Acute kidney injury after cardiac surgery and heart transplantation

Monitoring, prevention and treatment

Maria Tholén

Department of Anaesthesiology and Intensive Care
Institute of Clinical Sciences at
Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG

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maria.tholen@gu.se

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Department of Anaesthesiology and intensive care,
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Sahlgrenska Academy, University of Gothenburg
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ABSTRACT

Background: Acute kidney injury (AKI) is a common and serious complication after cardiac surgery and heart transplantation, leading to increased morbidity and mortality. To date, there are neither proven clinical real-time kidney monitoring techniques, nor effective preventive measures or treatments of AKI for these patients.

Aims and methods: This thesis evaluated whether the non-invasive technique, near-infrared spectroscopy (NIRS), can accurately assess renal tissue oxygenation in patients during cardiac surgery. Renal vein oxygen saturation ($SrvO_2$) was compared to renal tissue oxygenation (rSO_2) by NIRS. Furthermore, the effects of the inodilator, levosimendan (0.1 µg/kg/min, n=16), on renal blood flow (RBF) and glomerular filtration rate (GFR) were compared to placebo (n=13) in patients with AKI post cardiac surgery. In addition, the renoprotective effect of the atrial natriuretic peptide (ANP) was studied in patients undergoing heart transplantation. Seventy patients undergoing heart transplantation were randomized to receive either ANP (50 ng/kg/min) (n=33) or placebo (n=37) starting after induction of anaesthesia and continued for 4 days after heart transplantation. The primary end-point was measured GFR (mGFR) at day 4, assessed by plasma clearance of ^{51}Cr-EDTA. Finally, the correlation, accuracy and agreement between estimated GFR (eGFR) and measured GFR (mGFR) were tested after heart transplantation.

Results: Renal rSO_2, as assessed by NIRS, was correlated to (r=0.61. p<0.001), and in agreement with invasively measured $SrvO_2$ with an acceptable error of 17.6%. In hemodynamically stable patients with AKI after cardiac surgery, levosimendan increased RBF (p=0.011), but had little or no effect on GFR (p=0.079). During ongoing ANP infusion, median (IQR) mGFR at day 4 postoperatively was 60.0 (57.0) and 50.1 (36.3) mL/min/1.73 m^2 (p=0.705) and the need for dialysis was 21.6% and 9.1% (p=0.197) for the placebo and ANP groups, respectively. The incidences of AKI for the placebo and the ANP groups were 76.5% and 63.6%, respectively (p=0.616). The accuracy of eGFR to assess mGFR was 51%. The bias was 11.2 ± 17.4 mL/min/1.73 m^2, indicating that eGFR underestimated renal function (mGFR). The limits of agreement were -23.0 to 45.4 mL/min/1.73 m^2 and the error 58%. The concordance rate between eGFR and mGFR was 72%.

Conclusions: There is a good correlation and agreement between non-invasively measured renal tissue oxygenation and invasively measured renal vein oxygen saturation during cardiac surgery. In post cardiac surgery AKI, levosimendan induces a vasodilation of both afferent and efferent arterioles increasing renal blood flow with little or no effect on renal function. Prophylactic infusion of ANP during and after heart transplantation does not seem to attenuate postoperative renal dysfunction or decrease the incidence of AKI. eGFR underestimated mGFR and the agreement between eGFR and mGFR was poor. Furthermore, the ability of eGFR to assess changes in mGFR, postoperatively, was low. Thus, eGFR is not a good enough marker to assess renal function after heart transplantation.

Keywords: Cardiac surgery, heart transplantation, acute kidney injury, near-infrared spectroscopy, levosimendan, atrial natriuretic peptide, glomerular filtration rate.

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SAMMANFATTNING PÅ SVENSKA

Akut njursvikt är en vanlig och allvarlig komplikation efter hjärtkirurgi och hjärttransplantation och innebär en avsevärt ökad morbiditets- och mortalitetsrisk. Vad den akuta njursvikten beror på är inte helt klarlagd och är troligen multifaktoriellt betingad. Nedsatt genomblödning och syresättning av njurarna under operationen anses vara en av de viktigaste orsakerna bakom den akuta postoperativa njursvikten. Under operationer där hjärt-lung-maskin vanligen används, uppstår en dålig syresättning till njurarna pga. utspädning av blodet tillsammans med en sammandragning av njurarnas blodkärl. Postoperativt kan njursvikten ytterligare förvärras på grund av instabil cirkulation och administration av njurtoxiska droger så som immunhämmande läkemedel. Det finns idag inga enkla och vedertagna metoder att mäta njurens syresättning under och efter hjärtkirurgi och hjärttransplantation och inte heller några bra sätt att förebygga njursvikten eller behandla den när den uppkommit.

Nära infraröd spektroskopi (NIRS) är en icke-invasiv teknik som används för att följa syresättning i mänsklig vävnad. I denna avhandling har vi kunnat konstatera att NIRS, placerat över njuren, på ett adekvat sätt kan följa syresättningen i njuren under hjärtkirurgi.

Levosimendan är ett hjärtstärkande och blodkärls-vidgande läkemedel. I denna avhandling har det visats att levosimendan motverkar kärlsammandragningen i njurarna och därmed ökar njurens genomblödning hos patienter med akut njursvikt efter hjärtkirurgi. Dock sågs ingen statistiskt signifikant ökning i njurens förmåga att rena blodet (GFR).

Förmakspeptiden ANP har i tidigare studier visats förbättra njurfunktionen och minska dialysfrekvensen hos patienter med akut njursvikt efter hjärtkirurgi. I ett av delarbetena studerades effekten av profylaktisk intravenös administration av ANP till patienter under och efter hjärttransplantation. ANP visade sig i vår studie inte ha någon statistiskt signifikant effekt på varken njurfunktion eller förekomst av akut njursvikt i jämförelse med placebo.

Ett sätt att mäta njurens funktion är att mäta njurens glomerulära filtrationshastighet (mGFR). Detta är dock tidsödande och krångligt. Estimerat GFR (eGFR) används därför vanligen som rutin. Vi har i denna avhandling jämfört eGFR mot mGFR hos patienter som nyligen genomgått hjärttransplantation. Vår slutsats är att eGFR inte är en tillräckligt bra markör för att följa njurfunktionen hos dessa utmanande patienter med hög förekomst av akut njursvikt.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Tholén M, Ricksten S-E, Lannemyr L

Renal near-infrared spectroscopy for assessment of renal oxygenation in adults undergoing cardiac surgery

Journal of cardiothoracic and vascular anesthesia, 2020:34(12): 3300-3305

II. Tholén M, Ricksten S-E, Lannemyr L

Effects of levosimendan on renal blood flow and glomerular filtration in patients with acute kidney injury after cardiac surgery: a double blind, randomized placebo-controlled study

Critical care 2021:25:207

III. Tholén M, Kolsrud O, Dellgren G, Karason K, Lannemyr L, Ekelund J, Ricksten S-E

Atrial natriuretic peptide (ANP) in the prevention of postoperative acute renal dysfunction and acute kidney injury in patients undergoing heart transplantation - a single-center randomized placebo-controlled blinded trial

Manuscript

IV. Tholén M, Lannemyr L, Ricksten S-E

The correlation, accuracy and agreement between estimated (eGFR) and measured glomerular filtration rate (mGFR) and the ability of eGFR to track changes in mGFR early after heart transplantation

Manuscript

CONTENT

ABBREVIATIONS

AKI	Acute Kidney Injury
BMI	Body Mass Index
BSA	Body Surface Area
CABG	Coronary Artery Bypass Grafting
CKD	Chronic Kidney Disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CPB	Cardiopulmonary Bypass
CRRT	Continuous Renal Replacement Therapy
ECC	Extra Corporeal Circulation
ECMO	Extra Corporeal Membrane Oxygenation
eGFR	Estimated Glomerular Filtration Rate
GFR	Glomerular Filtration Rate
GUCH	Grown Up with Congenital Heart Disorder
ANP	Atrial Natriuretic Peptide
Htx	Heart transplantation
ICU	Intensive Care Unit
KDIGO	Kidney Disease: Improving Global Outcome
LVEF	Left Ventricle Ejection Fraction
mGFR	Measured Glomerular Filtration Rate
NIRS	Near Infrared Spectroscopy

PAH	Para-Amino Hippuric Acid
RIFLE	Risk, Injury, Failure, Loss and End stage kidney disease
RRT	Renal Replacement Therapy
rSO2	Regional Tissue Oxygenation
ScvO2	Central Venous Oxygen Saturation
SrvO2	Renal Vein Oxygen Saturation
SvO2	Mixed Venous Oxygen Saturation

DEFINITIONS IN BRIEF

Declaration of Helsinki	Regarding Clinical trials: International agreement meant to set a standard for the balance between the interests of humanity and the individual patient who is part of a clinical trial.

1 INTRODUCTION

The human body has two kidneys, located in the retro-peritoneal space, each with approximately a weight of 160 g and dimensions of 10 x 5 x 5 cm.

The role of the kidneys is to excrete toxins and waste products from the body. The kidneys are also involved in arterial blood pressure control, as well as the regulation of the salt and fluid levels and the acid-base balance. Furthermore, the kidneys produce erythropoietin and renin and also activate vitamin D.

Around 20% of the cardiac output is distributed to the kidney. In an adult with a cardiac output of approximately 5 L/min, this means that approximately 1 L of blood/min passes thorough the kidneys.

The renal arteries branch from the aorta right below the diaphragm, between the superior and inferior mesenteric arteries. The renal veins connect to the inferior vena cava at the same level. Most of the blood, about 90%, enters the outer portion of the kidney, the cortex, where the major part of glomerular filtration takes place. The relatively low blood flow to the inner part of the kidney, the medulla, creates an osmotic gradient and enhances concentration of the urine. This, together with the counter-current exchange of oxygen in the medullary vasa recta and the fact that the re-absorption of electrolytes (e.g., sodium), which is a highly O_2-demanding process, takes place in the medulla, leaves the medulla in a borderline hypoxic state.

1.1 The kidney

1.1.1 The glomeruli

The glomeruli represent a network of capillaries that is completely surrounded by mesangial cells inside the capsule of Bowman, which is located in the proximal portion of the nephron. Water and small soluble substances are filtered through the barrier to Bowman's space and then pass through to the tubules of the nephron. This filtrate is called the primary urine, and the kidneys filter approximately 180 L of primary urine per day.

Blood enters the glomeruli via afferent arterioles and exits via efferent arterioles (not venules). These arterioles are also denoted as pre- and post-glomerular resistance vessels. At a certain mean arterial pressure (MAP), the hydrostatic pressure within the glomerular capillaries is determined by the pre-glomerular resistance to post-glomerular resistance ratio. Thus, an increase in

the pre-glomerular resistance or a decrease in the post-glomerular resistance will cause a drop in the glomerular hydrostatic pressure, while a decrease in the pre-glomerular resistance or an increase in the post-glomerular resistance will increase the glomerular hydrostatic pressure. In addition to the glomerular hydrostatic pressure, the glomerular ultra-filtration is dependent upon the plasma colloid osmotic pressure and the hydrostatic pressure in the capsule of Bowman (Figure 1).

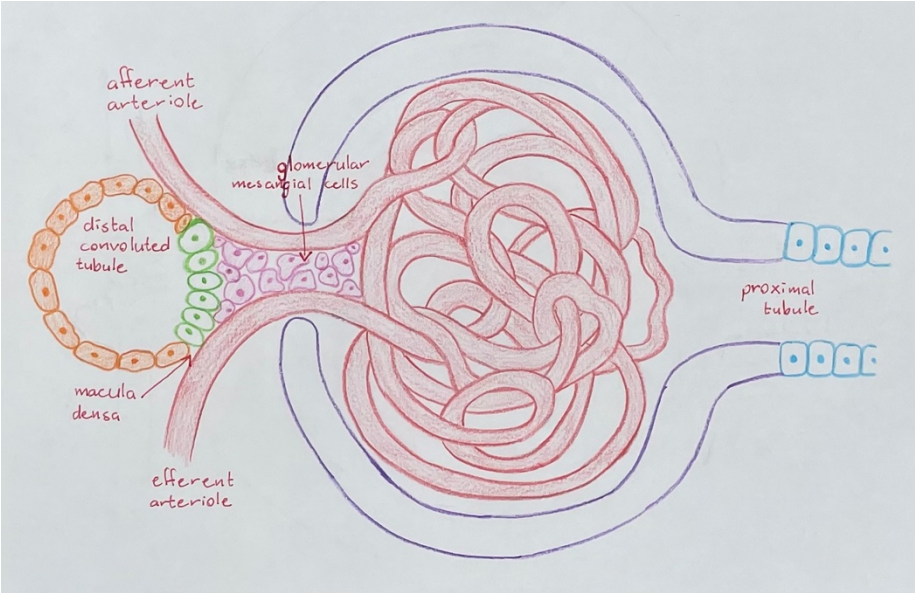


Figure 1. The glomeruli. Illustration by Rebecka Tholén.

1.1.2 The Nephron

The functional unit in the kidney is the nephron, and each kidney has a million nephrons located in 10–15 so-called ‘pyramids’. The nephron consists of the glomerulus, proximal tubules, loops of Henle, the distal tubules and the collecting tubes. Overall, 66% of the sodium and water that is filtered in the glomerulus is re-absorbed in the proximal tubule and approximately 25% is re-absorbed in the thick ascending limbs of the loops of Henle (Figure 2).

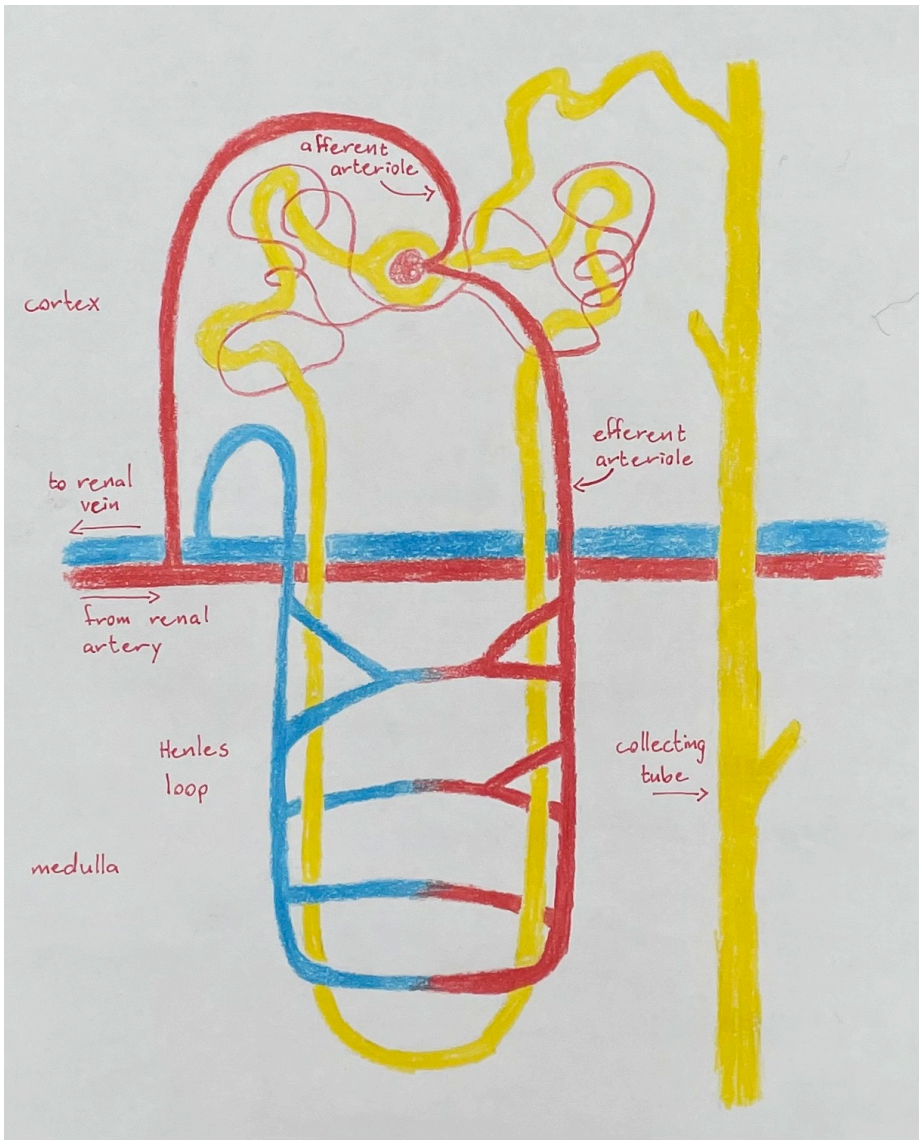


Figure 2. The nephron. Illustration by Rebecka Tholén.

1.1.3 The Renin angiotensin system

Sympathetic activation of beta-1 receptors, systemic hypotension and low levels of sodium ions (Na^+) in the distal tubules stimulate renin release from the kidney. Renin stimulates the formation of angiotensinogen in the blood and

tissues, leading to the release of aldosterone from the cortex of the adrenal gland, the release of ADH, and the retention of Na^+ and H_2O.

1.2 Kidney injury and kidney failure

1.2.1 Definition

Kidney injury and kidney failure are defined as being due to pre-renal, post-renal or renal events of different aetiologies that lead to pathophysiological changes to the kidney. These changes can result in decreased kidney function, which may range from mild dysfunction, with no clinical impact, to complete depletion of kidney function, necessitating dialysis to prevent death.

1.2.2 Clinical impact

When renal function is decreased by ≥50%, there is an accumulation of nitrogen waste products, as evidenced by an increase in the serum level of creatinine. The human body can survive without any noticeable symptoms until the kidney function drops to approximately 20%. Kidney function of <10% usually means that there is a need for dialysis. On the other hand, development of even mild renal dysfunction in connection with kidney injury is associated with increased mortality and morbidity.

1.2.3 Definition and staging of acute kidney injury

There exist several systems for the definition, classification and staging of acute kidney injury (AKI). One system for the classification for AKI involves the RIFLE (Risk, Injury, Failure, Loss of kidney function and End-stage kidney disease) criteria [1] (Table 1). Another system applies the KDIGO criteria (https://kdigo.org)[2], which are used in this thesis (Table 1). AKI according to the KDIGO criteria is defined as an increase in the serum level of creatinine over less than 2 days of >26.5 μmol/L from baseline or an increase in creatinine by >150% from baseline, or a urine output of <0.5 mL urine/kg/hour for more than 6 hours.

RIFLE

	eCCl	Urine output
Risk	Decrease by 25%	<0.5 mL/kg/hour for 8 hours
Injury	Decrease by 50%	<0.5 mL/kg/hour for 16 hour
Failure	Decrease by 75% or <35 mL/min/1.73m^2	<0.3 mL/kg/hour for 24 hour or anuric for 12 hour
Loss	Persistent renal failure > 4weeks	not applicable
End stage	Persistent renal failure >3 months	not applicable

KDIGO

	SCr	Urine output
Stage 1	Increase 1.5-1.9 x baseline or increase ≥27µmol/L	<0.5 mL/kg/hour for 6-12 hour
Stage 2	Increase 2-2.9 x baseline	<0.5 mL/kg/hour for ≥12 hour
Stage 3	Increase >3 x baseline **OR** SCr ≥354 µmol/L **OR** Initiation of RRT **OR** eGFR <35 mL/min/1.73m^2	<0.3 mL/kg/hour for ≥24 hour **OR** anuric for ≥12 hour

Table 1. The RIFLE and KDIGO criteria[3]. eCCI; estimated creatinine clearance, SCr; serum creatinine

1.3 Measuring and estimating kidney function

The glomerular filtration rate (GFR) is the sum of the rate at which blood is filtered through all of the kidney's glomeruli, i.e., the overall kidney function. GFR is the recommended variable for defining, screening, monitoring and classifying kidney disease. GFR is assessed through the rate of renal clearance of an exogenous marker after it has been injected into the blood-stream. The marker has to be completely filterable by the glomeruli and must not be re-absorbed or secreted from the primary urine by the renal tubules. Examples of such markers are inulin, ^{51}Cr-EDTA and iohexol [4-7].

Accurately measuring the GFR, through the use of the above-mentioned filtration makers, is expensive, complex and time-consuming. Therefore, various other markers/methods are used to estimate and follow kidney function, e.g., after surgery and in the intensive care unit. One of these markers is serum creatinine. Creatinine is a waste product derived from ingested food and our own muscles. Creatinine is filtered from the blood in the kidney and cleared from the body via the urine. If kidney function is reduced, the serum creatinine levels are increased. However, there are several problems associated with the use of serum creatinine as a marker of kidney function. The concentration of creatinine in the serum is affected by several non-renal factors, such as creatine release from the muscles and the metabolic transformation of creatine to creatinine. In addition, race, gender and age all affect muscle mass and, thereby, the serum concentration of creatinine [8, 9]. A young patient with large muscle mass will have a higher creatinine level compared to an elderly cachectic patient with reduced muscle mass. The serum creatinine level can also be low or high depending on the patient's hydration status. This is especially important after heart surgery and cardiopulmonary bypass, in that the patients often have excess body water. Furthermore, early post-operative immobilisation will decrease the serum creatinine concentration and, thus, attenuate the AKI-induced increase in serum creatinine.

One way of overcoming the limitations associated with the use of serum creatinine for the evaluation of renal function is to use estimating equations for the assessment of GFR, also known as 'estimated GFR' (eGFR). These equations use, in addition to serum creatinine, variables such as gender, age, body weight and race, as a surrogate for muscle mass [10-13].

In the management of patients with post-operative AKI, it is important to evaluate *changes* in the early post-operative level of renal function from the pre-operative level. The question that arises is whether *changes* in eGFR can be used for the assessment of *changes* in true mGFR in critically ill patients or in patients who are undergoing major surgery. Whether or not eGFR can be used to adequately assess the post-operative changes in true renal function has not yet been determined.

1.3.1 Estimating GFR using creatinine, CKD-EPI

The CKD-EPI equation was created to have an easy and as accurate as possible way to follow the true GFR using markers that are readily available, e.g., serum creatinine. It was developed and validated with data from 10 different studies, including >8,000 patients with and without chronic kidney disease (CKD). Thereafter, 16 studies with approximately 4,000 patients were used for the external validation [13].

The CKD-EPI equation, expressed as a single equation, is:

$GFR = 141 * \min(Scr/\kappa,1)^{\alpha} * \max(Scr/\kappa, 1)^{-1.209} * 0.993^{Age} * 1.018$ [if female]

where 'Scr' is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, 'min' indicates the minimum of Scr/κ or 1, and 'max' indicates the maximum of Scr/κ or 1.

1.3.2 Measurements of renal plasma flow (RPF) by infusion clearance of para-amino hippuric acid (PAH)

Infusion clearance of PAH is used to measure renal plasma flow [14]. Overall, 90% of the PAH is both filtered and secreted by the kidney in a single round of circulation. To obtain accurate values of RPF, without urine collection, enough time must be allowed for the steady state to be achieved, i.e., when the infused levels of PAH equal the urinary excretion levels of PAH, also regarded as stable levels of serum PAH. Ideally, the renal extraction of PAH should be measured with an in-dwelling renal vein catheter, as it has been shown that renal PAH extraction is <90% after uncomplicated cardiac surgery and is as low as 50%–80% in patients with AKI after complicated cardiac surgery [15]. If such low values of renal PAH extraction are not taken into account, the RPF and renal blood flow (RBF) will be severely under-estimated.

1.4 Extra corporal circulation (ECC)

To be able to perform open-heart surgery safely, the heart is arrested, disconnected from the circulation, and connected to an extra-corporeal circulation (ECC) apparatus, also known as a "heart and lung machine". This is done by inserting a cannula via the right atrium down into the inferior caval vein or alternatively, depending on the type of surgical procedure, by performing bi-caval cannulation of both the superior and inferior vena cava. The venous return is drained to the ECC machine, where it is oxygenated and CO_2 is removed, followed by return of the arterialised blood to the ascending aorta. The tubing system for an adult surgery patient has a volume of approximately 1,300 m. The tubing system is routinely filled with crystalloid, acetated Ringers' solution, before being connected to the patient (Figure 3).

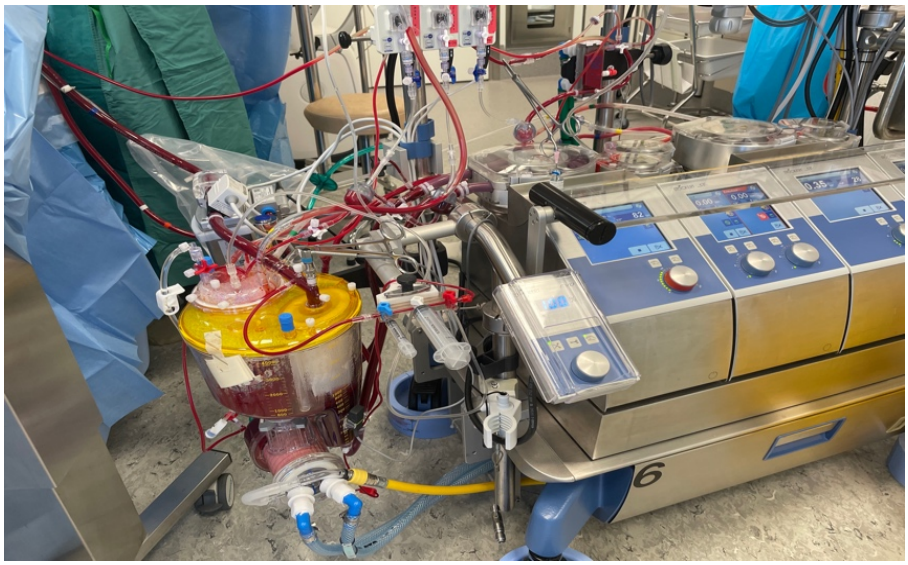


Figure 3. The CPB machine.

1.4.1 Cardiac surgery, ECC and the kidney

The kidney does not react favourably to the ECC treatment, for multiple reasons. One such reason is that when the blood cells are exposed to the tubing system of the ECC there is release of cytokines and other pro-inflammatory markers [16]. Another reason is the hypoxia induced by hypo-perfusion during surgery, intra-operative hypotension, haemodilution, and renal vasoconstriction [17, 18]. Post-operatively, there is haemodynamic instability due to bleeding or low cardiac output caused by heart failure, which in turn induces a renal O_2 supply/demand mismatch. In addition, there is renal vasoconstriction following cardiac surgery, which leads to impaired oxygenation [15]. Other factors contributing to the less-than-favourable environment for the kidney after ECC are the various medications, such as antibiotics and calcineurin inhibitors, administered to the patient [19-21]. The incidence of post-cardiac surgery AKI is 15%–30% and the frequency of need for dialysis after heart surgery is 2%–5% [22]. The mortality rate associated with dialysis-dependent AKI is high, being reported at >40% in one study [23].

1.5 Near-infrared spectroscopy (NIRS)

As light passes through a medium it is absorbed and scattered. Near-infrared light shows good penetration of human tissues, without causing damage, in

contrast to x-rays. Moreover, unlike microwaves, near-infrared light does not raise the temperature within the tissue.

Near-infrared light is absorbed by oxygenated blood. The capacity of the blood to carry oxygen is 19-20 mL O_2/100 mL blood, and almost all the oxygen is bound to haemoglobin. The software in the NIRS monitor uses an algorithm with the spectral extinction coefficients (absorptivity) of the chromophores in question (in this case, oxygenated and de-oxygenated haemoglobin), and it is based on the Beer-Lambert law, which states that there is a linear relation between the absorbance of a solution and its concentration.

The NIRS sensor consists of a transmitter and two detectors, all of which are placed in a row on a tape and connected to a monitor (Figure 4). The transmitter sends photons, in the near infrared-spectrum, down into the tissue. This light is scattered within the tissue, and some fractions of the light will reach the surface once again and can be picked up by the detector.

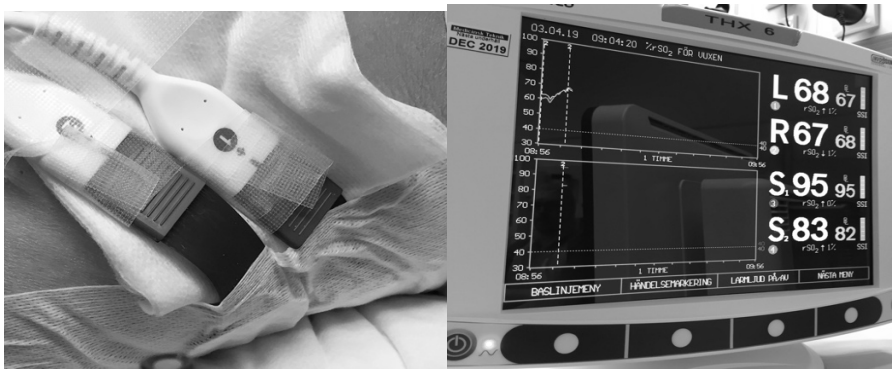


Figure 4. NIRS sensor and monitor.

The photons can take many different and multiple scattering pathways within the tissue, between the transmitter and the detector. Some pathways are more likely than others. If the light takes a path close to the tissue surface the photons are likely to be scattered out through the surface before they can reach the detector. Deep pathways are likely to be absorbed before they can reach the surface again and be detected [24].

For infrared light in human tissues, the most likely pathway is in the form of a "curved ellipse", resembling a banana (Figure 5).

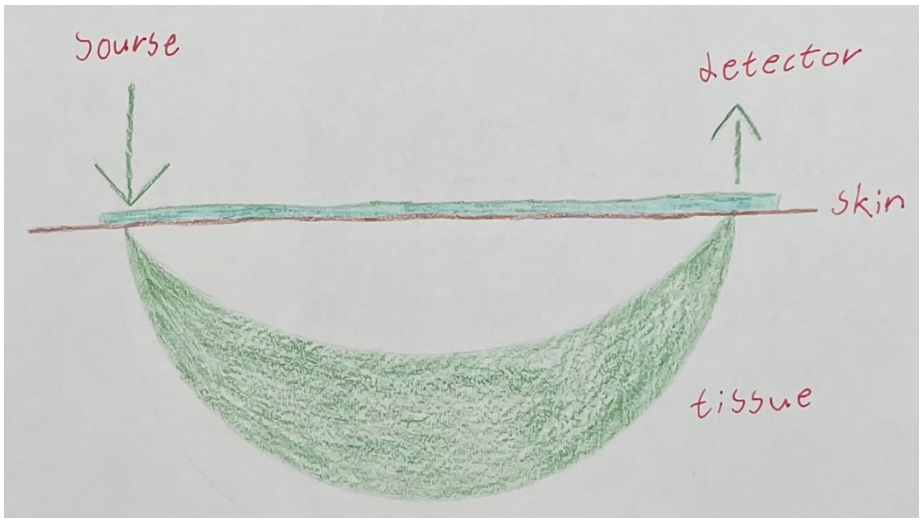


Figure 5. NIRS signal in tissue. Illustration by Julia Tholén.

This gives us the "visit probability", meaning the tissue that the light has passed through. The number of photons that will be absorbed, and thus do not reach the detector, is directly proportional to the visit probability.

If we have two detectors, as is the case with the NIRS receptor used in this study (Paper I), we can subtract one signal from the other, thereby eliminating the shallower path, which is the signal from the skin and subcutaneous tissue (or bone).

The NIRS technology offers a non-invasive way to study tissue oxygenation [25-28].

1.6 Inotropic agents and the kidney

It has been suggested that AKI after cardiac surgery is caused by insufficient renal oxygen delivery (RDO_2), which is induced by haemodilution-induced anaemia, hypotension, and low cardiac output due to heart failure or hypovolemia [17, 18]. In post-operative AKI, renal oxygenation (i.e., the renal oxygen demand/supply ratio) is impaired owing to a pronounced reduction in RBF, as explained by a high renal vascular resistance (RVR) [15].

In the management of post-cardiac surgery AKI, the main goal is to improve the GFR. However, any agent that increases the GFR will also increase the tubular sodium load and re-absorption, which, in turn, will increase renal

oxygen consumption (RVO_2). This would further impair renal oxygenation. Ideally, a renal vasodilatory agent that acts mainly on the afferent arterioles (pre-glomerular resistance vessels) would increase not only the RBF but also the GFR. Such a vasodilator would meet the increased metabolic demand (RVO_2) by increasing RDO_2. The commonly used inotropic agents dopamine, milrinone and levosimendan all have vasodilatory activities, potentially increasing the RBF. This does not imply that these inodilators also increase the GFR. A renal vasodilatory effect on GFR is dependent upon the agent's actions on afferent and efferent arterioles. Low-dose dopamine and milrinone have been shown to increase the RBF with no significant effects on the GFR [29, 30], suggesting vasodilatory effects on both the afferent *and* efferent arterioles [31]. Interestingly, Bragadottir et al. [32] have shown that levosimendan increases both the RBF and GFR after uncomplicated cardiac surgery without impairment of renal oxygenation, suggesting a vasodilatory effect, mainly on the afferent arterioles.

However, the effects of levosimendan on RBF, GFR and renal oxygenation have not been explored in patients with AKI after cardiac surgery. In addition to its inotropic effect, levosimendan exerts a vasodilatory effect on both the systemic and pulmonary circulatory systems. This vasodilatory action is mediated via the opening of ATP-sensitive potassium channels in the vascular smooth muscle cells [33].

1.7 Heart transplantation

Heart transplantation (Htx) is a method of treatment for end-stage heart failure. The Htx procedure, in un-complicated cases, takes only a couple of hours to perform. In the more complex cases, it can take the better part of 24 hours. The longer the operation, the greater the risks for the patient [34]. Complicated cases are: GUCH patients, who often have undergone multiple heart surgeries prior to Htx; patients with high pulmonary arterial pressure; patients with different types of heart-assisting devices; and patients with thoracic anomalies. Other aggravating factors include the taking of medications that affect blood coagulation or wound healing. Spending a long time on cardiopulmonary bypass (CPB) is linked to AKI [18]. After Htx, there are other factors that affect the patient and the kidney, some of which are the same as those seen after any cardiac surgery procedure, i.e., right heart failure, volume overload, and low cardiac output due to stunning of the heart. Other factors are specific to Htx, such as graft dysfunction/failure and immunosuppressive medications. After Htx, the AKI risk is 15%–40% and the frequency of need for renal replacement therapy (RRT) is 5%–6% [35, 36].

1.8 Atrial natriuretic peptide (ANP) and the kidney

ANP, which is a hormone that comprises 28 amino acids, is produced in the atria of the heart. While there is continuous release of ANP from the atria, the rate of release increases in response to stretching of the atrial walls. ANP is involved in body fluid and blood pressure homeostasis [37]. Released ANP induces both vasodilation and venodilation, mediated by cyclic GMP, natriuresis, inhibition of the sympathetic nerve activity and the renin–angiotensin–aldosterone axis. Exogenous administration of ANP to healthy volunteers increases the GFR, and inhibits tubular water and sodium re-absorption [38-42].

In the *treatment* of AKI after cardiac surgery and in cyclosporine-induced acute renal dysfunction after Htx, it has been shown that intravenous administration of ANP increases both the RBF and GFR, suggesting preferential dilation of the afferent arterioles [40, 43]. Furthermore, in a randomised, placebo-controlled trial, it was demonstrated that *treatment* with ANP decreased the likelihood of RRT and improved dialysis-free survival for patients with cardiac surgery-associated AKI [44]. In randomised placebo-controlled trials, ANP has also been shown to be reno-protective when used for the *prevention* of acute renal impairment after cardiac surgery with CPB [45-48]. In more-recent meta-analyses of patients who underwent surgery, it was shown that ANP, when used for the prevention or treatment of AKI, reduced the incidences of AKI and mortality, as well as the need for RRT [49, 50].

However, there is a scarcity of studies that have examined the effects of ANP used for the prevention of post-operative AKI in patients undergoing Htx.

2 AIMS

- To evaluate the levels of agreement and correlation between renal tissue oxygenation obtained by near-infrared spectroscopy (NIRS) and renal venous oxygen saturation measured using renal vein catheterisation in patients undergoing cardiac surgery with cardiopulmonary bypass.

- To investigate, in a double-blind randomised, placebo-controlled study, the effects of the inodilator levosimendan on renal blood flow (RBF) and glomerular filtration rate (GFR) in patients with acute kidney injury (AKI) early after cardiac surgery.

- To determine whether intra- and post-operative administration of atrial natriuretic peptide (50 ng/kg/min) can prevent a post-operative decrease in renal function after heart transplantation (Htx), in a double-blind, randomised, placebo-controlled, investigator-initiated, single-centre trial.

- To assess the correlation, agreement and accuracy between the estimated GFR (eGFR) and true measured GFR (mGFR), and the ability of eGFR to track changes in mGFR early after Htx.

3 PATIENTS AND METHODS

3.1 Ethics and registration

The Swedish Ethical Review Authority (*Etikprövningsmyndigheten*) approved all the study protocols presented in this thesis. Inclusion of patients was performed after written informed consent or after informed consent was received from the patients or their next of kin. The studies were performed in accordance with Good Clinical Practice and the recommendations set out by the Declaration of Helsinki, regarding ethics and scientific quality standards. All the studies were registered in ClinicalTrials.gov, with the following identifier numbers: Paper I, NCT02549066; for Paper II, NCT02531724; and for Papers III and IV, NCT02665377. Approval from the Swedish Medical Products Agency (*Läkemedelsverket*) was granted for the Levo-AKI study (described in Paper II) and the ANP-Htx study (Paper III). Approval was also granted from the Radiation Committee at Sahlgrenska University Hospital in Gothenburg for the use of Cr^{51}EDTA and fluoroscopy (for placement of renal vein catheters).

3.2 Patients

3.2.1 Paper I

This study was a pre-planned secondary analysis of data obtained from of the ICAROX investigation (Impact of Cardiopulmonary Bypass Flow on Renal Oxygenation) [51], which was a prospective observational study performed in the cardiothoracic surgical theatre at Sahlgrenska University Hospital in Gothenburg, Sweden. In this study, 13 patients where included after they provided written informed consent. The patients were scheduled for coronary bypass surgery, valve surgery or a combination of these two procedures. The inclusion criteria for the ICAROX study were: (1) age >18 years; (2) LVEF ≥50%; (3) a normal pre-operative serum creatinine level (women, 45–90 μmol/L; men, 60–105 μmol/L); (4) elective cardiac surgery with CPB; and (5) normothermia during CPB (bladder temperature >34°C). The exclusion criteria were: (1) acute/emergency surgery; (2) allergy to radiographic contrast medium; (3) BMI >32 kg/m^2; and (4) previous cerebrovascular lesion. Added for this sub-study was the exclusion criterion: (5) a distance from the skin to the kidney of >4 cm, as assessed by ultra-sound imaging.

3.2.2 Paper II

This was a single-centre, prospective, randomised, blinded cohort study on patients who developed AKI after cardiac surgery. Twenty-nine patients were included after written informed consent or consent from their next of kin (two patients). The inclusion criteria were: (1) a normal pre-operative serum creatinine level (women, 45–90 μmol/L; men 60–105 μmol/L); (2) post-operative AKI, defined as an increase in serum creatinine >27 μmol/L or an increase > 50% within 48 hours from surgery; (3) written informed consent, or consent from next-of-kin; (4) age >18 years; and (5) post-operative treatment after cardiac surgery with cardiopulmonary bypass (CPB). The exclusion criteria were: (1) ongoing treatment with an inotropic agent other than norepinephrine; (2) a central venous oxygen saturation (ScvO2) <60%; and (3) affected RBF due to vascular obstruction, e.g., from a dissection membrane (Fig. 9).

3.2.3 Paper III

This was a single-centre, prospective, randomised, double-blind trial. Eligible patients on the waiting list for Htx were informed about the study and enrolled into the study after they provided written informed consent and fulfilled the inclusion (but not the exclusion) criteria. They were then randomised and treated according to study protocol at the time of the transplant operation. The study was conducted in the cardiothoracic surgery theatre, the cardiothoracic intensive care unit (ICU), and the transplant ward, all at Sahlgrenska University Hospital, Gothenburg, Sweden. The inclusion criteria were: (1) patient accepted for de novo Htx; (2) age >18 years; (3) Swedish resident; and (4) signed written informed consent. The exclusion criteria were: (1) planned multi-organ transplantation; (2) ongoing ECMO treatment; (3) ongoing pre-operative dialysis; (4) age of donor >75 years; (5) graft ischemia time >6 hours; (6) ABO-incompatible donor heart; (7) known renal disease other than nephropathy caused by hypertension, diabetes or nephrosclerosis; (8) mGFR ≤30 mL/min/1.73 m^2; and (9) inability to understand the Swedish language. In total, 107 patients, were screened for eligibility. After enrolment, 15 patients were excluded. In total, 73 patients were randomised in this study. Seventy patients were ultimately analysed after the occurrence of two cancelled transplant operations and one intraoperative death (Fig. 11).

3.2.4 Paper IV

This study was a secondary analysis of the Htx-ANP trial (Paper III). Patients who required dialysis during the first four post-operative days, i.e., missing

measured mGFR on Day 4, were excluded, as were those patients who had a time interval of more than 4 days between the pre-operative measurements of eGFR and mGFR (except for one patient who had 24 days between the measurements, and who was considered to have stable renal function with repeated stable values of serum creatinine). Overall, pre- and post-operative data on eGFR and mGFR from 55 patients were analysed in this study.

3.3 Methods

3.3.1 Anaesthesia and CPB

Anaesthesia was conducted at the discretion of the attending anaesthesiologist. Hemodynamically stable patients were induced with fentanyl (5–10 μg/kg), propofol (1–1.5 mg/kg) and orally intubated after rocuronium administration (0.6 mg/kg). Hemodynamically unstable patients (Paper III) were induced by the addition of esketamine (0.25–1 mg/kg), and in a few cases after pre-oxygenation with nitic oxide (NO). Anaesthesia was maintained with sevoflurane before and after CPB, and with propofol during CPB.

After sternotomy and treatment with heparin at 400 IU/kg, the patients were cannulated in the aortic root, followed by caval cannulation, which was mono- or bi-caval depending on the surgical technique used. The target mean arterial pressure (MAP) was 60–80 mmHg and infusion of norepinephrine or nitroprusside was used when needed. The target haematocrit was 25%–35% and target body temperature was 35°–36°C. If there was no contra-indication or ongoing infection, the patients were treated with prophylactic cloxacillin 2 g × 3 (a total of 7 doses) starting 30 min prior to surgery.

The CPB machine used was the Stöckert S5® with the Stöckert Heater Cooler system 3T® (Stöckert GmbH, Germany). The circuit consisted of a Primox® or Inspire 8® oxygenator (Sorin Group, Italy), the HVR Hard-shell reservoir (Sorin Group), and the Sorin Adult® tubing system. The priming solution consisted of 1,300 mL Ringer´s solution and 10,000 IU heparin.

CPB pump flow:

In Paper I, renal oxygenation was monitored at different CBP pump flow rates. All patients were exposed to three different pump-flows; 2.4, 2.7, and 3.0 L/min/m^2. The patients were randomised as to the order in which the different pump flows were delivered. For the patients in Paper I and III, the CPB pump flow was operated in accordance with the clinical standard at our department, i.e., a CPB flow of around 2.4 L/min/m^2.

3.3.2 Measurements of renal oxygenation by Near-Infrared Spectroscopy (NIRS) and renal vein catheterisation (Paper I)

The NIRS sensor (INVOS Cerebral/Somatic Oximetry Adult Sensor; Medtronic Inc., Minneapolis, MN) was placed, with ultrasound guidance, over the left kidney and connected to a monitor (INVOS 5100C; Medtronic). An 8F renal vein catheter (Webster Laboratories, Irvine, CA) was inserted into the left renal vein via the left femoral vein, guided by fluoroscopy, prior to surgery. Since the cross-sectional area of the catheter is 25-times smaller than the area of the renal vein, the risk for partial occlusion of the vein is negligible. Blood samples from the renal vein were analysed for oxygen saturation, according to protocol, and data from the NIRS electrode were collected.

3.3.3 Measurements of renal blood flow (RBF) (Paper II)

RBF was assessed by the technique of infusion clearance for para-aminohippuric acid (PAH; Merck & Co. Inc. Rahway, NJ,). After blood blanks were drawn, PAH was administered as a bolus, followed by a constant infusion. The plasma concentration of PAH was measured with the Beckman DU 530 UV/Vis spectrophotometer (Beckman Coulter Inc., Brea, CA). Renal plasma flow (RPF) was calculated as the amount of infused PAH divided by the concentration in the arterial blood, corrected for an assumed PAH extraction of 0.7, which is the assumed extraction factor for patients with AKI after cardiac surgery [15]. RBF was calculated as: renal plasma flow (RPF) divided by (1 – the haematocrit). Renal vascular resistance (RVR) was calculated as: (MAP-CVP)/RBF.

3.3.4 Measurements of glomerular filtration rate (GFR) (Papers II - IV)

Infusion clearance with labelled chromium-ethylenediamine tetra-acetic acid (^{51}Cr-EDTA, GE HealthCare Ltd., Hatfield, UK) [4] or iohexol (Omnipaque®300 mgI/mL; GE HealthCare, Sweden) was used to measure the glomerular filtration rate (mGFR) [52]. After blood blanks was collected, an intravenous priming bolus dose of either ^{51}Cr-EDTA or iohexol was administered, followed by a constant infusion, individualised according to body surface area (BSA) and serum creatinine level. The serum concentration of iohexol was measured by ion mass spectroscopy (Xevo TQMS; Waters Corp., Milford, MA) and the serum concentration of ^{51}Cr-EDTA was measured in a gamma counter (Wizard 3" 1480 Automatic Gamma Counter; Perkin Elmer Oy, Turku, Finland). The GFR was calculated as the amount of infused ^{51}Cr-EDTA or iohexol divided by the arterial concentration of the respective substance. The filtration fraction (FF) was calculated as: RPF/GFR.

3.3.5 Administration of levosimendan (Paper II)

The patients in Paper II with post-operative acute kidney injury (AKI), according to the KDIGO criteria, were randomised 1:1 to placebo (Ringer-Acetat Baxter Viaflo) or levosimendan (Simdax; Orion Pharma, Espoo, Finland). A bolus dose of levosimendan (12 μg/kg), or an equivalent volume of placebo, was administered over 30 min, followed by a continuous infusion at an infusion rate of 0.1 μg/kg/min for 5 hours (or placebo in the same volume). The mean arterial pressure (MAP) was maintained at a target level of 70–80 mmHg, with norepinephrine infusion if needed. Ongoing diuretic treatment was unchanged.

3.3.6 Administration of atrial natriuretic peptide (Paper III)

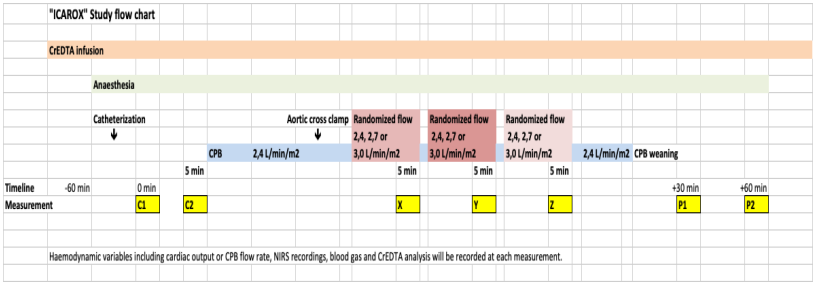
After induction of anaesthesia and placement of a central intravenous line, an infusion of atrial natriuretic peptide (HANP1000®; Daiichi Sankyo Propharma, Tokyo, Japan) was started at a rate of 50 ng/kg/min or with an equivalent dosage of placebo (saline). The treatment continued for 4 post-operative days or until renal replacement therapy was initiated. On post-operative Day 5, the study drug was gradually tapered off over a period of 24 hours. GFR was measured on post-operative Day 4, by infusion clearance of ^{51}CrEDTA or iohexol (Omnipaque 300 mg I/mL; GE HealthCare).

3.3.7 Postoperative care (Papers I - IV)

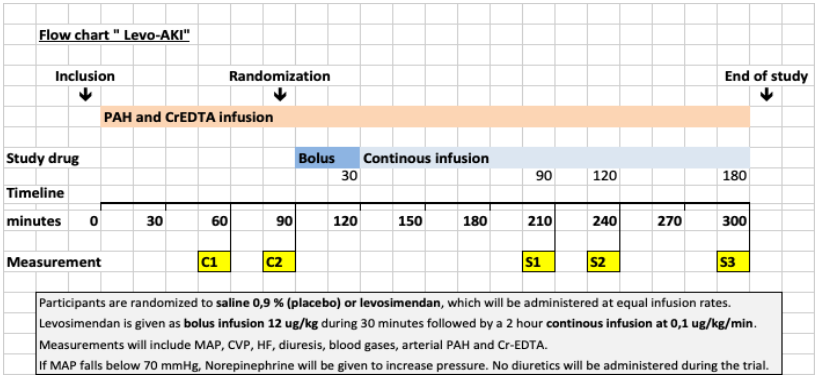
All the patients were transported from the cardiothoracic surgical theatre, in a sedated and intubated state, to the cardiothoracic ICU, where they are weaned from the respirator when FiO2 <40% and they were haemodynamically stable. The patients remained in the ICU overnight and if there was no further need for intensive care, they were moved to a post-cardiothoracic surgery ward (Papers I and II) or a transplantation ward (Papers III and IV).

3.4 Study protocol

Paper I



Paper II



3.5 Statistical analyses and sample size

All statistical analyses were made using Predictive Software Statistics ver. 18-25 (SPSS Inc., Chicago, IL).

3.5.1 Paper I

All variables were tested for normality using the Shapiro-Wilk test. Data are presented as means ± standard deviation (SD). Repeated measures correlation analyses [53] were used to assess the correlation between the regional renal tissue oxygenation saturation (rSO_2), as measured by NIRS, and the renal vein oxygen saturation ($SrvO_2$). To assess the strength of the association between rSO2 and SrvO2, the repeated measures correlation coefficient was calculated. Agreement between the two measurements was analysed with the Bland-Altman methodology [54-56]. The difference between the two methods (bias)

and the error (2 × SD of the difference divided by the mean of the two methods) were calculated, as were the limits of agreement, defined as the mean difference ± 2 SD. A p-value of <0.05 was considered statistically significant.

3.5.2 Paper II

At least 12 patients in each group were required to detect a between-group difference in GFR of 12 mL/min, according to Bragadottir et al. [32]. Group differences at baseline were tested with the Mann-Whitney *U*-test (continuous data) or Fisher's exact test (categorical data). To test time and group interactions, a linear mixed model was used and the differences between baseline and treatment within the groups were tested with the Wilcoxon signed-rank test. Data are presented as means ± SD. A p-value of < 0.05 was considered statistically significant.

3.5.3 Paper III

A power analysis was performed based on blinded measurements of GFR at Day 4 after surgery on the first 20 included patients, showing a mean ± SD mGFR of 70 ± 25 mL/min/1.73m^2. To detect a 30% higher mGFR in the ANP group, 31 patients per group would be required, assuming an 80% power and a two-sided error of 0.05. To detect a 30% group difference in the mean change of mGFR, expressed as the post-operative to pre-operative mGFR ratio with a SD of 0.44, 34 patients per group would be required. To compensate for drop-outs and for the use of non-parametric tests, we intended to include 70 patients. Normally and non-normally distributed variables are presented as the mean ± SD and the median (inter-quartile range; IQR), respectively. Depending on the distributions of the continuous variables, the *t*-test or Mann-Whitney *U*-test were used for comparing the groups. Fisher's exact test was used for comparison of binary outcomes. The difference in mGFR on Day 4, between the groups, was tested by the Mann-Whitney *U*-test. A mixed model for repeated measurements (interaction group vs time) was used to evaluate between-group differences in serum creatinine levels over time (patients requiring dialysis were not included). P-values <0.05 were considered statistically significant and two-sided significance tests were used throughout.

3.5.4 Paper IV

Normally distributed variables are presented as the mean ± SD, and non-normally distributed variables are presented as the median (IQR). To test the peri-operative changes in renal function, a paired *t*-test was used. A linear regression analysis was performed to assess the relationship between post-

operative eGFR and mGFR. The accuracy of eGFR to predict true mGFR was calculated as the percentage of patients with an eGFR within 30% of the mGFR, which was also denoted as P30. A P30 >75% is the level of accuracy for estimating GFR recommended by the National Kidney Foundation (https://www.kidney.org). The agreement between eGFR and mGFR was assessed according to Bland and Altman [54-56], as described above. An acceptable between-method error, according to Critchley and Critchley, is 30% or less [57].

A four-quadrant plot was constructed to evaluate the ability of eGFR to track changes in mGFR. The concordance rate was defined as the percentage of eGFR and mGFR values that changed in the same direction (increase or decrease) [58, 59]. An exclusion zone at the centre of the plot for absolute changes in mGFR <5 mL/min/1.73 m^2 was used to exclude statistical noise [59]. A concordance rate >90%, excluding the data in the exclusion zone, corresponds to a good trending ability [58].

4 RESULTS

4.1 Paper I

Thirteen patients (four females and nine males) were included and analysed. The mean age was 69 ± 8 years. The skin-to-kidney distance was 3 ± 1 cm. Five patents underwent CABG plus valve surgery, three patients underwent CABG only, and five patients underwent CABG plus Maze surgery. The pre-operative serum concentration of creatinine was 83 ± 13 µmol/L.

Figure 6 shows the values for rSO_2 and $SrvO_2$ at the various time-points during cardiac surgery. The renal vein oxygenation saturation levels (red bars) are higher at all measuring points compared to the renal regional oxygen saturation values obtained from the NIRS (blue), although they change in parallel, reflecting the same ups and downs.

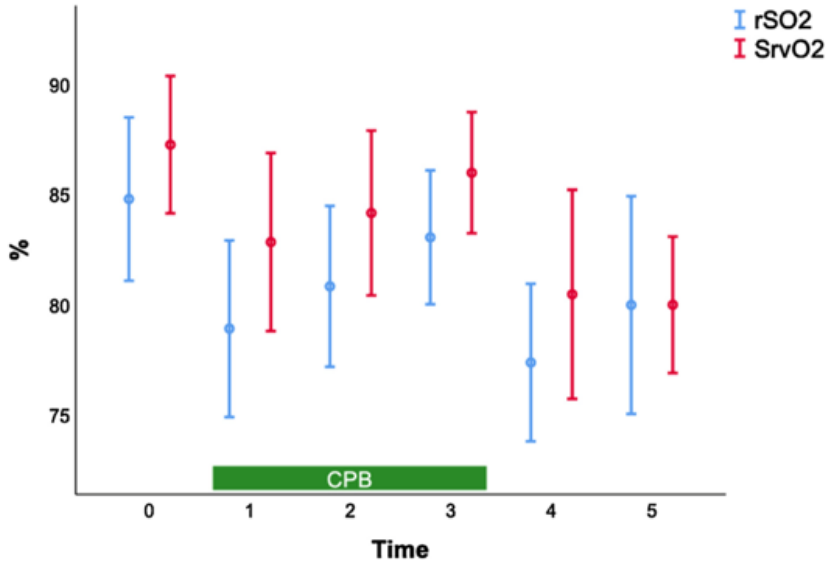


Figure 6. Renal regional oxygen saturation (rSO_2), assessed by NIRS, and the renal vein oxygen saturation ($SrvO_2$) at the various time-points. Data shown are mean ± 95% CI. CPB, cardiopulmonary bypass.

Figure 7 shows the individual correlation of all measurement points for each patient.

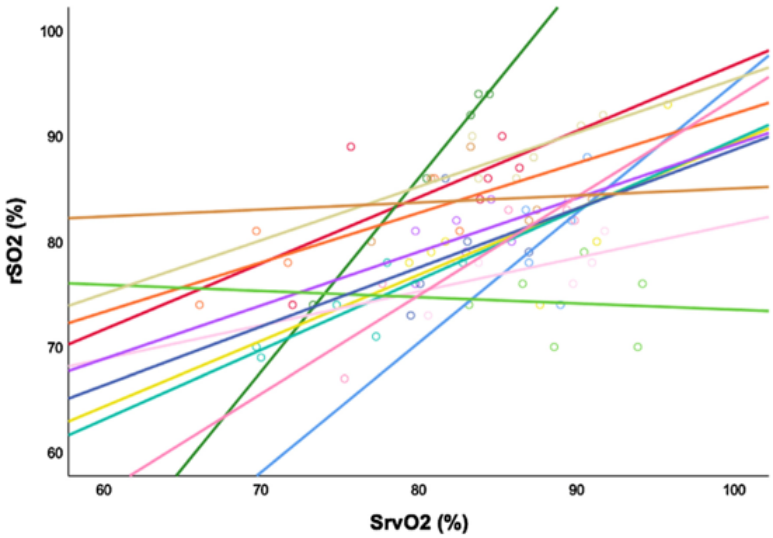


Figure 7. Individual correlation plots of the renal regional oxygen saturation (rSO_2), as obtained by NIRS, versus the renal vein oxygen saturation ($SrvO_2$).

In figure 8 the Bland-Altman plot [54-56] shows the agreement between the two methods for all measuring points. The mean difference (bias) was -2.71 ± 7.22, p = 0.002 with an error of 17.6%.

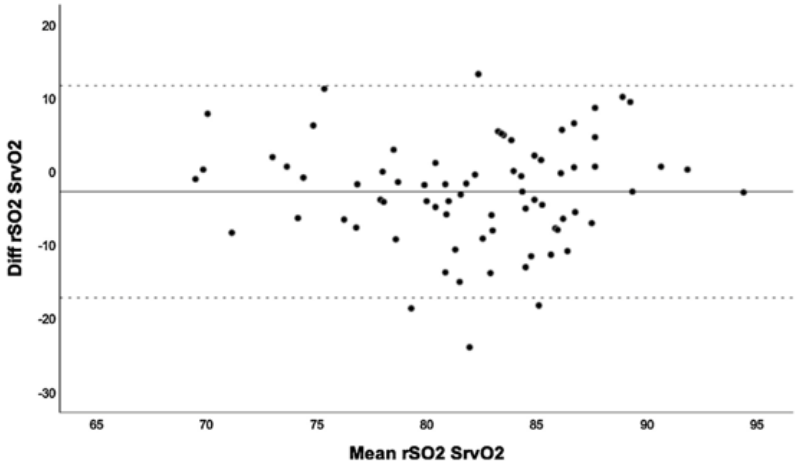


Figure 8. Bland-Altman plot of renal regional oxygen saturation (rSO_2) and renal vein saturation ($SrvO_2$), with all time-points included. The solid line indicates the mean difference, and the dotted lines indicate the limits of agreement.

4.2 Paper II

In total, 29 patients were included in Paper II, the majority were men (Fig 9).

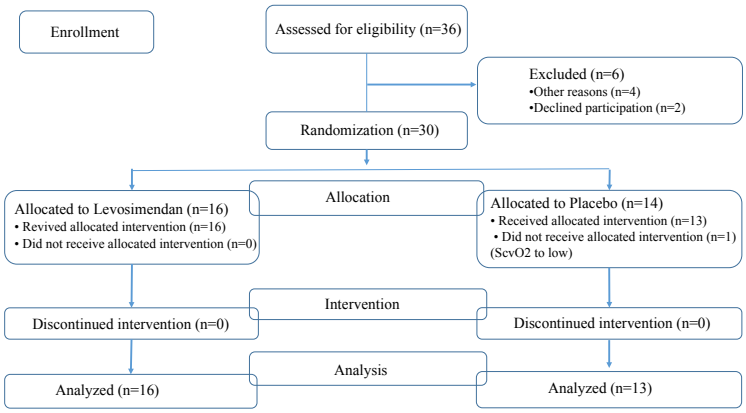


Figure 9. CONSORT flow diagram of the enrolment and randomization phases of the Levo-AKI study.

Mean pre-operative creatinine was 83 ± 14 µmol/L. The mean post-operative serum creatinine was higher in the levosimendan group (148 ± 29 µmol/L) at inclusion compared to the placebo group (127 ± 22 µmol/L). In the levosimendan group, 5 patients had AKI grad 2 at inclusion, all other patients in both groups had AKI grade 1. Table 2 shows the renal variables and p-value between the groups. The levosimendan group had significantly lower RBF and GFR at inclusion compared to the placebo group. A significant increase in RBF (+15%, $p < 0.001$)) and a significant reduction in renal vascular resistance (RVR) (-18%, $p = 0.001$) was seen in the levosimendan group. No significant changes were seen in the placebo group.

	Placebo (n=13)		Levosimendan (n=16)		BGD (LMM)
Variable	**Pre**	**Post**	**Pre**	**Post**	**P value**
Renal blood flow (L/min)	441 ± 159	455 ± 166	327 ± 148	375 ± 156 ***	0.011
Glomerular filtration rate (mL/min)	63.7 ± 19.7	63.8 ± 18.9	47.9 ± 15.2	50.0 ± 16.1	0.440
Renal prefusion pressure (mmHg)	70.3 ± 12.9	69.1 ± 7.3	66.0 ± 7.1	64.0 ± 5.5	0.907
Renal vascular resistance (mmHg/mL/min)	0.178 ± 0.067	0.170 ± 0.061	0.253 ± 0.131	0.208 ± 0.104**	0.043
Diuresis (mL/min)	1.89 ± 1.07	1.83 ± 0.61	1.71 ± 0.87	1.82 ± 0.96	0.637
Filtration fraction	0.104 ± 0.026	0.101 ± 0.018	0.117 ± 0.044	0.104 ± 0.034**	0.056

*Table 2. Between group difference (linear mixed model, LMM) = time × group interaction. BGD, Between-group difference. * p < 0.05, **p<0.01, *** p<0.001 for within-group differences.*

Figure 10 shows the RBF and GFR for the two groups, at baseline and after intervention. Shown in red (the lower of the lines) is the levosimendan group, and in black (the upper lines) the placebo group. In the levosimendan group, the GFR increased after the intervention, although the difference was not statistically significant (p = 0.079).

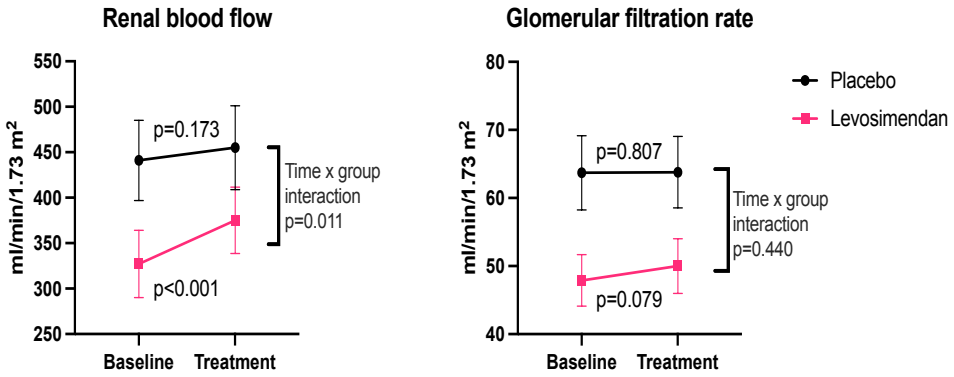


Figure 10. Effects of levosimendan or placebo on the renal blood flow and glomerular filtration rate of patients with AKI after cardiac surgery. The p-values indicate the between-group differences (time vs. group interaction from linear mixed model) and the differences within-groups (Wilcoxon signed-rank test).

4.3 Paper III

In the period of 2016–2020, 107 patients were assessed for eligibility, 86 were enrolled into the study, 73 were randomised, and the data for 70 patients were analysed (Figure 11).

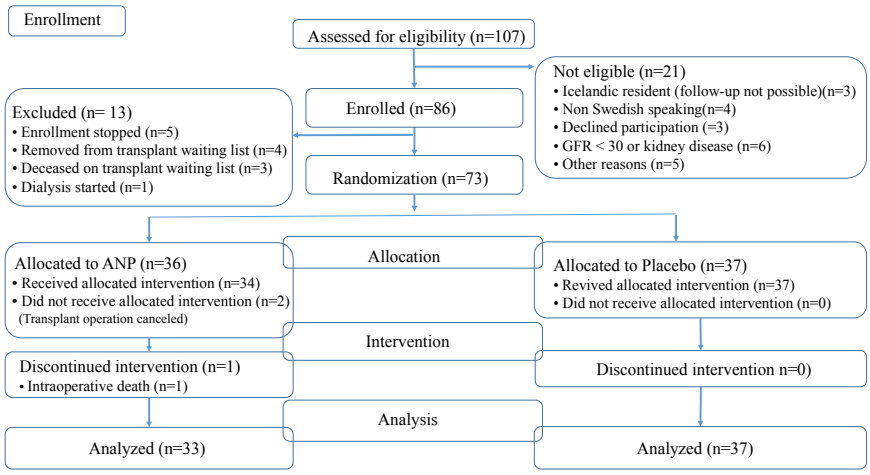


Figure 11. CONSORT flow diagram of the enrolment and randomization phases of the ANP-Htx study.

The ANP group and the placebo group were well-matched with respect to pre-operative demographics, morbidity and renal variables. Furthermore, the intra-operative data were without significant differences between the two groups. The post-operative data, such as MAP, CVP, CO, showed no differences between the two groups. The furosemide dose, urine output volume and the incidences of atrial fibrillation and right heart failure did not differ between the two groups. The post-operative renal data are shown in Table 3.

Variable	Placebo (n=37)	Atrial natriuretic peptide (n=33)	P-value
mGFR postop day 4 (mL/min/1.73 m^2), median (IQR)	60.0 (57.0)	50.1 (36.3)	0.705
Absolute change in mGFR (mL/min/1.73 m^2), median (IQR) (preop to day 4)	-2.7 (52.0)	-9.6 (51.0)	0.764
Percentage change in mGFR, median (IQR) (preop to day 4)	-4.5 (83.2)	-21.4 (77.4)	0.866
Absolute change in serum creatinine (mmol/L), median (IQR) (preop to day 4)	26.0 (74.0)	55.5 (133)	0.439
Acute kidney injury no (%):	26 (76.5)	21 (63.6)	0.616
Stage 1	12 (32.4)	7 (21.2)	0.420
Stage 2 or 3	14 (37.8)	14 (42.4)	0.808
Incidence of dialysis the first five postoperative days	8 (21.6)	3 (9.1)	0.197

Table 3. mGFR; measured glomerular filtration rate; IQR; inter-quartile range

The median (IQR) mGFRs on Day 4 post-operatively were 60.0 (57.0) and 50.1 (36.3) mL/min/1.73 m^2 for the placebo and ANP groups, respectively (p = 0.705). During ongoing ANP infusion, the frequencies of the need for dialysis were 21.6% and 9.1% for the placebo and ANP groups, respectively (p = 0.197). The incidences of AKI for the placebo and the ANP groups were 76.5% and 63.6%, respectively (p = 0.616). The incidences of AKI stage 1 were 32.4% and 21.2% for the placebo and ANP groups, respectively (p = 0.420) and for AKI stage 2 or 3, the corresponding incidences were 37.8% and 42.4%, respectively (p = 0.808).

4.4 Paper IV

Data from the 70 patients in the ANP-Htx study were available for this study. In all, 15 patients were removed because of missing data, either missing mGFR post-operatively due to post-operative dialysis or missing eGFR pre-operatively due to a too-long interval between the pre-operative creatinine and mGFR measurements. Finally, data from 55 patients were analysed in this study. The renal data are shown in Table 4.

Variable	preoperative (n=55)	postoperative (n=55)	p-value
Measured GFR, mean (SD), mL/min/1.73 m^2	60.3 (15.8)	64.3 (28.9)	0.259
Serum creatinine, mean (SD), µmol/L	108.3 (29.7)	172.2 (100.6)	<0.001
Estimated GFR, mean (SD), mL/min/1.73 m^2	71.3 (22.9)	53.2 (30.0)	<0.001

Table 4. GFR, glomerular filtration rate. SD, standard deviation.

There was a significant positive correlation between the post-operative values of eGFR and mGFR ($r = 0.825$, $p < 0.001$), although the accuracy of eGFR compared to mGFR was 51%. The level of agreement is shown in the Bland-Altman plot (Figure 12), where the bias is 11.2 ± 17.4 mL/min/1.73 m^2. The limits of agreement (LOA) were -23.0 to 45.4 mL/min/1.73 m^2 and the error was 58%.

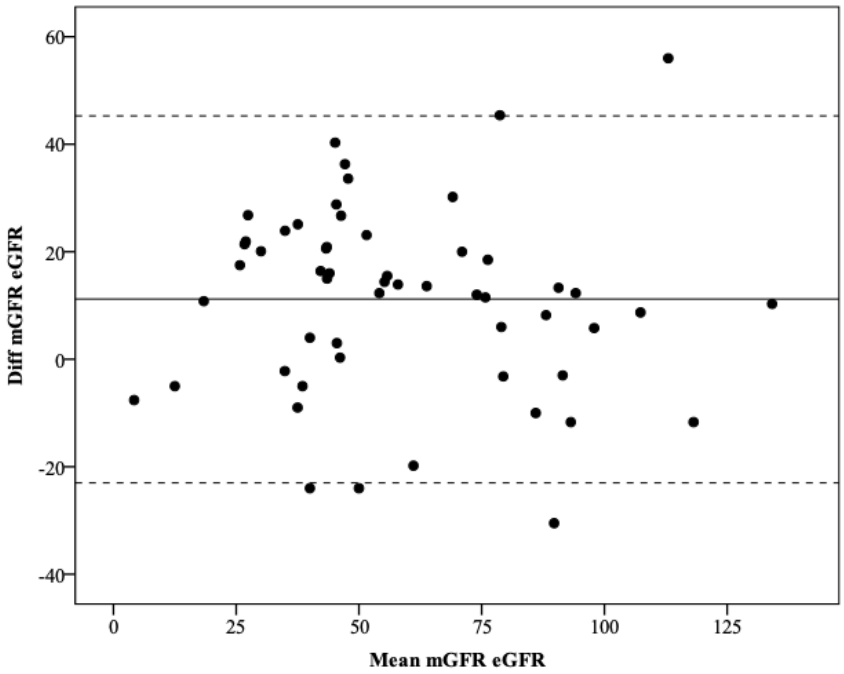


Figure 12. Bland-Altman plot showing the agreement between measured GFR (mGFR) and estimated GFR (eGFR). Solid line indicates mean difference and dotted lines indicates limits of agreement.

The ability of eGFR to track changes in mGFR is illustrated in the four-quadrant plot (Figure 13). The concordance rate was 72%.

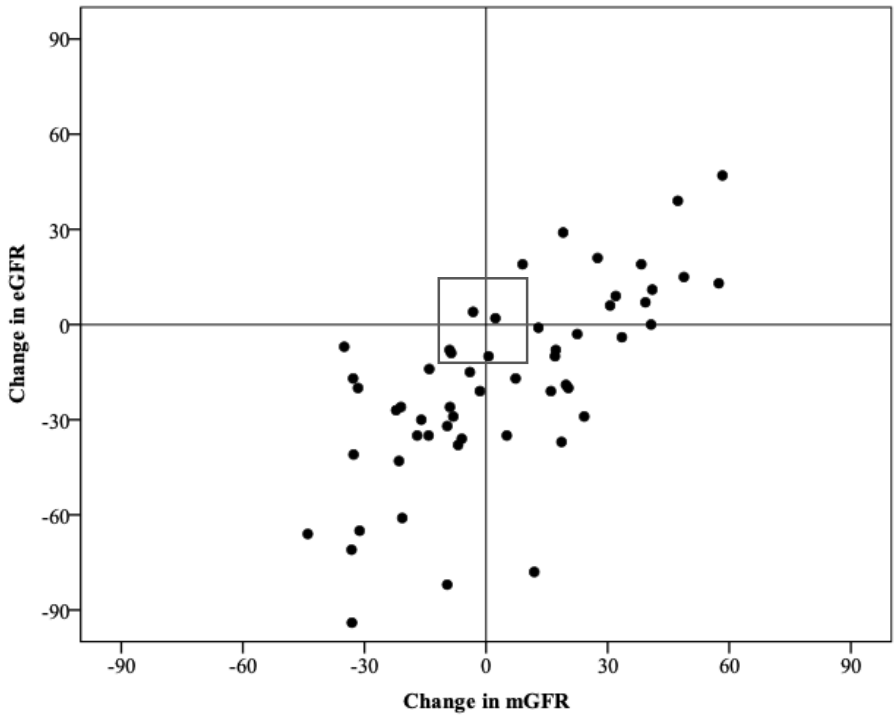


Figure 13. Four quadrant plot showing the concordance between changes in eGFR and changes in mGFR. The dots represent the change in eGFR and mGFR noted for each patient. The small square in the middle is an exclusion zone.

5 DISCUSSION

5.1 Ethical issues

Ethical approval was obtained for all the studies. For Papers II and III, approval was also obtained from the Swedish Medical Products Agency. Furthermore, for Papers I, II and III, approval was obtained from the Radiation Safety Committee of our hospital. All the studies were registered at ClinicalTrials.gov and the patients in the different studies were cared for in accordance with Good Clinical Practice and the Declaration of Helsinki.

In Papers I, III and IV, the patients were informed and enrolled in the study after they signed informed, written consent, before the surgery. They were given time to ask questions and consult with relatives.

In Paper II, the patients were informed and included in the study after they signed written informed consent, on Day 1 or 2 after the surgery. Two patients were not able to give informed consent, as they were sedated and treated with mechanical ventilation. In these two cases, informed consent was obtained from the next of kin.

In Paper I, a renal vein catheter was inserted through the left femoral vein. Renal vein catheterisation is a procedure that has been used in several studies performed at our institution, without any serious complications in experienced hands. The femoral vein was punctured using the guidance of vascular ultrasound, and the catheter was positioned in the renal vein by fluoroscopic guidance, through the use of small amounts of radio-contrast (Omnipaque) and a blood-gas analysis (renal vein oxygen saturation is very high at 85%–92%). Removal of the catheter was performed at the end of the experimental procedure according to standard procedure in our ward, i.e., manual pressure exerted for several minutes over the puncture site after removal of the catheter, followed by visual inspection for potential bleeding.

In Paper II, levosimendan or placebo was given as a standard intravenous continuous infusion. The line was checked for back flow and was pressure-controlled before initiation of infusion, to verify intravenous placement. Patients were in the cardiothoracic ICU and monitored continuously throughout the study. Since levosimendan is a commonly used inotropic drug that is known for its vasodilatory properties, the patients had norepinephrine connected to the central venous catheter for immediate treatment if hypotension should occur.

In Paper III, the patients were given ANP for 4–5 days as a continuous infusion, which was started immediately after induction of anaesthesia. ANP is an endogenous peptide that is produced by the human heart. It should be noted that ANP is not a commonly used drug in Sweden; it is used under individual license. Our department has performed several studies regarding the renal effects of ANP and has extensive experience with using this drug, also for clinical purposes. The infusion was delivered through a central venous line, which was checked for back flow and pressure-controlled before infusion initiation.

5.2 Methodological considerations

5.2.1 Infusion clearance of ^{51}Cr-EDTA and iohexol for GFR measurement

^{51}Cr-EDTA and iohexol are filtered freely in the nephron into the primary urine and are neither re-absorbed nor secreted at the tubular level. This means that the amount of ^{51}Cr-EDTA excreted in the urine is equivalent to the amount that is filtered in the glomeruli. By knowing the excreted amount of the filtration marker, the GFR can be calculated as: GFR = urinary excretion of the filtration marker divided by the serum concentration of the filtration marker. This is denoted as the urinary clearance technique, which is the gold standard for measurements of GFR. In Paper II, we used instead the infusion clearance technique, which assumes that, at steady state (i.e., when the serum concentration of the filtration marker is not changing during repeated measurements), the infused amount of the filtration marker is the same as the excreted amount. Thus, the GFR can be calculated as: GFR = infused amount of the filtration marker divided by the serum concentration of the filtration marker. Thus, the infusion clearance technique obviates the need for urine sampling. The infusion clearance technique has been validated against the urinary clearance technique, showing a high level of agreement, although the level of reproducibility was considerably higher with the former technique [60]. We confirmed the steady state before study drug administration using two baseline measurements without any difference in the serum level of ^{51}Cr-EDTA/iohexol. The renal clearance of iohexol has been found to be almost identical to that of ^{51}Cr-EDTA [61].

5.2.2 Infusion clearance of para-aminohippuric acid for measurement of renal blood flow

PAH is both filtered through the glomeruli and secreted at the tubular level. This means that almost all the PAH is eliminated from the blood as the blood

passes through the kidneys. Using the infusion clearance technique, the RPF can be calculated as follows: RPF = infused amount of PAH / (arterial PAH concentration-renal vein PAH concentration). In patients with healthy kidneys, the extraction of PAH is 90%, which means that the renal vein concentrations can be ignored. However, in patients who are undergoing uncomplicated cardiac surgery or in patients with post-cardiac surgery AKI, the extraction of PAH by the kidneys is considerably lower in the early post-operative phase (85% and 68%, respectively), most likely due to tubular injury/dysfunction [15, 44]. If not taking into account these low values of renal extraction of PAH, the RPF would have been severely underestimated. In Paper II, we assumed that the renal extraction of PAH was 70%. We also assumed that the treatment agent (levosimendan) itself did not affect the extraction of PAH, as supported by the unpublished findings of Bragadottir et al. [32]. The infusion clearance technique has been validated against the urinary clearance technique and found to have 20% higher RBF values, which suggests that PAH is eliminated by the extra-renal route. As for the GFR, the reproducibility of the infusion clearance technique was higher than that of the urinary clearance technique for PAH [44]. We confirmed the steady state before study drug administration with two baseline measurements without any difference in serum PAH level.

5.2.3 Near-infrared spectroscopy in medical use

The NIRS sensors used in our study were originally devised for monitoring oxygenation of the frontal lobe of the brain and, therefore, were equipped for placement on the forehead with penetration depths and exclusion zone designed for the brain tissue and the surrounding bone. The cerebral NIRS has been validated with invasive central venous saturation from the jugular vein, in a study to determine the arterial/venous ratio [62].

The expected mean penetration depth of the NIRS signal is <2 cm in commercially available systems, whereas in adult patients the kidney is often located more than 2 cm from the skin surface [63]. However, we do not know how deep into the tissue the signal actually travels. If it takes a shallow path, the signal could give us mixed information from both the kidney and overlying tissue, or it could be just a signal from the sub-cutaneous tissue. If the signal penetrates too deeply, it could pass through the renal medulla with its low oxygen content. Oxygenation of the renal medulla is over-estimated by measurements made following renal vein blood sampling because the renal vein blood sample is a mixture of blood from the cortex and medulla, and the cortex is hyper-perfused with low oxygen consumption, as compared to the medulla [64]. Furthermore, in patients with a longer distance from the skin to

the kidney, there is a risk of the sensor sliding out of position when the operating table is slanted.

5.3 Assessment of renal oxygenation by renal near-infrared spectroscopy during cardiac surgery with cardiopulmonary bypass (Paper I)

In this study of adult patients during open cardiac surgery with CPB, we compared the values of invasively measured renal vein oxygen saturation ($SrvO_2$) with those obtained with the non-invasive regional tissue oximetry sensor (NIRS) placed over the kidney. The two measurement methods correlated well both on and off CPB, showed good agreement with low biases, and had a low between-method error. The NIRS monitor was also able to track the same changes in O_2 saturation that were detected by the renal vein blood gas sampling, at various CPB pump flow rates [65]. The renal rSO_2 was lower than the $SrvO_2$, suggesting that the NIRS signal passed, at least partly, thorough the less-oxygenated medulla. This is highly interesting and important, as the renal medulla is highly sensitive to a reduction in oxygen delivery [64].

Several previous studies have investigated the use of NIRS to assess renal oxygenation. In a study conducted by Ortmann et al. [66], renal rSO_2, as measured by the NIRS technique, was compared with invasive measurements from the renal vein during cardiac catheterisation in small children. They found a strong correlation in small children weighing <10 kg but no correlation in children who weighed >10 kg. In small children, the skin-to-kidney distance is short, which would explain why no correlation was seen in the larger children. However, there are studies on the use of renal NIRS in adults for the measurement of renal oxygenation. Thus, Choi et al. [67] have shown that an intra-operative decrease in the renal NIRS signal can be a good predictor of post-cardiac surgery AKI in adults. Furthermore, Ortega-Loubon et al. [68] have shown that post-operative renal oxygenation, evaluated by NIRS, is associated with the development of cardiac surgery-associated AKI.

5.4 Effects of levosimendan in acute kidney injury after cardiac surgery (Paper II)

In this randomised, blinded study, we tested the effect of the inodilator (with inotropic and vasodilating effects) levosimendan on patients with AKI early after cardiac surgery. To the best of our knowledge, this is the first study on the effects of levosimendan on RBF and GFR in patients with AKI. We concluded that levosimendan increased RBF and reduced RVR, but had no

significant effect on the GFR. Furthermore, levosimendan caused a decrease in the renal filtration fraction. These findings suggest that in patients with post-cardiac surgery AKI, levosimendan causes dilatation of both afferent and efferent glomerular arterioles. These findings are in contrast to the results of previous studies on the renal effects of levosimendan in patients after uncomplicated cardiac surgery without AKI [32], in patients with acute heart failure [69], and in patients with congestive heart failure and cardio-renal syndrome [29]. Those three reports have shown that the increase in cardiac output (CO) is accompanied by renal vasodilation and an increase in GFR, indicating dilatation mainly of the afferent arterioles.

Why did we not see an increase in the GFR with levosimendan, as previously demonstrated? In AKI after cardiac surgery, there is a pronounced increase in the RVR [15], and although we did see a reduction in resistance in the levosimendan group, it could be that the levosimendan dosage chosen was not sufficiently high to overcome intense constriction of the afferent arterioles, as seen in the patients with AKI [15]. There was also, despite randomisation, an imbalance between the groups with respect to the level of renal function before the start of the experimental procedure. The levosimendan group had 25% lower GFR at baseline, and AKI grade 2 was seen in five patients (the remaining included patients all had AKI grade 1). Thus, some patients in the levosimendan group may have had an ongoing decline in GFR, during the study procedure, which could have masked an increase in GFR induced by levosimendan. In addition, the renin-angiotensin system is activated in AKI [70], which may, at least partly, explain the increased RVR seen in post-cardiac surgery AKI [15]. Angiotensin II causes, aside from vasoconstriction, contraction of the glomerular mesangial cells, leading to a decrease in glomerular filtration. Zager et al. [71] have shown that levosimendan, in experimental studies, inhibits the angiotensin II-induced mesangial cell contraction, leading to an increase in the glomerular ultra-filtration rate. One can speculate that the dose of levosimendan used in Paper II was too low to counteract this angiotensin II-induced mesangial cell contraction.

Nonetheless, our findings are in line with the outcomes of the LICORN [72] and LEVO-CTS [73] studies, which concluded that levosimendan did not exert a renoprotective effect in cardiac surgery patients.

5.5 Effects of ANP, used as a prophylactic drug, on kidney function after heart transplantation (Paper III)

In this placebo-controlled, blinded, randomised study, we tested whether ANP given as a prophylactic infusion at the time for heart transplantation exerted

reno-protective effects. The study did not show any difference in the two groups regarding the primary outcome, i.e., mGFR on Day 4 after heart transplantation. Thus, ANP did not attenuate renal dysfunction or decrease the incidence of AKI.

Previous studies on ANP used for the treatment of AKI, after cardiac surgery and acute kidney dysfunction caused by cyclosporine, and after heart transplantation [40-43], have shown that ANP infusion (50–100 ng/kg/min) increases RBF and GFR, suggesting ANP-mediated dilatation mainly of the afferent arterioles of the glomeruli. However, these studies were conducted on patients with existing AKI, i.e., ANP was given therapeutically rather than prophylactically (as in the present study).

There was a high incidence of AKI in our study (67%) and a high frequency of the need for dialysis (16%), although the mGFR on Day 4 was only marginally reduced compared to the pre-operative measurements. This is most likely due to the increase in cardiac output (CO) and the overall haemodynamic improvement that results from a new heart. The post-operative CO in our material was 40% higher than the pre-operative measurements.

Why did we not see any ANP-induced beneficial effect on renal function? This may be because a high number of our patients had reduced kidney function before their heart transplantation surgery (56% had a pre-operative GFR <60 mL/min/1.73 m^2), i.e., they suffered chronic renal failure. Chronic renal failure has a different aetiology than AKI and may not be susceptible to vasodilatation agents in the same way or at the same dosage as patients with AKI. In chronic kidney failure, renal oxygenation is impaired [74-76]. Chronic hypoxia leads to fibrogenesis and renal fibrosis [76]. This, in turn, causes a reduction in renal vascular density (rarefaction) [77, 78], with a consequent increase in renal vascular resistance and lower RBF. Such structural changes to the renal vascular bed may not be adequately amenable/accessible to vasodilator treatment.

High doses of ANP cause systemic vasodilatation and, thereby, hypotension [79, 80]. Hypotension may compromise renal perfusion, which is especially damaging in cases of acute renal failure when the renal blood flow auto-regulation is lost [81]. In the present study, we used low doses (four-fold lower than the dose needed to achieve systemic vasodilatation). The dose needed to induce systemic dilatation is about 200 ng/kg/min. There was a tendency in the present study toward a slightly higher norepinephrine dose (total over the first 3 days, including the time in the operating room) in the ANP group, although the difference was not statistically significant. There was no difference in mean

arterial pressure (MAP), intra-operatively or on arrival to the ICU), and there was no difference in the reported incidence of hypotension.

5.6 Evaluating the use and accuracy of estimated GFR for assessment of the true, measured GFR after heart transplantation (Paper IV)

In this study, we evaluated the agreement between eGFR and mGFR early after heart transplantation. The eGFR was calculated using the CKD-EPI formula and mGFR was assessed with the ^{51}Cr-EDTA/iohexol clearance technique. For critically ill patients with AKI, the CKD-EPI formula has, to our knowledge, been tested in only one previous study [82]. Patients after cardiac surgery and heart transplantation are often haemodiluted due to the use of CPB and peri-operative fluid treatment, which leads to dilution of the serum creatinine. This means that any eGFR formula based on serum creatinine would over-estimate the GFR. In our study, however, we noted that eGFR underestimated mGFR by approximately 11 mL/min/1.73 m^2. This may be explained by the fact that the level of serum creatinine lags behind a decline or improvement in renal function. Furthermore, the agreement between eGFR and mGFR was poor, and there was a large between-methods error of 58%. Critchley and Crichley, in an effort to define the criteria for a good level of agreement between two methods, proposed that the between-group error should be <30% [57]. All of these findings indicate that although there is a significant correlation between eGFR and mGFR, the low-level accuracy, the wide LOA, and the high error tell us that there is poor agreement between eGFR and mGFR early after heart transplantation

Estimating equations for the assessment of renal function should also have the ability to track *changes* in renal function. In the present study, such a direction of change analysis was performed by calculation of the concordance rate, defined as the percentage of all GFR measurements (eGFR and mGFR) that correctly change in the same direction, i.e., appear in the right-upper or left-lower quadrant of the four-quadrant plot in Figure 13. Low concordance was particularly seen when true renal function was improved post-operatively. As can be seen in Figure 13, 26 patients improved their renal function (mGFR) post-operatively by >5 mL/min/1.73 m^2 (upper- and lower-right quadrants). This improvement in mGFR was tracked by eGFR in only 12 patients (46%) (upper-right quadrant), while, if anything, a dis-concordant fall in renal function was tracked by eGFR in 13 patients (50%) (lower-right quadrant). Thus, there is a high risk (≈50%) that a post-operative improvement in renal function is not captured by eGFR, but may instead even signal a decrease in GFR. The clinical consequence of using eGFR for the assessment of renal

function early after Htx is, therefore, that there is a high risk of under-dosing drugs whose elimination is dependent upon renal function, and this is particularly important for the early initiation of immunosuppressive agents. On the other hand, eGFR performed better in the assessment of a decrease in mGFR, post-operatively. In the lower-left quadrant of Figure 13, it can be seen that mGFR was impaired by >5 mL/min/1.73 m^2 in 24 patients early after surgery. This decrease in mGFR was tracked by a decrease in eGFR in all the patients.

6 CONCLUSION

- Renal tissue oxygenation, as assessed by near-infrared spectroscopy, correlates well with renal venous saturation before, during and after cardiopulmonary bypass. There is good agreement between the two methods, with low-level bias and low between-method errors.

- Levosimendan induces renal vasodilation and improves renal blood flow in patients with acute kidney injury after cardiac surgery, whereas it has little or no effect on the glomerular filtration rate.

- ANP administered prophylactically during heart transplantation and continued for 4 days post-operatively does not seem to attenuate the renal dysfunction or decrease the incidence of AKI often seen after heart transplantation.

- After heart transplantation, the eGFR underestimates the mGFR, and the level of agreement between the two methods is low with large between-group errors and low-level accuracy. The ability of the eGFR to track changes in the mGFR was also poor. Thus, eGFR is not a valid marker for the assessment of renal function in patients who have undergone heart transplantation.

7 FUTURE PERSPECTIVES

Since acute kidney injury (AKI) is such a common and serious problem after cardiac surgery and heart transplantation it is important to have ways to monitor the kidneys during and after surgery and also find ways to prevent and treat AKI. This is essential for the care of cardiac surgery and heart transplant patients.

In this thesis we have contributed to the knowledge on the use of near-infrared spectroscopy (NIRS) to assess renal oxygenation during cardiopulmonary bypass (CPB). It was shown that the NIRS signal accurately tracked changes in renal oxygenation both on and off CPB. Whether intra-operative changes in renal oxygenation, traced by the NIRS sensor, can be correlated to AKI needs to be studied further. Furthermore, it would be interesting to investigate whether intra-operative monitoring of renal oxygenation, guiding potential treatments of low values of renal oxygen saturation, could decrease the incidence of postoperative acute kidney injury.

In patients with AKI after cardiac surgery, it was shown that the inotropic agent, levosimendan, reduced renal vascular resistance and increased renal blood flow. This is interesting, as it is known that the kidneys in post-cardiac surgical AKI are hypo perfused because of a high renal vascular resistance. Whether the treatment of post-cardiac surgical AKI, with levosimendan, may improve renal outcome should be tested in a randomised trial.

In Paper III the human atrial peptide (ANP) was given prophylactically for reno-protection to heart transplant patients at the time of the transplantation surgery. It was concluded that ANP failed to detect an attenuation of renal dysfunction. As this was a relatively small single-centre trial, a larger multi-centre study should be performed to finally assess the role of ANP for reno-protection in patients undergoing heart transplantation.

The findings that estimated glomerular filtration rate (eGFR) could not accurately estimate true measured GFR (mGFR) or accurately track postoperative changes in mGFR are important. These findings imply that for the clinical management of these patients or for scientific purposes, e.g. in the evaluation of therapeutic interventions to improve renal function, GFR should be measured and not estimated from serum creatinine concentrations by the use of mathematical formulas.