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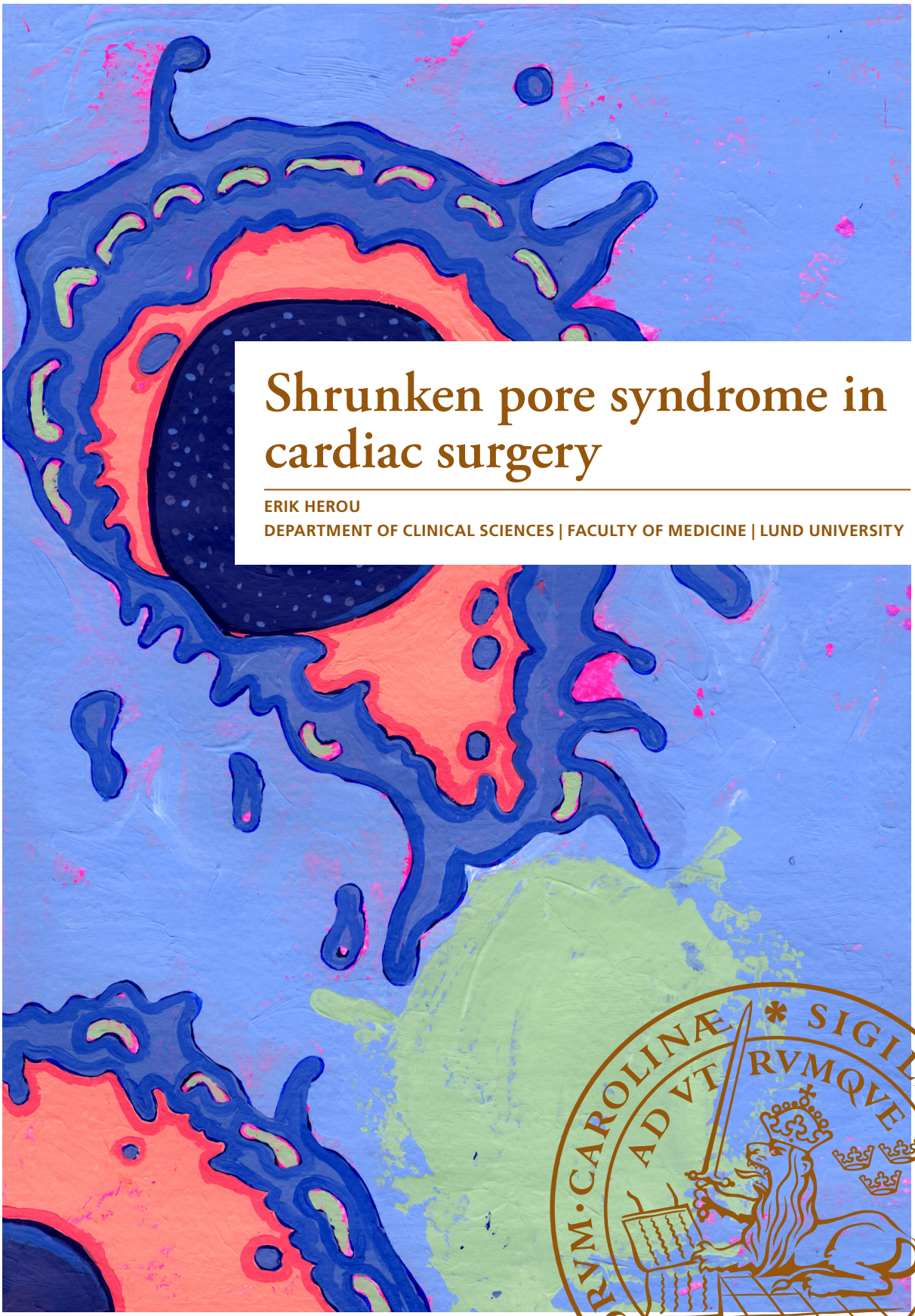
Herou, Erik

2025

Document Version:
Publisher's PDF, also known as Version of record



Shrunken pore syndrome in cardiac surgery

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Printed by Media-Tryck, Lund 2025 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

FACULTY OF MEDICINE

Department of Clinical Sciences

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2025:17
ISBN 978-91-8021-670-8
ISSN 1652-8220

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LUND
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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on the 4th of April at 09.00 in Segerfalksalen, BMC, Lund

Faculty opponent
Professor Alexander Wahba, MD, PhD

Norwegian University of Science and Technology, Trondheim, Norway

Organization: LUND UNIVERSITY

Document name: 2025:17 **Date of issue:** 2025-04-04

Author(s): Erik Herou **Sponsoring organization:** Region Skåne

Title and subtitle: Shrunken pore syndrome in cardiac surgery

Background: Shrunken pore syndrome (SPS) is defined as the estimated glomerular filtration rate (eGFR) of cystatin C <60% of $eGFR_{creatinine}$ and was postulated in 2015. It seems to afflict 8% of most studied populations and is associated with higher mortality and morbidity in the same studied populations.

Aims: I. Evaluate whether early and midterm mortality following elective cardiac surgery varies with different cut-off values for the ratio used to diagnose SPS. **II.** Investigate the impact SPS has on survival and how SPS compares to other risk factors for premature death. **III.** SPS influence on mortality and other known risk factors in a cohort of newly heart transplanted patients. **IV.** If the sampling location affects the creatinine or cystatin C levels in humans.

Methods and results: I. Retrospective cohort, 4719 consecutive patients undergoing elective cardiac surgery. Mortality correlated markedly and progressively with lower SPS ratio. **II.** Retrospective cohort, 3993 consecutive patients undergoing elective cardiac surgery. 1-, 5- and 10-year survival for patients with SPS was 90%, 59% and 45%, and without SPS 98%, 88% and 80% ($p < 0.001$). SPS was found to be an independent predictor for mortality with an HR of 1.96. SPS negatively affected survival regardless of pre-operative renal function. **III.** Retrospective cohort, 253 consecutive patients undergoing heart transplantation. Patients with SPS rose over time, from 7.5% the day after surgery to 71% in week 4. 5- and 10-year survival for patients diagnosed with SPS postoperative week 4 was 73% and 63%, respectively, and 93% and 90% for patients without SPS ($p<0.05$). **IV.** Prospective cohort of 170 patients undergoing transcatheter aortic valve replacement (TAVR). We observed a higher level of creatinine in femoral, peripheral, and jugular venous blood compared to arterial blood, and a higher level of cystatin C in peripheral, jugular, atrial, and femoral blood compared to arterial blood.

Conclusions: I. Short- and midterm mortality increases markedly with a decrease in the $eGFR_{cystatin\ C}/eGFR_{creatinine\ Ratio.}$ II. SPS significantly increases mortality for patients from the first year of follow-up to 10 years postoperatively. SPS independently predicts a decreased survival rate. III. 71% of heart transplanted patients develop SPS postoperatively, and they exhibit a worse prognosis compared to patients who do not. IV. The level of creatinine and, to some degree, cystatin C is affected by blood sampling location, highlighting the importance of sampling location.

Keywords: Shrunken pore syndrome, cardiac surgery, glomerular filtration, creatinine, cystatin C

Classification system and/or index terms (if any) Supplementary bibliographical information

Language English **ISSN and key title**: 1652-8220

Lund University, Faculty of Medicine

ISBN: 978-91-8021-670-8 Doctoral Dissertation Series 2025:17

Recipient's notes **Number of pages**: 80

Price Security classification

Signature Date 2025-01-01

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ISBN 978-91-8021-670-8
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2025

För barnen

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Abstract

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Populärvetenskaplig sammanfattning

Krympt por-syndrom är en nyupptäckt sjukdom där vissa genomsläppliga porer i njurarna förminskas och därmed inte kan släppa genom medelstora molekyler vilket är deras syfte.

Njurarnas uppgift är, bland annat, att filtrera vårt blod från molekyler som inte ska vara i blodbanan och få oss att kissa ut dessa molekyler. Tillvägagångssättet som njurarna gör detta är till huvudsak genom att filtrera vårt blod genom en beståndsdel i njurarna som heter glomerulus. Där finns små hål, porer, som molekylerna filtreras genom. En teori sager att det finns två olika storlekar på dessa porer, en liten där de små molekylerna filtreras och en stor där de större molekylerna filtreras.

För att kontrollera njurarnas funktion så används i första hand ett blodprov där man mäter koncentrationen av en mycket liten molekyl, kreatinin, i blodet. Är koncentrationen hög så har man försämrad njurfunktion. Sedan några decennier tillbaka så har även koncentrationen av en annan molekyl, cystatin C, som är större, använts för att kontrollera njurarnas funktion.

I de flesta fall överensstämmer de två blodproven, det vill säga att om man har sänkt njurfunktion uppmätt av koncentrationen för den ena molekylen så stämmer det om man mäter koncentrationen av den andra molekylen. 2015 upptäcktes det att en del av befolkningen hade nedsatt njurfunktion i testet baserat på cystatin C men normal njurfunktion baserat på kreatinin. Förklaringen kan vara att de större porerna som normal skulle släppa genom molekyler i cystatin C storlek har krympt – krympt por-syndrom.

I den här avhandlingen har vi i tre delarbeten som avhandlar krympt por-syndrom och dess effekt på patienters överlevnad samt ett delarbete om betydelsen av olika ställen i blodbanan som vi tar blodprover från.

I delarbete I försökte vi definiera krympt por-syndrom utifrån vilken ratio av koncetrationen av cystatin C jämfört med kreatinin som gav bäst sensitivitet och specificitet. Vi fann att ju lägre ratio desto sämre överlevnad hade patienterna samt at en gräns på 0.57 i ratio gav en hög specificitet.

Delarbete II undersökte vi en kohort på 3993 patienter som genomgått elektiv hjärtkirurgi och undersökte effekten av krympt por-syndrom hos dessa patiener. Vi fann att ca 8% av patienterna var drabbade av krympt por-syndrom samt att de hade en mycket sämre överlevnad jämfört med patienter som inte led av krympt por-syndrom. Vi fann även att krympt por-syndrom var lika dåligt, eller sämre, för överlevnaden som traditionella riskfaktorer vid hjärtkirurgi som diabetes eller sänkt hjärtfunktion.

Delarbete III tittade vi på en kohort av nyligen hjärttransplanterade patienter för att utröna hur de påverkades av krympt por-syndrom. Vi fann att innan

transplantationen så var 7,5% av populationen drabbade av krympt por-syndrom men att den proportionen steg kraftigt efter transplantation. Vi såg att de som diagnosticerades senare under vårdförloppet, det vill säga hade krympt por-syndrom längre tid efter transplantationen, hade sämre överlevnad jämfört med de andra.

I delarbete IV undersökte vi hur provtagningsställe influerade koncentrationen av såväl kreatinin som cystatin C. Bakgrunden är att kreatinin förekommer främst i muskulatur men cystatin C finns i samtliga celler som har en cellkärna. Vi fann att kreatinin, som förväntat, var högre i blod som kom från muskler men vi såg även att blod som kom från hjärnan hade en lätt förhöjd halt av kreatinin i sig. Mer förvånande var att även cystatin C varierade beroende på provtagningsställe men I mindre utrsträckning generellt sett än kreatinin. Detta kan vi dock inte förklara från denna studien.

List of Publications

This thesis is based on the following publications, which are referred to in the text by their roman numerals (I-IV)

I

Herou E, Dardashti A, Nozohoor S, Zindovic I, Ederoth P, Grubb A, Bjursten H.

The mortality increase in cardiac surgery patients associated with shrunken pore syndrome correlates with the eGFRcystatin C/eGFRcreatinine-ratio

Scand J Clin Lab Invest. 2019 May;79(3):167-173

II

Herou E, Grubb A, Dardashti A, Nozohoor S, Zindovic I, Ederoth P, Bjursten H.

Reduced renal elimination of larger molecules is a strong predictor for mortality

Sci Rep. 2022 Oct 20;12(1):17517

III

Herou E, Mortsell E, Grubb A, Nozohoor S, Zindovic I, Ederoth P, Dardashti A, Bjursten H.

Shrunken Pore Syndrome in heart transplantation; a pore ready to close?

Submitted.

IV

Sigurjonsson J, **Herou E**, Grubb D, Viterius B, Bjursten H.

Variations in serum levels of creatinine and cystatin C depending on the sampling location.

In manuscript.

Abbreviations

ACHD	Adult congenital heart disease
ACR	Acute cellular rejection
ADQI	Acute dialysis quality initiative
AKI	Acute kidney injury
AKIN	Acute kidney injury network
AMR	Antibody-mediated rejection
ASD	Atrial septal defect
ATG	Anti-thymocyte globulin
AVSD	Atrioventricular septal defect
BMI	Body mass index
CABG	Coronary artery bypass graft
CAPA	Caucasian asian pediatric adult
cAVSD	Complete atrioventricular septal defect
CI	Confidence interval
CKD-EPI	Chronic kidney disease epidemiology collaboration initiative
CNI	Calcineurin inhibitor
COPD	Chronic obstructive pulmonary disease
CPB	Cardiopulmonary bypass
CRP	C-reactive protein
CRRT	Continuous renal replacement therapy
CVI	Cerebrovascular insult
ECC	Extracorporeal circulation
ECMO	Extracorporeal membrane oxygenation
eGFR	Estimated glomerular filtration rate
ESKD	End-stage kidney disease
EPS	Elongated pore syndrome
FP	Foot processes
GBM	Glomerular basement membrane

GFR	Glomerular filtration rate
HR	Hazard ratio
IABP	Intra-aortic balloon pump
IQR	Inter quartile range
KDIGO	Kidney disease: improving global outcomes
LCOS	Low cardiac output syndrome
LMrev	Lund Malmö revised
LMR18	Lund Malmö revised 2018
LVAD	Left ventricular assist device
LVEF	Left ventricular ejection fraction
MDRD	Modified diet in renal disease
mGFR	Measured glomerular filtration rate
MI	Myocardial infarction
MMF	Mycophenolate mofetil
NOAC	Novel oral anticoagulants
PAPVC	Partial anomalous pulmonary venous connection
PA/VSD	Pulmonary atresia with ventricular septal defect
RECITA	Renal clearance in TAVI-patients
ROC	Receiver operating characteristic
RRT	Renal replacement therapy
SD	Standard deviation
SGHS	Selective glomerular hypofiltration syndrome
SIRS	Systemic inflammatory response syndrome
SPS	Shrunken pore syndrome
TAVI	Transcatheter aortic valve implantation
TOF	Tetralogy of Fallot
VSD	Ventricular septal defect

1 Introduction

1.1 Overview of kidney function

1.1.1 The Nephron

The kidneys' main function is to filter blood, and the primary structure of that is the glomeruli located in Bowman's capsule (1). The glomeruli are a capillary network in the kidney, supplied with blood from an afferent arteriole, which filters the blood through distinct but closely intertwined layers to form the filtrate: the fenestrated endothelium, the podocytes with their slit diaphragms and foot processes and the negatively charged glomerular basement membrane. This constitutes the glomerular filtration barrier. Blood that enters the glomeruli exits after the capillary network through an efferent arteriole and the filtrate that filters into Bowman's capsule, enters the renal tubule and is collected into the collection duct. This constitutes the smallest functional unit of the kidney – the nephron.

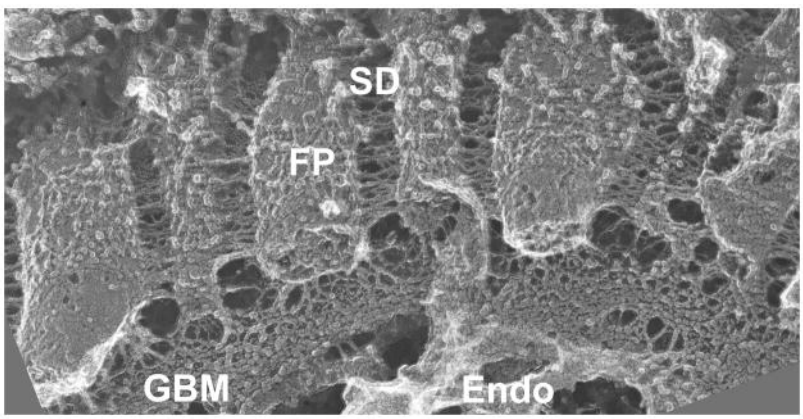

Figure 1.1 Scanning electron microscope picture showing the glomerular basement membrane (GBM), the fenestrated endothelium (Endo) and the foot processes (FP) and slit diaphragms (SD) of the podocytes. Reproduced from Curr Opin Nephrol Hypertens. 2009 May; 18(3): 226–232, Jarad and Miner, with permission.

1.1.2 Filtration, excretion, clearance, glomerular filtration rate

Different molecules filter differently over the glomerular filtration barrier. Negatively charged molecules filter less readily due to the negatively charged glomerular basement membrane, and a molecule's filterability is inversely related to its molecular weight. Some substances are reabsorbed back into the circulation, such as glucose, which filters freely but is fully reabsorbed in normal circumstances, and substances that are actively excreted directly into the tubules, thus bypassing the glomeruli. Finally, the urine is formed, and urinary excretion of a molecule can be expressed as (1):

> Urinary excretion = glomerular filtration – tubular reabsorption + tubular secretion

Urinary excretion rate of a molecule is the amount excreted per unit of time and is expressed as

> Excretion rate = U_s x V

U_s is the urine concentration of a molecule and V is the urine flow rate.

The clearance of a molecule from a specified volume of plasma in a unit of time, known as renal clearance, is perhaps the most common way of quantifying kidney function, it can be expressed as the urinary excretion rate of a molecule divided by its concentration in plasma (2):

> $C_s = U_s \times V / P_s$

C_s refers to the clearance of the molecule s, U is the urine concentration, V is the urine flow rate and P is the plasma concentration.

Glomerular filtration rate (GFR), the preferred way of quantifying kidney function, is the rate at which plasma flows from the glomerulus to Bowman's capsule over a specified period of time. It is equal to the clearance of a molecule if the molecule is freely filtered in the glomeruli and not reabsorbed, secreted or metabolized in the nephron and can in those cases, identical to renal clearance above, be calculated as follows (Guyton):

> $GFR = U_s \times V / P_s$

GFR is the glomerular filtration rate, U the urine concentration, V the urine flow rate and P the plasma concentration.

1.1.3 Measurement of glomerular filtration rate

We rely on clearance of substances that are exogenous to the body, that we add to the bloodstream, to give a measurement of GFR. As noted above, for a substance to be considered a good marker for GFR it should be eliminated solely by filtration in the glomeruli and not secreted nor reabsorbed in the tubuli. Inulin, a plant polysaccharide, is the classic substance used to measure GFR and its renal clearance has been in use for decades after it was found to meet these criteria in 1935, (3). Its measurement method is somewhat involved, which has led to the development of other techniques, substances, and markers for GFR. Of noteworthy example is iohexol, iothalamate and ^{51}Cr-EDTA, which are in clinical use as successors to inulin clearance (4).

1.1.4 Estimated glomerular filtration rate

In routine clinical practice, measured GFR (mGFR) has been overshadowed by estimated GFR (eGFR) in test volume due to ease of use and being cheaper. eGFR relies on endogenous substances that are measured in plasma and fed through a more or less complicated formula to translate a value of a substance concentration in plasma to that person's eGFR. eGFR relies on substances that are evenly produced endogenously without much difference in production in the same individual over time or between individuals. They should also filter freely and not be subject to absorption or secretion in the tubuli. The reasoning is that if GFR drops by 50%, the kidneys will only filter half of the substance as it used to, leading to a higher concentration of the substance in plasma.

1.1.5 Creatinine and cystatin C

Creatinine, a quite small molecule of 113 Dalton (5), has a long and studied past as a marker for kidney function. It was first postulated by Poul Brandt Rehberg in 1926 as a marker for glomerular filtration (6). He administered an oral load of 5 grams of creatinine, measured creatinine in both blood and urine afterwards, and created the first clearance formula – an unknown term at the time. It was believed that endogenous creatinine did not exist in detectable quantities in blood, which made an exogenous dose of creatinine, as Rehberg thought, necessary, (7). The first endogenous creatinine clearance formula was created in 1934 by Giovanni Ferro-Luzzi who used Rehberg's formula and a new method of detecting creatinine in blood, making the exogenous dose unnecessary. The first comparison of creatinine clearance, and thus its suitability for determination of glomerular filtration, to inulin was published in 1940 by Kurt Steinitz (8). The first equation to estimate GFR solely from a blood sample of creatinine was developed by Cockroft and Gault in 1976, (9) who created the Cockroft-Gault formula. The properties of creatinine that make

it suitable for estimating glomerular filtration are its freely filtered in the glomeruli and not reabsorbed in the tubuli. It also is produced at a constant rate in the breakdown of creatine. Its disadvantages are that it fluctuates with meat intake, a person's muscle mass influences it. Further, it is secreted to a variable degree in the tubuli (9).

Cystatin C (or γ-trace as it was known by at its inception due to its migration in electrophoresis to the gamma region) was discovered in 1979 by H Löfberg and A Grubb (10) and in the original paper it was established that cystatin C in patients with renal failure had values up to 13 times the normal value. Cystatin C has been used as a marker for GFR since 1985 (11, 12) and since then, myriad papers have described different estimating equations for GFR based on cystatin C (11). Cystatin C weighs 13.3 kDa, is freely filtered in the glomeruli, is not influenced by muscle mass or meat intake, but its levels are elevated if the patient is on glucocorticoids (12).

Optimal estimation of GFR based on creatinine or cystatin C has long been the subject of research. It has been suggested that equations including both creatinine and cystatin C provide the highest accuracy of eGFR (11).

1.1.6 Pore model for glomerular filtration

According to the pore model for glomerular filtration (13, 14) glomerular filtration can be understood to occur through two different types of pores, one small (37 Å) and one large (99 Å) with a selective decrease in filtration of molecules in sizes 5-30 kDa can occur by a shrinking of the pores.

1.2 Shrunken pore syndrome

1.2.1 What is in a name?

Shrunken pore syndrome (SPS) was first postulated in 2015 (15) and since then the accumulated body of work has referred to the new syndrome as shrunken pore syndrome. This lasted up until 2023 and 2024 when cases were made for calling the syndrome for elongated pore syndrome (EPS) and/or selective glomerular hypofiltration syndrome (SGHS) (9, 16, 17). In this thesis, we will tentatively refer to the syndrome as shrunken pore syndrome.

1.2.2 The beginning

SPS is defined as $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$ and tentatively as a selective impairment of glomerular filtration for molecules with sizes 5-30 kDa. When SPS was postulated in 2015 as a distinct kidney condition the idea came from studies on pregnant women in the third trimester where it was noticed as early as 2002 that eGFR based on cystatin C was markedly lower than both eGFR based on creatinine and invasive measurement based on iohexol which conversely has a low mass of 900 Da (18). These results were interpreted to originate from a shrinking of the glomerular pores. Further findings of an accumulation of different molecules in the 5-30 kDa size range in the same patient population supported the interpretation (19). The hypothesis that this was a condition that was not exclusive for pregnant women in the third trimester led the same group to explore in 1349 consecutive patients who arrived at the clinical chemistry laboratory at SUS Lund with a request for eGFR if the same tendency to have a lower $eGFR_{cystatin\ C}$ versus $eGFR_{creatinine}$. In that population, 8 % displayed $eGFR_{cystatin\ C} \leq 60\%$ of $eGFR_{creatinine}$. No survival data was reported.

1.2.3 The evidence accumulates

The first article on SPS with clinical outcomes was published in 2016 and showed a markedly worse survival in a cohort of elective coronary artery bypass graft (CABG) patients with a 5-year survival of 65% for patients with SPS and 90% for patients without SPS. They could also show that the survival was worse for patients with SPS regardless of their preoperative kidney function and that SPS afflicted 6% of their population. In all subsequent investigations, the presence of SPS has correlated with a higher mortality or morbidity, (20-25) where such data is available.

This includes sub-cohort analyses of patients with normal measured GFR and with or without other diagnoses, both showed a markedly high hazard ratio (HR) of 4.1 and 7.3 respectively. It has also been found in children but unfortunately without survival data (26).

1.2.4 The pathophysiology

Earlier studies have indicated that the glomerular filtration of molecules 5-30 kDa can be selectively decreased while the filtration of molecules with a lower size is unimpeded (27-29). About one third of the normal human proteome is estimated to have a molecular weight <30 kDa. One hypothesis regarding the high mortality in patients with SPS is that they accumulate proteins in the 5-30 kDa range which in healthy kidneys are cleared. This is supported by a study that found that patients with SPS had elevated proteins in the 5-30 kDa range, including 18 promoting atherosclerosis (23). Recently it has been showed that the permeability for cystatin C, but not creatinine, was impeded in patients with diabetic kidney disease and that the thickness of the glomerular basement membrane was inversely correlated with the ratio of $eGFR_{cystatin\ C}/eGFR_{creatinine}$ (30). In a similar animal model, diabetic rats had smaller glomerular pores than control animals, indicative of shrunken pores (31).

Even though the evidence tentatively points in the direction of impeded filtration, one could hypothesize that the relative elevation of cystatin C compared to creatinine in SPS could be due to increased production of cystatin C. That does not explain the accumulation of proteins in the 5-30 kDa range, if not production for all proteins that size is increased, nor do we have a pathophysiological model for this physiological state but much about the pathophysiology of SPS is still unknown.

2 Cardiac surgery

2.1 History of cardiac surgery

2.1.1 The beginning

Cardiac surgery is a quite young specialty with, if we disregard earlier and primarily unsuccessful attempts, its first documented case of intracardiac repair was performed by John Gibbon Jr in 1953 utilizing for the first time a heart-lung machine to oxygenate and circulate the patient during the time it took Dr Gibbon to close the patient's atrial septal defect (ASD) (32) with a running suture. Dr Gibbon did perform two more operations with his heart-lung machine but unfortunately both patients died during the procedure prompting Dr Gibbon to leave the field of cardiac surgery that he was instrumental in creating for other clinical and academic pursuits.

The gauntlet was passed to two men who became the first giants in our field, Dr Walton Lillehei at the University of Minnesota in Minneapolis and Dr John Kirklin at the Mayo Clinic. Dr Lillehei pioneered controlled cross circulation where a matching donor, often a parent, was used as an oxygenator. The patient's caval veins were cannulated and the blood pumped to the donor's femoral vein and from the donor's femoral artery to the patient's carotid artery, (33). Dr Lillehei performed corrective surgery on ventricular septal defects (VSD), tetralogy of Fallot (TOF), Pulmonary atresia with VSD (PA/VSD) and complete AV commune (cAVSD) with frankly astounding results. For the first 106 Fallot patients that were corrected, both using cross circulation and later a heart-lung machine, they reported a 30-year survival of 77% with a 91% freedom from reoperation (34). Controlled cross circulation did not catch on, mainly because of the risk it poses to the donor and Dr Lillehei switched in 1955 from cross circulation as a means to oxygenate the patient to a heart-lung machine (34, 35). Dr Lillehei was the first to use a patch to close a VSD, the first to utilize an outflow patch in correcting a Fallot and the first to correct pulmonary atresia with valvulotomy and transannular patch – techniques we use to this day. These inventions and more have made Dr Lillehei known as the "father of open-heart surgery" (36-38).

Figure 2.1 Picture of Dr Walton Lillehei of the University of Minnesota in Minneapolis taken in the operating theatre in his characteristic pose and a face full of scowl. Reprinted from Global Cardiology Science and Practice 2018:11, Cooper DKC, Christiaan Barnard—The surgeon who dared: The story of the first human-to-human heart transplant, with permission from the author.

At the Mayo Clinic, Dr Kirklin adopted the schematics for Dr Gibbon's heart-lung machine and built one that he refined and used in the beginning of his career in cardiac surgery. He was the first to correct a VSD with the aid of a heart-lung machine in 1955 (39). If Dr Lillehei was a patients surgeon then Dr Kirklin was a surgeons surgeon, he was one of the first to publish comprehensive articles of how to correct a VSD (40), how to perform patient selection in the setting of pulmonary hypertension (41), AVSD (42, 43), partial anomalous pulmonary venous connection (PAPVC) with ASD (44, 45) and together with Sir Barratt-Boyes he wrote and rewrote what is currently known as the comprehensive and authoritative textbook in our specialty – *Cardiac Surgery*.

Figure 2.2 Picture of Dr. John Kirklin taken early in his career. Reprinted from J Thorac Cardiovasc Surg. 2007 Jul;134(1):225-8, Stephenson LW, Historical perspective of The American Association for Thoracic Surgery: John W. Kirklin, MD (1917-2004), with permission from Elsevier.

The beginning of cardiac surgery in Sweden is perhaps not as well-known but no less distinguished. Clarence Crafoord performed the first operation for aortic coarctation in 1944 in Stockholm, a year ahead of Dr. Gross who popularized the operation. This was not a one-off success; Dr Crafoord and his team set a string of impressive surgical milestones. He performed the second successful ECC case in the world in 1954 inspired by Dr Gibbons machine, his younger colleague Viking Olov Björk co-invented the first successful tilting disc mechanical aortic valve – the Björk-Shiley valve, Åke Senning was in 1957 the first to invent a successful operation for transposition of the great arteries, the atrial switch or Senning procedure. Up to this day, we still perform the Senning procedure in certain complex cases at our institution, 67 years after it was invented (46, 47).

2.1.2 Extracorporeal circulation

The problem with cardiac surgery is that the heart is basically a rhythmically beating muscle with four blood-filled caverns in it. To gain access to the heart, its intricacies and defects one usually needs to drain it of blood and some of the time stop its beating. Cross circulation, as pioneered by Dr Lillehei, was, even if it was revolutionary at the time, an evolutionary dead end. From 1955 to the present-day, cardiac surgery has relied on heart-lung machines to accomplish extracorporeal circulation (ECC) or cardiopulmonary bypass (CPB). There were two different machines in the beginning. The one developed by Dr Gibbon with the help of International Business Machines (IBM) and later adopted by Dr Kirklin (48, 49) and the one designed by Dr Dewall and Dr Lillehei (50, 51). They both used the same

principle of a bubble oxygenator to oxygenate the blood. A simple heart-lung machine circuit would consist of a draining cannula in either the right atrium or the caval veins, an oxygenator to oxygenate the oxygen depleted blood, a pump and an aortic cannula to deliver the oxygenated blood to the patient.

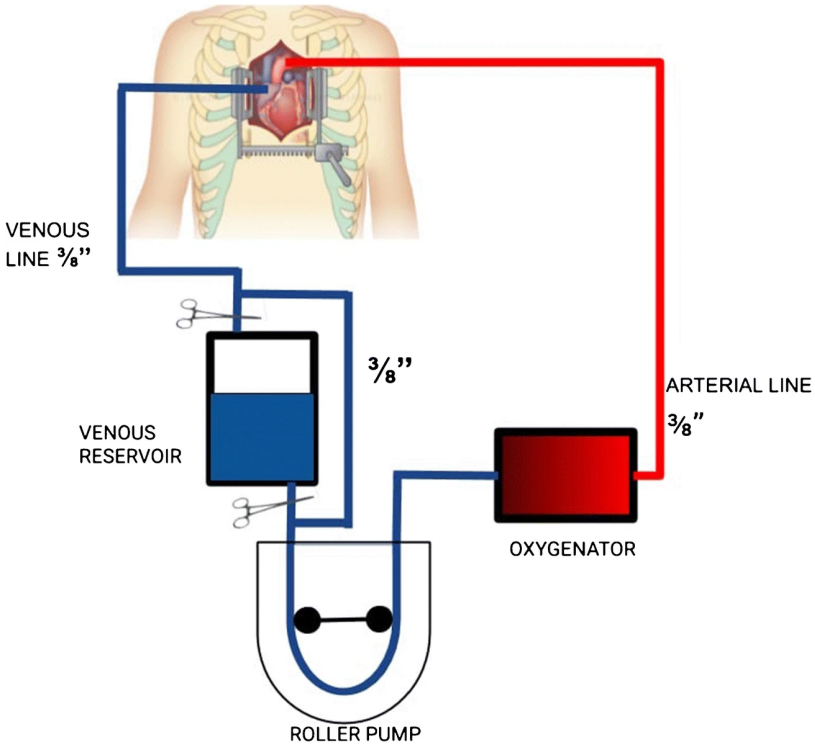

Figure 2.3 Diagram of a simple extracorporeal circulation circuit. Reprinted from George, B., Kandaswamy, M., Jaganathan, U. et al. Extension of cardiopulmonary bypass outside the operating room as a short-term bridge to recovery "the poor man's ECMO". Indian J Thorac Cardiovasc Surg 37, 108–111 (2021). With permission from Springer.

The heart-lung machine was, and still is, instrumental for cardiac surgeons to gain access to the heart, but it is not a machine without consequences and drawbacks. Its use damages red blood cells due to mechanical and immunological stress (52), it makes the blood both hypocoagulable due to the administering of heparin and hypercoagulable (53) which predisposes for both ischemic and hemorrhagic stroke (54, 55). Air embolism can occur due to problems with the circuit (56) and when the patient's blood encounters the tubing of the ECC circuit, an inflammatory response starts, which can develop to the systemic inflammatory response syndrome (SIRS), which in turn correlates with multi-organ dysfunction (56). There are more potential complications from ECC, but important for this dissertation is that renal complications and failure have been studied since the early 60's with regard to ECC (57-59).

2.2 Present techniques

2.2.1 Coronary artery bypass grafting

Cardiac surgery was in the beginning primarily for congenital defects. It soon became apparent that there was acquired disease that needed surgical treatment. Myocardial revascularization for coronary artery disease was one of these and in 1964 Dr Kolesov in the Soviet Union was the first to anastomose the left internal thoracic artery (LITA) to the left anterior descending artery (LAD). The same year Dr DeBakey sutured a saphenous vein graft to a coronary artery (60) and the basis for a modern CABG procedure was invented – LITA to the LAD and saphenous vein grafts to the other coronary arteries in need of revascularization. The field has evolved since then, with a trend over the last decades of multi arterial revascularization, but 90% of all CABG performed follows the procedure outlined above (61).

2.2.1.1 Surgical technique for CABG in Lund

The procedure is performed under general anesthesia in the prone position. It usually starts with the assistant harvesting the saphenous vein from the right or left leg. The main operator performs a median sternotomy and harvests the LITA, which, after its harvest, remains connected to the left subclavian artery proximally. Heparin is administered, the pericardium is opened and ECC is established with an aortic cannula in the ascending aorta and venous cannula in the right atrial appendage. Aortic cross clamp proximal of the aortic cannula is applied and antegrade cold blood cardioplegia is administered in the aortic root to achieve diastolic arrest. The target coronary vessels are incised and the distal anastomoses are completed. After the last distal, typically the LITA to the LAD, the aortic cross clamp is released, and the heart is started. A side biting clamp is applied to the ascending aorta and the proximal anastomosis for the vein grafts is completed. The patient is weaned of ECC, protamine is given to reverse the heparin effect, and the patient is closed after receiving temporary pacing wires and chest tubes.

2.2.2 Surgical aortic valve replacement

Aortic valve disease can be either congenital or acquired. The congenital type range in severity from intrauterine death at one extreme to a valve which is formed with a defect, but which never needs an intervention at the other. Acquired aortic valve disease is mostly stenotic in nature, with a usually decades-long, asymptomatic calcification of the valve, with risk factors of diabetes and hyperlipidaemia (62). The first direct vision commissurotomy to treat a stenotic congenital valve was performed in 1955 by Dr Kortz and Dr Swan (co-inventor of Swan-Ganz catheter)

(63) and the first surgical aortic valve replacement (sAVR) in anatomic position was performed by Dr Harken in 1960 (64). Since then, there has been a plethora of different valve designs and different types of valves. Mechanical valves were the first to be implanted and are still used predominantly in adults in working age, a drawback is that the patient needs to be on warfarin to lower the risk of thrombus forming on the valve (65). Biological valves are usually constructed from bovine or equine pericardium. Their use, which once was restricted for the geriatric population, is now creeping downwards in age due to the low risk of repeat aortic valve replacement and/or the increasing availability of transcatheter approaches. A drawback of biological prostheses is their limited lifespan, making a lifetime management necessary if implanted in the non-geriatric patient. The third option would be the Ross procedure, which is mostly performed in pediatric patients, where the patient's pulmonary valve is harvested and sutured into the anatomic place of the excised aortic valve. The pulmonary valve is replaced with a conduit of some sort, most commonly a pulmonary homograft. Advantages of the Ross procedure are that it makes it possible to replace a growing child's aortic valve and its excellent hemodynamics, drawbacks would be that the autograft tends to develop regurgitation in the aortic position, and that you have replaced a one-valve problem for a two-valve problem (66).

2.2.2.1 Surgical technique for sAVR in Lund

The procedure is performed under general anesthesia in the prone position. A median sternotomy is performed, heparin is administered, and the pericardium is opened. ECC is established with an aortic cannula in the ascending aorta and venous cannula in the right atrial appendage. Aortic cross clamp proximal of the aortic cannula is applied and antegrade cold blood cardioplegia is administered in the aortic root to achieve diastolic arrest, repeat cardioplegia is given in the retrograde manner through a cannula in the coronary sinus. The aorta is opened with an oblique incision and the aortic valve is inspected and excised in its entirety. The prostheses are sutured into place in the anatomic position and the aortotomy is closed. The cross clamp is removed, the heart started, and the patient is weaned of ECC. Protamine is given to reverse the heparin effect, and the patient is closed after receiving temporary pacing wires and chest tubes.

2.2.3 Heart transplantation

Dr Barnard performed the first heart transplantation in December 1967 (67, 68). Still, it was Dr Shumway at Stanford and a few other groups who persevered in the years following the first heart transplantation when the survival was if not abysmal then at least so bad that most heart transplantation programs were abandoned (69, 70). The problem was threefold: surgical technique, preservation of the donor heart, and perhaps, the most crucial, graft rejection.

2.2.3.1 Surgical technique

The surgical technique for heart transplantation began developing in the early 20th century. Dr Carrel at the University of Chicago was the first to transplant a donor heart in a canine model, although he transplanted the heart into the neck of the dog. The technique became more refined over time with increasingly standardized anastomoses of the great vessels during the 1940s and 1950s. How to anastomose the right and left atria was somewhat unclear but eventually the field settled on the biatrial approach by Dr Shumway (71). The biatrial approach retains both left and right atria from the recipient, which is then used in the anastomoses. This method reigned until the bicaval approach was spearheaded by Dr Yacoub in the late 1980s (72) which leaves just a posterior cuff of left atrial tissue and the vena cavae to perform the anastomoses. The fear was that the biatrial approach distorts the atria, gives rise to both supraventricular arrhythmias and tricuspid regurgitation (73).

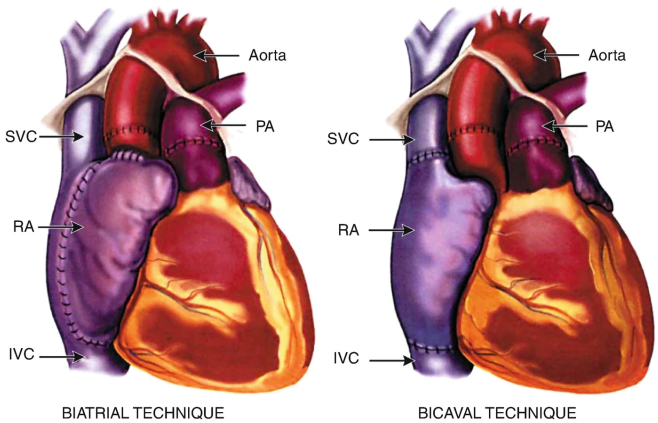

Figure 2.4 Biatrial and bicaval approach. IVC, inferior vena cava; PA, pulmonary artery; RA, right atrium; SVC, superior vena cava. Reprinted from Postoperative Critical Care for Adult Cardiac Surgical Patients. 2018 Dabbagh, A., Esmailian, F., Aranki, S. Springer, Cham. With permission from Springer.

In Lund we use the bicaval approach. After establishing ECC and aortic cross clamp, the recipient's heart is removed, leaving an ample left atrial cuff and good length of ascending aorta, main pulmonary artery, inferior and superior vena cava. When the donor heart arrives, cold blood cardioplegia is administered every 20 minutes until we can resume flow to the donor heart implanted in the following order: left atrium, inferior vena cava, main pulmonary artery, aorta, and superior vena cava. We typically reperfuse one hour on ECC before we wean, close the patient, and transfer to our cardiovascular intensive care unit.

2.2.3.2 Preservation of donor heart

During procurement of the donor heart, the heart is arrested, and it is first when the donated heart is sutured into place in the recipient's body that it is reperfused, the whole time during arrest there is a slow but steady decay of the myocardial cells.

The accepted upper limit for static cold storage of a donated heart is 4 hours (74). In the early days, arrest of the heart was achieved by topical hypothermia based on experiments by Shumway's group (75). During the 1970s, different cardioplegic solutions that accomplish a chemically induced arrest of the heart were in development and are in use since then. In Lund we routinely use 2000 ml of PLEGISOL® (Hospira, Inc., Lake Forest, IL) to help achieve cardiac arrest during heart procurement. Plegisol is also known as St. Thomas' Hospital cardioplegic solution No. 2, the successor to one of the earliest and more successful cardioplegic solutions made in the 1970s, it is an extracellular cardioplegic solution. It induces arrest through inhibition of the Na/K cell membrane pump (76).

The quest to safely prolong ischemic time has recently led to experimental ways of continuous perfusion of donor organs. They have shown promise to safely extend the time from harvest to recipient although there is, for now, a lack of evidence supporting superior outcomes (77, 78).

2.2.3.3 Graft rejection and immunosuppression

The single most significant contributing reason that heart transplantation programs were shut down after an initial burst of new programs during the 1970s was the high rejection rates and conversely low survival rates. Graft rejection can be either hyperacute or antibody mediated rejection (AMR) which is caused by preformed antibodies against HLA antigens on the graft or ABO blood group antigens. These two are somewhat more uncommon, the more common rejection is acute cellular rejection (ACR), a T cell-mediated response.

Initially, immunosuppression was limited to corticosteroids, 6-mercaptopurine, and, later, azathioprine. When cyclosporine was discovered and made clinically available, we gained a clinically effective immunosuppressor, and survival started to rise.

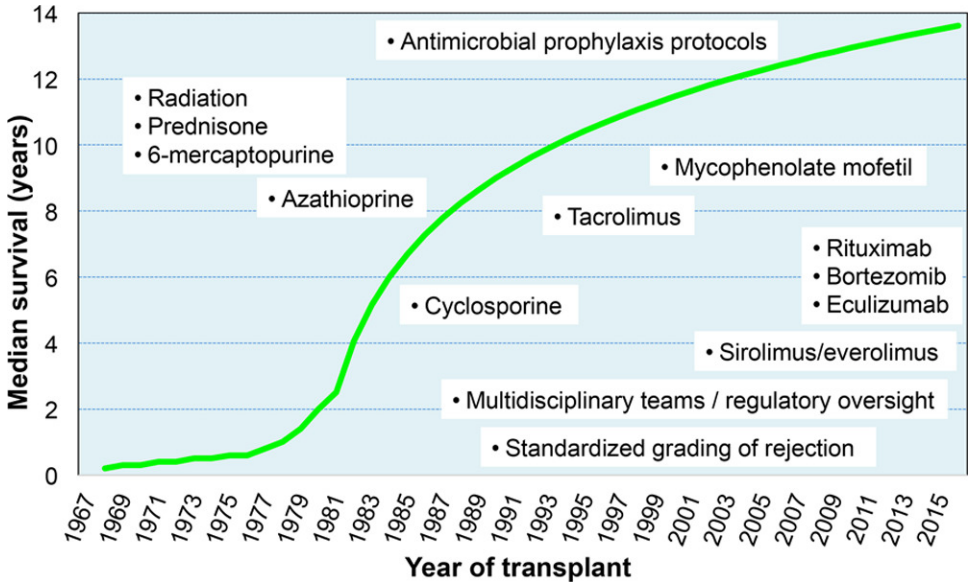

Figure 2.4 Median survival according to year of transplant with introduction of different immunosuppressive agents and other key changes. Reprinted from Stehlik J, Kobashigawa J, Hunt SA, et al. Honoring 50 Years of Clinical Heart Transplantation in Circulation: In-Depth State-of-the-Art Review. Circulation. 2018;137(1):71-87. With permission from American Heart Association.

Modern day immunosuppression may differ a bit between transplantation centres regarding which exact drug is used but it follows a common theme. Induction agents are used in about half of the world's heart transplantation centres and started perioperatively to decisively and early modulate the recipient's immune response. The two most common drugs work by either depleting the recipient of T-cells (anti-thymocyte globulin (ATG)) or are interleukin-2 receptor antagonists (basilixmab). Induction therapy has been shown to lower mortality and treated rejection episodes but may be associated with increased malignancy-related mortality (79, 80). After induction therapy, the bulk of patients will be prescribed a long-term maintenance regimen consisting of three drugs: a corticosteroid, an antimetabolite, and a calcineurin inhibitor (CNI). Corticosteroids are a mainstay of immunosuppression, and their immunosuppressive action is multifactorial. Initially, the steroids were continued indefinitely and in high dose. Concerns started to rise due to the side effects of corticosteroids; diabetes, osteoporosis, vasculopathy, and Dr Yacoub was the first to describe steroid-free immunosuppression, while Dr Pritzker reported the first steroid tapering protocol. Steroid tapering with the aim of steroid withdrawal within one year seems to be feasible for most patients and is the contemporary treatment of choice (81). Antimetabolites inhibit the cell cycle of T- and B-cells with the classic antimetabolite being azathioprine which has been largely replaced by mycophenolate mofetil (MMF) since MMF was shown to reduce mortality and rejection in the first year following heart transplantation compared to azathioprine

(82). CNIs work by inhibiting calcineurin, an enzyme in T-cells, and prevent the differentiation and proliferation of T-cells. Cyclosporine was the first CNI and as earlier described was a revolution in terms of survival after heart transplantation. Tacrolimus, a newer CNI, has largely replaced cyclosporine with its lower rate of side effects such as hypertension, hyperlipidemia, and hirsutism (83). The TICTAC-study has also shown that tacrolimus as monotherapy had similar rejection rates and long-term survival as the standard combination therapy, although with a non-significant trend of better survival with combination therapy, paving way for more tailored approaches to immunosuppression (84-87).

2.3 Cardiac surgery and renal damage

2.3.1 History

Renal damage was quite quickly recognized as a complication from open heart surgery. It was also recognized to occur at a greater rate than during or after other major surgery. Dr Lillehei and his group published a report of his first 1000 patients in 1962, both children and adults, describing a prevalence of 3% of acute renal failure and an ominous 87% mortality in their cohort for patients afflicted with acute renal failure. The one correlation they found was between acute renal failure and a period of hypotension or low cardiac output syndrome (LCOS) (58). The early accumulated body of evidence pointed to a correlation of hypotension, either from ECC or postoperatively, and acute renal failure (57, 59). It was also recognized that preexisting renal disease often became exacerbated after ECC and that the longer the time on ECC, the higher the incidence of renal failure.

2.3.2 Acute kidney injury

The diagnostic criteria for acute renal failure have changed, and varying criteria have been in use earlier (88-90). In 2004, the Acute Dialysis Quality Initiative (ADQI) group published a consensus definition of what became known as Acute Kidney Injury (AKI), which eclipsed both the term acute renal failure and earlier diagnostic criteria. It provided both diagnostic criteria to demonstrate AKI and a classification to assess the severity and progression of kidney injury termed the RIFLE criteria. The classification is dependent on two variables, serum creatinine and/or urine output levels, and there is a temporal aspect as well, the changes should occur within 7 days (91).

RIFLE stands for:

- **Risk**: elevated serum creatinine by 50% or a decrease in GFR by more than 25% or a urine output that is less than 0.5 ml/kg/h for 6 hours
- **Injury**: elevated serum creatinine by 100% or a decrease in GFR by more than 50% or a urine output that is less than 0.5 ml/kg/h for 12 hours
- **Failure**: elevated serum creatinine by 300% (or serum creatinine >350 μmol/l) or a decrease in GFR by 75% or a urine output that is less than 0.3 mg/kg/h for 24 hours or anuria for 12 hours
- **Loss**: complete loss of kidney function for more than 4 weeks

- **End-stage kidney disease (ESKD)**: complete loss of kidney function for more than 3 months

Since it was proposed, several studies investigated the RIFLE criteria's validity to classify AKI and it was generally found to be a good outcome predictor with a progressively worse outcome with higher RIFLE class (92-94). The classification was, however, considered to suffer from two shortcomings: the first being the reliance on prior knowledge of serum creatinine, its use of measured GFR, and the second that it was unclear how renal replacement therapy (RRT) influenced the stages before Loss or ESKD (95, 96). This led to a different classification of AKI published in 2007 by the Acute Kidney Injury Network (AKIN) which refined the RIFLE-criteria. The AKIN classification consists of three stages with a classification dependent on serum creatinine and/or urine output with a temporal aspect of that the changes should occur within 48 hours (97).

AKIN classification:

- **Stage 1**: elevated serum creatinine by 50-100% or a urine output that is less than 0.5 ml/kg/h for 6 hours
- **Stage 2**: elevated serum creatinine by 100-200% or a urine output that is less than 0.5 ml/kg/h for 12 hours
- **Stage 3**: elevated serum creatinine by >200% or a urine output that is less than 0.3 mg/kg/h for 24 hours or anuria for 12 hours

The main differences between RIFLE and AKIN are that RIFLE allows for diagnosis up to 7 days, while AKIN focuses more on rapid diagnosis. RIFLE includes Loss and end-stage kidney disease, which are long-term outcomes, while AKIN does not have criteria for long-term outcomes. AKIN does not require a baseline creatinine, but one can assume a baseline creatinine by using the Modified Diet in Renal Disease (MDRD) equation in AKIN (98, 99).

More recently in 2012, the Kidney Disease: Improving Global Outcomes (KDIGO) published guidelines that harmonized the RIFLE and AKIN criteria (100). It includes diagnostic criteria for AKI which are an increase in serum creatinine by >26,5 μmol/l within 48 hours or an increase in serum creatinine by >50% from baseline to have occurred within 7 days or a urine output ≤0,5 ml/kg/h for 6 hours.

The staging of AKI within KDIGO is similar to the staging in AKIN but with a slight difference in cutoffs:

- **Stage 1**: elevated serum creatinine by 50-90% or ≥26.5 μmol/L or a urine output that is less than 0.5 ml/kg/h for 6-12 hours
- **Stage 2**: elevated serum creatinine by 100-190% or a urine output that is less than 0.5 ml/kg/h for ≥12 hours
- **Stage 3**: elevated serum creatinine by >200% or serum creatinine ≥ 354 μmol/L or initiation of renal replacement therapy (RRT) or a urine output that is less than 0.3 mg/kg/h for 24 hours or anuria for 12 hours

Since its publication the KDIGO guidelines have in many cases become the preferred tool to diagnose AKI, eclipsing both RIFLE and AKIN (101).

3 Aims of this research

3.1 Background for study initiation

3.1.1 Study I

From the beginning the value of the $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio used to identify SPS was set to 0.6. This value was derived from studies of preeclamptic women (15, 18, 102, 103). The same cut-off value was used for both the equation pair used in the Chronic Kidney Disease Epidemiology Collaboration study (CKD-EPI), CKD-$EPI_{cystatin\ C}$ and CKD-$EPI_{creatinine}$, and the equation pair Caucasian Asian Pediatric Adult (CAPA) and Lund Malmö Revised (LMrev), based upon cystatin C and creatinine, respectively (104-106). Further research showed that the presence of SPS was strongly associated with mortality in a cohort undergoing elective CABG using the same cut-off value (107). However, the same article showed mortality was increased even when a ratio of 0.7 was used to diagnose SPS. Therefore, we decided to perform a study to try to elucidate if and if so, how the mortality varies with different cut-off values for SPS.

3.1.2 Study II

SPS has been found to correlate with higher mortality in all hitherto studied populations: from a mixed patient population (25) to healthy seniors (21) and patients undergoing elective CABG (107). It has also been shown to correlate with the need for sAVR for patients with aortic stenosis (24) and with right heart failure in patients with heart failure (22). All studies are either relatively small or heterogeneous. We set out to study SPS in a quite large (3997 patients) and well-defined cohort with multiple known risk factors undergoing elective cardiac surgery to investigate mortality of SPS in relation to other risk-factors and laboratory parameters. This in hope to elucidate patterns and get to know SPS as a new entity better.

3.1.3 Study III

Heart transplantation continues to be the standard of care for patients with end-stage heart failure who meet inclusion criteria (86, 108). Preoperative severe renal dysfunction often leads to postoperative chronic dialysis (108) and even with good preoperative renal function, postoperative renal failure often mar the postoperative course and contribute to morbidity and mortality (109, 110). SPS has been shown to influence mortality regardless of preexisting kidney disease (107) and as renal failure is prevalent in this population and SPS hasn't been studied in a heart transplanted population, we set out to study its prevalence and its influence on mortality on a cohort of newly heart transplanted patients.

3.1.4 Study IV

In clinical practice renal function is almost exclusively measured by determining serum levels of creatinine and/or cystatin C, and these absolute levels can be transformed into an estimated GFR with the use of a few different equations (111-114). These levels and/or estimations are used widely in clinical practice to closely follow trends in renal function over time.

Creatinine forms from the non-enzymatic conversion of creatine phosphate and creatine which mainly can be found in muscle (115-118) and cystatin C is produced at a constant rate in all investigated nucleated cells (119-122). From this one could assume that sampling from a vein in the extremities should yield a higher concentration of creatinine compared to cystatin C, which should not vary depending on sampling location.

3.2 Specific study aims

3.2.1 Study I

To evaluate whether early and midterm mortality following elective cardiac surgery varies with different cut-off values for the ratio used to diagnose SPS.

3.2.2 Study II

To investigate the impact SPS has on survival in our cohort and how SPS compares to other risk factors for premature death over a follow-up period of 10 years.

3.2.3 Study III

To study the SPS and its influence on mortality as a standalone risk factor and in relation to other known risk factors in a cohort of newly heart transplanted patients.

3.2.4 Study IV

Does sampling location affect the levels of creatinine or cystatin C in humans?

4 Material and methods

4.1 Patients and study design

4.1.1 Study I

The plasma levels of cystatin C and creatinine were measured preoperatively in 4719 patients undergoing CABG, sAVR or CABG + sAVR from 1 January 2010 to 31 December 2015, at the Department of Cardiothoracic Surgery at Skåne University Hospital in Lund, Sweden. Emergency operations (defined as surgery within one hour from decision), redo operations, and patients with missing preoperative creatinine or cystatin C data were excluded from the study, leaving 4007 patients for the final analysis. Perioperative data were obtained from an in-house quality database. Laboratory data were obtained from the hospital clinical chemistry department. Survival data were obtained from the national tax registry in January 2017. Follow up was virtually 100% complete with a mean follow up of 3.6 ± 1.8 years. The plasma level of cystatin C was determined by an automated particle-based immunoassay, adjusted to the international reference preparation ERM-DA 471/IFCC and that of creatinine by an enzymatic colorimetric assay with an IDMS-traceable calibrator. Both assays were run on a Cobas C-system (Roche Diagnostics, Basel, Switzerland). The assays were performed according to the manufacturer's instruction. The CAPA and the $\text{CKD-EPI}_{\text{cystatin C}}$ estimating equations, based on cystatin C, were used to estimate GFR, as were the LMrev and $\text{CKD-EPI}_{\text{creatinine}}$ estimating equations based on creatinine (104-106).

4.1.2 Study II

This was a retrospective single-center cohort study with prospective sampling of cystatin C and creatinine, as well as prospectively collected perioperative data. We listed the study at ClinicalTrials.gov with ID NCT04141072. We also reviewed the literature concerning SPS using a search strategy of "SPS" and "Shrunken Pore Syndrome" on pubmed.gov. The plasma levels of cystatin C and creatinine were measured simultaneously in 4719 consecutive patients undergoing CABG, sAVR, or CABG + sAVR from 1 January 2010 to 31 December 2015, at the Department of Cardiothoracic Surgery at Skåne University Hospital in Lund, Sweden (4.1.1 Study

I). 25 patients were excluded for missing survival data, 547 for missing cystatin C or creatinine preoperatively and 154 patients were excluded for redo surgery, emergency surgery or surgery for endocarditis leaving 3993 patients for final analysis.

4.1.3 Study III

This was a single center retrospective cohort study with prospective blood sampling and perioperative data collection. Included in the study were 253 consecutive adult patients undergoing heart transplantation from 1 January 2011 to 31 December 2020 at the Department of Cardiothoracic Surgery at Skåne University Hospital in Lund, Sweden. The plasma levels of creatinine and cystatin C were measured simultaneously in the above-described population. Laboratory and perioperative data were obtained from the hospital clinical chemistry department and our in-house quality database. International calibrators were used for the analyses of cystatin C and creatinine. Survival data were obtained from the national tax registry in June 2023. Median follow-up was 6.3 years (IQR 4.0-9.0 years). The serum cystatin C and creatinine analyses have been described previously (4.1.1 Study I). The CKD-EPI equations were used for estimating GFR based on creatinine or cystatin C (104, 123). The creatinine-based Lund Malmö revised 2018 (LMR18)- and cystatin C-based CAPA-equations were used in a separate analysis to estimate GFR (105, 124). Due to the lack of preoperative cystatin C samples, the first sample used for analysis was 1 day after the index operation.

4.1.4 Study IV

This was a prospective single center study conducted at Skåne University Hospital in Lund, Sweden, with patients undergoing an elective transcatheter aortic valve implantation (TAVI) between December 2021 and June 2022 as its subjects. The study population was high- to intermediate-risk patients. Given their advanced age, they often had comorbidities such as hypertension, peripheral vascular disease, diabetes, and chronic obstructive pulmonary disease (125-128).

This was a sub study in the RECITA study (REnal Clearance In TAVI-patients). RECITA was undertaken to describe renal one-pass elimination of proteins in detail (129). Blood sampling was performed from the renal vein and the femoral artery simultaneously, and in a subset of these patients, we performed sampling of venous blood from the femoral artery, liver vein, right atrium, and internal jugular vein. For all patients in the RECITA cohort, we had peripheral sampling of creatinine and cystatin C according to a clinical routine, which is performed either in cubital vein, vein on the back of the hand, or a vein between these locations.

Sampling at different locales was performed using a 100 cm long 5 Fr SIM 1 catheter (Cordis Corporation, Hialeah, FL, USA) inserted in the 6Fr introducer in a femoral vein. This catheter was advanced cervically into the inferior vena cava with the help of a guidewire under fluoroscopic guidance. Fluoroscopy used to determine the exact location for sampling. If there were uncertainty on exact location of the catheter tip, 3-4 mL of intravenous contrast (Visipaque, GE Healthcare AS, Oslo, Norway) was injected.

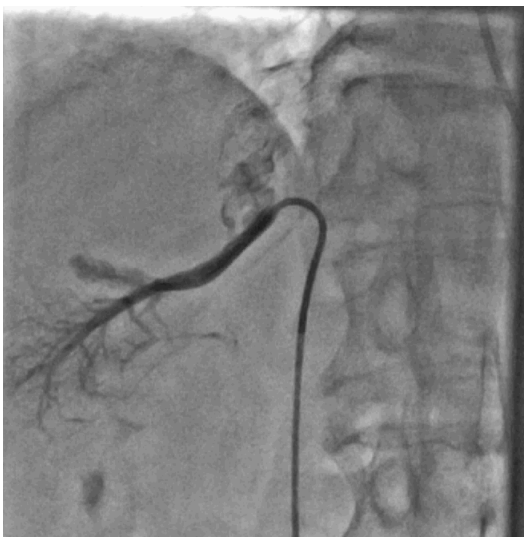

Figure IV.1 Injection of contrast into a liver vein during blood sampling for RECITA.

4.2 Ethical aspects

4.2.1 Study I-II

All studies were conducted in compliance with the declaration of Helsinki and the research protocol was approved by the regional ethics committee in Lund (LU EPN 2016/53). The need for patient consent was waived.

4.2.3 Study III

The study was conducted in compliance with the declaration of Helsinki and the research protocol was approved by the Swedish ethical review authority (Dnr 2020-0423, 2022-03545-02). The need for patient consent was waived.

4.2.4 Study IV

The study was conducted according to the guidelines of the Helsinki Declaration and was approved by the Swedish ethical review authority (approval number 2021-03618 with amendment 2022-04433-02). All participants gave informed consent, both oral and written, before inclusion in this study.

4.3 Statistical analysis

4.3.1 Study I

Categorical data were given as proportions, and continuous variables were expressed as the mean ± standard deviation (SD). In skewed distributions, medians and IQR were reported. Receiver operating characteristic (ROC) curves were used for the CKD-EPI$_{\text{cystatin C}}$/CKD-EPI$_{\text{creatinine}}$-ratio and the CAPA/LMrev-ratio. Youden's index was used to determine the optimal cut-off from the ROC curves. Testing of proportions was performed using Chi-square tests. Test of equality for ROC curves was performed according to DeLong et al. (130). Statistical analysis was performed using Statistica software version 13.1 (StatSoft Inc., Tulsa, OK) and Stata version 14.0 (StataCorp LLC, College Station, TX).

4.3.2 Study II

Categorical data were given as proportions, and continuous variables were expressed as the mean ± SD. In skewed distributions, median and IQR were reported. Survival rates during follow-up were estimated using Kaplan–Meier curves, and comparisons were performed using a log-rank test. Cox multivariable proportional hazards model was used to determine independent predictors for mortality. The variables entered in the Cox proportional hazards regression model were age, sex, body mass index, chronic obstructive pulmonary disease (COPD), creatinine, cystatin C, diabetes, previous cerebrovascular lesion, peripheral vascular disease, anemia (hemoglobin level < 120 g/l), C-reactive protein (CRP), unstable angina pectoris, previous myocardial infarction and ejection fraction ≤ 30%. The variables were chosen as they were readily available and previously shown or hypothesized to influence mortality in cardiac surgery. A backward, stepwise elimination method yielded the risk factors found in Table II.2 and these predictors became the final model. This model was used to determine the independent effect of Shrunken Pore Syndrome by adding SPS to the model. The adjusted Hazard Ratio (HR) and 95% confidence intervals (95% CI) were calculated for Shrunken Pore Syndrome as a predictor. P-values < 0.05 (two-tailed) were considered statistically significant. Statistical analysis was performed using Statistica software version 13.1

(StatSoft Inc., Tulsa, OK), Stata version 14.0 (StataCorp LLC, College Station, TX) and SPSS version 27 (IBM Corp, Armonk, NY).

4.3.3 Study III

Categorical data were given as proportions, and continuous data were expressed as the mean ± SD. In skewed distributions, median and interquartile range (IQR) were reported. Survival rates during follow-up were estimated with Kaplan-Meier curves, and comparisons were performed using log-rank test or Gehan's Wilcoxon test if proportional hazards were not found. Cox uni- and multi-variable proportional hazards regression were used to determine independent predictors for mortality. The variables entered in the Cox model are shown in Table III.3. The variables were chosen as they were readily available and previously shown or hypothesized to influence mortality in cardiac transplantation or cardiac surgery. The inclusion criterion for the full regression model was $p \leq 0.20$, and the limit for stepwise backward elimination was $p < 0.10$. A backward, stepwise elimination model yielded the risk factors that became the final multivariable model. This model was used for determining the independent effect of SPS by adding SPS to the model. The adjusted Hazard Ratio (HR) and 95% Confidence Interval (95% CI) were calculated for SPS as a predictor. *P*-values < 0.05 (two-tailed) were considered statistically significant. Statistical analysis was performed using Statistica software version 13.1 (StatSoft Inc., Tulsa, OK) and Stata version 14.0 (StataCorp LLC, College Station, TX).

4.3.4 Study IV

We used the REDCap software (Research Electronic Data Capture) hosted at Lund University, Lund, Sweden for data capture. Outliers were identified by undertaking a manual verification of the data file, excluding data points that met either of the following criteria: First, if arterial or venous test results fell below the detection limit of the test, and/or if test results suggested an abnormal production of a substance, indicating that samples had been incorrectly labelled or analyzed.

Categorical data were given as proportions, and continuous variables were expressed as the mean ± SD. In skewed distributions, median and IQR were reported. A comparison of groups was performed with Wilcoxon signed-rank test. No adjustments were made for multiple comparisons. Statistical analyses were made with Statistica® ver. 14 (TIBCO Software, Palo Alto, CA).

4.4 AI Models

4.4.1 Introductory summary

The introductory summary of this thesis was written in Microsoft Word version 16.93 (Microsoft Corp, Redmond, WA). Grammarly version 1.106.1.0 (Grammarly Inc., San Francisco, CA) and the auto-correct feature of Microsoft Word corrected spelling in the introductory summary; no other AI tools were utilized.

4.4.2 Study I-IV

Study I-IV was written in Microsoft Word version 16.93 (Microsoft Corp, Redmond, WA). The auto-correct feature of Microsoft Word corrected spelling; no other AI tools were utilized.

5 Results

5.1 Study I

5.1.1 Study population and patient characteristics

Of the 4007 patients studied, 3181 (79%) underwent CABG, 535 (13%) underwent sAVR, and 291 (7%) underwent both CABG and sAVR. The mean preoperative $eGFR_{cystatin\ C}$ using $CKD\text{-}EPI_{cystatin\ C}$ and CAPA equations was 63.6 ± 21.8 and 66.0 ± 20.9 mL/min/1.73m^2, respectively. The mean preoperative $eGFR_{creatinine}$ for $CKD\text{-}EPI_{creatinine}$ and LMrev equations was 74.9 ± 20.1 and 66.7 ± 17.5 mL/min/1.73m^2, respectively. The 1- and 3-year all-cause mortality was 2.9 and 6.8%, respectively.

5.1.2 CKD-EPI estimates

The prevalence of SPS in the population was 299 (7.5%) using the equation pair $CKD\text{-}EPI_{cystatin\ C}$/$CKD\text{-}EPI_{creatinine}$ with the cut-off of <0.6. Using this cut-off, the 1- and 3-year mortality was 10 and 21%, respectively. To study the influence of the $CKD\text{-}EPI_{cystatin\ C}$/$CKD\text{-}EPI_{creatinine}$-ratio used to diagnose SPS on 1- and 3-year mortality, the mortality at different intervals of the $CKD\text{-}EPI_{cystatin\ C}$/$CKD\text{-}EPI_{creatinine}$-ratio was calculated. There was an increase in mortality with a decrease in the ratio. A statistically significant increase in mortality was noted between cut-off values of 0.6–0.7 *versus* 0.7–0.8 for 1-year mortality and at cut-off values of <0.5 *versus* 0.5–0.6, 0.6–0.7 *versus* 0.7–0.8 and 0.8–0.9 *versus* 0.9–1.0 for 3-year mortality.

ROC curves plotting $CKD\text{-}EPI_{cystatin\ C}$/$CKD\text{-}EPI_{creatinine}$-ratios and survival for 1 and 3 years were produced. The area under the curve (AUC) was 0.713 for 1-year survival and 0.689 for 3-year survival. Youden's index for the ratio was 0.74 both for 1- and 3-year follow-up. Sensitivity and specificity at a ratio of 0.74 were 64%/74% and 57%/73% for 1- and 3-year follow-up, respectively, and the prevalence of SPS was 1181 (29%) at this cut-off. Mortality at 1- and 3-year follow-up was 6.6% and 14%, respectively, using 0.74 as the cut-off value for identifying SPS.

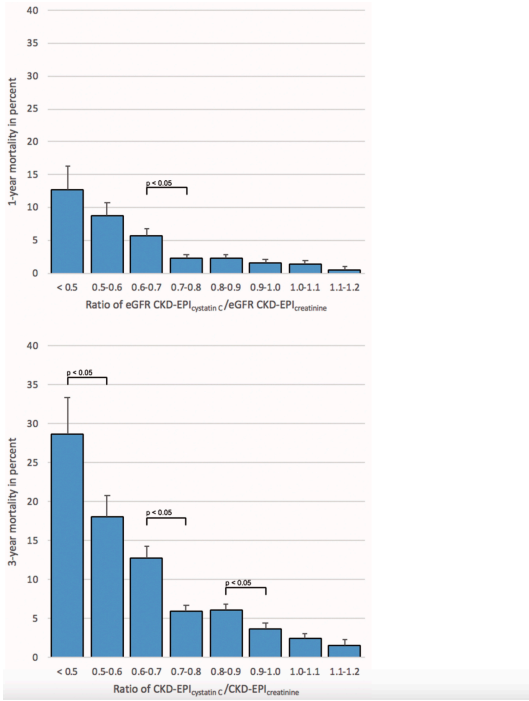

Figure I.1 One- and 3-year mortality in percentage at different ratios of CKD-EPI$_{cystatin\ C}$/CKD-EPI$_{creatinine}$. Error bars indicate standard error of the mean. Horizontal bars indicate statistical significance in Chi-square tests. CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration.

A high-specificity cut-off was selected to identify the subgroup that might stand most to gain from active intervention preoperatively. A specificity of 95% was chosen that provided sensitivities of 22 and 23% for 1- and 3-year follow up, respectively, with corresponding SPS ratios of 0.56 and 0.58. A mean cut-off value of 0.57 corresponded to a prevalence of 219 (5.5%) for patients with SPS and to 1- and 3-year mortalities of 11 and 24%, respectively.

5.1.3 CAPA LMrev estimates

The prevalence of SPS in the population was 94 (2.3%) using the equation pair CAPA/LMrev with the cut-off of <0.6. Using this cut-off, the 1- and 3-year mortality was 12 and 27%, respectively. To study the influence of the CAPA/LMrev-ratio used to diagnose SPS on 1- and 3-year mortality, the mortality at different intervals of the CAPA/LMrev-ratio was calculated. There was a general increase in mortality with a decrease in the ratio. A statistically significant increase in 1- and 3-year mortality was noted between the groups with SPS of 0.7–0.8 *versus* 0.8–0.9 and 0.8–0.9 *versus* 0.9–1.0.

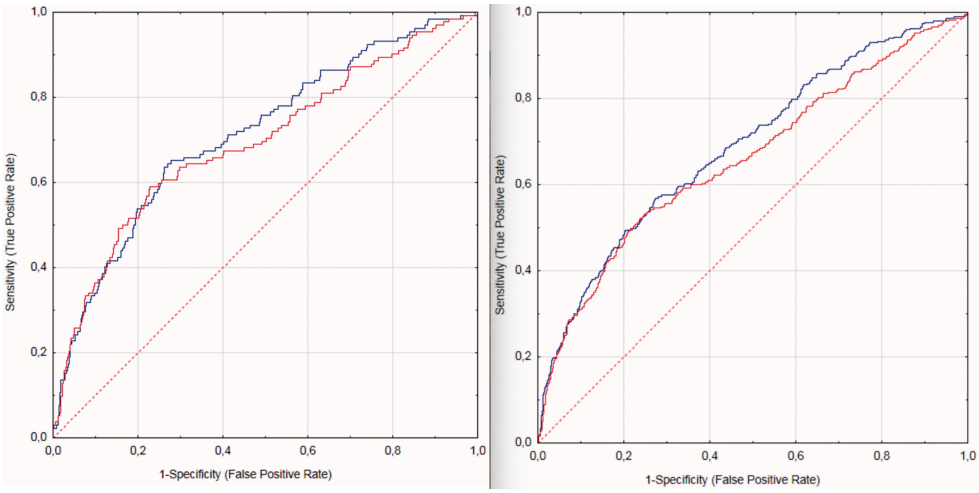

Figure I.2 ROC curve plotting ratios of the equation pair CKD-EPI$_{cystatin\ C}$/CKD-EPI$_{creatinine}$ (blue line) and the equation pair CAPA/LMrev (red line) for 1-year mortality on the left panel and 3-year mortality on the right panel. CAPA, Caucasian Asian Pediatric Adult; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; LMrev, Lund Malmö Revised; ROC, receiver operating characteristic.

ROC curves plotting CAPA/LMrev-ratios and survival for 1 and 3 years were produced. The AUC was 0.694 for 1-year survival and 0.660 for 3-year survival. Youden's index for the ratio was 0.85 both for 1- and 3-year follow-up. Sensitivity and specificity at a ratio of 0.85 were 59%/77% and 52%/76% for 1- and 3-year follow-up, respectively, and the prevalence of SPS was 908 (23%) at this cut-off. Mortality at 1- and 3-year follow-up was 7.0 and 15%, respectively, using 0.85 as the cut-off value for identifying SPS.

A high specificity cut-off with a specificity of 95% provided sensitivities of 25 and 22% for 1- and 3-year follow-up, respectively, with corresponding SPS ratios of 0.68 and 0.69. A mean cut-off value of 0.685 corresponded to a prevalence of 216 (5.4%) for patients with SPS and 1- and 3-year mortalities of 12 and 23%, respectively.

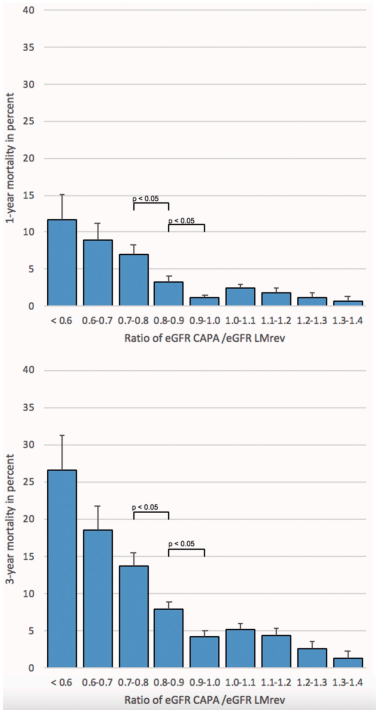

Figure I.3 1- and 3-year mortality in percentage at different ratios of CAPA/LMrev. Error bars indicate standard error of the mean. Horizontal bars indicate statistical significance in Chi-square tests. CAPA, Caucasian Asian Pediatric Adult; Lmrev, Lund Malmö Revised.

5.1.4 Comparison of different equations concerning prevalence and mortality for SPS

Comparison using the cut-off levels derived from the Youden indexes of ROC curves of 0.75 for the CKD-EPI$_{\text{cystatin C}}$/CKD-EPI$_{\text{creatinine}}$-ratio and of 0.85 for the CAPA/LMrev-ratio showed that of the 1181 patients identified as suffering from SPS by the CKD-EPI$_{\text{cystatin C}}$/CKD-EPI$_{\text{creatinine}}$-ratio, 868 also suffered from SPS by the CAPA/LMrev-ratio. Using the high-specificity cut-off levels of 0.57 for the CKD-EPI$_{\text{cystatin C}}$/CKD-EPI$_{\text{creatinine}}$-ratio and 0.685 for the CAPA/LMrev-ratio, we found that, of the 219 patients afflicted by SPS according to the CKD-EPI$_{\text{cystatin C}}$/CKD-EPI$_{\text{creatinine}}$-ratio, 185 patients also suffered from SPS by the CAPA/LMrev-ratio. The highest mortalities were observed for those patient populations identified as suffering from SPS by both ratios.

5.2 Study II

5.2.1 Study population and patient characteristics

Of the 3993 patients in the study, 296 had SPS as defined by eGFR CKD-EPI$_{cystatin\ C} \leq 60\%$ of eGFR CKD-EPI$_{creatinine}$, yielding a prevalence of 7.4%. Occurrence of SPS changed according to different eGFR strata with the highest prevalence of SPS in those with moderately reduced eGFR (30–45 ml/min/1.73 m2). The 1-, 5- and 10-year survival for the entire cohort was 97%, 86% and 77%; for patients with SPS it was 90%, 59% and 45%; and for patients without SPS it was 98%, 88% and 80% ($p < 0.001$ in 1-, 5- and 10-year survival).

Variable	All (3993)	SPS (296)	No SPS (3697)	P-value (T-test/ χ^2 test)
Age (years)	68 (±10)	73 (±9.4)	68 (±10)	0.000
Anemia	915 (23%)	131 (44%)	784 (21%)	0.000
Female	930 (23%)	67 (23%)	863 (23%)	0.781
Mean eGFR CKD-EPI	69 (±20)	54 (±16)	71 (±19)	0.000
eGFR CKD-EPI$_{creatinine}$	75 (±20)	70 (±20)	75 (±20)	0.000
eGFR CKD-EPI$_{cystatin\ C}$	64 (±22)	37 (±12)	66 (±21)	0.000
Previous stroke	375 (9.4%)	50 (17%)	325 (8.8%)	0.000
Diabetes	990 (24.8%)	121 (41%)	869 (24%)	0.000
COPD	449 (11.2%)	55 (19%)	394 (11%)	0.000
Previous vascular surgery	146 (3.7%)	26 (8.8%)	120 (3.2%)	0.000
Leucocytes (x10^9/L)	8.0 (±3.6)	8.5 (±2.9)	8.0 (±3.6)	0.021
Unstable preoperative state	131 (3.3%)	12 (4.1%)	119 (3.2%)	0.452
Peripheral vascular disease	405 (10%)	63 (21%)	342 (9.3%)	0.000
LVEF <30%	255 (6.4%)	55 (19%)	200 (5.4%)	0.000
Previous myocardial infarction	1575 (39%)	150 (51%)	1425 (39%)	0.000
EUROSCORE I	4.7 (±3.0)	7.0 (±3.4)	4.5 (±2.9)	0.000
Hemoglobin (g/L)	135 (±16)	128 (±20)	136 (±16)	0.000
Thrombocytes (x10^9/L)	236 (±69)	242 (±86)	235 (±68)	0.096
CRP (mg/L)	7,8 (±20)	18 (±37)	7 (±18)	0.000
IABP preoperative	45 (1.1%)	9 (3%)	36 (1%)	0.001
Peri-/post-procedural variables				
CABG	3179 (80%)	212 (72%)	2967 (80%)	0.000
sAVR	532 (13%)	47 (16%)	485 (13%)	0.179
CABG and sAVR	282 (7.1%)	37 (13%)	245 (6.6%)	0.000
Heart-lung machine time (min)	80 (±37)	80 (±39)	86 (±37)	0.007
Cross clamp (min)	51 (±24)	55 (±29)	51 (±24)	0.004
Time on ventilator (h)	10	9.7	16	0.001
Atrial fibrillation	889 (24%)	77 (30%)	812 (24%)	0.027
IABP postoperatively	57 (1.4%)	12 (4%)	45 (1.2%)	0.000
Renal replacement therapy	48 (1.3%)	8 (2.9%)	40 (1.2%)	0.01
On warfarin or NOAC	893 (22%)	85 (29%)	808 (22%)	0.006
Aspirin	3452 (86%)	256 (86%)	3196 (86%)	0.985
On clopidoprel or ticagrelor	710 (18%)	59 (20%)	651 (18%)	0.729
Perioperative MI	24 (0.6%)	0 (0%)	24 (0.7%)	0.177
Mediastinitis	53 (1.4%)	11 (4.2%)	42 (1.2%)	0.000
Stroke	12 (0.3%)	0 (0%)	12 (0.3%)	0.341
Reexploration for bleeding	95 (2.4%)	23 (7.8%)	72 (1.9%)	0.000

Table II.1 Variables used in the analysis presented whole and grouped on the presence of SPS. Described as mean (±SD) or number (%). P-levels from Student's T-test or χ2-test depending on the data. CABG, coronary artery bypass surgery; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate and in ml/min/1.73 m2; IABP, intra-aortic balloon pump; LVEF, left ventricular ejection fraction; MI, myocardial infarction; NOAC, novel oral anticoagulants; sAVR, surgical aortic valve replacement; SPS, shrunken pore syndrome.

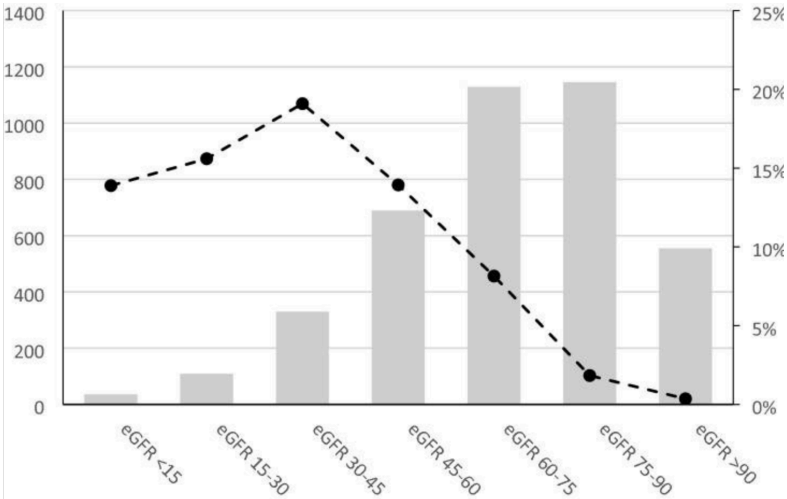

Figure II.1 The study population in different mean eGFR strata, all in ml/min/1.73 m2, number of patients on the y-axis to the left. The dotted line depicts the distribution of patients with SPS in the different mean eGFR strata in percent on the y-axis to the right. eGFR estimated glomerular filtration rate, SPS shrunken pore syndrome. eGFR, estimated glomerular filtration rate; SPS, shrunken pore syndrome

5.2.2 SPS as a risk factor for death

In univariable analysis, SPS yielded a HR for death of 3.49 (95% CI 2.94–4.14). In the multivariable Cox analysis, SPS was found to be an independent predictor for mortality with an HR of 1.96 (95% CI 1.63–2.36). Mean eGFR CKD-EPI < 60 ml/min/1.73 m2, chronic obstructive pulmonary disease (COPD), preoperative anaemia, peripheral arterial disease, leucocytosis and left ventricular ejection fraction (LVEF) < 30% were also identified as independent predictors of mortality. We performed a test for multicollinearity which showed no collinearity between SPS, Mean eGFR CKD-EPI < 60 ml/min/1.73 m2 or any of the other variables included in the Cox model.

	Univariable analysis		Multivariable analysis	
Variable	P-level	HR (95% CI)	P-level	HR (95% CI)
SPS	<0.001	3.49 (2.94-4.14)	<0.001	1.96 (1.63-2.36)
Mean eGFR <60 ml/min/1.73 m^2	<0.001	3.71 (3.25-4.22)	<0.001	2.82 (2.45-3.24)
Diabetes	<0.001	1.57 (1.37-1.80)	0.118	1.12 (0.97-1.30)
COPD	<0.001	1.79 (1.50-2.12)	<0.001	1.37 (1.14-1.63)
Anemia	<0.001	2.86 (2.41-3.40)	<0.001	1.53 (1.27-1.84)
Peripheral arterial disease	<0.001	2.38 (2.02-2.80)	<0.001	1.58 (1.33-1.87)
LVEF <30%	<0.001	2.43 (1.99-2.96)	<0.001	1.55 (1.26-1.90)
Leucocytosis	<0.001	1.48 (1.29-1.70)	0.026	1.24 (1.08-1.42)
Female sex	<0.001	1.43 (1.24-1.65)	0.070	1.15 (0.99-1.33)
Age, continuous	<0.001	1.08 (1.08-1.09)		
CRP	<0.001	1.01 (1.01-1.01)		
Previous CVI	<0.001	1.84 (1.53-2.21)		
LVEF <50%	<0.001	1.54 (1.34-1.78)		
Mean eGFR, continuous	<0.001	0.97 (0.96-0.97)		
Mean eGFR <30 ml/min/1.73 m^2	<0.001	4.45 (3.58-5.52)		

Table II.2 Cox univariable and multivariable analysis of risk factors for mortality. COPD, chronic obstructive pulmonary disease; CVI, cerebrovascular insult; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; SPS, shrunken pore syndrome.

5.2.3 SPS in different strata of eGFR, sex and diabetes

The presence of SPS affected survival negatively regardless of strata for pre-operative renal function (mean eGFR < 30, 30–60 and > 60 all in ml/min/1.73 m2). Our Cox regression model was employed to test the significance of SPS in the different eGFR strata as well as in each sex and diabetics selectively. The respective Hazard Ratios for SPS with different mean eGFR were 2.74 (95% CI 1.59–4.72), 1.81 (95% CI 1.42–2.31) and 2.16 (95% CI 1.54–3.03), respectively.

	Mean eGFR <30 ml/min/1.73 m2		Mean eGFR 30–60 ml/min/1.73 m^2		Mean eGFR >60 ml/min/1.73 m2	
Variables	P-level	HR (95% CI)	P-level	HR (95% CI)	P-level	HR (95% CI)
SPS	<0.001	2.74 (1.59-4.72)	<0.001	1.81 (1.42-2.31)	<0.001	2.16 (1.54-3.03)
Diabetes	0.429	0.84 (0.54-1.30)	0.650	1.05 (0.85-1.30)	0.085	1.21 (0.97-1.52)
COPD	0.793	0.92 (0.50-1.70)	0.033	1.32 (1.02-1.71)	0.004	1.49 (1.14-1.96)
Anaemia	0.696	1.10 (0.69-1.74)	0.006	1.42 (1.10-1.82)	<0.001	1.97 (1.40-2.77)
Peripheral arterial disease	0.005	1.93 (1.22-3.06)	0.013	1.37 (1.07-1.76)	<0.001	1.74 (1.31-2.29)
LVEF <30%	0.008	2.38 (1.25-4.52)	0.009	1.46 (1.10-1.93)	0.014	1.56 (1.09-2.23)
Leucocytosis	0.371	1.22 (0.79-1.89)	0.026	1.26 (1.03-1.55)	0.180	1.16 (0.93-1.43)
Female sex	0.286	1.26 (0.82-1.94)	0.971	1.00 (0.81-1.25)	0.055	1.25 (0.99-1.58)

Table II.3 Cox multivariable analysis of risk factors for mortality in different eGFR strata. COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; SPS, shrunken pore syndrome.

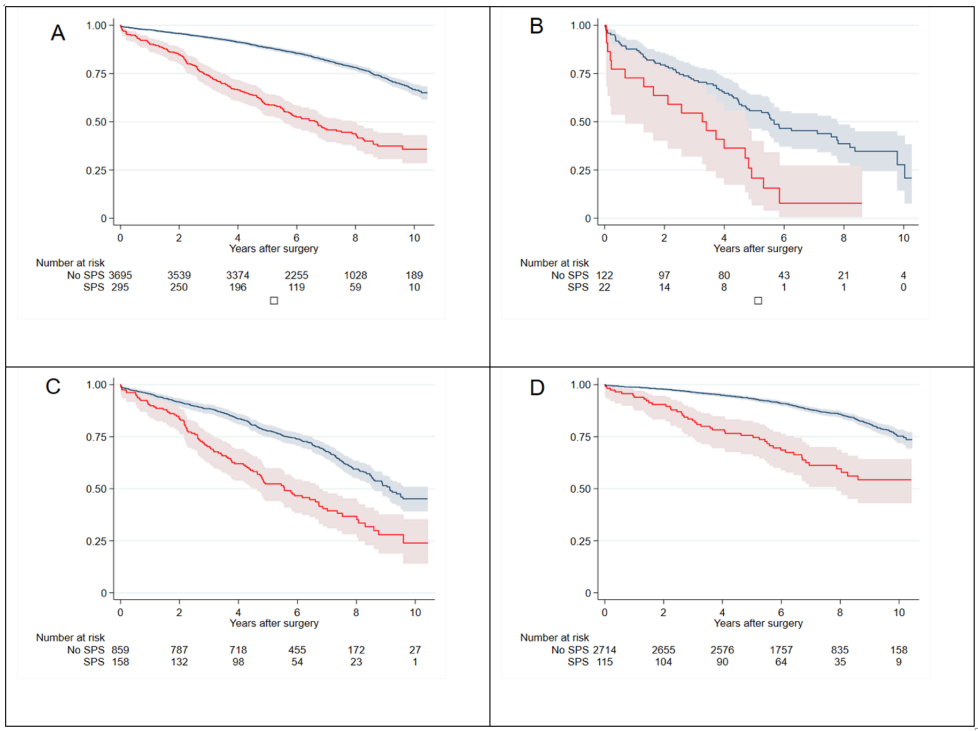

Figure II.2 A: survival after elective cardiac surgery for all patients, irrespective of eGFR, with Shrunken Pore Syndrome (red solid line) or without SPS (blue solid line). B: patients with eGFR < 30 ml/min/1.73 m2. C: patients with eGFR 30–60 ml/min/1.73 m2. D: patients with eGFR > 60 ml/min/1.73 m2. Faded area represents 95% CI. eGFR, estimated glomerular filtration rate; SPS, shrunken pore syndrome.

There was no difference in frequency of SPS between sexes: 67 females (7.2%) were afflicted by SPS and 229 males (7.5%). Our main Cox model was employed with males presenting with a HR of 1.95 (1.58–2.43) and females with a HR of 1.94 (1.36–2.76).

We observed a greater proportion of SPS in diabetics, where 121 of all diabetics (12.2%) had SPS while 175 of all non-diabetic patients (5.8%) had SPS ($p < 0.001$). The difference persisted numerically regardless of strata for pre-operative renal function. Our Cox regression model was employed to test the significance of SPS in diabetics and non-diabetics, respectively. The hazard ratios for SPS in diabetics and non-diabetics were 1.76 (95% CI 1.32–2.35) and 2.09 (95% CI 1.65–2.65), respectively. Of patients with mean eGFR <30 ml/min/1.73 m2, 13 (20%) of those with diabetes presented with SPS while 9 (11.25%) of non-diabetic patients presented with SPS (p=0.14). Of patients with mean eGFR 30-60 ml/min/1.73 m2, 63 (20%) of those with diabetes presented with SPS while 96 (13.62%) of non-diabetic patients presented with SPS (p=0.009). Of patients with mean eGFR >60

ml/min/1.73 m2, 45 (7.36%) of those with diabetes presented with SPS while 70 (3.16%) of non-diabetic patients presented with SPS (p<0.001).

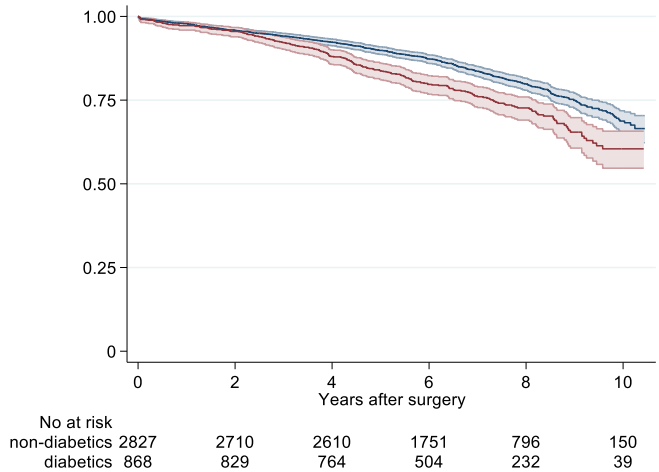

Figure II.3 Survival after elective cardiac surgery for patients with diabetes, red solid line, and without diabetes, blue solid line, and without Shrunken Pore Syndrome. Faded area represents 95% CI. SPS, shrunken pore syndrome.

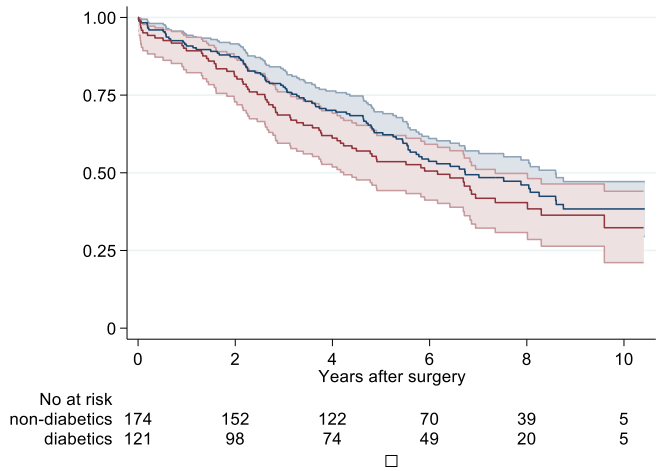

Figure II.4 Survival after elective cardiac surgery for patients with diabetes, red solid line, and without diabetes, blue solid line, with Shrunken Pore Syndrome. Faded area represents 95% CI. SPS, shrunken pore syndrome.

5.2.4 CAPA/LMrev estimates

When GFR estimating equations CAPA and LMrev were employed with the same diagnostic cut-off ratio of eGFR CAPA ≤ 60% of eGFR LMrev, 92 patients were afflicted by SPS, yielding a prevalence of 2.3%. Overall, 1-, 5-, and 10-year survival was 88%, 46%, and 38% respectively for patients with SPS and 97%, 87%, and 78% for patients without SPS. The difference in survival was statistically significant ($p<0.001$ in 1-, 5- and 10-year survival). Our primary Cox model was used which showed that the two risk factors with highest hazard ratios and significance were SPS (HR 1.66 (1.25-2.21) and mean eGFR CAPA LMrev <60 ml/min/1.73 m2 (HR 3.11 (2.70-3.58)).

5.3 Study III

5.3.1 Study population and patient characteristics

Overall, 1-, 5-, and 10-year survival was 94.1%, 85.8%, and 80.6%, respectively. Seventeen (7.5%) patients were found to be afflicted by SPS as measured the day after the index operation (D1) and defined as eGFR CKD-EPI$_{\text{cystatin C}}$ ≤ 60% of eGFR CKD-EPI$_{\text{creatinine}}$. Their eGFR CKD-EPI$_{\text{cystatin C}}$ and eGFR CKD-EPI$_{\text{creatinine}}$ were 32 (±13.0) and 75 (±26.8) mL/min/1.73 m2, respectively. In the group with SPS, we observed a numerically higher mean eGFR, except on the day after transplantation, compared to the non-SPS group.

	SPS	**No SPS**	**P-value (x^2/T-test)**
AKI	9 (53%)	135 (64%)	0.35
CRRT	2 (12%)	52 (25%)	0.23
Mean eGFR day after surgery	53.6 (+-18.7)	54.6 (+-27.0)	0.87
Mean eGFR 1 week postoperatively	48.5 (+-22.4)	45.0 (+-24.7)	0.23
Mean eGFR 2 week postoperatively	47.7 (+-24.4)	40.5 (+-23.5)	0.20
Mean eGFR 3 week postoperatively	43.2 (+-21.8)	42.1 (+-22.1)	0.37
Mean eGFR 4 week postoperatively	44.1 (+-23.0)	43.3 (+-23.0)	0.17

Table III.1 Kidney function and kidney related complications postoperatively. AKI, Acute Kidney Injury (RIFLE); CRRT, Continuous Renal Replacement Therapy; eGFR, estimated Glomerular Filtration Rate (CKD-EPI); SPS, Shrunken Pore Syndrome.

	Overall (n=253)	SPS D1 (n=17)	No SPS D1 (n=211)
Age	50 (+-12.6)	48 (+-11.6)	50 (+-12.6)
Female	65 (26%)	6 (35%)	54 (26%)
Diagnosis			
Dilated Cardiomyopathy	131 (52%)	5 (29%)	112 (53%)
Ischemic Heart Disease	44 (17%)	2 (12%)	40 (19%)
Hypertrophic Cardiomyopathy	21 (8%)	1 (6%)	20 (9%)
Restrictive Cardiomyopathy	10 (4%)	3 (18%)	6 (3%)
ACHD	10 (4%)	2 (12%)	5 (2%)
Other	37 (15%)	4 (24%)	28 (13%)
LVAD	103 (41%)	4 (24%)	89 (42%)
Ischemic Stroke preop	47 (19%)	5 (29%)	37 (18%)
Diabetes Mellitus	32 (13%)	3 (18%)	28 (13%)
Hypertension	36 (14%)	1 (6%)	32 (15%)
COPD	7 (3%)	0 (0%)	6 (3%)
ECMO preop	11 (5%)	4 (36%)	6 (3%)
Preoperative CKD-EPI eGFR creatinine (mL/min1.73m^2)	73.6 (+-26.6)	86.9 (+-26.4)	72.5 (+-26.4)
Waiting list GFR Iohexol (mL/min1.73m^2)	59.3 (+-18.6)	57.4 (+-14.8)	59.3 (+-19.0)
Ischemic time for donor's heart (min)	187 (+-55.3)	187 (+- 47.9)	186 (+-55.7)
Cardiopulmonary bypass time (min)	193 (+-59.0)	178 (+-49.6)	192 (+-59.2)
Recipient aortic cross-clamp time (min)	87.8 (+-37.6)	89.2 (+-50.9)	87.5 (+-36.3)
ECMO postoperative	16 (6.3%)	1 (0.9%)	13 (6.7%)
Ventilator time postop (days)	1.54 (IQR 0.92-2.78)	1.79 (IQR 1.15-2.92)	1.40 (IQR 0.85-2.58)
Reoperation for bleeding	16 (11%)	3 (25%)	11 (9.6%)

Table III.2 Pre- and perioperative variables. Attrition is due to a lack of D1 blood samples. ACHD, Adult Congenital Heart Disease; COPD, Chronic Obstructive Pulmonary Disease; ECMO, Extracorporeal Membrane Oxygenation; eGFR, estimated Glomerular Filtration Rate; GFR, Glomerular Filtration Rate; LVAD, Left Ventricular Assist Device; SPS, Shrunken Pore Syndrome.

5.3.2 SPS at Day 1 as risk factor

The 1- , 5-, and 10-year survival rates for patients with SPS diagnosed D1 after transplantation were 100%, 82%, and 76%, respectively. For patients without SPS, it was 94%, 85%, and 80%, respectively, with no statistically significant difference between the groups. In univariable analysis, the HR for mortality in the SPS group at D1 was 1.00 (95% CI 0.35-2.80), and in multivariable analysis, it was 1.22 (95% CI 0.15-10.0). Ventilator time was the only variable found to be an independent predictor of mortality, with an HR of 1.27 (95% CI 1.22-1.37) in multivariable analysis.

5.3.3 SPS and renal function over time

The proportion of patients with SPS rose markedly over time during hospitalization, from 7.5% the day after surgery to 71% in postoperative week 4. Both $eGFR_{cystatin\ C}$ as well as $eGFR_{creatinine}$ decreased successively from index operation to postoperative

week 4. The $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio of patients diagnosed with SPS at the same time points did not change over time.

	n (%)	1 year survival	5 year survival	10 year survival
SPS day 1 after surgery	17 (7.5%)	17 (100%)	14 (82%)	13 (76%)
SPS week 1 postoperatively	151 (69%)	145 (96%)	128 (85%)	118 (78%)
SPS week 2 postoperatively	115 (53%)	109 (95%)	96 (83%)	90 (78%)
SPS week 3 postoperatively	89 (63%)	81 (91%)	68 (76%)	62 (70%)
SPS week 4 postoperatively	71 (71%)	65 (92%)	52 (73%) *	45 (63%) *
No SPS day 1 after surgery	211 (93%)	197 (93%)	180 (85%)	211 (80%)
No SPS week 1 postoperatively	68 (31%)	62 (91%)	60 (88%)	57 (84%)
No SPS week 2 postoperatively	100 (47%)	95 (95%)	88 (88%)	83 (83%)
No SPS week 3 postoperatively	52 (37%)	50 (96%)	46 (88%)	43 (83%)
No SPS week 4 postoperatively	29 (29%)	28 (97%)	27 (93%) *	26 (90%) *

Table III.3 1-, 5-, and 10-year survival for patients diagnosed with SPS at different time points duringindex hospitalization. * depicts significant difference (x2-test). SPS, Shrunken Pore Syndrome.

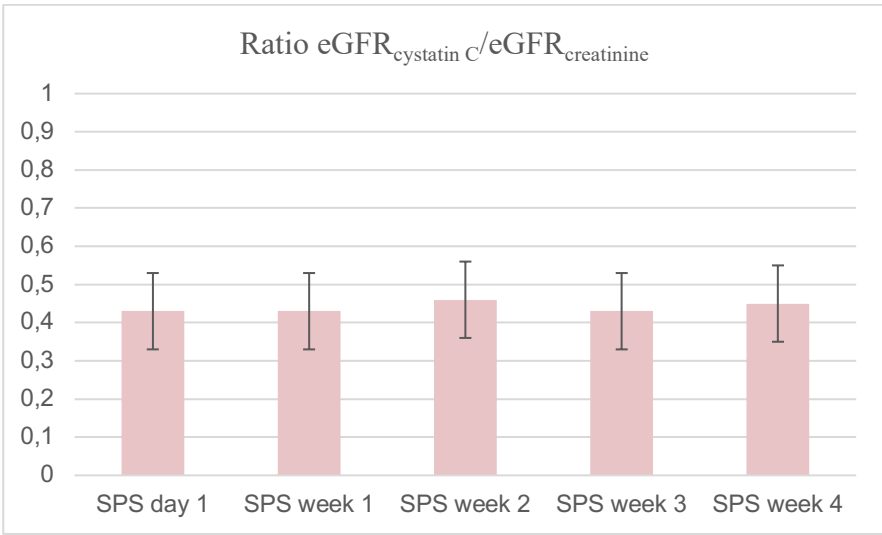

Figure III.1 Ratio of $eGFR_{cystatin\ C}/eGFR_{creatinine}$ at different time points postoperatively. Error bars depict standard deviation. eGFR, estimated Glomerular Filtration Rate.

We did observe a decreased and increasingly divergent survival for patients afflicted by SPS at later time points during hospitalization. Statistical significance was found in 5- and 10-year survival for patients diagnosed with SPS postoperative week 4 with a survival of 73% and 63%, respectively, for patients with SPS, and 93% and 90% for patients without SPS ($p=0.02$ for 5-year survival and $p=0.005$ for 10-year survival).

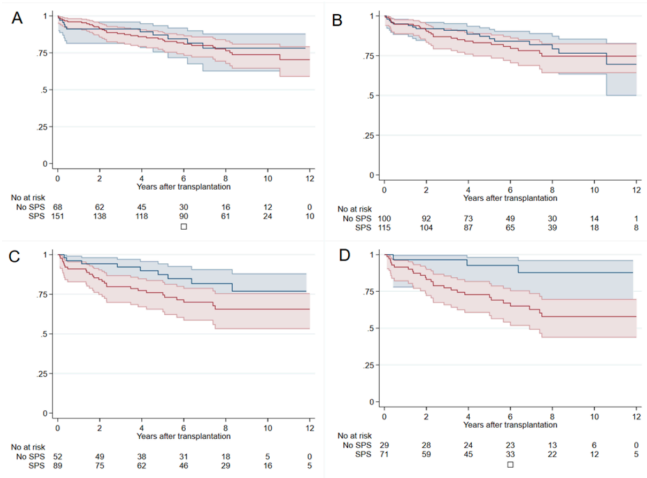

Figure III.2 Kaplan Meier estimates of survival after heart transplantation. The red line depicts patients with SPS, the blue line patients without SPS. Faded area represents 95% CI. A: SPS diagnosed 1 week postoperatively. B: SPS diagnosed 2 weeks postoperatively. C: SPS diagnosed 3 weeks postoperatively. D: SPS diagnosed 4 weeks postoperatively. SPS, Shrunken Pore Syndrome.

In our univariable analysis, SPS at week 4 was significantly associated with mortality, with an HR of 4.38 (95% CI 1.32-14.5). In multivariable analysis, the HR for SPS at week 4 was 4.65 (95% CI 1.36-15.8).

Pre- peri- postoperative variables	Univariable analysis		Multivariable analysis with SPS at week 4	
	P-level	HR (95% CI)	P-level	HR (95% CI)
SPS at day 1	1.00	1.00 (0.36-2.80)		
SPS at week 1	0.58	1.21 (0.61-2.40)		
SPS at week 2	0.63	1.16 (0.63-2.13)		
SPS at week 3	0.09	1.92 (0.90-4.09)		
SPS at week 4	0.01	4.38 (1.32-14.5)	0.01	4.65 (1.36-15.8)
COPD	0.99	0.00 (0.00-0.00)		
DM	0.21	1.59 (0.77-3.28)		
CVD	0.45	1.30 (0.66-2.53)		
LVAD	0.24	1.40 (0.80-2.43)		
Hypertension	0.44	1.33 (0.64-2.73)		
Preop ECMO	0.52	0.52 (0.07-3.78)		
Preop eGFR CKD-EPI <60	0.06	1.72 (0.99-2.99	0.08	2.09 (0.92-4.72)
eGFR CKD-EPI continuous	0.13	0.99 (0.98-1.00)		
GFR Iohexol continuous	0.26	0.99 (0.97-1.01)		
Haemoglobin continuous	0.62	1.00 (0.99-1.02)		
Albumin continuous	0.60	0.98 (0.91-1.05)		
CRP continuous	0.22	0.99 (0.97-1.01)		
Dilated Cardiomyopathy	0.53	0.84 (0.48-1.46)		
Ischemic Heart Disease	0.35	1.38 (0.71-2.69)		
Hypertrophic Cardiomyopathy	0.53	1.35 (0.53-3.40		
Restrictive Cardiomyopathy	0.51	1.62 (0.39-6.75)		
ACHD	0.95	1.05 (0.26-4.32)		
Other	0.36	0.65 (0.26-1.63)		
Donor Heart Ischemic time	0.86	1.00 (0.99-1.00)		
CPB time	0.38	1.00 (1.00-1.01)		
Cross clamp time	0.66	1.00 (0.99-1.01)		
AKI	0.07	1.76 (0.95-3.27)	0.02	3.70 (1.24-11.1)
CRRT	0.005	2.30 (1.28-4.12)	0.98	1.01 (0.39-2.65)
Ventilator time continuous (days)	0.001	1.09 (1.05-1.13)	0.33	1.00 (0.99-1.00)
Packed red blood cell concentrate (units)	0.001	1.14 (1.06-1.21)	0.82	1.02 (0.89-1.16)

Table III.4 Cox uni- and multi-variable analysis for mortality. ACHD, Adult Congenital Heart Disease; AKI, Acute Kidney Injury (RIFLE classification); COPD, Chronic Obstructive Pulmonary Disease; CPB, Cardiopulmonary Bypass; CRRT, Continuous Renal Replacement Therapy; CRP, C Reactive Protein; ECMO, Extracorporeal Membrane Oxygenation; eGFR, estimated Glomerular Filtration Rate; GFR, Glomerular Filtration Rate; LVAD, Left Ventricular Assist Device; SPS, Shrunken Pore Syndrome.

5.3.4 Alternative equations LMR18 and CAPA

Twelve (5.3%) patients were found to be afflicted with SPS as measured the day after index operation and defined as eGFR CAPA $\leq$ 60% of eGFR LMR18. The overall 1-, 5-, and 10-year survival for patients with SPS diagnosed day 1 after transplantation was 100%, 75%, and 67% respectively. For patients without SPS it was 94%, 86%, and 81%, respectively. There was no statistically significant difference between the groups.

As in the CKD-EPI group, the proportion of SPS rose over time, from 5.2% to 58% week 1, 43% week 2, 57% week 3, and 60% week 4. We observed lower survival

for those patients diagnosed with SPS later during their hospitalization with 1-, 5-, and 10-year survival of 95%, 84%, and 77%, respectively, for those diagnosed after 1 week. For those who were diagnosed after 2 weeks, survival was 93%, 82%, and 77%, respectively; for those diagnosed after 3 weeks, survival was 91%, 75%, and 72%, respectively; and for those diagnosed after 4 weeks, survival was 90%, 72%, and 63%, respectively. The 5- and 10-year survival for patients diagnosed week 4 with SPS was significantly lower ($p<0.03$ and 0.02 respectively) than those without SPS, largely mirroring our results with CKD-EPI.

5.4 Study IV

5.4.1 Study population and patient characteristics

We included a total of 170 patients with complete sampling of peripheral venous blood and arterial blood was done on the day of the procedure. In 22 of these patients, we performed sampling on different venous locations.

Variable	All (170)	Multiple sampling (22)
Age (years)	80,9 (±8,9)	80,9 (±7,8)
Female	70 (41,7%)	7 (31,8%)
Length (cm)	170,4 (±9,7)	171,6 (±8,7)
Weight (kg)	77,9 (±15,5)	79,6 (±15,9)
BMI	26,8 (±4,5)	27,0 (±4.9)
Hypertension	110 (67,5%)	15 (71,4%)
Smoker	5 (3,2%)	1 (4,8%)
COPD	13 (8,1%)	1 (4,8%)
Atrial fibrillation	49 (30,2%)	6 (28,6%)
Peripheral vascular disease	9 (5,6%)	0 (0,0%)
Previous stroke	13 (8,0%)	1 (4,8%)
Diabetes	41 (24,8%)	6 (30%)
Previous cardiac surgery	20 (12,4%)	2 (9,5%)
Aortic mean gradient	44,5 (±11,8)	45,7 (±13,8)

Table IV.1 Variables presented whole as well as grouped for multiple sampling. Described as mean (±SD) or number (%). BMI, body mass index; COPD, chronic obstructive pulmonary disease.

5.4.2 Variations in serum creatinine

We observed a mean level of 87,4 ± 43,8 µmol/l of creatinine in arterial blood. A higher level of serum creatinine in femoral blood as compared to arterial blood with a difference of +9,4% (±6%) ($p<0.001$) was noted. More notable, creatinine level in both peripheral blood and jugular venous blood was significantly higher than arterial blood ($p<0.001$ for peripheral and $p<0.04$ for jugular).

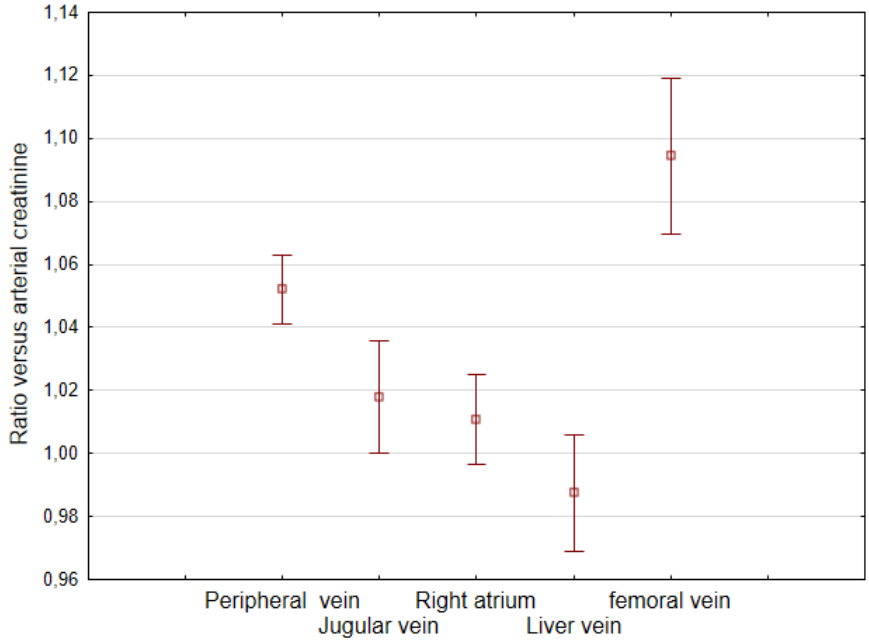

Figure IV.2 Ratio versus arterial creatinine in sampling of different locales. Error bars depict 95% CI.

5.4.3 Variations in serum cystatin C

We observed a mean level of 1,6 ± 0,5 µmol/l of cystatin C in arterial blood. A small but significant higher level of serum cystatin C was seen in jugular (1,03 (1,00-1,04)), atrial (1,04 (1,01-1,07)) and femoral (1,03 (1,00-1,06)) blood compared to arterial ($p<0,015$ for all). Notably, cystatin C level in peripheral blood was significantly higher than arterial blood (1,08 ((1,02-1,15)) and $p<0,001$).

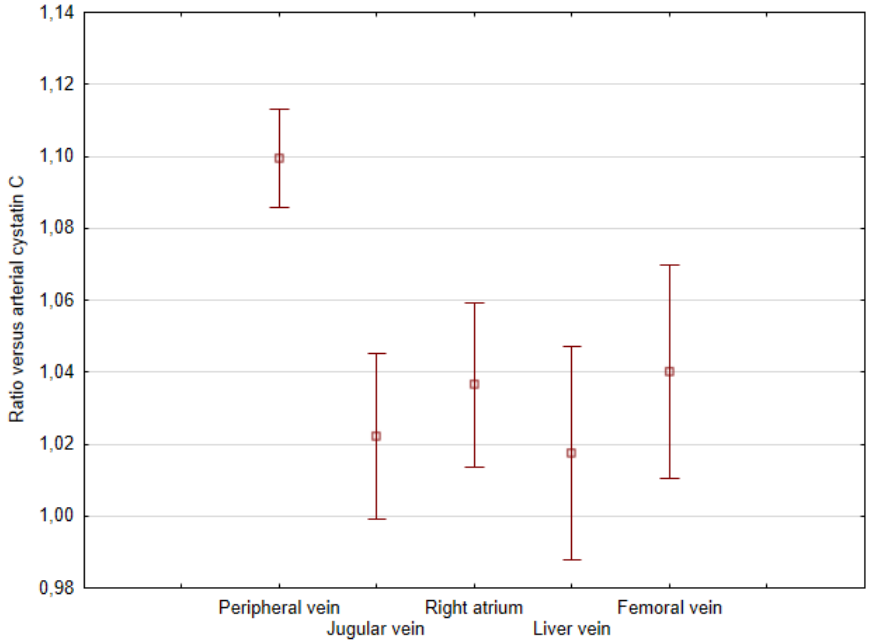

Figure IV.3 Ratio versus arterial cystatin C in sampling of different locales. Error bars depict 95% CI.

6 Discussion

Shrunken Pore Syndrome is a newly discovered condition of selective glomerular hypofiltration that increases mortality and morbidity. The understanding of this condition, and indeed all selective glomerular hypofiltration conditions are emerging through the diligent effort of colleagues worldwide and not least the conceiver of SPS – Prof. Anders Grubb. Our present studies are but a small part of that work.

In **study I** we evaluated the effect on mortality different levels of the $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio to gain a better understanding of SPS. We also set out to find an optimal cut-off value for SPS. We found that a progressive decrease in the $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio results in a substantial and progressive increase in mortality.

We also set out to find an optimal cut-off value for SPS. However, varying clinical situations require different cut-off levels. One commonly used rule is that the cut-off value for clinical decision-making should represent the Youden index of ROC curves, i.e., the value associated with the highest combined sensitivity and specificity of the test. However, in many clinical situations, the cut-off value requires much higher specificity to limit the risk of treating patients without the disorder. Both these requirements were employed to find suitable cut-off values for diagnosing SPS using the two pairs of cystatin C- and creatinine-based GFR-estimating equations. The cut-off values, whether based upon Youden index or to generate a high specificity of 95%, varied between the two pairs of GFR-estimating equations. Youden index yielded a cut-off of 0.74 for the equation pair $CKD\text{-}EPI_{cystatin\ C}/CKD\text{-}EPI_{creatinine}$ and 0.85 for the equation pair CAPA/LMrev in this cohort while the high specificity cut-off yielded a cut-off of 0.57 for the equation pair $CKD\text{-}EPI_{cystatin\ C}/CKD\text{-}EPI_{creatinine}$ and 0.69 for the equation pair CAPA/LMrev. The high specificity cut-off for the CKD-EPI equations was notably close to the 0.6 inferred in the beginning (15). The ratios generated by the two equation pairs differed and the main reason that $CKD\text{-}EPI_{creatinine}$ produced values about 15% higher than the mean estimated GFR-values of the other three GFR-estimating equations, CAPA, LMrev and $CKD\text{-}EPI_{cystatin\ C}$. A similar relative overestimation by the $CKD\text{-}EPI_{creatinine}$-equation has been noted in previous investigations of Scandinavian populations (106).

In **study II** we observed a markedly increased mortality rate for patients with SPS from the first year of follow-up to 10 years postoperatively. Further, we demonstrated that SPS independently predicts a decreased survival rate, with a magnitude like well-known risk factors such as diabetes, COPD, renal dysfunction and decreased left ventricular function (131-134). In addition to our main finding, our results seem to hold regardless of mean eGFR. The HR for SPS as a predictor of mortality ranged from 1.8 to 2.7 in the different eGFR strata, suggesting that SPS is not a marker for decreased GFR and chronic kidney disease but is a condition of its own. In patients with normal eGFR, the HR for patients with SPS was markedly increased, proposing that SPS is a strong, independent predictor for mortality after elective cardiac surgery.

We could show as a novel finding that SPS occurs significantly more frequently in diabetic patients. In our Cox model without diabetes, SPS had a HR of 2.09 (95% CI 1.65–2.65). When we added diabetes to the model, the HR for SPS was 1.76 (95% CI 1.32–2.35). More surprisingly, in patients with SPS, the survival for those with diabetes did not differ significantly from patients without diabetes: it was equally poor for both groups. The pathologic mechanism of SPS and its relation to diabetic glomerulopathy is unclear. Still, it is possible that the thickening of the glomerular basement membrane in diabetes is related to the development of SPS. This could explain why SPS occurs more frequently in diabetic patients and be a possible shared pathway for both SPS and diabetes for mortality (30).

Patients afflicted by SPS were significantly older, often had other classical risk factors and had longer cross-clamp and bypass times, all of which may serve as surrogate markers for intraoperative complexity. We could not detect a significant difference between the sexes, as both male and female patients seem to be afflicted in essentially the same ratios and with similar HR. The global burden of chronic kidney disease is substantially higher in women, further underscoring that SPS may have another pathophysiologic background than other types of chronic kidney disorders. The alternative cystatin C- and creatinine based GFR-estimating equations CAPA and LMrev showed similar results as when the CKD-EPI estimating equations were used.

Since 2015, when SPS was first postulated, patients with SPS regardless of population have shown increased mortality and/or morbidity (21-23, 25, 107, 135-141) and as these reports demonstrate, SPS seems to be whenever a material is analyzed, it is reasonable to assume that SPS is far more widespread than has been documented. Indeed, in **study III** we could for the first time report the prevalence of SPS in a new patient population - the postoperative period following heart transplantation.

We found that patients who developed SPS later during hospitalization, i.e. more time since the patient's transplantation, exhibited lower 5- and 10-year survival compared to patients without SPS. SPS was also found to be an independent risk

factor for death with an HR of 4.65 in multivariable analysis. Our finding that SPS lowers long-term survival may have a few possible explanations. The postoperative period for a heart-transplanted patient is critical for the patients and their physiology as they adapt to the new organ. It is possible that during this time, a latent SPS is unmasked, and when comparing these patients with those who have healthy kidneys and did not develop SPS, we see a lower survival. Further, it can be argued that the lack of increased mortality in the group diagnosed early with SPS may be that their kidney affliction was pre-renal and therefore improved with a heart transplantation. Evidence against this would be that all patients diagnosed early with SPS exhibited SPS at discharge from the hospital (results not shown), i.e., they were not cured of SPS.

In addition, we observed that SPS did not correlate with increasing use of Continuous Renal Replacement Therapy (CRRT) or development of AKI, supporting our assumption that SPS constitutes a novel pathophysiologic pathway separate from other renal diseases. Further, we observed that none of our pre-, peri, and postoperative variables influenced mortality, except for time on ventilator postoperatively, which is typically a combined surrogate marker for a poor preoperative state and/or complications during surgery. This might speak to the robustness of heart transplantation in the current era, where we have mitigated the earlier identified risk factors.

Another surprising finding is the high proportion of our cohort developing SPS postoperatively. Earlier studies of SPS report that it afflicts 6-8% of the population, which is consistent with the findings in our cohort the first day after surgery. This rose to above 70% at its peak four weeks after transplantation. However, administering cortisone, a standard treatment after heart transplantation, increases Cystatin C, leading to a false decrease of GFR estimated as $eGFR_{cystatin\ C}$ (9). We did not observe any significant change (results not shown) in the ratio of $eGFR_{cystatin\ C}/eGFR_{creatinine}$ in patients who exhibited SPS at day 1 after surgery compared to the same patient's postoperative week 4, which one would expect to see in that case. Earlier reports have shown that Cystatin C is a reliable marker for eGFR in renal transplant patients where most of the reported population is treated with steroids (142) and that glucocorticoids did not affect the performance of Cystatin C in identifying AKI (143). We assume, therefore, that the cortisone administration is not a source of error in estimating GFR based on Cystatin C. Cortisone is not the only drug that influences eGFR in the early postoperative period after heart transplantation; the nephrotoxicity of tacrolimus is well known (144, 145). In our material, the prevalence of SPS rises with time during the postoperative course, as does the concentration of tacrolimus. The nephrotoxicity of tacrolimus is mediated through the vasoconstriction of afferent and efferent arterioles (146). A possible explanation is that the larger pores that shrink are more susceptible to renal hypoperfusion.

In **study IV** we switched focus and performed a sub study under the RECITA umbrella to investigate the importance of blood sampling location for creatinine and cystatin C levels. We observed that the creatinine level was, as expected due to the excretion of creatinine from muscles, higher in femoral and peripheral blood. However, we observed a small but significant elevation of creatinine in jugular venous blood. The brain is a metabolically voracious organ that expresses its own creatine kinase (BB-CK) for conversion of creatine to creatinine that may explain the slight increase of creatinine in jugular blood (147, 148). More surprisingly, we observed a high ratio of cystatin C in peripheral blood compared to arterial. This defies our understanding of the life of cystatin C but may be due to stasis and leakage of cystatin C from damaged cells when the blood was collected (personal communication with Prof. A. Grubb). We observed slight variation in the levels of cystatin C, although we saw a small but significant increase in atrial, jugular, and femoral blood compared to arterial blood.

6.1 Study limitations

6.1.1 Study I

Due to its nature of being a retrospective cohort study, it exhibits all inherent limitations in this type of study – information bias, selection bias, unmeasured confounders, among others. We also have a limited long-term follow-up, although a strength is the reliability of the follow-up. This study is based on a dataset of a large and homogenous cohort, the results do not give any indication of how generalisable the findings are. A heterogeneous population of simply a different population could yield different results. The study may also be limited using equations estimating GFR instead of measuring GFR by injecting substances only excreted by glomerular filtration, such as Cr-EDTA, Inulin, or Iohexol.

6.1.2 Study II

The current retrospective study has both inherent strengths and weaknesses, and its limits are those of other retrospective cohorts, as described above. Among its strengths is that it is a large, well-defined population from a single centre, yielding data with high granularity. In addition, this population has both a high incidence of SPS and a high yearly risk for death, resulting in strong material from a statistical standpoint. Another advantage is the extended and reliable follow-up. A homogenous patient population, however, also limits this study, as a larger and more heterogeneous population may yield different results.

6.1.3 Study III

This retrospective cohort study has both strengths and weaknesses with some weaknesses detailed above in 6.1.1. The single-centre design may affect the generalizability of findings but provides a well-defined population that is thoroughly examined with a long and consistent follow-up.

6.1.4 Study IV

As a prospective observational study, it has some inherent weaknesses. There is risk for confounding and concern for losing subjects during follow up. The main advantage is its nullification of selection bias versus retrospective cohorts. We also have a well-defined population that is thoroughly examined, adding to its strengths.

7 Conclusions

7.1 Study I

We found that short- and midterm mortality increases markedly with a decrease in the $eGFR_{cystatin\ C}/eGFR_{creatinine}$-ratio. Also, the mathematically optimal cut-off for predicting short- and mid-term mortality with SPS was 0.74 using the equation pair $CKD\text{-}EPI_{cystatin\ C}/CKD\text{-}EPI_{creatinine}$ and 0.85 for the equation pair CAPA/LM-rev. More clinically valuable may be a high specificity (95%) cut-off, which corresponds to a cut-off of 0.57 using the equation pair $CKD\text{-}EPI_{cystatin\ C}/CKD\text{-}EPI_{creatinine}$ and 0.69 for the equation pair CAPA/LM-rev.

7.2 Study II

We observed a substantial and statistically significant increased mortality for patients with SPS from the first year of follow-up to 10 years postoperatively. Further, we demonstrated that SPS independently predicts a decreased survival rate, with a magnitude like well-known risk factors such as diabetes, COPD, renal dysfunction and decreased left ventricular function.

7.3 Study III

In this study we showed that a large proportion of heart-transplanted patients develop SPS shortly after their heart transplantation, and that patients developing SPS have a worse prognosis compared to patients who do not.

7.4 Study IV

We found that the level of creatinine and to some degree cystatin C is affected by blood sampling location, highlighting the importance of sampling location.

8 Future perspectives

It is reasonable to hypothesize that SPS exists in a multitude of populations that has not been studied yet. One that it has been found in but that no reliable survival data has been reported in are the children. An observational study with prospective data collection and very long follow up in a paediatric setting would enhance our understanding of the disease, when it originates and if children with SPS have a higher morbidity and mortality.

The pathophysiology of SPS needs to be elucidated and some promising studies has been conducted to try just that. A step forward would be to perform kidney biopsies on patients with SPS, measure the glomerular basement membrane thickness, and compare that to normal kidneys. SPS and its influence on the plasma proteome is a developing field where it's been found that patients with SPS accumulate plasma proteins in the 5-30 kDa range, where a few have been linked to atherosclerosis. A more detailed map of the accumulated plasma proteins and their effect in patients with SPS compared to patients without would be desirable.

New compounds that lower the levels of the accumulated proteins that might be responsible for mortality and/or morbidity in SPS could be a future treatment option.

9 Acknowledgements

I would like to express my heartfelt gratitude to everyone who has supported me in my studies and the completion of this thesis. In particular, I want to thank:

Associate Professor Henrik Bjursten, my senior supervisor. I am deeply grateful for your enthusiasm, support, and patience with me. Without you, none of this would have happened! Your sharp and pragmatic scientific approach serves as a role model for us all.

Professor Anders Grubb, my co-supervisor, is not only the father of SPS but also of cystatin C. Your enthusiasm for teaching and deep knowledge of all the topics I have explored in this thesis never cease to amaze me.

Associate Professor Shahab Nozohoor, my co-supervisor and dear friend. We´ve been on quite a few adventures together, and no matter the circumstances or how far removed from our regular jobs we are, you never hesitate to bring up my thesis and do your best to push me forward. Does that goading end now?

Associate Professor Per Ederoth, my co-supervisor. I am grateful to have had access to your knowledge of all things regarding AKI, intensive care and heart transplantation during my work on this thesis.

I thank all my coauthors, Alain Dardashti, MD, PhD, Associate Professor Igor Zindovic, MD, Emilie Mörtsell, MD, David Grubb, MD, PhD, Johann Sigurjonsson, MD, and Benedicte Viterius, RN, for their valuable contributions and support.

I express my sincere gratitude to Associate Professor Per-Ola Kimblad, the former head of the Department of Cardiothoracic Surgery and current Deputy CEO for Skåne Universitetssjukhus, for hiring me as a resident and supporting my academic efforts. I'd also like to express my heartfelt gratitude to my friend Associate Professor Kiet Tran, the current chief of surgery at the Pediatric Cardiac Surgery section, for believing in me, giving me a chance in pediatric cardiac surgery, and emphasizing the value of research in our clinical work.

I want to thank my friends and colleagues Edin Omerbasić, Shahriar Anvariazar and Jens Johansson Ramgren at the Pediatric Cardiac Surgery section for supporting me and teaching me the art of congenital cardiac surgery.

In the Cardiothoracic Surgery section, I'd like to thank my colleagues and friends, starting with the current chief, Associate Professor Johan Sjögren. We are a

generation of cardiac surgeons who did our first CABG with your assistance – and since then, we have followed you around like your ducklings. Per Wierup, your career and surgical advice are widely known to work in 99 cases out of 99; I'm deeply thankful for all your teachings. Mårten Larsson, many are the hours we have discussed all things clinical, and if I ever want support in buying a new expensive and unnecessary gadget, I can rely on you. Igor Zindovic, it seems like it was not that long ago you handed me the red on-call phone on my first day, but evidently, it's been 11 years – and I still ask you questions from time to time. Jacob Ede, you never stop to amaze or inspire me with how hard-working you are, thank you for being my friend.

Alain Dardashti, my dear friend, you've taught me everything about SPS, pool robots, and cardiac anesthesia. I'm honored to be your friend.

My friends Totte Stankovich, Petter Halling, Oskar Lagerwall, and Viggo Lagerwall, I would like to thank you all for still being there! Despite the colossal physical distance between us, we still manage to meet each other from time to time, and I always look forward to it immensely.

To my mother, Kerstin, my father, Bo, and my stepfather, Anders. Without you, none of this would be possible. You've all been instrumental in different ways in shaping me into the person I am today. I'm so very proud to be your son.

Ylva and Martin. I have always looked up to you as role models for how to conduct myself and live my life; I'm profoundly fortunate to have you as my older siblings.

Hanna, my best friend and the love of my life, we met for the first time over a faulty Swan-Ganz catheter. Since then, I look forward to every minute I can spend with you, regardless of the status of the Swan-Ganz catheters. Like so many other things in my life, this would not be possible without your support.

Theo, Harry, Frank. It's all for you.

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