Renal Function in Cardiac Surgery

Clinical and Experimental Studies

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Cover illustration:

The Heart, the Kidney and the Machine.

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Effort counts twice

-A. Duckworth

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ABSTRACT

Background: Impaired renal function (measured as glomerular filtration rate (GFR)) is a well-known problem after heart surgery and heart transplantation (HTx), affecting both short-term and long-term survival. The reason for this is multifactorial, but the use of heart-lung machine and cardiopulmonary bypass (CPB) is thought to be one of the causes. Whatever the cause, impaired kidney function after heart surgery is an important clinical problem.

Aims: We wanted to investigate whether estimated GFR could replace measured GFR in the follow-up of HTx recipients and to assess the renal and survival outcome in our entire cohort of HTx patients. Also, we wanted to investigate the potential renoprotective effects of ANP in an experimental model of CPB, and to compare the renal effects of a colloid-based CPB-prime versus a crystalloid-based prime in adult patients undergoing heart surgery.

Methods: Retrospective registry studies were performed to evaluate the agreement of three major estimation formulas for GFR to the measured values in about 400 HTx recipients. An animal study on 20 pigs was designed to compare the renal effects of ANP during CPB. A randomized controlled trial with 80 adult patients undergoing cardiac surgery was performed to compare the renal effects of a dextran 40-based fluid to a conventional crystalloid-based fluid (Ringer-Acetate and mannitol) when used as priming solutions in the CPB circuit.

Results: The agreement between estimated and measured GFR was very low, with a percentage error around 100%. Moreover, pre-HTx GFR did not predict mortality in our cohort. In our pig model, ANP increased GFR during CPB ($p<0.0001$) without increasing renal oxygen consumption. The patients receiving dextran 40-based priming solution in the heart-lung machine had lower levels of the tubular injury marker NAG in their urine than the patients receiving crystalloid prime ($p=0.045$).

Conclusions: Measured, not estimated, GFR should be used when assessing kidney function in HTx-patients. A GFR <30 ml/min/1.73m^2 should not automatically exclude heart failure patients from HTx-evaluation. ANP is a drug with potential renoprotective properties that should be investigated further. A dextran 40-based priming solution seems to induce less renal tubular damage than crystalloid-based prime, and should be investigated further, specifically in patients with a preoperatively impaired kidney function.

Keywords: Cardiopulmonary bypass, kidney function, acute kidney injury

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SAMMANFATTNING PÅ SVENSKA

Bakgrund: Njursvikt efter hjärtkirurgi med hjärt-lungmaskin är ett vanligt och allvarligt problem, som kan påverka överlevnaden efter en hjärtoperation. Orsakerna till den negativa effekten på njurfunktionen är många, men en av dem anses vara hjärt-lungmaskinen i sig. Hos hjärttransplanterade patienter anses även den livslånga behandlingen med mediciner som behövs mot avstötningen ha skadliga effekter på njuren på lång sikt.

Mål: Vi ville studera njurfunktionen hos hjärtopererade patienter ur flera synvinklar: Kan enklare metoder för att *beräkna* njurfunktion ersätta den dyrare metoden att *mäta* njurfunktion hos patienter som hjärttransplanterats? Och hur påverkar patienternas njurfunktion innan hjärt-transplantation överlevnaden och njurfunktionen efter transplantationen? Förmakspeptid (ANP) är ett läkemedel som används för att förbättra njurfunktionen vid njursvikt efter hjärtkirurgi. Kan ANP givet redan innan och under operationen förhindra att njursvikt uppkommer? Kan man reducera njurskadan om man i hjärt-lungmaskinen använder en priming-vätska med högre kolloidosmotiskt tryck, och som därmed liknar kroppens egna vätskor mera?

Metoder: Vi använde retrospektiva registerstudier för att jämföra beräknad och uppmätt njurfunktion hos hjärttransplanterade patienter, samt för att undersöka njurfunktionens betydelse för överlevnaden. För at undersöka ANPs effekter på njuren under hjärtoperation med hjärt-lungmaskin, utvecklade vi en stordjursmodell på gris. I en randomiserad studie på patienter som behövde hjärtoperation jämförde vi hur två olika vätskor i hjärt-lungmaskinen påverkade njurfunktionen.

Resultat: Den beräknade njurfunktionen stämde mycket dåligt överens med den uppmätta njurfunktionen. Vi fann även att njurfunktionen innan hjärttransplantationen inte påverkade överlevnaden efteråt. I vår studie på grisar fann vi tecken på att ANP har en njur-skyddande effekt, och en vätska med högre kolloidosmotiskt tryck i hjärt-lungmaskinen orsakade mindre njurskada än den vanliga vätskan.

Konklusioner: När man fattar beslut om patienter som skall bli, eller har blivit, hjärttransplanterade, bör njurfunktionen mätas, inte beräknas. Dessutom bör en dålig njurfunktion inte utesluta en patient med svår hjärtsvikt från transplantationsutredning. ANPs möjliga njur-skyddande effekt bör utredas närmare, och priming-vätskor med högre kolloidosmotiskt bör även provas på patienter med sämre njurfunktion.

LIST OF PAPERS

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

I. Kolsrud O, Ricksten SE, Holmberg E, Felldin M, Karason K, Hammarsten O, Samuelsson O, Dellgren G.
Measured and not estimated glomerular filtration rate should be used to assess renal function in heart transplant recipients.
Nephrol Dial Transplant. 2016 Jul;31(7):1182-9.

II. Kolsrud O, Karason K, Holmberg E, Ricksten SE, Felldin M, Samuelsson O, Dellgren G.
Renal function and outcome after heart transplantation.
J Thorac Cardiovasc Surg. 2018 Apr;155(4):1593-1604

III. Kolsrud O, Damén T, Nygren A, Ricksten SE, Tholén M, Hjärpe A, Laffin A, Dellgren G.
The effects of atrial natriuretic peptide on renal function during cardiopulmonary bypass: A randomized blinded study in a pig model.
In manuscript.

IV. Kolsrud O, Barbu M, Dellgren G, Sigvardsson AL, Björk K, Corderfeldt A, Jeppsson A, Ricksten SE.
Renal effects of dextran-based versus crystalloid-based priming solution in cardiopulmonary bypass: A randomized controlled study in adult cardiac surgery patients.
In manuscript.

CONTENTS

ABBREVIATIONS

AKI	Acute kidney injury
AKIN	Acute kidney injury network
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
ARDS	Acute respiratory distress syndrome
ATN	Acute tubular necrosis
BSA	Body surface area
CKD	Chronic kidney disease
CKD-EPI	Chronic kidney disease epidemiology collaboration
CNI	Calcineurin inhibitors
CONSORT	Consolidated standards of reporting trials
CPB	Cardiopulmonary bypass
^{51}Cr-EDTA	Chromium ethylenediaminetetraacetic acid
CRRT	Continuous renal replacement therapy
CVP	Central venous pressure
eGFR	Estimated GFR
ESRD	End-stage renal disease
FF	Filtration fraction
GFR	Glomerular filtration rate
HTx	Heart transplantation

ISHLT	International Society for Heart and Lung Transplantation
kDa	KiloDalton
KDIGO	Kidney disease improving global outcomes
KTx	Kidney transplantation
LDF	Laser Doppler flowmetry
MAP	Mean arterial pressure
MDRD	Modification of diet in renal disease
mGFR	Measured GFR
NAG	N-acetyl-β-glucosaminidase
RBF	Renal blood flow
RDO_2	Renal oxygen delivery
RIFLE	Risk, injury, failure, loss of kidney function, and ESRD
RO_2Ex	Renal oxygen extraction
RPF	Renal plasma flow
RVO_2	Renal oxygen consumption
RVR	Renal vascular resistance
SIRS	Systemic inflammatory response syndrome
VAD	Ventricular assist device

INTRODUCTION

Heart surgery

Background

Since its wavering beginnings in the late 40'ies, cardiac surgery has gone through a tremendous development, both technologically and demographically. The high gains and eventually good outcomes have turned it into a surprisingly safe routine procedure performed on hundreds of thousands of patients of all ages annually. The range of heart diseases now accessible to surgical correction spans over a wide variety of both congenital and acquired diseases, among them ischemic heart disease, the single most common cause of death worldwide[1]. When performed on the correct selection of patients, heart surgery prolongs life, reduces morbidity and relieves symptoms[2-4]. Figure 1 on opposite page depicts a typical operating room while preparing for heart surgery.

Regular heart surgery

The high prevalence of heart diseases in the population, in particular the acquired diseases like ischemic heart disease or aortic valve stenosis, continues to be the foundation for the majority of the surgical heart procedures, e.g. coronary artery bypass grafting or aortic valve replacement. Also, the correction of other heart valve diseases like stenosis or regurgitation of the mitral valve or of aneurysms of the ascending aorta, all potentially deadly afflictions, adds to the number of diagnoses that can be successfully remediated.

The correction of congenital heart disease, often in very young patients like new-borns or toddlers, is numerically performed in smaller volumes than surgery on the acquired diseases are, but the gain in life-years is, not surprisingly, much more pronounced when correcting congenital defects than in surgery aimed at the acquired diseases in a much older population[5,6].

Heart transplantation

The possibility to remove a terminally failing heart and replace it with a new, healthier one, stands in a special position within surgical treatment for heart diseases. The first human-to-human heart transplantation was performed by Christiaan Barnard in Cape Town, South Africa, 1967. It is important to remember that initially, this ultimate surgical procedure did not perform particularly well, and with discouraging results with few patients surviving more than a year after transplantation, the procedure quickly fell out of favour. However, with the advent of modern immunosuppressant therapy, in particular the calcineurin inhibitors (CNI), heart transplantation was

transformed into an established treatment for terminal heart failure during the 1980'ies[7].

Though this treatment is performed in much smaller volumes than other heart surgery, the immunosuppressive regimen of today have improved long-term survival dramatically, adding 10 life-years or more to 60 % of patients who were otherwise expected to live only for a few years, or even months, with the agonizing symptoms of a terminally failing heart, and has thus become a treatment of tremendous importance for the individual patient[8].

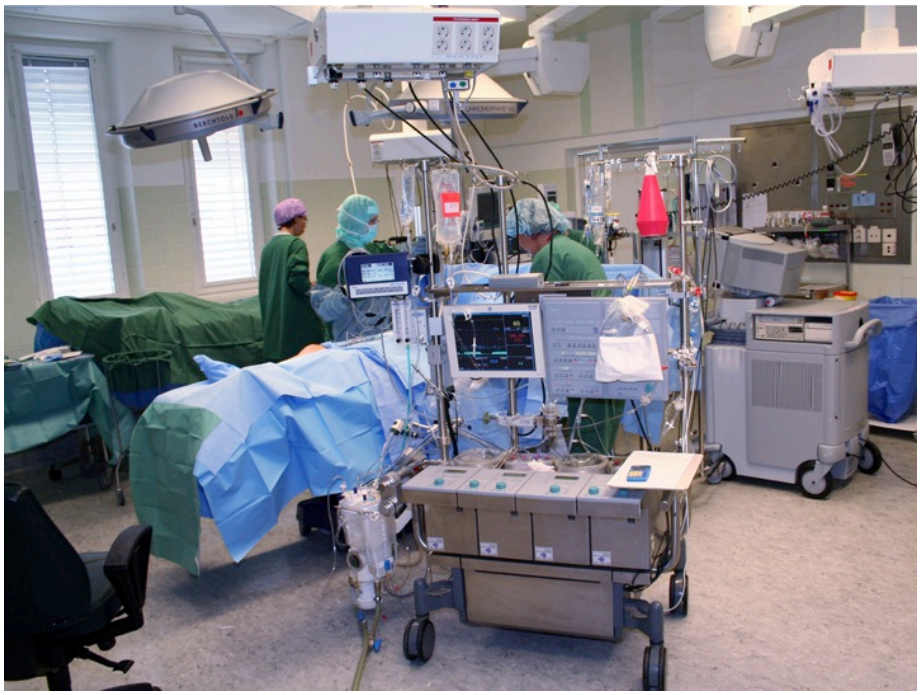

Figure 1: Picture from the author's workplace showing a typical operating room while preparing for heart surgery. The heart-lung machine is in the front, with reservoir and oxygenator (in white) on lower left side. (Photo by Carl Johan Malm, 2012).

The heart-lung machine

History

Modern day heart surgery would be unconceivable without the invention of the heart-lung machine. John Gibbon is credited with developing the first heart-lung machine, and performed the first successful operation using such a machine in 1953 in Philadelphia, USA, when he corrected a congenital heart defect, an atrial septal defect, on an 18-year-old girl[9]. This technological breakthrough provided the surgeons with the possibility to operate on an empty, flaccid heart for a prolonged period of time, while the rest of the patient's body was perfused with oxygenated blood. This opened up the field of heart surgery to much more complex and delicate procedures than had previously been possible. Needless to say, heart transplantation would be impossible without such an invention.

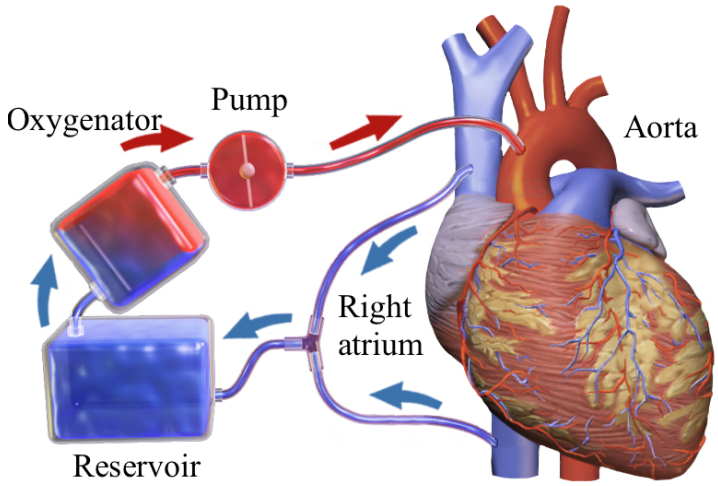

Figure 2: General principle of the heart-lung machine. Desaturated (oxygen-poor) blood is drained from the right atrium of the heart, collected in a reservoir, passed through an oxygenator and finally pumped back into the aorta. (Ill: Blausen medical communications, Inc., CC Creative Commons.)

Technique

During the typical cardiac surgery procedure, after opening the chest, the surgeon connects the heart-lung machine by cannulas to the ascending aorta and the right atrium of the heart (though other variants for cannulation are also possible). The desaturated (oxygen depleted), venous blood is drawn from the right atrium, drained into the reservoir of the machine, passed through an oxygenator and finally pumped back into the ascending aorta, completing a standard cardiopulmonary bypass (CPB) circuit (Figure 2). The dimensions of the cannulas and tubes in the system, as well as the pump speed, are chosen and adjusted to provide a "full CPB blood flow", i.e. totally alleviating the heart from the requirement of performing any pumping action to keep the tissues of the body oxygenated. Thereafter, the surgeon can exclude the heart from the systemic circulation by clamping the ascending aorta, induce asystole by injecting cardioplegia (usually a solution with a high concentration of potassium), and perform the planned surgery on a bloodless, motionless heart. Whether the heart-lung machine should mimic the heart and deliver a pulsative flow, or whether it suffices to have a steady, non-pulsative flow, is not known. The effects of pulsative versus non-pulsative flow on the organs are still, more than sixty years after the invention of the machine, being debated[10]. (At the Sahlgrenska University Hospital, non-pulsative flow is routinely being used during CPB.) At completion of the corrective procedure, the surgeon removes the aortic clamp to allow the blood to flow into the coronary circulation again, and through the ensuing washout of cardioplegia from the myocardium the beating action of the heart will automatically return. When the heart has regained it's rhythm and strength, the patient is weaned from the heart-lung machine, and the surgeon can remove the cannulas from the right atrium and the ascending aorta and close the chest.

The priming

Before commencing CPB, the reservoir and tubes of the heart-lung machine obviously have to be pre-filled, *primed,* with fluid of some sort to avoid the pumping of air into the circulation. To prime the system about 1200 ml of fluid is usually required. At initiation of CPB, this fluid will be pumped into the patients' circulation, and will mix with the patients' blood. Consequently, it seems reasonable that the composition of this priming fluid should to some degree mirror that of the blood and bodily fluids. Most priming fluids therefore consist of a solution with a physiological concentration of electrolytes (i.e. a crystalloid solution), such as the Ringer solution, often with additives such as heparin or mannitol. Since the composition of human plasma is much more complex, and also contains a whole range of *colloids*

(larger molecules unable to pass freely over the cell- or basal membranes), the addition of colloids to the priming solution is also used in many centres[11,12].

Such colloids generate oncotic (or *colloid osmotic*) pressure in the fluids in the body. The higher the concentration is of these molecules, the higher the *oncotic pressure*. Somewhat semantically confusing, the oncotic pressure is actually a negative pressure, sucking water and electrolytes across a semipermeable barrier from solutions with a *lower* oncotic pressure to solutions with a *higher* oncotic pressure. In the body, the semipermeable barriers separating these solutions are the cell- and basal membranes of the capillaries, such as the glomeruli in the kidneys (see kidney physiology section). In order to exert oncotic pressure, the molecules have to be bigger than the gaps in these semipermeable borders. Of particular interest in this thesis is the role of oncotic pressure in the renal circulation (see Paper IV). The glomerular membrane allow molecules smaller than 6 kDa (kiloDalton) to pass unhindered, but is virtually impenetrable to molecules larger than 60 kDa[13]. The most important natural colloid is albumin, a 69-kDa protein synthesized in the liver that constitutes almost 80% of the oncotic pressure of plasma[14].

However, systematic and definitive comparisons of crystalloid-based versus colloid-based priming solutions are scarce, and further studies that could help determine the composition of the optimal priming solution are warranted[15].

Concerns

However marvellous the invention of the heart-lung machine, the exposure of the blood to this kind of *extracorporeal circulation*, also has numerous negative side effects. Not being lined with vascular endothelium, the inner surfaces of the tubes and reservoirs of the machine are perceived as a giant foreign body by the several litres of blood that passes through the system each minute. This blood–to-foreign-body contact unleashes a host of inflammatory responses[16]: the intrinsic coagulation pathway; the fibrinolytic system; and the complement system are all activated. Cytokines like TNF, IL-1, IL-6 and IL-8 are released, and there is cellular activation of platelets, endothelial cells and leukocytes. The ensuing systemic inflammatory response is believed to be the cause of the multiple adverse effects observed in several different organ systems after CPB. These are described in brevity below:

Vasodilatory shock/vasoplegia

A profound loss of vascular tone and accompanying hypotension (MAP<50mmHg) poorly responsive to conventional vasopressor treatment is called vasoplegia, and seen in 5%-25% of patients immediately after CPB. The condition is thought to be caused by cytokine-induced activation of iNO-synthase and ATP-dependent potassium channels, resulting in hyper-polarization of endovascular membrane and release of the vasodilator substance nitric oxide (NO)[17]. The mortality has been reported to be as high as 10%[18].

Coagulopathy

Needless to say, bleeding is a common and important problem in heart surgery. Adding harm to injury, the use of CPB affects and impairs haemostasis on several levels. The CPB priming volume causes haemodilution and reduction of platelet count, and the CPB itself induces thrombocyte dysfunction and increased fibrinolysis[19]. Around 5 % of patients undergoing heart surgery in Sweden need reexploration for bleeding[20], and excessive bleeding is, not surprisingly, associated with increased risk for mortality[21,22].

Neurocognitive dysfunction

Postoperative delirium is a condition that engages medical staff of all categories that are involved in the postoperative care of heart surgery patients. The patients that are affected can experience delusions, hallucinations or paranoia that luckily usually subsides spontaneously within days. Different studies have shown that it affects between 3-31%[23] of heart surgery patients and believed to be at least partially caused by the systemic inflammation disrupting the blood brain barrier, leading to a neuroinflammatory process[24].

A subtler but more long-standing cognitive impairment than delirium is the effects on memory, attention and language comprehension, on both short-term and long term[25]. 40% of patients have been reported to have measurable cognitive decline 5 years after surgery. The aetiology of this negative cognitive effect is believed to be cerebral microembolisation of lipid-, gas- or other particulate origin[26].

Pulmonary dysfunction

After CPB, impaired lung function is seen in as many as 12%, with another 1.3% developing the more severe *adult respiratory distress syndrome* (ARDS)[27]. About 5% of patients in Sweden need mechanical ventilation for

more than 48 hours after heart surgery[20]. Again, an inflammatory response seems to be the cause. Activated neutrophils and matrix metalloproteinases, increased myeloperoxidase, IL-8 and elastase levels all contribute to destruction of lung tissue[28-30]. The majority of these patients recover, but severe lung injury has been shown to have a mortality of over 50%[31].

Kidney dysfunction

Impaired renal function after heart surgery and CPB, acute or chronic, is seen in up to almost a third of patients, and is associated with prolonged hospitalization, ICU stay and even increased mortality[32-36].

The effect of heart surgery on renal function lies at the heart of this thesis, and kidney dysfunction and physiology are therefore described in more detail in the following sections.

Kidney dysfunction after cardiac surgery

Background

The development of acute kidney injury (AKI) is a well-known complication after heart surgery and cardiopulmonary bypass (CPB). Up to 30% of patients is affected and the condition is associated with a fivefold increase in mortality[32-35]. If a patient develop an AKI so severe that renal replacement therapy (RRT/dialysis) is needed, the condition is associated with a 30-day mortality of up to 50 %[36]. In Sweden, 2.5% of patients undergoing cardiac surgery in 2016 developed this most severe form of AKI[20].

Even if heart transplant recipients do not develop AKI in the immediate conjunction with the transplantation, they are still at risk for developing *chronic* kidney disease later on, often after several years. The prevalence of this condition increases over time[37] and consequently becomes an increasingly important cause of death as time goes by. After 10 years, almost 10% of deaths are attributed to renal failure[8].

The special importance of GFR in heart transplantation

Renal function plays a particular role in solid-organ transplantation of any kind, not only in patients undergoing kidney transplantation[37]. The problem of renal dysfunction after solid-organ transplantation (i.e. not including blood transfusion or bone marrow transplantation) was well known already at the beginning of the HTx era, based on experiences from liver-, lung- and kidney transplantation. With this in mind, renal dysfunction was already from the beginning considered a relative contraindication for HTx. The ISHLT guidelines for HTx still have a lower limit for renal function, below which the renal function is considered a relative contraindication for HTx. This means that when a patient is evaluated for HTx, a low GFR will usually be weighed in as a negative factor, implying that the patient could be denied listing for HTx. This is an understandable strategy, since the early HTx-attempts in the 60-ies and 70-ies had demonstrated discouraging results in long-term survival, and excluding patients with renal dysfunction was conceived as one way to improve the results. However, controversy still remains about the exact level at which a reduced GFR (Glomerular Filtration Rate, see 1.4.2) should be considered a risk factor for transplantation. At the 24th Bethesda conference in 1993, a creatinine clearance less than 50 ml/min was considered secondary exclusion criteria for HTx[38]. In 2006, the International Society of Heart and Lung Transplantation (ISHLT) recommended that a pre-transplant GFR of 40 ml/min/1.73m^2 or less should

be considered as a relative contraindication to HTx[39]. In the ISHLT 2016 guidelines[40] this level was further reduced to 30 ml/min/1.73m^2.

Complicating the consideration of pre-HTx renal function is the possibility of a pre-renal cause of the condition, i.e. the heart failure itself. The kidneys might be suffering from the low cardiac output, but may be otherwise unaffected, and will regain their normal function once the failing heart is exchanged for a new, healthier one.

More research regarding the importance of pre-HTx renal function and its effect on outcome after transplantation is needed to facilitate decisions regarding which patients should be listed for HTx, and how to handle their renal function post-transplantation, both short-term, immediately after HTx and long-term, in the years afterward. The importance of pre-HTx kidney function is a particular subject of interest in Paper I and Paper II.

Acute Kidney Injury

Definition

The general definition of acute kidney injury (AKI) is a syndrome featured by a relatively rapid loss of renal excretory function diagnosed by decreased urine output or accumulation of nitrogen metabolites, or both. However, the more specific definition of this condition has undergone several revisions over the years, and the lack of a uniform definition has made the epidemiology of AKI difficult to assess with precision. The earlier RIFLE criteria[41] (Risk, Injury, Failure, with Loss of kidney function and End-stage kidney disease as outcome criteria) and AKIN-criteria[42] (the Acute Kidney Injury Network) have now been combined into the KDIGO criteria (Kidney Disease: Improving Global Outcomes). What they all have in common is a defined increase in serum creatinine from baseline within a defined timespan[43]. The KDIGO criteria now defines AKI according to the following criteria[44]:

- Increase in serum creatinine by ≥26.5 µmol/l within 48 hours; or
- Increase in serum creatinine by ≥1.5 times baseline which is known or presumed to have occurred within the prior 7 days; or
- Urine volume <0.5ml/kg/hour for 6 hours

Pathophysiology

The development of surgery-associated AKI is considered to be a multifactorial process. In *cardiac* surgery the patient is not only exposed to a major surgical trauma, but also to the use of cardiopulmonary bypass. The use of heart-lung machine/CPB has been shown to trigger the systemic inflammatory response syndrome (SIRS) and to contribute to haemolysis and micro-embolization[45], all with negative renal effects. In addition, haemodilution, hypotension, renal vasoconstriction and impaired auto-regulation of renal blood flow during CPB also have the potential to impair oxygen delivery to the kidneys[46-48]. The renal medulla, utilizing large amounts of oxygen for tubular sodium reabsorption (renal physiology section), is on the verge of hypoxia already under normal conditions and therefore particularly susceptible to acute renal ischemia[49].

Ischemic damage to the endothelial cells in the renal microvasculature leads to cellular and interstitial oedema, and to cell debris being shed into the lumen[50]. This causes further impairment of renal perfusion, and the resulting medullar ischemia can cause the tubular epithelial cells to die, a condition known as acute tubular necrosis (ATN). The normal functions of the tubules are consequently disrupted as they are no longer are able to perform their reabsorptive or excretory functions. Necrotic tubular cells are shed into the tubular lumen, causing obstruction of the nephrons[51].

In the pursuit to find a clinical biomarker to detect ATN, N-acetyl-β-glucosaminidase (NAG) has been identified, amongst others. NAG is a lysosomal enzyme produced in tubular cells, mostly in the proximal tubules in the renal medulla. Urinary secretion of NAG has been shown to be associated with tubular necrosis in cardiac surgery patients, and analysis of urinary NAG can thus be used for detecting tubular damage[52-55].

Chronic kidney disease

According to KDIGO, chronic kidney disease (CKD) is defined as a GFR less than 60 ml/min/1.73m2 (if no other markers of kidney disease)[56], and kidney failure is defined as GFR <15. However, over the years these definitions have varied.

As mentioned above, chronic renal failure is a problem in all solid-organ transplantations[37]. After heart transplantation, renal failure (in this case defined as GFR <30 ml/min/1.73m^2) has been reported in as many as 41% of patients after 10 years[57], and renal failure becomes an increasingly important cause of death as the years pass by[8]. In comparison, the prevalence of such a low GFR (<30) is less than 1% in the total population[58,59].

The aetiology of chronic kidney disease after heart transplantation is multifactorial[60]. A biopsy study identified both sequelae from the perioperative acute kidney injury as well as long-term effects of hypertension and diabetes (which are also the most common causes in the population at large), as well as treatment with nephrotoxic immunosuppressant drugs like calcineurin inhibitors (CNI). CNI is believed to have both acute and chronic nephrotoxic effects[61], though the exact mechanisms and their relations to the other non-CNI factors like hypertension and diabetes is still debated[60].

Nevertheless, due to the well documented risk of developing CKD after transplantation, the pre-transplantation GFR level has, since the beginning of the HTx era, been considered an important factor to consider when deciding whom to accept, or not to accept, for HTx listing.

The importance of pre-HTx GFR for long-time survival after HTx is one of the questions investigated in Paper II.

Treatment

Acute kidney injury

In addition to the injury the kidney sustains from the CPB and the associated traumas mentioned above, the kidney might also suffer under a low cardiac output from a failing heart. Therefore, in treating AKI after heart surgery, a "pre-renal" cause of the impaired kidney function must also be suspected, and detected early. The heart function should therefore be monitored closely, and signs of heart failure should be treated with inotropic agents such as isoprenaline, dopamine, milrinone or norepinephrine. Postoperative pulmonary hypertension leading to a right ventricular failure could be treated with inhaled prostacyclin or nitric oxide.

The clinical results of pharmacological treatment of acute renal failure targeting the kidney directly have been contradictory at best, but mostly disappointingly unsuccessful, though several regimens has been tried, and

tested. The idea is that diuretics could protect the kidneys from AKI through a decrease in renal oxygen demand, and increased diuresis; the latter protecting the tubules from tubular occlusion. However such effects have been difficult to demonstrate[62].

- Loop diuretics derive their name from their effect on the part of the nephron called the Loop of Henle where it inhibits the reabsorption of sodium, chloride and potassium. Although they have several other effects on the nephron, the main diuretic effect stem from the inhibition of sodium reabsorption, and the concomitant decreased reabsorption of water from the tubuli. However, even if loop diuretics increases the volumes of urine, no reduction in morbidity, mortality or need for dialysis has been found[63].

- Mannitol is a small 0.182 kDa monosaccharide that is not metabolized and is eliminated from the circulation only by glomerular filtration. Thus, due to its small size, it freely passes over the glomerular membrane into Bowman's capsule. It works as an osmotic diuretic, and has been shown to increase GFR in postoperative cardiac surgery patients[64] and probably also deswelling of endothelial cells and tubular cells injured by hypoxia[65,66]. However, like loop diuretics, no effect on AKI outcome has been demonstrated[67].

- Dopamine increases diuresis through renal vasodilatation, inhibiting the reabsorption of sodium in the proximal tubules of the nephron, and increasing GFR. However, it has not been shown to affect the renal outcome of AKI[68,69].

- Atrial natriuretic peptide (ANP) is a hormone peptide with diuretic effects. The possible renoprotective effect of ANP has been discussed, and a systematic review and meta-analysis in 2009 recommended further studies particularly in patients undergoing cardiac surgery[70]. ANP is described in more detail in 1.4.2 and 3.3.

Given the inability of diuretics to show any improvements in clinical outcome, the 2012 KDIGO Guidelines state that diuretics should not be used to treat AKI, except as a way to treat fluid overload[71].

If the AKI progresses to renal failure, dialysis, in the form of continuous renal replacement therapy (CRRT) might be necessary as a last resort.

Fortunately, the renal function in most patients that develop post-CPB AKI recovers, but as mentioned above, the condition still is associated with a fivefold increase in mortality, and around 2.5 % develops the need for CRRT, which carries a staggering 30-day mortality of up to 50 %[36].

Avoiding the AKI and the ensuing renal failure with some prophylactic strategy would be a better option, both from the patient's perspective, and from an economical perspective. But as pointed out above, no convincing such renoprotective therapy has been identified, but mannitol is often added to the CPB priming or during the CPB[11,12]. At the Department of Cardiothoracic Surgery at Sahlgrenska University hospital, ANP is often applied postoperatively in the treatment of patients with AKI.

Chronic kidney disease

The principal strategies for treating chronic kidney disease involves, apart from treating the cause, amending the metabolic consequences, adjusting the blood pressure and level of hydration, and correcting electrolyte levels. In particular, any nephrotoxic drug should be removed or the dosage reduced. In transplanted patients, the problem is of course that the most effective immunosuppressive therapy is based on CNI, which is well known for its nephrotoxic effects[61]. The reduction of CNI, while not compromising with the immunosuppressive effect, is a subject of much research. As with AKI, diuretics are not compulsory unless the patient suffers from over-hydration, or needs correction of potassium levels. As a last resort, dialysis, and maybe even kidney transplantation, must be considered. When chronic kidney failure finally develops into such a severe state that dialysis or kidney transplantation is needed, the patient is often said to have reached end stage renal disease (ESRD)[41].

Kidney function

Renal physiology

The kidney is a complex organ that has several important functions; excretion of waste products, secretion of hormones, gluconeogenesis (producing glucose) and the homeostasis of pH and blood components. It is far beyond the scope of this thesis to describe renal physiology in any depth, and only what is most relevant in the context of this thesis will be mentioned briefly.

The kidney performs its production of urine through filtration, reabsorption and secretion. The functional units of the kidney are the nephrons (see Figure 3) that produce and carry the filtrated fluid from the blood to the calyx of the kidney. When blood enters the kidney it is first passed through the glomerulus, a small sphere of winding capillaries. The glomerulus is surrounded by Bowmans's capsule into which massive amounts of fluid are filtrated from the blood. This primary urine, or ultrafiltrate, is then passed down the nephron where over 99% of the filtrate is reabsorbed in the tubules.

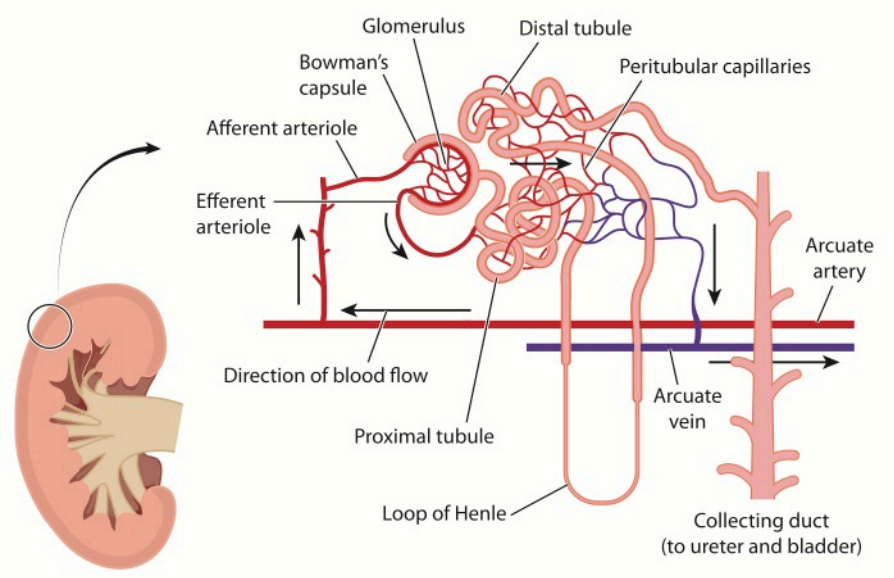

Figure 3: Kidney anatomy: on the left, the cortex (light brown) and the medulla (pink), and on the right the functional unit of the kidney; the nephron. (Shutterstock, Standard license.)

The filtration of fluid from the glomerulus into Bowman's capsule through several layers of semipermeable membranes (the capillary endothelial cells, the basement membrane and the epithelial cells of Bowman's capsule, which allow only small molecules to pass[13]. Molecules with a molecular weight of 5 kDa pass through this glomerular membrane unhindered. In this situation the permeability of the membrane is defined as 1.0. The permeability for 30 kDa molecules are only 0.5. Above 60 kDa, the permeability is very low. To albumin, the most common of the serum proteins, and also the smallest, with a molecular weight of 69 kDa, the permeability is only 0.005. To other important serum proteins, like immunoglobulins (150 kDa), essential for the immune defense, and fibrinogen (340 kDa), essential for blood clotting, the glomerular membrane is, for all practical purposes, impenetrable. In effect the primary urine produced in the corpuscle, the glomerular filtrate, is the same as plasma, except that it contains negligible amounts of proteins.

The amount of fluid filtrated from the blood into Bowman's capsule is determined by the differences in net filtration pressure over the semipermeable membranes. This pressure-gradient is generated both by the blood hydrostatic pressure itself, but also by the colloid osmotic (or oncotic) gradient between the glomerulus and Bowman's capsule.

Accordingly, the filtration pressure, and thus the amount of fluid filtrated, can be regulated by modulation of any of these pressures.

The afferent and efferent arterioles: Each glomerulus is supplied by a single afferent arteriole, and on the opposite end the capillaries of the glomeruli converges back to one single efferent arteriole. Both the afferent and the efferent arteriole has the ability to constrict or dilate, which can be use to regulate the impediment to blood flow in these segments. When the afferent arteriole dilates, the glomerulus is exposed to a higher pressure, which can be further accentuated by a constriction of the efferent arteriole. By this mechanism, and regulated through both myogenic mechanisms and by tubuloglomerular feedback mechanisms, the blood flow and the hydrostatic pressure in the glomeruli can be regulated.

The glomerular oncotic pressure: The glomerular oncotic pressure is generated by the colloid particles unable to cross the semipermeable barrier lining the capillary walls of the glomerulus. Thus, the nature and amount of colloid particles will influence the filtration pressure, and the GFR.

About 20% of the cardiac output goes through the kidneys, which amounts to about 1200 ml/min in an adult person. The rate of production of ultrafiltrate

each minute in both kidneys, the glomerular filtration rate (GFR), is approximately 125 ml/min. This means that each day almost 2000 litres of blood is passed through the kidneys, and almost 180 litres of primary urine is produced. Since almost all of the fluid that enters the tubuli is reabsorbed, the final amount of urine is only 0.5-2 litres (0.5-1 ml/kg/hour) a day.

As the massive amounts of ultrafiltrate are passed through the tubuli, the fluid and its constituents is processed in a number of important ways, both passive (i.e. not requiring energy) and active (i.e. requiring energy). The fact that the processing of certain substances requires energy is an important point in renal physiology. Energy expenditure also increases oxygen consumption, and thus the oxygen demand of the renal tissues. If this increased demand is not matched by an increased delivery, the ensuing oxygen supply/demand-mismatch may result in renal hypoxia and tubular damage or necrosis.

Substances are selectively absorbed or secreted during the tubular passage, and this allows the kidneys to separate substances that are to be conserved in the body, from substances that are to be eliminated in the urine. The most important substance to be actively reabsorbed in the tubuli is sodium ions (Na^+). Also, glucose, amino acids, chloride ions, phosphate, calcium, magnesium and hydrogen ions are actively co-transported, coupled to the energy-consuming action of the pumping of sodium ions into the interstitium, and reabsorbed from the fluid together with sodium. The water-molecules themselves move passively out of the tubuli through osmosis as the concentration of osmotic active substances (like Na^+) decreases in the tubuli and increases in the interstitium.

Substances that are actively secreted include hydrogen ions, potassium ions and urate ions

The kidneys play an important role in regulating the acid-base balance by secreting hydrogen ions into the tubular lumen of the nephrons, balancing the bicarbonate ions that are continuously filtered from the glomerulus into the proximal tubules. Any impairment of the tubular epithelial cells ability to excrete hydrogen ions into the tubular lumen will result in a build-up of hydrogen ions in the body, thereby threatening to lower the pH-level, which is normally tightly regulated within the range of 7.35-7.45.

Atrial natriuretic peptide

Several substances and hormones are involved in regulating renal function, such as endothelin, angiotensin II, aldosterone, antidiuretic hormone (ADH), parathyroid hormone (PTH), bradykinin and natriuretic hormones. However,

only natriuretic hormones have direct relevance for this thesis, and accordingly this text will focus mainly on them, in particular the atrial natriuretic peptide.

The natriuretic peptide system is primarily an endocrine system that maintains fluid and pressure homeostasis by modulating cardiac and renal function. At least eight natriuretic peptides have been discovered so far, of which the most well known are the atrial natriuretic peptide (ANP), the brain natriuretic peptide (BNP) and the C-type natriuretic peptide (CNP)[72].

ANP was the first of the natriuretic peptides to be discovered, and is a 28-amino acid peptide hormone produced in the cardiac muscle cells in the atrial wall (Figure 5). Already in the 1950'ies, electron microscopy suggested that a substance was generated in the cardiomyocytes of the atria of guinea pig's hearts[73], though the effects of the substance were not discovered until 1979[74,75]. ANP is released continuously from the heart and the rate of release increases in response to atrial stretch, will cause vasodilation, natriuresis, and inhibition of the sympathetic nervous system and the renin–angiotensin–aldosterone axis[76]. In the distal convoluted tubuli and the cortical collecting duct, ANP decreases sodium reabsorption, thereby reducing the reabsorption of water, which induces natriuresis and diuresis. In healthy volunteers and in post-cardiovascular surgery patients with normal renal function, the majority of studies have shown that the natriuretic response to ANP is associated with an increase in GFR[76-80]. Such an increase in GFR will also increase the tubular sodium load, renal sodium reabsorption and consequently renal oxygen consumption (RVO_2). On the other hand, experimental data suggest that ANP inhibits tubular sodium reabsorption in the collecting ducts of the medulla, which would decrease RVO_2.[81,82]

Thus, ANP seems to have several effects on kidney function, not all of them very well understood.

Clinically, recombinant human ANP has been approved in Japan to treat patients with heart failure since 1995[83]. At the Sahlgrenska University Hospital, ANP is used under licence for treating AKI after cardiac surgery and heart transplantation.

The effects of ANP on renal function during cardiopulmonary bypass are the focus of the investigations presented in Paper III.

How do we assess kidney function?

At the most fundamental level, the definition of "kidney function" depends on the perspective of the investigator, i.e. which of the renal "functions" is to be measured. However, a universally accepted definition of kidney function is to state the glomeruli's capability to filter fluid out of the passing blood. In the practical setting, to measure the amount of ultrafiltrate from the glomeruli per time unit, (ml/minute, i.e. the glomerular filtration rate, GFR) one would need a particle with some ideal properties for the task: It should be filtered freely across the semipermeable membranes, and not be reabsorbed or excreted in the tubuli. Also, it should not be metabolized or eliminated in any other way than through the urine. The measurement of the concentrations of this substance in blood or urine could then be used to calculate the amount of fluid filtrated. Different exogenous and endogenous substances and techniques are being used to this end, like inulin, iohexole and ^{51}Cr-EDTA, and even substances with less ideal properties, like creatinine and cystatin C.

Creatinine in blood

Creatinine is a final breakdown-product of muscle metabolism that is chiefly removed from the blood through the kidneys[84]. Being a small molecule (0.113 kDa) it is easily filtrated into Bowman's capsule. However, very little reabsorption of creatinine occur (but some active excretion). If the kidney function is impaired in some way, the serum levels of creatinine will rise accordingly. This rise in levels of serum creatinine can thus be used to assess the kidney function. In women, the normal level of creatinine in serum is 45-90 µmol/L, whereas in men it is 60-105. Above these levels, some degree of impaired renal function should be suspected. However, due to the several non-renal factors influencing the levels of creatinine, like age, sex, weight and muscular mass, the level of creatinine in serum or plasma is only a rough indicator of renal function[85]. Since creatinine is a breakdown product of muscle cells, the amount of muscle will affect the concentrations. Also, non-renal metabolism of creatinine plays an increasingly important role with increasingly impaired kidney function. Furthermore, the creatinine levels can not be guaranteed to be raised above the normal range until 60% of the total renal function is lost[84].

Even though it is a relatively rough estimate of renal function, the plasma creatinine concentration holds the advantage of being a cheap routine blood sample analysis, and is therefore widely used in the daily clinical setting of decision-making in hospitals or outpatient receptions.

When a more precise description of the kidney function is needed though, some way of assessing GFR is necessary.

Measured GFR (mGFR)

Measuring the clearance of infused exogenous substances (with the ideal properties mentioned above) will give the most accurate assessment of GFR. Substances used for this purpose include inulin, iohexole and ^{51}Cr-EDTA. This is called measured GFR, or simply mGFR.

Inulin is a 5.2 kDa polysaccharide that easily passes through the glomerular membrane but is neither excreted nor reabsorbed in the tubuli, and has been considered the gold standard of GFR measurement. However, inulin is expensive and difficult to measure, and is rarely used clinically. Other substances with similar properties exist that are more practical in the clinical setting (though still more cumbersome and expensive than the measurement of creatinine).

Iohexole is a 0.821 kDa contrast agent used for x-ray examination that also has ideal properties and can be analysed with high performance liquid chromatography-technique. EDTA is a metal ion binding chelating agent that, when loaded with the radioactive chromium isotope ^{51}Cr forms ^{51}Cr-EDTA, a 0.342 kDa molecule, that is readily detected with radioisotopic methods. Both iohexole and ^{51}Cr-EDTA have been shown to perform as well as inulin in measuring GFR[86].

Both iohexole and ^{51}Cr-EDTA were used when measuring GFR in the studies included in this thesis.

Estimated GFR (eGFR)

Since measuring GFR with one of the methods mentioned above is both cumbersome and expensive, several methods for *estimating* GFR (eGFR) has been developed. The cheap and readily available routine blood sample analysis of creatinine levels (see above) is heavily influenced by non-renal factors, and does not describe GFR, but through mathematical equations that take these non-renal factors into account (like age, sex, weight, and in some cases even race) several formulas for *estimating* GFR from serum creatinine levels have been developed[87-89]. Similar formulas have also been developed for the Cystatin C, a 0.013 kDa protein that can be used as a biomarker for kidney function in a similar fashion as creatinine[90], but since analysis of creatinine is far more common and ubiquitous in medical institutions, and is the biomarker of focus in this thesis, the role of Cystatin C will not be discussed further.

Three of the most well known creatinine-based formulas for estimating GFR are:

- The **Cockcroft-Gault** formula.
- The **MDRD** formula (Modification of Diet in Renal Disease).
- The **CKD-EPI** formula (Chronic Kidney Disease-Epidemiology collaboration).

See Table 1 for the mathematical description of these formulas. Other formulas also exist, like the Swedish LM-rev formula (Lund-Malmö revised formula)[91]. The LM-rev formula seems to have promising estimating power[92] in a Swedish population, but has so far not been widely applied internationally and has not been included in the formulas studied in this thesis.

For the day-to-day use in the clinical setting, these formulas often give sufficiently exact estimates of GFR. All of these formulas have their advantages and disadvantages, and their relevance is continually being evaluated in different groups of patients[93,94]. A 2013 report from the Swedish Council on Health Technology Assessment concluded that more studies are needed in several patient groups, e.g. patients undergoing organ transplantation or heart surgery[93].

In this thesis, the level of agreement between estimated GFR and measured GFR in heart-transplanted patients was studied to decide whether eGFR could replace mGFR at our department (see Paper I).

Table 0: Equations for estimating GFR (mL/min/1,73m^2) based on age, sex, weight (kg) and creatinine (µmol/L).

1. ***Cockcroft-Gault*** original formula[87]; **(140 - age) x [(weight)/(72 x creatinine/88.4)] (x 0.85 if woman**)
2. ***Cockcroft-Gault*** (original formula) **x 0.80** (IDMS traceable creatinine calibration (since 2004-06-01))
3. ***MDRD*** abbr formula; caucasian men[88]: **186 x (creatinine/88.4)$^{-1.154}$ x (age) $^{-0.203}$ (if woman: x 0.742)**
4. ***MDRD*** abbr formula; caucasian men: **175 x (creatinine/88.4)$^{-1.154}$ x (age) $^{-0.203}$ (if woman: x 0.742)** (IDMS traceable creatinine calibration (since 2004-06-01))
5. ***CKD-EPI*** formula[89]:
   i. **Woman, creatinine ≤ 62: 144 x [crea/(0.7x88.4)] $^{-0.329}$ x (0.993)^{age}**
   ii. **Woman, creatinine > 62: 144 x [crea/(0.7x88.4)] $^{-1.209}$ x (0.993)^{age}**
   iii. **Man, creatinine ≤ 80: 141 x [crea/(0.9x88.4)] $^{-0.411}$ x (0.993)^{age}**
   iv. **Man, creatinine > 80: 141 x [crea/(0.9x88.4)] $^{-1.209}$ x (0,993)^{age}**

The terminal consequence of renal failure

This basis of this thesis is the clinical problem of renal failure after heart surgery and cardiopulmonary bypass. As described above, renal failure is a potentially lethal complication. In brief, the following is the terminal pathophysiological consequences of renal failure[95]:

Denied the eliminating services of the kidneys, the patient's body will start to accumulate salt and water, causing swelling oedemas. The concentration of end products of protein metabolism, like creatinine, uric acid and urea also increases, causing the condition of uraemia. Since the kidneys are essential to the regulation of the acid-base balance through elimination of H^+ and normal acidic products, acidosis will ensue as the pH drops below the normal physiological levels of 7.35-7.45.

Within a week of total renal failure, the patient will slip into uremic coma as the cerebral neurons stops functioning, probably due to the build-up of hydrogen ions

Death will occur when the pH of the blood reaches 6.8, if not before.

AIMS OF THE STUDY

This thesis is based on four studies, all of which investigates renal function in relation to heart surgery. These studies were designed and performed with the intention and hope that they might help to answer some of the questions that arise for clinicians when involved in the day-to-day clinical decision-making regarding these patients.

In particular, the following aims were identified:

1. To investigate whether estimated GFR is an acceptable substitute for the more cumbersome, and expensive, methods of measuring GFR with either ^{51}CrEDTA or Iohexole-clearance methods in patients eligible for heart transplantation (Paper I).

2. To investigate how renal function both pre- and post-transplantation affects mortality and morbidity after heart transplantation (Paper II).

3. To investigate if, and possibly how, ANP affects renal function during CPB when given as a continuous prophylactic treatment starting before CPB is commenced (Paper III).

4. To investigate whether priming the heart-lung machine with a solution with a higher-than-normal oncotic pressure could have any effect on kidney injury sustained during heart surgery and CPB (Paper IV).

PATIENTS AND METHODS

Patients

Paper I and Paper II

At the Sahlgrenska University Hospital, heart transplantations have been performed since 1980-ies, generating 478 HTx procedures between 1988 and 2010. As part of the pre-HTx evaluation, the patients' renal function have been assessed by measuring their GFR with the ^{51}Cr-EDTA or iohexol methods. These patients have received annual follow-ups at our centre, which also have included the measurement of GFR through the same methods. We wanted to study the survival and the renal outcomes in the adult population (≥18 years) undergoing their first HTx, a total of 416 patients. Between 1988 and 2010, 2190 GFR measurements were performed on these patients, of which 383 were preoperative and 1807 gathered during follow-up. These kidney function measurements registered in the HTx registers at the Sahlgrenska University Hospital, are the basis of the retrospective cohort studies performed in Paper I and II.

In Paper II, the occurrence of renal replacement therapy was checked with the Swedish Dialysis Registry.

Ethical approval to review the clinical data was obtained from the institutional review board at the University of Gothenburg (ethical approval no Dnr 728-12).

Paper IV

We wanted to assess, in humans, the renal effects (if any) of a colloid-based priming solution versus a standard crystalloid-based priming solution. PrimECC® is a new dextran 40 based priming solution (XVIVO AB Gothenburg, Sweden). An investigator initiated double-blinded randomized study in adult patients undergoing heart surgery was designed. The study protocol was approved by the Gothenburg Regional Ethics Committee (Dnr T 847-16 Ad 1003-15). The study was registered at ClinicalTrials.gov (identifier: NCT02767154). Written informed consent was obtained from all patients.

The inclusion criteria were: all patients aged 50-80 years accepted for elective cardiac surgery with an expected CPB time above 75 minutes. Exclusion criteria were: previous cardiac surgery, coagulation disorder, malignancy, kidney failure, liver failure, on-going septicaemia, on-going antithrombotic treatment (other than acetylsalicylic acid), systemic

inflammatory disorders (treated with corticosteroids), or not able to understand Swedish language.

In total, 39 patients were randomized to the colloid group (PrimECC-solution, dextran 40 based) and 41 to the crystalloid group (see Figure 8).

Animals

In order to address the questions outlined in the third aim, we developed a pig-model for studying renal function during CPB (Paper III). The choice of using pigs in our experimental model depended on several factors. Pigs have hearts and kidneys whose anatomy and physiology are more similar to humans' than many other animals. Also, we needed animals large enough for the cannulas and tubing fitted in our heart-lung machine system. Last, but not least, the animals had to be of practical size for the author to operate on. The pigs used in our study had a mean weight of 56 kg (range 47-64 kg) facilitating the feasibility of the surgical procedure, particularly the dissection of the renal hilum, and it´s structures (see Methods section).

Figure 4: The author visiting the animal housing at the animal research facility. The housing conditions for research animals in Sweden are regulated in strict detail.

Pigs are prone to several infections that among other things can cause pleuritis, generating severe pleural adherences. To avoid these problems, pigs can be bred in special farms controlled and guaranteed to be free of these pathogens, generating Specific Pathogen Free (SPF) animals. The animals used in our study were female Yorkshire pigs (Vallrum farm, Ransta, Sweden, specialized in producing SPF pigs) (Figure 4).

In Sweden, performing animal experiments requires the primary investigator to pass an exam in Laboratory animal science and technique. Animal research is regulated in The Animal Protection act and the Animal Protection Ordinance. Ethical approval for this study was obtained from the Animal Research Ethical Committee of the University of Gothenburg (ethical approval no 107-2016). All animals received care in compliance with the Swedish Board of Agriculture regulations concerning research animals (SJVFS 2015:38). Pigs

are social animals, and always require at least one other animal for company to avoid experiencing agonizing stress. In our study design, this meant that no pig could be left alone overnight for the last experiment, and that the last two pigs had to be anaesthetized simultaneously. Since we did not have two heart-lung machines at our disposal to perform two parallel CPBs, the “companion pig” was used for sham-operation with continuous infusion of ANP.

In total, 28 animals were operated on, of which twenty were randomized in the study (the other eight established the model, or were sham-operated).

Atrial natriuretic peptide (ANP)

The atrial natriuretic peptide (ANP) used in this thesis is a recombinant human ANP drug used under license for treating AKI at the Intensive Care Unit at the department of Cardiothoracic surgery at the Sahlgrenska University Hospital, but otherwise not well known or used in Sweden. The drug has been approved for treating heart failure in Japan[83], where it is also produced (Daiichi Sankyo, Japan). In the treatment of AKI, ANP has been shown to increase renal blood flow (RBF) and GFR and to decrease the need for dialysis in post-cardiac surgery patients. The effects of ANP on RBF, GFR and RVO_2, when used for prevention in cardiac surgery with CPB are, to our knowledge, not well studied nor understood. In order to investigate the effects of ANP on kidney function during CPB we developed a pig model for invasive cardiothoracic and renal studies (Paper 3).

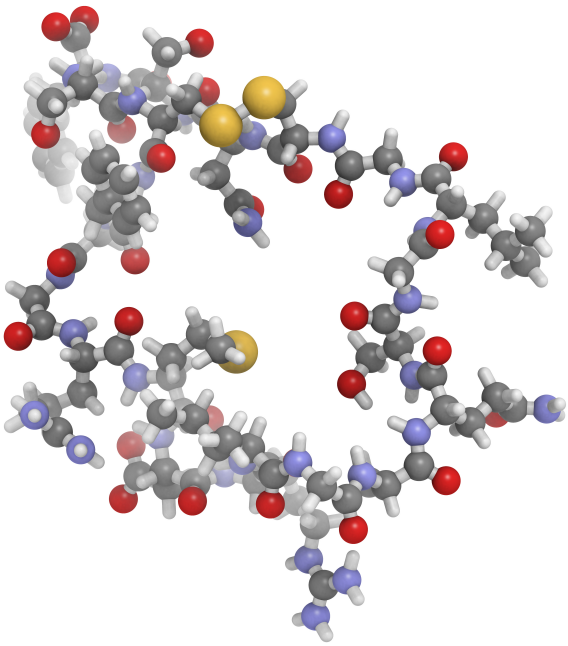

Figure 5: The molecular structure of atrial natriuretic peptide (ANP) (Shutterstock, Standard license)

Dextran 40

Dextrans are glucans, i.e. polysaccharide molecules made of long chains of glucose molecules, and come in a wide variety of lengths, from 3 to 2000 kDa. First discovered by Lois Pasteur, the dextrans were introduced into medicine by Swedish scientists in the 1950's, as colloids with properties making them suitable as possible plasma substitutes for treatment of hypovolemia, a much sought for product at the time[96,97]. Dextrans have a prolonged anti-thrombotic effect and have been used for anticoagulation therapy[98,99]. However, this anticoagulant effect may in some situations be unwanted, particular in the perioperative setting, and the colloid osmotic properties and anticoagulant effect has to be weighed against each other.

The renal excretion of dextrans with a molecular weight over 50 kDa is very low[100]. Dextran 40 (also known as Rheomacrodex) however, is a 40-kDa glucan of which 70% is excreted in urine within 24 hours. This gives dextran 40 distinct colloid properties, while at the same time being eliminated relatively rapidly compared to heavier dextrans (like dextran 70, also known as Macrodex) that can remain in the circulation for weeks.

The effects of dextrans have been investigated in organ preservation studies with encouraging results[101-103], leading to a recommendation that Dextran 40 should be included in such solutions[104]. The oncotic properties in conjunction with the possible preservation effects and relatively short half-life, makes dextran 40 an interesting component in a CPB priming solution. We therefore wanted to compare a dextran 40-based priming solution to our standard crystalloid solution in a randomized, blinded study on adult patients undergoing heart surgery (Paper IV).

Methods

This thesis is based on several studies of different design. Paper I and II are both retrospective longitudinal cohort studies, whereas Paper III describes a blinded, randomized study in animals, and Paper IV describes a double-blinded, randomized controlled trial in humans. The principals of these studies, with their strengths and weaknesses are outlined below.

Randomized controlled trials

A controlled trial compares the outcomes of two groups when one group receives a specified intervention, and the other group do not. When designating patients to one of the two groups, there is always a risk that the investigators, or the patients themselves, consciously or inadvertently, affects the choice, or even other treatments given later on in the trial. The selection problem of this systematic error, or *selection bias*, can be minimized by randomly assigning the study subjects to either group. When possible, in order to further strengthen the internal validity of the study, either the subjects or the investigators, or both, can be blinded to the results of the randomization. This blinding reduces the placebo effect and observer bias. However, such a blinding often presents practical problems and is not always possible (e.g. when comparing a surgical treatment to a medical treatment).

The first randomized controlled trial (RCT) in medicine was published in 1948, regarding the treatment of tuberculosis with streptomycin[105]. Since then, RCT has become the gold standard method for investigating the effect of medical therapies. To improve and standardize the reporting of RCTs, the CONSORT 2010 Statement (Consolidated Standards of Reporting Trials) has been widely accepted. This includes the CONSORT-diagram that visualises the flow of the study subjects through the trial, and the number of subjects at each stage. Also, RCTs should be registered in the ClinicalTrials.com run by United States National Library of Medicine to increase transparency. In this thesis, CONSORT-diagrams were included in Paper II and IV.

Disadvantages

Even though the RCT is regarded gold standard in clinical research trials, it has certain disadvantages. It can be very expensive studies to conduct, and take several years from planning of the study to the results being published. Studying interventions that are aimed at reducing infrequent events may demand very large numbers of study subjects, adding to the costs and time, and observational studies may therefore be a more practical alternative[106], as in the case of renal failure and heart transplantation, mentioned above.

Due to the costliness of running RCTs, the investigators might be tempted to let the trials be sponsored by the pharmaceutical industry, which obviously may create concerns about conflict of interests. It has been shown that industry-sponsored RCTs are more likely to produce pro-industry findings[107], and that industry-sponsored RCTs have lower odds for publication of completed trials than nonindustry-sponsored[108]. The possibility of publication bias cannot be ruled out as a cause of these differences. Conducting an *investigator initiated* study, i.e. with minimal funding from the industry, and where the results of the trial will be published regardless of the results, can reduce this problem.

Another interesting problem is the *ethics* of such RCTs. Usually the reason for conducting a RCT is a well-grounded suspicion that a treatment could have some beneficial effect. But difficulties might arise as to decide the exact level of suspicion at which it becomes unethical to deny half of the study group the treatment. The final decision of such ethical problems lies with the ethics committees, weighing in all aspects of such a study.

In this thesis, the design of an investigator-driven, double-blinded, randomized controlled trial was chosen when comparing the renal effects of a colloid-based priming solution to a standard crystalloid-based priming solution (see Paper IV).

Registry studies

Though randomized controlled trials are considered gold standard for comparing patient groups in a clinical setting (see above), certain circumstances can make these studies difficult to conduct from a practical point of view. In particular, studying patients receiving a relatively rare treatment (such as a heart transplantation) and the developing complications (such as death or ESRD) over a prolonged period of time (such as decades) would generate an immensely costly and logistically complicated study if it were to be designed as a prospectively, randomized follow-up study. A retrospective study however, can be conducted on existing data, or at least data that can be extracted afterwards. Thus, data that have been gathered continuously over the years and entered into an existing registry can make analyses possible, for e.g. descriptive purposes, which would otherwise not have been practically feasible. The limitations of such a retrospective study, however, are several. There will be multiple parameters that one does not know of, and that cannot be controlled. If part of the data retrieval is based on human memory, there will be the problem of selective memory, or recall bias. Patients may be lost in follow-up, or the data may simply not exist. Thus,

care must be taken when interpreting the results from such retrospective studies. Specifically, no causal links can be ascertained, at best only suggested.

Despite the limitations of such retrospective studies, they have the advantage of being practical to perform, particularly when it comes to relatively rare diagnoses and complications during long-term-follow up[106]. Thus, in this thesis, two *retrospective longitudinal cohort studies* were conducted: One used existing data regarding renal function in an HTx cohort to compare the estimated GFR to the measured GFR over 10 years (see Paper I), a *method comparison study* (see statistics section). The other study compared the impact of different levels of pre-HTx GFR on survival and ESRD up to 20 years after HTx (see Paper II). In this latter study risk factors for death and ESRD were also analysed. The statistical methods involved in such analyses include Kaplan-Meier survival analysis, the logrank test and Cox proportional hazard regression (see Statistics section).

Animal studies

For the study described in Paper III, we developed a large-animal model to study the effects of ANP on the kidneys during CPB in pigs. The invasive character of the planned renal investigations, necessitated the use of animals, not humans, to pursue the aim outlined above: to investigate if and possibly how, ANP affects renal function during CPB when given as a continuous prophylactic treatment starting even before CPB is commenced. In Sweden, the use of animals in experimental studies is regulated in The Animal Protection act (Djurskyddslagen) and the Animal Protection Ordinance (Djurskyddsförordningen). The surgical procedure is briefly described below. For further details and dosages, see Paper III. The study was designed as a blinded, randomized controlled study, in which 10 animals received a continuous infusion of ANP during CPB, and 10 animals received only saline (NaCl 0.9%). The results of the randomizations were not revealed to the investigators until the whole study was finished. Flow-chart of the experiment is depicted in Figure 6.

Anaesthesia

The animals were pre-medicated with an intramuscular injection of tiletamin, zolazepam and dexmedetomidin-hydrochloride. Anaesthesia was induced with thiopental and morphine infusions. An endotracheal intubation was performed and anaesthesia was maintained with a combination of thiopental, isoflurane and morphine. Fentanyl was given when needed. At the induction

methylprednisolone and amiodarone were given in order to provide stable cardiovascular function.

Surgical preparation

In the supine position, the right carotid artery and the right jugular vein were cannulated for measurements of arterial and central venous pressures, for blood sampling and for administration of fluids, respectively. The left jugular vein was catheterised for continuous infusion of ^{51}Cr-EDTA (see below). Mean arterial pressure (MAP) and central venous pressure (CVP) were measured via pressure transducers at the level of the heart and calibrated to atmospheric pressure. A Foley catheter inserted in the urinary bladder sampled urine. The animals were laparotomised in order to expose the left kidney and the renal hilum. The renal artery and vein were identified and a Doppler flow probe was applied around the left renal artery and an 18 Gauge catheter was inserted into the renal vein for repeated blood sampling. A laser Doppler flow probe was sutured to the kidney surface for measurements of cortical perfusion. For medullary measurements, a needle laser Doppler probe was inserted 10-12 mm into the kidney.

After completing the abdominal preparation, a sternotomy was performed. After heparinisation the arterial and the venous cannulas were placed in the ascending aorta and the right atrium and connected to a standard cardiopulmonary bypass circuit.

Measurement of renal variables

RBF and laser Doppler flowmetry (LDF) of the cortex and the medulla were continuously measured. The total RBF was assumed to be twice the blood flow to the left kidney. An intravenous priming dose of ^{51}Cr-EDTA (0.6 MBq/m2 BSA), (GE Healthcare Limited, The Grove Center, Amersham, UK), was given immediately after the introduction of the central venous catheter. This was followed by an infusion at a constant rate individualized to body weight. Serum concentrations of ^{51}Cr-EDTA activity from arterial and renal vein blood were measured using a well counter (Wizard 300, 1480, Automatic Gamma Counter, Perkin Elma LAS, Turkuu, Finland). Renal vascular resistance was calculated from the formula: RVR=(MAP-CVP)/RBF. Renal plasma flow (RPF) was calculated as: RBF (1-haematocrit). GFR was calculated using the infusion clearance technique as the amount of infused 51Cr-EDTA divided by the arterial 51Cr-EDTA concentrations. Filtration fraction (FF) was calculated from the formula: GFR/RPF. RVO_2 and renal oxygen extraction (RO_2Ex) were derived from the formulas $RVO_2 = RBF \times (CaO_2 - CvO_2)$ and $RO_2Ex = (CaO_2 - CvO_2 / CaO_2)$

(x100), respectively, where CaO_2 and CvO_2 are the arterial and renal vein oxygen contents. Renal oxygen delivery (RDO_2) was calculated as RBF x CaO_2. All renal data were normalised to the body surface area (BSA, m^2) of the animals, which was calculated according to the female swine specific formula[109] for BSA = 734 x (bodyweight in kg) $^{0.656}$.

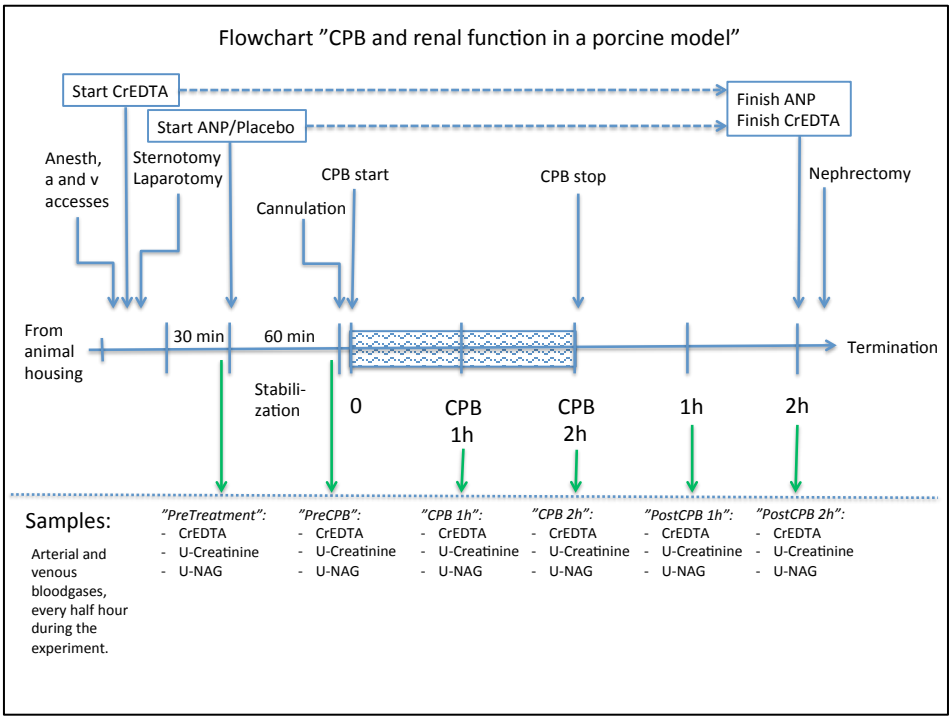

Figure 6: Flowchart depicting the timeline of the animal experiment. First sampling (PreTreatment) was immediately before ANP/NaCl-infusion was started, the next was 1 hour later (immediately before CPB was commenced), then at regular intervals during 2 hours of CPB, and finally at regular intervals during 2 hours after CPB-weaning. Blood-gases were drawn every 30 minutes.

Statistical analysis

Since this thesis is based on several different studies with different scientific designs, a range of different statistical methods has been applied. These will briefly be described below together with their applications, and their strengths and weaknesses. However, the actual mathematical equations will not be described. Mathematically interested readers are recommended further reading in Altman DG: "Practical statistics for medical research", Campbell MJ: "Medical statistics" or Björk J: "Praktisk medicin för medicin och hälsa".

Correlation and Agreement

When studying the association between to continuous variables, the method of correlation can be applied. The degree of correlation is stated in the correlation coefficient called *r*, and can take any value from -1 to +1, i.e. from a perfect negative correlation to a perfect positive correlation, and where 0 denotes no correlation at all.

However, in medicine, a common clinical question is whether two different methods for measuring the same variable are equally good, and therefore potentially interchangeable. This question may arise out of practical reasons: One method might be more expensive or more cumbersome, and the clinician would like to know if it could be exchanged for a cheaper, quicker method. When comparing the two different methods for measuring the same variable, the most interesting question is of course to what degree the two methods come up with the *same result*, not only how well they *correlate*. It would be tempting to use correlation, but that would not be sufficient: whereas the correlation coefficient measures the degree of *association* between two groups, it says nothing about how closely they *agree*. Thus, in method comparison studies, agreement analysis is needed.

The Bland-Altman agreement analysis[110] calculates the mean difference between the two methods (called the *bias*, but not to be confused with the term "bias" in clinical experiments), as well as the standard deviation of these differences. The area around this bias up to ±2 standard deviations is called *the limits of agreement*. However, the practical importance of these limits of agreement might be difficult to interpret in a clinical setting. To get some idea about the degree of error between the methods, the width of the limits of agreement can be divided by the mean of the two measurements. The resulting *percentage error* gives an indication of how large the spread of the differences are, in relation to the absolute values of the method tested[111]. If the percentage error is 100%, the width of the limits of agreement (which is 2

standard deviations, or equal to the 95% confidence interval), is as wide as the absolute value of the mean of the methods being tested. Most clinicians would consider a percentage error of 100% unacceptably inaccurate, but there is actually no statistically defined cut-off at which the percentage error becomes unacceptable. What level of error should be considered unacceptable is therefore a strictly clinical consideration, which must take into account the gravity of the clinical consequences of the error for the patients.

In this thesis, the method of *measuring* GFR was compared to the method of *estimating* GFR, using both Pearson product-moment correlation and Bland-Altman agreement analysis (see Paper I).

Student's t-test

In the process of improving the quality of stout, William Gosset, a statistician employed at the Guinness' brewery in Dublin, invented a statistical method for describing a special case of normal distribution, which was later to be known as the t-distribution[112]. Since he was restricted from publishing corporate secrets, his method was published in 1908 under the pseudonym "The Student". The difference between the standardised normal distribution and the t-distribution is that the t-distribution needs a wider interval to cover a certain percentage (e.g. 95%) of all the values in a population.

The Student's t-test tests to what degree the means of two sample groups reflect the same population. It returns the probability, p, that the means reflects the same population, provided that the null hypothesis is true (the null hypothesis being "they are the same"). Usually, a probability lower than 5% (0.05) is considered statistically significant, and is presented as $p<0.05$. However, this implies that when performing this calculation repeatedly, 1 result out of 20 could, statistically, still be a mere coincidence.

Strictly speaking, the Student's t-test is only valid provided the variances of the two populations are also equal. When this assumption of equal variances is dropped a slightly different test, the Welch's t-test, is often employed. Also, the t-test assumes a normal distribution in the first place, which requires population samples of some size. Test that assumes a normal distribution of the study population are called parametric. In this thesis, comparing means with Welch's t-test was performed when comparing the group of 39 patients that received colloid-based priming in the heart-lung machine, to the 41 that had crystalloid-based priming (see Paper IV).

Mann-Whitney U-test

When comparing small sample groups, or groups where one cannot assume normal distribution, medians should be compared, instead of means. Using medians reduces the effect of a possible skewed population, and without the assumption of normal distribution these tests are called non-parametric tests. The non-parametric equivalent of the t-test is the Mann-Whitney test (also called Wilcoxon rank sum test, but not to be confused with the Wilcoxon *signed* rank sum test). This test is recommended when comparing two groups containing less than 20 individuals each. In this thesis, the Mann-Whitney test was used when comparing the two small-sized groups of pigs that were treated with either ANP or saline only, containing only 10 animals in each group (See paper III).

Kaplan-Meier survival analysis

In longitudinal studies, the occurrence of a specific incident (or hazard, when it comes to survival studies) in a population over time, can be plotted as a Kaplan-Meier plot. The plot depicts the proportion of the population in which this incident has *not* occurred yet. On the Y-axis, the plot starts out on 1 (maximum) or 100%. Then, as the incident occurs in the population, only those *not* affected are plotted. This generates a sloping curve that stops at the end of the follow-up period, at which time it may or may not have reached zero on the Y-axis. At each time-point only those individuals left in the population are included in the calculation, which means that the Kaplan-Meier curve can be constructed despite individuals leaving the study for some reason, e.g. lost during follow-up. Since individuals are being censored with time (either because they die, or because they leave the study for some other reason) the resultant number of individuals left at each time point (the number at risk) should always be noted on the Kaplan-Meier plot.

The hazards of interest in the retrospective longitudinal studies in this thesis were death and end-stage renal disease (see Paper II).

Log-rank test

The log-rank test is a non-parametric method for testing the null hypothesis that the groups being compared are samples from the same population. It is particularly designed to compare survival results over time, and the principle of the test is to divide the survival time scale into intervals, the intervals being defined by the observed occurrence of the hazard studied, and at the same time ignoring survival times of censored individuals (see Kaplan-Meier above). For each time interval the observed result is compared to an expected result, i.e. what we would expect if the null hypothesis were true (that they

are samples from the same population). The log-rank test can detect the existence of a difference between groups; however, it does not contain information about *how* different the groups are. In this thesis, the log-rank test was used when analysing survival in patients undergoing heart transplantation (see Paper II).

The log-rank test does not account for competing risks, e.g. death *and* renal failure. In the presence of such competing risks, Pepe-Mori's test should be used[113]. This test compares the cumulative incidence functions for the event of interests, while taking into account competing risks. In this thesis, Pepe-Mori's test was applied when comparing the development of later end stage renal disease in patients that received CRRT due to AKI in the immediate post-operative phase (see Paper II).

Cox proportional hazard regression model

As opposed to correlation analysis, regression analysis not only states the strength of the relation between to parameters, but also gives a *description* of the relation. It enables us to make predictions about a value, when we only have the other. The principle is the generation of equations that describe the relations. Several regression techniques exist and can be applied depending on the values that are to be studied. The Cox proportional hazard regression model is a model particularly suited for survival data, and to assess *hazard ratios*. (If odds ratios are studied, logistic regression should be applied instead).

The hazard ratio (HR) is a measure that describes the relative risk of death between two groups. It is the ratio between the hazard *rates* in the two groups, calculated for each time point. This gives an estimate of the relative event rates in the groups. If the relative event rate in the first group is equal to that of the other group, the hazard ratio will be 1. If the rate is lower in the first group the hazard ratio will be <1, and vice versa.

In survival studies, the hazard ratio is calculated for the entire time period studied. It is however possible that the hazard actually varies over time. The visual impression from the survival plot (Kaplan-Meier) is therefore an important complement. The term "Hazard ratio" implies that the calculations are not based on *actual* survival times (for which the term relative risk (RR) may be used), but rather on the *proportions* alive at the particular time-points.

For continuous variables (like age), the Cox regression gives a hazard ratio that describes how the hazard is increased (or decreased) each added year of age. Univariate regression analysis handles only one variable at the time, but

when performing survival studies, several variables are usually of interests. Therefore multivariable regression analysis are usually applied, to take into account several variables and assessing their individual hazard ratios.

Cox regression analysis is complex, and the results should be interpreted with care. It is generally recommended that a professional statistician be consulted when performing this analysis.

In this thesis, Cox proportional hazard regression model was applied when analysing pre-transplant factors affecting post-transplant survival (see Paper II).

ANOVA

When studying variances between groups, it is also important to decide how much of this variance comes from variance *within* the groups, and how much arises from actual variances *between* the groups. The analysis of variance (ANOVA) deals with this problem. The larger the proportion of the total variance that depends on the variance *between* the groups, rather than within the groups, the stronger one can assume that the groups are different.

ANOVA is used for structured data sets, which typically arise from designed experiments. In this thesis, ANOVA was used when studying differences between groups receiving ANP or saline in the animal study (see Paper III), and between groups receiving colloid-based or crystalloid-based prime in the randomized control study (Paper IV).

RESULTS

Agreement of eGFR and mGFR in HTx (Paper I)

The aim of Paper I was to compare *measured* GFR to the cheaper and simpler *estimated* GFR. The results of such a comparison could aid in the decision as to whether estimated GFR is an acceptable substitute for the more cumbersome and expensive method of measuring GFR with either ^{51}CrEDTA or Iohexole-clearance methods in patients eligible for heart transplantation. Three of the most common formulas for estimating GFR were to calculate the estimated value, namely: Cockcroft-Gault, MDRD and CKD-EPI. Then both Pearson product-moment correlation and Bland-Altman Agreement analyses were performed to compare the results from the estimation formulas to the measured values (see Figure 7).

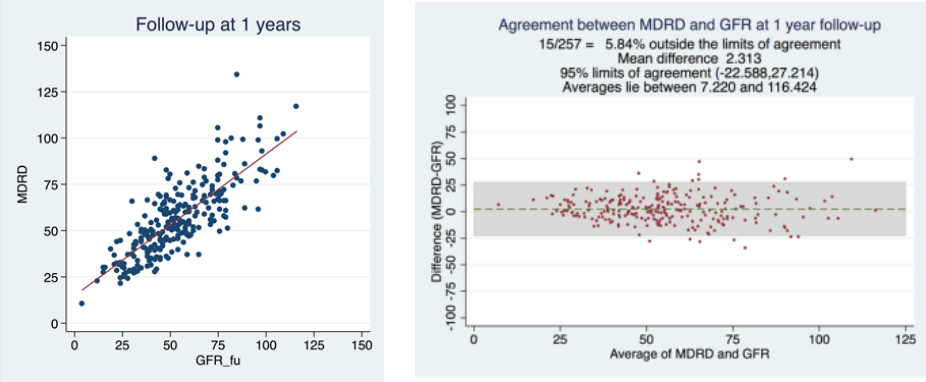

Figure 7: Typical scatter-plots from Pearson product-moment correlation analyses (left) and Bland-Altman agreement analyses (right). This particular example illustrate correlation and agreement for GFR *estimated* with the MDRD formula, compared to *measured* GFR, at the 1st year of follow-up

The measured and the estimated GFR values were compared at defined time points: Both prior to transplantation (Pre HTx) and then at 1, 5 and 10 years after the procedure. Also, the *bias*, the *percentage error* and *P30* were calculated. The results of these analyses are summarized in the tables 1 through 5 below.

Table 1: Correlation coefficients between estimated and measured GFR

	Pre HTx	1 year	5 years	10 years
Cockcroft-Gault	0.547	0.7	0.674	0.748
MDRD	0.53	0.798	0.705	0.771
CKD-EPI	0.53	0.8	0.7	0.815

Table 2: Bias between estimated GFR

	Pre HTx	1 year	5 years	10 years
Cockcroft-Gault	9.9	10.5	12.8	14.4
MDRD	1.7	2.3	2.1	3.1
CKD-EPI	4.9	5.1	3.4	4.1

Table 3: Limits of agreement between estimated and measured GFR

	Pre HTx	1 year	5 years	10 years
Cockcroft-Gault	-30.4, 50.1	-25.4, 46.4	-23.4, 49.0	-15.4, 44.3
MDRD	-32.4, 35.8	-22.6, 27.2	-25.9, 30.2	-22.5, 28.8
CKD-EPI	-31.4, 41.2	-21.1, 31.3	-23.7, 30.5	-19.7, 28.0

Table 4: Percentage error (%) between estimated and measured GFR

	Pre HTx	1 year	5 years	10 years
Cockcroft-Gault	126.0	134.5	157.3	144.6
MDRD	107.6	93.3	121.7	124.2
CKD-EPI	114.4	98.1	117.6	115.5

Table 5: Percentage of estimated GFR results within 30% of measured GFR (P30)

	Pre HTx	1 years	5 years	10 years
Cockcroft-Gault	59.1	26.9	25.5	22.4
MDRD	77.3	70.3	77.0	68.2
CKD-EPI	67.4	64.3	73.3	65.9

GFR and outcome after HTx (Paper II)

The aim of Paper II was to investigate to what degree measured glomerular filtration rate (mGFR) is a risk factor for death and/or end stage renal disease (ESRD) after heart transplantation (HTx). Also, we wanted to identify other possible risk factors, and investigate the impact of AKI early after the transplantation. All adult patients (n=416) who underwent HTx between 1988 and 2010 were included; see CONSORT-diagram below.

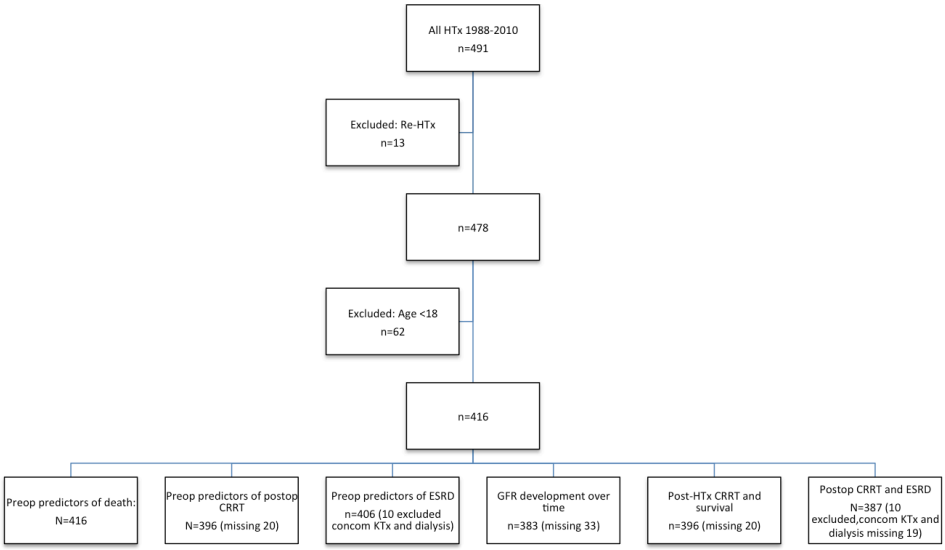

Figure 8: CONSORT-diagram illustrating inclusion and exclusion of patients within the study population.

Eight patients concomitantly received a kidney transplant (KTx) at the same occasion as the HTx. Fifteen more patients had a late KTx due to severe chronic renal failure. The survival at 1, 5 and 10 years was 83%, 74% and 59% respectively.

In the multivariable Cox analysis, only age (as a continous variable) and the need for a ventricular assist device (VAD) were preoperative predictors of death (Table 6).

Preoperative mGFR however, was not a predictor for long-term survival, neither grouped (Figure 9, Table 6) or as a continuous variable (Table 6).

Table 6: Preoperative predictors of death (excerpt)

			Univariable Cox-regression			Multivariable Cox-regression		
		n	Hazard ratio	95% CI	p	Hazard ratio	95% CI	p
Age	Cont.	416	1.02	1.01-1.03	<0.001	1.03	1.02-1.04	<0.001
VAD	No	323	1.00			1.00		
	Yes	57	1.48	0.98-2.23	0.060	2.23	1.43-3.46	<0.001
mGFR	<30	10	1.24	0.51-3.04	0.637			
(groups)	30-59	154	1.15	0.86-1.52	0.345			
	>60	219	1.00					
mGFR	Cont.	393	1.00	0.99-1.00	0.283			

CI: confidence interval; VAD: ventricular assist device; mGFR: measured glomerular filtration rate. The Hazard ratio for age is per year.

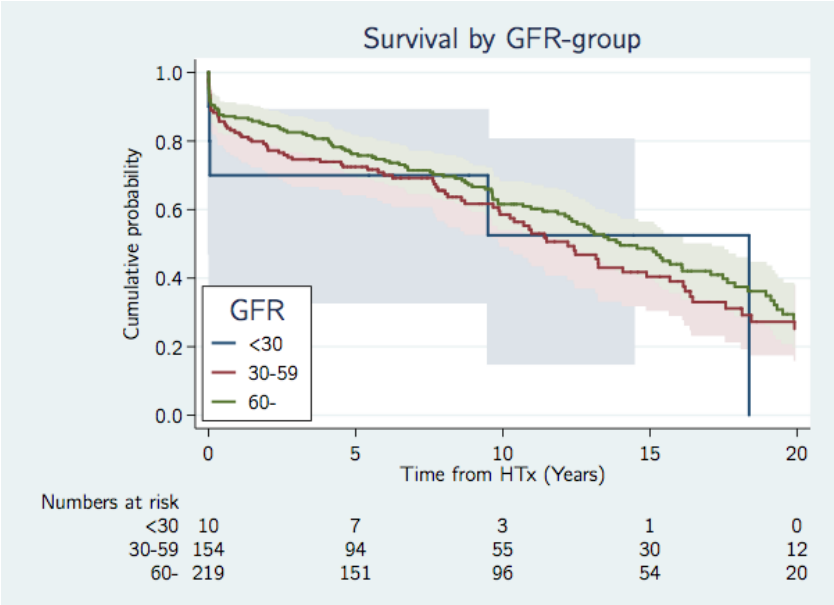

Figure 9: Survival after HTx. Patients stratified for preoperative mGFR: <30, 30-59 and 60-. The differences were not significant (log rank p=0.6).

Preoperative predictors of later end-stage renal disease (ESRD) were diabetes mellitus and mechanical ventilation prior to HTx. However, preoperative mGFR did not predict ESRD (Table 7).

Long-term survival was significantly worse for those experiencing a decrease in mGFR >25% during the first year after transplantation (Figure 10).

Table 7: Preoperative predictors of late ESRD (excerpt)

			Univariable Cox-regression			Multivariable Cox-regression		
		n	Hazard ratio	95% CI	p	Hazard ratio	95% CI	p
Age	Cont.	406	1.01	0.99-1.04	0.27			
Diabetes	No	239	1.00			1.00		
	Yes	40	2.56	1.31-4.98	0.006	2.39	1.15-4.99	0.02
MV prior to	No	337	1.00					
HTx	Yes	23	3.08	1.21-7.82	0.018	3.49	1.36-8.99	0.009
mGFR	<30	6	4.13	0.97-17.5	0.06			
(groups)	30-59	151	1.20	0.67-2.16	0.55			
	60-	218	1.00					
mGFR	Cont.	375	0.99	0.97-1.00	0.15			

ESRD: end-stage renal disease. CI: confidence interval. MV: Mechanical ventilation.

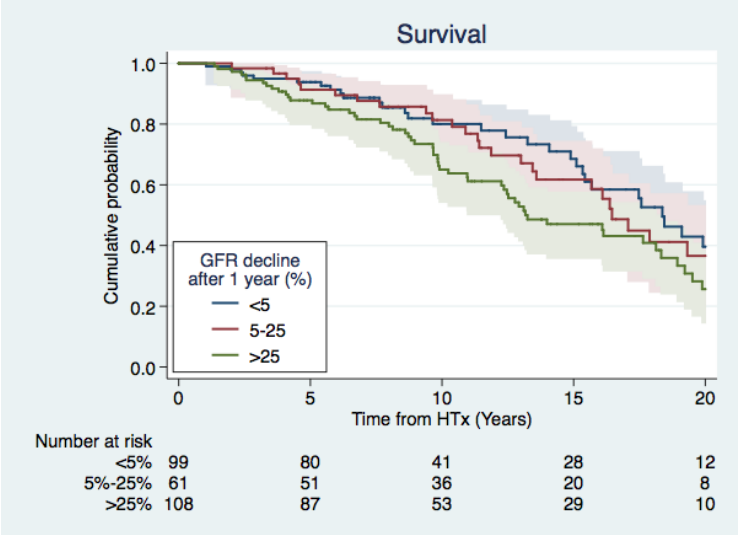

Figure 10: Long-term survival conditioned on 1 year and stratified for different levels of GFR decline during first postoperative year (log rank p=0.073). In this analysis only patients were included that were alive after 1 year, and thereafter stratified according to GFR decline. The shaded area around the curves illustrates the 95% confidence interval.

The need for acute postoperative continuous renal replacement therapy (CRRT/dialysis) was associated with impaired survival (Figure 11) but did not predict ESRD among survivors (Figure 12).

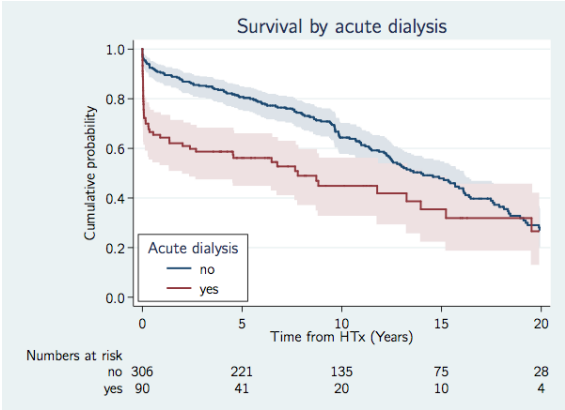

Figure 12: Survival of patients requiring early postoperative continuous renal replacement therapy (CRRT) in the ICU after HTx. Mortality was significantly higher in this group than in those without early renal failure, particularly during the first weeks/months (log rank p=0.0009, Hazard ratio 1.71, 95%CI 1.24-2.36).

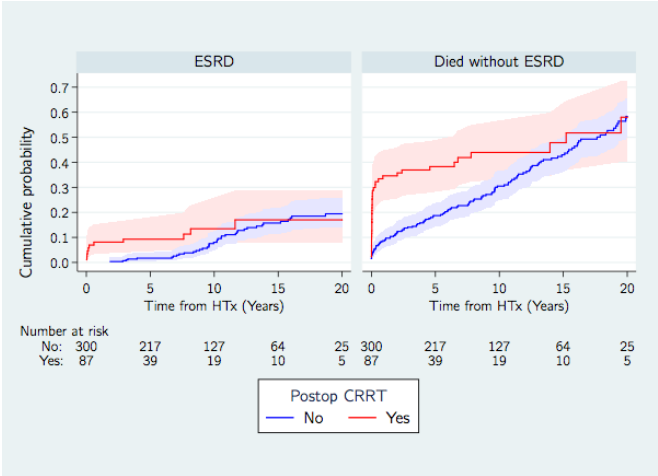

Figure 11: Pepe and Mori test comparing the cumulative incidence of events in the group that received early postoperative continuous replacement treatment in the ICU (CRRT), and those who did not. Those requiring early CRRT had a significantly increased risk for mortality (p=0.006) but the difference in later development of end-stage renal disease ESRD was not significant (p=0.09). Thus, those patients who survive early dialysis-dependent acute renal failure do not have an increased risk for later development of ESRD.

ANP and kidney function during CPB (Paper III)

In Paper III, we aimed to study the renal effects of extracorporeal circulation in the form of standard cardiopulmonary bypass (CPB), as routinely performed during heart surgery. In particular, we wanted to investigate whether atrial natriuretic peptide (ANP) could influence renal physiology if given during CPB. We designed an animal experiment that allowed for substantial invasive measurements of renal physiological variables, as described in Methods section.

During the initial phase of establishing the procedure as a functioning model for CPB, three animals died before the intended protocol was completed. During the entire experiment, a total of five pigs were sham-operated, of which two also received ANP-infusion. When the experimental model was established, 20 pigs (mean weight 56 kg, range 47-64) were included and randomized to continuous infusion of either ANP or saline solution before, during and after CPB.

We found no differences in mean arterial pressures (MAP) or renal perfusion pressures (RPP) between the groups during the procedure (Table 4-8).

There were significant increases in GFR, diuresis and sodium excretion in the ANP group compared to the control group ($p<0.001$). Interestingly, there was no difference between groups during the first hour after start of the ANP-infusion. Only after initiation of CPB did GFR increase significantly more in the ANP group at 60 ($p=0.003$) and 120 minutes ($p=0.002$), and even at 60 minutes after weaning from CPB ($p=0.023$) (Figure 13, left). Diuresis and sodium excretion followed this same pattern, with a CPB-related increase, though even more pronounced ($p<0.001$ for both) (Figure 13 and 14).

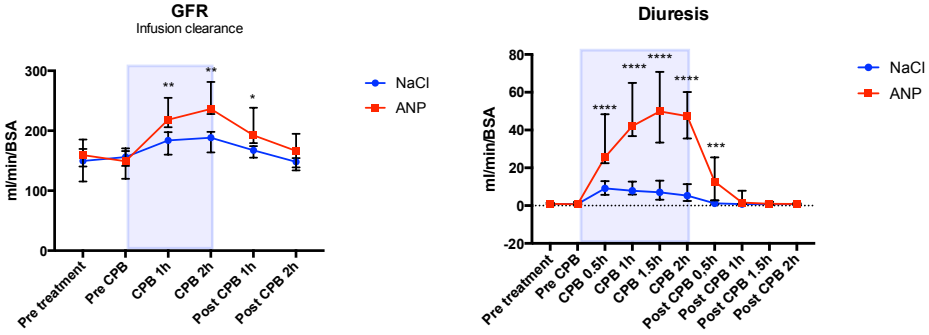

Figure 13: GFR and diuresis during the experiment. Period on CPB is marked in light blue shading. Note the absence of difference between the groups until CPB

RBF and RVR differed after weaning from CPB, but not during CPB.

For calculation of RVR, FF, RVO_2, RDO_2 and RO_2Extr see Methods section.

As opposed to GFR and diuresis, filtration fraction (FF) rose in the ANP group already from the initiation of ANP-infusion, and stayed higher than the control group during the entire procedure ($p<0.0001$) (Table 8).

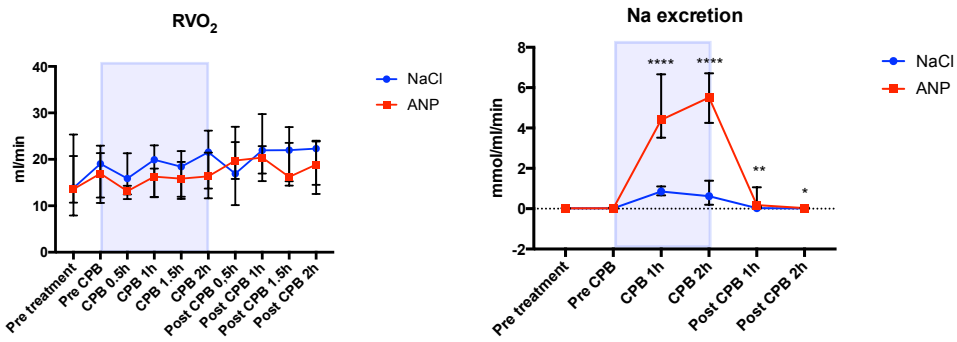

Figure 14: Renal oxygen consumption (RVO_2) and sodium excretion. There was no significant difference between groups with respect to RVO_2. Sodium excretion followed the pattern that diuresis and GFR did.

Renal oxygen consumption (RVO_2) (Figure 14) and renal oxygen delivery (RDO_2) did not differ between groups, while renal oxygen extraction (RO_2Extr) did ($p<0.001$). RO_2Extr was significantly higher in the ANP group 60 minutes after end of CPB ($p=0.002$).

In the present study on the renal effects of ANP during CPB, ANP did not affect RVO_2, despite the increase in GFR.

Laser Doppler Flowmetry (LDF):

Cortical perfusion measured with the LDF technique did not differ significantly between groups, and neither did medullary perfusion. However, when only NaCl was infused, the medullar perfusion did fall significantly *relatively* to the cortical perfusion during CPB (ANOVA $p=0.036$). In the ANP-group, no such reduction in medullar flow was observed (cortical/medullar flow $p=0.88$) (Table 9 and Figure 15).

Table 8: Renal and circulatory parameters. The table has been shortened for brevity. Where the ANOVA did not show significant difference between groups, the p-values for the repeated measures have been omitted. GFR, diuresis, sodium excretion and RBF are illustrated in figures above.

	NaCl	ANP	p-value
MAP (mmHg)	ANOVA interaction:		0.21
RPP (mmHg)	ANOVA interaction:		0.13
GFR (ml/min/BSA)	ANOVA interaction:		<0.0001
Diuresis (ml/min/BSA)	ANOVA interaction:		<0.0001
Na-excr (mmol/ml/min)	ANOVA interaction:		<0.0001
RBF (ml/min/BSA)	ANOVA interaction:		<0.01
Pre-treatment	554 (370-965)	534 (294-912)	0.74
ANP/Placebo 1h	647 (386-945)	451 (372-571)	0.22
CPB 1h	730 (497-1066)	720 (497-834)	0.74
CPB 2h	856 (519-1260)	692 (552-902)	0.28
Post CPB 1h	694 (428-909)	389 (313-451)	<0.01
Post CPB 2h	519 (346-605)	314 (217-455)	<0.05
RVR (mmHg/ml/min)	ANOVA interaction:		<0.01
Pre-treatment	0.145 (0.082-0.178)	0.132 (0.079-0.259)	0.76
ANP/Placebo 1h	0.113 (0.076-0.166)	0.141 (0.109-0.189)	0.17
CPB 1h	0.099 (0.065-0.149)	0.090 (0.078-0.135)	0.70
CPB 2h	0.089 (0.089-0.148)	0.099 (0.079-0.139)	0.56
Post CPB 1h	0.084 (0.071-0.145)	0.134 (0.111-0.173)	<0.05
Post CPB 2h	0.110 (0.092-0.158)	0.131 (0.108-0.212)	0.128
FF	ANOVA between animals:		<0.0001
Pre-treatment	0.271 (0.239-0.321)	0.312 (0.260-0.343)	0.28
ANP/Placebo 1h	0.279 (0.248-0.301)	0.380 (0.329-0.413)	0.01
CPB 1h	0.254 (0.240-0.274)	0.309 (0.282-0.343)	0.0004
CPB 2h	0.227 (0.222-0.243)	0.352 (0.317-0.406)	0.0019
Post CPB 1h	0.313 (0.294-0.320)	0.432 (0.394-0.450)	<0.0001
Post CPB 2h	0.294 (0.208-0.341)	0.377 (0.282-0.435)	0.065

Table 9: Laser Doppler Flow (LDF) measurements.

	Cortex	Medulla	p-value
LDF (NaCl)	ANOVA interaction:		0.036
Pre-treatment	100	100	NA
ANP/Placebo 1h	116 (98-127.5)	94 (90-117)	0.25
CPB 1h	106 (84-130)	82.5 (48.5-89.8)	0.027
CPB 2h	119 (75.5-162)	69.5 (45-136.8)	0.28
Post CPB 1h	106 (77.5-138)	93 (66-108)	0.39
Post CPB 2h	103 (76.5-124)	98 (66-118)	0.68
LDF (ANP)	ANOVA interaction:		0.88
Pre-treatment	100	100	NA
ANP/Placebo 1h	90 (78-105.5)	107 (92-161.5)	
CPB 1h	91 (75-124.5)	107 (92-161.5)	
CPB 2h	103 (78.5-119)	121 (35-157.5)	
Post CPB 1h	80 (72-105.5)	92 (61-102)	
Post CPB 2h	75 (60.5-94.5)	84 (64.5-157.5)	

LDF measurements from renal cortex and medulla. The results of LDF measurements are unitless "Flow Units", and therefore normalised to value "100" at initiation of experiment. All numbers are medians (IQR). Where ANOVA is significant, p-values for individual time points are shown. NA: Not applicable.

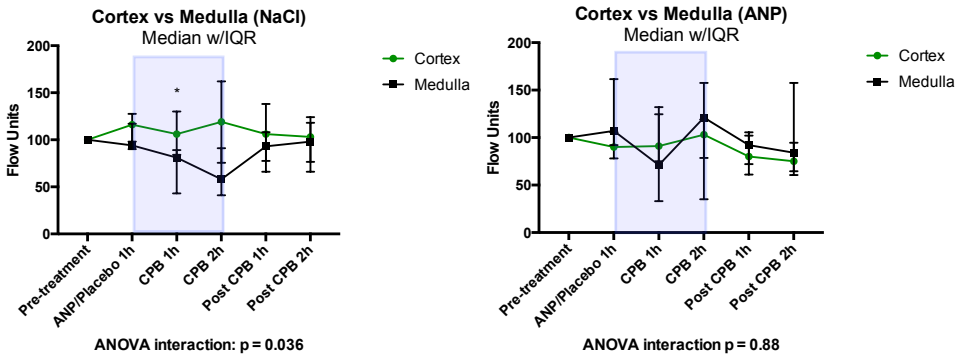

Figure 15: LDF measurements from renal cortex and medulla. When only saline solution is given, the flow in the medulla drops during CPB (left), and then recovers. This pattern disappears when ANP is infused (right).

There was no accompanying significant difference in the release of tubular-injury marker u-NAG between groups (Figure 16).

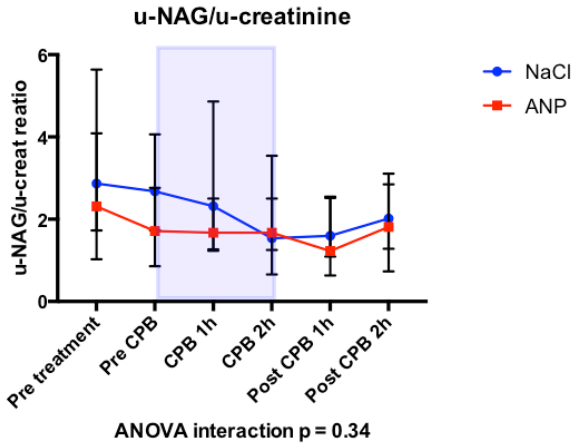

Figure 16: Tubular injury marker u-NAG/u-creatinine ratio during the experiment. There was no significant difference between groups. Period on CPB is marked in light blue shading.

Colloid- vs crystalloid-based prime (Paper IV)

The aim of Paper IV was to compare the renal effects of two different priming solutions for the heart-lung machine: A colloid-based and a standard crystalloid-based priming solution, respectively. The colloid of choice was dextran 40. The PrimECC study was designed as an investigator initiated, double-blinded, randomized controlled trial in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB). In all, 39 patients were randomized to the colloid-based prime and 41 to the crystalloid-based prime.

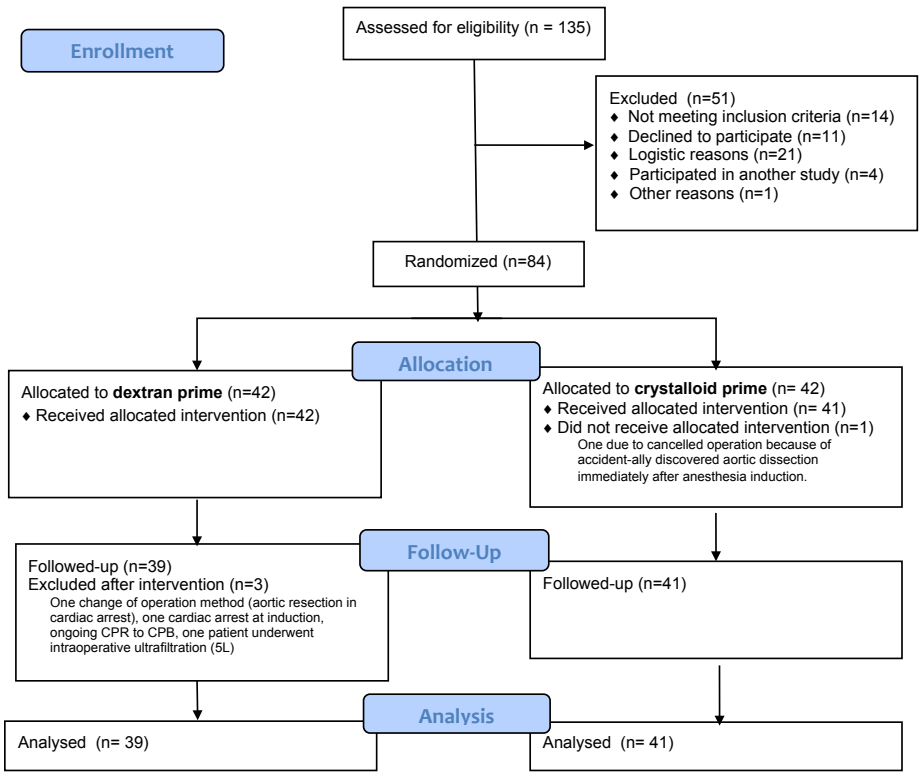

Figure 17: CONSORT diagram showing the inclusion and exclusion of patients in the PrimECC study.

The baseline characteristics between the groups did not differ significantly; neither did the intraoperative data, except for the time on CPB, which was 91±25 minutes for the dextran 40-group versus 107±39 minutes for the crystalloid group (p=0.042). Aortic clamp time and total operation time did not differ. Nor was there any difference in mean arterial pressure (MAP) during CPB or the type of procedures.

As expected, colloid osmotic (oncotic) pressure was higher in the dextran 40-group during and immediately after CPB. Serum haemoglobin and haematocrit followed an inverse pattern (Table 10). Within 2 hours however, these effects had already disappeared.

Table 10: Results: non-renal parameters

	Dextran 40 (n=39)	Crystalloid (n=41)	p-value
Oncotic pressure (mmHg)	ANOVA interaction:		<0.0001
Preoperative	19.9±2.8	20.7±3.1	0.24
During CPB	18.8±2.9	16.4±2.9	<0.001
Post CPB 10 min	19.2±2.7	16.8±2.9	<0.001
Post CPB 2h	19.5±3.1	18.6±3.2	0.20
Post CPB 24h	20.7±3.1	21.0±3.5	0.62
Haemoglobin (g/L)	ANOVA interaction:		0.008
Preoperative	125±14	126±14	0.80
Post CPB 2h	107±10	115±12	0.001
Post CPB 24h	109±11	109±13	0.79
Haematocrit	ANOVA interaction:		0.03
Preoperative	0.36±0.05	0.37±0.04	0.43
Post CPB 2h	0.31±0.03	0.34±0.04	0.002
Post CPB 24h	0.32±0.03	0.32±0.04	0.78

There was no difference in 30-day mortality, ICU stay, reoperation for bleeding or postoperative AKI (measured as a rise in serum creatinine according to KDIGO criteria).

The crystalloid group required significantly more volume substitution during CPB, and the total fluid balance (which was net positive in both groups) after the first 12 hours was 24 % higher in the crystalloid group (Table 11).

Table 11: Fluid balance (ml) peroperatively until first 12 hours postoperatively

	Dextran 40 (n=39)	Crystalloid (n=41)	P-value
Peroperative	555±595	841±574	0.031
Postoperative first 12h iv	876±677	1060±745	0.251
Postoperative first 12h iv + oral	1221±808	1360±797	0.440
Total preop +postop iv	1431±741	1901±922	0.014
Total preop +postop iv + oral	1775±866	2201±1011	0.047

During CPB, diuresis was significantly lower in the dextran 40-group than in the crystalloid group. Since CPB time differed between groups, the diuresis was also calculated per minute on bypass (ml/min/BSA) but was significantly lower nonetheless (Table 4-12).

Renal tubular injury, assessed by analysing the u-NAG/u-creatinine ratio, was less pronounced in the group receiving dextran-40 based colloidal solution as a pump prime compared to the crystalloid group (Figure 18, Table 12).

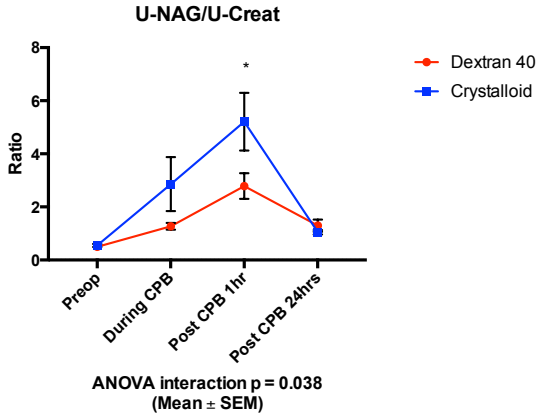

Figure 18: Ratio of NAG to creatinine in the urine. At 1 hour after CPB, this tubular injury marker was higher in the group that received crystalloid priming solution.

Table 12: Renal parameters

	Dextran 40 (n=39)	Crystalloid (n=41)	p-value
Diuresis (ml/BSA)	ANOVA interaction:		0.014
Perop, no CPB	221±172	177±113	0.18
During CPB (ml/BSA)	47±62	126±94	<0.0001
During CPB (ml/min/BSA)	0.53±0.76	1.23±0.83	0.0002
Post CPB 12h	800±261	730±220	0.31
Post CPB 24h	416±160	456±144	0.83
S-creatinine (µmol/L)	ANOVA interaction:		0.35
Peak s-creatinine	110.6±43.5	113.8±49.56	0.76
eGFR (ml/min/1.73m^2)	ANOVA interaction:		0.60
Lowest eGFR	60.6±20.3	59.9±21.2	0.87
Urinary Sodium (mmol/L)	ANOVA interaction:		0.022
Preop	96.1±41.0	95.8±35.5	0.97
During CPB	73.7±34.9	54.0±27.3	0.007
Post CPB 1 hr	89.5±36.4	68.0±32.5	0.007
Post CPB 24 hrs	44.3±25.7	45.5±23.3	0.83
U-NAG/U-Creatinine	ANOVA interaction:		0.038
Preop	0.5±0.3	0.5±0.3	0.35
During CPB	1.2±0.7	2.8±5.7	0.11
Post CPB 1h	2.5±2.7	4.7±6.3	0.045
Post CPB 24h	1.2±1.2	1.0±0.5	0.33

CPB: Cardiopulmonary bypass. S-creatinine: Serum creatinine. U-NAG/Ucreatinine: urinary NAG/creatinine ratio

DISCUSSION

Paper I

Main findings

The main finding in the study presented in Paper I was that the *estimated* GFR values using three of the most common estimating equations for this purpose (the Cockcroft-Gault, the MDRD or the CKD-EPI formulas), agrees poorly with the *measured* values, in a HTx cohort. This poor agreement was found both before HTx and at follow-up 1, 5 and 10 years after transplantation.

The Pearson's product-moment correlation analysis showed strong positive *correlations* of the follow-up comparisons of eGFR and mGFR ($r > 0.7$), but poor *agreement* according to the Bland-Altman agreement analyses that consistently yielded percentage errors around or above 100%. The finding that P30, the percentage of the estimated GFR results that comes within 30% of the measured GFR values, with only two exceptions was below 75% confirmed a low level of precision[114].

As clinicians, we argue that the level of agreement between estimated and measured GFR in HTx-patients is unacceptably low, and that measured GFR should be used in this patient group.

Study in perspective

Our study included 383 adult patients receiving HTx, and the comparison of GFR-estimating formulas and measured GFR comprised both pre-HTx values and follow-up values, with an average follow up time of 8.9 years (SD±6.96 years) years. We thus could conduct both pre-HTx comparisons, as well as comparisons at 1, 5 and 10 years after HTx.

To our knowledge, this is the first study to specifically address both the pre-HTx and long-term follow-up applicability of eGFR in a complete cohort of heart transplanted patients.

The study was performed on a very specific group of patients: Those with a severely failing heart who underwent heart transplantation. These patients have several risk exposures that may influence their kidney function, which separates them from other patients: Their pre-transplant renal function may be affected by their severe heart failure, they are exposed to major surgery and the heart-lung machine, and finally their kidneys are exposed to long-

term immunosuppressive treatment, drugs that have documented nephrotoxic effects. Thus, the results of this study should only be interpreted in the context of this particular patient group.

On the other hand, a comprehensive review[93] of the current status of the precision of the GFR estimation formulas in 2013 concluded that very little data existed concerning organ-transplanted patients. In particular, one could only identify one study concerning heart-transplanted patients; and this only included 27 patients[115]. That study indicated that the estimated GFR agreed poorly with measured GFR. Another study included 43 HTx patients[116], but only used Pearson correlation, not Bland-Altman agreement analysis. None of those studies analysed pre-HTx GFR, and there were no longitudinal data. We believe that our study contributes with important knowledge about the (lack of) agreement between estimated and measured GFR in this particular group, and we consequently decided to continue with mGFR in our clinical routine.

Study limitations

Even though our study is the largest in this patient group so far, it has several limitations. The general limitations of a retrospective cohort study are outlined in the statistics section 3.5.2. In our study, there were missing data at all time-points; both before HTx and during follow up. Thus, missing data made it impossible to analyse 7.9% of patients before transplantation, 18% at 1 year, 34% at 5 years and 42% at 10 years. This of course generates increasing uncertainty about the results as the time from HTx increases. However, since the results of the agreement analyses stay within the same intervals, we believe that a complete data set would not alter the results.

Another limitation is the formulas not explored: The LM-rev equation was not included in the study since this was not regarded as one of the formulas applied worldwide. There also exist formulas that are based on cystatin C, but no cystatin C samples was drawn from any of our patients, and therefore no such analyses could be performed in our cohort.

Paper II

Main findings

The main finding in this study of heart transplantation patients was that the patients' pre-transplantation kidney function did not predict mortality or later end stage renal disease, not even in the patients with a pre-transplant GFR less than 30 ml/min/1.73m^2. These results were achieved in a transplant program offering simultaneous or later kidney transplantation. We argue that this result indicates that a severely impaired renal function should not automatically exclude a patient from HTx-consideration, if otherwise eligible for HTx.

However, we also found that a drop of more than 25% during the first year after transplantation had a poorer prognosis than those with a first-year drop of < 5%. (HR 1.62; 95 % CI 1.04-2.53; p=0.03). Whether this finding is just describing a co-variance with some other factor affecting the survival in this group, or whether there exists some sort of causality, this observational study cannot answer.

In consistency with other studies, patients that developed postoperative acute kidney injury requiring renal replacement therapy (dialysis) had a significantly shorter long-term survival (HR 1.71; 95% CI 1.24-2.36; p=0.0009).

Study in perspective

Over the years, pre-transplant renal function has been considered an important factor to weigh in when assessing a patient's eligibility for HTx. This is a reasonable policy, since the incidence of chronic renal failure accumulates with increasing time after all solid-organ transplantation. The rationale behind a restrictive policy regarding the lowest acceptable level of pre-HTx kidney function is of course to maximise survival and minimise the risk of later end stage renal disease. However, controversy still remains about the exact level at which a reduced GFR should be considered a risk factor for transplantation. This is reflected in the development of the ISHLT guidelines regarding eligibility for HTx. At the 24th Bethesda conference in 1993, a creatinine clearance less than 50 ml/min was considered secondary exclusion criteria for HTx[38]. In 2006, the International Society of Heart and Lung Transplantation (ISHLT) recommended that a pre-transplant GFR of 40 ml/min/1.73m^2 or less should be considered as a relative contraindication to

HTx[39]. In the ISHLT 2016 guidelines this level was further reduced to less than 30 ml/min/1.73m^2. Below this level, the renal function is considered a relative contraindication for HTx[40].

The problem with impaired renal function in patients eligible for HTx might be a pre-renal problem since the kidneys might be suffering under the low cardiac output from the failing heart, but may be otherwise unaffected, and will have the potential to regain their normal function once the failing heart is exchanged for a new, healthier one. Therefore, a better procedure for pre-HTx evaluation of the actual GFR should be developed.

Despite the limitations described below, we believe that this study adds important information regarding clinical decision-making in HTx-recipients: both during the pre-HTx evaluation and later, during follow-up. We argue that this study demonstrates that a poor pre-HTx renal function should not automatically exclude a patient from HTx. Also, during follow-up, the GFR should be especially closely monitored during the first 12 month, since a drop in GFR of more than 25% in this period is associated with a shorter long-term survival.

However, we realise that these arguments only are valid if the HTx centre is capable of offering a wide range of treatments, including that of kidney transplantation if needed. This study cannot give any definitive answers about causality, or if any intervention actually can alter the prognosis. To answer such questions will need a prospective randomized controlled trial, and that might be difficult to perform, since it will be a very expensive and time-consuming study.

Study limitations

This single-centre retrospective longitudinal cohort study suffers from the same limitations mentioned for Paper I, chapter 5.1 above. Though 416 HTx recipients were included in the study, missing data reduced the numbers actually possibly to calculate risk factors on by as much as 7.9 % (see Figure 4-2). There is also most certainly a selection bias, particularly in the group with the lowest pre-HTx GFRs. These patients have probably been accepted for HTx due to other factors strongly in favour of transplantation.

Also, even if the log-rank analysis of the Kaplan-Meier survival curve was significant, the number of patients with a pre-HTx GFR < 30 ml/min/1.73m^2 was low, only 10 individuals, and after 5 years of follow-up so few that conclusions are difficult overall. This necessitates caution when interpreting the results.

Paper III

Main findings

The main finding in Paper III was that a continuous infusion of atrial natriuretic peptide could increase GFR during cardiopulmonary bypass. This was caused by pre-glomerular vasodilation and post-glomerular vasodilation, as reflected by the increase in renal filtration fraction. As the GFR is the major determinant of renal oxygen consumption, one would have expected that ANP should have increased renal oxygen consumption, which was not the case.

Study in perspective

The frustrating lack of existing modalities for effective prevention or treatment of postoperative AKI, calls for investigation of any substance that can potentially exhibit renoprotective properties[71]. Studies have indicated that ANP may be such a substance[81,117,118], but the drug has mostly been administered after CPB, or when the AKI already is manifest. The existing evidence is contradictory.

During and after CPB, the renal oxygen extraction increased with ANP, suggesting an impairment of the renal oxygen supply/demand relationship. Despite this, there was no increase in the tubular injury marker NAG. These findings indicate a potential renoprotective effect of ANP during CPB.

This lack of difference in renal injury despite the ANP-induced impairment of renal oxygenation (=impaired renal oxygen supply/demand relationship) could be explained by an ANP-induced inhibition of tubular sodium reabsorption in the medullary collecting duct, which would decrease RVO_2. This would protect the tubuli from the increased energy demand and oxygen consumption caused by the ANP-induced increase in sodium filtration and increased tubular sodium load.

This animal study thus supports the notion that ANP not only improves renal function but also seem to protect the kidney from the potential negative consequences of the increase in GFR, by blunting the increase in renal oxygen demand, otherwise seen when an agent increases GFR.

It is well known that the normal medullar perfusion is only a fraction of the cortical blood flow[119,120]. However, the CPB-related reduction of the blood

flow in the renal medulla was a new and unexpected finding. In the animals that only received saline infusion, the medullar perfusion seemed to decrease relative to the cortical perfusion, an effect that was not seen when ANP was infused. The ANP-induced maintenance of medullary flow perfusion during CPB could be another renoprotective effect of ANP, as the medulla is particularly sensitive to impaired perfusion. The mechanisms by which the CPB should induce such an effect are as yet unclear to us, as well as the pharmacological mechanisms behind the registered effect of ANP. An obvious difference between the normal circulation and the CPB-circulation is the non-pulsatile character of circulation induced by the CPB. However, it is unlikely that this pulsatility even under normal circumstances reaches the glomerular capillaries[121,122]. The findings in this study that CPB and ANP affect medullar perfusion will have to be repeated in larger animal studies, and in humans, before one can draw any certain conclusions from it. However, if these results stand up to repeated scrutiny, they may point to a hitherto unknown aspect of renal physiology.

To investigate the possible renoprotective effects of ANP further, randomized controlled studies in humans are necessary, particularly in the setting of heart surgery and CPB.

Study limitations

An inherent uncertainty in all animal studies is of course whether or not the findings also apply to human physiology. However, the fact that the animals reacted with increased GFR and increased diuresis to this human peptide hormone, points to at least some similarities in renal physiological response. The question of applicability of our results in humans can only be fully answered by studies in humans.

Another limitation of this study is that he numbers of animals included was limited, comprising only 10 animals in the treated group and 10 in the control group. Consequently, even if the statistics used have been adjusted to take this into account, the possibility that either a statistical error type I or type II may have occurred cannot be ruled out.

One could also question the use of human ANP for the evaluation of its renal effects in pigs. However, human ANP has been extensively used in experimental animal research, including pigs, using similar infusion rates as in the present study. Also, as mentioned above, the animals in this study responded to the human drug in an at least partly expected fashion, with increased GFR, diuresis and natriuresis.

Paper IV

Main findings

The main finding in paper IV was that the patients that received dextran 40-based priming solution had significantly lower release of the tubular injury marker U-NAG than those who received standard crystalloid priming solution ($p=0.038$). Also, their oncotic pressure was significantly higher during and immediately after CPB ($p<0.0001$). These findings suggest that a colloid-based priming solution might induce less kidney damage than a crystalloid-based one.

However, and importantly, there were no differences between groups regarding clinical renal outcomes, such as AKI or CRRT, or even s-creatinine or eGFR.

Study in perspective

The use of colloids in the priming solution of heart-lung machines has been debated for decades, and the results have been contradictory. Studies on colloid infusion in general have raised concerns about the safety of colloids[123-125]. Currently, crystalloid-priming solution seems to be the most common choice, with colloid priming being used in less than 25% of cardiac surgery centres around the world[11,12].

However, considering the looming clinical problem of post-CPB acute kidney injury and renal failure, the fact that colloid solutions have at least theoretically favourable properties when it comes to preserving renal function, makes continued research in this area necessary.

Dextran 40 is a molecule with colloid properties that at the same time are eliminated relatively quickly from the circulation. The potential advantage of such a molecule is that it could provide the assumed beneficial effects of a preserved microcirculatory colloid osmotic pressure during CPB, while at the same time avoiding prolonged negative effects on haemostasis thanks to the shorter half-life than larger dextrans.

This is, to our knowledge, the first randomized controlled study comparing the renal effects of a dextran 40-based and crystalloid-based priming solution.

What then, could be the reason for the less pronounced level of the tubular injury marker in the colloid group in this study? NAG has been shown to be a sensitive detector of AKI in cardiac surgery[52,53]. It has recently been shown that both colloid and crystalloid plasma volume expansion induces haemodilution. However, neither the colloid nor the crystalloid increases oxygen delivery, despite the fact that they both increased RBF[126]. However, GFR and renal oxygen consumption increased in the crystalloid but not in the colloid group. This may be because of the higher oncotic pressure in the glomeruli, preserving more fluid in the intravascular space and thus reducing the fluid load on the tubuli. Since the major determinant of renal oxygen consumption is GFR, the crystalloid solution induces a renal oxygen supply/demand mismatch, since the oxygen delivery was not increased proportionally, which was not seen with the colloid. It is plausible that the more pronounced renal tubular injury seen with the crystalloid, in the present study, could be explained by a more pronounced impairment of renal oxygenation, when compared with the colloid. In our study, serum creatinine was not sampled until 24 hours after CPB, and hence eGFR could not be calculated during or immediately after CPB. Thus, a possible explanation for the lower NAG/u-creatinine-ratio seen in the colloid group in this study might be that the higher oncotic pressure had a renal protective effect in reducing the renal oxygen consumption, and thus reducing renal hypoxia and tubular damage.

Also, in our study, the crystalloid required more fluid substitution during CPB, and the total accumulated fluid administration and fluid balance during surgery and the first 12 hours afterwards was significantly higher in the crystalloid group, indicating a higher level of tissue oedema also in the kidneys. Haemodilution with crystalloid solution has earlier been shown tin experimental studies to be associated with hypoxia-inducible factor 1α and renal tissue oedema[127].

Thus, both oxygen supply/demand mismatch and renal tissue oedema formation could be the reason for increased tubular injury in the crystalloid group in our study.

The fact that we could not demonstrate any differences in clinical outcome could have at least four different explanations:

- There might be no connection between the elevated NAG-levels and any clinically significant renal damage, expressed as increased levels of serum creatinine.

- The tubular injury was not large enough to detect a renal dysfunction, assessed by an increase in serum creatinine.
- There might be too few patients included in the study to identify a true difference in clinical outcome (type II error).
- The patients included in the study might have had a too low risk for the development of AKI for this strategy (colloid-based priming) to have any effect on renal outcome.

Since patients with a GFR <30 were excluded from inclusion into this study, it follows that those with the most vulnerable kidney function were not studied. Also, the mean estimated GFR of the patients included was 77 (77.11±7,9 in the dextran 40 group vs 77.07±18.04 in the crystalloid group, p=0.85), which is quite good. One might speculate that patients with a worse kidney function, already on the verge of kidney failure or dialysis might benefit the most from a strategy to protect kidney function during CPB, and would generate a difference even in clinical outcomes. To answer this question one would need to repeat the study in a high-risk population with a lower mean GFR, including even those with the worst kidney function.

Study limitations

The main limitation of this study was that the number of patients included was too low to detect any differences in renal outcome. Also, patients with a pre-operative GFR <30 were excluded altogether, and the mean GFR of the patients included was 75-80, indicating that the renal function in this group might be to good to be significantly affected by any strategy to increase their colloid osmotic pressure. Also, a future study should have some strategy for measurements of GFR during and immediately after CPB.

CONCLUSIONS

Based on the results from papers included in this thesis, we conclude the following:

- In heart transplantation recipients, a method comparison analysis of three of the most common equations for estimating GFR revealed that the estimated values are in unacceptably poor agreement with the actual measured values. We therefore recommend that measured GFR, not estimated, should be used when assessing renal function in heart transplantation recipients.

- Offered optimal therapy, including kidney transplantation, kidney function before heart transplantation does not predict mortality or later end stage renal disease, not even in the patients with a pre-transplant GFR less than 30 ml/min/1.73m^2. We argue that a severely impaired renal function should not automatically exclude a patient from HTx-consideration, if otherwise eligible for HTx.

- Atrial natriuretic peptide (ANP) increased glomerular filtration rate in an experimental model of cardiopulmonary bypass (CPB) caused by a pre-glomerular vasodilation and a post-glomerular vasoconstriction. Results also suggested that ANP might have a renoprotective effect. To investigate these results further, we recommend further studies in both animals and humans.

- A colloid-based priming fluid for heart-lung machines containing dextran 40 reduced renal tubular damage, assessed by urinary NAG/creatinine ratio. We recommend that further studies investigating the effect of dextran 40 based priming solution on patients with preoperatively impaired kidney function undergoing heart surgery and CPB, should be performed.

.

FUTURE PERSPECTIVES

The results of the studies described in this thesis, and the conclusions we draw from them, not only points to future studies but may also have immediate clinical implications for patients undergoing heart surgery:

Based on our studies, we recommend that renal function in heart transplantation recipients should be assessed with measured GFR, not estimated. It is worth noting that at the start of the study on estimated GFR (Paper I) our interest lay in exchanging our tradition of measuring GFR with the cheaper and quicker method of estimation. However, our study has convinced us to continue with measurements instead of estimations of GFR. We believe this strategy will give the most exact assessment of the patients' kidney function, and thus give us, their physicians, the best basis to decide on the most optimal treatments at the most optimal time.

Also, the results from Paper II have an immediate clinical application. When evaluating the eligibility for heart transplantation in patients with a severely reduced kidney function, our results indicate that no one should be excluded solely on the basis of their GFR. In the future, pre-transplant GFR should be considered important for designing a treatment strategy for the individual patient, such as concomitant kidney transplantation, but not for excluding them from heart transplantation.

Paper III and Paper IV points towards further studies: We need to establish with certainty whether ANP really has renoprotective properties, and if so, elucidate the mechanisms behind this property. Also, we need to design further studies to investigate how cardiopulmonary bypass affects medullar perfusion, and by what mechanisms.

Paper IV indicates that colloid-based priming with dextran 40 induces less tubular damage than crystalloid. Yet we need more studies to establish whether this effect also has clinical impact. Therefore, new randomized controlled trials in humans are warranted, particularly in patients with a preoperatively impaired kidney function, even with a GFR as low as <30 ml/min/1.73m^2.

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