The cardiorenal syndrome: Structural and functional aspects including associations with the shrunken pore syndrome

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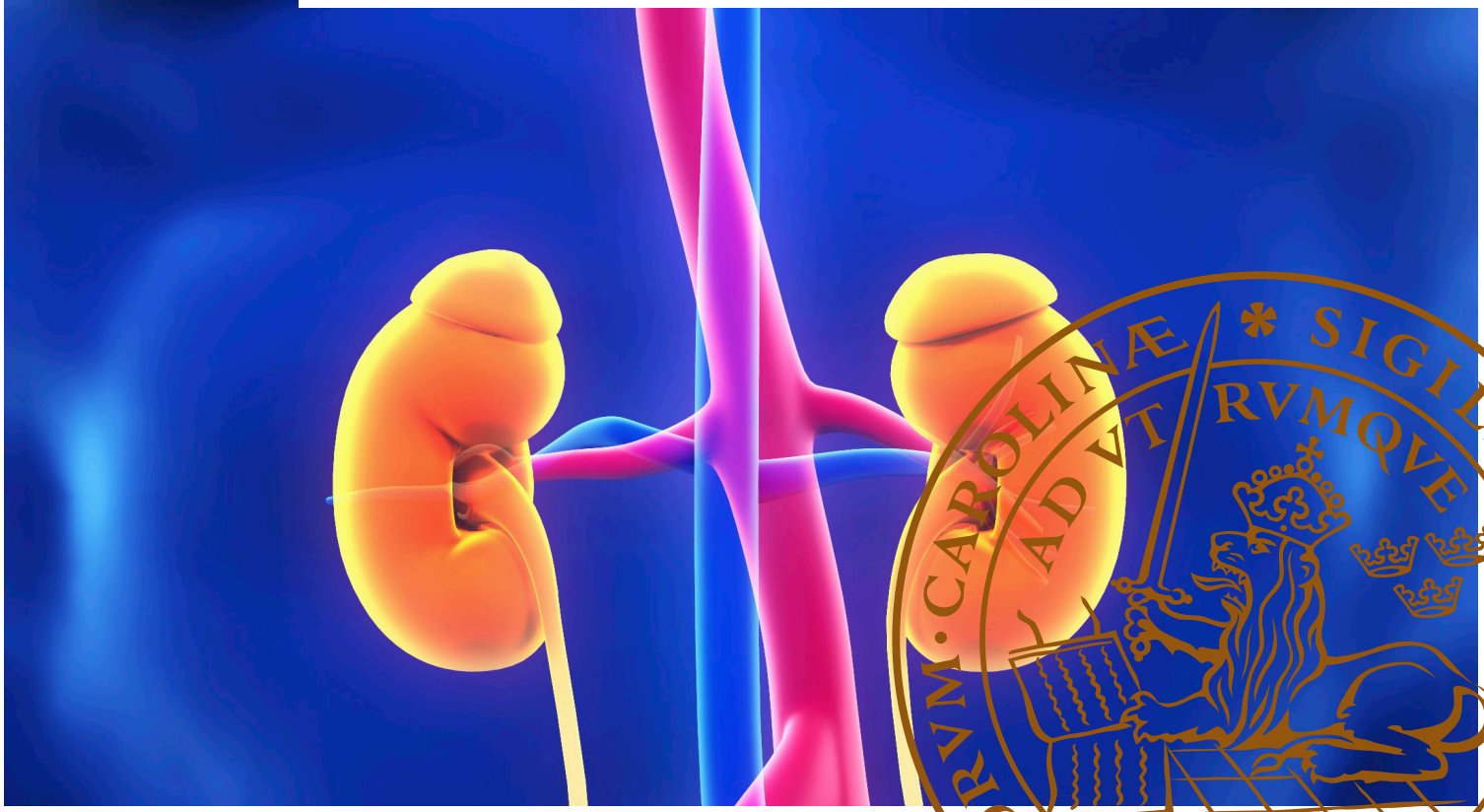

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LIANA XHAKOLLARI is a specialist in Internal and Nephrology since 2015. She works at Skånes University Hospital, Malmö Sweden. Her thesis explore the interplay between the heart and the kidneys in the general population and illustrate a new pathophysiological mechanism behind increased morbidity and mortality in patients with heart failure and individuals in the general population called "shrunken pore syndrome"

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Abstract

Mild to moderate renal impairment affects 10% of the general population. Renal impairment is difficult to detect because of the lack of symptoms, but it can be estimated by calculating the estimated glomerular filtration rate (eGFR) on the basis of plasma creatinine and/or cystatin C concentrations. Chronic kidney disease (CKD) is associated with an increased risk of development of cardiovascular disease and an increase in the mortality rate. As kidney function decreases, structural and functional changes in the heart increase. Cardiovascular disease also affects renal function, leading to CKD. This pathophysiological association between the two organs is referred to as cardiorenal syndrome (CRS). Mortality and morbidity rates are increased in patients with CRS, and early detection of this syndrome can lead to a reduction in the disease burden. To more accurately stage CKD and calculate the mortality risk, the eGFR based on creatinine ($eGFR_{CR}$) and cystatin C ($eGFR_{CYS}$) is recommended for use in clinical practice. The $eGFR_{CYS}$ and $eGFR_{CR}$ usually correspond well with each other. In some individuals, the $eGFR_{CYS}/eGFR_{CR}$ ratio is < 0.7, and it is associated with increased morbidity and mortality. A selective decrease in renal filtration of cystatin C is thought to cause the difference between $eGFR_{CYS}$ and $eGFR_{CR,}$ and this condition is called shrunken pore syndrome (SPS). This thesis presents studies on the early detection of CRS and the association of SPS with mortality and morbidity in patients with heart failure (HF) and in individuals who were randomly chosen from a population-based cohort. In **Paper I,** data from 1504 individuals without HF from the Malmö Prevention Project, which is a population-based cohort, showed significant associations between mild to moderate impairment of renal function and echocardiographic markers of cardiac structure and diastolic function. These findings support the hypothesis that there is an interaction between the kidney and heart, even in the early to moderate stages of renal impairment. In **Papers II and III**, data from approximately 300 hospitalized patients with HF from the HeARt and brain failure inVESTigation trial showed an association between SPS and a doubled risk of all-cause mortality, the risk of 30-day rehospitalization, and impaired quality of life. Proteomic analyses showed that in patients with HF, SPS was associated with proteins related to atherosclerosis and cell proliferation. These findings may help identify pathophysiological pathways involved in the known adverse effects of SPS. In **Paper IV**, data from 5061 individuals from the Malmö Diet and Cancer cardiovascular cohort, which is a population-based cohort, showed that SPS affected as much as 8% of the general middle-aged population, and that individuals with SPS had a 38% higher risk of all-cause mortality. In conclusion, structural changes in the heart can develop as early as when the eGFR is 45–60 mL/min/1.73 m^2. SPS is an important predictor of mortality in patients with HF and in individuals in the general population. Further studies are required to investigate the pathophysiology of SPS and the mechanism behind the association between SPS and mortality.

Key words: cardiorenal syndrome, cardiovascular disease, chronical kidney disease, cystatin C, mortality, shrunken pore syndrome

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“Po nise një udhë,
do kaptosh sheshe,
male, dhe gurë”

(Albanian folklore quote - If you start a journey,
you will pass squares, mountains, and rocks)

To my wonderful family

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Original papers

1. Xhakollari L, Leosdottir M, Magnusson M, Nilsson P, Holzmann M, Christensson A. **Echocardiographic Findings in Patients with Mild to Moderate Chronic Kidney Disease without Symptomatic Heart Failure: A Population-Based Study.** Cardiorenal Med. 2019;9(5):284-296
2. Xhakollari L, Grubb A, Jujic A, Bachus E, Nilsson PM, Leosdottir M, Christensson A, Magnusson M. **The Shrunken pore syndrome is associated with poor prognosis and lower quality of life in heart failure patients: the HARVEST-Malmö study.** ESC Heart Fail. 2021;8(5):3577-3586
3. Xhakollari L, Jujic A, Molvin J, Nilsson P, Holm H, Bachus E, Leosdottir M, Grubb A, Christensson A, Magnusson M. **Proteins linked to atherosclerosis and cell proliferation are associated with the shrunken pore syndrome in heart failure patients: Shrunken pore syndrome and proteomic associations.** Proteomics Clin Appl. 2021;15(4):e2000089. doi: 10.1002/prca.202000089
4. Åkesson A, Xhakollari L, Laučytė- Cibulskiene A, Grubb A, Larsson L, Jujic A, Magnusson M, Björk J, Christensson A. **Shrunken Pore Syndrome and Mortality in the Population Based, Malmö Diet and Cancer Cohort: A Generalized Propensity Score Approach.** (Manuscript unpublished)

Abbreviations

51Cr	EDTA- Chromium-51 ethylenediaminetetraacetic
^{99m}Tc	Technetium 99 isotope
A	doppler measurement of peak velocity of the blood flow through the mitral valve in late diastole
ADH	antidiuretic hormone
Álat	peak velocity of the lateral wall of the basal myocardium of the left ventricle in late diastole.
Ámean	the mean value of the peak velocity of the lateral and septal walls of the basal myocardium of the left ventricle in late diastole.
Ásept	peak velocity of the septal wall of the basal myocardium of the left ventricle in late diastole
AXL	tyrosine-protein kinase receptor UFO
BIS	Berlin Initiative Study
BMI	Body mass index
BP	Blood pressure
BSA	body surface area
CAPA	Caucasian, Asian, Pediatric and Adult
CD163	scavenger receptor cysteine rich type 1 protein M130
CI	confidence interval
CKD	Chronic Kidney Disease
CKD – EPI	Chronic Kidney Disease Epidemiology Collaboration
CKD-EPI$_{CR}$	CKD-EPI formula based on creatinine
CKD-EPI$_{CYS}$	CKD-EPI formula based on cystatin C
CKD-EPI$_{CR-CYS}$	CKD-EPI formula based on both creatinine and cystatin C
CG	Cockcroft-Gault
CO	Cardiac output
CVD	Cardiovascular diseases
DM	Diabetes mellitus
DTPA	Dietylentriaminpentaacetat
E	doppler measurement of peak velocity of the blood flow through the mitral valve in early diastole
Élat	peak velocity of the lateral wall of the basal myocardium of the left ventricle in early diastole

E/Élat	the ratio of: doppler measurement of peak velocity of the blood flow through the mitral valve in early diastole/peak velocity of the lateral wall of the basal left ventricular myocardium in early diastole
Ésept	peak velocity of the septal wall of the basal myocardium of the left ventricular in early diastole
E/Ésept	the ratio of: doppler measurement of peak velocity of the blood flow through the mitral valve in early diastole/peak velocity of the septal wall of the basal left ventricular myocardium in early diastole
EF	ejection fraction
eGFR	Estimated glomerular filtration rate
$eGFR_{CR}$	eGFR based on creatinine
$eGFR_{CYS}$	eGFR based on cystatin C
$eGFR_{CR-CYS}$	mean eGFR of $eGFR_{CR}$ and $eGFR_{CYS}$
Émean	the mean value of the peak velocity of the lateral and septal walls of the basal myocardium of the left ventricle in early diastole.
ERM471	European Reference Material 471
FAS	Full age spectrum
FG	fasting glucose
GFB	glomerulus filtration barrier
GFR	glomerular filtration rate
GPS	Generalized propensity score
HDL	high density lipoprotein
HF	Heart failure
HFmrEF	HF with midrange EF
HFpEF	HF with preserve EF
HFrEF	HF with reduced EF
HR	hazard ratio
HT	Hypertension
IDMS	Isotope dilution mass spectrometry
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IHD	ischemic heart disease
IL2RA	Interleukin 2 Receptor Subunit Alpha
KDIGO	Kidney Disease Improving Global Outcomes
K/DOQI	The National Kidney Foundation's Kidney Disease Outcomes Quality Initiative
LAarea	left atrium area
LASd	left atrium systolic diameter
LAsdm2	Left atrial dimension/body surface area
LDL	low density lipoprotein
LMR	Lund Malmö revised

LMR-CAPA	Mean value of both LMR and CAPA formula
LV	left ventricular
LVEF	left ventricular ejection fraction
LVMI	Left ventricular mass index
MDC	Malmö Diet and Cancer study
MDC-CC	Malmö Diet and Cancer Cardiovascular cohort
MDRD	Modification of Diet in Renal Disease
meanÉ/Á	É_mean/Á_mean ratio
mE/Élatsep	the mean value of E/Ésept and E/Élat
mGFR	measured GFR
MM	Molecular mass
MPP-RES	Malmö Preventive Project re-examined
MRB	Mineralocorticoid receptor blockade
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NYHA	New York Heart Association
OPG	osteoprotegerin
OR	odds ratios
pCr	plasma creatinine
QoL	quality of life
RAAS	renin-angiotensin-aldosterone-system
SCr	Serum creatinine
SCys	Serum cystatin C
SD	standarddeviation
SE -radius	Stokes-Einstein radius
SGLT2	sodium-glucose cotransporter-2
SPS	Shrunken pore syndrome
TAPSE	Tricuspid annual plane systolic excursion
TDI	tissue doppler imaging
TNFR1	tumor necrosis factor receptor 1
TNFR2	tumor necrosis factor receptor 2

Introduction

Even in ancient times, the kidneys were considered to be of great importance. The kidneys and the heart were the only organs that were left in the body during mummification rites because they were thought to determine the fate of the soul. This belief was shared by the Egyptians, the Jews of Egypt, and the Chinese (Salem 1999, Chan 1994). In ancient Babylon, physicians started diagnosing and treating conditions (mostly to relieve urinary obstruction) on the basis of urine turbidity, color, and sediment (Mujais 1999). The term nephrology is derived from the Greek word "nephos", which means "cloud" (which produces rain). The Greek physician Galen of Pergamum (150 BC) was the first to observe the kidney's ability to produce urine and thought it functioned as a filter (Diamandopoulos 1999). Areteus of Capadocia (130–200 AD) was the first to diagnose diabetes by examining urine (Diamandopoulos 1999). Avicenna was a great Persian philosopher who lived from 980 to 1037 and played an important role in conveying ancient medical knowledge with a more modern touch in Europe. His book "Canon of Medicine" served as a code of medical knowledge of anatomy, physiology, and clinical practice in Europe up to the 17th century (Mujais 1987).

During the late 19th and early 20th centuries, some milestones in nephrology included the description of microscopic anatomy of the kidney by Marcello Malpighi, followed by Jacob Henle and William Bowman (Mezzogiorno 1997; Kinne-Saffran 1994; Eknoyan 1996). In 1827, Richard Bright realized that blood urea concentrations increase as kidney function decrease, and that edema and albuminuria could be symptoms of renal disease (Black 1980). Nephrology developed rapidly after the second half of the 20th century owing to technological development. Some of the more prominent developments were renal biopsy, dialysis, and transplantation. Nils Alwall who worked in Lund Sweden was the first to perform an aspiration needle biopsy in 1944 (Cameron 1997). He was a technical genius and was the first to develop a dialysis machine that not only performed hemodialysis, but could also treat a patient's edema by ultrafiltration. George Haas was thought to be the first clinician to attempt to treat uremic patients with hemodialysis in 1923. Willem Johan Kolff was the first to develop a "rotating drum dialyzer" that had sufficient capacity for treatment of these patients (Alwall 1986). Further development in the treatment of terminal kidney disease was achieved by Joseph Murray who was the first to successfully transplant a kidney from an identical twin to another (Merrill 1956). Murray then performed the world's first

successful living donor kidney transplant in 1959. Additionally, in 1962, Murray performed the world's first diseased renal transplant, thus earning the Noble prize in Physiology and Medicine in 1990 (Nobelprize.org).

Anatomy of the kidney

Kidneys are paired organs, large as a fist, approximately 11 cm long, and shaped as beans (http://ovid.visiblebody.com/atlas). They are located on each side of the spine in the retroperitoneal space. The left kidney lies between the T12 and L3 vertebrae, and the right kidney is slightly lower because of the position of the liver (Figure 1). The kidneys are surrounded by three layers comprising the renal capsule, fat layer, and renal fascia. These layers help the kidney to maintain its shape, protect it from infection (renal capsule) and trauma (renal capsule and fat layer), and anchor the kidney to other abdominal structures (renal fascia). The 11th and the 12th ribs also protect the upper parts of the kidneys from trauma.

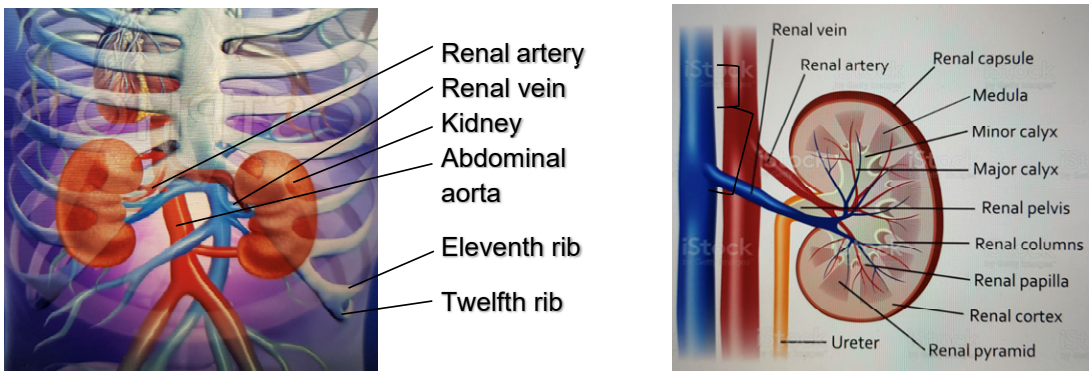

Figure 1: Gross anatomical features of the kidneys. The left panel shows the kidney's position in the abdomen and the right panel shows a dissected kidney with distinct regions within the kidney.
Modified from mostphotos.com and *istockphoto.com*

The cortex, medulla, and pelvis are the constituents of the kidney. The cortex is in the outer kidney and the medulla is in the inner kidney. The pelvis connects the kidney with the ureter (Figure 1). Nephrons are found mostly in the cortex, but also in the medulla. The nephrons are the functional unit of the kidney where urine is produced (Figure 2). The pelvis serves as an excretory channel for the kidney. Renal columns are part of the cortex and serve as pillars for anchoring it. The medulla has several striated conical masses called pyramids that transport the urine from the cortex and medulla to the papilla, and then to the calyxes of the pelvis.

Each kidney is usually supplied by a single renal artery. The renal artery then divides into further branches and forms a network of arterioles called afferent arterioles. These arterioles turn into a specialized capillary network called glomeruli. The

glomeruli merge again to form the efferent arterioles. Blood from the efferent arterioles runs through peritubular capillaries in the cortex and the vasa recta in the cortex, and flows to the peritubular venules. Blood then leaves the kidney by the renal vein (Figure 2). (http://ovid.visiblebody.com/atlas).

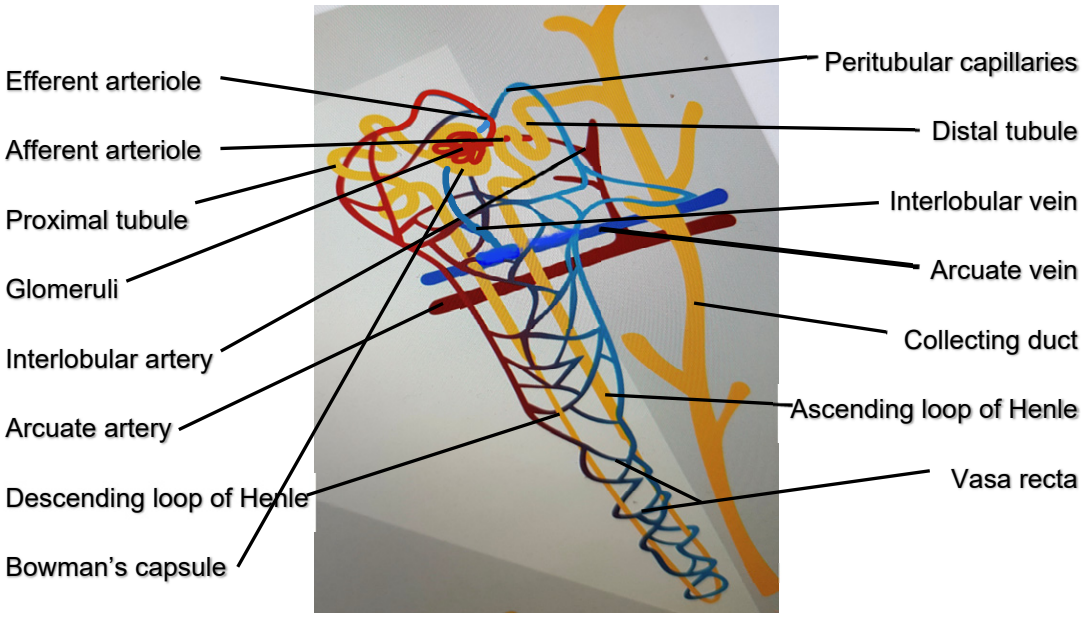

Figure 2: Simplified picture of nephron
Modified picture from pixabay.com

Physiology of the kidney

The kidney has several functions. The three most important functions of the kidney are the excretion of waste products, the regulation of salt, minerals, and water balance, and maintaining acid–base homeostasis. These functions are achieved during the production of urine. Blood pressure regulation and hormone secretion are two other functions of the kidney.

Urine formation: The nephron, which is the smallest functional unit of the kidney, is responsible for urine formation. Every kidney has approximately one million nephrons. Approximately 20%–25% of the blood leaving the heart each minute or the cardiac output (CO) is filtered by the nephrons. Blood enters the nephron through the afferent arterioles and into the glomeruli. Small and middle-sized substances, such as water, urea, creatinine, and cystatin C, are filtered and move within the glomeruli. Large substances, such as cells and proteins, cannot pass the glomerular membrane; therefore, they continue to the efferent arterioles. Filtrated compounds then move from Bowman's capsule to the proximal tubule. Most (70%) of the water, important ions such as sodium, potassium, and bicarbonate, and small molecules such as glucose and amino acids are reabsorbed in the proximal tubule through osmosis, and this does not require much energy. The remaining fluid is then transported to the descending loop of Henle where the reabsorption of the water continues. The ascending loop of Henle is not impermeable to water. In this loop, sodium, potassium, and chloride are actively reabsorbed owing to a concentration gradient that requires a lot of energy. This hypotonic fluid enters the distal tubule. In the distal tubule and the collecting ducts, regulation and fine-tuning of water, electrolyte balance, and pH occurs. This process is regulated by hormones, such as aldosterone, antidiuretic hormone (ADH) and parathyroid hormone. The final urine is then drained across the renal pyramid and papilla to the calyx. During the formation of urine, the secretion of waste products, such as urea, creatinine, and ions (e.g., potassium, ammonium, and calcium) continues throughout the different parts of the nephron in the opposite direction compared with reabsorption (Hall 2020).

Blood pressure regulation: CO and systemic vascular resistance determine blood pressure. An increase in CO leads to dilation of the atria and ventricles. This dilation leads to production of atrial natriuretic hormone and brain-type natriuretic peptide. Both of these hormones dilate the afferent arterioles, causing an elevation in the glomerular filtration rate (GFR). Along with hormones signaling the hypothalamus to reduce the secretion of ADH, an increase in the GFR causes a loss of sodium and

fluid into the urine, thus reducing blood volume and CO. (Hall 2020). Besides volume and salt control, the kidneys affect blood pressure by the renin–angiotensin system. Afferent arterioles have a special type of cells called juxtaglomerular cells that release renin into the blood. The release of renin is induced by a low blood pressure owing to prostaglandin secretion from the macula densa of the kidney when blood sodium concentrations are low, and by the release of beta-1 receptors when the sympathetic nervous system is activated. Renin catalyzes the breakdown of angiotensinogen produced by the liver to angiotensin I. Angiotensin I has a mild vasoconstrictor effect. It is converted to angiotensin II by angiotensin-converting enzyme (ACE), which is produced by the kidneys and the lungs. Angiotensin II is a potent vasoconstrictor and activates the sympathetic nervous system, and stimulates the secretion of aldosterone from the adrenal glands. These processes increase the reabsorption of sodium, chloride, and water in the tubules. Finally, this stimulates secretion of ADH, which increases the reabsorption of water in the collecting duct (Hall 2020).

Kidney as an endocrine organ: The kidney is responsible for the production, activation, and metabolism of several hormones besides renin. Erythropoietin is produced by fibroblasts in the cortex in response to hypoxia. Thrombopoietin is produced in small amounts in cells of the proximal tubule. Vitamin D which is produced by the skin when in contact with the sun or is contained in some foods, undergoes its final transformation and become active due to 1-alpha hydroxylase, which is produced by the proximal tubule. Only in its active form can vitamin D regulate calcium and phosphate balance and insulin secretion (Bland 2004). The production of parathyroid hormone is regulated by how the kidney affects calcium and phosphate homeostasis. Many hormones are metabolized by the kidney through renal clearance (e.g., insulin) (Hall 2020) or by renal catabolism (e.g., gastrin) (Davidson 1973).

GFR

One of the main functions in the kidney is glomerular filtration, and it is considered to be the best way to evaluate kidney function. The GFR cannot be measured directly in the kidneys because the filtration occurs simultaneously in millions of glomeruli and the urine changes its volume and consistency. The GFR is measured by calculating the velocity of the plasma clearance of endogenous or/and exogenous substances through the urine (Hall 2020).

$$Clerance\ of\ X\ = \frac{Urine\ concentation\ of\ X * Urine\ flow}{Plasma\ concentration\ of\ X}$$

The elimination of substances is constant in healthy subjects, which means that the amount of an eliminated substance (X) (urine concentration of X × urine flow) is the same every day. In chronic kidney disease (CKD) at steady state, the amount of the substance that is eliminated is also constant day by day, but it results in higher plasma concentrations at a low GFR.

The GFR can be measured (mGFR) or estimated (eGFR) by using exogenous or endogenous markers. Methods for the mGFR are more accurate than those for the eGFR, but they are also more cumbersome and expensive.

Measured GFR

The mGFR approach involves injection/infusion of an exogenous GFR marker. A description of several GFR markers and their development during the 20th century is provided below.

Exogenous GFR markers

Inulin renal clearance is the gold standard for the measurement of kidney function. Inulin is a polysaccharide produced by many plants and has a small molecular mass (MM) (3–5 kDa). The first use of inulin was reported in 1935 (Ferguson 1949). Inulin's constant and free glomerular clearance without secretion or reabsorption from other parts of the kidney makes it a perfect marker of the GFR. However,

measurement of inulin is complicated and expensive. Inulin is administered as an intravenous infusion to achieve a constant concentration of inulin in the blood. During the infusion period (usually 4 hours) and after plasma inulin concentrations have stabilized, urine is collected, and the urine concentration of inulin is measured. Because this process is complicated and expensive, other exogenous substances are usually used to evaluate the GFR.

Chromium-51 ethylenediaminetetraacetic (51Cr-EDTA*)* was first proposed by Bröchner-Mortensen *et al* in 1966. This marker is a chelate complex of a radioactive isotope of chromium. In a previous study, 17 subjects received a single injection of 51Cr-EDTA, and 11 plasma concentrations were measured from each subject between 15 minutes to 5 hours after exposure. The plasma clearance of 51Cr-EDTA closely corresponded with the clearance of inulin (Bröchner-Mortensen 1966). However, if plasma 51Cr-EDTA concentrations are measured too close to the injection time, the GFR can be overestimated, especially in individuals with a low GFR. Bröchner-Mortensen suggested using a correction factor for the steep first part of the time–concentration curve of 51Cr-EDTA. One disadvantage of this technique is that 51Cr-EDTA is radioactive.

Diethylenetriamine pentaacetic acid (DTPA) is a chelating agent that has been used for the mGFR since the 1960s. DTPA was first proposed for determining the mGFR by Funck-Brentano *et al* in 1967. The measurement procedure of DTPA is similar to that of 51Cr-EDTA (Funck-Brentano 1967). The technetium 99 isotope (^{99m}Tc) is usually used as a marker. ^{99m}Tc may dissociate from DTPA and binds to plasma proteins, which may cause an underestimation of the GFR. Therefore, this possible underestimation has led to controversy regarding whether ^{99m}Tc-DTPA is a reliable marker for the mGFR. A systematic review of methods for the mGFR by the Swedish Council on Health Technology Assessment (SBU) in 2013 and by Soveri *et al* in 2014 questioned the use of ^{99m}Tc-DTPA. Their conclusion has been disputed by other scientists. Two studies by Andersen *et al* in 2019 and Simonsen *et al* in 2020 that compared the two methods showed a similar mGFR using both methods. This issue is highly relevant because of the shortage of 51Cr-EDTA.

Iohexol is an iodine-based, low-osmolar, non-ionic contrast agent with a low MM of 821 Da, and it was invented in Malmö by Professor Torsten Almén (Almen 1985). The low osmolarity of iohexol is an improvement in relation to older contrast agents and it makes injection with contrast agents less painful. Similar to inulin, iohexol is a water-soluble, non-protein bound substance that is filtered mostly by the glomeruli, and it is not secreted or absorbed by the tubules. Iohexol was first introduced as a marker for the GFR in 1983 by Olsson *et al* and in 1984 by Krutzen *et al*. They compared the GFR of iohexol with that of 51Cr-EDTA. They analyzed plasma iohexol concentrations after an infusion or a single injection, several times on different occasions (1 minute to 5 days) and also analyzed urine iohexol concentrations (0.5 hours to 5 days). Plasma clearance of iohexol correlates well

with that of 51Cr-EDTA (Olson 1983; Krutzen 1984). Another study that compared iohexol with inulin showed similar results (Sterner 2008).

Iothalamate was first used by Sigman *et al* in 1965. Similar to iohexol, iothalamate is an iodine-based contrast agent, but it has a high osmolarity. The isotope form ^{125}I-iothalamate is mostly used to measure the GFR. ^{125}I-iothalamate can also be administered as a subcutaneous bolus, and then its concentration in blood and urine is measured four times from 1.5 to 2 hours. Seegmiller *et al* compared the plasma clearance of iohexol with the renal clearance of ^{125}I-iothalamate, and the mean proportional ratio was 0.85, which indicated a lower GFR when measured with iohexol. These findings are disputed by other scientists such as Sterner *et al.* They believe that the higher GFR of iothalamate is due to the tubular clearance of this agent (Sterner 2016). Earlier data also suggest that iothalamate has tubular clearance (Odlind 1985). This issue is important for the evaluation of different eGFR (discussed below).

Sampling techniques

The problem with the plasma clearance after a single injection is that it has two different clearance rates. During the first phase (distribution phase), the clearance is more rapid because agents also leave the plasma compartment owing to the distribution to various body tissues in addition to renal clearance. Therefore, if plasma concentrations of agents are measured too soon after the injection, the GFR can be overestimated (Bröchner-Mortensen 1972). Initially, measuring plasma concentrations of agents in several samples (usually four) during several hours (usually 3–5 hours) after the injection (multiple sample procedure) was common. To further simplify the procedure, most laboratories now only analyze the plasma concentration once (Jacobsson 1983; Sterner 1996). To avoid measurement during the distribution phase, the measurement of plasma concentrations of agents is performed after a specific amount of time based on the individual's body surface area and presumed GFR.

The mGFR obtained by agents, such as 51Cr-EDTA and iohexol, is relatively expensive and inconvenient for many patients, and the results take a longer time to obtain than those with the eGFR. The mGFR is used when knowledge of glomerular filtration is of particular importance (e.g., treatment with nephrotoxic cytostatic drugs and with living kidney donation.

Endogenous GFR markers

The eGFR by an endogenous marker is the recommended bedside method to evaluate renal function. The eGFR is based on plasma clearance of endogenous substances. Several endogenous GFR markers are described below.

Urea was the first substance that was formally used to measure kidney function in the early 1900s. Urea is the residual product of protein degradation. It has mostly renal elimination, but it is strongly dependent on the diet (McIntosh 1928, Cope 1993), diuresis, and medications. These factors are more determinant of the plasma concentration of urea than the renal clearance of urea. Therefore, urea is a weak marker of kidney function, and it is currently mostly used as marker of the degree of uremia (e.g., to determine the start of dialysis).

Creatinine was first suggested as a marker of kidney function by Rehberg in 1926 and it is still used to indicate kidney function (Rehberg 1926). Creatinine is a small molecule with a MM of 0.113 kDa and a Stokes-Einstein (SE) radius of 0.3 nm. Creatinine is produced by the muscles during normal breakdown of their cells and by protein metabolism (mostly meat) and is distributed in the entire water volume of the body. Only one-third of creatinine is in the extracellular fluid space. Creatinine is mostly eliminated by glomerular filtration but is also eliminated by proximal tubular secretion (Shannon 1935). Conditions that affect creatinine metabolism, such as a high protein intake or exercise, increase serum creatinine concentrations. Muscle mass varies depending on age, sex, and ethnicity. Children and older people have a lower muscle mass than young adults, women have a lower muscle mass than men, and Afro-Americans have a higher muscle mass than some non-black races (Gallagher 1997). There are many conditions that affect tubular secretion. Kidney failure increases tubular secretion, which increases creatinine clearance (Perrone 1992). Medicinal intake can alter creatinine clearance in different ways (Andreev 1999) (e.g., cimetidine (Dubb 1978) and trimethoprim (Berglund 1975) decrease creatinine clearance). Diseases in the thyroid gland can also affect serum creatinine concentrations (Fricker 2003).

The method by which a substance is measured is equally important in assessing the substance's credibility. Before the 1980s, the so-called Jaffe method was used for measuring creatinine in plasma. Creatinine reacts with picric acid at a basic pH and builds a complex that is proportional to creatinine concentrations in a blood sample (Delanghe 2011). The colored complex can be measured by absorbance at a wavelength of 500 nm. Before the 1970s, proteins were removed by dialysis or precipitation. When an increasing number of samples needed to be analyzed, laboratories started using creatinine from plasma without the removal of proteins. However, picric acid reacts with other proteins and forms pseudocreatinine, thus leading to an overestimation of creatinine concentrations in the plasma (Delanghe 2011). The Jaffe method was later replaced by an enzymatic method. Using this method, creatinine is transformed in several steps to a chromogen, which is measured dichromatically at 546 and 700 nm. The change in the absorbance is proportional to the creatinine concentration in the sample (Roche 2016/2018). Further improvement in the measurement of creatinine was followed by the introduction of isotope dilution mass spectrometry (IDMS). This standardized method facilitated comparison of creatinine values analyzed at different

laboratories. Regular comparison of the measurement results in all hospital laboratories in Europe is performed several times each year (external quality assessment).

Cystatin C is a recently introduced endogenous substance that began to be used in the 2000s to measure kidney function. Cystatin C was formerly known as gamma trace, post-gamma-globulin, or neuroendocrine basic polypeptide, and was first found in the urine of patients with proteinuria by Butler and Flynn in 1961. Cystatin C is part of the cystatin family, and it inhibits cysteine protease activity. This substance affects the response of the body to brain injury (Abrahamson 2003) by inducing autophagy of neurons (Tizon 2010) and by preventing amyloidosis (Kaeser 2007). Mutations in the cystatin C gene lead to amyloid deposits, causing stroke and death, as described by Levy *et al* in 1989 (Levy 1989). Cystatin C is thought to have a protective role in the prevention of atherosclerosis because of its inhibition of cathepsin S. Cathepsin S is a cysteine protease that, among other functions, degrades elastin and thus participates in vascular wall remodeling (Shi 1999, Eriksson 2004). In laboratory conditions, cystatin C affects the movement of human polymorphonuclear neutrophils (Leung-Track 1990). Therefore, cystatin C may play a regulatory role in the inflammatory process.

Löfberg and Grubb were the first to report the amino acid sequence of cystatin C (Grubb 1982). Furthermore, Grubb was the first to recognize cystatin C's role as a GFR marker. Cystatin C has a higher MM and SE radius than those of creatinine (13.4 vs 0.113 kDa and 0.3 vs 1.8 nm, respectively), and it is produced at a stable rate by all cells containing a nucleus (Abrahamson 1990). However, there is a genetic polymorphism in CST3 that affects cystatin C production in approximately 20%–40% of the population (Åkerblom 2014, O´Seaghdha 2014). Cystatin C is found only in the extracellular fluid space with the highest concentration in the seminal vesicles, and it regulates extracellular proteolysis (Abrahamson 1986). Cystatin C is less dependent of body composition, sex, and ethnicity than creatinine (Vinge 1999). Conditions affecting cellular metabolism, such as hyperthyroidism (Fricker 2003), cancer (Funda 2010, Oc 2014), inflammation (Harman 2009), and high cortisone intake (Cimerman 2000, Risch 2001), alter cystatin C concentrations.

Experimental data (mostly in rats) have shown that cystatin C, similar to creatinine, is mostly eliminated by glomerular filtration (94%), and its extrarenal elimination is much smaller than that of creatinine (Tenstad 1996). In individuals with a normal GFR, 100% of cystatin C is absorbed in the tubules and catabolized (> 99%) into amino acids (Tenstad 1996). In uremic rats, plasma cystatin C concentrations reached a steady state on day 2 after bilateral nephrectomy and did not continue to increase, unlike creatinine, in the following 5 days, suggesting changes in its metabolism (Bökenkamp 2001). How much of these changes depend on changes in production or a change in extrarenal clearance of cystatin C remain unclear.

Similar to creatinine, the methods for measuring cystatin C have changed over time. The first method used by Grubb and Löfberg in 1979 was the radial immunodiffusion assay (Grubb 1982). This method is time consuming and not particularly accurate. Since this time, other different methods have been developed for measuring cystatin C. This development has led to problems in comparing different cystatin C values analyzed at different laboratories. A comparison of cystatin C results conducted in Sweden in 2011 showed a large variation in intermediate laboratories by 9% (SBU 2013). After an initiative from Anders Grubb *et al* in 2008, a primary and a secondary reference preparation for cystatin C was produced and characterized by the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) working group on standardization of cystatin C (Blirup-Jensen 2008). The world calibrator is called European Reference Material 471/International Federation of Clinical Chemistry and Laboratory Medicine (ERM471/IFCC). Currently, cystatin C concentrations are compared between different laboratories as easily as creatinine concentrations after IDMS standardization. The most commonly used methods for measuring cystatin C in current practice are the particle-enhanced nephelometric immune-assay and the particle-enhanced turbidimetric immunoassay. The principle is the same in both analyses.

Estimated GFR

In the late 1990s, the incidence of CKD started to become a recognized public health problem (K/DOQI 2002) because of poor outcomes in patients with kidney failure and the high cost of treatment in these patients. An increasing amount of evidence has indicated that if CKD is diagnosed early and treated, the development of terminal kidney failure could be postponed or avoided. At this time, plasma creatinine concentrations were used for estimating the level of kidney function, but this has a low accuracy for detecting renal impairment (K/DOQI 2002). Early renal impairment of 50% of normal function results in an increase in creatinine concentrations within the normal reference range. Therefore, this level of renal impairment is frequently not detected by physicians. This fact in combination with the fact that plasma creatinine concentrations are affected by factors other than the GFR, led to analysis of creatinine clearance for a more accurate assessment of kidney function (K/DOQI 2002). This procedure is time consuming and collection of 24-hour urine is difficult to perform. In 2002, the National Kidney Foundation's Kidney Disease Outcomes Quality Initiative (K/DOQI) investigated whether the eGFR based on formulas using serum creatinine as a marker were as good as measurement of the creatinine clearance. After reviewing publications, the K/DOQI recommended using the Cockcroft Gault (CG) and Modification of Diet in Renal

Disease Study 4 (MDRD) formulas in adults, and the equations of Schwartz and Counahan–Barratt in children.

In 2012, Kidney Disease Improving Global Outcomes (KDIGO) published clinical practice guidelines for the evaluation and management of CKD. KDIGO recommended the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula for estimating the GFR. Other formulas based on creatinine were recommended if they could show an improved accuracy of GFR estimates compared with the CKD-EPI formula. For the first time, the measurement of cystatin C was recommended in some cases (KDIGO 2012). In September 2021, the National Kidney Foundation-American Society of Nephrology published their new recommendation of the use of eGFR equations based on creatinine and/or cystatin C that do not incorporate race as a variable (Inker 2021).

Only eGFR equations for adults are further investigated in this thesis.

Estimated GFR equations

There are many equations based on creatinine and cystatin C both for adults and children. To evaluate the accuracy of these formulas for estimating the true GFR I looked at the Accuracy, Bias and Precision parameters of the formulas. However, these parameters were not always available and were defined in different ways by different authors. For a summary of the reviewed formulas for eGFR please see Table 1.

The formulas that historically have been used in adults are:

Creatinine based GFR ($eGFR_{CR}$):

Cockcroft and Gault (CG)-formula. The CG formula was proposed in 1976 by Cockcroft who was a resident researcher in the field of pulmonary diseases. The project was supervised by Gault who was a nephrologist. Cockcroft and Gault compared their results of the eGFR based on creatinine with 24-hours creatinine excretions/kg in 249 patients. The correlations coefficient between CG formula and the creatinine clearance was 0.83. In 95% of the patients the predicted and mean measured values differed by ≤35%. In 67% of the patients, the values differed by ≤ 20%. The CG formula was widely used until late 1990s/early 2000s. This formula overestimates the GFR in patients with a reduced kidney function (GFR < 60 mL/min/1.73 m^2). No data were available on accuracy, bias or precision (Levey 1999; SBU 2013).

Modification of Diet in Renal Disease Study (MDRD) formula: The MDRD formula was proposed by Levey *et al* in 1999 to develop a formula that could estimate the eGFR better than the CG-formula. They compared the eGFR by the MDRD formula, GFR by ^{125}I-iothalamate, and creatinine clearance in 558 patients with chronic renal

disease. These patients were not on dialysis and did not have a kidney transplant. The mean mGFR was 39.8 ± 21.2 mL/min/1.73 m^2. In their study, the eGFR based on the CG equation was 16% higher than that with the mGFR. The creatinine clearance overestimated the GFR by 19%. The eGFR based on the MDRD formula showed a 90.3% concordance correlation coefficient (R^2) with the GFR measured by iothalamate (Levey 1999). The MDRD formula underestimates the GFR in patients with a GFR > 60 mL/min/1.73 m^2 (Stevens 2007). Even here there was no data on accuracy, bias or precision.

Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula based on creatinine ($CKD\text{-}EPI_{CR}$) was proposed by the research group of CKD-EPI, which was established by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The purpose of this new formula was to estimate the GFR even in patients without renal impairment. This research group used participants from 10 different studies, including 5504 to develop the formula, 2750 for internal validation, and 3896 for external validation. The $eGFR_{CR}$ was compared with urinary clearance of iothalamate in the development and internal validation phases, and by iothalamate and other filtration markers for the external validation. The bias was measured as the median of the difference between the mGFR and the eGFR. The precision was measured as the interquartile range of the difference (i.e., difference of the median of the upper half of the eGFR and the median of the lower half). The accuracy was measured as the root-mean-square error and as the percentage of estimates that differed by more than 30% from the mGFR (P_{30}). The mean mGFR was 68 (±39) mL/min/1.73 m^2. The bias was 2.1 mL/min/1.73 m^2 in patients with a GFR < 60 mL/min/1.73 m^2 and 3.5 mL/min/1.73 m^2 in patients with a GFR ≥ 60 mL/min/1.73 m^2. The mean difference for the MDRD formula was 3.4 and 10.6 mL/min/1.73 m^2, respectively (Levey 2009).

Lund Malmö revised (LMR) formula: The concern that the MDRD formula underestimated the GFR in healthy patients led to the development of the LMR formula. Two cohorts of patients in Sweden who were older than 18 years were used to predict the eGFR using a new formula in 2007. In the first group of 436 patients, creatinine concentrations were measured by an enzymatic method. In the second group, creatinine concentrations were measured by the Jaffe method in 414 patients. All patients had iohexol clearance measurement and their GFR varied from normal to low. The median (2.5 and 97.5 percentiles) mGFR in the first group was 63 (12–124) mL/min/1.73 m^2 and 42 (8–122) mL/min/1.73 m^2 in the second group. In the entire cohort, the median mGFR was 55 (9–121) mL/min/1.73 m^2 (Björk 2007). The bias, accuracy, and precision were defined in the same way as that in the CKD-EPI formula. The LMR formula was better regarding all three parameters of bias, accuracy, and precision compared with the CKD-EPI formula (Björk 2011).

Berlin Initiative Study 1 (BS1): The BS1 was developed to better estimate the GFR in older patients in 2012. The BS1 compared eGFR results with iohexol clearance

and the eGFR on the basis of the CG, CKD-EPI, and MDRD formulas in 570 individuals aged ≥ 70 years living in Berlin. The mean mGFR was 60.3 (15.5–116.7) mL/min/1.73 m^2. The bias was defined as the difference between the eGFR and the mGFR for each equation. The precision was measured as the interquartile range for the difference and an SD of the bias. The accuracy was assessed as the percentage of estimates within 30% of the mGFR. The BIS1 had better precision than the other formulas, especially when the mGFR was > 30 mL/min/1.73 m^2. All of the other formulas overestimated the GFR (Schaeffner 2012).

Full age spectrum (FAS) formula: In 2016, Pottel *et al* thought that an equation for the eGFR across the FAS was lacking. They studied 6870 individuals from databases from different countries (France, Norway, UK, Germany, and the USA) for the development and validation of the formula. In children and adolescents, the FAS and FAS-height equations were compared with the Schwartz equation. In adults, the FAS formula was compared with the CKD-EPI formula, and for the oldest adults, the BIS1 was added. In all cohorts, iohexol was used for the mGFR. The mean mGFR (mL/min/1.73 m^2) was 94.5 ± 31 in children, 78.6 ± 26.2 in individuals aged 18–70 years, and 55.7 ± 20.6 in individuals aged > 70 years. The bias was defined as eGFR – mGFR. Precision was defined as the root mean square error. Accuracy was assessed as the percentage of estimates within 30% of the mGFR. The FAS equation had the same bias as that for the Schwartz equation, but it had better accuracy in children aged < 14 years. The FAS-height equation was equivalent to the Schwartz equation in adolescences and young adults, but better than the CKD-EPI equation. In adults, the FAS and CKD-EPI formulas were similar. The advantage of the FAS formula is its continuity; therefore, the eGFR can be followed and compared, regardless of the age of the patient (Pottel 2016).

Cystatin C based eGFR formulas ($eGFR_{CYS}$):

CKD-EPI based on cystatin C ($CKD\text{-}EPI_{CYS}$) formula: The $CKD\text{-}EPI_{CYS}$ formula was developed to further improve the estimation of the GFR because creatinine-based formulas were still imprecise. The CKD-EPI funded by the NIDDK used participants' data from different databases, and the eGFR was compared with the mGFR of renal or plasma clearance of an exogenous filtration marker. A total of 3522 participants were used for the development of the formula, with 1830 participants for internal validation and 1119 participants for external validation The mean mGFR (± SD) was 68 ± 39 mL/min/1.73 m^2 in the development and internal validation group and 70 ± 41 mL/min/1.73 m^2 in the external validation group. The addition of race did not improve the equation. The bias for the cystatin C equation was 3.4 mL/min/1.73 m^2. The bias was lower in patients with a GFR < 60 mL/min/1.73 m^2, only 0.4 mL/min/1.73 m^2. This further strengthened a previous finding that cystatin C was better for estimating the GFR in patients with a reduced renal function (Inker 2012).

Caucasian, Asian, Pediatric and Adult (CAPA) equation: A total of 4960 individuals from CAPA cohorts were used to develop a formula for the eGFR using cystatin C, which was measured by the ERM-DA471/IFCC calibrator. The renal or plasma clearance of inulin or the plasma clearance of iohexol was used for measuring the GFR. The bias was defined as the median of the individual differences between the eGFR and the mGFR. The precision was measured as the interquartile range of the differences between the eGFR and the mGFR. The accuracy was assessed from the absolute error |eGFR-mGFR| and expressed as the percentage of the mGFR. The CAPA and CKD-EPI equations were also compared. The bias for the CAPA and CKD-EPI equations was similar. The advantage of the CAPA equation is that sex does not need to be included as a factor (Grubb 2014).

Combined creatinine and cystatin C based formulas for eGFR ($eGFR_{CR-CYC}$)

$CKD\text{-}EPI_{CR\text{-}CYS}$ formula: The same database as that used for the development of the $CKD\text{-}EPI_{CYS}$ equation was used for the development of the $CKD\text{-}EPI_{CR\text{-}CYS}$ equation. The bias for the equations was 3.9 mL/min/1.73 m^2. In the validation data, the $CKD\text{-}EPI_{CR\text{-}CYS}$ equation was better for predicting the GFR than the other CKD-EPI equations (Inker 2012).

BIS2: The same procedure was used to develop the BIS2 as that used for the BIS1. The only difference between procedures is that cystatin C is used as well as creatinine. Using cystatin C instead decreases the effect of age and sex and was recommended by the authors to be the best choice for the eGFR in older individuals (Schaeffner 2012).

LMR-CAPA equation: A total of 1112 adult Swedish patients with the mGFR obtained by plasma clearance of iohexol were used as a database for the development of the mean LMR-CAPA equation. The eGFR based on the LMR-CAPA equation was compared with the eGFR based on the $CKD\text{-}EPI_{CR\text{-}CYS}$ equation. The mean mGFR was 51 mL/min/1.73 m2, the mean eGFR was 49 mL/min/1.73 m^2) The eGFR based on $CKD\text{-}EPI_{CR\text{-}CYS}$ formula was 48 mL/min/1.73 m^2 (Björk 2014). The bias for LMR-CAPA equation was 2.2 mL/min/1.73 m^2 and for $CKD\text{-}EPI_{CR\text{-}CYS}$ equation was 1.6 mL/min/1.73 m^2. The accuracy (P30) was 91.3 and 91.1, respectively.

For an overview of the different formulas please see **Table 1:**

Table1. Overview of the different formulas for eGFR.

	Cohort (n)	Gender M/F (%)	Mean age	Reference method	mGFRmL/min/1.73 m^2	eGFR (mean±SD)	Precision	Bias mL/min/1.73m^2	Accuarcy P_{30}
CG	249	100/0	57	Urine crea. cl.	71.2 mL/min	73 ± 37 mL/min	?	?	?
MDRD	1,628	60/40	51	Iothalamate	39.8	40±21 mL/min/1.73 m^2	?	?	?
CKD-EPI_{CR}	8254	57/43	47	Iothalamate	68±40	???	16.6	2.5	84.1
LMR	850	56/44	60	Iohexol	55 (9-121)	56 (9-111)	12.3	0.3	85.8
BIS1	570	57/43	78.5	Iohexol	60.3 (16-117)	60.3 (14-92)	9.2	0.11	95.1
FAS a: b:	4371 1764	52/48 53/47	53.0 77.5	Iohexol, inulin, iothal-mate	78.6 55.7	78.6 55.6	17.2 11.2	5.0 1.1	81.6 86.1
CKD-EPI_{CYS}	5353	58/42	47	Iothalamate	68 (±39)	??	83.6	3.4	85.9
CAPA	1200	53/47	63	Iohexol	51 (10-114)	43 (9-1003)	12.0	5.2	82.8
BIS2	570			Iohexol	60.3 (16-117)	60.3 (12-105)	8.06	0.09	96.1
CKD-EPI_{CR-CYS}	5353	58/42	47	Iothalamate	68 (±39)	??	13.4	3.9	91.5
LMR-CAPA	1200	53/47	63	Iohexol	51 (10-114)	49 (10-103)	9.2	2.2	91.3

a: 18-70 years, b: >70 years

Equations

The formulas for eGFR used in this thesis are:

Creatinine based:

$$\text{CKD-EPI}_{CR}$$

Female and serum creatinine (SCr) ≤ 62 µmol/L

$$144\,x\,\left(\frac{SCr}{0.7}\right)^{-0.329}\,x\,0.993^{age}\,[x\,1.159\;if\;Black]$$

Female and SCr >62 µmol/L

$$144\,x\,\left(\frac{SCr}{0.7}\right)^{-1.209}\,x\,0.993^{age}\,[x\,1.159\;if\;Black]$$

Male and SCr ≤ 80 µmol/L

$$141\ x\ \left(\frac{SCr}{0.9}\right)^{-0.411}\ x\ 0.993^{age}\ [x\ 1.159\ if\ Black]]$$

Male and SCr >80 µmol/L

$$141\ x\ \left(\frac{SCr}{0.9}\right)^{-0.1.209}\ x\ 0.993^{age}\ [x\ 1.159\ if\ Black]]$$

LMR

$$e^{X-0.0158xage+0.438xln(age)}$$

Female and plasma creatinine (pCr) < 150µmol/L: X = 2.50 + 0.0121 × (150–pCr)

Female and pCr ≥ 150µmol/L: X = 2.50 – 0.926 x ln (pCr/150)

Male and pCr < 150µmol/L: X = 2.56 + 0.00968 x (180–pCr)

Male and pCr < 150µmol/L: X = 2.56 – 0.926 x ln (pCr/180)

Cystatine C based:

CKD-EPI$_{CYS}$

Female or male if serum cystatin C (SCys)≤ 0.8 mg/L

$$131\ x\ \left(\frac{SCys}{0.8}\right)^{-0.499}\ x\ 0.996^{age}\ [x\ 0.932\ if\ Female]]$$

Female or male if SCys > 0.8 mg/L

$$131\ x\ \left(\frac{SCys}{0.8}\right)^{-1.238}\ x\ 0.996^{age}\ [x\ 0.932\ if\ Female]]$$

CAPA

$$130\ x\ cystatinC^{-1.069}\ x\ age^{-0.117} - 7$$

Both creatinine and cystatin C based:

CKD-EPI$_{CR-CYS}$

If female and serum creatinine (SCr) ≤ 0.7 mg/dl and serum cystatin C (Scys) ≤0.8 mg/L

$$130\ x\ \left(\frac{SCr}{0.7}\right)^{-0.248}\ x\ \left(\frac{Scys}{0.8}\right)^{-0.375}\ 0.995^{age}\ [x\ 1.08\ if\ Black]$$

If female and SCr ≤ 0.7 mg/dl and serum Scys >0.8 mg/L

$$130\ x\ \left(\frac{SCr}{0.7}\right)^{-0.248}\ x\ \left(\frac{Scys}{0.8}\right)^{-0.711}\ 0.995^{age}\ [x\ 1.08\ if\ Black]$$

If female and SCr > 0.7 mg/dl and Scys ≤ 0.8 mg/L

$$130\ x\ \left(\frac{SCr}{0.7}\right)^{-0.601}\ x\ \left(\frac{Scys}{0.8}\right)^{-0.375}\ 0.995^{age}\ [x\ 1.08\ if\ Black]$$

If female and SCr > 0.7 mg/dl and Scys) > 0.8 mg/L

$$130\ x\ \left(\frac{SCr}{0.7}\right)^{-0.601}\ x\ \left(\frac{Scys}{0.8}\right)^{-0.711}\ 0.995^{age}\ [x\ 1.08\ if\ Black]$$

If male and SCr ≤ 0.9 mg/dl and Scys) ≤ 0.8 mg/L

$$135\ x\ \left(\frac{SCr}{0.9}\right)^{-0.207}\ x\ \left(\frac{Scys}{0.8}\right)^{-0.375}\ 0.995^{age}\ [x\ 1.08\ if\ Black]$$

If male and SCr ≤ 0.9 mg/dl and Scys) > 0.8 mg/L

$$135\ x\ \left(\frac{SCr}{0.9}\right)^{-0.207}\ x\ \left(\frac{Scys}{0.8}\right)^{-0.711}\ 0.995^{age}\ [x\ 1.08\ if\ Black]$$

If male and SCr > 0.9 mg/dl and Scys) ≤ 0.8 mg/L

$$135\ x\ \left(\frac{SCr}{0.9}\right)^{-0.601}\ x\ \left(\frac{Scys}{0.8}\right)^{-0.375}\ 0.995^{age}\ [x\ 1.08\ if\ Black]$$

If male and SCr > 0.9 mg/dl and Scys) > 0.8 mg/L

$$135\ x\ \left(\frac{SCr}{0.9}\right)^{-0.601}\ x\ \left(\frac{Scys}{0.8}\right)^{-0.711}\ 0.995^{age}\ [x\ 1.08\ if\ Black]$$

CKD

Classification

During several decades, there has been an increase in patients with end-stage renal disease (ESRD) receiving dialysis or/and a kidney transplant. In the late 1990s, a large increase in the rate of CKD was observed. At the same time, there were major developments in the understanding and treatment of many diseases leading to ESRD. Therefore, there was a chance to delay or inhibit the progression of CKD to ESRD. A problem at this time was that starting treatment for CKD varied by physicians and countries (K/DOQI 2002).

To facilitate early detection of CKD and thus start intervention early to prevent loss of kidney function, the Kidney Disease Outcomes Quality Initiative (KDOQI) based in the USA attempted to stage CKD (KDOQI.org). The first staging of CKD is shown in **Table 2.**

Table 2. Stages of CKD

Stages of Chronic Kidney disease		
Stage	Description	GFR (mL/min/1.73 m^2)
1	Kidney damage with normal or ↑ GFR	≥ 90
2	Kidney damage with mild ↓ GFR	60-89
3	Kidney damage with moderate ↓ GFR	30-59
4	Kidney damage with severe ↓ GFR	15-29
5	Kidney failure	<15 or dialysis

Kidney damage define as pathological abnormalities or marker of damage including abnormalities in blood or urine tests or imagining studies.

Over the years, there has been a lot of controversy about this staging system because it does not reflect the risk of death and development of cardiovascular disease (CVD). Patients with different causes of kidney disease, and thus a different risk for mortality, were categorized in the same stage of CKD. Important factors that predict mortality (e.g., age and proteinuria) better than the eGFR were not taken into account (Gerstein 2001; Smink 2012.).

A new staging system was published in 2012 (**Table 3**) in which the role of proteinuria as a risk marker was included. This system is better for physicians to determine which of their patients have a high risk of mortality and CVD.

Table 3. Mortality risk of CKD by GFR and albuminuria categories

Prognosis of CKD by GFR and albuminuria categories: **KDIGO 2012**			Presistent albuminuria categories		
			A1	A2	A3
			Normal to mildly increased	Moderately increased	Severely increased
GFR categories (mL/min/1.73 m^2)			<30 mg/g >3 mg/mmol	30-300 mg/g 3-30 mg/mmol	>300 mg/g >30 mg/mmol
Grade 1	Normal or high	≥ 90	Low risk	Moderately Increased Risk	
Grade 2	Mildly decreased	60-89			
Grade3a	Mildly to moderately decreased	45-59	Moderately Increased Risk	High risk	
Grade3b	Moderately to severely decreased	30-44		Very high risk	
Grade 4	Severely decreased	15-29			
Grade 5	Kidney failure	<15	Highest risk		

There is still controversy regarding the definition of CKD. In the current form, this definition includes a GFR of 60–90 mL/min/1.73 m^2 (Grades 1–2) and it does not take the patient's age into consideration. A GFR < 60 mL/min/1.73 m^2 is half of the normal kidney function in younger and healthy individuals. Studies performed in older kidney donators showed a lower pre-donation GFR compared with that in younger donators. Even the GFR threshold for which an increase in the mortality risk occurs varies with age (e.g., < 75 mL/min/1.73 m^2 in younger individuals and < 45 mL/min/1.73 m^2 in older individuals) (Delanay-19, Eriksen -20). Many healthy older individuals end up in Grade 3a/A1 without having kidney damage and without having an elevated risk of mortality and CVD. This leads to overdiagnosis and an increased rate of referrals to nephrologists, thus leading to an increased burden on primary and specialist care. Healthy individuals receive a misdiagnosis that can lead to unnecessary suffering and anxiety. An overestimated rate of CKD in older individuals can affect the planning of kidney care in the future, especially because the population is becoming older. Supporters of keeping the definition for CKD unchanged argue that this definition is well known worldwide, and it has improved the early detection of kidney disease and its treatment. Additionally, this definition has been used in clinical trials, and thus led to a better understanding of CKD, and has been a keystone in health care planning. Their solution to this controversy is that changes in the management of CKD should be age-adapted similar to other diseases such as hypertension (HT) (Levey 2020). The discussion of this issue is still ongoing. In the meantime, many centers have custom-made variants of KDIGO recommendations from 2012 to facilitate the care of individuals with CKD.

Epidemiology

CKD (eGFR < 60 mL/min/1.73 m^2) is reported to affect 9%–13% of the general population worldwide (GBD 2020). The same rate is thought to be true in Sweden, although population-based studies are rare. A population-based study by Hallan performed in Norway in 2006 showed the same prevalence of CKD at approximately 10% (Hallan 2006). Better data are available for patients who receive treatment for ESRD. The incidence of ESRD is higher in the USA compared with that in European countries, despite a relatively similar incidence of CKD (van Dijk 2001; USRDS 2001). In the 1990s, the incidence of ESRD markedly increased. In the USA, the incidence of ESRD increased by 14% during 1996 to 2001 (USRDS 2001). In Sweden, between 1991 and 2002, the number of patients with ESRD increased from 3736 to 6359 (70% increase), partly because nephrologists in this country started registering patients with CKD in a national register (SNR).

Mortality from CKD in 1999 increased faster than that in other diseases worldwide (except for HIV). This increase is estimated to increase from 12.2 deaths/100 000 people to 14/100 000 people by 2030 (WHO 2016). CKD is the 14th cause of death worldwide. (GBD 2020). In Sweden, the mortality rate of patients with ESRD decreased from 13.8% in 1991 to 9.5% in 2017. This finding is mostly due to improved survival in patients on dialysis (Svensk njurregister). Additionally, in the USA, the increased mortality observed in the 1990s markedly decreased in the 2000s (Collins 2015).

Patients with CKD have a poorer quality of life, and the incidence of this disease is higher in those with poorer socioeconomic circumstances. Even minorities in high-income countries (e.g., Hispanics in the USA, and Blacks and Asians in the UK) have a higher incidence of CKD. Suarez *et al* showed that although the rate of nephrology referral was higher in minority veterans than that in White veterans, the rate of progression to ESRD was higher in minority veterans. This finding suggested the importance of biological and socioeconomic circumstances.

Most patients receiving treatment for ESRD live in high-income countries. With further improvement of global health leading to an increase in the older population, and better survival from diseases (e.g., diabetes mellitus, HT, and CVD), and better economic conditions leading to more accessible and affordable health care, the demand for treatment of ESRD will increase (Mills 2015). The cost of offering medicinal care to patients with CKD is constantly growing and it is negatively correlated with kidney function. Therefore, the cost increases as kidney function worsen.

A problem regarding CKD is that it is difficult to detect owing to the lack of symptoms. However, early detection and treatment of CKD can reduce the rate of a reduction in the eGFR and slow down or avoid the progression from CKD to ESRD.

This can relieve the patient's suffering and can be economically beneficial for society.

Cardiorenal syndrome

As mentioned previously, patients with CKD have an increased risk for the development of CVD including heart failure (HF) even in the early stages of CKD (Foley 1998). The mortality rate due to CVD is as high as 50 % for all-cause mortality in these patients (Coresh 2003). The reverse is true as well, patients with CVD have a higher incidence of CKD than the general population (Heywood 2004). Incidence of CKD (eGFR<60 mL/min per 1.73 m^2) varies between 30-60% in patients with HF (Adams 2005; Ezekowitz 2004; McAlister 2012). The eGFR predicts mortality in patients with HF better than left ventricular ejection fraction (LVEF) (Hillege 2000). The close relationship between heart and kidney has been known for a long time. A simple search for "cardiorenal" on medical databases like Pubmed results in numerous published articles, one of which, published as early as 1913. The term "cardiorenal syndrome" (CRS) was used among physicians and it was known that patients suffering from diseases in both organs had higher mortality, morbidity, and cost of care (McAlister 2004; Forman 2004). Due to a lack of consensus on the definition and management of the syndrome, the work for prevention and treatment of CRS was disunited and often concentrated in only one of the organs (Ronco 2010). In 2008, a consensus conference was held to define, classify and recommend both management and therapy to prevent the development and further deterioration of the syndrome. Ronco *et al* published the results of this conference in 2010 (Ronco 2010). CRS was defined as an acute or chronic dysfunction of the heart or the kidney that induce an acute or chronic dysfunction in the other organ. Five subtypes of the syndrome were identified and classified, see **Table 4**

Table 4 Classification of CRS

Type 1	Acute CRS (cardio-renal)	Acute worsening of heart function leading to kidney injury and/or dysfunction
Type 2	Chronic CRS (cardio-renal)	Chronic abnormalities in heart function leading to kidney injury and/or dysfunction
Type 3	Acute RCS (reno-cardiac)	Acute worsening of kidney function leading to heart injury and/or dysfunction
Type 4	Chronic RCS (reno-cardiac)	Chronic kidney disease leading to heart injury, disease and/or dysfunction
Type 5	Secondary CRS	Systemic conditions leading to simultaneous injury and or dysfunction of heart and kidney

Since both HF and CKD are reaching epidemic levels especially in older patients and those with HT and/or DM (GBD 2020) it is clear that a multidisciplinary approach is very important for the detection and treatment of CRS. House *et al* published in 2019 KDIGO`s recommendation for detection, prevention, diagnosis and treatment (House 2019). Introduction of new medicines that protect both heart and kidney like sodium-glucose Cotransporter-2 (SGL2) inhibitors are used not only in patients with DM but also in patients with HF and in CKD patients especially if albuminuria is present. Treatment of hyperkalemia with new agents such as patiromer and sodium zirconium cyclosilicate enables continued treatment with renin-angiotensin-aldosterone system (RAAS) inhibitors and/or mineralocorticoid receptor antagonists (MRAs) and should improve outcomes in patients with HF and CKD.

All five types of CRS share similarities in pathophysiology and there are several mechanisms responsible for the development of CRS. Venous congestion that occurs in patients with acute HF is important in the development of renal failure especially in CRS type 1 (Testani 2011; Ronco 2010). Other hemodynamic derangements such as reduced CO, arterial underfilling and elevated arterial pressure that are present in HF with impaired LVEF trigger several neurohormonal adaptions that affect renal function. (Schrier 1999; Ronco 2010). Activation of RAAS is one of these neurohormonal adaptions. It leads to water and salt retention that if continued causes left ventricular hypertrophy, cardiac remodeling and myocardial fibrosis. In the kidney, RAAS elevate central venous pressure that lowers renal perfusion (Francis 1990; Levine 1982). High levels of norepinephrine seen in patients with HF indicate a rise in sympathetic nervous system activity (Levine 1982). Norepinephrine is directly toxic to myocardial cells and induces apoptosis (Mann 1992). Activation of the sympathetic nervous system decreases both the cortical and medullary blood flow of the kidney through the activation of the renal nerve (Hermansson 1984). In patients with HF and hyponatremia, there is an increase in vasopressin concentrations (Szatalowicz 1981) that leads to water and salt retention. Another vasoconstrictor, endothelin, is increased in some patients with HF and causes renal vasoconstriction. It helps in maintaining perfusion to the heart and the brain but reduce cardiac output and in turn further reduce renal perfusion (Francis 1990; Levine 1982). As a response to vasoconstriction several local vasodilatory elements like nitric oxide, prostaglandins and bradykinin are produced. The production of these elements doesn´t overwhelm the effects of vasoconstriction, instead, it can further damage kidney and/or heart tissue due to reduced renal flow resulting in renal ischemia (Vallon 2001; Roman 1991; Barros 2018). Increased intraabdominal pressure due to ascites and abdominal wall edema is another feature of HF especially in right ventricular dilatation and dysfunction (Mullens 2008). It can deteriorate renal function in the same way as venous congestion (Bradley 1947).

Shrunken pore syndrome

CKD-staging especially if it is age-adjusted has improved risk calibration of mortality for patients with CKD. Another way to further improve the calculation of mortality risk is by looking closer to eGFR based on both cystatin C and creatinine. A new syndrome called "Shrunken pore syndrome" is based on the difference between $eGFR_{CYS}$ and $eGFR_{CR}$ in the same individual.

Filtration of substance from glomerular capillaries to the Bowman´s capsule must pass through the glomerulus filtration barrier (GFB) (Hall 2020). This barrier has three layers: the endothelial layer of capillary; the glomerular basement membrane (GBM); and the layer of the epithelial cell (Figure 3). The permeability of GFB is 100 to 500 greater than that of a usual capillary because of the large number of pores on its three layers. The permeability of GFB to substances of different MM differs and depends on the sizes of the pores in the membrane and the electrical charge of the substance (Hall 2020).

Creatinine and cystatin C permeability through the GFB differs slightly because of their different MM and SE-radius. Creatinine´s sieving coefficient is 1.00, the same as that of other small molecules like inulin (Hall 2020) while cystatin C has a lower sieving coefficient of ~ 0.84 (Lund 2003).

The best way in clinical practice to estimate GFR is by combining $eGFR_{CYS}$ and $eGFR_{CR}$ (Inker 2012). When $eGFR_{CYS}$ and $eGFR_{CR}$ correspond well with each other, the mean value of $eGFR_{CYS\text{-}CR}$ reflects better the mGFR (Grubb 2012). In most patients, $eGFR_{CYS}$ and $eGFR_{CR}$ match well and the ratio $eGFR_{CYS}/eGFR_{CR}$ is close to one. There are patients where this ratio is <0.7. Many studies have examined the association between $eGFR_{CYS}/eGFR_{CR}$ ratio and mortality and morbidity (Grubb 2015, Prude 2016, Herou 2019, Åkesson 2020, Xhakollari 2021). The mortality in these patients was significantly higher even after adjustment for factors that can affect creatinine such as body mass index (BMI) or after adjustment for $eGFR_{CYS.}$ Grubb was the first to notice this in 2015. He called the syndrome for Shrunken pore syndrome (SPS) and suggested that the difference between $eGFR_{CYS}$ and $eGFR_{CR}$ reflects a change in permeability across GFB of middle size molecules like cystatin C compared to small molecules like creatinine due to the "shrinkage" of the pores of GFB (Figure 3). Öberg *et al* showed in their study that thickening of GBM results in selective reduction of the permeability of the middle-sized molecules resulting in different $eGFR_{CYS}$ and $eGFR_{CR}$ values and an $eGFR_{CYS}/eGFR_{CR}$-ratio <0.75 as suggested by Grubb (Figure 4) (Öberg 2021). The pathophysiology behind SPS remains unclear and requires further studies.

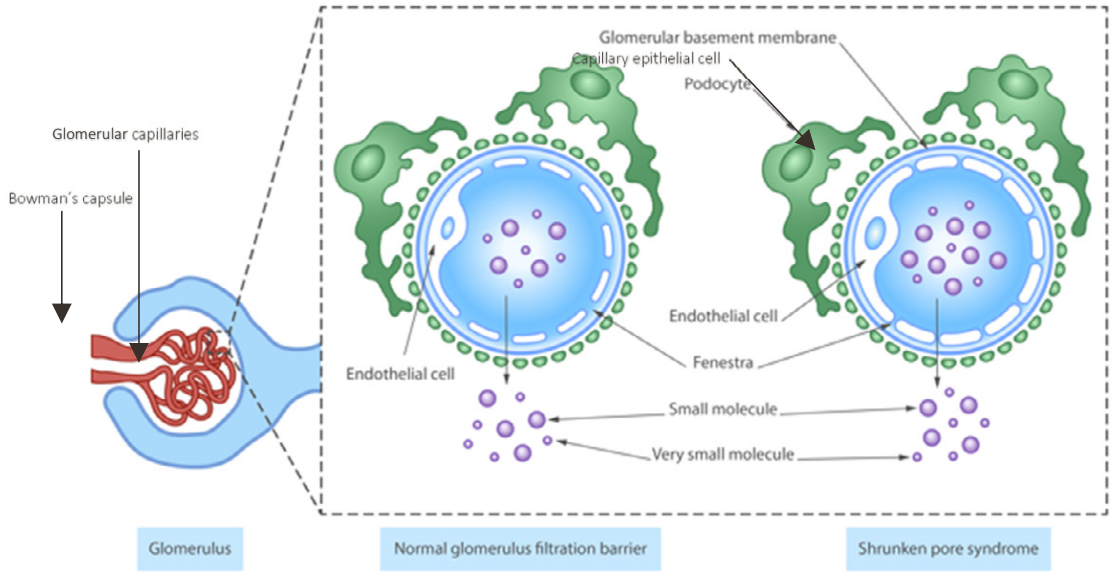

Figure3. Schematic view of possible pathophysiology of Shrunken pore syndrome showing a thickening of endothelial cells and a narrowering of the fenestra between endothelial cells. Publishen

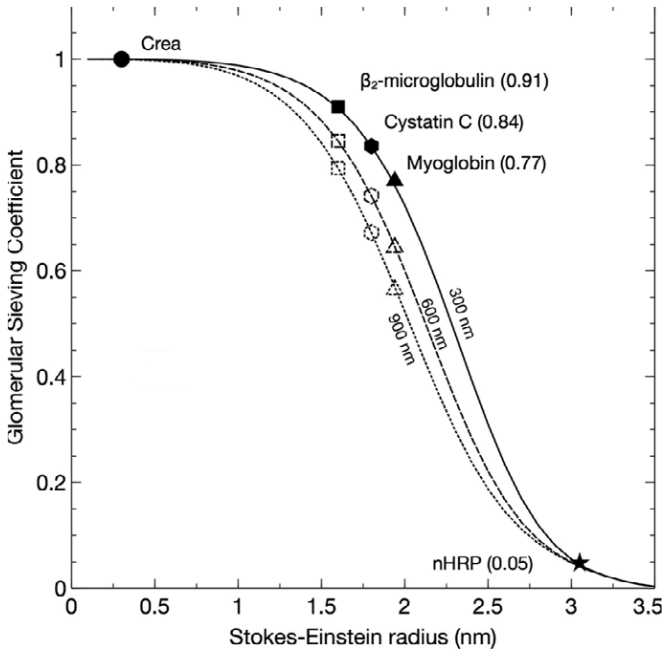

Figure 4. Glomerular sieving coefficients versus the Stokes- Einstein radii of the solute proteins for three different thicknesses of the glomerular basement membrane 300 nm (and an effective filtration pressure [EFP] of 10 mmHg), 600 nm (EFP 20 mmHg) and 900 nm (EFP 30 mmHg). Also shown are experimentally measured sieving coefficients for β2-microglobulin (Norden 2001) (black square) and myoglobin (Lund 2003) (black triangle). The sieving coefficient of a 3.05 nm protein (neutral horseradish peroxidase nHRP (Lund 2003) (solid star) will be practically unaltered by the thickening of the renal filter.

Figure by Öberg et al published in 2021 in Physiological Reports.

Proteome and proteomics

Proteome refers to all proteins expressed by an organism. The amount and composition of the proteome changes over time (Gerner 2002). Proteomics, the study of proteome, identifies proteins expressed in an organism, the amount and location of the proteins produced, changes of the proteins after their production, their role in the organism and their interaction with each other. It has revolutionized the measurements of proteins that can now be detected down to attomole rage (1 protein molecule per 10^{-18} molecules) and thus elucidated many processes for the development of disease and health at the protein level.

The first analysis was performed in 1976 with the development of 2D protein electrophoresis by Avital *et al* (Barritaul 1976). There are several techniques used to study proteome. Many of them have been used for several decades but their accuracy has increased markedly over the last years. Low throughput methods like ELISA, gel electrophoresis, mass spectrometry and chromatography-based methods are being replaced by high throughput methods like analytical, functional, and reverse-phase microarrays, and mass spectrometry-based proteomics. With high throughput methods, one can conduct a massive parallel analyses of proteome faster and with very little biological material like plasma.

Proximity Extension Assay technology is one of the high throughput methods used to analyze proteome. Oligonucleotide-labelled antibody pairs bind to the protein. These oligonucleotides, when are nearby each other, hybridize and are extended by a DNA polymerase that forms a unique code for each protein. A polymerase chain reaction (PCR) amplifies this new DNA code. The number of PRC amplified codes correspond to the concentration of the protein in the sample. The amount of each DNA code is then quantified by microfluid polymerase chain reaction. One microfluid polymerase chain reaction can read out 48 to 96 assays at the same time. With this technique, one can understand biological mechanisms that predict disease, stratify patients, discover new treatments by only using a small amount of plasma (Assarsson 2014).

The overall aim of the project

To describe the interaction between the kidneys and the heart in the early stages of kidney disease and to elucidate on a new pathophysiological mechanism behind increased morbidity and mortality in patients with HF and individuals in the general population

Aims of the studies:

I. To investigate whether the association between mild to moderate renal dysfunction and echocardiographic markers for cardiac structure and function both systolic and diastolic, exists in individuals from a population-based cohort without HF or asymptomatic reduced LV function

II. To investigate SPS in a heart failure cohort (the HARVEST cohort) and its association with mortality and morbidity.

III. To further explore the pathophysiological pathways behind the known adverse effects of SPS by using proteomics analysis in patients with HF.

IV. To investigate the prevalence of SPS and the association between SPS and morbidity and mortality in individuals randomly selected from a population-based cohort.

Material and methods

Study populations

The Malmö Preventive Project

The first paper in this thesis includes individuals from Malmö Preventive Project. It started in the early 1970s as a study of a middle-aged population in Malmö with the aim to screen and treat individuals with cardiovascular risk factors, impaired glucose tolerance, high levels of alcohol consumption and breast cancer. All men from certain prespecified birth cohorts (1921-1949), born in, and still living in Malmö were invited by letter to a medical examination with questionnaires, physical examination, and blood samples. A few years later women born between 1926-1949 were also invited. A total of 22444 men and 8676 women participated in the screening period. Men participated mostly between 1974-1982 and women participated mostly between 1981-1992. Participation across each age group was on an average 71,2% (range 64-78%) (Berglund 1996 and 2000). The non-responders had a less advantageous socio-economic situation compared to the responders and women had a shorter follow up time period.

During the follow-up years (2002-2006), 17284 individuals were re-examined and are referred to as MPP-RES-cohort. The attendance rate was 72% and 63% of the cohort were men. Among the MPP-RES participants, approximately 1800 of them were examined further with echocardiography with tissue Doppler imaging (TDI). The selection was done randomly from groups defined by glucometabolic status: Group one had individuals with normal fasting glucose (FG) (≤6.0 mmol/L). Groupe two included individuals with impaired FG (>6 and <6.7 mmol/L). Groupe three had individuals with new-onset type 2 DM (FG≥6.7 mmol/L). Groupe four included individuals with prevalent DM both type 1 and 2. To ensure enough subjects were studied from each group, an oversampling of individuals with glucometabolic disturbances was done (Leosdottir 2010).

In the study, only the individuals without a history of HF and an LVEF ≥40% were included. Individuals with LVEF between 41 and 55% were excluded if they reported being prescribed 2 or more drugs used for the treatment of heart failure (angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics, or beta-blockers). In addition, individuals with missing or an $eGFR_{CYS}$ ≤15

mL/min/1.73m^2 were excluded (Xhakollari 2019). A total number of 1504 individuals were included in the study.

The HeARt and brain failure inVESTigation study

For the second and third papers, data from the Swedish ongoing, prospective HeARt and brain failure inVESTigation (HARVEST) study was used. The patients that were admitted to medical or cardiology clinics in Malmö from Mars 2014 to January 2019 for the treatment of acute HF, chronically or newly diagnosed, and who were able to give written or oral consent, were enrolled in the study. When patients had a severe cognitive impairment diagnosed by the mini mental test examination score < 13 points, the consent was given by their relatives (Christensson 2016). In total there were 411 patients, but only 379 of them had complete data including blood sample donations, questionnaire on living habits and clinical examination, and thus were included (Xhakollari 2021).

The Malmö Diet and Cancer Study

For the fourth paper data from the population-based cohort, the Malmö Diet and Cancer Study were used. It started in the early 1990s to investigate the association between diet and the risk of cancer in middle-aged men and women living in Malmö between 1991 and 1996. Men born between 1923 and 1945 and women born between 1923 and 1950 were recruited via personal letters and public advertisements. Of all individuals 44-74 years old living in Malmö and eligible for the study, only 28 449 attended (attendance rate 40.8%). Baseline examination included blood samples, anthropometric measurement, dietary assessment, and a self-administered questionnaire (Berglund 1993).

Half of the individuals who entered the study between November 1991 and February 1994 were randomly invited to take part in a study to investigate the prevalence of carotid artery disease (MDC-CC). 5540 individuals accepted the invitation (Hedblad 2000). Individuals with prior myocardial infarction and stroke were excluded. Because of missing data, only 5061 individuals were included in my study.

Uppsala Cohort: is the reference cohort used to correct cystatin C for individuals in MDC-CC study. The 142 978 individuals in this cohort were 44-74 years old. They were referred to Uppsala Hospital´s laboratory for blood analysis from non-nephrology units during 2005-2015. Anonymous data on plasma creatinine, cystatin C, age and gender are available for these individuals.

Echocardiography

In paper I, a 3V2c (Acuson Sequoia) or an S3 (Sonos 5500 Philips) transducer was used for measurements with tissue doppler imaging (TDI) echocardiography. For evaluation of LVEF and cardiac dimensions, apical two- and four-chamber and parasternal long- and short axis views were used. Quantification of LVEF was done visually. Calculations for left ventricular (LV) mass were based on the parasternal long-axis view measurements at the end of the diastolic filling phase (diastole) at the level of the mitral tip. These calculations were indexed for body surface area (BSA). TDI in four chambers view and transmitral pulsed doppler flow were used for measurements of LV diastolic function. Peak velocities for LV lateral (lat) and septal (sept) walls during early (É) and late (Á) diastole were measured with TDI close to the mitral annulus in the apical 4-chamber view. The peak velocity of blood flow through the mitral valve in early (E) and late (A) diastole was estimated with doppler. If the registrations were homogeneous a single cycle was used. If the registrations weren´t homogeneous a mean of three to five cycles was used (Leosdottir 2010).

In Paper II and III, a Philips IE33 with a 1-5 MHz transducer or a GE Vingmed Vivid with a 1-4 MHz transducer ultrasound was used. Apical two- and four chambers and parasternal long axis were used to obtain cine loops. LVEF was calculated from end-diastolic volume (EDV) and end-systolic volume (ESV) according to the formula: $LVEF = \frac{\mathrm{EDV}-\mathrm{ESV}}{\mathrm{EDV}}$. Tricuspid annual plane systolic excursion (TAPSE) and pulsed TDI-derived tricuspid annular systolic velocity were measured for evaluation of right ventricular systolic function (Christensson 2016).

Laboratory tests

Hemoglobin (Hb), fasting blood glucose (B-glucose), total cholesterol, high-density lipoprotein (HDL), N-terminal prohormone BNP (NT-proBNP), creatinine, and c-reactive protein (CRP) were analyzed at the Department of Clinical Chemistry at Skånes University Hospital, Malmö Sweden according to standard routines (Leosdottir 2010, Svensson-Färbom 2014, Christensson 2016). Plasma levels of protein were analyzed by Olink Bioscience Uppsala Sweden.

An automated particle-enhanced immunoturbidimetric method was used to measure plasma cystatin C on paper I. Reagents from DakoCytomation, Glostrup, Denmark were used, and the total analytical imprecision was 2.1% when using a control sample of 1.0 mg/L and 1.7% when using a control sample of 4.0 mg/L. The analyses were performed before the introduction of the international reference calibrator (Leosdottir 2010).

In papers II and III plasma cystatin C was analyzed by an automated particle-based immunoassay adjusted to the international reference preparation ERM-DA 471/IFCC. The same reagents as in paper I was used, and the imprecision was the same as well (Christensson 2016).

In paper IV a particle enhanced immunonephelometric assay (N Latex Cystatin; Dade Behring, Deerfield, IL, USA) was used for measurements of cystatin C but the analysis was done before the introduction of the international reference preparation ERM-DA 471/IFCC (Svensson-Färbom 2014).

Plasma creatinine was not analyzed in paper I. In papers II and III, an enzymatic colorimetric assay was used to measure plasma creatinine, and the Jaffé method was used for measurements in paper IV. Both methods were traceable to IDMS (Svensson-Färbom 2014, Christensson 2016).

Clinical examination

In papers I and IV fasting blood samples were drawn. In papers II and III, fasting blood samples were mostly taken the day after. A self-administered questionnaire was given to the participants regarding medical history and lifestyle factors. Anthropometric measurements were carried out in all four studies. BMI was calculated. Blood pressure (BP) was measured twice after 10 minutes' rest in the supine position in papers II-IV. In paper I, blood pressure was measured twice in the supine position after only 5 minutes of rest (Leosdottir 2010, Svensson-Färbom 2014, Christensson 2016).

Definitions

HT was defined as a history of HT, diagnose of HT, use of antihypertensive medications or BP ≥ 140/90 mmHg. DM was defined as a history of DM, use of antidiabetic medications or as an FG >6 mmol/L (FG ≥ 7 mmol/L in papers II and III). Smoking was defined as current if participants were smoking when included in the study (in paper IV current smoking included smoking within the past year). Ischemic heart disease (IHD) was defined as physician diagnosis of myocardial infarction or angina pectoris or treatment with coronary artery bypass grafting or percutaneous coronary intervention. In papers II and III a pathological myocardial perfusion imaging, pathological coronary angiogram, or pathological exercise electrocardiogram were used as well to define IHD. In paper IV IHD and stroke were clustered together as CVD. CVD was defined as physician diagnosis of myocardial infarction and or stroke. HF was diagnosed as a physician diagnosis of

HF. In paper I HF was diagnosed even if LVEF <40 % as well (Leosdottir 2010, Svensson-Färbom 2014, Christensson 2016).

The eGFR was based on CKD-EPI$_{CYS}$ formula in paper I (Leosdottir 2010), based on CKD-EPI$_{CR\text{-}CYS}$ in paper II (Christensson 2016), based on LMR-CAPA formulas in paper IV (Svensson-Färbom 2014). In Paper III both CKD-EPI$_{CR\text{-}CYS}$ and LMR-CAPA formulas were used for eGFR (Xhakollari February 2021). CKD-staging was based on KDIGO classification only according to eGFR. SPS was defined as eGFR ratio ≤ 0.6 in paper II and III and eGFR ratio < 0.7 in paper IV.

Statistical analyses

IBM SPSS Statistics, version 24-25 was used for statistical calculations in papers I-III. Admission data is presented as mean with standard deviation (SD), absolute numbers with percentages, or as median (25th–75th inter-quartile range). In paper IV, STATA SE version 16.1 (30) and R v.4.1.0 was used for all statistical analysis.

Paper I

The subjects were divided into 6 categories based on their eGFR levels. Univariate general linear model analysis (UNIANOVA) was used to investigate the correlation between eGFR groups and echocardiographic variables. In the first analysis the model was adjusted for age and gender (Model 1). All echocardiographic variables associated with eGFR groups with a p-value <0.1 were tested with the same analysis (UNIANOVA) but with further adjustment for age, gender, medication blocking RAAS, HT, DM, and smoking (Model 2). All calculations were carried out for the whole cohort and then stratified by gender. For echocardiographic variables that were associated with eGFR groups with a p-value <0.05, we compared their mean effects for the pair eGFR group. Thus, we compared the mean value of different echocardiographic variables for:

45–60 mL/min/1.73 m2 vs >90 mL/min/1.73 m2

45–60 mL/min/1.73 m2 vs >60 mL/min/1.73 m2

30–60 mL/min/1.73 m2 vs >90 mL/min/1.73 m2

30–60 mL/min/1.73 m2 vs >60 mL/min/1.73 m2.

A p-value <0.05 was considered to be statistically significant.

Since the subjects were > 60 years old, subgroup analysis for those with eGFR >60 mL/min/1.73 m^2 was done. The association between echocardiographic variables

and eGFR as a continuous variable was analyzed with UNIANOVA adjusted for age, gender, HT, DM, RAAS blockade, and smoking.

Paper II

The subjects were divided into two categories based on $eGFR_{CYS}/eGFR_{CR}$-ratio. Those with an eGFR ratio ≤ 0.6 were considered having SPS and were compared with the subjects with an eGFR ratio > 0.6. To investigate the baseline differences between these groups, one-way ANOVA was used for continuous variables and χ^2 test for binary ones. P-value < 0.05 was considered statistically significant. Cox regression model was used to investigate the association between SPS and mortality and between SPS and 30-day hospitalization. Logistic regression analyses were used to investigate associations between SPS and quality of life (QoL). Three models were used. In Model 1 adjustments were done only for age and gender. Model 2a was adjusted for age, gender, BMI, atrial fibrillation, smoking, systolic BP at admission, DM, IHD, total cholesterol, log-transformed NT-proBNP, and New York Heart Association (NYHA) class at admission. In model 2B adjustments were done for age, gender, DM, ethnicity, employment status, NYHA class at admission, log-transformed NT-proBNP, HDL, and systolic BP at admission. At the request of reviewers on top of Model 2a, we added one at the time eGFR based on $CKD\text{-}EPI_{CR\text{-}CYS}$, LVEF, TAPSE and standardized diuretic dosage at discharge. At the request of reviewers, we investigated the association between SPS and mortality in subgroups of HF patients based on theirs LVEF (HFrEF-LVEF ≤ 35%; HFmrEF-LVEF >35% but ≤ 50%; and HFpEF >50%).

Paper III

Proximity Extension Assay technique using the Proseek Multiplex CVD III 96x96 reagents kit was used to analyze plasma levels of proteins. The CVD III panel include 92 proteins with known or suggested associations with CVD, metabolism, and inflammation. All variables that weren´t normally distributed were Ln-transformed. Data on proteins were further z-transform (how many standard deviations an observation moves away from the mean) for easier comparison. To see baseline differences, χ^2 test was used for binary variables and one-way ANOVA test for continuous ones. Logistic regression was used to investigate the association between SPS and plasma proteins. Since there were 92 proteins a p-value < $0.05/92=5.4\text{x}10^{-4}$ was considered significant (Bonferroni corrected). In the first analysis, adjustments were carried out for age and sex. If p-value < $5.4\text{x}10^{-4}$, besides age and sex, further adjustments for BMI, NYHA, systolic BT at admission, DM and smoking were done (Model 2). The third adjustment with age, sex, BMI, NYHA, systolic BT at admission, DM, smoking, β-blocker treatment, angiotensin-converting enzyme blockage, angiotensin II receptor blockage treatment, low-

density lipoprotein (LDL), HDL, and FG was carried out (Model 3). In these analyses a p-value <0.05 was significant.

Paper IV

Since plasma cystatin C was analysed before the introduction of the world calibrator, correction of cystatin C was done by using sex-specific linear regression analysis in the MDC-CC cohort and in the reference cohort from Uppsala where cystatin C was determined by an automated particle-enhanced immunoturbidimetric assay and calibrated against the international cystatin C reference material (ln (cystatin C) = age + ln(creatinine)).

The predicted data from the models were exponentiated and cystatin C was corrected as:

$$\text{cystatin C}_{corrected} = \text{cystatin C}_{\text{MDC–CC}} + \left(\text{cystatin C}^{predicted}_{\text{reference cohort}} - \text{cystatin C}^{predicted}_{\text{MDC–CC}}\right)$$

Subjects were divided into four groups based on their $eGFR_{CYS}/eGFR_{CR}$-ratio.

Generalized propensity score (GPS*)* was estimated by multinominal logistic regression analysis. Dependent value was $eGFR_{CYS}/eGFR_{CR}$-ratio and covariant were age, gender, education, employment status, living conditions, smoking, physical activity, BMI, HT, cancer, CVD, DM, and BP-lowering medicines. Using GPS, four groups of individuals with balanced risk factors were created. This cohort was referred to as matched cohort.

Cox regression analyses were performed to investigate the association between SPS ($eGFR_{CYS}/eGFR_{CR}$-ratio<0.7) and all-cause mortality, incident CVD, incident DM and incident CKD. The model was adjusted for $eGFR_{CYC}$.

Ethical approval

The MPP-RES was approved by the Ethics Committee of Lund University, Sweden (No. LU 244-02).

The HARVEST study was approved by the Ethical Review Board at Lund University, Sweden (Dnr. 2013/360)

The MDC-CC study was approved by the Regional Ethical Review Board at Lund, Sweden (ID 532-2006)

All studies complied with the Declaration of Helsinki. All participants signed an informed consent form before entering the study.

Results

Paper I

Baseline characteristics of participants are shown in Table 5 and are presented as means ±SD or percentages (%). There were twice as many men compared to women in the study. The prevalence of DM was higher among the participants in the study compared to the general population due to initial inclusion criteria.

Table 5. Baseline data in MPP RES.

	All	Included	Excluded
Number (n)	1792	1504	288
Age (year)	67.3±6	66.9±6	69.7±6
Gender Men (%)	70,6	**70,1**	73,3
Waist (cm)	98.5±12	97.8±12	102.4±13
BMI (kg/m^2)	28.3±4	28.1±4	29.6±5
Smoking (%)			
Now	18.6	19.2	14.4
Before	50.4	49.5	53.7
Never	31.0	31.3	28.1
NT-proBNP (pmol/L)	28±63	19.8±33	72±134
LVEF (%)	60.1±8	61.0±6	54.1±14
eGFR by CKD-EPI$_{cysC}$ (mL/min/1.73m^2)	68.6±17	69.7±16	62±19
eGFR categories (mL/min/1.73m^2) (n)			
>90 (mL/min/1.73m^2)	10.4	11.1 (167)	7
75-89.9 (mL/min/1.73m^2)	25.2	26.5 (399)	18.6
60-74.9 (mL/min/1.73m^2)	33	34.6 (520)	25.3
45-59.9 (mL/min/1.73m^2)	22.1	20.8 (313)	28.1
30-44.9 (mL/min/1.73m^2)	6.9	6.4 (97)	9.5
15-29.9 (mL/min/1.73m^2)	1.1	0.5 (8)	4.2
<15 (mL/min/1.73m^2)	0.2		1.1
HT (%)	62.9	64.6	54.4
RAAS (%)	23.5	20.5	40
DM (%)	35.5	**34.3**	42.1

BMI, body mass index; NT-proBNP , N-terminal pro B-type natriuretic peptide; LVEF, left ventricular ejection fraction; CKD, chronic kidney disease; CKD-EPI$_{cysC}$, CKD-Epidemiology Collaboration based on cystatin C; eGFR, estimated glomerular filtration rate; HT, hypertension; RAAS, renin-angiotensin -aldosterone system; DM, diabetes mellitus

Associations between eGFR categories and echocardiographic variables adjusted for age and gender are shown in table 6.

Echocardiography variable	F-value	p-value
LVEF (%)	3.249	0.006
E (cm/s)	1.624	0.151
A (cm/s)	3.608	0.003
TransmEA	1.088	0.365
Ésept (cm/s)	0.820	0.535
Ásept (cm/s)	0.418	0.837
ÉÁsep	1.562	0.168
Élat (cm/s)	1.019	0.405
Àlat (cm/s)	0.474	0.796
ÉÁlat	1.735	0.123
É_mean	1.055	0.384
Á_mean	0.522	0.760
E/Ésept	1.925	0.087
E/Élat	3.924	0.002
meanÉ/Á	2.051	0.069
mE/Élatsep	3.039	0.010
LAarea	0.551	0.738
LASd (mm)	2.634	0.022
LAsdm2	2.077	0.066
LVMI	0.551	0.738
LAarea/BSA	2.878	0.014

Table 6. Associations between eGFR categories and echocardiographic variables adjusted for age and gender.

LVEF, left ventricular ejection fraction; E, Doppler measurement of peak velocity of blood flow through the mitral valve in early E diastole; A, Doppler measurement of peak velocity of blood flow through the mitral valve in late diastole; TransmEA, the ratio between E and A; Ésept, Peak myocardial velocity of the basal LV wall in early diastole in the septal wall; Ásept, Peak myocardial velocity of the basal LV wall late diastole in the septal wall; ÉÁsept, Ésept/Ásept ratio; Élat, Peak myocardial velocity of the basal LV wall in early diastole in the lateral wall; Álat, Peak myocardial velocity of the basal LV wall in late diastole in the wall; ÉÁlat, Élat/Álat ratio; É_mean; the mean value of peak myocardial velocity of the basal LV wall in early diastole in the lateral and septal walls; Á_mean, the mean value of peak myocardial velocity of the basal LV wall in late diastole in the lateral and septal walls; E/Ésept, E/Ésept ratio; E/Élat, E/Élat ratio; meanÉ/Á, ratio of É_mean/Á_mean; mE/Élatsep, the mean value of E/Ésept and E/Élat; LAarea, left atrium area; LASd, left atrium systolic diameter; LAsdm2, Left atrial dimension / body surface area; LVMI, Left ventricular mass index; LAarea/BSA, Left atrial area / body surface area.

Of twenty-one analyzed echocardiographic variables, nine were associated with eGFR categories with a p-value <0.1. Association between these variables and eGFR categories adjusted for age, gender RAAS medication, DM, HT and smoking (Model 2) were analyzed. A p-value <0.05 was considered significant. The results are shown in table 7.

Table 7. Associations between eGFR categories and different echocardiography variables adjusted for age, sex, RAAS medication, diabetes mellitus, hypertension and smoking

Echocardiography variable	All		Women		Men	
	F-value	p-value	F-value	p-value	F-value	p-value
LVEF (%)	3.429	**0.004**	1.119	0.350	2.798	**0.016**
A (cm/s)	3.669	**0.003**	0.814	0.540	4.713	**<0.001**
E/Ésept	1.872	0.096	1.293	0.266	1.748	1.21
E/Élat	3.861	**0.002**	2.046	0.071	2.348	**0.039**
meanÉ/Á	2.536	**0.027**	0.512	0.768	2.719	**0.019**
mE/Élatsep	3.081	**0.009**	1.563	0.169	2.183	*0.054*
LASd (mm)	2.536	**0.027**	1.407	0.221	3.178	**0.007**
LAsdm2	2.037	0.071	1.104	0.358	1.842	0.102
LAarea/BSA	2.910	**0.013**	1.940	0.087	1.299	0.262

LVEF, left ventricular ejection fraction; A, Doppler measurement of peak velocity of blood flow through the mitral valve in late diastole; E/Èsept, ratio of doppler measurement of peak velocity of blood flow through the mitral valve in early diastole and peak myocardial velocity of the basal LV wall in early diastole in the septal wall; E/Èlat, ratio of doppler measurement of peak velocity of blood flow through the mitral valve in early diastole/peak myocardial velocity of the basal LV wall in early diastole in the lateral wall; meanÉ/Á, ratio of the mean value of peak myocardial velocity of the basal LV wall in early diastole in the lateral and septal walls/the mean value of peak myocardial velocity of the basal LV wall in late diastole in the lateral and septal walls; mEélatsep, the mean value of E/Ésept and E/Élat; LAarea, left atrium area; LASd, left atrium systolic diameter; LAsdm2, Left atrial dimension / body surface area; LAarea/BSA, Left atrial area/body surface area.

Echocardiographic variables for both systolic (LEVF) and diastolic (A, E/Èlat, meanÉ/Á, mE/Élatsep) function, as well as structural changes (LASd; LAarea/BSA), were statistically associated with eGFR groups for the whole cohort. After stratifying for gender, the association remained statistically only among men.

For the five echocardiographic variables that were statistically associated with eGFR categories according to model 2, one way ANOVA was used to compare the mean value of echocardiographic variables between eGFR categories in pairs. The most important statistically significant differences were found for LVEF (p=0.003), E/Élat (p=0.015), and mean É/Á (p=0.019) between categories of eGFR >90 mL/min/1.73 m^2 and eGFR 45-59.9 mL/min/1.73 m^2.

In the subgroup analysis of individuals with eGFR > 60 mL/min/1.73 m^2 there was a significant association between eGFR as a linear variable and E/Élat (p= 0,026), E /Ésept (p= 0,018) and mE/Élatsep (p=0,016)

Paper II

Baseline characteristics of the study population are shown in table 8. Values are means (±standard deviation (SD) or medians (25-75 interquartile range).

Table 8. Baseline characteristics of the study population.

	Total n=373	eGFR ratio ≤ 0.6 n=94	eGFR ratio >0.6 n=279	p-value
Age (years)	74.8 (±12.1)	77.4 (±11.1)	74.0 (±12.3)	0.017
Sex (female n (n, (%))	118 (31.6)	63 **(67.0)**	55 **(19.7)**	<0.001
Ethnicity				0.403
Nordic (n (%))	330 (88.5)	87 (92.6)	243 (87.1)	
Non-Nordic European (n (%))	36 (9.7)	7 (7.4)	29 (10.4)	
Non-European (n (%))	6 (1.8)		6 (2.2)	
Employment status				0.062
Employed	17 (4.6)	2 (2.1)	15 (5.4)	
Non-employed	49 (13.1)	6 (6.4)	43 (15.4)	
Retired for health reason	13 (3.5)	4 (4.3)	9 (3.2)	
Retired for non-health reason	294 (78.8)	82 (87.2)	212 (76.0)	
Smoking (n (%))	44 (11.8)	18 **(19.1)**	26 **(9.3)**	0.011
BMI (kg/m^2)	27.9 (±6.0)	29.1 (±7.3)	27.4 (±5.4)	0.018
New-onset heart failure	114 (30.5)	17 **(18.1)**	97 **(34.7)**	0.002
Diuretic dosage at discharge (mg, n=307)	60 (40-80)	70 (40-120)	60 (40-80)	0.083
SBP (mmHg)	137 (±27)	140.1(±27.2)	136.8 (±27.9)	0.308
β-blockers (n (%))	329 (88.2)	78 (83.06)	251 (90.0)	0.069
ACEi or ARB (n (%))	298 (79.9)	70 (74.50)	228 (81.7)	0.113
MRA (n (%))	24 (6.4)	2 **(2.1)**	22 **(7.9)**	0.048
Loop-diuretics (n (%))	359 (96.2)	92 (97.9)	267 (95.7)	0.338
Diabetes (n (%))	136 (36.5)	39 (41.5)	97 (34.8)	0.242
NYHA-class				0.342
I-II	46 (12.4)	7 (7.4)	39 (14.0)	
III-IV	327 (87.6)	87 (92.6)	240 (86.0)	
AF (n (%))	225 (60.3)	58 (61.7)	167 (59.9)	0.752
IHD (n (%)	145 (38.9)	31 (33.0)	114 (40.9)	0.175
Total cholesterol (mmol/L)	3.6 (±1.01)	3.5 (±1.0)	3.6 (±1.0)	0.336
HDL	1.2 (±0.4)	1.2 (±0.4)	1.2 (±0.4)	0.030
Cystatin C (mg/L)	1.8 (1.3-2.2)	2.1 (1.6-2.5)	1.7 (1.2-2.0)	<0.001
Creatinine (mmol/L)	105 (84-136)	103 (79-124)	120 (88-141)	0.007
eGFR $(mL/min/1.73m^2)_{CKD\text{-}EPI}$	43.2 (±18.6)	42.1 (±16.0)	43.6 (±19.3)	0.533
eGFR $CKD\text{-}EPI_{cystatinC}$	37.8 (±17.1)	30.4 (±12.2)	40.2 (±17.8)	<0.001
e-GFR $CKD\text{-}EPI_{creatinine}$	51.6 (±22.6)	60.6 (±22.3)	48.6 (±21.9)	<0.001
CRP (mg/L)	9.4 (4.9-22.0)	12.0 (5.1-25.0)	9.0 (4.8-21.0)	0.355
NT-pro-BNP (ng/L)	4141 (2237-8693)	4189 (2380-9708)	4141 (2178-8117)	0.389
LVEF (%)	38.5 (±16.1)	**42.7** (±16.2)	**37.4** (±15.9)	0.026
TAPSE (mm)	16.6 (±5.4)	17.2 (±5.3)	16.6 (±5.0)	0.426

BMI=body mass index; SBP=systolic blood pressure; ACEi=angiotensin converting enzyme inhibitors; ARB=angiotensin II receptor antagonists; MRA=aldosteronantagonist, HDL=high density lipoprotein; AF=atrial fibrillation; IHD=ischemic heart disease; GFR=glomerular filtration rate; CKD-EPI=chronic kidney disease epidemiology collaboration; CRP=c-reactive protein; NT-pro-BNP= N-terminal pro-brain natriuretic peptide; LVEF=left ventricular ejection fraction; TAPSE=tricuspidal annular plane systolic excussion.

The cohort was divided into two groups based on their $eGFR_{CYS}/eGFR_{CR}$-ratio. The prevalence for SPS was 25% and the syndrome was more common in women and in smokers. Patients without SPS were younger, had a higher occurrence of new-onset HF, lower NYHA class and LVEF, and used more aldosterone antagonist. During the follow-up, 124 patients died. Hazard ratio (HR) for all-cause mortality after adjustment for multiple risk factors was 1.99 (confidence interval (CI) 95% 1.23–3.21; p-value=0.005). The association between SPS an all-cause mortality continued to be statistically significant even after further adjustment (one at the time) for $eGFR_{CR\text{-}CYS}$, LVEF, TAPSE, and standardized diuretic dosage at discharge.

Seventy 30-days rehospitalizations events occurred during the follow up. SPS was associated with higher rehospitalization risk (HR 1.82; CI 95% 1.04–3.18; p-value=0.036) but also with lower QoL (Odds ratios (OR) 2.15 (CI 95% 1.03–4.49; p-value= 0.042). For more information, see table 9 and 10.

Table 9. Cox regression model of association between SPS based on the CKD-EPI formulas (n=94 of the total 373 subjects) and all-cause mortality (124 events)

	HR	CI95%	p
SPS	1.99	(1.23-3.21)	0.005
Age	1.06	(1.04-1.09)	$4.69x10^{-8}$
Sex	0.37	(0.23-0.61)	$8.89x10^{-5}$
Smoking	1.52	(0.82-2.81)	0.184
Atrial fibrillation	0.63	(0.42-0.94)	0.024
Diabetes	1.02	(0.67-1.55)	0.912
BMI	1.02	(0.98-1.06)	0.261
NYHA-class			
I-II	1.05	(0.51-2.13)	0.901
≥III	1.57	(0.96-2.56)	0.074
Systolic blood pressure	0.99	(0.98-1.00)	0.003
Total cholesterol	0.90	(0.73-1.11)	0.343
NT-proBNP	1.53	(1.25-1.88)	$4.23x10^{-5}$
Ischemic heart disease	0.72	(0.48-1.08)	0.114

ACEi= AF=atrial fibrillation, BMI=body massindex; NYHA= NYHA-class at admission, NT-proBNP=N-terminal prohormone BNP SPS=Shrunken pore syndrome

Table 10. Associations between SPS and 30-day re-hospitalization, and lower quality of life. Patients with SPS were compared to those with an $eGFR_{CYS}/eGFR_{CR}$-ratio >0.6

	30-day re-hospitalization (70 events)		KCCQ overall score <50 points (n=54)	
	HR (CI 95%)	p-value	OR (CI 95%)	p-value
Model 1	1.73 (0.99-3.02)	0.023	2.41 (1.21-4.81)	0.012
Model 2b	1.82 (1.04-3.18)	0.035	2.15 (1.03-4.49)	0.042

Values are hazard ratios (HR) or odds ratios (OR) and 95% confidence intervals (CI 95%). SPS=Shrunken pore syndrome; eGFR=estimation glomerular filtration rate; CKD-EPI=chronic kidney disease epidemiology collaboration;
Model 1: adjusted for age and sex
Model 2b: adjusted for age, sex, DM, ethnicity, employment, NYHA- class at admission, Nt-proBNP, HDL and SBP at admission.

Paper III

Baseline characteristics are shown in table 11. Although the population in papers II and III is the same, in paper III, fewer patients were included because the study was conducted beforehand, and the baseline data were not available for all individuals on the HARVEST cohort. SPS was defined as $eGFR_{CYS}/eGFR_{CR}$ ratio < 0.6 based on both CKD-EPI formulas and CAPA and LMR formulas. Data are expressed as means (±SD) or medians (25-75 interquartile range). As in Paper II SPS was more common among smokers and women. BMI was higher in patients with SPS. The use of betablockers and ACE-inhibitors differed between the patients with SPS and those without SPS.

There was a difference in SPS prevalence depending on which eGFR formulas were used. Thus 22.3% of the patients had SPS if CKD-EPI formulas were used but only 9% if CAPA and LMR formulas were used.

Table 11. Baseline Characteristics of the HARVEST Population

Study sample	*SPS*	*No SPS*	*p*	*SPS*	*No SPS*	*p*
	CKD-EPI<0.6	CKD-EPI>0.6		CAPA/LMR<0.6	CAPA/LMR>0.6	
	n=67	n=233		**n=27**	n=273	
Sex (women; n (%))	46 **(68.7)**	44 (18.9)	<0.001	20 **(74.1)**	70 (25.6)	**<0.001**
Age (years)	76.8 (±11.6)	74.3 (±11.4)	0.117	74.8 (±9.1)	74.8 (±11.7)	0.971
Systolic BP (mmHg)	141 (±30)	135 (±26)	0.150	139 (±30)	136 (±27)	0.574
BMI at admission (kg/m^2)	**29.4** (±7.4)	27.4 (±5.5)	0.016	**32.2** (±8.2)	27.4 (±5.6)	**<0.001**
BMI at discharge (kg/m^2)	28.5 (±6.6)	26.4 (±5.1)	0.007	30.4 (±7.0)	26.5 (±5.2)	<0.001
Weight at admission (kg)	81.8 (±22.9)	83.1 (±18.7)	0.608	88.9 (±21.5)	82.2 (±19.2)	0.089
Weight at discharge (kg)	79.6 (±20.0)	79.9 (±16.9)	0.886	84.0 (±18.9)	79.4 (±17.4)	0.196
Prevalent diabetes (n (%))	29 (43.3)	82 (35.2)	0.227	14 (51.9)	97 (35.5)	0.094
Smoking (n (%))	14 **(20.9)**	21 (9.0)	0.272	7 **(25.9)**	28 (10.3)	**0.016**
NYHA (III+IV;n(%)	62 (92.6)	196 (84.1)	0.080	25 (92.6)	233 (85.3)	0.301
Betablockers (n (%))	53 (79.1)	208 (89.3)	0.223	19 (70.4)	242 (88.6)	**0.007**
Statins (n (%))	35 (52.2)	135 (57.9)	0.407	14 (51.9)	156 (57.1)	0.597
ACE-inhibitors (n (%))	27 (40.3)	135 (57.9)	0.011	9 (33.3)	153 (56.0)	**0.024**
AII-antago-nist (n (%))	21 (31.3)	55 (23.6)	0.199	10 (37.0)	66 (24.2)	0.143
Glucose (mmol/L)	6.2 (5.4-7.5)	6.2 (5.5-7.3)	0.700	6.7 (5.3-7.7)	6.2 (5.5-7.5)	0.750
LDL (mmol/L)	2.1 (±0.9)	2.1 (±0.9)	0.904	2.1 (±0.9)	2.1 (±0.9)	0.715
HDL (mmol/L)	1.2 (±0.4)	1.2 (±0.4)	0.149	1.2 (±0.4)	1.2 (±0.4)	0.949
Cystatin C (mg/L)	1.8 (1.4-2.4)	1.6 (1.2-2.1)	0.002	2.1 (1.7-2.5)	1.6 (1.3-2.1)	<0.001
Creatinine (mmol/L)	87 (76-117)	108 (90-141)	0.004	89 (77-117)	106 (84-140)	0.159
CAPA	34.2 (±12.9)	42.6 (±17.0)	<0.001	29.8 (±10.9)	41.8 (±16.6)	<0.001
LMR	56.3 (±20.3)	43.6 (±19.7)	<0.001	57.0 (±19.9)	45.4 (±20.3)	0.005
CKD-EPI$_{CYS}$	32.6 (±12.9)	40.1 (±17.6)	0.001	28.5 (±10.5)	39.4 (±17.1)	0.001
CKD-EPI$_{CR}$	64.1 (±23.4)	47.8 (±21.5)	<0.001	64.0 (±22.9)	50.2 (±22.6)	0.003

BMI=body mass index, BP=blood pressure, CR=creatinine, CYS=cystatin C, NYHA=New York Heart Associations functional classification, ACE=angiotensin converting enzyme, AII=angiotensin II, LDL=low density lipoprotein cholesterol, HDL=high density lipoprotein cholesterol, eGFR=estimated glomerular filtration rate

Of 92 analyzed proteins, six of them were significantly Bonferroni-corrected with SPS based on both pairs of eGFR formulas in age and sex adjusted model. HR and p-value after adjustment according to models 2 and 3 are shown in table 12.

Table 12. Logistic Regression Analysis Examining Proteins relation to SPS

	SPS based on CKD-EPI formulas				SPS based on CAPA and LMR formulas			
	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
	Model 2		**Model 3**		**Model 2**		**Model 3**	
TNFR2	2.43 (1.62-3.68)	$2.1x10^{-5}$	2.38 (1.55-3.64)	$6.7x10^{-5}$	3.04 (1.73-5.34)	$1.2x10^{-4}$	2.98 (1.64-5.44)	$3.6x10^{-4}$
CD163	2.08 (1.43-3.01)	$1.1x10^{-4}$	2.11 (1.42-3.13)	$2.2x10^{-4}$	2.81 (1.65-4.79)	$1.4x10^{-4}$	3.06 (1.68-5.56)	$2.8x10^{-4}$
AXL	2.01 (1.39-2.90)	$1.9x10^{-4}$	1.95 (1.32-2.86)	0.001	3.69 (2.01-6.77)	$2.5x10^{-5}$	3.88 (1.98-7.56)	$7.1x10^{-5}$
TNFR1	2.01 (1.36-2.97)	$4.5x10^{-4}$	1.96 (1.30-2.95)	0.001	2.66 (1.53-4.63)	0.001	2.78 (1.52-5.07)	0.001
IL2RA	1.90 (1.33-2.72)	$4.4x10^{-4}$	1.85 (1.27-2.69)	0.001	2.90 (1.67-5.04)	$1.6x10^{-4}$	2.99 (1.65-5.44)	$3.3x10^{-4}$
OPG	2.20 (1.51-3.21)	$4.4x10^{-5}$	2.17 (1.47-3.21)	$1.0x10^{-4}$	2.81 (1.63-4.86)	$2.2x10^{-4}$	2.92 (1.60-5.33)	0.001

Logistic regressions for prevalent SPS based on the CAPA and LMR Formula (27 cases vs. 273 controls) adjusted for age and sex (Model 1) age, sex, BMI, NYHA, SBP, diabetes and smoking (model 2) age, sex, BMI, NYHA, SBP, diabetes, BB, smoking, ACE/AII, LDL, HDL and Glu (Model 3). TNFR2; Tumor necrosis factor receptor 2, CD163; Scavenger receptor cysteine rich type 1 protein M130, AXL; Tyrosine-protein kinase receptor UFO, TNFR1; Tumor necrosis factor receptor 2. IL2RA; Interleukin 2 Receptor Subunit Alpha, OPG; Osteoprotegerin

Five proteins are implicated in atherosclerosis: tumor necrosis factor receptor 1 (TNFR1), tumor necrosis factor receptor 2 (TNFR2), scavenger receptor cysteine rich type 1 protein M130 (CD163), interleukin-2 receptor subunit alpha (IL2-RA), and osteoprotegerin (OPG). One protein is involved in cell proliferation, cell survival, differentiation and migration (tyrosine-protein kinase receptor UFO (AXL). Figure 5 illustrate protein-protein pathways, protein-disease associations, and a possible disease pathway.

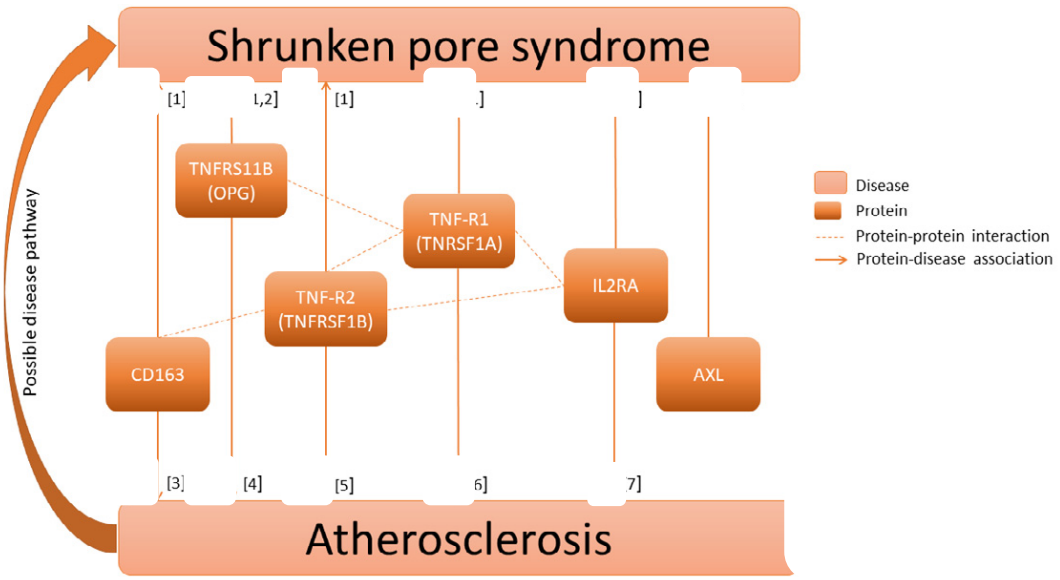

Figure 5. Illustration of protein-protein pathways, protein-disease associations, and possible disease pathway.

Paper IV

Baseline characteristics are shown in tables 13a and 13b. Continuous characteristics are presented as median (2.5-97.5 percentiles) and categorical values are presented as percent. SPS was present in 405 individuals (8% of the whole cohort). In the original cohort, there is a difference in the prevalence of several risk factors with known association with higher mortality risk. For example, the incidence of diabetes is 12.8 % among individuals with SPS and 7.3% among individuals with $CAPA_{CYS\ corr}$ / LMR_{CR} ratio ≥ 1

Table 13a. Baseline characteristics stratified by the ratio of estimated GFR according to $CAPA_{CYS\ corr}$ and LMR_{CR} in the original cohort

		$CAPA_{CYS\ corr}$ / LMR_{CR} ratio			
Original cohort	Total	<0.70	0.70-0.84	0.85-0.99	≥1.00
n	5 061	**405**	1 377	1 903	1 376
Age, years	58 (47-67)	60 (48-67)	59 (48-67)	58 (47-67)	59 (47-67)
Female, % (n)	59.1 (2 992)	58.5 (237)	52.4 (722)	60.1 (1 143)	64.7 (890)
Education, % (n)					
Primary	45.6 (2 234)	54.7 (209)	49.5 (655)	45.2 (836)	39.8 (534)
Secondary	35.1 (1 715)	29.3 (112)	34.7 (460)	35.4 (654)	36.5 (489)
Tertiary	19.3 (946)	16.0 (61)	15.8 (209)	19.4 (358)	23.7 (318)
Employment status, % (n)					
Employed	65.1 (3 208)	52.1 (201)	61.7 (821)	66.2 (1 230)	70.6 (956)
Retired	27.7 (1 366)	38.6 (149)	31.7 (422)	25.8 (480)	23.3 (315)
Unemployed	4.3 (213)	6.7 (26)	3.8 (51)	4.8 (90)	3.4 (46)
Housewife/Student	2.9 (141)	2.6 (10)	2.7 (36)	3.1 (58)	2.7 (37)
Current or latest job (SEI), % (n)					
Manual worker	38.6 (1 896)	46.2 (178)	43.6 (579)	36.8 (680)	34.1 (459)
Non-manual worker	51.3 (2 517)	41.3 (159)	46.4 (616)	53.4 (986)	56.1 (756)
Employer	10.0 (493)	12.5 (48)	9.9 (132)	9.8 (181)	9.8 (132)
Living alone. % (n)	22.6 (1 145)	22.7 (112)	24.1 (332)	22.0 (419)	20.5 (282)
Smoking habits, % (n)					
Current smoker	**22.0 (1 088)**	**40.8 (159)**	**28.1 (378)**	**19.0 (354)**	**14.5 (197)**
Ex-smoker /occasional smoker	38.0 (1 884)	27.7 (108)	38.2 (513)	38.8 (723)	39.8 (540)
Never smoker	40.0 (1 980)	31.5 (123)	33.7 (452)	42.2 (786)	45.7 (619)
Physical activity score	6 920 (515-23 900)	6 510 (0-25 547)	6 660 (13-24 645)	6 870 (686-23 490)	7 345 (867-23 743)
BMI, kg/m^2	25 (19-35)	26 (19-38)	26 (20-37)	25 (19-34)	24 (20-33)
Blood pressure lowering drugs, % (n)	16.4 (832)	26.4 (107)	18.6 (256)	14.8 (281)	13.7 (188)
Hypertension, % (n)	**38.4 (1 943)**	**47.9 (194)**	**41.7 (574)**	**36.7 (698)**	**34.7 (477)**
Cancer, % (n)	**7.9 (400)**	**8.9 (36)**	**8.6 (118)**	**8.3 (158)**	**6.4 (88)**
CVD, % (n)	5.7 (290)	11.9 (48)	7.2 (99)	4.8 (91)	3.8 (52)
Diabetes, % (n)	9.2 (467)	12.8 (52)	11.0 (152)	8.5 (162)	7.3 (101)

In the GPS-matched cohort, the difference is no longer present.

Table 13b. Baseline characteristics stratified by the ratio of estimated GFR according to $CAPA_{CYS\ corr}$ and LMR_{CR} in the GPS matched cohort

		$CAPA_{CYS\ corr}$ / LMR_{CR} ratio			
GPS matched cohort	Total	<0.70	0.70-0.84	0.85-0.99	≥1.00
n	1 332	333	333	333	333
Age, years	59 (48-67)	60 (48-67)	59 (48-67)	58 (47-67)	59 (48-67)
Female, % (n)	59.2 (788)	59.2 (197)	60.7 (202)	56.5 (188)	60.4 (201)
Education, % (n)					
Primary	53.1 (707)	53.8 (179)	55.3 (184)	49.2 (164)	54.1 (180)
Secondary	29.7 (396)	29.7 (99)	29.1 (97)	30.3 (101)	29.7 (99)
Tertiary	17.2 (229)	16.5 (55)	15.6 (52)	20.4 (68)	16.2 (54)
Employment status, % (n)					
Employed	59.1 (786)	55.1 (183)	59.2 (197)	60.1 (199)	62.2 (207)
Retired	32.7 (434)	35.2 (117)	33.3 (111)	29.3 (97)	32.7 (109)
Unemployed	5.3 (71)	7.2 (24)	4.8 (16)	6.9 (23)	2.4 (8)
Housewife/Student	2.9 (38)	2.4 (8)	2.7 (9)	3.6 (12)	2.7 (9)
Current or latest job (SEI), % (n)					
Manual worker	45.3 (603)	44.4 (148)	48.3 (161)	42.3 (141)	45.9 (153)
Non-manual worker	43.2 (575)	44.1 (147)	41.4 (138)	43.2 (144)	43.8 (146)
Employer	11.6 (154)	11.4 (38)	10.2 (34)	14.4 (48)	10.2 (34)
Living alone. % (n)	27.4 (365)	27.3 (91)	27.0 (90)	29.1 (97)	26.1 (87)
Smoking habits, % (n)					
Current smoker	**38.8 (517)**	**38.7 (129)**	**38.7 (129)**	**39.0 (130)**	**38.7 (129)**
Ex-smoker /occasional smoker	29.1 (388)	29.4 (98)	30.3 (101)	29.1 (97)	27.6 (92)
Never smoker	32.1 (427)	31.8 (106)	30.9 (103)	31.8 (106)	33.6 (112)
Physical activity score	6 805 (507-24 414)	6 810 (0-26 236)	6 990 (268-26 355)	6 270 (960-23 458)	7 080 (723-20 985)
BMI, kg/m^2	26 (20-35)	26 (19-36)	26 (20-36)	26 (19-34)	26 (20-36)
Blood pressure lowering drugs % (n)	23.4 (312)	24.6 (82)	24.3 (81)	22.8 (76)	21.9 (73)
Hypertension, % (n)	**45.0 (599)**	**42.3 (154)**	**45.1 (150)**	**43.8 (146)**	**44.7 (149)**
Cancer, % (n)	**7.5 (100)**	**7.5 (25)**	**8.4 (28)**	**6.6 (22)**	**7.5 (25)**
CVD, % (n)	**8.6 (115)**	**9.0 (30)**	**9.0 (30)**	**8.4 (28)**	**8.1 (27)**
Diabetes, % (n)	**9.8 (131)**	**10.2 (34)**	**8.4 (28)**	**10.5 (35)**	**10.2 (34)**

As shown in tables 14a and 14b the difference between groups for e GFR variables is similar in both the original cohort and in the GPS matched cohort. This is to be expected since GPS did not include eGFR variables. Creatinine levels and eGFR based on creatinine are similar between the groups while cystatin C and eGFR based on cystatin C differ.

Table 14a. eGFR-variables, stratified by the ratio of estimated GFR according to $CAPA_{CYS}$ corr and LMR_{CR} in the original cohort.

	Original cohort				
		$CAPA_{CYS\ corr}$ / LMR_{CR} ratio			
	Total	<0.70	0.70-0.84	0.85-0.99	≥1.00
n	5 061	405	1 377	1 903	1 376
Cystatin C corr, mg/L	1.14(0.80-1.73)	1.53 (1.19-2.64)	1.27 (1.02-1.67)	1.11 (0.89-1.46)	0.98 (0.71-1.35)
Cystatin C, mg/L	0.76 (0.55-1.11)	1.03 (0.86-1.74)	0.86 (0.73-1.04)	0.75 (0.65-0.89)	0.65 (0.49-0.81)
Creatinine, µmol/L	83 (60-117)	83 (57-139)	83 (61-118)	83 (59-112)	84 (61-125)
$CAPA_{CYS\ corr}$, mL/min/1.73 m^2	63 (38-97)	44 (22-61)	55 (39-74)	65 (47-85)	76 (51-112)
$CAPA_{CYS}$, mL/min/1.73 m^2	101 (65-148)	71 (40-90)	88 (70-108)	104 (84-125)	121 (94-168)
LMR_{CR}, mL/min/1.73 m^2	70 (49-92)	71 (42-95)	71 (50-92)	71 (51-92)	69 (44-90)
$CAPA_{CYS\ corr}$ / LMR_{CR} ratio	0.90 (0.61-1.29)	0.65 (0.41-0.70)	0.79 (0.71-0.85)	0.92 (0.85-0.99)	1.09 (1.00-1.48)
$CAPA_{CYS}$ / LMR_{CR} ratio	1.44 (0.97-2.16)	1.03 (0.78-1.25)	1.25 (1.05-1.49)	1.46 (1.26-1.73)	1.77 (1.49-2.50)

Table 14b. eGFR-variables, stratified by the ratio of estimated GFR according to $CAPA_{CYS}$ corr and LMR_{CR} in the GPS matched subset.

	Matched				
		CAPACYS corr / LMRCR ratio			
	Total	<0.70	0.70-0.84	0.85-0.99	≥1.00
n	1 332	333	333	333	333
Cystatin C corr, mg/L	1.2(0.82-1.89)	1.51 (1.19-2.44)	1.27(1.03-1.68)	1.11(0.88-1.49)	0.98 (0.66-1.40)
Cystatin C, mg/L	0.80(0.56-1.21)	1.02 (0.86-1.52)	0.85 (0.73-1.03)	0.75 (0.63-0.91)	0.65 (0.49-0.83)
Creatinine, µmol/L	83 (59-118)	83 (56-122)	82 (62-113)	84 (58-111)	84 (58-132)
$CAPA_{CYS\ corr}$ mL/min/1.73m^2	59 (34-94)	45 (24-61)	56 (39-73)	65 (46-86)	76 (60-122)
$CAPA_{CYS}$, mL/min/1.73 m^2	95 (59-143)	72 (44-90)	89 (71-108)	103 (81-126)	120 (91-165)
LMR_{CR}, mL/min/1.73 m^2	70 (48-92)	71 (46-94)	70 (51-92)	71 (50-93)	67 (42-90)
$CAPA_{CYS\ corr}$ / LMR_{CR} ratio	0.85 (0.54-1.27)	0.65 (0.43-0.69)	0.79 (0.71-0.84)	0.92 (0.85-0.99)	1.09 (1.00-1.56)
$CAPA_{CYS}$ / LMR_{CR} ratio	1.36 (0.86-2.16)	1.04 (0.78-1.25)	1.26 (1.08-1.52)	1.46 (1.24-1.75)	1.74 (1.48-2.56)

In the GPS matched cohort SPS and all-cause mortality were statistically significant associated even after adjustment for $eGFR_{CYS}$ based on the CAPA formula. HR was 1.38 (95% CI (1.04 - 1.87)). No association between incident CVD, DM or CKD and SPS was found. For more information see table 15.

Table 15. Results from Cox regression models* in GPS matched subset after adjustment for $eGFR_{CYS}$.

	A: All-cause mortality		**B:Incident CVD**	
	HR (95% CI)	p-value	HR (95% CI)	p-value
CAPACYS corr / LMRCR ratio		0.017		0.968
<0.70	1.38 (1.04 - 1.87)		1.10 (0.73 - 1.67)	
0.70-0.84	0.99 (0.76 - 1.29)		1.09 (0.75 - 1.59)	
0.85-0.99	1.04 (0.82 - 1.32)		1.05 (0.74 - 1.47)	
≥1.00	1.0 (Ref.)		1.0 (Ref.)	
Observations**	1 332		1 277	
	C:Incident CKD		D:Incident DM	
	HR (95% CI)	p-value	HR (95% CI)	p-value
CAPACYS corr / LMRCR ratio		0.681		0.051
<0.70	1.13 (0.69 - 1.86)		1.98 (1.20 - 3.28)	
0.70-0.84	1.03 (0.65 - 1.63)		1.35 (0.85 - 2.16)	
0.85-0.99	1.24 (0.83 - 1.86)		1.28 (0.84 - 1.94)	
≥1.00	1.0 (Ref.)		1.0 (Ref.)	
Observations**	1 331		1273	

CVD= Cardiovascular diseases, CKD - Chronic Kidney Disease, DM-Diabetes mellitus

* Cox regression with shared frailty.

** Prevalent cases of the outcome were excluded before analysis. 55 prevalent CVD, 1 prevalent kidney disease, and 59 prevalent DM cases were excluded.

Discussion

General discussion

Chronic CRS is challenging due to two main reasons; firstly, it is difficult to detect and secondly the treatment that benefits one organ can be harmful to the other one. Early detection and treatment of CRS are important for reducing mortality and morbidity in these patients but also for society overall because the cost for medical care is high in patients with CRS.

Most cardiologists only analyze creatinine in their patients. Early detection of renal impairment can thus be difficult to achieve. Treatment with RAAS and mineralocorticoid receptor blockade (MRB) can cause hyperkalaemia especially if both types of medicines are used and especially if the patients have reduced renal function (Beusekamp 2019). This can lead to decreased or the discontinuing of the treatment which in turn leads to increased mortality and morbidity (Beusekamp 2019). Treatment with new potassium-binding drugs can increase continuation of treatment with RAAS and/or MRB but is not generally used by cardiologists (Expert meeting between cardiologists and nephrologists in Stockholm in 2019 on the management of hyperkalaemia in patients with the cardiorenal syndrome).

Approvement of SGLT2 inhibitors in late 2020 and early 2021 for treatment of patients with heart and/or renal failure provided new opportunities for treatment of patients with CRS since these medicines have a protective effect for both organs. It is important that treatment with these medicines should be initiated early since there are no studies done in patients with GFR < 25mL/min.

Examination with echocardiography is not included in routine examination in patients with CKD stage 3-5 who are followed up by nephrologists regularly even though structural and functional changes of the heart exist in 17-21% of individuals with GFR< 60 mL/min (Kottgen 2007) and up to 44% of patients on hemodialysis (House 2019). The same applies to examination with BNP or other markers of reduced heart function.

The use of both creatinine and cystatin C in the last 20 years in Sweden for estimation of GFR was mostly performed by nephrologists. With recent studies on the outcome of patients with SPS, it is vital that $eGFR_{CYS}$ and $eGFR_{CR}$ are not only used to estimate CKD stages but also to further improve the estimation of mortality risk in all patients.

Paper specific discussion

Diastolic dysfunction of the heart in patients with CKD is not well studied as systolic dysfunction. Jain *et al* was the first study to show an association between worsening eGFR and degree of diastolic dysfunction in a population-based cohort in individuals with preserved LVEF (Jane 2017). However, eGFR was based on the MDRD formula and ranged from below 30 to above 90 mL/min/1.73 m^2. In the first work, I and my co-authors showed that structural and functional changes of the heart can be detected early, in the course of CRS development, already in individuals with mild to moderate decreased kidney function (eGFR 45-59.9 mL/min/1.73 m^2). eGFR was based on the CAPA formula. In the gender stratified analyses, the association between eGFR and echocardiographic variables was no longer significant. I believe that this was due to the low number of female participants in the study (n=450). Another explanation can be that women develop heart disease later in life when compared to men.

SPS has proven to be an important marker of increased mortality risk in different patient groups. Dardashti *et al* showed this in patients undergoing elective coronary artery bypass graft (Dardashti 2016). Herou showed that the lower the $eGFR_{CYS}/eGFR_{CR}$-ratio was, the higher was the mortality risk in patients undergoing elective cardiac surgery (Herou 2019). Lüders *et al* showed that cystatin c/creatinine ratio before elective heart catheterization was a predictor for mortality and the development of contrast medium induced acute kidney injury in patients (Lüders 2015). Prude *et al* showed that SPS was a strong predictor for mortality in healthy older individuals (Purde 2016). Åkesson *et al* showed that SPS was associated with a rise in mortality even in individuals with normal mGFR (iohexol clearance) and without any diagnoses. (Åkesson 2010). In the second and fourth work, I and my co-authors showed that SPS was also associated with a higher mortality risk in patients with HF and individuals randomly selected from a population-based cohort even after adjustment for eGFR based on CKD-EPI_{CR-CYS}, respectively $eGFR_{CYS}$ based on CAPA. In paper II, SPS was more common in women. This was reported by Lüders and Purde as well but was not seen in paper IV of this thesis or reported by Åkesson, Dardashti or Herou in their studies. In paper IV there was a tendency towards an association between SPS and incident DM. This is not seen in the other studies investigating SPS.

The prevalence of SPS in the fourth paper was 8% suggesting a high prevalence of SPS in the general population among middle-aged individuals. Prude studied the prevalence of SPS in healthy and physically active older individuals. The Prevalence of SPS was 0.2%. However, the definition for SPS was $eGFR_{CYS}/eGFR_{CR}<0.6$ in the study by Prude e*t al.* Another difference might be that individuals in Prude´s cohort might have been healthier. The prevalence of HT was similar in both studies but there is no information about the prevalence of DM among individuals studied by Prude *et al.*

In the third study, I and my co-authors showed elevated levels of protein with known or proposed associations with atherosclerosis and cell survival, proliferation, differentiation, and migration in patients with HF and SPS. Almen *et al* showed also an association with proteins associated with atherosclerosis (Almen 2019). I believed that findings from these two studies could explain the pathophysiological pathways behind the known adverse effects of the SPS. It came therefore as a surprise when SPS was not significantly associated with incident CVD in study IV. However, in the unmatched cohort, the incidence of CVD at baseline was higher in patients with SPS pointing out to some degree of association, nevertheless.

Strengths

The study populations were representative and similar to the clinical conditions that I encounter at my work. The populations in papers I and IV are based on large community-based cohorts. The population in papers II and III mimic the HF population at my hospital. Cystatin C and eGFR based on cystatin C were used in all four papers and as previously shown cystatin C and $eGFR_{CYS}$ have a better association with CVD and mortality. Definition of diagnosis (like DM, HT etc) was based on several criteria and thus harder to miss. The Swedish Diagnose and Cause of Death registers are of high quality and the identification for each individual was done through record linkage of the 10-digital personal identification number. The use of GPS in paper IV, significantly reduces the bias due to confounding factors. The adjustment for $eGFR_{CR\text{-}CYS}$ in papers II and III and adjustment for $eGFR_{CYS}$ in paper IV strengthen the theory that SPS is a new syndrome that should be studied further. The information available for BMI and adjustment for it in studies II-IV, reinforces the fact that changes in eGFR between creatinine and cystatin C in patients with SPS are not a result of low muscle mass and thus falsely low creatinine but a result of differences in the renal filtration of these markers. Echocardiography with doppler used in papers I-III is a relatively sensitive method for measurements of heart function and is generally used in clinical conditions. Creatinine and cystatin C are analyzed randomly as well. Thus the findings of this thesis should be easily implemented by the clinicians.

Limitations

The subjects of the study populations were mainly of European descent, and this may limit the generalization to other ancestries. Information on urinary albumin excretion was missing for all four papers and as mentioned before albuminuria is a very potent prognostic marker. In paper IV, cystatin C was not standardized but the correction of cystatin C levels should reduce its effects. BMI is not the best marker for measuring muscle mass in an individual, but it is sensitive enough to screen large groups and is often used in studies and clinical practice.

Clinical implementations

Since early detection of CRS is important to improve the outcome of the patients with CRS, I recommend calculating eGFR by using formulas based on both creatinine and cystatin C in patients frequently visiting cardiology clinics but not only. An early referral or consultation with a nephrologist is recommended because a multidisciplinary approach is the best way to treat these patients.

For nephrologists, I recommended a more liberal examination with echocardiography or measurements of markers of reduced heart function in their patients with CKD stage 3-5, especially when other risk factors for developing CVD like proteinuria, diabetes, HT etc are present.

For all patients with SPS, it is wise to have frequent return visits and optimize treatment for other cardiovascular risk factors.

Treatment with SGLT2 inhibitors and the use of potassium binders to facilitate titration and continuation of treatment with RAAS and MRB should further improve the outcome of patients with CRS.

Future research

Although SPS has been shown to be a marker for increased mortality and morbidity even after adjustment for mGFR or for eGFR based on cystatin C there are still those that believe that the rise in mortality seen in patients with SPS is due to higher levels of cystatin C measured in them. A study where patients with SPS are compared with those without SPS and where cystatin C levels are the same in both groups could be very interesting to conduct. This would mean that patients with different eGFR and probably in different CKD stages are compared. If such study would support the hypothesis that SPS is associated with a rise in mortality, the case of the SPS being a marker of elevated mortality risk, would be much stronger.

A study using electron microscopy examinations of kidney biopsy from patients with SPS and those without SPS, but with the same disease burden and other risk factors such as smoking etc. could determine the pathophysiology behind SPS.

Prospective studies on animals exposed to various harmful elements that may lead to the development of SPS can shed more light on the mechanisms behind SPS development. An understanding of the mechanisms leading to SPS can pave the way for the prevention and/or treatment of SPS.

Conclusion

Studies conducted by me and my collaborators have shown that early detection of CRS is possible if physicians use sensitive methods such as echocardiography or eGFR based on both creatinine and cystatin C, which show diseases in both organs ahead of the development of any symptoms. The eGFR based on both creatinine and cystatin C can not only detect early impairment of renal function but can also diagnose patients with SPS who have a higher mortality and morbidity risk. Early detection and better tailored treatment for these patients can lead to improved quality of life and an extended life expectancy.

Sammanfattning på svenska

Kardiorenala syndromet (KRS) är ett välkänt tillstånd som beskriver samspelet mellan hjärtat och njurarna. Sjukdomar i hjärtat och njurarna drabbar 20 % respektive 10 % av befolkningen. Personer med KRS har en sämre livskvalitet och lägre livslängd i jämförelse med patienter som har sjukdomar i endast hjärtat eller njurarna. Individer med svår njursvikt har oftare förändringar i hjärtats struktur och funktion. Omkring 30–60% av patienter med hjärtsvikt har en försämrad njurfunktion. De mediciner som används för att behandla hjärtsjukdomar, skyddar också njurfunktionen men personer som har svår njursjukdom, tål i vissa fall inte medicinerna. Även en försämring av hjärtfunktionen hos patienter med njursjukdom kan leda till ännu snabbare försämring av njurfunktionen. Det är därför viktigt att tidigt upptäcka försämring av funktionen i ena organet hos individer med sjukdom i andra organet. Detta kan vara svårt att åstadkomma om man inte aktivt kontrollerar och utreder patienterna. Förändringar i hjärtats struktur och funktion kan upptäckas genom att undersöka hjärtat med ultraljud. För att undersöka njurfunktionen analyseras kreatinin och cystatin C i blodet. Resultaten kan med hjälp av olika formler beräkna den glomerulära filtreringshastigheten (eGFR) vilket är det bästa sätet att utvärdera njurfunktionen. Glomeruli är njurens funktionsenhet och varje njure innehåller cirka 1 miljon glomeruli.

Hos de flesta patienter stämmer värdena av eGFR baserat på kreatinin ($eGFR_{CR}$) väl med eGFR baserad på cystatin C ($eGFR_{CYS}$). Det finns personer där kvoten mellan $eGFR_{CYS}/eGFR_{CR}$ ligger under 0,7. Flera studier har visat att dessa personer har en ökad dödlighet. Tillståndet kallas för Krympt-por-syndrom (KPS) och hypotesen bakom den är att det finns en skillnad i njurarnas filtrering av kreatinin och cystatin C som beror på storleksskillnad på dessa ämnen. Cystatin C är större än kreatinin och filtreras i mindre mängd av njurarna hos personer med KPS. Jag undersökte om det fanns skillnad i hjärtats struktur och funktion hos personer i befolkning med lätt till måttlig sänkning av njurfunktionen. Med hjälp av ultraljudundersökning av hjärtat kunde skadorna på hjärtat ses betydligt tidigare än forskare trodde. I studiens andra del undersökte jag närmare KPS. Jag fann att patienter med hjärtsvikt och KPS hade dubbelt så hög dödlighet än patienter med bara hjärtsvikt. Dessa patienter lades in på sjukhuset oftare och hade sämre livskvalitet också. I deras blod fanns det en ökad koncentration av äggviteämne (proteiner) som har koppling till utveckling av kärlförkalkning och cellförökning. Detta intressanta fynd kan vara en av förklaringarna till varför patienter med KPS har en ökad mortalitet.

Då lite är känt om förekomsten av KPS i befolkning studerade vi detta hos 5061 personer som valdes slumpmässigt från befolkningen i Malmö. 405 personer eller 8% hade KPS. Även dessa individer hade en ökad mortalitet.

Jag hoppas att min studie kommer att förbättra vården av patienter med hjärt- och njursjukdomar. Genom att upptäcka tidigt skador i dessa organ kan man sätta in skyddande behandlingar för hjärtat och njurar och förbättra patienternas livskvalité och livslängd. Det finns tyvärr fortfarande oklarheter kring utveckling av KPS som jag hoppas att kommande studier kommer att belysa.

Përmbledhje në shqip

Sindroma Cardiorenale (CRS) përshkruan ndërveprimin e ngushtë midis zemrës dhe veshkave. Sëmundjet në njërin nga organet, ndikojnë në funksionin e organit tjetër.

Sëmundjet e zemrës dhe të veshkave prekin përkatësisht 20% dhe 10% të popullsisë në Suedi. Në Shqipëri rreth 80 000 persona vuajnë nga sëmundjet e zemrës dhe infarkti në zemër është shkaku kryesor i vdekjeve me rreth 11 599 raste në 2018.

Personat me CRS kanë më shumë shqetësime të shëndetit si dhe një jetëgjatësi më të shkurtër në krahasim me pacientët që kanë sëmundje vetëm në zemër ose vetëm në veshka.

Pacientët që trajtohen me dializën e veshkave kanë shpesh ndryshime në strukturën dhe funksionin e zemrës. Gjithashtu, rreth 30-60% e pacientëve me insuficiencë kardiake tregojnë shenja të dëmit të veshkave. Shumë nga barnat që përdoren për trajtimin e sëmundjeve të zemrës, mbrojnë gjithashtu edhe veshkat. Fatkeqësisht, këto barna jo gjithmonë mund të tolerohen nga pacientët me funksion të ulët të veshkave. Diagnostikimi i hershëm i CRS e rrit mundësinë e mjekimit me këto barna dhe i mbron organet nga dëmtimi i mëtejshëm.

Që CRS-të të zbulohen herët, duhet që mjekët që kontrollojnë pacientët me sëmundje të njërit prej organeve, të kontrollojnë rregullisht edhe funksionin e organit tjetër. Ndryshimet në strukturën dhe funksionin e zemrës mund të zbulohen nëpërmjet ekokardiografisë. Për të kontrolluar funksionin e veshkave, duhen marrë analiza në gjak të kreatininës dhe të cystatin C. Rezultatet e këtyre dy analizave përdoren në formula të ndryshme për të llogaritur shkallën e filtrimit glomerular (eGFR), e cila është mënyra më e mirë për të vlerësuar funksionin e veshkave. Në shumicën e pacientëve, vlera e eGFR bazuara në kreatininë ($eGFR_{CR}$) përkon mirë me vlerën e eGFR të bazuar në cystatin C ($eGFR_{CYS}$). Ka pacientë ku raporti $eGFR_{CYS}$ / $eGFR_{CR}$ është nën 0.7. Shumë studime kanë treguar se këta njerëz kanë një shkallë të lartë të vdekshmërisë. Gjendja quhet ”sindroma e tkurrjes së poreve të glomerulave (SPS)” dhe hamendëson një ndryshim në filtrimin e veshkave midis kreatininës dhe cystatinës C, për shkak të ndryshimit të madhësisë qelizore të këtyre substancave. Cystatin C është më e madhe se kreatinina dhe filtrohet në sasi më të vogla nga veshkat tek personat me SPS.

Unë hulumutova nëse kishte një ndryshim në strukturën dhe funksionin e zemrës tek personat me një rënie të lehtë ose të moderuar të funksionit të veshkave. Me

ndihmën e ekokardiografisë, dëmtimi i zemrës mund të shihet shumë më herët sesa mendonin më parë studiuesit.

Në pjesën e dytë të studimit, unë shqyrtova me hollësi SPS-në . Tregova se pacientët me insuficiencë kardiale dhe SPS kishin një dyfishim të vdekshmërisë në krahasim me pacientët që vuanin vetëm nga insuficienca kardiale. Këta pacientë rishtroheshin në spital më shpesh dhe kishin gjithashtu një cilësi më të ulët të jetesës. Në gjakun e tyre, kishte një nivel më të lartë të pesë proteinave që ndikojnë në zhvillimin e sëmundjeve të enëve të gjakut si për shembull arteriosklerozа dhe lëvizjes së qelizave. Ky zbulim interesant mund të jetë një nga arsyet pse pacientët me SPS kanë një vdekshmëri të lartë.

Deri tani nuk ka asnjë njohuri për shpërndarjen e SPS -së në popullatë dhe nëse personat me SPS kanë të njëjtën rritje të vdekshmërisë si pacientët me SPS. Ne studiuam 5061 persona të cilët u përzgjodhën rastësisht nga popullata në Malmö, Suedi. 405 persona ose 8% e tyre kishin SPS. Edhe këta individë, ashtu si dhe pacientët e studiuar më parë, kishin rritje të vdekshmërisë.

Shpresoj që studimi im të përmirësojë përkujdesjen e pacientëve me sëmundje në zemër dhe në veshka. Sa më herët të zbulohet dëmtimi i këtyre organeve, aq më herët mund të fillohet edhe trajtimi i sëmundjeve në mënyrë që dëmtimi i mëtejshëm i organeve të ndalohet dhe që cilësia e jetës dhe jetëgjatësia e pacientëve të përmirësohet.

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