation)
NAG	N-acetyl-β-D-glucosaminidase
NKF	National Kidney Foundation
OR	Odds ratio
OTC	Over-the counter
PbB	Blood lead level
PEW	Protein energy wasting
PTH	Parathyroid hormone
RAS	Renin Angiotensin System
RCT	Randomized controlled trial
RR	Relative risk
RRT	Renal replacement therapy. Any method to replace kidney function. Usually hemodialysis, peritoneal dialysis or kidney transplantation
S-Cr	Serum creatinine
TGF	Transforming growth factor
TNF	Tumor necrosis factor
SD	Standard deviation

1 GENERAL INTRODUCTION

Chronic kidney disease (CKD) is a worldwide killer. Not only does a poor renal function cause patients to die of uremia, but there is also a strong association between CKD and cardiovascular disease. This association, which is present also in earlier stages of CKD, has increased the awareness of the importance of a normal kidney function. In industrialized countries, most patients with renal failure receive renal replacement therapy (RRT) with dialysis or kidney transplantation. In many developing countries, uremia is lethal because of the lack of economy and the necessary infrastructure needed to support any type of RRT. Although countries in the industrialized world are willing to pay for dialysis, the cost is increasing every year. One reason may be the increasing RRT demand. In the United States the incidence of end-stage renal disease (ESRD) was 354 per million population-years in 2007 compared to 300 million per population-years in 1996 and 150 million per population-years in 1986.[1] The incidence is increasing more among the elderly (10.4% annual increase for those age 75 years and older and 5.5% annual increase for those age 20-44 years). The overall incidence in Europe was 125 per million population-years in 2006 compared to 110 per million population-years in 1997.[2, 3] The annual increase was estimated to 4.8% during the 1990's [4] but seems to have stabilized recently at 0.6%. The stabilization is mainly due to lower incidence rates among the elderly.[5] In Sweden, the overall incidence was 117 per million population-years in 2006 and 115 per million population-years in 1997.[6] The RRT prevalence has increased even more during the latest decades; the annual increase in Europe is estimated to 2.7%.[5] The reason may be that survival has improved overall in RRT, and thereby treatment duration. Another reason may be a trend to initiate RRT at higher eGFR, which could lead to higher apparent incidence rates [7] and higher prevalence.

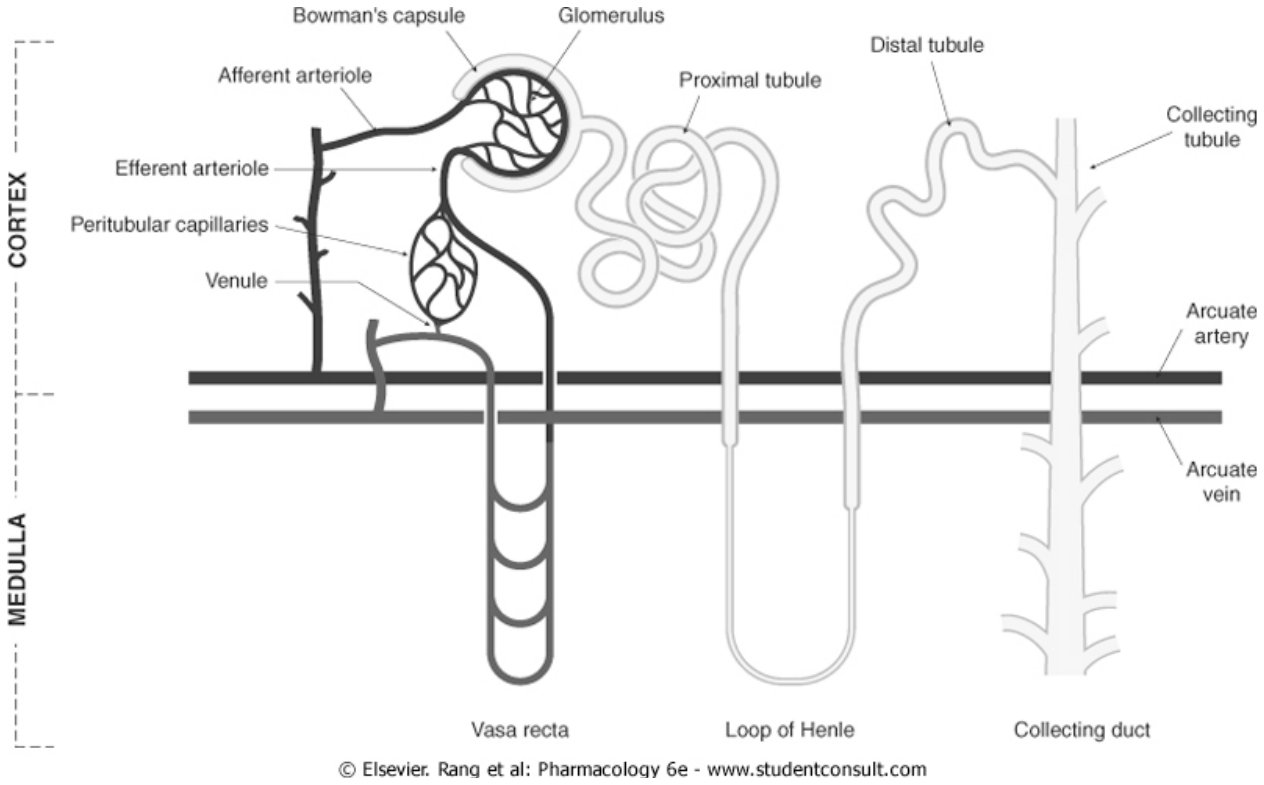
1.1 BASIC KIDNEY FUNCTION

The kidneys are central in both fluid management and the removal of toxic waste products (uremic toxins). The blood pressure and water balance is regulated through direct effects in the renal vessels (auto regulation, oncotic and hydrostatic pressure gradient) and through the renal endocrine system called the renin-angiotensin system (RAS). The kidneys produce the hormone erythropoeitin needed to stimulate the red blood cell production. They also express the enzyme 1,25 hydroxylase, which converts inactive D-vitamin to active D-vitamin. [8]

The renal blood flow is approximately 20% of the cardiac output (1.1-1.3 L/min). The blood volume is filtered through the renal capillaries and the renal glomerulus's to form the primary urine. About 20% of the plasma is filtered; the normal glomerular filtration rate (GFR) is approximately 125 ml/min. The primary urine is transported in the tubular system, in which most of the water and electrolytes are re-absorbed to form the final urine. The functional unit of the

kidney (the glomerulus's and its connecting tubular system) is called "the nephron". [9]

Figure 1. Schematic figure of the nephron



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Activation of the RAS is essential in renal diseases and hypertension. The renal hormone Renin is secreted from the juxtaglomerular cells near the afferent arteriole of the glomeruli. Renin promotes the transformation of angiotensinogen to angiotensin I. Further conversion to angiotensin II is enhanced by the enzyme ACE (angiotensin converting enzyme) in lung and endothelial cells. Angiotensin II activates the Angiotensin I receptor. The result is vasoconstriction, stimulation of sympathic nerves, salt and water retention, resulting in increased blood pressure, and decreased natriuresis.[10]

1.2 CLASSIFICATION OF CKD

The terminology for patients with declining renal function has varied over time and situation. Classification of renal diseases used to be categorized by the primary cause. In 2002, the National Kidney Foundation and Kidney Disease Outcomes Quality Initiative (NKF/KDOQI) published their guidelines with the purpose to unify the classification and definition of CKD.[11] The new

classification system is based on the level of glomerular filtration rate (GFR). The accepted definition of CKD is kidney damage for ≥3 months, defined by structural or functional abnormalities of the kidney (pathological abnormalities or abnormalities of imaging or the composition of blood or urine), *with or without* decreased GFR. CKD is also defined as GFR <60 ml/min/1.73m for ≥3 months, *with or without* kidney damage. There are currently five stages of CKD (Table 1).

Table 1. Definition and classification of CKD stages

Stage	Description	GFR (ml/min/1.73m^2)
1	Kidney damage with normal or ↑GFR	>90
2	Kidney damage with mild ↓ GFR	60-89
3	Moderate ↓ GFR	30-59
4	Severe ↓ GFR	15-29
5	Kidney failure with or without dialysis	<15 (D+/D-)

Based upon the current classification system, the overall population prevalence of CKD has been estimated to 10-13%.[12, 13] The prevalence is 3.1%, 3.4%, 4.5%, and 0.16% for Stage 2-5 respectively. This prevalence is similar in Norway and the United States in spite of the much higher incidence of ESRD in the United States. Age is strongly related to likelihood of having CKD, 17.9% and 0.71% of the subjects in the oldest age category (>70 years) had CKD stage 4 and 5, compared to 0.2% and 0.02% among 20-39 year olds.

Recently there has been a debate because some nephrologists believe that the current classification system over- and misdiagnoses CKD.[14-17] Observations seem to indicate that the reported prevalence of CKD in the general population is too high compared to the observed incidence of ESRD.[14] A recent meeting has suggested both a split of stage 3 at GFR 45 ml/min and stratification by albuminuria, because of its strong relationship to overall and cardiovascular mortality and better prediction of progress to ESRD.[18]

1.3 END-STAGE RENAL DISEASE

As renal function declines, it is eventually time to start RRT (hemodialysis, peritoneal dialysis or kidney transplantation). The decision to start is usually based upon a combination of the GFR, the patient's uremic symptoms (e.g. nausea, weight loss, vomiting, itching, diarrhea, fluid retention) and the patient's and doctor's preference. The US NKF/KDOQI guidelines from 2002 state that dialysis should be considered when Kt/V_{urea} is 2.0, which approximates a GFR of 10.5 ml/min.[11] In the up-dated version from 2006 it is suggested that dialysis

could start even at GFR above 15 ml/min if needed.[19] European guidelines state that dialysis should be prepared when GFR is 8-10 ml/min and initiated no later than eGFR 6 ml/min. [20] Before the introduction of these guidelines GFR was generally lower at dialysis initiation. Since then, the trend has been towards an earlier start. In the United States between 1996 and 2005, the percentage of patients starting at an eGFR above 10 ml/min/1.73 m^2 in the United States increased from 19% to 45%.[21] Although dialysis treatment is an option for almost everyone in the developed countries, some elderly patients with severe CKD are still treated conservatively. Overall, elderly patients who initiate dialysis show a better one year and two year survival compared to patients treated conservatively, but in selected groups with a higher overall co morbidity score, conservative treatment may be an equal alternative.[22, 23]

2 BACKGROUND

2.1 CAUSES OF CKD

There are many causes of CKD, the most common cause being diabetic nephropathy. [6] Diabetic patients represent 24% of incident ESRD patients and 19% of the prevalent dialysis population in Sweden. The second most common cause of CKD is hypertension. About 20% of the incident ESRD patients were classified as having nephrosclerosis, followed by glomerulonephritis (11%), hereditary diseases (9%), and pyelonephritis (4%). The cause of CKD is often unknown. In Sweden, the proportion of patients with unknown causes of uremia is 7%.[6] The primary renal disease diagnosis is most often based upon the pathology report of the kidney biopsy. However, there are many other factors contributing to the development of CKD; environmental toxins, occupational exposures, drug use, diet, smoking, and alcohol habits. These factors may have an additional negative effect on already established CKD, or contribute to the development of *de novo* CKD.

2.2 GLOMERULAR FILTRATION RATE ESTIMATIONS

Measurements of renal function or GFR are central in the classification of kidney diseases.[11] The gold standard for GFR measurement uses the fructose polysaccharide inulin.[24] Inulin is infused intravenously into the patient's blood and measured in blood and urine after a given time. The GFR is calculated using the formula

$$\mathbf{GFR = U_{in} \times V / P_{in}}$$

in which U_{in} is the urine concentration, P_{in} is the plasma concentration and V is the amount of urine excreted per time unit. There are also other methods that nowadays are more frequently used to measure glomerular filtration rate, like ^{125}I-iothalamate, ^{99m}Tc-DTPA, and iohexol.[25]

However, these methods are inconvenient and expensive to use in everyday clinical practice. Instead, endogenous substances such as creatinine, urea, and cystatin C are measured in urine or blood.[26] Serum creatinine (from the Greek *kreas*, flesh) is the most widely used marker. It is a cheap test included in almost every standard blood sampling for electrolyte balance. Unfortunately, there are several important limitations.[25] Serum creatinine (S-Cr) is a breakdown product of creatine phosphate in muscle and produced at a constant rate by the body under steady-state conditions. It is freely filtered in the kidneys, but a small amount is actively secreted and this proportion becomes more important if the filtration rate decreases. The S-Cr level is a general marker of the nutritional status of the patient. It can be affected by the individual muscle mass, recently ingested meat and the patient's fluid balance.

Several attempts have been made to produce a reliable equation to estimate GFR from the S-Cr value. The first more widely spread formula used to estimate renal function is the Cockcroft-Gault formula.[27]

Creatinine clearance = (140-age*bodymass [kg] /plasma-creatinine [mg/dL] * 72) *0.85 [if female]

The Cockcroft-Gault formula estimates the creatinine clearance, which is not corrected by body surface area, and thus the absolute value of the filtration rate. Due to the increased creatinine secretion, the creatinine clearance usually overestimates GFR when the GFR is low. In the year 1999, a new formula was presented which standardized the GFR to body surface area (ml/min/1.73m^2).[28] The formula was developed from the Modification of Diet in Renal Disease (MDRD) study, which consisted of 1,628 non-transplanted CKD-patients with non-diabetic renal disease.

eGFR= 186$*$[S-Cr]$^{-1.154}$ $*$[Age]$^{-0.203}$ $*$[0.742 if female]$*$[1.212 if African American]

The MDRD equation was re-expressed in 2006. The new equation is adjusted to a standardized creatinine calibration (eGFR = 175 x (Standardized S-Cr)$^{-1.154}$ x (age)$^{-0.203}$ x (0.742 if female) x (1.210 if African American) which gives approximately 5% lower values of the S-Cr.[29] There are many comparisons between the MDRD equation and the Cockcroft-Gault formula. Currently, the MDRD equation is more widely used in research, and it is believed to give a more accurate estimate of the GFR compared to the Cockcroft-Gault formula, especially for obese and older individuals.[26, 30] The bias compared to inulin clearance is quite large with both equations, but for CKD stage 5 it is less with the MDRD equation.[31] Lately, yet another equation has been developed. This is called the CKD-EPI and produces higher eGFR values in the high eGFR range (>60 ml/min/1.73 m^2), and lower eGFR values in the lowest range.[32]

The serum levels of Cystatin C (a proteinase inhibitor) depend on the glomerular filtration. Cystatin C is produced by all human cells and has become an increasingly more popular endogenous marker for GFR during the 21st century. It has better precision among patients with mild CKD and is less sensitive to food intake, age, sex, and body composition.[33] The validity of the GFR estimate is however highly dependent on the laboratory method and currently several estimating equations are used for the different analyses methods. An international calibrator is under development, which will improve the standardization of the analysis.[34]

2.3 RISK FACTORS FOR CKD AND DECLINE IN RENAL FUNCTION

The GFR deteriorates after the age of 20-30 years, with a normal rate of on average 1 ml/min per year. The decline in GFR (progression rate) may vary substantially between individuals. Among patients with CKD, the progression rate is usually faster than in the general population.[35, 36] Although it is

sometimes difficult to treat the primary renal disease itself, nephrologists try to reduce the progression rate, reduce albuminuria, and prevent the patient from need of dialysis.

Loss of renal function may be engraved by a number of causes: primary renal diseases, diabetes, hypertension, diet, nephrotoxic drugs and environmental toxins. The multi-factorial etiology of renal function loss is sometimes referred to as the "multi-hit hypothesis".[37] Loss of nefron mass results in glomerular hypertension in the remaining nephrons due to compensatory hyper filtration. [38]The consequence of renal hyper filtration is increased mesangial cell proliferation and over-expression of cytokines. A loss of more than 50% of the nephrons is associated with risk of developing proteinuria and severe CKD. The elevated intra-glomerular pressure correlates to increased urine albumin excretion. These proteins are reabsorbed in the proximal tubule, and induce activation of the intra-renal angiotensin converting enzyme and enlarged cytokine production.[39] Cytokines induce fibrosis, apoptosis and monocytic infiltration and ultimately leads to cell death and glomerulosclerosis. [40] It has been demonstrated that macrophage and myofibroblast infiltration in the interstitium correlates with the degree of renal function. [41] Macrophages produce more cytokines such as IL-6, TNF-α and express vascular endothelial growth factor while myofibroblasts express receptors for profibrogenic cytokines such as TGF-β and platelet-derived growth factor, which induces even more fibrosis and ultimately apoptosis.

2.3.1 Hypertension and proteinuria

One of the first risk factors found to increase the progression rate was hypertension. However, blood pressure is not merely a risk factor for progression; it is also a primary cause of CKD and a consequence of the disease. Due to the kidneys' close connection to the regulatory RAS system, up to 75% of CKD patients have elevated blood pressure. Lowering the blood pressure reduces the risk for progressive renal disease in CKD of different etiologies. [36, 42, 43] Fifteen years ago it was demonstrated that proteinuria is an important effect-modifier of hypertension-related progressive kidney disease among CKD patients.[36] The MDRD study showed that patients with greater baseline proteinuria excretion had a greater beneficial effect of a lower blood pressure target on the mean progression rate. This study, along with others formed the present KDIGO guidelines which have different goals for different levels of proteinuria.[19, 44] The target blood pressure for CKD patients with 0.25-1g proteinuria/24h is <130/80mmHg, a limit that is below the general goals for the treatment of hypertension. The target is even lower (<125/75mmHg) for patients with diabetes, and higher levels of proteinuria. Often multiple antihypertensive agents are required to reach the targets.

Proteinuria or albuminuria is also an independent risk factor for disease progression. Just as with hypertension, it is a consequence of CKD. Proteinuria may develop as a result of impairment of the renal glomerular membrane, or because of impairment of the renal tubular re-absorption. Whichever the starting

point, proteinuria induces complement activation and the inflammatory response in tubular epithelial and tubular-interstitial cells responsible for the progressive glomerulosclerosis.[39] In addition, in the general population albuminuria is an important marker for the risk of progressing CKD. The HR for progression to ESRD was 18.50 (95% CI 9.9-34.7) for individuals with microalbuminuria and 193.70 (95% CI 94.6-396.7) for individuals with macroalbuminuria compared to those with normal albumin/creatinine ratio.[45]

Angiotensin converting enzyme (ACE) inhibitors, and Angiotensin II receptor blockers (ARB), have emerged as corner-stone drugs in the treatment of both hypertension and proteinuria. First demonstrated to slow progression rate and prevent ESRD among patients with diabetes, these drugs were soon shown to have similar effects for CKD patients of different etiologies. [42, 46-48] Both ACE inhibitors and ARB lower the intra-glomerular pressure and reduce hyper-filtration, blood pressure, and proteinuria.[49] The protective effects seem to go beyond the blood pressure lowering, as has been demonstrated in comparisons with other antihypertensive agents.[43, 50] Some studies have investigated the effect of adding an ARB to an ACE-inhibitor and shown an additional effect on protein excretion and TGF-β levels[51] as well as progression rate in chronic glomerular diseases.[52]

2.3.2 Age and gender

Age contributes to the progress of CKD. However, the question of whether the definition for CKD should be different for older patients remains unanswered. Is CKD a part of the normal aging process, or is it a disease? Today, classification of CKD is the same regardless of age, although voices have been raised to change this.[16] Most studies have claimed that age is a risk factor for a faster progressing rate, but there are also results showing the opposite. Younger age has been associated with faster decline of GFR or progression to RRT in selected populations of type1 diabetes [53] and mesangio-proliferative glomerulonephritis[54]. In a study of patients with hypertension related kidney disease older patients progressed more slowly.[55] Male sex, on the contrary, has been established as a risk factor for faster progression in several epidemiological studies.[56] Among potential kidney donors, 20-50 years old, GFR fell by 8.7 ml/min/1.73 m^2 per decade for men but not for women.[57] The reason for this relationship is not obvious but differences in sex hormones have been suggested to affect renal function and progression rate.[58] It is however not clear if it is the lack of estrogen or the presence of testosterone that makes the difference. Overall, CKD is much more common among men than women. Men represent about 64% of the Swedish ESRD population in 2009.[6]

2.3.3 Obesity

Obesity is a risk factor for CKD, even if disregarding the closely associated diseases diabetes and hypertension.[59-62] High BMI is associated with a greater risk of developing nephropathy and proteinuria also among diabetics or hypertensives.[63, 64] Among subjects with non-diabetic metabolic syndrome,

waist circumference is an independent risk factor for the development of albuminuria.[65]Obesity may accelerate the progression rate among subjects with other types of primary renal diseases.[66] Reversely, weight reduction (after gastric bypass) has lead to a decline in proteinuria or stabilized GFR in small studies of both subjects with and without CKD.[67, 68] The patho-physiological disturbances seen in the kidneys with obesity are renal hyper-filtration and focal glomerulosclerosis.[69] The effects are probably mediated through vasodilatation of the afferent arterioles, leptin-induced cellular proliferation and stimulation of cytokines, and up-regulated RAS.[70, 71]

2.3.4 Smoking and alcohol intake

Smoking is now considered to be a risk factor for both the development, and for the progression of established CKD.[72, 73] The strongest associations have so far been noted among diabetics and hypertensives, [73, 74] but there are studies indicating that smoking is detrimental to renal function also in other primary renal diseases [75] and in the general population.[76, 77] In epidemiological case-control and cohort studies, smoking has been associated with albuminuria, rise in serum creatinine, CKD, and risk of ESRD.[72, 78-80] Smoking is one of the factors linked to lower socio-economic status responsible for the over representation of people with low educational level among ESRD patients. Other factors known to be influenced by socio-economic status and capable of modifying the relationship to CKD are presence of obesity, diabetes type II, alcohol use, occupational exposures, and dietary factors. The relationship between high alcohol consumption and CKD is not clear. Although one study showed increase in albuminuria [81] with heavy alcohol use, other studies have seen no effect [82] or favorable effects [81, 83] on the decline in GFR among healthy subjects.

2.3.5 Protein intake

It has been known for a long time that reduction in protein intake reduces uremic symptoms among patients with severe CKD. Before dialysis was invented and implemented, dietary treatment was the only option for most CKD patients. More than 50 years ago it was first suggested that a low-protein diet may be beneficial for patients with CKD.[84] Experiments in both animals and in humans has then demonstrated that a high protein load causes acute effects in the kidneys by increasing the renal blood flow and inducing hyper filtration.[38, 85-87] However, a long-term detrimental effect on the kidneys of a high chronic protein intake has been more difficult to show. In the Nurses health study, a high intake of animal protein was associated with presence of microalbuminuria but not decline in eGFR over 11 years. Two or more servings of red meat per week was associated with microalbuminuria (Odds ratio (OR) 1.51, 95% CI 1.01-2.26) compared to less than one serving per week.[88] Among CKD patients there have been several trials with low protein diets with diverse results. The MDRD study did not really show a convincing effect in the first publication.[89] In post-hoc analyses, results from that trial showed that protein reduction could have some effect on the progression rate among patients with severe CKD, but less effect in

earlier stages.[90] After that, there have been meta-analyses suggesting a beneficial effect of low protein diet in patients with moderate and severe disease.[91-93] The beneficial effect from protein restriction mainly appear to be a delay in time to dialysis rather than an actual decrease in the progression rate. The protein restricted diet possibly decreases the production and retention of uremic toxins, and thereby symptoms of uremia, prompting initiation of dialysis treatment. Today, protein restriction (0.6g/kg/24 hours) together with supplementary essential amino acids is used in the pre dialysis programs at many clinics.

2.4 RISK FACTORS FOR MORTALITY

2.4.1 Mortality among CKD patients

As in the general population, traditional risk factors such as smoking, dyslipidemia, diabetes, and hypertension predict mortality and morbidity among CKD patients in the early stages (1-3). However, for patients with severe kidney damage and patients with ESRD other risk factors (inflammation, endothelial dysfunction, protein-energy wasting (PEW), and vascular calcification) seem to play a far greater role in prediction of the cardiovascular risk.[94] A complicating fact is that the traditional risk factors are modified as renal function declines. Some of them, for example hypertension and volume-overload become increasingly frequent while others such as total cholesterol and protein intake decrease spontaneously.

Level of GFR is an independent risk factor for death. The increased cardiovascular risk starts already at GFR <75 ml/min and further increase as renal function declines.[95, 96] In the United States, the age and sex adjusted standardized mortality rate was 1.08 (eGFR 45-59 ml/min/1.73 m^2), 4.76 (eGFR 30-44 ml/min/1.73 m^2), 11.36 (eGFR 15-29 ml/min/1.73 m^2) and 14.14 (eGFR <15 ml/min/1.73 m^2).[97] In one study the relative risk (RR) of cardiovascular mortality associated with eGFR <70 ml/min/1.73m^2 was 1.68 (95% confidence interval [CI] 1.33-2.13) compared to eGFR >90 ml/min/1.73m^2.[98] In fact, the probability of dying from cardiovascular disease is much greater than the probability of progressing to ESRD for most CKD patients in early stages. [99, 100]

Albuminuria modifies the relationship between eGFR and mortality.[101] Albuminuria also seems to be an independent risk factor of death, cardiovascular events and heart failure regardless of the GFR.[102] The level of macroalbuminuria has been linked to increased mortality, but also microalbuminuria (<300mg/24h) increases the risk of fatal and non-fatal cardiovascular events regardless of renal function and presence of diabetes.[103] Lately it has been observed that the risk of death and cardiovascular events is increased also among individuals with cut-off levels less than the ordinary limits for microalbuminuria.[104] Since the relationship between urinary albumin-creatinine ratio and mortality is virtually linear it has been suggested that

microalbuminuria is more a marker of generalized endothelial damage than an expression for a kidney disease. [105] Chronic inflammation is also associated with mortality. Elevated markers of inflammation such as CRP and fibrinogen have been observed to correlate to fatal and non-fatal cardiovascular events among patients with Stage 3-4 CKD.[106] Notably, the CRP levels themselves do not seem to be a risk factor. Individuals with an elevated CRP due to gene polymorphism without the corresponding inflammation have not the same increased cardiovascular risk seen in inflammatory diseases. [107] Furthermore, there are studies showing that albuminuria and inflammation together have a greater association to hypertension, atherosclerosis, and metabolic abnormalities than albuminuria alone.[108-110] In the general population, the risk for all-cause mortality was four times greater among subjects in the highest quartile of both albumin-creatinine ratio and fibrinogen compared to the lowest.[111]

There are many plausible explanations for the link between reduced renal function, albuminuria, inflammation and mortality. Endothelial dysfunction seems to be one of the markers of cardiovascular injury that is of importance among CKD patients, and it has been linked to both albuminuria and inflammation.[112] Indeed, among CKD patients there is an imbalance between the levels of circulating endothelial cells and endothelial progenitor cells, which are mobilized from the bone marrow as a result of vascular injury.[113] Among CKD patients the number of endothelial progenitor cells is decreased which may cause impaired neo-vascularization of ischemic vascular tissue.[114] Surrogate markers of endothelial dysfunction, e.g. intracellular adhesion molecule 1, are strongly associated to mortality among ESRD patients.[115] Other factors, such as increased oxidative stress and the accumulation of post-synthetically modified proteins have also been suggested to promote CKD-associated inflammation.[94]

When GFR declines it is followed by a spontaneous decrease in protein and energy intake as well as falling serum total cholesterol and serum transferrin levels.[116] In normal skeletal muscle turnover, there is a balance between skeletal muscle synthesis and breakdown. In catabolic states and uremia the breakdown exceeds the synthesis which results in muscle wasting. The state of PEW seen among CKD patients is closely linked to both inflammation and cardiovascular outcomes.[117] Patients judged to be malnourished by subjective global assessment demonstrated higher CRP and fibrinogen levels as well as increased frequency of carotid plaques and calculated intima-media area.[117] The same malnourished patients also exhibit signs of more vascular endothelial activation, and associations to higher all-cause mortality[118]. A number of other factors promote PEW such as metabolic acidosis and excessive angiotensin II. Impaired insulin/IGF-1 signaling may also be of importance.[119] As S-Cr levels reflect muscle mass, these levels are generally lower among malnourished patients. Low S-Cr has been independently associated to mortality in epidemiological studies of patients initiating dialysis.[120]

Changes in the mineral and bone metabolism starts early in the course of renal function decline. Elevated levels of serum phosphate have been associated with increased mortality among CKD patients.[35] Prescence of vascular calcification

has been associated with serum phosphate within the normal range. Among Stage 3 CKD patients, each 1-mg/dl (0.32 mmol/l) increment in serum phosphate concentration was associated with a 21% greater prevalence of coronary artery calcification and 62% greater prevalence of mitral valve calcification.[121] An estimate of the vascular calcification is the abdominal aortic calcification (AAC) score, which relies on lateral lumbar radiographs. The AAC score is highly predictive of cardiovascular mortality in several studies including the general population and dialysis patients.[122, 123] [124]

2.4.2 Mortality among dialysis patients

Prevalent patients in dialysis, regardless of dialysis modality, have nearly seven times higher mortality compared to the general population. The expected remaining lifetime in US for a 50-54 year old patient in dialysis is 6.5 years, compared to 29 years for a person of similar age without ESRD. However, survival has improved over the years, adjusting for the aging dialysis population. Compared to 1993-1997, the 7-year survival has increased by 7.5% to 38%. [125] The overwhelming cause of death is cardiovascular disease, and this is especially noted for the elderly. Mortality is greatest during the third month after dialysis initiation. Although overall mortality has decreased among dialysis patients, mortality during the first 3-4 months is still higher today than during the 1980's.[125, 126] Traditional risk factors for cardiovascular disease and death are not obviously related to mortality and morbidity among dialysis patients. The reasons may be that many of these markers such as cholesterol levels, hypertension and obesity change along the disease trajectory because of the renal function decline.

The number of obese patients starting dialysis has increased and in the US today more than 30% have a BMI >30 kg/m^2 at initiation. The incidence of ESRD caused by diabetes has increased more than 55% among 30-39 year old African Americans since the year 2000.[126] Although obesity is associated with increased mortality in the general population, a paradoxical association between BMI and mortality has been found repeatedly among dialysis patients.[127, 128] The correlation between higher BMI and lower mortality has been observed across subgroups of diabetics, non-diabetics, ethnical groups, and dialysis modalities.[129] Furthermore, weight loss but not weight gain is associated with an increased risk of death.[130] On the other hand, the protective effect seems to be highest for obese patients with a normal or high muscle mass indicating that muscle protein also plays some role here.[131] Thus, PEW is a risk factor also among obese individuals in hemodialysis.[132] Obesity has been correlated to increased inflammation, oxidative stress and insulin resistance. Activated white adipose tissue increases the synthesis of pro inflammatory cytokines, such as IL-8, IL-6, IL-1, and TNF-α, while regulatory cytokines are decreased. In particular visceral fat correlates to cytokine levels, dyslipidemia and serum leptin levels.[133]

In contrast to the general population, the relationship with serum total cholesterol and low-density lipoprotein (LDL) cholesterol is the reverse. A low LDL-

cholesterol and total cholesterol is a prognostic marker for early death among hemodialysis patients.[134] The LDL particle arises when very low-density lipo protein (VLDL) is converted to an intermediate-density lipoprotein (IDL) and further to form LDL. High-density lipoprotein (HDL) is needed to perform the last step and formed from the IDL particle. In ESRD-patients, the clearance rate of both the highly atherogenic IDL [135] and LDL is slower, but since the formation of LDL also is reduced, there is an over-representation of IDL particles.[136] HDL-cholesterol is generally lower than normal in patients with ESRD, and the low values are associated with increased cardiovascular events just as in the general population.[137] Inflammation decreases the levels of both total cholesterol and HDL while LDL has been observed to increase following an acute infection.[138] Thus, chronic inflammation among patients in dialysis may attribute to their lower cholesterol levels, regardless of renal function.

The relationship between the uremic state and inflammation is even more pronounced among dialysis patients. Also in dialysis, inflammation is closely linked to PEW and death.[117] For many years, serum albumin was used as a proxy marker for malnutrition. Hypoalbuminemia has been a known risk factor for mortality among dialysis patients for a long time.[134] However, a low serum albumin is a poor nutritional marker as serum albumin decreases late in the course of starvation.[139] On the other hand, albumin is closely linked to inflammation, and when correcting for inflammation by using CRP, fibrinogen or IL-6, the relationship between serum albumin and mortality weakens.[140] It is believed that hypoalbuminemia among dialysis patients is caused by a lower albumin synthesis and a reduced possibility of down-regulating albumin degradation.[141] The intervention of hemodialyis may also contribute to the process as increased body protein breakdown has been observed but no subsequent increase in production during the hemodialysis session.[142] Apart from their effects on lipid metabolism, treatment with statins is generally believed to reduce inflammation and endothelial dysfunction among non-CKD patients. In a large randomized trial, there was however no significant effect of statin treatment on the rate of death, myocardial infarction or stroke among hemodialysis patients although both CRP and LDL-cholesterol levels were successfully lowered.[143, 144]

Another reason for the extra-ordinary cardiovascular mortality among patients with ESRD is their perturbed bone and mineral metabolism. Secondary hyperparathyroidism and reduced possibility of phosphorus elimination causes serum calcium, and phosphorus to rise. Observational studies show that a high plasma phosphorus and calcium are independent risk factors for cardiovascular events and all-cause mortality.[145] The relationship for parathyroid hormone (PTH) is more complex; both high and low values are associated with increased mortality. Dialysis patients who achieve the targets for calcium, phosphate and PTH have improved survival [146] Treatment includes the administration of active vitamin D and oral phosphate binders. Lately, the calcimimetic drug cinacalcet has shown favorable effects on both lowering of calcium, phosphate and PTH. [145] Observational studies have also demonstrated favorable effects on cardiovascular hospitalization, fractures, parathyroidectomy, and quality of

life with cinacalcet treatment.[147] The AAC score predicts mortality among dialysis patients. Although the score probably relates to early disturbances in calcium and phosphate metabolism, it is not directly associated with calcium, phosphate or PTH when investigating prevalent dialysis patients. [148] Variables linked to a higher AAC score were invariably age, dialysis vintage, and co-morbidity.

One of the factors considered having impact on survival for hemodialysis and peritoneal dialysis patients is timing of dialysis initiation. The reason is believed to be through known risk factors such as PEW, which increases when GFR declines. Other authors argue that the dialysis procedure itself increases the risk for inflammation and silent myocardial ischemia.[149] The earliest studies investigating the effect of timing of dialysis showed that early start (at higher GFR) was associated with improved survival compared to late start.[150, 151] However, when methodological issues were considered, it became obvious that lead-time bias may have affected the results of the first studies. Later epidemiological studies were in favor of dialysis start at lower GFR values. [152-155] However, it was found that timing of initiation was correlated to co-morbidity status and age; the results were thus subjected to a great probability of confounding by indication.[156, 157] The NKF/KDIGO Guidelines from 2002 based their recommendation on the limit for optimal dialysis dose, which was Kt/V 2.0 (approximately 10.5 ml/min)[19, 158] and the observation that nutritional values decreased as GFR declined. Knowing the strong relationship between PEW and mortality, they argued that the decision to initiate dialysis should be based upon a combination of renal Kt/V and nutritional status of the patient. The introduction of these guidelines made a big impression and led to a rising trend in GFR at dialysis start. [159] However, the guidelines have been questioned; in subsequent studies it became evident that the prognosis of patients in dialysis was not so much related to dialysis dose, as to residual renal function.[21, 160] In a large randomized control trial, it was found that neither dialysis dose, nor type of modality or membrane significantly affected mortality.[161] Recently, the results from a randomized controlled trial (IDEAL) investigating the timing of dialysis initiation on mortality was presented.[162] The study did not show any benefit of earlier dialysis start. However, the study also demonstrated the difficulty of performing such a trial. Although the investigators aimed at starting dialysis at 10-14 ml/min and 5-7 ml/min for early and late start respectively, 76% of the patients randomized to late start in fact started earlier, and 11% of the patients randomized to early start started later.

2.5 ANALGESIC DRUG USE AND CKD

Both acetaminophen (paracetamol) and phenacetin was discovered to have analgesic and antipyretic effects already in the 1880's. However, because of phenacetin's supposedly lower toxicity it was first introduced on the market as one of the compounds in "headache powder".[163] Phenacetin increased in popularity, and sales grew, especially in the footsteps of the large Spanish influenza pandemic of 1918-1919. Apart from its analgesic effects, it also had psychotropic effects, which was one of the causes behind the emerging analgesic abuse of phenactin-containing mixtures.

In 1953, it was first discovered that workers in a Swiss clock factory who abused analgesic drugs had a higher probability of hematuria, proteinuria and rise in serum creatinine.[164] Analgesic abuse was coupled to renal papillary necrosis, and interstitial nephritis. At the same time in Sweden, an increased incidence of death from uremia was noted in the Huskvarna and Jönköping areas. An investigation revealed that workers at the Huskvarna factory were affected to a large extent. Eventually, evidence was presented that pointed to the popular analgesic mixture "Dr Hjorton's headache powder" as the causative agent.[165] The powder contained a mixture of phenacetin, caffeine and phenazone. It was sold over the counter (OTC) and had reached a large popularity in the specific area. Phenacetin also had other side-effects, such as methemoglobinemia, anemia, and uroepithelial cancer[166, 167] and soon was banned in Sweden, followed by other countries. The renal damage caused by phenactein was named "analgesic nephropathy".

Later, in the 1980's, when it was noted that the incidence and prevalence of analgesic nephropathy did not decline as expected after the removal of phenacetin from the market, other types of analgesic drugs regained attention as causes of CKD.[168] In experiments, phenacetin alone had not demonstrated the expected toxic effects. It was the major metabolite, acetaminophen (paracetamol) that was accumulated in the renal papillae. Acetaminophen was suggested to increase free radical formation and thereby induce cell death. Aspirin, which also was a common compound in the analgesic mixtures, produced acute nephrotoxic effects and was suspected to contribute to renal damage.[168, 169] The term "analgesic-associated-nephropathy" was then introduced to refer to the possible negative effects by different types of analgesics on the progression and development of CKD.

One theory was that analgesic nephropathy was caused by the abuse of analgesic mixtures containing two or more analgesic substances plus caffeine.
This was later suggested by an expert ad-hoc committee of the National Kidney Foundation in 1996.[170] The mechanism behind the additive effect of analgesic mixtures was attributed to that aspirin caused reduced glutathione depletion of the tubular cells, hereby enhancing the acetaminophen-induced nephrotoxic effect by of reactive oxygen species formation. [171]Although some support was presented for this theory,[172-174] a peer-review in 2000 concluded that there

was not enough evidence for the association between analgesic mixtures and CKD.[175] Later, it was shown that the decrease in analgesic nephropathy was similar in both Australia and Belgium despite the fact that analgesic mixtures had overtaken the market in Belgium whereas in Australia single-substance drugs were used.[176] The Australian study showed that the incidence was actually decreasing and that the increased prevalence was caused by a greater number of older patients in dialysis. A recent autopsy study demonstrated that the morphological and pathological features of analgesic nephropathy has disappeared 20 years after the ban of phenacetin from the market.[177]

2.5.1 Acetaminophen

When phenacetin disappeared from the market, acetaminophen largely took its place as the most popular analgesic substance. Acetaminophen was first used as an analgesic drug already in 1893, but was not introduced to the US market until 1950 and in Australia in 1956.[178] The antipyretic action of acetaminophen is due to central inhibition of prostaglandins, but the analgesic effects are probably due to both central and peripheral actions.[179] Today, acetaminophen is the most sold OTC analgesic in Sweden with more than 40 Defined Daily Doses per 1000 inhabitants. [180]
Several epidemiological studies have investigated the relationship between acetaminophen and CKD. In almost every case-control study,[181-185] but not all, [186, 187]acetaminophen use was associated with a greater risk of developing either CKD or ESRD. Many of the earlier studies had problems due to residual confounding from phenacetin,[183, 184, 188-190] which was still in use in some countries. Other problems were methodological shortcomings such as recall bias, and most important prothopatic bias.[191] The cases in the studies had been selected late in their course of disease and analgesic use due to early symptoms from the renal disease itself or the primary cause (e.g. diabetes, rheumatoid arthritis), may have inflated the analgesic-CKD relationship. Patients late in the course of the disease may also have been advised to change their use of analgesic from aspirin or non-steroidal anti-inflammatory drugs, which are known to have acute effects in the kidneys, to acetaminophen. In a case-control study from our own research group, Fored et al found an association (OR 2.5, 95% CI 1.7-3.6) between acetaminophen regular use and risk of CKD, which was still present after the exclusion of analgesic use the closest 10 years before inclusion. [185]

Not many follow-up studies have analyzed the risk of declining renal function attributed to analgesic drugs *without* phenacetin. One large study in the US followed 4494 apparently healthy men over 14 years.[192] Analgesic use was assessed retrospectively by a questionnaire. There was no association between acetaminophen use (OR 1.02, 95% CI 0.55-1.90 among heavy users [>2500 pills] compared to never users) with >30 ml/min/1.73 m^2 decline in estimated GFR. However, in another study of 1697 healthy women there was a significant association between acetaminophen use and >30 ml/min/1.73 m^2 decline in GFR between 1989 and 2000 (OR 2.04, 95% CI 1.28-3.24). The OR increased with lifetime cumulative acetaminophen use. Analgesic use was registered retrospectively in 1999.[193]

2.5.2 Aspirin

The association between aspirin (acetylsalicylic acid) and CKD was explored due to the acute toxic effects seen when large doses of aspirin were administered in experimental trials[194, 195]**.** Aspirin, as well as non-steroidal anti inflammatory drugs act mainly through inhibition of prostaglandin synthesis, which is essential to regulate the renal blood flow[196]**.** Despite this, the overall epidemiological evidence of aspirin as a cause of CKD is weak[197]**.**
The case-control studies showing an increased risk of CKD with aspirin use are few.[185, 187, 189] In the case-control study by Fored et al regular use of aspirin had ha significant association to CKD (OR 2.5, 95% CI 1.9-3.3). The association was strongest among subjects with diabetic nephropathy (OR 2.9, 95% CI 1.9-4.5). However, the association failed to remain significant after the exclusion of aspirin use 10 years prior to inclusion.[185]

In the two longitudinal studies on healthy men and women there was no relationship between aspirin use and reduction in GFR.[192, 193] In fact, both studies presented OR's below one for this association, although non-significant. In the men's study the association was modified by presence of cardiovascular risk factors.

2.6 LEAD METAL EXPOSURE AND CKD

2.6.1 General lead toxicity

The heavy metal lead is a ubiquitous pollutant. It enters the body through ingestion or inhalation. The main source of environmental lead is from ingestion of contaminated food items and dust containing lead, secondary to lead emission into the atmosphere from smelters, incinerators and leaded gasoline.[198] Inhalation of lead in air may also contribute, especially if air concentrations of lead are high, for example during occupational exposure. Ambient air exposure to lead has decreased during the last decades mainly because of legislative actions aiming at removing lead in petrol. Blood lead levels has also decreased among workers occupationally exposed to lead.[199] Data from the NHANES study in the United States show a decline in the geometric mean blood lead (PbB) level in the general population from 13.1µg/dL in 1976-1988 to 1.6µg/dL in 1999-2002.[200, 201] Likewise, the average PbB concentration in the Swedish population has decreased considerably since the 1980's.[202]

Lead intoxication mainly gives rise to anemia, polyneuropathy, encephalopathy, and gastrointestinal symptoms. It is stored in the body in bone, and released during periods of increased bone turnover. The half-life in blood and soft tissues is about 30 days while the half-life in bone is 4-20 years.[203] Because of the short mean biological life in blood, PbB primarily reflects on-going exposure. However, if exposure to lead has been for long periods, giving rise to a high body burden of lead, PbB will remain elevated for extended periods.[204] To measure the accumulated lead (lead burden) one can perform a mobilization test using a

chelating agent. This is done by intramuscular or intravenous administration of $CaNa_2$ ethylene-diamine-tetraacetic acid (EDTA).[205]

2.6.2 Lead exposure and risk for CKD

In 1929 Nye first described a high incidence of young people with chronic nephritis in Queensland, Australia and linked it to the high frequency of childhood lead intoxication in the same area.[206] In later follow-up studies the children treated for lead intoxication showed a much higher age-adjusted mortality rate caused by chronic nephritis compared to the general population.[207, 208] Classical lead nephropathy, exhibited at toxic levels, is described to cause minimal proteinuria, a benign urinary sediment, hyperuricemia, and often hypertension. The kidneys are described as granular and contracted. Renal biopsies show tubular atrophy and interstitial fibrosis without cellular infiltration. The glomeruli are sclerotic and the arterioles often show intima proliferation and hyaline degeneration of the media. In the proximal tubules, acid-fast nuclear inclusion bodies, consisting of a lead-protein binding complex, can be seen.[209] (Figure 2) Increased urinary secretion of N-acetyl-β-D-glucosaminidase (NAG), lysozyme, and urinary α-1-microglobulin can be observed early among lead-exposed individuals.[210, 211]

2.6.2.1 Epidemiological studies

Occupational studies investigating lead exposure have been cross-sectional with few exceptions. In spite of all that has been reported on effects from acute or ongoing excessive exposure, there are only a few occupational studies where the GFR is significantly reduced.[212-214] Longitudinal studies provide no further evidence of lead causing CKD. The largest longitudinal study on 537 lead workers over 2.1 years showed no general effect of PbB (mean baseline 31.3 µg/dL) on creatinine clearance.[215] Mean creatinine clearance *increased* during the study period. In another longitudinal study of 30 lead workers over 10 years higher PbB at baseline was associated with a subsequent decrease in S-Cr, suggesting hyper filtration.[216]

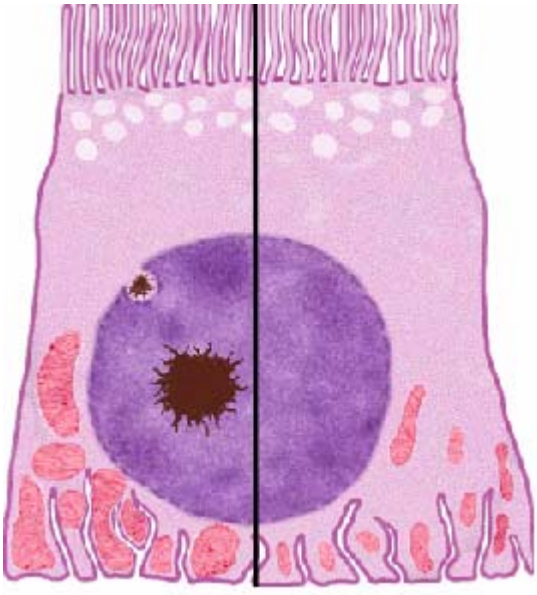
During recent decades several epidemiological studies have presented suggestive evidence that low levels of environmental lead cause kidney disease in large proportions of the general population.[200, 217] It has also been suggested that low levels of environmental lead exposure contribute to increased cardiovascular mortality and high blood pressure.[218, 219] Most of the general population studies are also cross-sectional. The majority of the studies with renal function outcomes use S-Cr or creatinine clearance, whereas some studies use Cystatin C.[220, 221] Although most of these studies showed an association between higher PbB and lower creatinine clearance or estimated GFR,[201, 217, 218, 220, 222] two studies (of which one was on children) instead presented an inverse correlation,[223, 224] one found an association only among hypertensives, [200]and the other lacked any association.[225]

Only three case-control studies investigating lead exposure and its association with CKD are published. One study measuring lead by the expert rating method found that the odds ratio (OR) for CKD associated with lead exposure was 2.1 (95% CI 1.2-4.4).[226] Another similar case-control study found no such association.[183] In a small study, 55 patients with end-stage renal disease had significantly higher PbB compared to 53 age and sex-matched controls whereas mean tibia lead did not differ significantly.[227] The OR for end-stage renal disease associated with tibia lead ≥20 µg/g was 1.6 (95% CI 0.6, 4.4) and thus showed a non-significant positive association.

2.6.2.2 Experimental studies

Animal studies evaluating lead exposure and renal function present similar results compared with the occupational reports in showing tubular effects and nuclear inclusion bodies, and to some extent glomerular hyper filtration.[228, 229] Rats fed with high doses of lead (mean PbB 125 µg/dL) increased their GFR at 3 months compared to control rats, but had lower GFR at 6 and 12 months.[230] The kidneys had proximal tubule inclusion bodies, interstitial fibrosis, tubular atrophy, and focal glomerular sclerosis. Rats fed with lower doses of lead (mean PbB 29 µg/dL) presented with glomerular hyper filtration and increased NAG secretion after 3 months, but after 12 months the renal biopsies showed almost no morphological changes. Chelate treatment improved GFR *both* among lead-fed rats and control rats.[231, 232]

Figure 2. Normal renal tubular cell to the right. On the left-hand side mitochondrial swelling and a lead-inclusion body in the cell nucleus.



3 AIMS

To define risk factors for start of renal replacement therapy and for death among unselected patients with severe chronic kidney disease

To define risk factors for decline in estimated glomerular filtration rate among patients with severe chronic kidney disease

To establish to what extent analgesic drug use affects decline in glomerular filtration rate among patients with chronic kidney disease

To determine to what extent occupational lead exposure is a risk factor for chronic kidney disease development, or increase in decline of glomerular filtration rate among patients with established chronic kidney disease

To establish how “timing of dialysis initiation” is associated with mortality in patients with chronic kidney disease

While performing these studies, to learn more about epidemiological methods

4 METHODS

4.1 SETTING

The study took place in Sweden where an important characteristic of the community is the use of personal identification numbers. A unique personal identification number is assigned to every Swedish citizen at birth or immigration and is consistent through all governmental systems, such as the tax registry, population registry, health care, and the school system. This enabled us to identify the study base, which consisted of all Swedish-born citizens living in Sweden between May 20 1996 and May 31 1998 (n=5.3 million).

Another characteristic of Sweden is that health care is administered by a county council, "Landstinget", which primarily is funded by taxes. The fees paid at each visit are low and affordable by most citizens. Above a certain low level health care and prescribed drugs are free of charge. Privately financed health care initiatives were few when the study began, but have become more common since 2000. Private health care insurances are very rare in Sweden and all costs for RRT are covered by the county council in which the patient resides. The result is that access and quality of health care are relatively equal and not related to socio-economic status or place of residence.[233]

4.2 STUDY SUBJECTS

Individuals, 18-74 years old, born and living in Sweden during the inclusion period (May 20 1996 and May 31 1998) were eligible. The case-selection was founded on individuals who had a blood sample of S-Cr which for the first time exceeded a predefined limit. The limit was 300µmol/l (3.4 mg/dL) for men and 250µmol/l (2.8 mg/dL) for women. Women have less muscle mass than men. Thus, the S-Cr is lower in women for the same corresponding eGFR (19 and 17 ml/min/1.73 m^2 for men and women respectively at our inclusion limit). The reason for the S-Cr elevation had to be CKD. Since the definition of CKD includes a time aspect, a second S-Cr test was taken after three months on those individuals who had not yet started RRT. To allow fluctuation of the values and regression towards the mean the second S-Cr limit was lower, 250µmol/l (2.8 mg/dL) for men and 200µmol/l (2.3 mg/dL) for women.

To ensure a complete case-finding structure, the chemical laboratories in Sweden (n=69) produced monthly lists of elevated S-Cr values. More than 10,000 values were evaluated by Fored, Ejerblad et al in the original case-control study.[185] The CKD diagnosis was performed by the collaborating nephrologists (n=60) at the hospital were the blood sample was taken through review of the patient's medical record. Post-renal obstruction, pre-renal volume depletion, septicemia, previous kidney transplantation, and terminal illnesses (malignances) were conditions that excluded the individual from further participation. The primary renal disease diagnosis was otherwise founded on ordinary clinical follow-up and

kidney biopsy, if necessary. The nephrologists also asked the patients about their final participation and informed consent.

Control subjects were selected from the study base of native Swedes 18-74 years old, using the national "registry of the total population". This registry is controlled by the Swedish Tax authority and continuously up-dated. On three occasions during the study inclusion period control subjects, frequency-matched for age (in 10-year strata) and sex, were randomly selected. Control subjects provided informed consent before enrolment.

4.3 EXPOSURE ASSESSMENT

After the decision of final eligibility the patients and the control subjects were mailed a questionnaire with a wide range of questions on anthropometric data, diet, smoking habits, and alcohol use. Thereafter, they were interviewed fact-to-face by professional interviewers from Statistics Sweden, a government agency responsible for producing official statistics. The interviewers were not aware of the study hypothesis, but we were unable to blind them from case and control status. The interviewers had computers to help them interview the subjects in a standardized manner. The questions covered medical history, medical drug use, occupational history and work-related exposures.

4.3.1 ANALGESIC USE

A life-long history of non-narcotic drug use was obtained. Both prescribed and non-prescribed drug use was recorded. During the interview the study subjects were shown a booklet with pictures of present and historical packages of non-narcotic analgesic drugs to help them remember earlier use. There were pictures of all analgesic drugs on the market since 1960 containing phenactein or acetaminophen and 78 pictures of the most sold other non-narcotic drugs. If a participant had used more than 20 pills of a certain type of drug, detailed questions followed to verify the frequency and dosage of tablets used during the participant's lifetime. Likewise, if the participant reported regular use, questions followed to investigate the dosage, frequency of use, and age of the subject when regular use began and discontinued. Lifetime cumulative dose was calculated for each substance knowing the amount each brand contained and given the subject's self-reported use.

We defined regular users as users of more than two tablets a week, for more than three months at the time of inclusion. Lifetime cumulative dose was divided into categories of non-users, 1-99 gram (g), 100-499g, 500-2999g, and ≥3000g. Non-users were defined as a lifetime cumulative use of less than 20 tablets. Analgesic use during follow-up was based on information in the medical records. If a subject had received at least one prescription of analgesics or if it was recorded on the drug list in the record, he/she was defined as a user. Patients prescribed low-dose aspirin, either self-reported or indicated in the medical record were considered regular users in all the follow-up analyses.

4.3.2 LEAD EXPOSURE

During the interview, all participants provided a complete life-time history of their occupations. This history included company name, occupational title, work tasks and duration of each employment period of at least one year. The answers were then evaluated by industrial hygienists knowledgeable about common exposures associated with different occupations. Participants at risk of work-related exposure were then subjected to a telephone interview. During that interview questions were asked on frequency and duration of the exposure/exposures, and about personal protective equipment and ventilation. Although the hygienists were unaware of the study hypothesis, it was impossible to blind them from case and control status since the cases sometimes revealed they had kidney disease.

The intensity of lead exposure was judged to be low, moderate or high for each employment period. The method used is the "expert rating method".[234-236] Low lead exposure intensity was 3-10% of the Occupational Exposure Limit ([OEL] defined by the Swedish Work Environment Authority in 1996 as 0.1 mg/m^3) moderate exposure was 10-30% of the OEL, and high exposure was >30% of the OEL. No exposure was defined as exposure below 3% of the OEL. In the analyses, we estimated a low intensity to 0.0067 mg/m^3, medium exposure 0.02 mg/m^3, and high exposure 0.075 mg/m^3 (75% of the OEL). The average level of lead exposure was calculated as the time-weighted average of the estimated OEL *x* proportion of workdays exposed during a subject's lifetime occupational history until inclusion in the study. Only employments with exposure to lead were included. Lifetime cumulative lead exposure was computed as the average lead exposure *x* duration. Categorization was made according to tertiles among exposed controls.

4.4 OUTCOME MEASURES AND FOLLOW-UP

4.4.1 Register data

At the beginning of year 2003 we asked the cases (CKD cohort) if they agreed to take part in the follow-up study. We then used the personal identification number and linked it to the National Population Register, the Swedish Causes of Death Register, and the Swedish Renal Registry (SRR, Svenskt Njur Register). The National Population Register is kept by the Swedish Tax Authority since 1991 when they took over from the Swedish State church. The population register holds information about the birth date, birth location, current residence, and dates of emigration/immigration. The accuracy of the registry is very good. Only 0.1% of the population is "over covered", i.e. registered but not living in Sweden.[237] The problem with "over coverage" is greater among immigrants. The cause of death register is kept by the Swedish National Board of Health and Welfare, and is continuously up-dated. It currently lags 18 months at the most. The number of unreported causes of deaths are <1%. The coding error is approximately 0.3%. [238]The Swedish Causes of Death Register was founded in its current form in

1952, although the history stretches as far back as 1749. The primary cause of death and contributing causes are registered according to the International Classification of Diseases (ICD) 10th version. The Swedish Renal Registry is a registry for health care quality assessment, run by the health care providers and at the time of the study located in Skövde. It was founded in 1994 and covers about 95% of the dialysis patients in Sweden after 1997.[6] This register contains data on timing of renal replacement therapy, type of therapy (hemodialysis, peritoneal dialysis and kidney transplantation), and primary renal diagnosis. We performed two linkages to the registries; one in 2003 (Study I), and one in 2007 (Study II, III, IV).

4.4.2 Information from medical records

To be able to estimate the decline in glomerular filtration rate (progression rate) we recorded up to six S-Cr values for each patient during the period from inclusion to either start of RRT, death, or June 1 2003. The S-Cr values were found through visual inspection of the patient's medical record. The blood samples were taken during the routine clinical follow up of the patient. With previous knowledge of the timing of RRT start and death date we divided the follow-up time (whichever came first of RRT start, death or June 1 2003 minus inclusion date) into five equal time intervals. Four dates, apart from inclusion date and end-of-follow up date, were then suggested. We registered values from the records that were closest to the suggested date. In addition to S-Cr, we abstracted information on blood pressure, weight at end of follow up, and prescribed drugs. We only registered prescribed and non-prescribed drugs at inclusion and during the follow up period. During the follow up, utilization for more than three months (one prescription) was considered to be "use" whereas less than three months was considered "non-use".

4.4.3 Categorization of covariates

In all the analyses age at inclusion was divided into predefined categories (<45, 45-64, ≥65 years) while body mass index (BMI) was categorized as proposed by the World Health Organization (<20, 20-24.9, 25-29.9, ≥30 kg/m^2). Estimated glomerular filtration rate, plasma albumin, hemoglobin, and mean arterial blood pressure at inclusion were categorized into quartiles. Smoking (lifetime cumulative pack-years) and alcohol use (gram/week) was divided into three groups; non-users, and below and above the median value among users. Self-reported level of education was categorized into predefined groups (≤9 years, 10-12 years, ≥13 years) corresponding to the different levels in the Swedish school system (elementary school, high school, and university). Proteinuria was categorized as either high or low, according to the first registered value at inclusion. High was defined as a value above 1500 mg total proteinuria/24 hours or above 1000 mg total albuminuria/24 hours or a dipstick quantitative value exceeding one. The primary renal disease diagnosis was assigned by the clinical nephrologists at inclusion and first categorized into systemic diseases, glomerulonephritis, interstitial nephritis, diabetic kidney disease, hereditary

diseases, hypertensive kidney disease, other renal diseases and unknown diseases (Paper I)
Later, these diagnostic groups were merged into diabetic kidney disease, glomerulonephritis, hypertensive kidney disease (nephrosclerosis), and other diseases (Paper II-IV).

4.4.4 Co-morbidity Index

From the medical records we recorded information on co-morbid diseases at inclusion (previous myocardial infarction, previous cerebrovascular lesion, peripheral arterial disease, congestive heart failure, ischemic heart disease, chronic obstructive pulmonary disease, hemiplegia, dementia, mild or moderately severe liver disease, diabetes with and without complications, metastatic cancer, solid tumor disease or leukemia, systemic inflammatory disease, and AIDS). The definition of a co-morbid condition was that it was diagnosed in the record. We used the comorbid conditions to calculate the Charlson co-morbidity index (CCI). CCI is the most frequently used and validated co-morbidity index in follow-up studies,[239] including studies of patients with end-stage renal disease.[240] CCI assigns a weight between 1 and 4 to each co-morbid condition. The sum of all weights comprises the index score. Chronic kidney disease has a score of 2, and thus 2 is the lowest score possible for a patient in the cohort. We categorized CCI in approximate tertiles (score 2, 3-4, >4).

4.5 STATISTICAL ANALYSIS

In the studies we used statistical analysis software (SAS) version 9 or higher (SAS Institute, Inc, Cary, NC, USA) (Study I) and STATA version 9 or higher (StataCorp LP, www.stata.com) (Study II-IV). In all the analysis of progression rate we defined end of follow-up to June 1, 2003 since we did not abstract medical record information beyond that date while we used later dates in some of the Cox proportional hazards regression models.

4.5.1 Analysis of the natural history (I)

Descriptive statistics was performed to characterize the study population and their renal diseases. Patients were followed from the date of inclusion (the date of the first S-Cr elevation) through the date of the outcome of interest (death or RRT onset) or the end of the study period (December 31, 2002), whichever came first. Cox proportional hazards regression models were used to explore the relationship between the independent variables under study (age, sex, BMI, primary renal disease, estimated GFR, transplanted during follow-up [only in mortality analysis]) and the outcomes of interest, RRT and death. Estimated GFR was calculated both with Cockcroft-Gault formula and the 4-variable MDRD equation. Only results using the MDRD equation are presented in this paper. Crude and adjusted relative rates (RR) and 95% Confidence Intervals (CI) were computed for each of the outcome variables, RRT and death. Mortality data were directly standardized against age and sex-specific all-cause mortality for Sweden by inclusion year (1996-1998).

4.5.2 Analysis of analgesics (II)

Summary statistics described the mean, median and range for the follow-up information (number of days, number of measurements) and eGFR at inclusion. Baseline characteristics were stratified by the main exposures (acetaminophen and aspirin). Proportions (categorical variables) and means (continuous variables) were produced and stratified by both regular use and lifetime dose of acetaminophen and aspirin. Parametric (t-test and chi-square) and non-parametric tests (Kruskall-Wallis) assessed statistical differences between groups. In the analyses of progression rates, we excluded individuals with less than 14 days of follow-up or those who lacked a second S-Cr estimation. To characterize the change in estimated GFR we first described the individual-specific trend by fitting a linear regression model for each individual estimating the beta coefficient (slope), which represents the rate of change in eGFR per year. The decline in eGFR was assumed to occur at a constant rate (linearly) which is the most common way to describe renal deterioration in the scientific literature.[36, 50] The estimated beta coefficients' distribution was summarized using both mean and median, and stratified by analgesic use and all other variables of interest. Patients with follow-up shorter than one year had their progression rate multiplied and presented by year to be comparable with the others in the univariate analysis.

In the further analyses, both the correlation among repeated measurements and the unbalanced data were handled in a linear mixed effects model, with both fixed and random intercept and slope. For each exposure of interest (regular use versus no regular use at inclusion and lifetime cumulative dose), the fitted model included the exposure and the linear time effects, sex and age at baseline. The study hypothesis was tested by adding appropriate interaction terms between the exposure and time of follow-up. Statistically significant or biologically relevant variables were left in the final model (age, sex, angiotensin converting enzyme inhibitor or angiotensin II receptor blocker use, and mean arterial pressure). The goodness of fit in the final model was assessed through visual inspection of the observed and fitted trajectories and study of the estimated standardized residuals. All the fitted models reproduced the observed trends, and the residual plots did not reveal any substantial deviation from the underlying assumptions. In the analysis of renal survival we used a Cox proportional hazard model, including the same confounding variables as in the mixed model. In addition, we included co-morbidity and primary renal disease. Patients were censored at the date of RRT onset or the date of death, whichever occurred first.

4.5.3 Analysis of occupational lead (III)

In addition to the above-mentioned covariates, we included self-reported information on ever diagnosed with gout, diabetes, or hypertension. We first compared cases and controls in univariate analyses using chi-square statistics (Fisher exact) for categorical variables and t-tests (normal distribution) or Kruskall-Wallis non-parametric tests (non-normal distribution) for continuous variables. We then produced age- and sex-adjusted Odds Ratios (OR) for CKD overall among ever lead exposed as well as in relation to average and life-time cumulative lead exposure using logistic regression modeling with non-exposed as the reference category. In sub-analyses, we also estimated the ORs for CKD due to each of the largest groups of primary renal diseases; glomerulonephritis, nephrosclerosis, and diabetic nephropathy. In the multivariate logistic regression model we first included all risk factors known to be associated with CKD and those that attained a p-value below 0.25 in the univariate analyses. Variables that changed the coefficient for the main exposure of interest by more than 10% were retained in the final model, which also included risk factors known *a priori* to be associated with CKD. The Goodness of fit was assessed by the Hosmer-Lemeshow test.

In the analysis of the follow-up data we estimated the individual progression rate along the disease trajectory using up to six S-Cr values and assuming a linear trend (see section 4.5.2). In the multivariate analysis we handled both the correlation among repeated measurements and the unbalanced data in a linear mixed effects model, with both fixed and random intercept and slope.[241] The fitted model included the exposure and the linear time effects. The study hypothesis was tested by adding appropriate interaction terms between the exposure and time of follow-up. Significant variables and potential confounders were retained in the final model. In addition, we also examined renal survival in relation to previous lead exposure using a Cox proportional hazards model. In this analysis it was possible to extend the follow-up time to Dec 31 2005 because of complete registry data. Patients were censored at start of RRT, at death before RRT, or end of follow-up whichever occurred first. The selection of the adjusting variables followed the same principles as in the logistic and linear regression.

4.5.4 Analysis of early versus late dialysis (IV)

For each patient we aimed at estimating GFR (from S-Cr) at least six times along the disease trajectory during follow-up. The 4-variable MDRD equation was used to estimate the GFR.[28] Since the S-Cr determinations were part of routine care, they varied in number and timing. The distribution of S-Cr values along follow-up was highly unbalanced. We estimated the individual progression rate by fitting a linear mixed effects model, with both fixed and random intercept and slope.[241] If we could not calculate an individual progression rate (if the patient had only a first eGFR value) the model imputed an estimate based upon similar patients in the total cohort.

4.5.4.1 Classification of early and late dialysis

The timing of dialysis start was defined in terms of attained eGFR value. We used the last value before initiation to decide whether timing was early or late. However, if the latest S-Cr determination was more than 30 days before initiation of dialysis we inferred the last eGFR value by using the last recorded eGFR and the individual progression rate of the patient according to the principle "last value carried forward". Patients were censored at the date of the latest eGFR recording if it was >2 years before end of follow-up. To be consistent with other studies, [153, 155] we defined early and late initiation as dialysis start with an eGFR ≥7.5 and <7.5ml/min/1.73 m^2, respectively. To allocate deaths or other censoring in patients without dialysis treatment to the correct eGFR stratum, we used the S-Cr closest to these events and otherwise followed the same principles as the allocation to early versus late dialysis start.

The individual progression curves were used to establish when patients moved between the predefined strata of eGFR. We classified a patient not yet subjected to dialysis with an eGFR 20 -7.5ml/min/1.73 m^2 as a "candidate for early dialysis". When eGFR fell below 7.5ml/min/1.73 m^2, the patient was moved to the "candidate for late dialysis" category. Patients who later improved and permanently regained an eGFR value of >20ml/min/1.73 m^2 were regarded as not being at risk for dialysis and did not contribute with person-time thereafter.

4.5.4.2 Analysis of mortality in early and late start dialysis

The study covariates were summarized with proportions and means stratified according to the last eGFR-status (early versus late). Differences between the groups were assessed by non-parametric (Kruskall-Wallis for continuous, Chi-Square for categorical variables) statistics. The variables were then studied according to their possible effect on the exposure early or late start of dialysis. Patients' survival time (in days) to dialysis was censored when they either died or reached the end of the study (June 1, 2003) alive without having started the treatment. Patients who received a renal transplant were censored at transplantation date. Patients with a pre-emptive renal transplantation were directly censored at transplantation date and did not contribute to the survival analysis of early and late dialysis mortality.

We fitted a time-dependent multivariate proportional hazards regression (Cox) model that included the survival time in all of the different strata (candidate for early dialysis, candidate for late dialysis, subject to early start dialysis, and subject to late start dialysis) and adjusted for the other covariates including eGFR at inclusion (as a continuous variable) and progression rate (rate of change of eGFR per year) divided into quartiles. Thus, most patients started generating person-time at the point in time when eGFR was observed or projected to be 20 ml/min/1.73 m^2, and were transferred to the "candidate for late dialysis" when eGFR passed 7.5 ml/min/1.73 m^2. The final model included variables significantly associated with the outcome as well as *a priori* suspected confounding factors, while the variables plasma albumin, hemoglobin, mean

arterial pressure, and proteinuria at inclusion were dropped due to trivial effects on the associations of interest. The proportionality assumptions, checked through visual inspection of the log of the incidence rates were satisfactorily met.

4.5.4.3 Analysis of mortality before and after start of dialysis

Kaplan Meier curves described the survival among patients, still without RRT. The patients were divided into four strata (eGFR ≥15, 10-14.9, 7.5-9.9, and <7.5 ml/min/1.73 m^2) according to the actual dates of the recorded S-Cr values. Patients who moved from one eGFR stratum to another were censored in the old stratum and re-started from time 0 in the new. Definite censoring occurred at initiation of RRT. We further produced a Kaplan Meier survival curve without stratification for eGFR level and compared it to a corresponding curve for patients after having started dialysis, starting on the day of dialysis initiation. Censoring occurred when a patient got a renal transplant. We also fitted a time-dependent Cox model where we started follow-up registration when eGFR passed 15 ml/min/1.73m^2 and with dialysis as a dichotomous no/yes variable. The selection of adjusting variables followed the principles described previously.

5 RESULTS

5.1 BASELINE CHARACTERISTICS

Between the years 1996 and 1998, 1189 eligible patients were identified; 69 patients died shortly after diagnosis, 83 were too ill to be interviewed, and 111 refused to participate. Of the remaining 1120 patients 926 (83%) agreed to participate in the case-control study. Out of 1330 population controls, 998 (75%) participated (221 declined, 56 could not be reached, and 55 were too ill to be interviewed). In the follow-up study another 6 patients declined to participate and thus 920 were included. The age and sex distribution is presented in Table 2. As expected from the frequency matching, there was no difference in age and sex among the cases and controls. There were more men than women.
Mean age was 58.0 years for men and 56.8 years for women.

Table 2. Age and sex among cases and controls

	Case (N=926)	**Control** (N=998)
Age (mean)(SD)	57.6 (13.6)	57.6 (13.5)
Sex (n)		
Men (%)	597 (64.5)	653 (65.4)
Women (%)	329 (35.5)	345 (34.6)

The median serum creatinine among the patients was 336µmol/l among men and 281µmol/l among women. The overall mean estimated creatinine clearance using Cockcroft-Gault formula was 21.1ml/min (SD 7.3), range 2.2 – 53.0 at inclusion. The mean estimated creatinine clearance was 22.3ml/min for men (range 2.2 – 53.0) and 19.2 ml/min for women (3.4- 34.7). Using the 4-variable MDRD equation (1999) the mean estimated GFR (eGFR) was 16.5ml/min/1.73 m^2 (range 1.7-31.34) for men and 15.4ml/min/1.73 m^2 (range 2.4 – 27.4) for women.

5.1.1 Primary renal diseases among patients

The most common renal diagnose was diabetic nephropathy, followed by glomerulonephritis, and nephrosclerosis (Table 3). About 30% of the renal diagnoses were founded on renal biopsies. Among patients with glomerulonephritis as many as 61% (n=135) had performed a biopsy. Most of the patients (n=798) had known renal disease before they were included in the study, while 120 patients reported that they were not diagnosed before study inclusion. Most of these previously unknown patients had diabetic nephropathy (42%) or nephrosclerosis (20%).

Table 3. Renal diseases among the patients

Primary renal disease	Number	Percent (%)
Systemic disease	81	8.8
Glomerulonephritis	220	23.9
Interstitial nephritis	28	3.0
Diabetes	284	30.9
Hereditary disease	98	10.7
Nephrosclerosis	138	15.0
Other renal diseases	27	2.9
Unknown	44	4.8

5.1.2 Baseline covariates among the patients

The covariates at the beginning of follow-up are presented in Table 4. Most of the 920 patients (58%) with CKD had ≤9 years of education, whereas a smaller proportion (18%) had attended university. Twenty-five percent were non-users of alcohol and 40% had never smoked. There were significantly fewer smokers, and more alcohol users among patients in the highest educational level. Mean BMI at inclusion was 25 kg/m^2, range 15.4-49.2 kg/m^2. There were 13.7% obese (BMI >30 kg/m^2) patients at beginning of follow-up, while 34.5% were overweight (BMI 25-30 kg/m^2).

Although 38.4% of the patients lacked any anti-hypertensive medication, 23.3% were on two different drugs, and 6.6% were on three. Beta blockers and calcium channel antagonists were the most prescribed antihypertensive drugs (33.6 and 33.9% respectively), whereas ACE/ARB were less common; only about 30% were on any of those substances. However, 40.5% of the diabetic patients were prescribed ACE/ARB, probably reflecting the prescription pattern among nephrologists in Sweden during the 1990's. The use of ACE/ARB was also significantly associated with educational level. Patients in the highest educational level were prescribed ACE/ARB to a larger extent compared to patients with the lowest educational level (40.4% versus 25.3%). The mean arterial blood pressure at inclusion was however similar across educational level, BMI strata, age categories and gender. Patients prescribed more antihypertensive drugs had significantly higher blood pressure; the mean arterial blood pressure among patients with no anti hypertensive drugs was 120 mmHg (SD 18.3) compared to 126.4 mmHG (SD 21.1) among those prescribed three medications.

Plasma albumin at the beginning of follow-up was correlated to both BMI and eGFR; patients with lower plasma albumin tended to have lower BMI and eGFR. Plasma albumin was also significantly correlated to albuminuria; patients with <500 mg/24h had plasma albumin 36.7g/L (SD 5.8) compared to 33.9g/L (SD 6.1) among those with >2000 mg/24h. Moreover, albuminuria was correlated to mean arterial pressure and smoking; subjects with more albuminuria were more likely to have higher blood pressure and higher lifetime cumulative smoking.

Table 4. Baseline covariates at inclusion among the patients

Characteristic		Number	Percent/Mean
Education (%)	≤9 years	534	58.0
	10-12 years	206	22.4
	≥13 years	168	18.3
Alcohol consumption	Non users (%)	231	25.1
	Mean g/week among users (SD)	679	76.4 (176.6)
Smoking history	Never (%)	367	39.9
	Mean Cum pack-years among users (SD)	540	21.8 (15.9)
Body Mass Index	Mean kg/m^2 (SD)	894	25.4 (4.5)
ACE/ARB (%)	Non-users	645	70.1
	Users	275	29.9
Beta blocker (%)	Non-users	611	66.4
	Users	309	33.6
Calcium channel antagonists (%)	Non-users	608	66.1
	Users	312	33.9
Number of anti hypertensive drugs (%)	None	353	38.4
	One	292	31.7
	Two	214	23.3
	Three	61	6.6
Mean arterial pressure	mmHg (SD)	798	110.4 (15.0)
B-Hemoglobin	Mean g/L (SD)	820	114.3 (17.6)
P-Albumin	Mean g/L (SD)	756	35.9 (6.2)
Albuminuria	<500 mg/24 h	111	17.8
	500-<1000 mg/24 h	98	15.7
	1000 - <2000 mg/24 h	130	20.8
	≥2000 mg/24 h	285	45.7

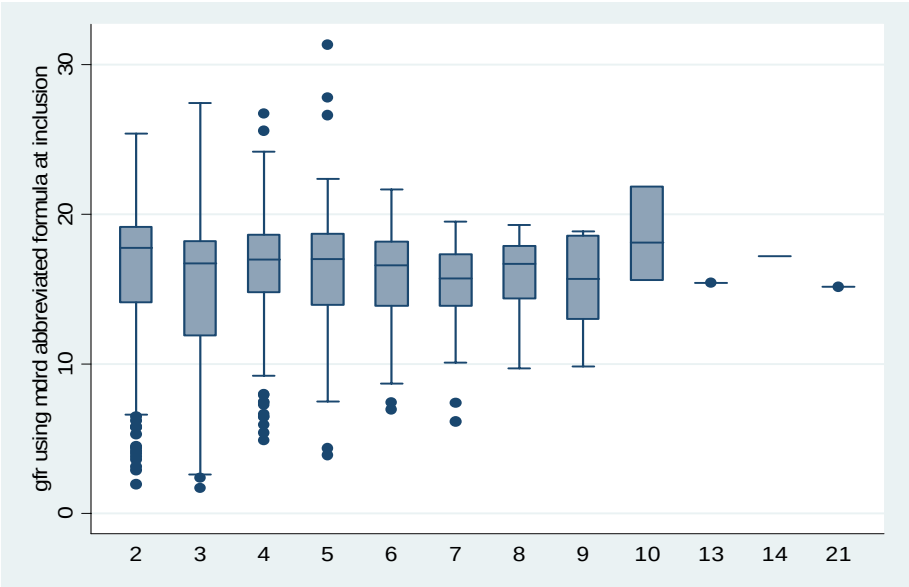
5.1.3 Co-morbid diseases

We were unable to find medical records for 57 subjects. Based upon information in the records there were 62 (7.2%) patients with chronic pulmonary disease or asthma, 153 (17.8%) with coronary heart disease, 163 (18.9%) with congestive heart failure, 100 (11.6) with previous cerebrovascular lesion, 94 (10.9%) with previous myocardial infarction, 111 (12.9%) with peripheral arterial disease or amputation, 86 (10%) with systemic inflammatory diseases, 308 (35.8%) with type 1 or type 2 diabetes, 3 with hemiplegia (0.4%), 3 (0.4%) with dementia, 17 (2%) with mild liver disease, 7 (0.8%) with severe liver disease, 67 (7.8%) with a solid tumor disease or leukemia, 6 with metastatic cancer (0.7%), and 51 (5.9%) with gastric ulcer disease.

At the time of inclusion, 40.5% were diagnosed with cardiovascular disease, 42% men and 38% women. 86% of the patients with diabetes had diabetic nephropathy, whereas 3-5% of the patients with other primary renal diseases also had diabetes. Almost one third of the patients (31.3%) had no other disease diagnosed than CKD. The median CCI score was 4.0 (range 2-21).

The CCI score was unrelated to eGFR at inclusion (Figure 2). It was also not associated with, sex, BMI, blood pressure and albuminuria at inclusion but positively correlated with age, alcohol consumption, and cumulative smoking. It was negatively correlated with plasma albumin, blood hemoglobin and years of education.

Figure 3. Box plot of estimated glomerular filtration rate (using the 4-variable MDRD equation) at the different Charlson co-morbidity index scores at inclusion.



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5.1.4 Analgesic drugs

Eight patients did not give a complete history of analgesic use at inclusion. Lifetime consumption and regular analgesic use is presented in Table 5.

Table 5. Analgesic use at the beginning of follow-up and during follow-up

Acetaminophen		**Number (%)**	**Regular use[1] (%)**	**Regular use during follow-up[2] (%)**
	Non users	329 (36.1)	-	42 (12.7)
	≤99 gram (g)	294 (32.2)	14 (4.8)	51(17.3)
	100-499 g	160 (17.5)	35 (21.9)	40 (25.0)
	500-2999 g	71 (7.8)	52 (73.2)	32 (45.0)
	≥3000 g	58 (3.4)	43 (74.1)	32 (55.2)
	Missing	8	8	57
Aspirin				
	Non users	280 (30.7)	-	47 (16.8)
	≤99 gram (g)	289 (21.7)	63 (21.8)	72 (24.9)
	100-499 g	212 (23.2)	89 (42.0)	88 (41.5)
	500-2999 g	81 (8.9)	47 (51.9)	26 (32.1)
	≥3000 g	50 (5.5)	27 (54.0)	18 (36.0)
	Missing	8	8	57

[1] *Regular use at the beginning of follow-up*
[2] *Regular use at any time during the follow-up for more than three months*

When comparing regular users of acetaminophen at inclusion with non-regular users, the regular users had significantly higher CCI score, lower alcohol intake, lower level of education, more cardiovascular disease and diabetes, and a larger proportion were women and regular users of aspirin. Patients with higher cumulative acetaminophen use were older, had lower level of education, higher CCI score, used ACE/ARB more frequently, smoked more, and were more often women.

Regular users of aspirin were older, had higher CCI score, lower level of education, higher frequency of cardiovascular disease, diabetes and nephrosclerosis, and smoked more. Patients with a high lifetime cumulative dose of aspirin had higher CCI score and more cardiovascular disease, and used more alcohol per week. Most of the patients with aspirin use were users of low-dose aspirin (88.5%).

5.1.5 Occupational lead exposure

Almost all participants gave a complete occupational history (n=913 patients and 991 controls). There were 81 (8.7%) patients and 95 (9.5%) controls ever occupationally exposed to lead. The average lead exposure among the exposed was 0.016 mg/m^3 (range 0.000056 – 0.075 mg/m^3, standard deviation [SD] 0.023) while mean lifetime cumulative lead exposure was 0.21 mg*year/m^3 (range 0.000375-2.85, SD 0.42). This corresponds to lead exposure at the OEL every workday for approximately two years. There was no difference in the mean average or lifetime cumulative exposure to lead among the patients compared to the controls.

The CKD patients smoked more than the control subjects; they had a lower average level of education and a higher mean BMI at 20 years of age. The number of subjects with gout, diabetes, and hypertension was as expected significantly higher among cases than among controls. Lead exposed were more often men, had significantly higher alcohol intake, smoked more, had lower level of education and were diagnosed with gout more frequently.
Among the CKD patients who were exposed to lead there was no difference in the length of follow-up, number of S-Cr measurements or eGFR at inclusion between exposed and non-exposed, but the lead exposed had higher blood pressure in addition to the above mentioned differences.

5.1.6 Early and late dialysis initiation

At June 1 2003, 736 patients had started RRT, 90 had died before any RRT was initiated, and 56 were still alive and without RRT. Of the patients who started RRT, 28 had been given a pre-emptive renal transplantation, and so were not included in any of the dialysis groups, thus leaving 708 patients. There were 323 patients who initiated dialysis early (≥7.5 ml/min/1.73m^2) and 385 who initiated dialysis late (<7.5 ml/min/1.73m^2). Mean of the observed or inferred last eGFR before start of dialysis was 7.6 (median 7.0, SD 3.3, range 1.8-30.1) ml/min/1.73m^2. The mean eGFR in the early start group was 10.8ml/min/1.73m^2 (SD 3.2) and in the late start group 5.5 ml/min/1.73m^2 (SD 1.2).

Compared to early dialysis starters, late starters were, on average, younger, had higher plasma albumin and lower co-morbidity index, had less diabetes and cardiovascular disease, were more likely to receive a kidney transplant during the follow-up, and had a faster decline in eGFR per year. The patients initiating dialysis early were followed for 1.55 years before RRT was started compared to 1.65 years for the late startes (p=0.5). Thus, in spite of a lower eGFR at inclusion, there was no statistically significant difference in time from initial inclusion to dialysis start among late starters compared to early starters. This was probably was due to the faster progression rate among late starters.

5.2 PROGNOSIS OF CHRONIC KIDNEY DISEASE

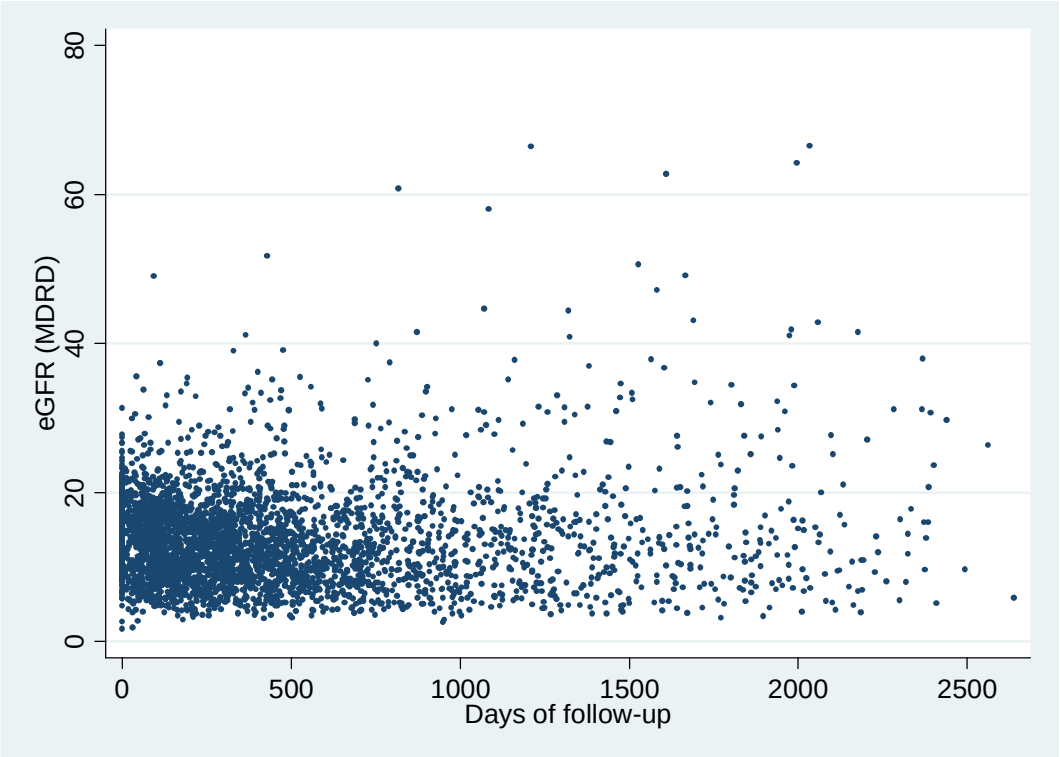
Most of the patients included in the follow-up study started RRT. By Dec 31, 2005, 756 patients had initiated RRT while 46 were still alive and without RRT. After one and three years, the proportion of patients alive and without RRT was 64% and 29%, respectively.

5.2.1 Risk factors for progressive disease (I, II)

5.2.1.1 Decline in glomerular filtration rate

In the analysis of progression rate we excluded 57 patients for whom we were unable to find medical records or a second S-Cr measurement, and another 62 patients who either started RRT or died within 14 days from inclusion leaving 801 patients in the analyses. Until June 1 2003, mean follow-up time was 768 days (2.1 years) with a range of 15 to 2637 days (7.0 years). The 801 patients generated 4291 S-Cr observations (Figure 4). Most of the patients (68%) had a complete record with six measurements whereas 5% had only two. The mean number of S-Cr measurements was 5.2. The inter-measurement variation was substantial (range 2-954 days) and the data were unbalanced due to the differences in follow-up time. The median inter-measurement time varied from 118 to 136 days (SD 130-146, mean 160-184 days) in the five different time intervals.

Figure 4. Estimated glomerular filtration rate (eGFR) during at the most 7 years of follow-up for 801 patients, included when their serum creatinine first passed 300µmol/l and 250µmol/l for men and women respectively.



With linear regression, we estimated the individual progression rate from the eGFR values. The distribution of the progression rates was skewed; most of the subjects (25^{th} to 75^{th} percentile) progressed between -9.7 to -2.3 ml/min/1.73 m^2 per year. The mean unadjusted decline in GFR was 9.0 ml/min/1.73 m^2 per year, while the median decline was 5.1 ml/min/1.73 m^2 per year. Patients with a very rapid progression rate started RRT early and thus were followed more shortly, whereas patients who progressed slowly and sometimes even improved were followed until end of follow-up. Patients with a more rapid progression rate were also more likely to have a higher eGFR at inclusion than patients with lower progression rates. The unadjusted progression rates stratified for the baseline covariates are shown in Table 6.

In this univariate analysis, younger patients, those with higher blood pressure, more albuminuria, lower plasma albumin, diabetics, and men had a faster decline in glomerular filtration rate.

Table 6. Unadjusted decline in glomerular filtration rate per year (n=801)

Characteristic	**Groups (Number)**	**Median Progression rate in ml/min/1.73m^2 per year §**	**Significance Test #**
Age (years)			<0.01
	<45	-6.6	
	45-64	-5.6	
	≥65	-3.8	
Sex			<0.01
	Male	-6.0	
	Female	-4.0	
Estimated GFR at first inclusion*			
	1.7-14.8	-4.1	<0.01
	14.8-17.2	-4.8	
	17.2-18.7	-4.9	
	18.7-31.3	-6.6	
Primary Renal Disease			<0.01
	Diabetes	-6.5	
	Glomerulonephritis	-5.7	
	Nephrosclerosis	-4.6	
	Other	-4.7	
Co-morbidity			0.21
	CCI <3	-5.3	
	CCI 3-4	-4.9	
	CCI >4	-5.7	

Characteristic	Groups (Number)	Median Progression rate in ml/min/1.73m^2 per year §	Significance Test #
Education			0.68
	≤ 9 years	-4.9	
	10-12 years	-5.2	
	≥13 years	-5.5	
Smoking			0.53
	Never smokers	-4.7	
	≤20 pack years	-5.1	
	> 20 pack years	-5.5	
Alcohol use			0.55
	No use	-4.9	
	≥32.6 grams /week	-4.8	
	>32.6 grams/week	-5.5	
Body mass Index			0.68
	<25 kg/m^2	-5.2	
	≥25 kg/m^2	-5.1	
Mean arterial pressure			<0.01
	<100 mmHg	-3.4	
	100-110 mmHg	-4.7	
	110.1-118 mmHg	-5.7	
	>118 mmHg	-6.4	
ACE-inhibitors/ARB			0.25
	No use	-4.9	
	Regular use	-5.5	
Albuminuria			<0.01
	<500 mg/24 h	-4.4	
	500-<1000 mg/24 h	-3.2	
	1000 - <2000 mg/24 h	-4.4	
	≥2000 mg/24 h	-6.4	
Plasma albumin			<0.01
	≤31 gram/L	-6.8	
	32-36 gram/L	-6.7	
	37-39 gram/L	-4.9	
	≥40 gram/L	-4.3	
Blood hemoglobin			0.66
	≤102 gram/L	-5.7	
	103-113 gram/L	-5.1	
	114-125 gram/L	-5.3	
	≥126 gram/L	-4.7	

** ml/min/1.73 m^2, CCI (Charlson co-morbidity index score)*
 # P-value for comparisons of patients belonging to different categories was estimated from quintile regression

We also estimated the progression rate using a mixed effects model with random intercept and slope. In this model we were able to include all 920 patients since the estimate for those with only a first eGFR value is based upon the slopes of patients with similar characteristics in the cohort. The mixed effects model also takes account of the different length of follow-up time between subjects and thus makes the estimate more stable.

The unadjusted progression rate using the mixed effects model (n=920) was -4.2ml/min/1.73 m^2 per year (SD 2.5). The same covariates (age, sex, primary renal disease, albuminuria, plasma albumin, blood pressure) were significantly associated with differences between categories as in the linear regression analysis. However in multiple regression analysis, only age, blood pressure and albuminuria remained significantly associated with the progression rate. All of these variables also showed a significant trend associated with progression rate; higher age was associated with a slower progression rate whereas higher blood pressure and albuminuria was associated with a more rapid progression rate.

5.2.1.2 Risk of Renal Replacement therapy

Patients with low eGFR at the beginning of the follow-up had, as expected, higher risk of receiving RRT than patients with high eGFR. Age was inversely related to risk of RRT (adjusted hazard ratio (HR) for patients ≥65 years relative to patients <45 years 0.7; 95% CI 0.6-0.9). Men had a higher risk of RRT than women (HR 1.6; 95% CI 1.4-1.9), and patients with diabetic nephropathy higher risk of RRT compared to subjects with glomerulonephritis (HR 1.24; 95% CI 1.02-1.51) and other primary renal diseases.

5.2.2 Analgesic drugs (II)

5.2.2.1 Acetaminophen

Of the 920 patients eligible for the follow-up we excluded 62 with follow-up shorter than 14 days and 57 for whom we were unable to find the medical records or a second S-Cr measurement, leaving 801 (68%) patients for inclusion in our analysis. The excluded patients (n=119) did not differ significantly with respect to age, sex, primary renal disease, or acetaminophen and aspirin use compared with the patients included in the analyses. The unadjusted progression rate based on simple linear regression among regular users of acetaminophen at the time of inclusion in the cohort was -5.2 ml/min/1.73m^2 per year. Non-regular users of acetaminophen at inclusion progressed at -5.1 ml/min/1.73m^2 per year. The results from the multivariate mixed effects model is shown in Table 7.

72 patients were regular users of acetaminophen at inclusion and continued to use the drug during follow-up. The mean adjusted progression rate of these patients was -2.9 ml/min/1.73 m^2 per year, which was significantly lower compared to patients with no regular use at inclusion or during follow-up. Exclusion of 50 patients who were users both of acetaminophen and aspirin and thus restricting

the analysis to solely acetaminophen users, did not make the estimates change substantially; the adjusted progression rate among regular users was -4.0 ml/min/1.73 m^2 per year (95% CI -5.1, 2.9)

Table 7. Progression rate for CKD patients with different levels of lifetime cumulative acetaminophen use

Acetaminophen		**Progression rate[§] (95% CI)**	**P-value**
	Never used	-4.5 (-5.0, -4.0)	Ref.
	1-99 g	-4.7 (-5.2, -4.1)	0.7
	100-499 g	-3.9 (-4.7, -3.1)	0.2
	500-2999g	-3.9 (-5.1 -2.6)	0.3
	≥3000g	-4.3 (-5.6, -2.9)	0.7
			P-value trend 0.24
	No regular use	-4.5 (-4.9, -4.2)	Ref.
	Regular use	-3.6 (-4.4, -2.8)	0.04

§ ml/min/1.73 m^2 per year; mixed effects model, adjusted for age, sex, ACE/ARB use, and blood pressure at inclusion.

Regular acetaminophen use was associated to a slower progression rate regardless of whether we stratified on eGFR at inclusion, primary renal disease, sex or age. For patients who were older and had nephrosclerosis, the difference in retardation in progression rate was statistically significant. The stratified analysis on acetaminophen regular use is shown in Table 8.

Table 8. Difference in progression rate compared to non-regular users of acetaminophen among patients with different baseline characteristics

Acetaminophen Regular use	**Coefficient[§] (95% CI)**	**P-value**
<45 years	0.32 (-2.1, 2.7)	0.79
45-64 years	0.25 (-1.3, 1.8)	0.75
≥65 years	1.6 (0.4, 2.7)	0.006
Men	1.2 (-0.2, 2.5)	0.10
Women	0.34 (-0.8, 1-5)	0.55
Single substance acetaminophen use	0.53 (-0.6, 1.7)	0.37
Diabetic nephropathy	0.45 (-1.1, 2.0)	0.6
Nephrosclerosis	3.2 (1.3, 5.3)	0.002
Glomerulonephritis	0.93 (-1.7, 3.5)	0.48

§ Difference in progression rate (ml/min/1.73 m^2) per year compared to the reference group (non-regular users). Negative values mean faster progression, and positive values mean slower progression compared to the reference.

Lifetime cumulative dose of acetaminophen overall did not show any effect on the progression rate. For patients who had used >3000 gram acetaminophen and were non-regular users of aspirin the fully adjusted progression rate was 0.43ml/min/1.73 m^2 per year faster (-2.2, 1.3) compared to non-users. For patients with diabetes who had used >3000 gram acetaminophen the difference in progression rate compared to non-users with diabetes was -1.2 ml/min/1.73 m^2 per year (-4.3, 1.9) whereas it was associated with slower progression rates both among patients with glomerulonephritis and nephrosclerosis. Stratification on sex or age did not change the pattern of a slight, non-significant effect of acetaminophen cumulative dose on the progression rate.

5.2.2.2 Aspirin

Based upon the linear regression, the unadjusted progression rate for patients who used aspirin regularly was -4.4ml/min/1.73 m^2 per year compared to -5.3 ml/min/1.73 m^2 per year among non-regular users. In Table 9 the adjusted progression rates (mixed effects model) associated with different lifetime cumulative doses of aspirin is presented. There was no difference in progression rate among subjects with different lifetime cumulative aspirin use, but patients who used aspirin regularly at inclusion had a significantly slower decline in eGFR compared to non-regular users. When we stratified these results on whether the patients were low dose aspirin users or regular aspirin users, we found that it was low dose aspirin use that was associated with slower progression. Restricting the analysis to regular users of only aspirin and not acetaminophen made the coefficient no longer significant, but still positive (0.61, 95% CI -0.1, 1.4).

Table 9. Progression rate for CKD patients with different levels of lifetime cumulative aspirin use and aspirin regular use

Aspirin	**Progression rate[§] (95% CI)**	**P-value**
Never used	-4.8 (-5.4, -4.3)	Ref.
1-99 g	-4.3 (-4.9, -3.7)	0.2
100-499 g	-4.1 (-4.8, -3.5)	0.1
500-2999g	-4.1 (-5.2 -3.0)	0.3
≥3000g	-4.1 (-5.6, -2.7)	0.4
		P-value trend 0.12
Aspirin regular use		
No regular use	-4.6 (-5.0, -4.3)	Ref.
Regular use	-3.8 (-4.4, -3.3)	0.02
Only low-dose	-3.7 (-4.3, -3.1)	0.01
Also during follow-up	-3.5 (-4.2, -2.9)	0.004

§ ml/min/1.73 m^2 per year; mixed effects model, adjusted for age, sex, ACE/ARB use, and blood pressure at inclusion.

For patients without cardiovascular disease who used aspirin regularly, the progression rate was 0.74 ml/min/1.73 m^2 per year (-0.3, 1.9) slower compared to non-regular users while the progression rate among regular aspirin users with cardiovascular disease only differed by 0.24ml/min/1.73 m^2 per year (-0.8, 1.2) compared to non-regular users. Analyses stratified by gender demonstrated that women that used aspirin regularly had a 0.41 ml/min/1.73 m^2 per year (-0.6, 1.4) slower progression rate compared to non-regular users; men who used aspirin regularly slowed their progression rate by 1.08 ml/min/1.73 m^2 per year (0.2, 2.0) compared to non-regular users. Comparing the effect of regular aspirin use in patients with different primary renal disease we saw a slight positive effect in all the investigated groups, but among patients with glomerulonephritis the effect on progression rate was even more pronounced (difference compared to non-regular users 1.95 ml/min/1.73 m^2 per year, 95% CI 0.5, 3.4, p=0.007).

For aspirin users who continued to use aspirin during follow-up, the protective effect was most evident among patients with the highest lifetime cumulative dose. Patients who had used >3000 g aspirin and continued to use aspirin had a 1.95 ml/min/1.73 m^2 per year (95% CI -0.3, 4.2) slower progression rate compared to never users. Restricting the analysis to users of only aspirin and not acetaminophen did not change the estimates substantially. Also here, patients with glomerulonephritis benefited the most of continued aspirin treatment; all coefficients were 1.0 - 4.3 ml/min/1.73 m^2 per year slower for aspirin users of different cumulative dose.

5.2.3 Occupational lead exposure (III)

5.2.3.1 Risk for CKD development

The unadjusted Odds ratio (OR) for CKD was 0.94 for lead exposed compared to never-exposed. In the final multivariate analysis adjustments for age, sex, alcohol consumption, diabetes, level of education and BMI did not change the OR substantially (OR 0.97, 95% CI 0.7-1.4). The OR for subjects exposed to lead at the highest average level (7.5-75% of OEL) did not reach significance level (OR 1.09, 95% CI 0.6-1.9). For subjects who were exposed at the highest lifetime cumulative exposure level (>0.15mg/m^3*year) the odds for CKD was below unity (OR 0.69, 95% CI 0.4, 1.3). Restricting the analysis to subjects exposed at the highest level we found that there were 9 CKD patients and 14 controls that had been exposed at an average level >50% of OEL. The OR associated with CKD in this high average exposure group was 0.73 (0.3-1.8). Among subjects with a lifetime cumulative dose at the OEL for at least 5 years the OR for CKD was 0.54 (95% CI 0.2, 1.6). The OR associated with different subgroups are presented in Table 10.

Table 10. Odds Ratios for different diagnosis of CKD associated with occupational lead exposure

Subgroup[1]	**Odds Ratio (95% CI) [§] Lead exposure**	**Odds Ratio (95% CI) [§] Highest average lead exposure**
Diabetes nephropathy	0.85 (0.5-1.4)	1.1 (0.5-2.2)
Glomerulonephritis	0.93 (0.6-1.5)	0.99 (0.5-2.1)
Nephrosclerosis	1.17 (0.6-2.1)	1.36 (0.6-3.2)
>60 years at inclusion	1.03 (0.7-1.7)	1.05 (0.5-2.1)

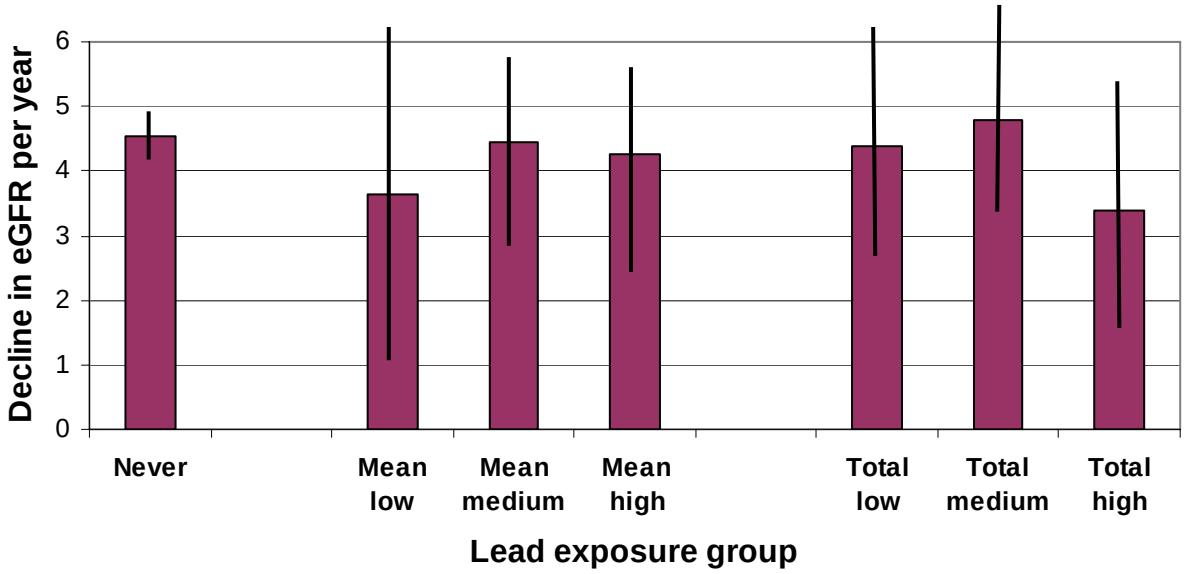
[1] *Restricted analysis*
§ Adjusted for age, sex, alcohol use, smoking and BMI

5.2.3.2 Risk for progressive disease and RRT

The progression rate did not differ significantly for any of the lead exposure groups we studied compared with non-exposed (Figure 5). In stratified analyses by primary renal disease the decline in eGFR among patients with diabetic nephropathy was 0.39ml/min/1.73m^2 per year (95% CI -2.7, 1.9) faster among lead exposed compared to non- exposed. The difference in progression rate was 0.17 ml/min/1.73m^2 per year faster among lead exposed patients with nephrosclerosis while the coefficient was positive in lead exposed patients with glomerulonephritis. Among younger patients (<45 years) the lead exposed patients progressed 1.79 ml/min/1.73m^2 per year faster (95% CI -7.3, 3.7) but due to the very limited number of exposed patients in that group the confidence intervals were very wide. There was no difference in progression rate among lead exposed with different smoking habits, or eGFR at inclusion.

In a Cox proportional hazards regression model we estimated the adjusted hazard rate (HR) for RRT start associated with occupational lead exposure and the different groups of lead exposure levels. In an analysis adjusting for age, sex, smoking and ACE/ARB use the HR was 0.92 (95% CI 0.7, 1.2) for lead exposed, HR 0.83 (95% CI 0.5, 1.3) for patients with the highest cumulative lead exposure, and HR 0.89 (95% CI 0.6, 1.3) for patients with the highest average lead exposure compared to non-exposed patients.

Figure 5. Decline in eGFR (ml/min/1.73 m^2 per year) and 95% confidence interval for patients never exposed to occupational lead, compared to groups of patients with different levels of mean and total cumulative exposure to lead.



Adjusted for age, sex, ACE/ARB use and smoking. NOTE! The confidence intervals in the published Ms IV are wrong for the mean exposure group, these are the correct values.

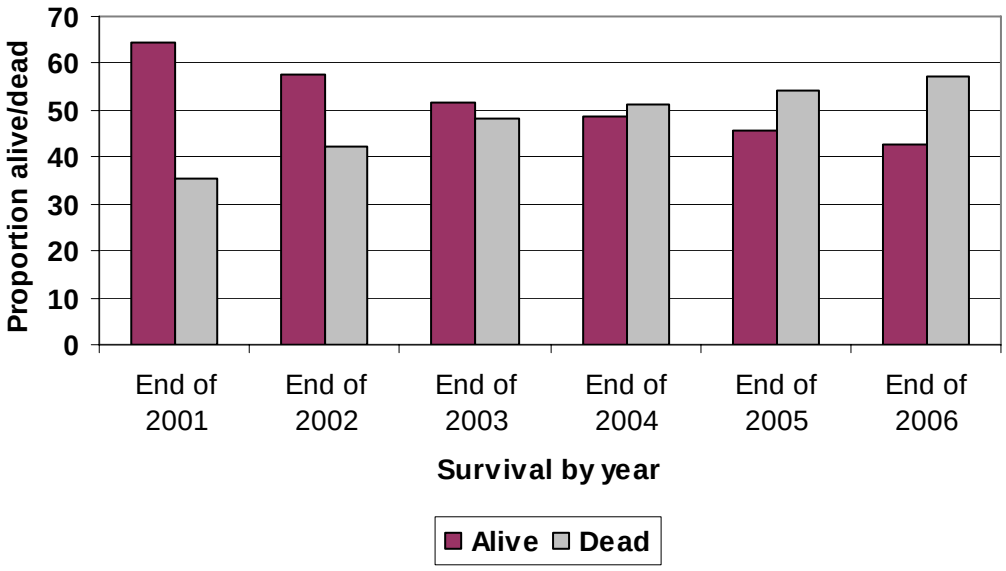
5.3 MORTALITY (I, IV)

The all-cause mortality was high in the cohort. One-year survival was 97% and five-year survival 61%. By the end of 2005, only 45.6% of the patients were still alive (Figure 6). We were able to find the registered primary cause of death in the National Registry for causes of death for 456 (91%) of the 500 deaths by 31 Dec 2005. The most common cause of death was from cardiovascular disease (45.8%), followed by diabetes (22.5%), kidney diseases (10.3%) and malignancies (9%). The proportion of patients with an infection as the primary cause of death was very low, only 3.1% of the patients in the cohort.

In the first descriptive paper (I) we analyzed risk of death until 31 December 2002 associated with a small number of patient characteristics at inclusion. We found a significantly higher mortality with older age (HR for age 65 or older versus age <45 years =5.2; 95% CI =3.1-9.0), BMI ≤ 20 kg/m^2 versus 20.1-25 kg/m^2 (HR=1.96; 95% CI =1.4-2.8), and underlying diagnosis of diabetes relative to glomerulonephritis (HR=3.1; 95% CI =2.3-4.3) when adjusting for all other variables and transplantation during follow-up. Obesity (BMI > 30 kg/m^2) at inclusion was borderline significantly protective (HR=0.7; 95% CI =0.5-1.0). GFR at entry was unrelated to mortality. In further analyses we extended the analysis time to 31 December 2005 and included all other baseline variables (level of education, smoking, alcohol, blood pressure, albuminuria, CCI, plasma

albumin and blood hemoglobin, use of ACE/ARB and other antihypertensive drugs) and we censored transplanted patients at transplantation date.

Figure 6. Survival in a cohort of CKD patients, who were included when the serum creatinine first passed 300µmol/l for men and 250µmol/l for women



In univariate analysis, patients with diabetic nephropathy, lower levels of plasma albumin and blood hemoglobin, lower level of education, alcohol intake and BMI, and patients with higher CCI, blood pressure, cumulative smoking and albuminuria had a significantly elevated HR of death. The results from the multivariate analysis are presented in Table 11.

Compared with the general Swedish population, the members of this cohort had a substantially higher risk of dying as the (Standardized Mortality Ratio [SMR] = 8.3; 95% CI=7.5-9.2). This excess risk was even more pronounced in the younger age categories (SMR=20.6 in age < 45 [95% CI 11.0-35.3]) and among women (SMR=12.3 [95% CI 10.3-14.5]) compared with men (SMR=7.2 [95% CI 6.3-8.1]).

Table 11. Adjusted Mortality for 920 CKD patients included when their serum creatinine first passed 300µmol/l for men and 250µmol/l for women, followed through Dec 31 2005 and censored at date of kidney transplantation.

Variable	Category	Hazard Ratio*	95% Confidence Interval	P-value for trend
Age	< 45 years	**1**	**Ref.**	
	45-64	3.0	1.6-5.7	
	≥65	4.2	2.2-8.3	<0.0001
Sex	Men	1.1	0.8-1.5	
Body mass index (kg/m^2)	<20	**1**	**Ref**	
	20-24.9	0.4	0.3-0.7	
	25-29.9	0.3	0.2-0.5	
	≥30	0.2	0.1-0.4	<0.0001
Alcohol use	No use	**1**	**Ref**	
	Low	0.7	0.5-1.0	
	High	0.9	0.6-1.3	0.72
Smoking	Never smoked	**1**	**Ref**	
	Low	0.8	0.6-1.1	
	High	1.0	0.7-1.4	0.89
Education	≤9 years	**1**	**Ref**	
	9-12	0.7	0.4-1.0	
	≥ 13	0.9	0.5-1.4	0.18
Mean arterial pressure (mmHg)	<100	**1**	**Ref**	
	100-109.9	0.9	0.6-1.3	
	110-117.9	1.0	0.7-1.6	
	≥118	0.7	0.5.1.1	0.26
ACE/ARB use	Yes	1.3	1.0-1.8	
Co-morbidity score	2 (no other)	**1**	**Ref.**	
	3-4	2.2	1.4-3.6	
	>4	4.4	2.5-7.2	<0.0001
Blood hemoglobin (g/L)	≤102 gram/L	**1**	**Ref**	
	103-113 gram/L	0.7	0.5-1.0	
	114-125 gram/L	1.0	0.7-1.4	
	≥126 gram/L	1.0	0.6-1.5	0.78
Plasma albumin (g/L)	≤31	**1**	**Ref**	
	32-36	0.8	0.5-1.1	
	37-39	0.7	0.5-1.1	
	≥40	1.0	0.7-1.5	0.91
Albuminuria	High level	1.7	1.2-2.4	
Primary renal disease	Glomerulonephritis	0.3	0.2-0.5	
	Diabetes	**1**	**Ref**	
	Hypertension	0.8	0.6-1.2	
	Other/Unknown	0.8	0.6-1.2	

**Adjusted for all other variables and eGFR at inclusion*

5.3.1 Mortality before Renal Replacement Therapy was initiated

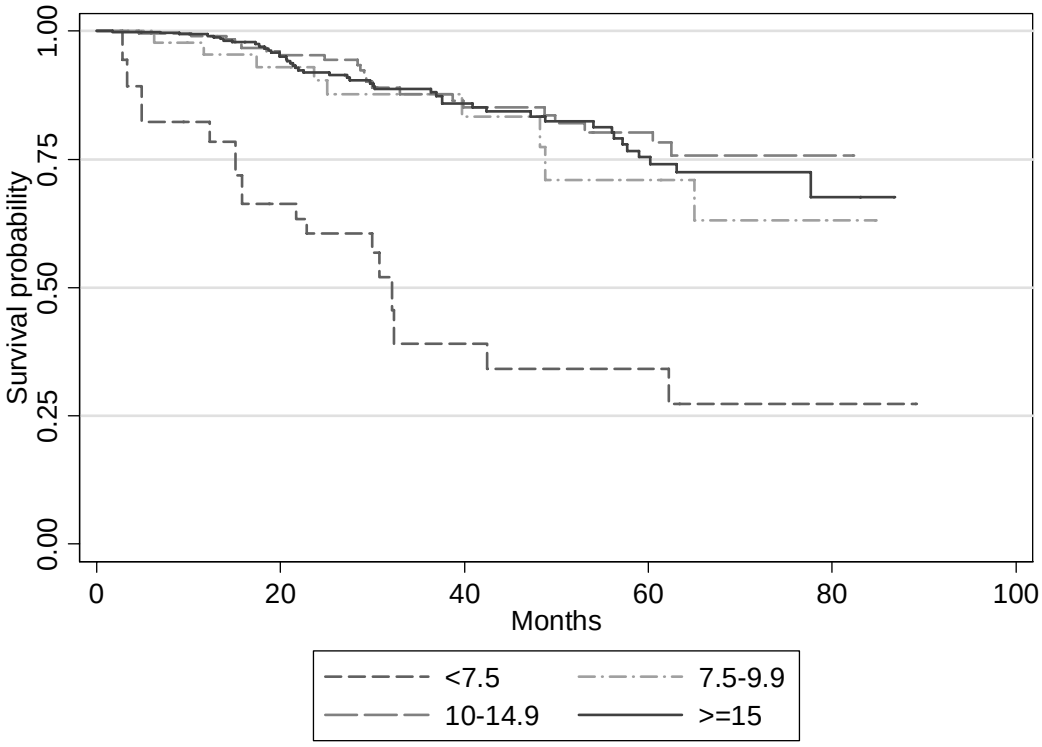
By December 31 2005, 100 patients (9.2%) died before RRT was initiated. We found the cause of death for 94 of these patients. The most common cause of death among patients who died before initiation of RRT was cardiovascular diseases (51%), followed by malignancy (20%) and diabetes (12%). Renal failure or uremia was considered the primary cause of death in 8.5% of the patients who died before RRT.

Not surprisingly, patients who died before RRT was initiated were older, had lower levels of education and more often diabetes, cardiovascular disease, and hypertension and thus a higher CCI score. Plasma albumin, blood hemoglobin was not different at inclusion compared to patients who survived without RRT and higher than patients who started RRT. Albuminuria was significantly higher among patients who died before RRT was initiated compared to those who survived without RRT, but lower than among those who initiated RRT. During follow-up, the patients who died before RRT lost significantly more weight (mean weight loss 2.4 kg (SD 7.7)) compared to patients who started RRT (mean weight loss 0.8 kg (SD 5.3)) and patients who survived without RRT (mean weight *gain* 2.1 kg (SD 6.4)).

In a multivariate analysis, the covariates significantly associated with an increased risk of death before RRT was initiated was higher age, low BMI (BMI <20 kg/m^2 HR 5.9 [95% CI 1.6-21.6] compared to BMI>30 kg/m^2) and high CCI (CCI score >4 HR 3.2 [95% CI 1.1-9.3] compared to CCI score 2). Patients with glomerulonephritis had a significantly lower risk of death before RRT was initiated compared to patients with diabetes nephropathy.

Estimated GFR was also significantly associated to mortality before RRT was initiated (Figure 7). In a Cox proportional Hazards regression model where eGFR was treated as a time-dependent covariate (each time a patient was moved from one eGFR strata to another we censored and restarted the patient's survival time in the new strata) the HR for eGFR <7.5 ml/min/1.73 m^2 was 9.0 (95% CI 4.4-18.1) compared to eGFR ≥15 ml/min/1.73 m^2. The HR for eGFR 7.5-9.9 ml/min/1.73 m^2 was 1.21 (95% CI 0.5-3.0) and for eGFR 10-14.9 ml/min/1.73 m^2 1.1 (95% CI 0.6-1.9) relative to eGFR ≥15 ml/min/1.73 m^2 adjusting for age, sex, CCI, BMI, diabetes, smoking, alcohol use, blood hemoglobin, plasma albumin level, and level of education. In a time-dependent model where only patient survival time was counted when eGFR had fallen to eGFR 10 ml/min/1.73 m^2, the HR for eGFR <7.5 ml/min/1.73 m^2 was 4.65 (95% CI 1.28, 9.49) compared with non-RRT patients of 10≥eGFR≥7.5 ml/min/1.73 m^2.

Figure 7. Kaplan-Meier patient-survival curves by level of estimated glomerular filtration rate (eGFR, ml/min/1.73 m^2) for 901 stage 4-5 CKD patients not yet subjected to renal replacement therapy (RRT). Patients who moved from one eGFR stratum to another were censored in the old stratum and re-started from time 0 in the new stratum. Censoring also occurred at initiation of RRT

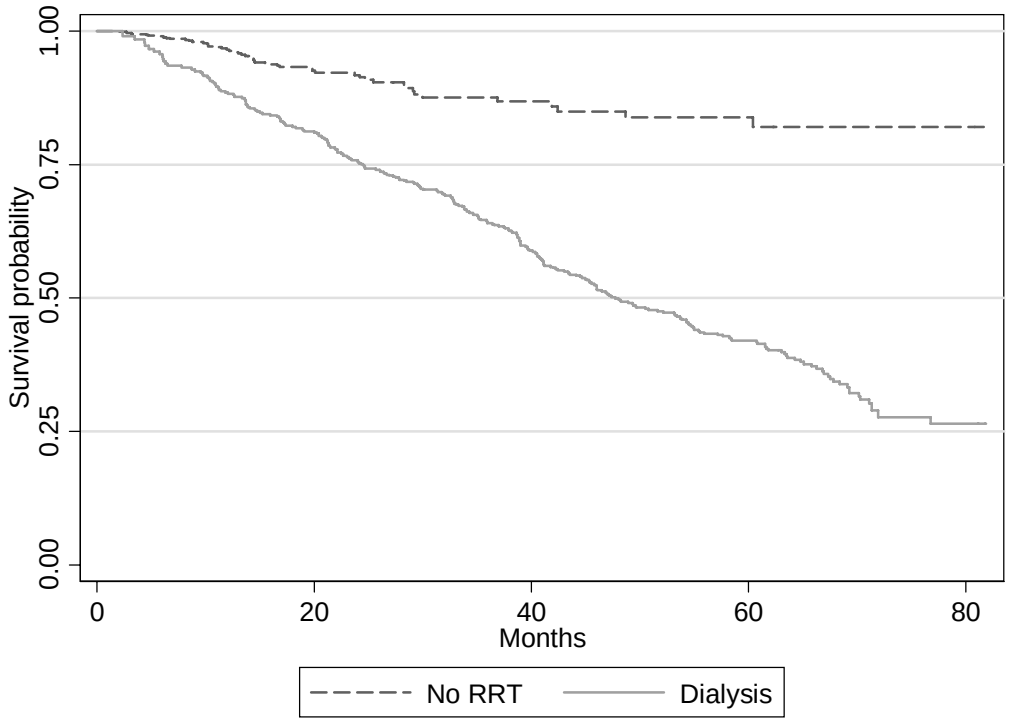


5.3.2 Mortality in relation to dialysis initiation

5.3.2.1 Initiation of dialysis

In a first analysis we investigated mortality for patients who started versus not started RRT. Dialysis start was treated as a time-dependent covariate, and survival time was censored at the date when a patient was either transplanted or ended follow-up (June 1, 2003) without having started RRT. The Kaplan-Meier curve comparing the mortality rates for patients without and with dialysis is presented below (Figure 8). In the Cox proportional hazards regression model, the HR for starting dialysis was 2.64 (95% CI 1.80, 3.89) relative to patients who had not started dialysis after adjustments were made for the differences in baseline characteristics.

Figure 8. Kaplan-Meier survival curves for 901 stage 4-5 CKD patients without renal replacement therapy (RRT) and after initiation of such therapy. In the "No RRT" category, censoring occurred at initiation of RRT. If the RRT was dialysis, the patients started at time 0 in the "Dialysis" category, in which censoring occurred at renal transplantation, if any.



5.3.2.2 Timing of initiation of dialysis

In further analyses we treated both eGFR and dialysis initiation as time-dependent variables. The analysis compared patients who had eGFR ≤20 ml/min/1.73m^2, and started dialysis at different eGFR levels along the disease trajectory. Since late start was defined as dialysis initiation at eGFR <7.5 ml/min/1.73 m^2 we regarded any patient time within eGFR 20-7.5 as "candidate for early dialysis" and any time after 7.5 as "candidate for late start dialysis". The moment the patient started dialysis the patient's survival time was restarted in the "early dialysis start" or "late dialysis start" group. To be able to properly adjust for the differences in progression rates, which in turn affected the probability of reaching the late start dialysis stage, we included in our models both progression rate and eGFR at start of follow-up.

The results of the multivariate analysis are presented in Table 12. Relative to patients who started early, late starters had a non-significantly lower risk of death during follow-up.

Table 12. Time-dependent analysis of timing of dialysis initiation in a cohort of 901 unselected patients with CKD

Variable	Category	Hazard Ratio	95% Confidence Interval	P-value for trend
Clinical status	**Candidate for early dialysis†**	0.35	0.23-0.51	
	Subject to early start dialysis‡	**1**	**Ref.**	
	Candidate for late dialysis§	0.95	0.53-1.67	
	Subject to late start dialysis[a]	0.84	0.64-1.10	
eGFR* when first identified (ml/min/1.73m^2)	(Continuous variable)	0.97#	0.94-1.00	0.12
Decline in eGFR* (ml/min/1.73m^2 per year)	<2.77	**1**	**Ref.**	
	2.77 - 4.60	1.81	1.19-2.73	
	4.61 – 6.01	1.50	1.02-2.20	
	≥6.02	1.54	1.06-2.21	

**ml/min/1.73m^2 estimated by the 4 variable MDRD [28]*
† 20≥eGFR≥7.5 ml/min/1.73 m^2 and not subjected to renal replacement therapy (RRT)
‡ 20≥eGFR≥7.5 ml/min/1.73 m^2 at initiation of dialysis
§ eGFR <7.5 ml/min/1.73 m^2 and not subjected to RRT
[a] eGFR <7.5 ml/min/1.73 m^2 at initiation of dialysis
 # HR per ml/min/1.73 m^2 change of eGFR, adjustments are made for age, sex, BMI, alcohol use, cumulative smoking, primary renal disease, level of education, eGFR first inclusion, progression rate, and CCI.

We also did a sensitivity analysis where we included only patients who had passed ≤15ml/min/1.73m and ≤10ml/min/1.73m^2 to see if the risk associated with late start dialysis changed. The risk estimate did not change substantially; HR (late start) 0.86; (95% CI 0.66, 1.12), and HR (late start) 0.88; (95% CI 0.65, 1.18) respectively compared to early dialysis start. The same pattern of a slight non-significant protective effect of late dialysis start was observed in both sexes, in patients with or without diabetes nephropathy, and for different age categories. We also excluded all patients with imputed eGFR values without any substantial change in the results.

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

When testing a hypothesis the best method to investigate whether an exposure causes an outcome is to conduct an experiment. In medical research however, there are ethical considerations; sometimes an experiment is not possible to perform without putting one of the exposure-groups at risk. There are also economical considerations as randomized controlled trials sometimes are costly. [242]Observational studies investigate exposures that occur within a population without an experimental design. This means that exposed and non-exposed individuals are most likely different. In a perfectly conducted randomized controlled trial, the experimental design results in two equal groups in every aspect be the exposure. In observational studies one has to consider the differences between the two groups and adjust for them in the analyses. Another aspect of the observational study is the study setting and selection of study participants. If the study subjects are selected more frequently because they are exposed, or if they belong to certain risk populations the results will be invalid.

6.1.1 Study design

Most of the studies we performed were cohort studies (I, II, III, IV), but we also included a case-control study in one of the papers (III). Case-control studies are usually performed in medical research when the studied disease is rare but the exposure quite common. The principle is to select cases that have developed the disease and compare with control subjects without the disease that derive from the same study base. Differences between cases and controls have to be adjusted for by statistical methods, usually in logistic regression analysis. Unmeasured differences between cases and controls cannot be adjusted for, and thus may result in so-called “residual confounding”. A common pitfall with case-control studies is in the flawed selection of study subjects; both for cases and controls.[243]

6.1.1.1 Selection of cases and controls

The cases in our study (patients with CKD) have to be as close to “incident” as possible. By “incident” we mean, “newly diagnosed”. It may be easy to find incident cases with appendicitis or fractures, but it is problematic to find a subject with incident CKD; there are so few early symptoms. We decided to define incident as the first time a patient passed a predefined S-Cr value. Although S-Cr is not the perfect measure of renal function it is a common test performed at a wide range of suspected clinical conditions as well as screening in certain risk groups such as hypertensives and diabetics. Since the S-Cr varies with muscle mass, we had different cut-offs for men and women. The serum creatinine limit was set high enough to ensure inclusion of as many true positive

individuals as possible (high sensitivity), but low enough to find the patients before they had reached ESRD with need for dialysis. The reason one wants to find incident patients in case-control studies instead of prevalent (all living patients with CKD) is that prevalent CKD patients are different. Among prevalent cases, there is an over-representation of those with milder and slowly progressive disease. In Sweden the prevalence of diabetes nephropathy and glomerulonephritis among all patients in RRT is 19% and 26% respectively, while the yearly incidence of patients with diabetic nephropathy is 24-27% and glomerulonephritis 11%.[6] We aimed at finding all incident patients in Sweden. While the true incidence of CKD in Sweden is not known, our study results suggest 102-115 per million population year (pmp). This is lower than the incidence of ESRD based upon the Swedish Renal Registry, where the incidence rate was 118 pmp in 1997, and 124 in 1998. Considering that 13% of the prevalent dialysis population was more than 74 years old in 1997 and that the yearly incidence of patients >65 years old was 367 pmp in 1997,[6] we believe we found most patients with incident CKD.

The controls in a case-control study also have to be carefully selected. In a population-based study such as ours it is essential that they derive from the same study base of native Swedes, 18-74 years, living in Sweden May 1996-1998. To obtain this was rather easy giving the structure and accuracy of the up-dated Swedish population register. We calculated the study base to 5.3 million people, and controls were randomly selected from the study-base on three occasions during the study period (1996-1998). Because we wanted to obtain the same age and sex distribution, the controls were frequency matched on these variables. The definition of a control is that he or she does not have the studied disease. We did not measure S-Cr on the controls. The prevalence of existing CKD stage 4-5 (as the cases selected for the study) in a population has been shown to be 0.16%[12] and none of the controls had known CKD based on the interview data. Even supposing some of the controls in fact had CKD it would at most be 1 or 2 subjects and that would not have affected the results to any great extent.

6.1.1.2 Cohort studies

In a prospective cohort study a group of individuals are followed forward in time and events that occur along the path are registered. In medical research, cohort studies may be used when studying unusual exposures and relatively common diseases. The problem with cohort studies is that they usually require a large number of participants for enough events to happen, and that they are expensive and time-consuming to conduct. One can make internal comparisons; comparisons of individuals within the cohort – those who developed the event and those who did not. Another way is to make external comparisons, for example with another cohort or the general population. Our cohort consisted of patients with incident CKD and we made internal comparisons. The advantage with analyses in our cohort was that although we studied relatively few patients, virtually all had a disease progression, and mortality and RRT incidence was high.

It is important that the registration of events is complete and not depend on exposure or disease status. In our survival analyses we used the continuously updated population register, and the Swedish Renal Register, which resulted in no follow-up losses. The eGFR was estimated repeatedly during follow-up so we could compute an individual progression rate. We used S-Cr values taken in regular clinical follow-up abstracted from the medical records during 2003-2005. The number of measurements varied between individuals in the medical records, but we wanted to register the same number of measurements for all participants regardless of disease or exposure status; we hypothesized that "sicker" patients would have more values than "healthier" patients giving rise to different accuracy of the progression rates and differential misclassification (see section 6.1.2.3). The resulting dataset was unbalanced since all individuals had unequal inter-measurement intervals. The repeated, correlated and unbalanced data could be managed using advanced statistical models, now available in most statistical software packages. We tried to find all medical records but were unable to find some (n=57). If the medical records were missing completely at random (not because of any relation to disease or exposure status) they should not affect the result of the estimate. We studied the missing records and found that there was no significant difference in age, sex, analgesic use, and time to RRT or death among patients with a missing record compared to those with a record. Patients with missing records though, had slightly lower eGFR and BMI at inclusion. However, none of the variables were independently associated with missingness. Thus, we do not think that the missing medical records affected our results importantly.

6.1.2 Validity

By validity one means that the results adhere as close to the true results as possible; inferences are said to possess internal validity if a causal relation between two variables is properly demonstrated.[243] External validity is how the results apply to settings other than those studied, generality. Internal validity may be low due to systematic errors, bias, and these one must consider when designing and analyzing the study. There are several types of bias that may apply to both case-control and cohort studies.

6.1.2.1 Selection bias

Selection bias refers to the problem that differences between groups may exist already before the study takes place, and that preferential recruitment of patients belonging to these groups may be responsible for the observed effect. In case-control studies, one example could be the choice to use hospital controls. Subjects belonging to certain socio-economic groups or exposures may be over- or under represented in some hospitals and clinics resulting in selection-bias. In our study we would have had problems with selection bias in the case-control study (IV) if patients or controls that declined to participate or suffered from early death were different in for example lead exposure compared to those included in the cohort. The non-participation rate was however low, and comparable between cases and controls. Selection bias relating to differential recruitment of subjects with low socio-economic status is unlikely because the

health system in Sweden gives essentially equal access to health care regardless of residence or income, [233]and virtually no private actors were involved here.

Another selection problem that mainly pertains to the cohort studies (I-IV) would be if there was a preferential inclusion of patients who had either severe or mild disease; *selection on the outcome*. However, even though the range of the eGFR was wide at inclusion, most of the patients were included close to the eGFR corresponding to the S-Cr limit. With a pre-defined cut-off at S-Cr 250-300 µmol/l we aimed at an eGFR level of approximately 20 ml/min. We assumed that with such levels of S-Cr, the cause of the elevation was most likely to be kidney disease. Also, patients with such values were more likely to have been referred to a Renal Unit or Department. The timing when patients were included depended both on their progression rate, and the interval between their S-Cr measurements. Patients who were unaware of their renal disease and had high progression rates were sometimes included at a very late stage while patients with a more severe disease would probably have their serum creatinine checked more often, and thus were more likely to be included close to the pre-defined limit. Looking at our inclusion data, we find that patients with high eGFR at inclusion more often had a faster progression rate, except for the group with the lowest eGFR values, in which the mean progression rate also was almost as high. In the studies of analgesics (II) and lead exposure (III), there were however no difference in eGFR at inclusion for the different analgesic or lead exposure groups and the CCI score was not associated to eGFR at inclusion. We excluded those with a follow-up less than 14 days because we thought that it was not meaningful to calculate a progression rate with lesser time (II, III). This could have introduced bias if the "sickest" (and with highest exposure levels) were excluded. To analyze the effect of possible "selection of the fittest" we performed sensitivity analyses where we excluded individuals with <3 months of follow-up or eGFR <7ml/min/1.73 m^2 at inclusion. The results of these additional analyses were virtually the same. Since we obtained the other outcome data (mortality and start of RRT and transplantation) from linkages to registries, there was no difference in this information between the different exposure groups.

To avoid *selection on the exposure* we chose to use self-reported information at inclusion. Since 42% of the patients in the cohort were dead by the time we did the first linkage in 2002, introducing a second exposure assessment during follow-up would have resulted in selective information on follow-up data for the surviving proportion. To have equal information from the patients on drugs used during follow-up we used information from the medical records.

In survival analyses there are other selection problems to keep in mind. One is called "*survivor treatment selection bias*", and refers to the common problem that the longer a patient survives the greater is the probability to receive a treatment.[244] If you want to study treatment effects, this bias will most likely favor the treatment group if you not adjust for it by study design. One way to adjust is the use of time-dependent models where the survival probability among treated is compared to the survival probability of non-treated who have survived

thus far. Another problem linked to survival studies is *"lead-time bias"*. To illustrate lead-time bias, one can think of cancer screening. A woman (A) who is screened for breast cancer is diagnosed with a small sub-clinical tumor. Another woman (B) discovers a palpable tumor in her breast and is later diagnosed with breast cancer. If these two women are included in a study at the "date of diagnosis", woman A will have longer survival because she was detected "earlier in the course of disease". The studies of early and late dialysis start faced the same problem as they included incident dialysis patients. The patients included early were "detected" earlier and were favored in the survival analyses.

In our study of early and late dialysis start (IV) we believe that lead-time bias was unlikely to affect the estimates because we included the patients when they passed a predetermined S-Cr level before dialysis start. Although estimated GFR differed significantly between the two groups at first inclusion, late starters had lower eGFR than early starters and lead-time bias, if any, would in that case increase the benefit of late start.

6.1.2.2 Confounding

If exposed and non-exposed differ in some other respect related to the studied disease there is a possibility of confounding. For a variable to be a true confounder is has to be associated to both the exposure and the disease, and not a consequence of the exposure. In our studies we have several potential confounders. Lead exposed patients are for example more often smokers, and smoking is also independently associated to progression rate. The ways to deal with confounding in analyses are regression or stratification. If we had not adjusted (by regression analysis) for smoking in our model we may have observed a faster progression rate for lead exposed that was really caused by smoking. It is only possible to adjust for measured confounders, and unmeasured factors may still be present and obscure a true relationship. In our models we tried to adjust for as many covariates as possible known to us to be associated to our examined exposures and our outcomes.

Confounding by indication is common in observational studies.[245] Patients who start dialysis early don't do that solely by chance, but because they possess some characteristics that may have made their nephrologists chose an early start. These characteristics are often related to severity of disease. Sicker patients are often treated differently to healthier patients. If we do not adjust (by regression or stratification) for the factors relating to severity of disease the results will be confounded. Sometimes it is not possible to adjust for *everything* that the nephrologists may have in mind when assigning early or late dialysis start. There may not be enough resolution in the data to measure small clinical differences, and these small differences will cause "residual confounding". In our analysis of early versus late start of dialysis (II) there may be residual confounding due to insufficient clinical information on co-morbid conditions and other laboratory parameters. Since late starters more often are healthier compared to early starters,

correction for residual confounding will most likely dilute the differences between early and late start even more.

Another type of confounding is *protopathic bias* - factors or symptoms linked to precursor stages of the disease cause patients to have a greater chance to be exposed. In the case-control study by Fored et al [185]investigating analgesic exposure and risk for CKD it was found that the odds for regular users of acetaminophen of developing CKD was 2.5 compared to non users. If early symptoms of CKD (or the disease that gave rise to CKD e.g. diabetes or vasculitis) caused the subject to use more analgesic drugs it would cause protopathic bias. Indeed, the greatest risk for CKD in Fored's study was found among diabetics and patients with systemic diseases - diseases that may cause painful symptoms. However the OR exceeded one in all subgroups studied, and remained significant overall in analyses excluding any exposure within the closest ten years before study inclusion.

In our follow-up of the same patients (II), neither regular nor a high cumulative analgesic use was associated with a faster progression rate. One of the explanations of the differences in results may be that protopathic bias was responsible for the results in the first case-control analysis. Another explanation is that the mechanisms that initiate the early renal damage, and that make the renal disease progress in the advanced stages are different. Protopathic bias is not likely to obscure a true relationship in our follow-up study of analgesic exposure. In order to do that, patients with slowly progressing disease had to consume more analgesic drugs.

Protopathic bias in the occupational lead investigation (III) would mean that patients with early symptoms of CKD became less likely to become lead workers or to continue the exposure. It is possible that a patient with diabetes or vasculitis would have a lesser chance of getting a job and that would indeed cause under-representation of lead exposed among the cases. Lead workers have their blood checked regularly and there is a chance of discovering CKD, which could cause a recommendation to change job. However, this would reduce the OR in the analysis of cumulative exposure, but it does not explain our lack of association of ever versus never exposure and average lead exposure since we recorded a lifetime work history. Protopathic bias cannot explain that we found no difference in progression rate when we performed internal comparisons of exposed and non-exposed CKD patients.

In case-control studies collection of information regarding the exposure is often done retrospectively. A subject who has recently been diagnosed with a disease may then be more likely to remember and give weight to earlier exposures. When cases and controls are compared this *"recall bias"* may result in spurious associations. In our lead exposure study (III) we do not feel that recall bias explained our results because then the CKD patients would have been less likely to remember earlier work-place exposures. In the follow-up studies the information on analgesic drugs, lead exposure and other covariates was collected before we started follow-up, prospectively, and the outcome could not have influenced the information given. Exposed and non-exposed

were interviewed in a standardized manner, and the patients were not aware of the study hypotheses.

6.1.2.3 Misclassification

There are two types of misclassification, differential and non-differential. By *differential misclassification* we mean that information on exposure or outcomes was collected or classified differently for exposed and non-exposed in cohort studies, or cases and controls in case-control studies. Differential misclassification can give rise to bias affecting the results in any direction. We believe that our standardized protocol, interviews, and registry data prohibited differential misclassification in our studies.

Non-differential misclassification is when exposure and covariates are not assigned properly, but if this happens in a non-structural manner then cases, controls, exposed and non-exposed are affected to the same extent. Non-differential misclassification usually causes the results to approach the null-hypothesis.

We do not believe we had any misclassification of the CKD outcome in the case-control study because nephrologists were involved in the diagnostic assignment. The thorough information at inclusion on analgesic exposures (which included both prescribed and non-prescribed use), occupational exposures and other covariates minimized the risk for substantial misclassification. The information on analgesic use during follow-up was however difficult to retrieve from the medical records, and potentially subject to non-differential misclassification. Therefore we chose a restricted approach in the analysis (II), comparing only subjects with regular use both at inclusion and during follow-up, to subjects with no analgesic at inclusion or during follow-up. In the occupational lead investigation there is a certain risk of non-differential misclassification. The risk of misclassification is related to the reliability of the expert rating method. This method, although not perfect, is believed to be the best method to assess occupational exposures retrospectively in community based investigations.[236] We tried to improve misclassification by the combination of experts and self-reported information on work tasks, duration, frequency, ventilation, and protective equipment, which has been shown to increase sensitivity. We also tried to validate our method by doing another expert rating among 53/52 randomly selected cases/controls up to three years after the first. The kappa-value for agreement between the two analyses was 0.87, which is almost perfect. In the study of rare exposures (like lead) a high specificity is needed to reduce the possibility for misclassification. In earlier studies the specificity for the expert rating method has been high (0.91-0.98).[235] In studies of occupational exposures it is found that the more exposed a subject is (high level), the more likely is it that he/she is classified correctly. In our sub-analysis of those with the highest exposure we did not see any higher risk of CKD or higher progression rate compared to the non-exposed.

To validate our use of co-morbid conditions from the medical records we analyzed the kappa statistics comparing the information in the medical records with self-reported information given at the interview. The agreement was 97% (κ=0.94) for presence of diabetes. We also had self-reported information on angina, which we compared to our registration of coronary heart disease from the record. Here the agreement was slightly lower, 91% (κ=0.69). Our overall interpretation is that the medical records gave valid information on the different co-morbid conditions.

In the progression studies (II, III) we acknowledge that there is a risk of non-differential misclassification of the outcome. Here we base our estimates on estimated GFR from the MDRD equation. One of the strengths is that we rely upon serial measurements of eGFR and that our main outcome, except in paper IV, is progression, not the eGFR values per se. The S-Cr values were analyzed at different chemical laboratories all over Sweden and not in a standardized manner. When the study started the Jaffe method was used, but over the following years some laboratories may have used the enzymatic method for some periods. Although there is a difference in serum creatinine with the two methods before the standardized assay was introduced, the resulting bias after introduction into the GFR estimating equation is likely to be small. At the level of serum creatinine where patients started dialysis the difference in eGFR (caused by the use of different methods) is 0-1 ml/min/1.73 m^2. The advantage with a progression rate outcome is that even though creatinine calibration differed slightly between the laboratories, most patients had their tests taken at the same place. The inter-measurement variation is thus most likely very small. In 2003 the laboratories started to use the standardized calibration, regardless of whether they used the enzymatic method or not. Patients initiating dialysis after the calibrated method was introduced have somewhat lower last eGFR compared to those who initiated dialysis before. The differences are, however, small in the range of S-Cr the patients of the cohort had. A 57-year-old man with S-Cr 800µmol/l has an eGFR of 6ml/min/1.73 m^2 regardless of whether the standardized method was used or not. The difference was 1ml/min/1.73 m^2 at S-Cr values 300-700µmol/l. In our data, we find statistical associations for most of the previous known risk factors for disease progression (age, sex, smoking, diabetes, blood pressure, and albuminuria). This speaks, in our opinion, against major non-differential misclassification of the outcome. We have been careful not to extend our analyses to subgroups since the in-born bias from the MDRD equation may be greater and introduce differential misclassification.

6.1.3 Precision

Statistical precision is achieved if the random sampling results in point estimates that are close. Information on precision is obtained from the confidence intervals. Results may have a high precision but nevertheless be biased and so invalid. The size of the standard error is dependent on the size of the population, the number of exposed, the number of individuals who develop the measured outcome, and the distribution of the exposed and events across the study population. Due to the

large number of participants in our cohort, as well as the number of events, we have good precision in most of our survival analyses. In the analyses of lead exposure about 10% of the patients in the cohort were exposed. At this exposure level the case-control study is powered to detect a 40-50% risk increase of CKD incidence (OR ~1.5). Lower risks may not have been detected, but this is also a lesser problem in absolute terms. In the highest exposure groups there were only a few subjects which limit the precision further. The same applies to the analysis of lead exposure as a risk factor for different primary renal diseases where limited precision prohibited us from any elaborate interpretation of the results.

6.2 FINDINGS AND IMPLICATIONS

6.2.1 Risk of Renal Replacement therapy and Mortality (I, IV)

Incident patients with severe CKD (Stage 4-5) in Sweden have a high probability of progressing to ESRD and dialysis. In our population-based study, 82% of the patients eventually initiated RRT. Although all patients had severe renal failure at inclusion, the median time to RRT initiation was 2.2 years. International comparisons show that the prevalence of CKD Stage 3 far outnumbers that of CKD Stage 4-5[12]. We demonstrate that the proportion of patients with a progressive disease seems to be higher for patients who have reached eGFR around 20ml/min/1.73m^2. We found that the risk for RRT was greatest among the youngest patients and those with diabetic nephropathy indicating that these patients will benefit more from timely referral to a nephrologist and preparation of vascular access. Another implication of our results would be to stress the importance of finding and treating patients with earlier stages of CKD since it seems too late to prevent future need for dialysis for most patients with eGFR 20 ml/min/1.73m^2. Although 10% of the patients died before RRT was initiated, the cause of death was usually not uremia but cardiovascular diseases. We did not find any evidence of a large number of untreated patients dying from uremia as has been speculated upon in earlier articles.[246] Patients with the highest risk of death were those of high age, low BMI, diabetes nephropathy, high cumulative co-morbidity score, and high level of albuminuria. Environmental factors such as smoking and alcohol intake showed no independent effect on mortality after adjustments for other related factors. Our findings imply that albuminuria is a strong prognostic factor, both for risk of RRT and for mortality – long after dialysis has been initiated. These results are in line with those of other researchers who have shown that level of albuminuria affects the cardiovascular mortality also in otherwise healthy individuals[102, 103]. Interestingly, we demonstrate that this applies also to patients with severe renal failure where the level of albuminuria may serve as a marker of the vascular/endothelial damage[112], and not only a marker of the severity of the renal disease.

6.2.2 Risk factors for progression of Chronic Kidney Disease (II)

The median progression rate (-5.1 ml/min/1.73 m^2 per year) in our unselected population-based cohort of CKD patients in Sweden was remarkably similar to progression rates estimated in other more selected cohorts. It is however worth noting that the follow-up of this study was undertaken during a time (1996-2003) when the use of ACE-inhibitors was restricted to certain risk groups and the awareness of a strict anti-hypertensive management were not as profound as today. Among the risk factors for decline in eGFR we could confirm several of the previously known risk factors such as gender, elevated blood pressure and proteinuria. We also saw that presence of diabetes and low plasma albumin was associated with a faster progression rate, although the later association disappeared after correction for albuminuria. At this stage of disease, other life-style related risk factors such as smoking and alcohol consumption did not seem to affect the progression rate. Thus, the major effort to retard disease progression among patients with Stage 4-5 CKD should be focused on treatment of hypertension and proteinuria. Treatment with ACE inhibitors/ARB has been demonstrated to slow the progression rate in selected patients with more preserved renal function.[47] In our study however, treatment with ACE inhibitors/ARB was not independently associated with a slower progression rate. On the contrary, in multivariate analyses we saw a faster decline in eGFR with such treatment. This relationship is difficult to interpret in the observational setting since the choice to treat a patient with ACE/ARB to a large extent is subjected to confounding by indication and since the treatment itself tends to increase S-Cr.

6.2.3 Analgesic drug use and risk for decline in estimated GFR (II)

Contrary to our previous beliefs, we found that neither acetaminophen nor aspirin use was related to a faster decline in eGFR. Although previous observational data have been divergent, the case-control study from our own reseach group showed a significantly increased risk with both acetaminophen and aspirin use.[185] The associations in the formar study were strongest for acetaminophen where the OR continued to be significantly elevated when subtracting exposure ten years before the study inclusion. For asprin, the association weakened in this “lagged” analysis. In our 5-7 years of follow-up of the same patients, the decline in eGFR was no different for acetaminophen users compared to non-users, and a slightly protective effect was noted among users of low-dose aspirin. Restricting the analysis to various subgroups did not change the results to any great extent. The results of our study (II) were first regarded with some skepticism by editors and reviewers, but other recent studies have come to the same conclusion[247, 248]. Our interpretation is that nephrologists may continue to use acetaminophen and

low-dose aspirin for patients with CKD Stage 4-5 without risking a faster progression or initiation of RRT. The difference in our results compared to the case-control study may be that the mechanism initiating the first renal damage is different from that causing a more rapid progression. It may also be that the results in the case-control study still were influenced by protopathic bias, although precautions had been made to minimize this in the study design. We also acknowledge that there is some risk of non-differential misclassification affecting the results of our follow-up study. The number of exposed in the highest category were few, and this means lower precision. Moreover, based on our results, one may hypothesize that low-dose aspirin could be used to prevent disease progression among certain CKD risk groups. This must however be further evaluated with a proper randomized controlled trial.

6.2.4 Occupational lead exposre and risk for CKD, RRT and decline in estimated GFR (III)

Workers who had been occupationally exposed to lead did not develop CKD to a greater extent than the age and sex-matched general population. The OR was very close to one after multiple adjustments were made for confounding factors such as age, sex, alcohol consumption, diabetes, level of education and BMI. Neither did patients occupationally exposed to lead progress faster nor start RRT more often compared to non-exposed. Sub-group analyses restricted to those exposed at the highest levels (more than 50% of the Occupational Exposure Limit for at least five years) did not change the interpretation of no effect of lead exposure on renal function. The exposure levels in Sweden are quite low though. In a Swedish occupational cohort the mean blood lead level was 31.8µg/dL for workers who were exposed daily at a lead smelter factory[249]. The blood lead levels in the few other studies associated with renal damage have often been more than 80µg/dL[212, 214, 250]. In Sweden, workers who are occupationally exposed to lead check their PbB regularly and are removed from exposure if the levels rise above 43µg/dL[251]. Our study implies that the present blood-lead limits in Sweden are defined at a level that precludes severe kidney complications. However, our study says nothing about other possible health effects seen with lead exposure at these levels. In addition, we confirmed a relationship for lead exposure with both hypertension and gout as has been demonstrated in several other studies.

6.2.5 Survival and timing of dialysis initiation (IV)

Our data confirm that CKD patients have a high mortality both before and after dialysis initiation. To our knowledge, our study is the first to compare survival rates before and after start of dialysis. We show that mortality increases with declining eGFR, and that the highest mortality is when eGFR moves below 7.5 ml/min/1.73m^2. In spite of the increasing mortality before dialysis, the timing of

dialysis initiation does not significantly affect survival. We see no survival benefit from earlier start of dialysis. Our results thus are coherent with those of the recently published RCT in which there was no difference in survival between the early and late dialysis group[162]. However, it is worth noting that accoring to the MDRD equation, their level of eGFR at dialysis initiation was higher than the mean level in our study. The early group in the IDEAL-study started dialysis at 9.0 ml/min/1.73m^2 compared to 7.2 ml/min/1.73m^2 in the late start group. In our study, the eGFR was 10.8 and 5.5 ml/min/1.73m^2 at dialysis initiation for early and late starters respectively. Thus, although the difference between the groups was larger in our study, there was still no benefit from early initiation of dialysis. On the contrary, there was a small non-significant survival benefit from late start. Maybe the reason lies in the increased mortality seen after dialysis initiation. Of course, there may be a selective transfer of "sicker" patients into the dialysis group, but it could be that the dialysis procedure is demanding and increases inflammation and risk of arrhythmia. Our results show that although we save the patients from uremic death, our substitute treatment (dialysis) does not improve mortality. Based on our observations, a timely start of dialysis (where the survival benefits from dialysis out-weigh complications from treatment) is around eGFR 7.5 ml/min/1.73m^2.

We can also demonstrate that the progression rate is strikingly related to the timing of dialysis initiation. A patient with a faster progression rate has a greater probability of starting dialysis at lower eGFR than a patient with a more slowly progressing disease. Although most of the patients were already recognized at a renal clinic, there is always a delay between the recognition of the future need for RRT and the actual initiation of the dialysis process. First, there is the need to prepare the patient psychologically – not everyone wants to realize their need before they start to feel more distict symptoms. Then there is the need for vascular access or peritoneal cathether surgery, which sometimes delays the process. Thus, there are many reasons why the individual progression rate determines the timing of dialysis initiation. The progression rate is also independently associated to mortality, even after RRT is initiated. This relationship is more difficult to understand, but perhaps the progression rate is a marker of the severity of the disease that goes beyond albuminuria. The results of our studies indicate that the progression rate is another marker that predicts the long-term prognosis of CKD stage 4-5 patients.

6.3 FUTURE RESEARCH

In our study, we saw that patients who used low-dose aspirin regularly progressed somewhat more slowly. Other cohort studies have indicated the same results. Today, low-dose aspirin is used as secondary prevention against ischemic heart disease. Whether low-dose aspirin really does reduce decline in glomerular filtration rate among CKD patients in general or among those with glomerulonephritis, is not well studied. To investigate this association in the observational setting would require follow-up of a large number of patients with early stages of CKD. The problem is however confounding by indication. The only way to know if low dose aspirin is beneficial is to conduct a proper randomized controlled trial.

Another question raised by our research is whether our results on lead exposure also apply to occupational cohorts. The number of lead exposed in our CKD cohort was only about 10%. Few exposed means poor precision, especially in the subgroup analyses. Information on PbB has been collected in Sweden among occupational lead workers for a couple of decades. The values are kept in a register at Arbetsmiljöverket. Linking these data (by using the personal identification numbers) to the Swedish Renal Registry would provide further knowledge of the risk of ESRD associated with different PbB-levels. We have also information on lifetime cumulative organic solvent exposure that we intend to analyze with regard to progression of eGFR and possibly by linkages to the SRR. There are conflicting data of the influence of organic solvents on the progression rate and development of CKD. Whereas some studies indicate that organic solvents increase the progression rate among certain primary glomerulonephritis, [252, 253] others fail to demonstrate the same relationship.[254]

The renal registries in Sweden are important tools making high quality epidemiological research possible. These registries could be further developed to facilitate follow-up of large CKD groups. It is important to link the renal registries to databases with biologic material, such as kidney biopsies and blood, to increase the translational research. One interesting research question is if older patients benefit from RRT or if these patients are better managed with conservative treatment. Using the CKD registry it would be possible to define an eGFR (for example 10 ml/min/1.73m^2) where follow-up is started, and then analyze survival for those treated conservatively versus those treated with dialysis. It would also be possible to apply advanced statistical models[255] (marginal structure models) and adjust for repeated information on co-morbidity and laboratory parameters. Our research has demonstrated that most patients with eGFR <20 ml/min/1.73m^2 eventually start RRT. Another interesting challenge would be to develop a decision-making model to determine the risk factors for future progressive renal disease. Such a model would make it easier to determine which Stage 3 CKD patients in our aging population would most benefit from nephrological follow-up, and which measures would prevent further progression.

7 CONCLUSIONS

I. Most patients with eGFR <20 ml/min/1.73m^2 eventually start RRT. Gender, elevated blood pressure and proteinuria are the most important determinats of future need for dialysis. Risk factors for early death are age, low BMI, diabetes nephropathy, high cumulative co-morbidity score, and high level of albuminuria.

II. Use of acetaminophen or asprin does not increase the decline in estimated renal function for patients with stage 4-5 CKD.

III. Low level occupational lead exposure does not cause advanced CKD or a faster decline in estimated renal function for patients with stage 4-5 CKD.

IV. Early initiation of dialysis does not improve survival. Mortality increases below estimated GFR 7.5 ml/min/1.73m^2 and increases further after initiation of dialysis treatment.

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