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Aspects of Acute Kidney Injury in Thoracic Surgery and Intensive Care

With special interest in extracorporeal circulation and transplantation

Edgars Grīns, MD

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DOCTORAL DISSERTATION

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Faculty opponent
Professor Jan van der Linden
Karolinska Institute, Karolinska University Hospital, Stockholm, Sweden

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Title and subtitle: Aspects of Acute Kidney Injury in Thoracic Surgery and Intensive Care with special interest in extracorporeal circulation and transplantation

Abstract

Background: acute kidney injury (AKI) is a common clinical problem in cardiac surgery, lung-transplanted, and severely ill intensive-care patients, especially when extracorporeal (ECC) treatment modalities are utilized. The proposed mechanisms of AKI include ischemia-reperfusion injury and augmented inflammatory response. There is no specific treatment for AKI, so early diagnosis is crucial for timely interventions; therefore, search for early biomarkers of AKI is highly warranted. In experimental studies, AKI can be prevented by cyclosporine (CsA) given before the ischemic insult. CsA's cytoprotective effect has been attributed to the prevention of ischemia-reperfusion injury; however, as an immunosuppressive substance, CsA could potentially decrease cytokine production in response to surgery and ECC.

Aims: to investigate the potential of preventing AKI in elective cardiac surgery, delineate associated factors, and investigate the incidence of AKI and its impact on mortality in extracorporeal membrane oxygenation (ECMO) treated and lung-transplanted patients.

Methods: In studies, I and II, a prospective, placebo-controlled study in elective coronary artery bypass grafting (CABG) surgery, 154 patients were randomized either to 2.5 mg/kg cyclosporine or placebo. Study III is a sub-study to study II; cytokine levels were measured after induction of anesthesia and four hours after the end of cardiopulmonary bypass. In study IV, an observational study, serum cytokine levels were analyzed in 100 VA-ECMO-treated patients at pre-cannulation, 48 hours, and 8 days. Study V, a retrospective observational study on early postoperative AKI in 569 lung-transplant patients in Sweden between 2011 and 2020.

Results: Study I and II; cystatin C increase was more pronounced in the CsA group, with a difference of 20% (95% CI, 10.2 to 31.2, $p<0.001$). Study III; cyclosporine does not affect cytokine production ($p>0.005$) in response to CABG with ECC. Study IV; increased pre-cannulation IL-10 levels are associated with the development of AKI during ECMO support ($p=0.0025$, OR=1.2(1.02-1.32)). Study V: the incidence of AKI was 43% (n=243), and 54% (n=132) developed AKI grade 1.

Conclusions: Study I and II; CsA pre-treatment does not prevent CABG patients from AKI but causes a decrease in postoperative renal function compared to placebo. Study III; in CABG surgery, CsA pre-treatment does not affect the perioperative cytokine production. Study IV; increased pre-cannulation IL-10 levels are associated with the development of AKI during VA ECMO support. Study V; AKI after lung transplantation is a common complication; however, most patients develop mild AKI grade 1.

Key words: Acute Kidney Injury, Extracorporeal circulation, Cyclosporine, Interleukiens, Lung transplantation
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Aspects of Acute Kidney Injury in Thoracic Surgery and Intensive Care

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extracorporeal circulation and transplantation

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Cover photo of the Inaccessible Island in the Southern Ocean, taken by the author when the probability of finalizing this thesis felt as remote and inaccessible as this island.

To my Latvian and Swedish family

Table of Contents

List of Papers

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

I. Ederoth P, **Grins E**, Dardashti A, Brondén B, Metzsch C, Erdling A, Nozohoor S, Mokhtari A, Hansson MJ, Elmér E, Algotsson L, Jovinge S, Bjursten H. Ciclosporin to Protect Renal function In Cardiac Surgery (CiPRICS): a study protocol for a double-blind, randomized, placebo-controlled, proof-of-concept study. British Medical Journal Open. 2016 Dec 15;6(12): e012299.

II. Ederoth P, Dardashti A, **Grins E**, Brondén B, Metzsch C, Erdling A, Nozohoor S, Mokhtari A, Hansson MJ, Elmér E, Algotsson L, Jovinge S, Bjursten H. Cyclosporine Before Coronary Artery Bypass Grafting Does Not Prevent Postoperative Decrease in Renal Function: A Randomized Clinical Trial. Anesthesiology. 2018 Apr;128(4):710-717.

III. **Grins E**, Ederoth P, Bjursten H, Dardashti A, Brondén B, Metzsch C, Erdling A, Nozohoor S, Mokhtari A, Hansson MJ, Elmér E, Algotsson L, Shresta NM, Jovinge S. Effect of Cyclosporine on Cytokine Production in Elective Coronary Artery Bypass Grafting: A Sub-Analysis of the CiPRICS (Cyclosporine to Protect Renal Function in Cardiac Surgery) Study. Journal of Cardiothoracic and Vascular Anesthesia. 2022 Jul;36(7): 1985-1994.

IV. **Grins E**, Leacche M, Shresta NM, Bjursten H, Ederoth P, Jovinge S. Interleukin-10: A Potential Pre-Cannulation Marker for Development of Acute Kidney Injury in Patients Receiving Veno-Arterial Extracorporeal Membrane Oxygenation. Submitted.

V. **Grins E**, Wijk J, Lannemyr L, Bjursten H, Ricksten SE, Lindstedt S, Ederoth P, Dellgren G. Acute kidney injury after lung transplantation: incidence, pre- and intraoperative risk factors -a Swedish nationwide retrospective study. In manuscript.

Sammanfattning på Svenska

Akuta njurskador är vanliga hos hjärtopererade och svårt sjuka intensivvårdspatienter och är förknippade med ökad sjuklighet och dödlighet. Akut njursvikt är särskilt vanligt vid användning av hjärtlungmaskin, en mekanisk pump som ersätter hjärtats och/eller lungornas funktion och som används vid hjärtoperationer, transplantation av hjärta och ibland lungor, samt för att upprätthålla livsviktiga funktioner hos de allra svåraste kritiskt sjuka intensiv-vårdspatienter.

Tidigare forskning har visat att upp till 30% av hjärtopererade patienter drabbas av njursvikt. Att just användning av hjärtlungmaskin är förknippat med njurskador är känt sedan länge, trots det har risken inte minskat genom åren. Delvis beror det på att det inte är en, utan sannolikt flera samverkande faktorer som bidrar till njurskadan. Faktorer som ofta nämns är tillfällig minskning av blodtryck och blodflöde till njurar med syrebrist som följd, en alltför kraftig reaktion i immunsystemet hos patienterna och användandet av nödvändiga men njurskadliga mediciner.

Lungtransplanterade patienter måste ta en medicin som dämpar kraften i immunsystemet, ciklosporin, mot avstötning av de transplanterade lungorna hela livet. Vi vet att ciklosporin skadar njurarna på sikt. Samtidigt har man i djurstudier visat att ciklosporin kan skydda njurcellerna mot skada efter tillfällig syrebrist, om ciklosporin givits före skadan.

I de tre första delarbetena undersöker vi om ciklosporin givet före operationen kan minska njurskador hos patienter som genomgår planerad hjärtoperation i form av kranskärlskirurgi. Patienterna lottades till att behandlas strax före operationen med antingen ciklosporin eller placebo. Vilka patienter som fått vad var dolt (blindat) för både patienterna och personal. Våra resultat visade, tvärtemot vår hypotes, att njurfunktionen omedelbart försämrades hos patienterna som förbehandlades med ciklosporin. Vid kontroll efter en månad hade njurfunktionen normaliserats igen.

I tredje delarbetet, från samma studie som ovan, har vi studerat hur kroppens immunförsvar reagerar vid hjärtkirurgi och om denna reaktion påverkas av ciklosporin. Detta är intressant eftersom en alltför kraftig reaktion i immunsystemet tidigare visats öka risken för akut njursvikt. Våra resultat visade att kranskärlsoperation med hjärtlungmaskin orsakade en påtaglig aktivering av patienternas immunsystem, men att behandlingen med ciklosporin inte påverkade

graden av reaktion jämfört med placebo. Vi kunde inte heller påvisa något samband mellan hur olika delar av immunförsvaret regerar och ökad risk för njurskador.

I delarbete fyra har vi studerat svårt sjuka intensivvårdspatienter som behandlas med en typ av hjärtlungmaskin för upprätthållandet av livsviktiga organfunktioner, d.v.s. för att överleva. Genom att studera olika delar av immunförsvaret kunde vi visa att det går att förutsäga vilka patienter som kommer att utveckla njursvikt, och det till och med innan behandlingen med hjärtlungmaskin hade startats. Vi kunde också visa att patienter som utvecklade akut njursvikt och som har överlevt de första 30 dagarna, har lika god överlevnad på ett års sikt som de utan njursvikt.

Konsekvenserna av akut njursvikt är stora båda för patienter, vården och samhället. Patienterna löper risk att utveckla kronisk njursvikt, som ibland kräver dialysbehandling, eller behöva njurtransplantation, medan vården och samhället drabbas av kraftigt ökade kostnader.

Konsekvenserna av njursvikt är särskild stora för patienter som har genomgått lungtransplantation på grund av livslång behandling med njurskadliga mediciner, t.ex. ciklosporin. Det är inte känt hur många patienter som drabbas av akut njursvikt efter transplantation av lungor i Sverige.

I delarbete fem undersökte vi hur många av alla patienter som lungtransplanterades i Sverige mellan åren 2011–2020 som drabbades av njursvikt och sökte efter riskfaktorer för att utveckla njursvikt.

Våra slutsatser är att akut njursvikt är vanlig hos patienter som genomgår lungtransplantation i Sverige men samtidigt är låga i ett internationellt perspektiv. Vidare fann vi att behov av blodtransfusion under operationen är starkt bidragande faktorer till njursvikt. Bland patienter som utvecklar njursvikt, utvecklar merparten den lättaste graden av njursvikt men, patienter som drabbas av svåraste graden av den njursvikt har större risk att avlida inom ett år.

Sammanfattningsvis har vi i denna avhandling visat att behandling med ciklosporin givet före operation inte förhindrar utvecklingen av akut njursvikt och inte påverkar immunsvaret vid hjärtkirurgi där hjärtlungmaskin används. Hos kritiskt sjuka intensivvårdspatienter kan utvecklingen av akut njursvikt förutsägas genom att studera immunförsvaret redan innan livsuppehållande behandling med hjärtlungmaskin har påbörjats. Slutligen visade vi att akut njursvikt är en vanlig komplikation efter lungtransplantation i Sverige men något lägre än i andra jämförbara länder, samt att blodtransfusion är en riskfaktor för njursvikt.

Abbreviations

AE	adverse events
AKI	acute kidney injury
AKIN	acute kidney injury network
ATP	adenosine triphosphate
CKD	chronic kidney disease
CKD-EPI	chronic kidney disease epidemiological collaboration
CNI	calcineurin inhibitors
CRRT	continuous renal replacement therapy
CsA	Cyclosporine A
CVP	central venous pressure
DSMB	drug safety monitoring board
ECC	extracorporeal circulation
ECLS	extracorporeal life support
ECMO	extracorporeal membrane oxygenation
G-CSF	granulocyte colony stimulating factor
ICU	intensive care unit
IQR	interquartile range
IRI	ischemia reperfusion injury
IL	interleukin
IL-1β	interleukin 1 beta
IFN-γ	Interferon gamma
KDIGO	kidney disease improving global outcome
LTx	lung transplantation
MCP-1	monocyte chemoattractant protein 1

MDRD	modification of diet for renal disease
MIP-1β	macrophage inflammatory protein 1 beta
mPTP	mitochondrial permeability transition pore
NF-AT	nuclear factor of activated T-cells
SD	standard deviation
SCr	serum creatinine
RFR	renal functional reserve
RIFLE	risk injury failure loss end-stage
RRT	renal replacement therapy
ROS	reactive oxygen species
SAE	serious adverse events
SD	standard deviation
TNF-α	tumor necrosis factor alfa
TR	tricuspid regurgitation

Introduction

Modern cardiac surgery and intensive care are unimaginable without the use of extracorporeal circulation (ECC). Initially only used in cardiac surgery, ECC is now an integral part of modern intensive care, and the list of indications is growing. Today ECC is also used as rescue therapy in patients with severe cardiac and respiratory failure and for resuscitation in cardiac arrest.

However, ECC remains a high-risk procedure associated with several side effects, among others, increased risk of acute kidney injury (AKI). Despite recent advances in ECC, AKI remains a serious complication affecting morbidity and mortality. Long-term consequences of AKI include chronic kidney disease (CKD), dialysis, and kidney transplantation[1, 2].

The presumed ECC-related causes of AKI are many, including ischemia-reperfusion injury, augmented inflammatory response, and hemolysis. In addition to the presumed nephrotoxic effect of ECC in itself, cardiothoracic intensive care per se includes elements that are known or believed to be dangerous for the kidneys. For example, hemodynamic instability and nephrotoxic pharmacotherapy. A group of patients at particularly high risk of postoperative AKI are lung transplant recipients, as they are exposed to both ECC and mandatory treatment with nephrotoxic medications.

Here, calcineurin inhibitors (CNI) are of particular interest since CNI are both required immunomodulators to prevent rejection of the transplanted organs and known nephrotoxic drugs. The timing and dosing of CNI are often a compromise between desired pharmacological effects and undesired side effects. Although the nephrotoxic effects of CNI are explored to some extent, the clinical effects are complex and not yet fully understood.

Paradoxically, in animal studies, the administration of CNI before an insult result in decreased ischemia-reperfusion injury, an effect attributed to the inhibition of mitochondrial permeability transition pore (mPTP). Consequently, the CNI cyclosporine (CsA) has emerged as a potential candidate for organ protection when administered before surgery and ECC. In addition, as an immunosuppressive substance, it could potentially decrease the inflammatory response after surgery and ECC. However, the prophylactic administration of CsA in patients to prevent renal failure is controversial, since long-term CsA treatment induces renal insufficiency.

On the other hand, CsA's organ-protective effect on the heart and brain has been tested in human studies without reported renal side effects[3-5].

The reason AKI has such an impact on outcomes has not been fully explained. This highlights the importance of delineating factors associated with AKI and searching for treatment strategies to prevent it. This thesis aims to study various aspects of AKI in patients exposed to ECC treatment in cardiac surgery, intensive care, and lung transplant recipients.

Background

The history of extracorporeal circulation
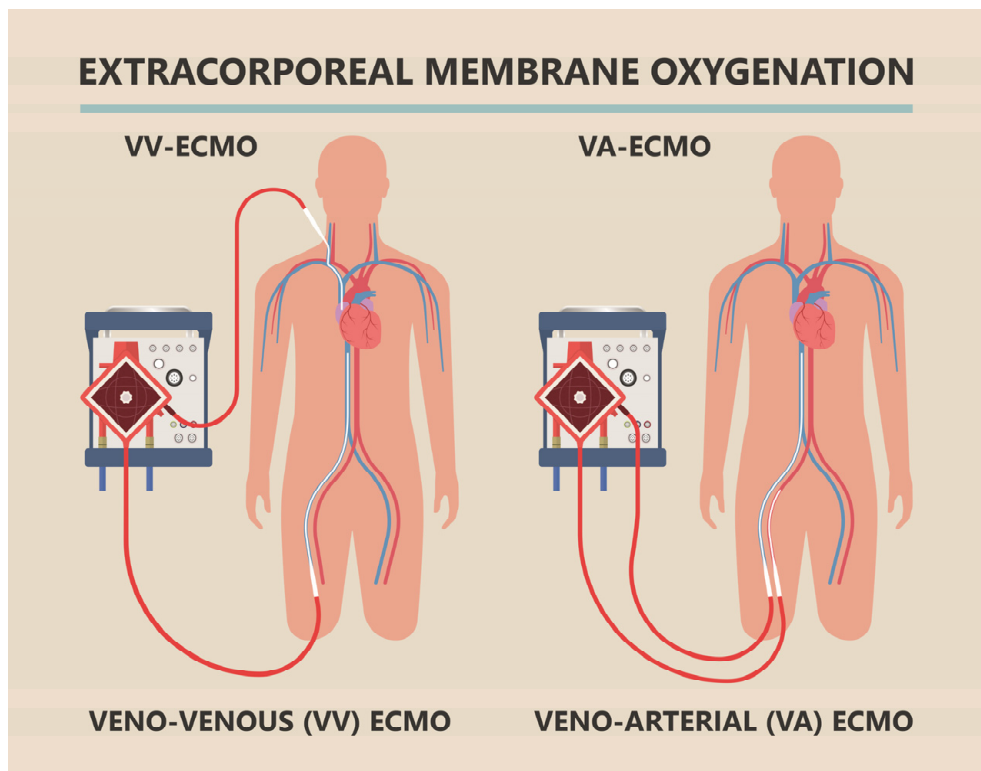
Figure 1. Extracorporeal circulation
Veno-venous ECMO and Veno-arterial ECMO systems. Adobe Stock license.

Extracorporeal circulation or "heart-lung machine" is a collective name for treatment modalities where venous blood is drained from the patient, carbon dioxide removed, oxygen added, and blood returned to the circulation via a vein or artery. In cardiac surgery, it is called a cardiopulmonary bypass (CPB), while in intensive care, it is called ECMO (Extracorporeal Membrane Oxygenation) or ECLS (Extracorporeal Life Support).

ECMO is typically used in cases of severe respiratory or circulatory failure, such as pneumonia or cardiogenic shock. CPB, on the other hand, is a surgical technique that uses a machine to take over the function of the heart and lungs during open-heart surgery. Both techniques share several similarities; however, there are important distinctions. The most obvious one is the duration of support; in contrast to CPB, ECMO can be used for weeks or even months. Another difference is the use of cardiotomy suctioning and air/blood interface in CPB, which renders higher levels of pro-inflammatory cytokines[6-8].

There are also differences in perfusion in CPB-treated patients compared to ECMO. Usually, the blood flow during CPB is non-pulsatile, in contrast to ECMO treatment, where pulsatile flow is generated depending on the residual function of the native heart.

The concept of ECC in humans in the 1930s-1940s was introduced by Dr Gibbon Jr, the individual first credited for successfully using cardiopulmonary bypass in humans. However, the prerequisite for using ECC was the discovery of heparin by Jay McLean in 1916, who, as a medical student, isolated from a canine liver an anticoagulant substance later named heparin[9, 10]. To this day, heparin remains the cornerstone of anticoagulation for almost all ECC procedures.

The clinical introduction of ECC in patients started in the late 1950s, initially functioning as a support device in cardiac surgery[11]. Oxygenators used in the early 50s were so-called bubble oxygenators, and their clinical use was limited due to foaming and bubble formation.

By the 1960s, the ECC was reliable enough to support circulation during short open-heart surgeries; however, further modifications were required for more prolonged use of ECC in intensive care.

The first reported successful use of ECC outside the operating room was in 1971 when ECC was used to treat a young man with respiratory failure after trauma[12]. This can be said to be the birth of ECMO. After that, numerous reports demonstrating the success of ECMO were published in the 1970s. However, the first randomized controlled trial in patients with respiratory failure was conducted in 1979[13], reporting 90% mortality in both groups, and the initial enthusiasm stalled over the next 30 years.

Today's ECMO landscape differs entirely from the early days of 90% mortality. Technological improvements and advances in other aspects of critical care have led to significant expansion of ECMO use. For example, the treatment of patients during H1N1 influenza and, most recently, the COVID-19 pandemic has led to the expansion of ECMO use. Since 2006, the use of ECMO in the United States has increased by over 400%, and the list of indications is growing[14].

Despite the increased use of ECMO, to this day there are no randomized clinical trials prove ECMO treatment to be superior to not using ECMO[15, 16]. ECMO

treatment is also fraught with complications; so, it should only be used when other treatment options have failed.

Cyclosporine; historical perspective and current indications of use

The discovery of the CNI cyclosporine revolutionized the field of organ transplantation 50 years ago, and even today, CNI remains a cornerstone of immunosuppression in solid organ transplantation[17].

Although initially derived from filamentous fungus in the antibiotic screening program, CsA showed an immunosuppressive activity on T lymphocytes which stimulated the interest to investigate CsA as an immunosuppressive substance in animals. The discovery of CsA in the 1980s led to a dramatic improvement in the survival of solid organ recipients.

In the mid-1980, another molecule from a soil fungus was discovered and named tacrolimus. CsA and tacrolimus share the same property of activated T-cell suppression via inhibition of calcineurin, and both drugs possess similar effects on cell-mediated and humoral immune responses. Calcineurin is stimulated by activated T-cells, which leads to activation of nuclear factor of activated T-cells (NF-AT) and increased production of IL-2 and other pro-inflammatory cytokines[18].

CNI binds intracellular proteins called immunophilins, forming a cyclosporin-cyclophilin complex in the case of CsA or FK-binding proteins in the case of tacrolimus. This complex binds to calcineurin, inhibiting its activity and leading to inhibition of IL-2, TNF-α, IFN-γ, and IL-4, all of which are involved in the inflammatory process[19]. Specifically, inhibition of IL-2 is believed to be responsible for CNI's immunosuppressive properties, as IL-2 is necessary for T-cell activation and proliferation.

With the multitude of effects of CsA, new treatment options targeting mechanisms of ischemia-reperfusion and inflammatory response may be hypothesized. Firstly, experimental evidence indicates that ischemia-reperfusion injury in the kidneys may be attenuated by CsA when administered before an ischemic event[20]. The renoprotective mechanism is attributed to CsA's inhibitory effect on mPTP[21]. Secondly, CsA also exhibits potent anti-inflammatory effects, the mechanism ascribed to an inhibitory effect on neutrophils and mononuclear cells[19]. Here the possible renoprotective effect could simply be attenuation of the inflammatory reaction in response to surgery and ECC.

Definition of Acute kidney injury

AKI is characterized by rapid loss of the kidney's excretory function. It refers to a clinical syndrome characterized by an accumulation of serum creatinine (SCr) and decreased urinary output. Over the years more than 35 definitions have been used to define AKI in clinical studies. However, the initial lack of a standard definition resulted in significant variation in reported incidence and the associated morbidity and mortality of AKI[22]. The introduction of RIFLE criteria (Risk, Injury, Failure, Loss, End-stage) was a new approach that included a variation of SCr and urinary output accordingly to three severity grades and has been validated in more than 500,000 patients[23].

The RIFLE criteria were further modified into AKIN (Acute Kidney Injury Network) criteria to include a small increment of SCr of at least 26.5 µmol/l, reached in 48-hour time window and proposed stages in 1.2 and 3. The comparison of RIFLE and AKIN classifications does not reveal clear superiority of one to another, although AKIN includes more patients with minor SCr change[24].

The most recent consensus definition of AKI has emerged from the Kidney Disease: Improving Global Outcomes (KDIGO) group[25] in 2012, which merged the RIFLE and AKIN criteria and aimed to establish a uniform definition and classification of AKI. According to KDIGO, AKI is determined as any of the following: increase in SCr by ≥ 26.5 µmol/l within 48 hours or increase in SCr to ≥ 1.5 times baseline within the 7 days; urinary volume < 0.5 ml/kg/hour for 6 hours or need for renal replacement therapy (RRT) (Table 1).

Table 1 Staging of Acute Kindey Injury according to KDIGO criteria

Stage	Serum creatinine	Urinary output
1	1.5 to 1.9 times baseline or ≥ 26.5 µmol/l increase within 48 hours	< 0.5 ml/kg/hour for 6 to 12 hours
2	2 to 2.9 times baseline	< 0.5 ml/kg/hour for ≥ 12 hours
3	3.0 times baseline or increase in serum creatinine to ≥ 353.6 µmol/l or initiation of renal replacement therapy	< 0.3 ml/kg/hour for ≥ 24 hours or anuria for ≥ 12 hours

Extracorporeal circulation and the kidney
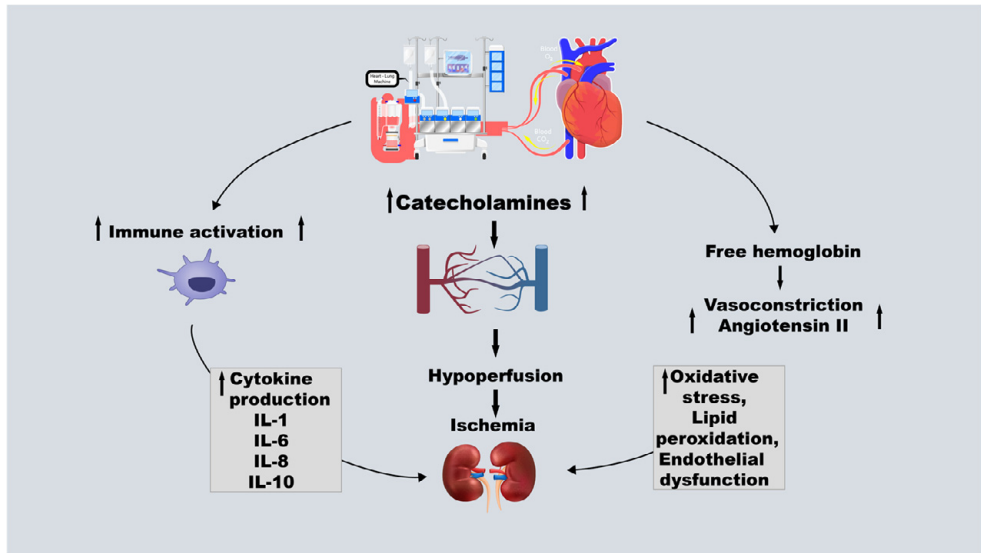
Figure 2.
Schematic overview of the pathophysiology of AKI in ECC-treated patients. IL; interleukin. Created by Edgars Grins 2023.

The relationship between the use of extracorporeal treatment modalities and AKI is well-established[26, 27]. For example, in elective coronary artery bypass grafting (CABG) patients, the perioperative use of CPB is associated with a higher incidence of AKI than compared to surgery without CPB[28]. The incidence is even higher in patients treated with ECMO, where up to 60% of the patients are reported to develop AKI[26, 29]. Patients treated with veno-arterial ECMO represent the sickest patient population in the intensive care unit (ICU) and the critical illness per se results in increased incidence of AKI, irrespectively of the use of ECC. Moreover, many risk factors for AKI, such as pre-existing kidney dysfunction, hypertension, or diabetes, are not modifiable and may contribute to the high incidence of AKI in this patient population[30].

Unfortunately, despite a significant increase in the use of ECC over the past decade and continuous technological developments, in-hospital mortality remains high [26, 31], and the long-term consequences of AKI include reduced lifespan, chronic renal failure, and dialysis[32, 33].

In patients exposed to ECC, the proposed causes of kidney injury are multifactorial, including ischemia-reperfusion, augmented inflammatory response, reduced pulsatility, renal vasoconstriction, and hemolysis[34-36]. Preventive pharmacological

and nonpharmacological strategies, including ischemic preconditioning, have largely failed to reduce the incidence of AKI after cardiac surgery[36, 37].

Therefore, despite advances in our understanding of the pathogenesis of AKI, the true impact of ECC on the development of AKI is challenging to assess.

Current evidence supports careful management of fluid balance and the avoidance of venous congestion while maintaining intravascular volume, cardiac output, and renal perfusion[38-40]. However, the proposed treatment options remain sub-optimal, and the main focus is on prevention and early diagnosis. Therefore, new studies are needed to understand the complex relationship between ECC and AKI.

Effect of cyclosporine on ischemia-reperfusion injury
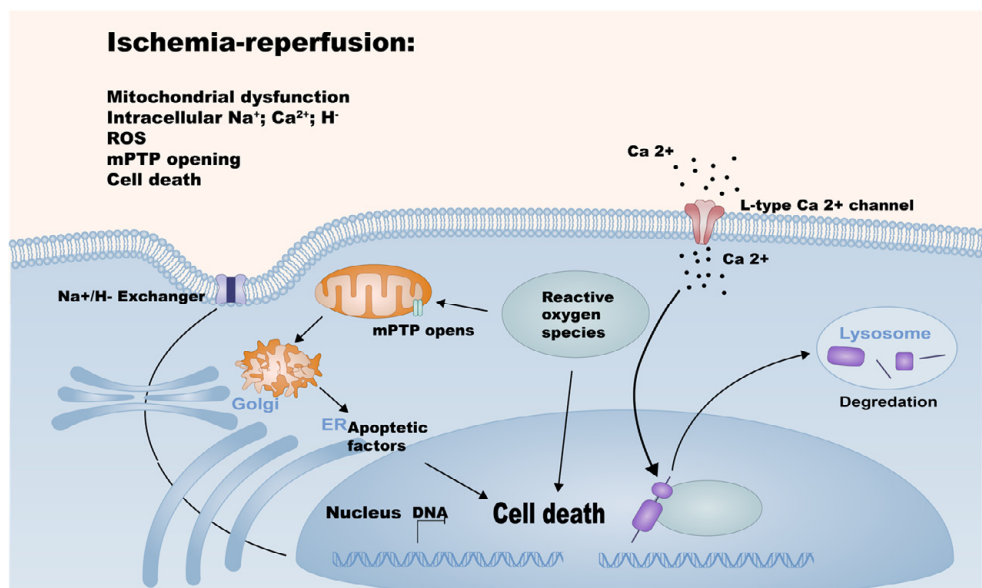
Figure 3.
Schematic overview of the molecular mechanisms involved in ischemia-reperfusion injury. mPTP: mitochondrial premeability transition pore, ROS: reactive oxygen species. Created by Edgars Grins 2023.

The obstruction of blood flow and following reperfusion is common in clinical conditions such as circulatory arrest, ischemic stroke, myocardial infarction, cardiogenic shock, etc., and is inevitable in organ transplantation.

The re-establishment of blood flow is essential to salvage ischemic tissues, however, the following reperfusion causes paradoxical exacerbation of cell dysfunction called ischemia-reperfusion injury (IRI). The term IRI describes the experimental and

clinical findings when restoring blood flow to ischemic tissues aggravates the local injury and may even induce impairment of remote organ functions. IRI is a complex pathophysiological phenomenon involving activation of cell death programs, inducing inflammation, and activating innate and adaptive immune system[41].

The hypoperfusion causes cell hypoxia and leads to anaerobic metabolism, lower levels of adenosine triphosphate (ATP), and mitochondrial dysfunction. In addition, electrolyte disbalance caused by failure of sodium-potassium and calcium pumps leads to intracellular accumulation of calcium, sodium, and hydrogen and consequently cell swelling.

During reperfusion, when intracellular pH returns to pre-ischemic values, the restoration of oxygen levels leads to production of large amounts of reactive oxygen species (ROS). The high levels of ROS together with elevated calcium are believed to lead to opening of mPTP, the final step in reperfusion injury[42, 43].

The mPTP remains closed during ischemia and opens soon (~2 min) after reperfusion[44], which causes loss of membrane potential, ATP breakdown and ultimately, cell death. A key component in the opening of the mPTP after an ischemic event is cyclophilin-D. It was discovered that the opening of mPTP can be inhibited pharmacologically by the calcineurin inhibitor CsA through its binding to cyclophilin-D[45]. Cyclophilin-D is a mitochondrial receptor for cyclosporine, but not itself a pore component[46].

In animal studies, kidney injury can be reduced by the administration of cyclosporine before the ischemic event [21, 47], thus, promoting the idea of preventing AKI with CsA administered before surgery also in humans. Importantly, in experimental settings CsA has been administered before the ischemic event and subsequent reperfusion.

Renal ischemia-reperfusion injury in cardiac surgery

In cardiac surgery with ECC the perioperative causes of kidney injury are multifactorial, including IRI, augmented inflammatory response, renal vasoconstriction, and hemolysis[34, 35]. Whether the treatment strategies directed specifically to prevent IRI in elective cardiac surgery patients is of clinical importance is unknown. Theoretically, ischemia reperfusion injury[35], could be prevented by preoperative administration of CsA.

However, the administration of CsA to prevent renal failure in cardiac surgery is controversial; long-term cyclosporine treatment is associated with the development of chronic progressive kidney disease, which is usually irreversible[48]. Nevertheless, the impact of a single dose of CsA on postoperative kidney function is not known.

In experimental studies, the short-term effects on kidneys following CsA exposure include reduced renal blood flow and glomerular filtration by vasoconstriction of the afferent and efferent glomerular arteries, which is mainly reversible and dose dependent[49-51]. On the other hand, CsA's protective effect against ischemia-reperfusion induced myocardial injury has been tested in several human studies without reported renal side effects. All these studies administered CsA as bolus injections of 2.5 mg/kg in cardiac patients before the reperfusion. However, renal outcomes were not the primary endpoints in these studies[3, 4, 52-55].

Role of inflammation and coagulation in AKI development

Augmented inflammatory response, the so-called cytokine storm, is not only seen in cardiac surgery or as a response to ECC treatment. Severe critical illness is also associated with acute inflammation secondary to increased cytokine production leading to multiorgan failure[56]. However, more than 150 clinical trials tested the effect of impeding sepsis associated individual mediators without success[57].

Inflammation is a physiological process that protects the organs against injurious stimulus of ischemia, infections, or toxins. In hemodynamically unstable patients, kidneys are usually the first organ to fail, as they require 25% of the cardiac output. The kidney detects these stimuli through intrarenal immune cells and native renal cells, which respond to stimuli by secreting cytokines, such as IL-10 and IL-6, and recruiting leucocytes to the area of damage[58]. The relation between renal cytokine production and AKI is complex. Although renal cytokine production has been shown to aggravate a renal injury, under some conditions, cytokines ameliorate renal tubular injury and protect kidneys from further damage[59, 60].

The role of augmented inflammatory response in AKI's development in patients treated with extracorporeal circulation is well established, as blood exposure to artificial surfaces induces complement activation and increased cytokine production[27, 61]. Nevertheless, the specific pathogenesis of AKI in ECC treatment is inadequately understood and involves multiple pathological processes, including coagulation and inflammation. Furthermore, there is a bidirectional relationship between coagulation and inflammation, whereby inflammation leads to the activation of coagulation, and coagulation affects inflammatory activity[62]. Increased levels of proinflammatory cytokines, mainly IL-6, stimulate mononuclear cells to express tissue factor, which on exposure to blood, contributes to increased levels of thrombin[63].

In the vascular system, endothelial cells maintain equilibrium by producing pro- and anticoagulation factors. When a patient's blood comes into contact with an ECC

circuit, the coagulation pathways, divided into extrinsic and intrinsic, are activated[61]. The intrinsic pathway is initiated by the exposure of blood cells to the negatively charged surfaces of the ECC and the activation of factor XII. Activated factor XII leads to generation of thrombin which subsequently converts fibrinogen to fibrin. The extrinsic pathway is activated by tissue trauma and the exposure of blood vessels to tissue factor, which promotes the activation of factor X[64]. The generation of activated factor X is the point where both coagulation pathways converge, leading to coagulation and inflammation[65-67].

Despite recent improvements in ECC circuits, oxygenators, and the introduction of heparin-bounded surfaces, the inflammatory response remains a clinical concern. A number of pharmacological and non-pharmacological interventions have been evaluated in clinical and experimental trials with the aim to ameliorate inflammatory responses in ECC treated patients[68]. Although the perioperative administration of steroids, statins, and volatile anesthetics have been associated with the attenuation of inflammation, their effect on clinical outcomes remains modest[61].

However, even though ECC treatment modalities are implicated in an intense inflammatory response, in some cases, ECC treatment can reduce inflammation by improved perfusion and gas exchange. For example, in patients with ARDS or cardiogenic shock, the initiation of ECMO treatment can reduce stress induced by mechanical ventilation or hypoperfusion and thus reduce the inflammatory response[69].

Cytokines as a potential biomarkers of acute kidney injury

AKI is a sudden loss of excretory kidney function within 7 days after renal insult conventionally diagnosed by an increase in SCr, a decrease in urinary output, or the use of renal replacement therapy (RRT). However, SCr levels and urinary output may be affected by the patient´s volume status and use of diuretics. Furthermore, a sudden deterioration in renal function will not be reflected by an immediate increase in SCr; typically, it takes 2-3 days from renal insult to SCr maximum[70]. Therefore, there is an unmet need for earlier identification of patients with AKI, and the search for new biomarkers is ongoing and highly warranted.

Clinically applicable biomarkers should ideally be non-invasive, easily accessible, and rapidly measurable. In recent years several novel biomarkers have been tested for the ability to detect AKI earlier than an increase in SCr and a decrease in urinary output. In addition, three randomized trials have confirmed the benefit of biomarker-guided preventive strategies after kidney insult in patients after cardiac surgery and after major abdominal surgery[38, 71, 72].

In cardiac surgery patients, several cytokines have been reported to be early biomarkers for postoperative AKI development in pediatric and adult patients[73-76]. The results are, however, contradictory. For example, some studies have reported IL-6 and IL-10 as early biomarkers for postoperative AKI development; at the same time, other groups did not find such an association[77, 78].

The correlation between increased levels of IL-10 and acute and chronic kidney diseases has been described earlier[60]. Increased serum levels of IL-10 on admission were associated with an increased risk for AKI and mortality in septic patients[79]. Furthermore, high IL-10 levels at the time of ECMO installation and during the first 6 hours after ECMO support were associated with a grave prognosis[80].

Cytokines are attractive as biomarkers since they are easily measurable and might signal an upcoming or resolving AKI earlier than SCr. However, so far, the evidence for cytokines as AKI biomarkers is limited and more research is required to adopt them into clinical praxis.

Acute kidney injury in lung transplanted patients

Lung transplantation is a high-risk procedure associated with various perioperative complications, including AKI. In addition, the characteristics of lung transplant recipients have changed over time, with recipients becoming older and sicker and with more patients on mechanical support at the time of transplantation[81, 82].

The reported incidence of AKI after lung transplantation is 40 to 68%, depending on the definition, and long-term consequences are chronic kidney disease, dialysis, and increased mortality[83, 84]. Furthermore, the effects of AKI are also associated with an increased burden on the health care system and elevated costs[85]. In addition, kidney impairment in lung-transplant recipients is especially problematic, considering life-long treatment with CNI, which further decreases renal function[86, 87]. Therefore, searching for potentially modifiable preoperative and intraoperative factors associated with AKI is highly warranted.

Unfortunately, the preoperative risk factors are not easily modifiable; however, they could be used to modify further the individual patient's lung allocation score, which was developed to prioritize the sickest candidates and thus reduce the waiting list mortality. For example, by replacing waiting time as a primary determinant for lung allocation, the waiting list mortality was reduced by 40%[88].

On the other hand, the modifiable AKI-associated intraoperative factors could be used to choose the optimal strategy regarding intraoperative circulatory support, management of volume status, use of diuretics, blood transfusions, vasopressors, etc.[89].

Most studies investigating postoperative AKI in lung transplant recipients are of the retrospective design[83, 84, 90]. While retrospective studies can be useful for generating hypotheses and identifying potential risk factors, they have several limitations that can affect the validity and reliability of the results. This can make it difficult to establish cause-and-effect relationships and precludes making definitive conclusions about the effectiveness of a particular treatment.

Aims

The overall aim of this thesis was to explore various aspects of acute kidney injury associated with the use of extracorporeal circulation in thoracic surgery, lung transplantation and intensive care.

Papers I & II

To investigate if cyclosporine pre-treatment can reduce the level of kidney dysfunction in patients scheduled for elective CABG surgery where ECC is utilized.

Paper III

To evaluate cyclosporine's effects on cytokine production in response to CABG surgery and ECC and delineate factors associated with postoperative kidney impairment.

Paper IV

To investigate if cytokine levels before the initiation of veno-arterial extracorporeal support can predict the development of AKI in hemodynamically severely compromised patients. To investigate the impact of AKI on 30-day and 1-year mortality.

Paper V

To investigate the incidence of early AKI in LTx recipients in the Swedish LTx program between 2011 and 2021 and to delineate the pre- and intraoperative risk factors associated with AKI.

Material and methods

Overview of this thesis

Table 2 Overview of the five studies included in this thesis

	Study I	Study II	Study III	Study IV	Study V
Aims	To describe the scientific background, rationale, methods, and sample size calculation for study II and III	To investigate if cyclosporine pre-treatment can reduce the level of kidney dysfunction in elective CABG surgery	To investigate cyclosporine`s effects on inflammatory response in elective CABG surgery patients and factors associated with AKI	To investigate if pre-ECMO cytokine levels can predict AKI development and to study the incidence of AKI after ECMO start and its impact on survival	To investigate incidence, and factors associated with early AKI in lung transplant recipients in Sweden between 2011-2020
Design	Descriptive study protocol for study II	Double blind, randomized, prospective,placebo-controlled, investigator-initiated proof-of-concept study	Sub-study to study II, a predefined analyses of cyclosporine's effect on inflammatory response	Observational study	Retrospective study
Study population		154 elective CABG patients	67 randomly selected patients from study II	100 V-A ECMO treated patients	569 Lung transplanted patients
Statistical analyses	Power and samples size calculations	Student´s t-test, Mann-Whitney's U-test, linear mixed model	Student's t-test, Wilcoxon´s test, Fischer´s exact test, Univariate and multivariate linear regression analysis	Student´s t-test, Mann-Whitney's U-test, Univariable and multivariable regression analysis, Kaplan-Meier survival analyses, log-rank analysis	Descriptive statistics, univariable and multivariable regression analysis, Kaplan-Meier survival analysis, log-rank analysis

AKI=acute kidney injury; CABG=coronary artery bypass grafting; V-A ECMO=veno-arterial extracorporeal membrane oxygenation

Patients and study design

Paper I & II

Paper I is a pre-published peer-reviewed study protocol for the CiPRICS (Cyclosporine to Protect Renal Function in Cardiac Surgery) study (Paper II), where study's endpoints, safety analysis, and statistical analysis plan were pre-specified[91].

Paper II, the CiPRICS study, was performed at the Department of Cardiothoracic Surgery Anesthesia and Intensive Care at Skåne University Hospital, Lund. This study was an investigator-initiated, prospective, double-blind, randomized, placebo-controlled, parallel-design, single-center study.

Study registration and ethical approval

The study was registered under EudraCTNo. 2014-004610-29 and ClinicalTrials.gov (NCT02397213), and approved by Regional Ethical Review board, Lund (LU 2014/777), and Swedish Medical Products Agency (Uppsala, Sweden). The study was conducted in accordance with the current version of to the Declaration of Helsinki, and the European Guidelines for Good Clinical Practice.

Study population

Men and women scheduled for elective CABG surgery including the use of ECC were eligible for the study. All patients were informed at the pre-operative evaluation and written informed consent was obtained before the enrollment. The study protocol dictates two strata were preoperative eGFR 15 to 59 or 60 to 90 ml/min/1.73m^{-2}.

Inclusion criteria

Men and women scheduled for elective CABG surgery including the use of ECC were eligible for the study. Preoperative eGFR 15-90 ml/min/1.73m^2, calculated using both cystatin C based on the Chronic Kidney Disease Epidemiological collaboration (CKD-EPI) and creatinine based on Modification of Diet for Renal Disease (MDRD) formula. The lowest eGFR value were used as inclusion criteria.

Study endpoints

Primary endpoint: relative P-cystatin C change from baseline to day 3. Secondary endpoints included markers of inflammatory response and biomarkers of kidney, heart, and brain injury.

Definition of AKI

Risk Injury Failure Loss End-stage (RIFLE) criteria, based on changes in plasma creatinine, were used to assess the incidence of AKI.

Study drug

Lipid emulsion of cyclosporine (CicloMulsion 5 mg/mL) or matching placebo (NeuroVive Pharmaceutical AB, Lund, Sweden), given as a single intravenous bolus dose of 2.5 mg/kg. The difference between the active drug and placebo was the presence or absence of cyclosporine.

Study protocol and intervention

After induction of anesthesia and before surgery the study drug/placebo corresponding to 2.5 mg/kg cyclosporine was administered as a 10-minute infusion. Efficacy data were collected preoperatively and daily until postoperative day 4. The study was terminated after a follow-up phone call after day 30.

Safety measurements

An independent Drug Safety Monitoring Board (DSMB: Lund, Sweden) assessed the safety of the study after 50 and 100 patients and adverse events (AE) and serious adverse events (SAE) were collected daily. In addition, a follow-up telephone call was made 30 days after the surgery to determine if any new adverse event had occurred after the discharge.
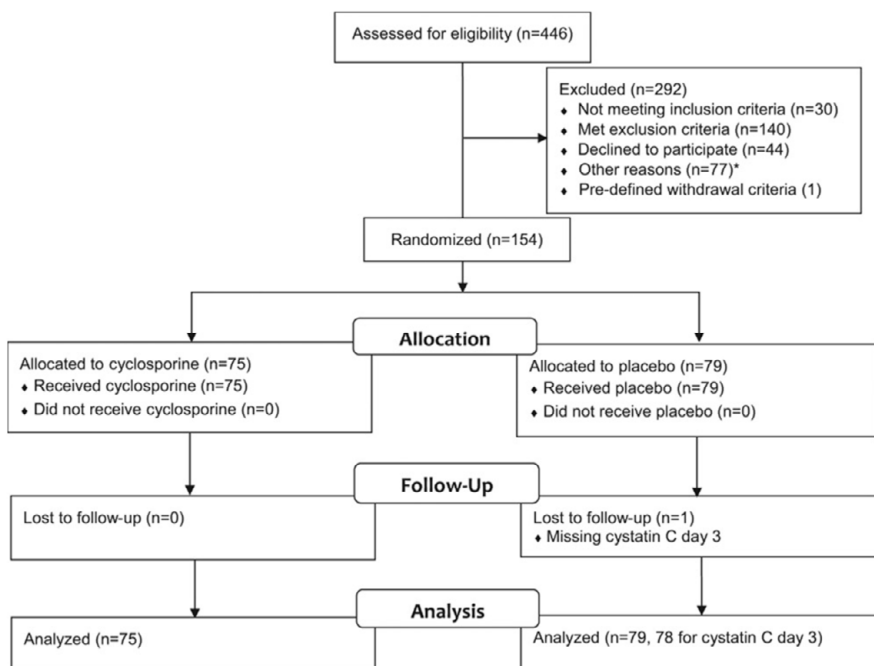

Figure I.1. Study flowchart

Paper III

This is a pre-defined sub-study to the main CiPRICS study (Paper II)[70], also described in the paper I, and a collaborative work between the Department of Cardiothoracic Surgery Anesthesia and Intensive Care at Skåne University Hospital, Lund, and Van Andel Institute MI, US. The cytokine analyses were performed in the US.

Cyclosporin's effect on inflammatory response was a pre-defined endpoint in the CiPRICS study. However, after the commencement of the study, we temporarily stopped the cytokine sampling, as, unexpectedly another ethical approval was requested for sample analysis outside the European Community. Cytokine sampling was resumed as soon as this additional approval was obtained, and following consecutive patients were included in the study. The study population represents patients recruited after the approval. Out of 154 patients in the main CiPRICS study, 67 were included in this sub-study.

Study population

67 randomly selected patients from the original CiPRICS study were included.

Ethical vetting and registration

No additional registration was required for this sub-study, as cytokine analysis was pre-specified secondary endpoint. However, additional ethical approval was obtained for sample analysis outside the European Community.

Study endpoints

The endpoint was plasma cytokine concentration change from preoperative to four hours after the end of ECC. Secondary endpoints were factors associated with a 30% increase in cystatin C on postoperative day 3.

Definition of renal dysfunction

The 30% increase in plasma cystatin C defined postoperative renal dysfunction. The KDIGO classification was used to assess the incidence and grade of AKI.

Paper IV

This is a single-center clinical observational study on 100 VA ECMO treated patients. Patients included in the study were treated at Meijer-Heart Center/Spectrum Health, Grand Rapids, MI, US. The study concept and design, data acquisition, analysis and interpretation was a collaborative work between the Department of Cardiothoracic Surgery, Anesthesia, and Intensive Care at Skåne University Hospital, Lund, and Spectrum Health/Van Andel Institute, GR, MI, US.

Registration and ethical approval

All ECMO treated patients at Meijer Heart Center/Spectrum Health are included in a local database registered at ClinicalTrials.gov (NCT02748668). Clinical data for the study were collected from this local ECMO database. The study protocol was reviewed and approved by the local Institutional Review Board at Spectrum Health and Van Andel Institute, Grand Rapids MI, USA, approval number 2016-171.

Study population

One hundred consecutive patients requiring veno-arterial circulatory support were included. Both men and women were included in the study. Patients were excluded from the analysis if they were treated with renal replacement therapy before the initiation of circulatory support, were < 18 years of age, or were treated for autoimmune diseases.

Study endpoints

The primary endpoint was correlation between pre-cannulation cytokine levels and AKI after start of VA ECMO treatment. The secondary endpoints were the temporal pattern of serum concentrations of cytokines over time, and the relation between the incidence of AKI and all-cause mortality at 30-days and 1-year.

Definition of AKI

KDIGO criteria were used to define the incidence of AKI. Patients were stratified into two sub-groups based on pre-cannulation renal function mildly to moderate decreased (eGFR $\geq$ 45 ml/min/1.73m2) or moderately to severely decreased (eGFR < 45 ml/min/1.73m2).

Paper V

A retrospective, observational, nationwide study of all lung-transplanted patients in Sweden between 2011 and 2020. Patients included in the study were transplanted either at Lund University Hospital or Sahlgrenska University Hospital, Gothenburg.

Study population

Five hundred ninety-four patients who underwent either a single or double lung transplantation were assessed. Patients who were under 18 years of age, receiving preoperative renal replacement therapy, receiving concomitant transplantation of another organ, and patients who died within 48 hours after transplantation were excluded from the study; five hundred sixty-nine patients were included in the study.

The study was approved by the Swedish Ethical Review Authority (Dnr 2020-00423), and the need for informed consent was waived because of the retrospective design of the study.

Study endpoints

The early incidence of AKI days 1 to 7 after the surgery and association of AKI with pre- and intraoperative variables. AKI's impact on 30-day and 1-year mortality. The KDIGO criteria were used to assess the incidence and grade of AKI.

Statistical analysis

Paper I & II

The predefined statistical analysis plan was pre-published in the study protocol[91]. The power and sample size calculations were performed by a statistician and based on a previous study in our department in which an increase in Cystatin C on day 3 was normally distributed with a SD of 27% [92]. With statistical power of 80% and a significance level of 5% to detect the half of SD change (13%) in plasma cystatin on day 3, the estimated sample size was 75 patients in each arm.

Student´s t-test or Mann-Whitney U test was used for testing single measurements depending on data distribution. Data are presented as mean ± SD, number (%), or median with interquartile range, p values less than 0.05 was considered statistically significant.

A linear mixed model was used for testing the primary and secondary endpoints with preoperative eGFR as the covariate, and a log-transformation was used for skewed variables. All statistical analyses were performed by an independent statistician according to predefined statistical analysis plan.

Paper III

Statistical analysis plan

There are no previous studies on cyclosporine's effect on cytokine release in cardiac surgery. Therefore, we used IL-6 for sample size calculations as it is the most well-documented cytokine in cardiac surgery. Previously published cardiac surgery data on IL-6 showed an increase of 340±250 pg/ml 6 hours after surgery[93]. With statistical power of 80% and a significance level of 5%, to detect 50% decrease in

IL-6 plasma concentrations in cyclosporine group, the estimated sample size was 68 patients.

Normally distributed continuous data were compared between groups in an unpaired Student's t-test, while data with non-normal distribution were analyzed with Wilcoxon's test. The categorical data were compared between groups with Fisher's exact test. Paired comparisons were performed before and after ECC and un-paired proportions were compared with Fisher's exact test. Normally distributed data are described as mean ± SD and non-normally distributed data as median with interquartile range (IQR).

A multivariable regression analysis was used to test secondary endpoints, factors associated with a 30% increase in cystatin C on postoperative day 3. The analysis was initiated with univariate regression analysis and criteria for selecting variables $p < 0.2$. All statistical analyses were performed by a statistician affiliated with Spectrum health/Van Andel institute.

Paper IV

Normally distributed and continuous data were compared using Student's t-test, while ordinal, or data with non-normal distribution, were analyzed with Mann-Whitney's U-test. Paired continuous variables were tested in a paired t-test. Proportions were compared with Fisher's exact test. Normally distributed data are described as mean ± standard deviation and non-normally distributed data as median with IQR.

A multivariable regression analysis was used to find predictors of AKI. The analysis was initiated with a univariable analysis, and the criterion for selecting variables was set at $p < 0.1$. All p-values < 0.05 were considered as significant. The ROC analysis was used to test the ability of IL-10 to predict AKI development.

Kaplan-Meier curves presented survival analyses, and a log-rank test was used to determine the statistical significance of differences between AKI and no-AKI patients.

All statistical analyses were performed by a statistician affiliated with Spectrum health/Van Andel institute.

Paper V

We used descriptive statistics to describe the study population, and values were presented as median and interquartile range (IQR) for continuous variables and as frequency rates and percentages n(%) for categorical variables. Statistical analysis was initiated with univariable regression analysis, and variables with a significance level of $p < 0.3$ were introduced in a multivariable regression model. Separate

analyses were performed for preoperative and intraoperative risk factors associated with AKI. The impact of AKI on 30-day and 1-year mortality was presented as a Kaplan-Meier survival analysis and log-rank test was used to test the differences in survival between the groups. Statistical analysis was performed by an independent statistician affiliated with the Region Skåne research center.

Results

Paper I and II

Patients scheduled for elective CABG surgery at the Department of Cardiothoracic Surgery at Skåne University Hospital Lund, Sweden were eligible for the study. Between April 2015 and June 2016, we assessed 456 patients for eligibility, and 154 were enrolled. Seventy-five patients were assigned to the cyclosporine group and 79 to the placebo group. All enrolled patients were analyzed.

Preoperative characteristics.

The preoperative characteristics and demographics are presented in Table II.1.

There were no significant differences in patient baseline characteristics between the study cohorts. In addition, the preoperative eGFR were similar in cyclosporine group and the placebo.

Table II.1 Baseline sharacteristics

Baseline characteristics	Placebo (N=79)	Cyclosporin (N=75)
Male sex - no. (%)	68 (86.1)	62 (82.7)
Age (year)	69.1 ± 8.3	69.7 ± 8.1
Height (cm)	174.7 ± 7.9	174.1 ± 8.6
Weight (kg)	86.1 ± 14.7	82.3 ± 13.3
Systolic Blood Pressure (mmHg)	137.4 ± 18.5	134.4 ± 17.4
Diastolic Blood Pressure (mmHg)	74.9 ± 8.1	72.5 ± 9.1
Medical History – no. (%)		
Hypertension	62 (78.5)	54 (72.0)
Congestive heart failure	15 (19.0)	10 (13.3)
LVEF < 30%	3 (3.8)	2 (2.7)
LVEF 30–50%	14 (17.7)	9 (12.0)
LVEF > 50%	59 (74.7)	62 (82.7)
COPD	0 (0)	4 (5.3)
Diabetes	26 (32.9)	14 (18.7)
Peripheral Vascular Disease	5 (6.3)	4 (5.3)
Previous CVI	5 (6.3)	6 (8.0)
Thyroid Disease	8 (10.1)	3 (4.0)
Chronic AF	4 (5.1)	1 (1.3)
Paroxysmal AF	6 (7.6)	5 (6.7)
Medication use - no. (%)		
Diuretics	23 (29.1)	10 (13.3)
ACE/ARB	59 (76.0)	60 (78.7)
Beta-Blocker	64 (82.7)	62 (81.1)
Statins	76 (96.2)	69 (92.0)
Warfarin	2 (2.5)	1 (1.3)
ASA	73 (92.4)	68 (90.7)
Clopidrogel/prasurgel	3 (3.8)	5 (6.7)
Antithrombotic treatment	20 (25.3)	16 (21.3)
Antibiotics	4 (5.1)	1 (1.3)
Pre-op eGFR CKD-EPI (ml/min/1.73m^2)		
All patients	65.1 ± 18.9	69.0 ±20.0
Subgroup eGFR 15-59	51.1±11.2	54.4±11.9
Subgroup eGFR 60-90	79.9±10.0	81.5±10.1

Characteristics of patients at baseline. Values are presented as mean ± SD or no (%). LVEF=Left ventricular ejection fraction. COPD=chronic obstructive pulmonary disease, AF = atrial fibrillation, ACE = angiotensin conversion enzyme inhibitors, ARB = angiotensin receptor blockers, ASA = acetyl salicylic acid, eGFR = estimated glomerular filtration rate, MDRD = modification of diet in renal disease, CKD-EPI = the chronic kidney disease-epidemiology collaborative group.

Clinical outcome

The primary endpoint was relative plasma cystatin C concentration change from preoperative to day 3 postoperatively. No protective effect of cyclosporine pre-treatment on kidney function was found. The increase in cystatin C was more pronounced in the cyclosporine (136.4±35.6%) than in the placebo (115.9±30.8%) group (Figure II.2). The difference between two groups was 20.6% (95% CI, 10.2 – 31,2%, p<0.001)
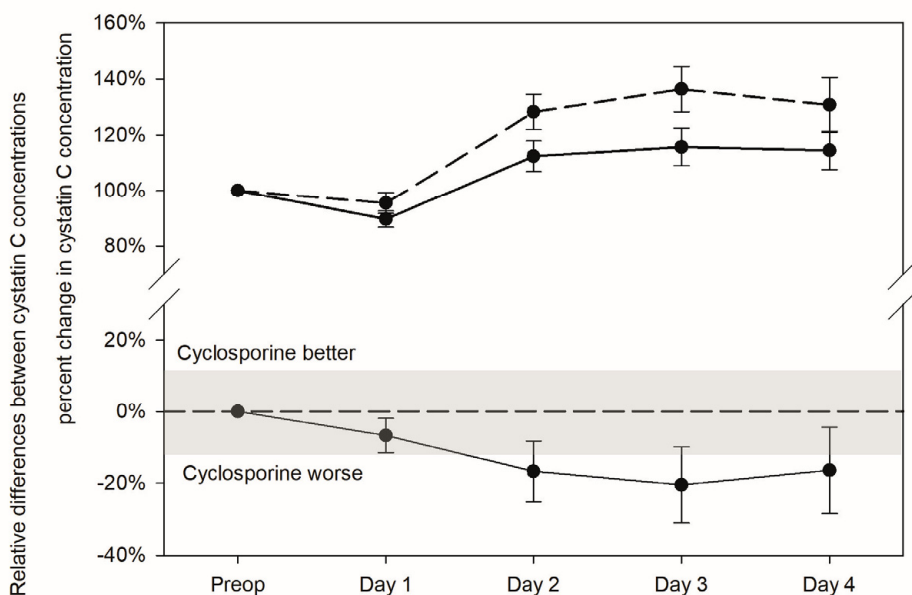

Figure II.2 Cystatin C changes from preoperative to day 4.
Changes in plasma cystatin C in the cyclosporine group (dashed line) and placebp grpup (solid line) from preoperative to day 4 postoperatively. Relative differences between cystatin C concentrations for the groups with 95% CI. The gray area reflects 13% changes in the primary endpoint used in power calculations. Preop=preoperative.

Changes in eGFR for two subgroups (eGFR 15 to 59 or 60 to 90 ml/min/1.72m^{-2})

The study protocol dictated two predefined subgroups eGFR 15 to 59 or 60 to 90 ml/min/1.73m^{-2}. The increase in plasma cystatin C in patients with an eGFR 60 to 90 ml/min was 1.34±0.36 in the cyclosporine group and 1.18±0.34 in the placebo group. In patients with preoperative eGFR 15 to 59 ml/min/1.73m^{-2}, the increase in plasma cystatin C was 1.39±0.35 in the cyclosporine group and 1.11±0.24 in the placebo group. No differences were found between the two subgroups in the statistical analysis (p=0.858). Shown in Figure II.3.

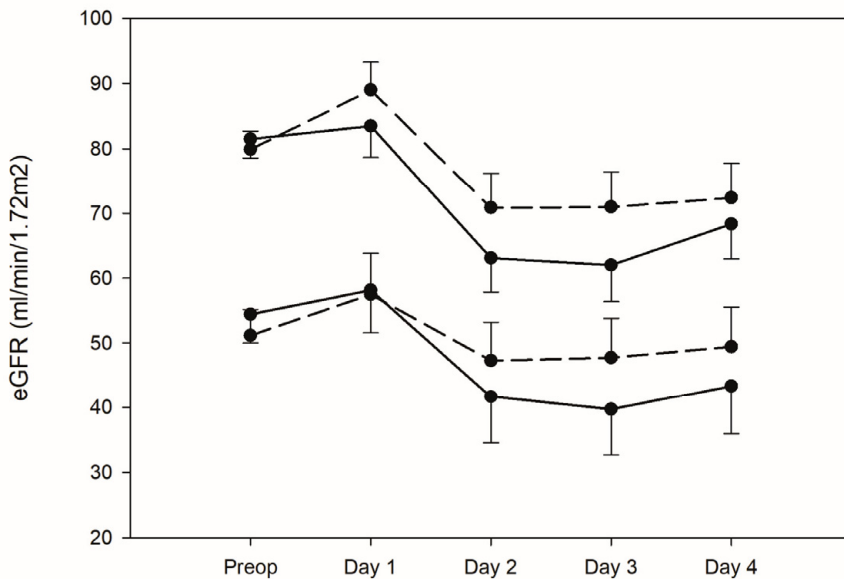**Figure II.3 changes in eGFR**
Changes in eGFR from preoperative to day 4 postoperatively for two subgroups (eGFR 15 to 59 and 60 to 90 ml/min/1.72m^{-2}).

Post hoc evaluation of renal function after 1 to 3 month and 3 to 6

In both groups, plasma creatinine was normalized 1 to 3 and 3 to 6 months after end of the study. Creatinine values were obtained in 86% of study patients. Shown in Figure II.4.
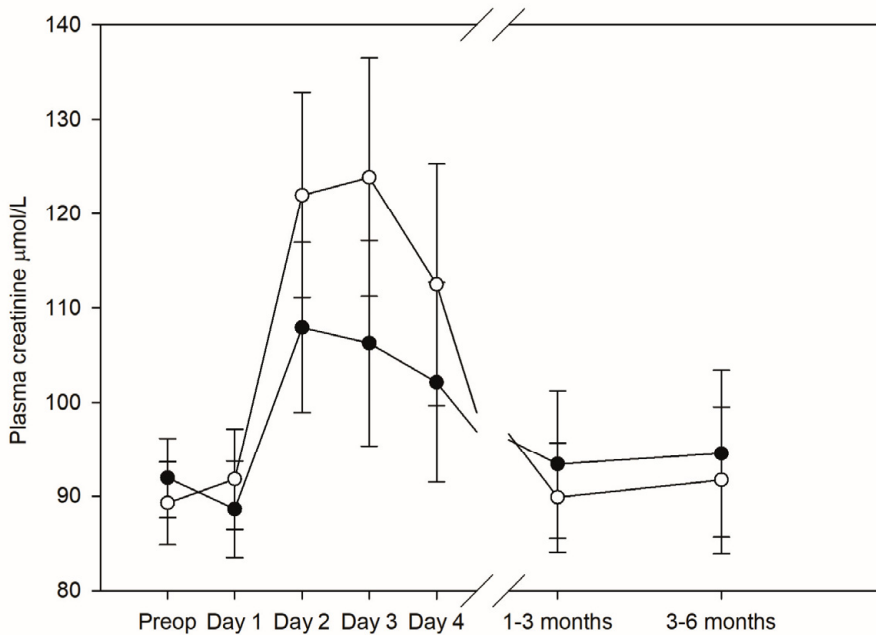
Figure II.4.
Mean values with 95% CI for plasma creatinine in the cyclosporine (dashed line) and placebo (solid line) groups. The broken axis denotes that post hoc analysis was performed in the period 1 to 6 month after surgery.
Preop=preoperative. Days 1 to 4 = days after surgery.

General outcome

Two patients suffered a stroke in the placebo group, and one died, the only death in the study. No protective effect on the heart was found, and no patients in either group were treated with CRRT. Time to extubation was shorter in the cyclosporine group, but there was no difference in ICU time.

Paper III

To evaluate the effect of cyclosporine on perioperative inflammatory response we included 67 randomly selected patients from the original CiPRICS trial (Paper II).

The cohort analyzed consisted of 36 patients treated with a placebo and 31 patients given a single dose of cyclosporine. The preoperative baseline demographics and characteristics are presented in Table III.1

Table III.1 Baseline demographics and characteristics of the patients

Baseline characteristics	Placebo (N=36)	Cyclosporin (N=31)	p-value
Male sex - n(%)	31 (86.1)	28 (90.3)	0.716
Age (year)	67.9 ± 6.7	70.9 ± 8	0.112
Height (cm)	175.8 ± 9.	174.74 ± 7.9	0.579
Weight (kg)	86 ± 13.5	81.23 ± 10.9	0.062
Systolic Blood Pressure (mmHg)	135 ± 19.2	135 ± 14.4	0.887
Diastolic Blood Pressure (mmHg)	75.2 ± 8.4	72 ± 7.7	0.332
Medical History – no. (%)			
Hypertension	28 (77.7)	24 (77.4)	0.972
Congestive heart failure	8 (22.2)	7 (22.6)	0.972
LVEF < 30%	1 (2.7)	1 (3.2)	0.914
LVEF 30–50%	8 (22.2)	7 (22.5)	0.972
LVEF > 50%	26 (72.2)	23 (74.1)	0.856
COPD	0	1 (3.23)	0.463
Diabetes	10 (27.7)	3 (9.6)	0.072
Peripheral Vascular Disease	2 (5.5)	1 (3.2)	0.645
Previous CVI	3 (8.3)	2 (6.4)	0.770
Thyroid Disease	2 (5.5)	0	0.495
Chronic AF	1 (2.7)	0	0.349
Paroxysmal AF	4 (11.1)	4 (12.9)	0.821
Antithrombotic treatment	9 (25)	8 (25.8)	0.939
Antibiotics	1 (2.7)	1 (3.2)	0.914
Pre-op eGFR CKD-EPI (ml/min/1.73m2)			
All patients	64.3 ± 17.1	65.96 ± 21.2	0.73
Subgroup eGFR: 15-59	47.3 ± 11.1	44 ± 13.0	0.515
Subgroup eGFR: 60-90	73.9 ± 11.3	78 ± 13.8	0.488
Perfusion time, ECC (min)	70.14 ± 22.73	73.81 ± 26.87	0.477
Aortic cross-clamp duration (min)	44.81 ± 14,95	45.48 ± 16.58	0.861

Abbreviations: ASA, acetylsalcylic acid; ACE, angiotensin-converting enzym inhibitor; AF, atrial fibrillation; ARB, angiotensin-receptor blockera; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; COPD, chronic obstructive pulmonary disease; CVI, cerebrovascular icident; ECC, extracorporeal circulation; eGFR, estimated glomerular filtration rate; LVEJ, left ventricular ejection fraction; SD, standard deviation.
*n (%); plus-minus values are means ± SD for all other variables.

Cyclosporine's effect on cytokine production

As a response to CABG surgery with ECC, the postoperative levels of tissue-aggressive (IL-1β, MIP-1β, G-CSF, IL-6, IL-8, IL-17, MCP-1) and tissue-lenient (IL-4) cytokines were increased. However, there were no significant differences between placebo and cyclosporine treated patients (Figure III.1).
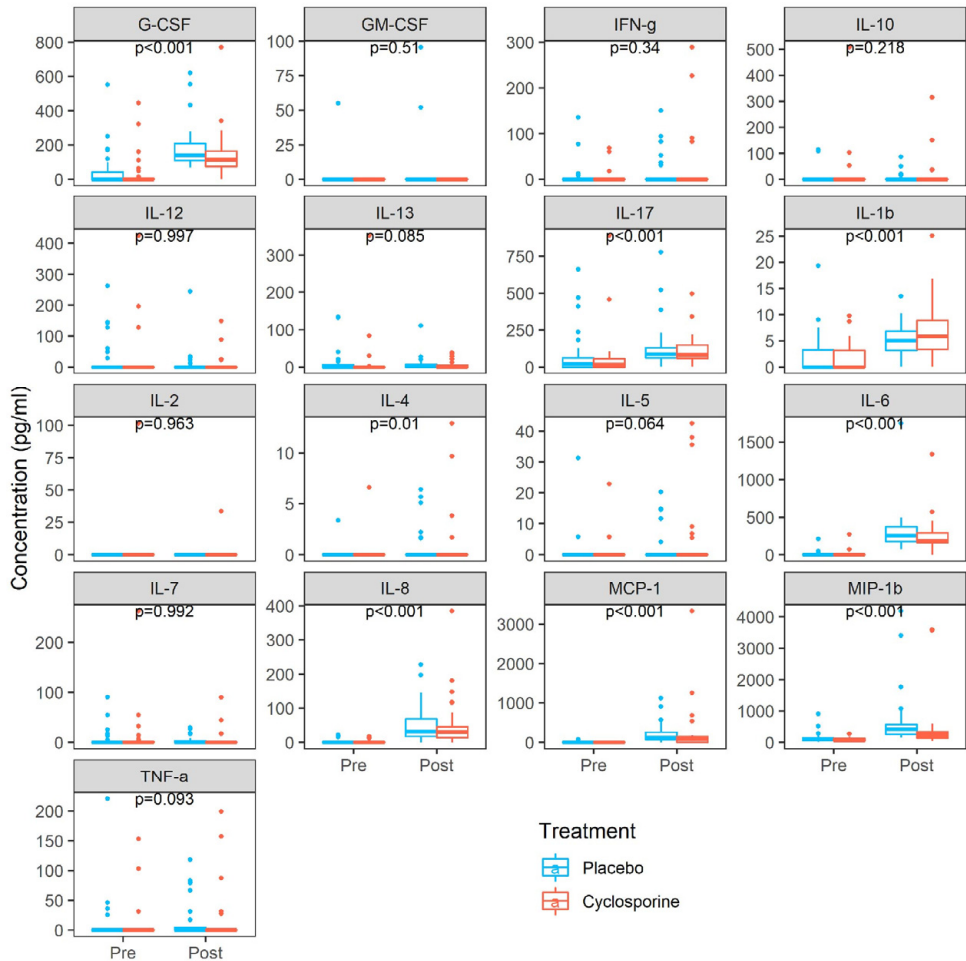
Figure III.1. Comparison of plasma cytokine levels before and after CABG surgery in cyclosporine and placebo treated patients.

Shown are plasma cytokine levels pre-and postoperatively after CABG surgery in the placebo and cyclosporine-treated patients. Presented p values (Wilcoxon's test) represent differences pre-and postoperatively when placebo and cyclosporine treated patients are pooled together.

Cyclosporine's effect on postoperative increase in IL-6

Although, IL-6 levels were significantly increased (p<0.001), there was no difference between cyclosporine treated patients and the placebo (p=0.336). Shown in Figure III.2.
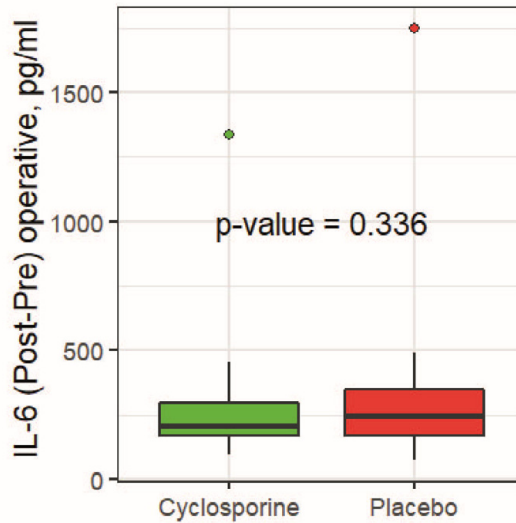
Figure III.2 Comparison of post-operative rise in IL-6 levels.
Postoperative increase in IL-6 concentrations in the cyclosporine and placebo treated patients after CABG surgery, p>0.336 (Wilcoxon's test).

Factors associated with 30% increase in cystatin C at day 3 postoperatively

The secondary outcomes of the study, factors associated with a 30% increase in cystatin C, were analysed in a multivariable regression analysis. The variables with a $p<0.05$ were total perioperative norepinephrine dose ($p<0.001$) and age ($p<0.04$). Shown in Table III.2

Tabell III.2 Multivariable regression analysis, outcome 30% increase of P-cystatin C postoperative day 3 from preoperatively

Variable	Estimate	Std.Error	t-value	p-value
Intercept	-0.439	0.553	-0.794	0.43
Age (yr)	0.024	0.008	2.95	0.004
Norepinephrine (day 0)	0.072	0.012	5.852	<0.001

Variables (p<0.05) associated with a postoperative increase of P-cystatin C.

Correlation Between Postoperative Increase in Cystatin C and IL-6

The Figure III.3 shows the correlation between IL-6 and cystatin C as non-significant. The initially significant correlation in the placebo group at day 3 and 4 become non-significant when outlier was removed from the analysis r=0.29 [0.03,0.56]; p<0.09) and day 4(r=0.3[-0.04,0.57]; p<0.08).
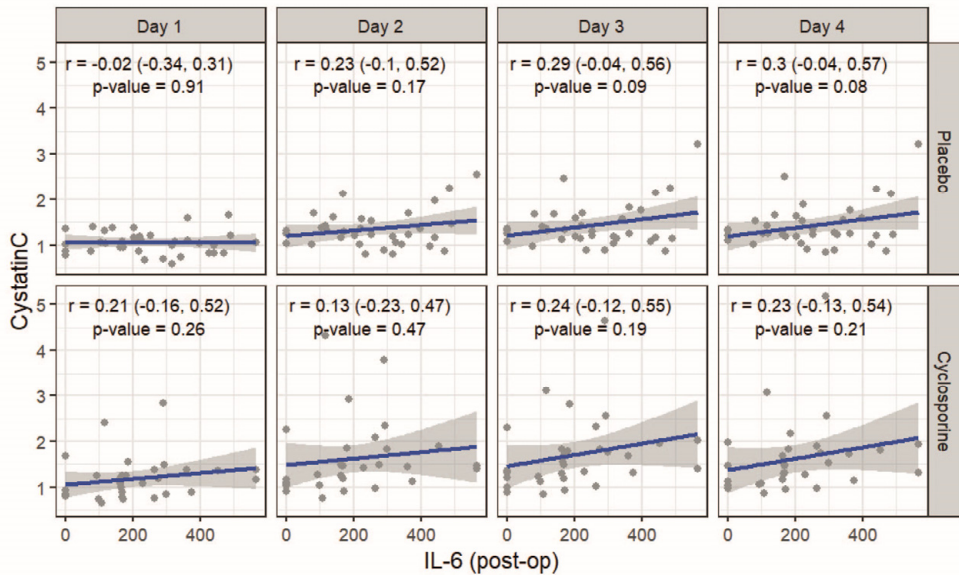
Figure III.3 Correlation between cystatin C and IL-6 levels.
Cystatin C levels as function of post-operative IL-6 levels. Outliers in the placebo and cyclosporine groups are removed from the statistical analysis in this figure. Days 1 to 4 = days after surgery.

Maximal stage of AKI

Maximal stage of AKI (KDIGO classification) from day 0 to day 4 in the in cyclosporine and the placebo group (Table III.3).

Table III.3 Acute Kidney Injury by KDIGO classification

Treatment group (n)	AKI n (%)	Stage 1 n (%)	Stage 2 n (%)	Stage 3 n (%)
Cyclosporine (31)	15(48)	10(32)	5(16)	0
Placebo (36)	8(22)	5(14)	1(3)	2(6)
p-value (t-test)	0.008	0.007	0.005	0.1

Presented are n (%) of patients developing AKI and stage of AKI by KDIGO classification from post-operative day 1 to day 4 in the cyclosporine and the placebo treated groups after CABG surgery with ECC

In summary, we found that CABG surgery with ECC induces cytokine production. However, this cytokine activation was not impacted by cyclosporine pretreatment. We also found that none of the investigated cytokines correlate with postoperative kidney function decline. The initial correlation between IL-6 and cystatin C became non-significant when the outlier was removed.

In conclusion, preoperative use of cyclosporine does not impact cytokine inflammatory response to surgery and ECC.

Table IV.1 Demographics of patients that did and did not develop AKI after start of VA-ECMO according to KDIGO criteria.

Variable	No-AKI (n=58)*	AKI (n=42)	p-value
Age (yr)	58.22 ± 12.75	56.76 ± 12.43	0.569
Sex: Female	18(31)	9(21.4)	0.401
Race: Caucasian	32(55.1)	22(52.3)	0.942
Height (cm)	171.88 ± 9.43	175.34 ± 9.91	0.079
Weight (kg)	91.98 ± 21	89.42 ± 17.16	0.519
BMI (kg/m^2)	31.09 ± 6.56	29.04 ± 4.94	0.092
Albumin (g/dL)	3.08 ± 0.64	2.97 ± 0.66	0.403
AST (IU/L)	338.95 ± 878.66	270.36 ± 756.86	0.487
ALT (IU/L)	264.09 ± 717.72	170.52 ± 524.54	0.63
Bilirubin Total (μmol/L)	16.93 ± 17.78	19.15 ± 17.27	0.246
Arterial pH	7.32 ± 0.18	7.33 ± 0.18	0.657
Lactate (mmol/L)	4.47 ± 3.57	4.5 ± 3.74	0.617
Creatinine(mg/dL)	1.52 ± 1.43	1.24 ± 0.41	0.964
MDRD eGFR (ml/min/1.73m^2)	57.69 ± 29.74	58.82 ± 24.3	0.71
Sodium (mmol/L)	137.43 ± 6.17	141.07 ± 6.5	0.01
Calcium (mmol/L)	2.12 ± 0.24	2.26 ± 0.4	0.029
Potassium (mmol/L)	4.44 ± 0.65	4.28 ± 0.65	0.223
Magnesium (mmol/L)	0.91 ± 0.21	0.98 ± 0.26	0.151
Glucose (mmol/L)	8.57 ± 6.05	11.64 ± 6.79	0.019
Central venous pressure (mmHg)	15.7 ± 8.33	12.68 ± 6.88	0.076
Mean Arterial pressure (mmHg)	84.1 ± 55.35	64.64 ± 24.96	0.074
Systolic Blood Pressure (mmHg)	110.07 ± 41.15	96.12 ± 24.66	0.047
Diastolic Blood Pressure (mmHg)	69.98 ± 40.34	62.92 ± 17.77	0.487
Hemoglobin	12.49 ± 2.57	12.17 ± 2.81	0.558
Troponin ng/ml	3.35 ± 9.62	5.39 ± 9.99	0.141
INR	1.71 ± 1.21	1.6 ± 0.96	0.817
Inotrope score	16.07 ± 11.7	16.05 ± 13.01	0.831
SOFA score	10.04 ± 2.76	9.95 ± 2.71	0.882
ECMO indications*			0.008
eCPR	4(6.9)	8(19.05)	
Acute myocardial infarction	2(3.45)	1(2.38)	
Bridge to VAD/Transplantation	15(25.86)	7(16.67)	
PGD (Heart)	0	3(7.14)	
Post-cardiotomy	8(13.79)	13(30.95)	
ADHF	23(39.66)	6(14.29)	
Cardiac arrest with ROSC	2(3.45)	2(4.76)	
Other	4(6.9)	2(4.76)	

Values are presented as n(%); means ± SD for all other variables.

* Fisher's test for distribution in indications between the AKI and no AKI cohorts.

Abbreviations: KDIGO = Kidney Disease Improving Global Outcome; BMI = body mass index; MDRD eGFR = Modification of Diet in Renal Disease estimated Glomerular Filtration Rate; eCPR = extracorporeal Cardiopulmonary Resuscitation; Cardiac arrest = cardiopulmonary resuscitation with return of spontaneous circulation (ROSC); ECMO = extracorporeal membrane oxygenation; INR = international normalized ratio; PGD = primary graft dysfunction; ADHF = acute decompensated heart failure; AST = aspartate aminotransferase; ALT = Alanine aminotransferase.

Paper IV

Patient Characteristics

Forty-two percent (n=42) of 100 patients included in the study developed AKI after the start of VA ECMO. SOFA (Sequential Organ Failure Assessment) score (AKI 9.95 ± 2.71 vs. no AKI 10.04 ± 2.76; p=0.88) and pre-cannulation eGFR (AKI 58.8 ± 24.3 ml/min/1.73m2 vs. no AKI 57.6 ± 29.7 ml/min/1.73m2; p=0.71) were not significantly different between groups. The levels of IL-10, sodium, glucose, and calcium at pre-cannulation were significantly (p<0.05) different between the two cohorts. Table IV.1

Pre-cannulation IL-10 Concentrations Predict AKI

In patients who developed AKI during V-A ECMO treatment, IL-10 levels were higher than in no AKI patients (212 [38.9, 620.7] pg/ml vs. 49.05 [25.8, 102.2] pg/ml; p=0.007) (Table IV.2 and Fig. IV.1). A ROC analysis showed an AUC of 0.71 (Fig. IV.1.b.), with the best separation at 112 pg/ml. After 48 hours of V-A ECMO treatment, the levels of IL-10 returned to baseline (Fig. IV.2).

Table IV.2 Serum cytokine concentrations at pre-cannulation, 48 hours, and day 8

Day 0	No-AKI (n=58)	AKI (n=42)	p value
IFN-γ	5.67(0, 26.48)	16.7(5.05, 43.79)	0.031
IL-10	49.05(11.9, 191.8)	212.1(38.94, 620.7)	0.007
IL-1β	0.64(0, 2.98)	1.54(0, 3.03)	0.58
IL-6	141.8(41.17, 568.8)	227.8(74.54, 709.5)	0.3
IL-8	48.94(17.11, 210.7)	51.27(20.37, 154.1)	0.922
TNF- α	13.79(5.73, 101)	21.64(7.83, 75.41)	0.777
48 hours	**No-AKI (n=58)**	**AKI (n=42)**	
IFN-γ	0(0, 14.74)	3.66(0, 24.78)	0.362
IL-10	9.88(0.85, 20.87)	6.53(0, 36.18)	0.979
IL-1β	0(0, 1.015)	0(0, 1.61)	0.517
IL-6	105.5(30.78, 301.1)	86.25(38.42, 458.4)	0.767
IL-8	29.52(13.16, 76.1)	37(18.5, 260.6)	0.114
TNF- α	13.45(2.45, 69.94)	13.46(0.34, 79.91)	0.94
Day 8	**No-AKI (n=58)**	**AKI (n=42)**	
IFN-γ	0(0, 22.92)	2.033(0, 17.99)	0.479
IL-10	4.35(0, 14.94)	0(0, 9.63)	0.289
IL-1β	0(0, 1.5)	0(0, 1.753)	1
IL-6	44.94(11.62, 139.1)	66.07(29.05, 112.6)	0.6
IL-8	23.86(15.45, 76.78)	34.46(15.74, 119.2)	0.365
TNF- α	14.93(0, 64.03)	19.14(0, 86.59)	0.673

Serum cytokine concentrations (pg/ml) at the time of cannulation, at 48 hours and day 8. Median, (Q1, Q3). P values (Mann Whitney's U-test).
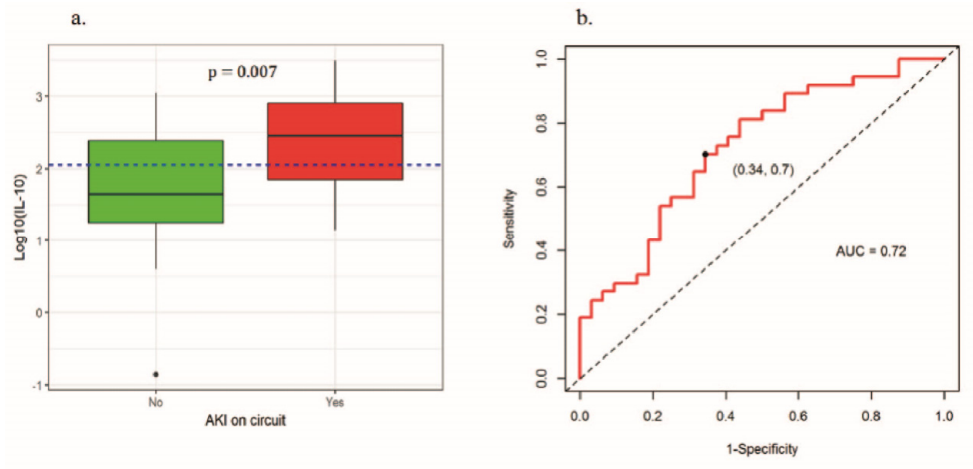
Figure IV.1 Pre-cannulation IL-10 concentrations and performance of IL-10 in discriminating AKI in VA ECMO treated patients
a) a Box plot of pre-cannulation serum IL-10 levels (pg/ml) of patients who did not (green) and those who developed (red) AKI on VA-ECMO.
b) ROC analysis for the separation of those who developed AKI on VA-ECMO vs those who did not. AUC =0.72. Best separation at 112 pg/ml. AUC: Area Under the Curve, AKI: Acute Kidney Injury, VA-ECMO: Veno-Arterial Extracorporeal Membrane Oxygenation
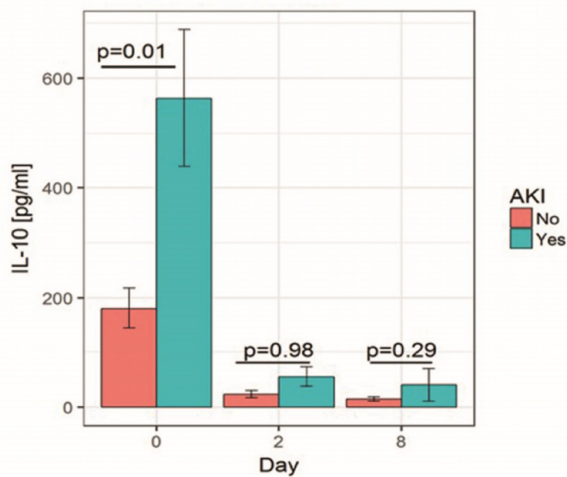

Figure IV.2 Serum IL-10 levels over time.
Shown are IL-10 serum levels (pg/ml), in patients that developed AKI on VA ECMO vs. those who did not at Day 0, 2, 8. AKI: Acute Kidney Injury, VA ECMO: Veno-Arterial Extracorporeal Membrane Oxygenation

48

30-days and 1-year mortality in VA ECMO AKI and no AKI patients

The mortality at 30 days and 1 year were not different between AKI and no AKI cohorts. Nevertheless, when sub-groups of no-AKI patients (eGFR < or > 45 ml/min) were compared to AKI patients, the mortality at 30-days (p<0.049; Fig. IV.4a) was significantly increased in AKI patients compared to patients with pre-cannulation eGFR ≥ 45 ml/min, who did not develop AKI.

Moreover, the survival at 30 days (p=0.886; Fig. IV.4a) and 1-year (p=0.826; Fig. IV.4b was not different in patients with decreased kidney function at pre-cannulation, (eGFR < 45 ml/min) who did not develop AKI.

For patients who survived the first 30 days of VA ECMO treatment, the survival at 1 year was not different from those without AKI (Fig. IV.4b).
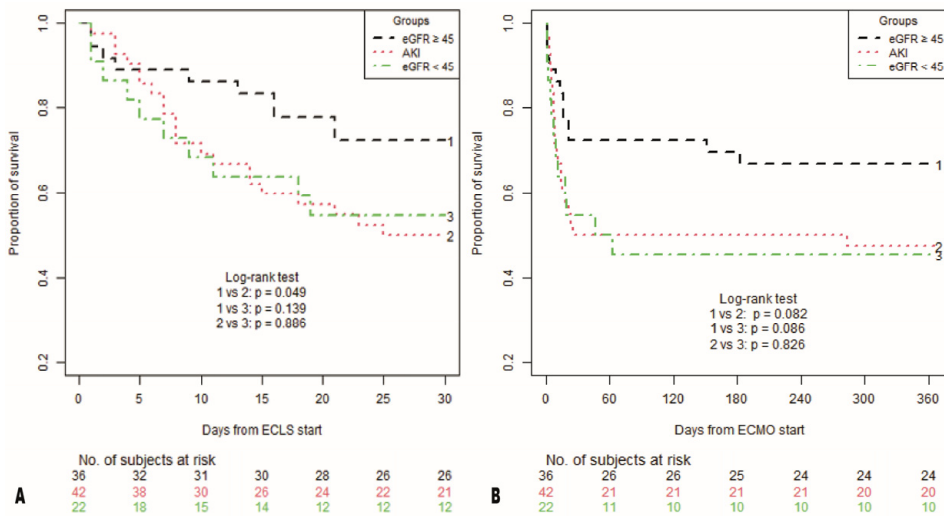

Figure IV.4 Kaplan-Meier survival analysis and log-rank test, 30-day and 1-year mortality
A) Fig. IV.4.. shows Kaplan Meier curves with 30-day mortality as an outcome. Log rank analysis shows significantly increased mortality in patients who developed AKI vs. those with eGFR ≥ 45 ml/min without AKI development after start of VA ECMO, p=0.049.
B) Fig. IV.4. shows Kaplan Meier curves with 1-year mortality as an outcome of patients who developed AKI on VA-ECMO vs. those who did not - either with normal to moderately decreased kidney function (eGFR ≥ 45 ml/min/1.73m^2) or severely decreased kidney dysfunction (eGFR <45 ml/min/1.73m^2) before the cannulation. Log-rank analysis shows no statistical difference in survival neither in AKI patients vs. non-AKI with eGFR ≥ 45 ml/min (p=0.082), nor in AKI patients vs. non-AKI with eGFR< 45 ml/min (p=0.826).

Paper V

A total of 594 patients underwent lung transplantation in Sweden between 2011 and 2020 and were assessed for eligibility. Twenty-five of these patients were found non-eligible due to age < 18 years, preoperative CRRT treatment, or simultaneous transplantation of another organ. We included 569 eligible patients, intending to study the incidence of early postoperative AKI and delineate pre- and intraoperative associated factors.

Preoperative baseline characteristics with or without AKI and grade of AKI (Table V. 1.)

P-values were not calculated for the baseline characteristics; to compare AKI and no AKI patients, we used p-values from the univariate analysis.

The median age of patients who developed AKI was 56 [47.0, 63.0] years and 57 [49.1, 63.0] in no AKI patients. The body mass index (BMI) was 23.6 [21.0, 27.8] kg/m2 in the AKI patients vs. 23.2 [19.9, 26.3] in no AKI, and chronic kidney disease (CKD) 13% in AKI vs. 6% in no AKI cohort.

Fifty-six percent of those who developed AKI were of male gender compared to 50% in the no AKI group. Waiting list time for LTx was 1.8 [0.8, 4.3] month in the no AKI cohort vs. 2.3 [0.8, 4.3] in the AKI cohort. The AKI was more common in patients who underwent retransplantation of the lungs, 13.6% vs. 7.7%.

Eighty-three percent of patients had another primary diagnosis leading to transplantation than COPD, and 17% had COPD. Patient categories with other diagnosis than COPD were idiopathic pulmonary fibrosis (28%), cystic fibrosis (CF)(13.2%), primary pulmonary hypertension (PPH) (7%) and alpha 1-antitrypsine deficiency (9.9%). In patients who developed AKI 18% had diabetes, 18% had hypertension, and 13% had CKD. Tricuspid regurgitation was present in 51% of the AKI patients and 15% had a tricuspid regurgitation grade 2-3. Five percent of AKI patients were treated with ECMO and 8% received mechanical ventilation. Prior to LTX 23% of AKI patients were treated with CNI and 21% in no AKI group, shown in Table V.1.

Table V. 1. Preoperative baseline characteristics with or without AKI, AKI grade

Variable	No AKI (n=326)	AKI (n=243)	AKI 1 (n=132)	AKI 2 (n=46)	AKI 3 (n=65)
Age (years)	57.0 [49.1, 63.0]	56.0 [47.0, 63.0]	56.0 [46.0, 63.0]	55.0 [48.0, 63.0]	60.0 [47.3, 63.0]
Female gender	165 (50.6)	107 (44.0)	55 (41.7)	17 (37.0)	35 (53.8)
BMI	23.2 [19.9, 26.3]	23.6 [21.0, 27.8]	23.0 [20.8, 26.6]	23.9 [21.2, 28.5]	24.8 [21.7, 28.5]
Primary diagnosis leading to LTx					
COPD	89 (27.3)	42 (17.3)	23 (17.4)	5 (10.9)	14 (21.5)
Idiopathic pulmonary fibrosis	84 (25.8)	68 (28.0)	35 (26.5)	13 (28.3)	20 (30.8)
Alfa-1 deficiency	34 (10.4)	24 (9.9)	18 (13.6)	4 (8.7)	2 (3.1)
Cystic fibrosis	31 (9.5)	32 (13.2)	18 (13.6)	5 (10.9)	9 (13.8)
Primary pulmonary hypertension	14 (4.3)	17 (7.0)	10 (7.6)	4 (8.7)	3 (4.6)
Other	74 (22.7)	60 (24.7)	28 (21.2)	15 (32.6)	17 (26.2)
Diabetes	38 (11.7)	44 (18.1)	26 (19.7)	7 (15.2)	11 (16.9)
Hypertension	54 (16.6)	45 (18.5)	22 (16.7)	7 (15.2)	16 (24.6)
CKD	21 (6.4)	31 (12.8)	12 (9.1)	9 (19.6)	10 (15.4)
Preoperative smoking	190 (58.3)	126 (51.9)	70 (53.0)	20 (43.5)	36 (55.4)
Re-transplantation	25 (7.7)	33 (13.6)	18 (13.6)	10 (21.7)	5 (7.7)
P creatinine start waiting list	69.0 [58.0, 80.0]	74.0 [59.0, 86.5]	73.0 [59.0, 89.2]	72.5 [54.8, 81.0]	74.0 [60.0, 85.0]
mGFR start waiting list	86.0 [75.0, 98.0]	78.0 [69.0, 92.0]	77.0 [69.0, 90.0]	80.0 [64.2, 92.2]	79.0 [69.0, 98.5]
eGFR start waiting list	94.0 [84.0, 104.0]	93.0 [80.0, 103.2]	93.1 [78.8, 104.2]	98.0 [84.4, 106.8]	92.0 [79.0, 100.0]
eGFR day of surgery	94.0 [82.0, 105.0]	94.0 [78.0, 104.0]	93.0 [77.0, 104.0]	94.0 [79.2, 104.5]	97.0 [81.0, 105.0]
Preoperative ACE/ARB	33 (10.1)	30 (12.3)	18 (13.6)	3 (6.5)	9 (13.8)
Time on waiting list (month)	1.8 [0.8, 4.3]	2.3 [1.0, 6.5]	2.7 [1.1, 6.6]	1.9 [0.7, 6.3]	2.2 [0.9, 7.4]
TI (grade)					
No TI	194 (61.0)	118 (49.2)	69 (52.7)	20 (44.4)	29 (45.3)
TI grad 1	99 (31.1)	85 (35.4)	43 (32.8)	17 (37.8)	25 (39.1)
TI grad 2 or 3	25 (7.9)	37 (15.4)	19 (14.5)	8 (17.8)	10 (15.6)
No TI	194 (61.0)	118 (49.2)	69 (52.7)	20 (44.4)	29 (45.3)
TI grad 1	99 (31.1)	85 (35.4)	43 (32.8)	17 (37.8)	25 (39.1)
TI grad 2 or 3	25 (7.9)	37 (15.4)	19 (14.5)	8 (17.8)	10 (15.6)
Preoperative ECMO	8 (2.5)	13 (5.3)	2 (1.5)	4 (8.7)	7 (10.8)
Preoperative mechanical ventilation	11 (3.4)	20 (8.2)	6 (4.5)	6 (13.0)	8 (12.3)
EVLP	11 (3.4)	12 (4.9)	4 (3.0)	2 (4.3)	6 (9.2)
Preoperative O2 treatment	189 (58.0)	153 (63.0)	76 (57.6)	30 (65.2)	47 (72.3)
6 min walk test	279.5 [189.0, 385.5]	250.0 [162.0, 373.5]	249.0 [160.0, 400.0]	244.5 [196.2, 310.8]	275.5 [123.2, 380.8]
Preoperative CNI	70 (21.5)	56 (23.0)	32 (24.2)	15 (32.6)	9 (13.8)

Values are presented as n (%); median and IQR for all other variables

Abbreviation: BMI = body mass index, EVLP = ex vivo lung perfusion, CNI = calcineurin inhibitors, ACE = angiotensin inhibitors, ARB = angiotensin blockers, CKD = chronic kidney disease, LTx = lung transplantation, mGFR = measured glomerular filtration rate, eGFR = estimated glomerular filtration rate (CKD-EPI), ECMO = extracorporeal membrane oxygenation. other lung diagnosis = CLAD (chronic lung allograft dysfunction), bronchiolitis obliterans, chronic pulmonary embolism, bronchiectasis, graft versus host disease of the lungs.

Incidence of AKI

Of 569 patients included in the study, 241(43%) developed AKI within seven days of surgery. Most of the patients 132(55%) developed grade 1 AKI, 46(19%) grade 2, and 63(26%) grade 3 AKI. CRRT was used in 25 patients of AKI grade 3 patients. The majority of patients developed AKI within the first two days after surgery. Shown in Figure V. 1. and V.2.
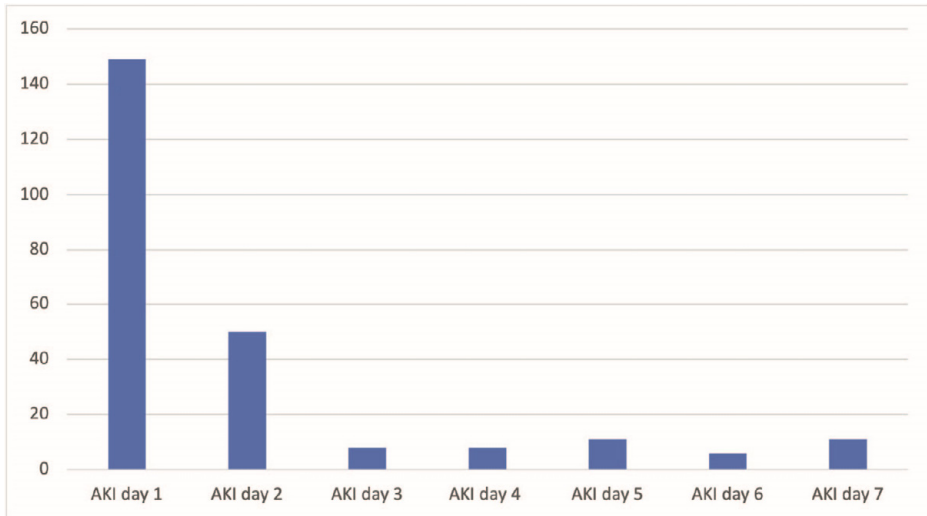

Figure V. 1. Bar chart diagram of number of patients developing AKI stratified by day after surgery
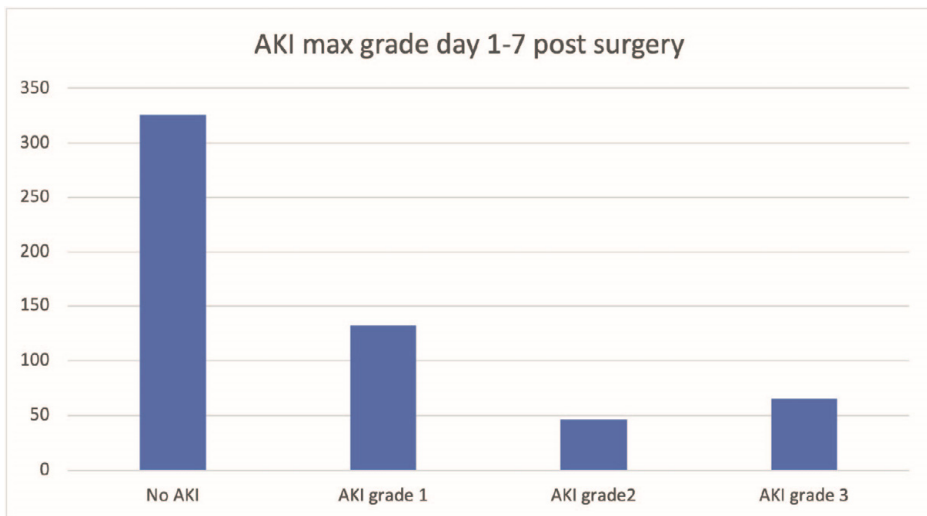

Figure V. 2. Bar chart diagram of number of patients with different grades of AKI within the first seven days after surgery

Intraoperative variables with or without AKI and grade of AKI. Table V. 2.

Most LTx patients underwent double-lung transplantation 83% and 17 % single-lung transplants. Out of patients who developed AKI, 86% underwent double-lung transplantation and 16% single-lung transplantation. In 42% of AKI patients, the bilateral sequential thoracotomy was used; in 24%, clamshell incision and in 34%, sternotomy.

In 44% of patients ECMO or CPB was used as intraoperative extracorporeal support. The median CPB duration in AKI patients was 222 [185.2, 268] minutes, vs. 179.5 [160.5, 228.5] minutes in the no AKI group, and median ECMO time 353 [225.2, 480.0] min in AKI cohort vs. 272 [230.2, 351.2] in the no AKI group.

Intraoperative fluid use and red blood cell transfusions (RBC) were more common in AKI patients, as well as the use of induction immunosuppression treatment other than ATG.

Table V. 2. Intraoperative variables of lung transplant recipients with and without AKI

Variable	No AKI (n=326)	AKI (n=243)	AKI 1 (n=132)	AKI 2 (n=46)	AKI 3 (n=65)
Single-lung transplantation	62 (19.0)	33 (13.6)	17 (12.9)	10 (21.7)	6 (9.2)
Double-lung transplantation	264 (81.0)	210 (86.4)	115 (87.1)	36 (78.3)	59 (90.8)
Type of incision					
Thoracotomy/bilateral sequential thoracotomies	237 (72.7)	102 (42.0)	57 (43.2)	20 (43.5)	25 (38.5)
Sternotomy	40 (12.3)	83 (34.2)	42 (31.8)	16 (34.8)	25 (38.5)
Clamshell	49 (15.0)	58 (23.9)	33 (25.0)	10 (21.7)	15 (23.1)
Intraoperative use of cardiopulmonary bypass	56 (17.2)	110 (45.3)	54 (40.9)	21 (45.7)	35 (53.8)
Cardiopulmonary bypass time (min)	179.5 [160.5, 228.5]	222.0 [185.2, 268.0]	199.0 [183.2, 246.8]	232.0 [190.0, 280.0]	246.0 [201.0, 304.5]
Intraoperative use of ECMO	34 (10.4)	53 (21.8)	22 (16.7)	16 (34.8)	15 (23.1)
Time on ECMO (min)	272.0 [230.2, 351.2]	353.0 [225.2, 480.0]	318.0 [243.2, 369.0]	424.5 [234.8, 500.0]	310.0 [216.2, 636.5]
Surgical time (hours)	6.2 [5.0, 7.4]	6.5 [5.3, 8.4]	6.4 [5.3, 8.4]	6.9 [5.8, 8.3]	6.5 [5.3, 8.4]
Anesthesia time (hours)	9.2 [7.9, 10.6]	9.6 [8.1, 11.4]	9.7 [8.0, 11.3]	9.6 [8.7, 11.5]	9.6 [8.0, 11.7]
Diuresis/body weight/anesthesia time (ml/kg/h)	1.0 [0.7, 1.8]	1.2 [0.7, 2.0]	1.1 [0.6, 1.9]	1.4 [0.8, 2.4]	1.1 [0.6, 2.3]
Intraoperative bleeding (ml)	400.0 [200.0, 700.0]	600.0 [300.0, 1300.0]	500.0 [300.0, 1000.0]	900.0 [400.0, 2000.0]	1000.0 [300.0, 2350.0]
Transfused units of red blood cells during first 24 hours	0.0 [0.0, 2.0]	4.0 [1.0, 9.0]	3.0 [1.0, 7.0]	5.0 [3.0, 9.0]	5.0 [2.0, 13.5]
Intraoperative use of HAES	24 (7.4)	15 (6.2)	7 (5.3)	2 (4.4)	6 (9.4)
Intraoperative use of aprotinin	0 (0.0)	3 (1.2)	1 (0.8)	2 (4.4)	0 (0.0)
Intraoperative fluid use (liter)	2.7 [1.7, 4.7]	5.9 [2.7, 9.3]	5.1 [2.4, 7.8]	8.1 [4.7, 11.6]	7.2 [3.2, 12.1]
Intraoperative fluid balance (liter)	1.0 [0.4, 2.0]	2.4 [1.0, 4.3]	1.8 [0.9, 3.5]	3.1 [0.8, 5.2]	3.4 [1.2, 5.2]
Other induction immunosuppression than ATG	29 (8.9)	57 (23.5)	19 (14.4)	14 (30.4)	24 (36.9)
Perioperative antibiotics used					
Cefotaxim/cephalosporin	211 (64.9)	116 (47.9)	62 (47.0)	25 (54.3)	29 (45.3)
Other	42 (12.9)	30 (12.4)	13 (9.8)	7 (15.2)	10 (15.6)
Carbapenem	72 (22.2)	96 (39.7)	57 (43.2)	14 (30.4)	25 (39.1)
Inotropic score	16.0 [10.0, 25.0]	20.0 [10.0, 32.0]	18.0 [10.0, 30.0]	20.2 [10.8, 44.1]	21.0 [10.0, 36.0]
Use of inhaled nitric oxide intra-/postoperatively	53 (16.3)	41 (16.9)	18 (13.6)	9 (19.6)	14 (21.5)
Ischemia time lungs (hours)	6.0 [4.5, 7.3]	5.7 [4.4, 7.3]	5.3 [4.3, 7.2]	6.4 [4.5, 7.8]	6.3 [4.6, 8.0]

Values are presented as n (%); median ± Interquartile range [Q1, Q3] for all other variables.
ECMO = extracorporeal membrane oxygenation, ATG = antithymocyte globuline, HAES = hydroxyethyl starch solution.

Univariate and multivariate analysis of preoperative variables as predictors of AKI. Table V. 3.

In the univariate analysis, the following preoperative variables were significantly associated with AKI; BMI (p=0.013),), time on the waiting list (p=0.015), re-transplantation (p=0.023), diagnosis of PPH (p=0.020), idiopathic pulmonary fibrosis (p=0.030) or CF (p=0.013), tricuspid regurgitation grade 2-3 (p=0.004), preoperative diabetes (p= 0.031), preoperative CKD (p=0.011), creatinine at the day of surgery (0.020) and preoperative mechanical ventilation (p=0.014).

In the multivariate analysis the following variables were significantly associated with AKI: BMI (kg/m2) (OR 1.07, p=0.004), time on transplantation waiting list (months) (OR 1.05, p=0.005), re-transplantation (OR 2.3, p=0.034) and higher grade (2–3) of tricuspid regurgitation (OR 2.5, p=0.005).

Table V. 3. Preoperative variables associated with acute kidney injury

Variable	Univariate regression			Multivariable regression		
	OR	95% CI	P-value	OR	95% CI	P-value
Age	0.992	0.979-1.01	0.223	0.996	0.975-1.02	0.728
Gender (female)	0.768	0.55-1.07	0.12	0.769	0.524-1.13	0.182
Body Mass index (kg m^2)	1.05	1.01-1.09	0.013	1.07	1.02-1.12	0.0047
Time on waiting list (month)	1.04	1.01-1.07	0.0152	1.05	1.01-1.09	0.00539
Re-transplantation	1.89	1.09-3.28	0.0228	2.3	1.07-4.97	0.0336
Preoperative smoking	0.771	0.552-1.08	0.127	0.925	0.584-1.47	0.74
Diagnosis lung disease (ref: COPD)			0.0675			0.5
Idiopathic pulmonary fibrosis	1.72	1.05-2.79	0.0298	1.2	0.687-2.08	0.527
α 1-Antitripsin deficiency	1.5	0.79-2.83	0.216	1.49	0.77-2.89	0.236
Cystic fibrosis	2.19	1.18-4.05	0.0127	1.91	0.803-4.53	0.143
Primary pulmonary hypertension	2.57	1.16-5.71	0.0201	1.09	0.398-3	0.863
Other	1.72	1.04-2.83	0.0341	0.948	0.478-1.88	0.879
Diabetes	1.68	1.05-2.68	0.0314	1.31	0.744-2.32	0.347
Hypertension	1.14	0.74-1.77	0.543			
CKD	2.12	1.19-3.8	0.0111	1.44	0.7-2.95	0.323
eGFR day of surgery	0.995	0.988-1	0.243	0.997	0.986-1.01	0.632
Creatinine day of surgery	1.01	1-1.02	0.0196			
Tricuspid regurgitation (ref: no TI)			0.00376			0.011
TI grade 1	1.41	0.976-2.04	0.0673	1.4	0.94-2.08	0.0977
TI grade 2 and 3	2.43	1.39-4.25	0.00174	2.55	1.32-4.91	0.00521
Preoperative ECMO treatment	2.25	0.916-5.51	0.0769	1.02	0.296-3.53	0.971
Preoperative mechanical ventilation	2.57	1.21-5.47	0.0144	1.92	0.684-5.39	0.216
Preoperative ACE or ARB treatment	1.25	0.74-2.11	0.404			
EVLP	1.49	0.645-3.43	0.352			
Preoperative O$_2$ treatment	1.23	0.876-1.73	0.23	1.14	0.785-1.65	0.498
Ischemia time lungs	0.987	0.917-1.06	0.724			
Preoperative CNI treatment	1.1	0.735-1.63	0.655			

ACE = angiotensin converting enzyme inhibitors, ARB = angiotensin receptor blocker, ECMO = extracorporeal membrane oxygenation, EVLP = ex vivo lung perfusion, TI = tricuspid regurgitation, other lung diagnosis = CLAD (chronic lung allograft dysfunction), bronchiolitis obliterans, chronic pulmonary embolism, bronchiectasis, graft versus host disease of the lungs.

Univariate and multivariate analysis of intraoperative variables as predictors of AKI. Table V. 4.

Out of the intraoperative variables analysed in the univariate analysis, sternotomy ($p<0.001$), clamshell incision ($p<0.001$), and the use of CPB ($p<0.001$) or ECMO ($p<0.001$), as well as longer surgery time ($p= 0.01$), were significant variables associated with AKI. Increased intraoperative bleeding ($p<0.01$) and number of transfused units of red blood cells the first 24 hours ($p<0.001$) were also associated with increased risk of AKI, as well as higher inotropic score at arrival in the ICU ($p=0.045$) and intraoperative diuresis ($p=0.01$).

In the multivariable analysis intraoperative diuresis (ml/kg/hour) (OR 0.68, $p<0.01$), the number of transfused RBC units ($p<0.001$), and the use of induction immunosuppression other than ATG and methylprednisolone (OR 2.91, $p<0.001$) were significantly associated with AKI. Intraoperative use of CPB, although significant in the univariate analysis, did not reach significance level in multivariate analysis (OR 2.3, $p<0.052$). Table V. 4.

Table V. 4. Intraoperative variables associated with acute kidney injury

Variable	Univariate regression			Multivariable regression		
	OR	95% CI	P-value	OR	95% CI	P-value
Type of surgery, double vs. single	1.49	0.944-2.37	0.0866	1.18	0.661-2.12	0.57
Type of incision (ref: Thoracotomy/bilateral sequential thoracotomy)			<0.001			0.527
Sternotomy	4.82	3.1-7.51	<0.001	1.12	0.446-2.81	0.811
Clamshell	2.75	1.76-4.29	<0.001	0.713	0.305-1.67	0.434
Intraoperative extracorporeal circulation			<0.001			0.146
Cardiopulmonary bypass	5.39	3.59-8.09	<0.001	2.3	0.993-5.31	0.052
ECMO	3.79	2.29-6.29	<0.001	1.53	0.755-3.08	0.239
Surgical time	1.08	1.01-1.15	0.0161	0.962	0.88-1.05	0.392
Anesthesia time	1.08	1.01-1.14	0.0158			
Diuresis/body weight/anesthesia time (ml/kg/h)	1.12	0.971-1.3	0.116	0.688	0.56-0.846	<0.001
Intraoperative bleeding ml (ref: >600)			<0.001			0.578
601-800	2.11	1.08-4.14	0.0298	1.02	0.45-2.3	0.966
801-1000	1.65	0.872-3.13	0.123	0.697	0.327-1.48	0.349
1001-2000	2.11	1.28-3.49	0.0035	0.689	0.353-1.34	0.275
>2000	5.26	2.69-10.3	<0.001	1.3	0.489-3.44	0.602
Units of red blood cells transfused during first 24 hours (ref: 0 units)			<0.001			<0.001
1-2 units	2.27	1.37-3.77	0.00148	2.3	1.3-4.06	0.00421
2-3 units	5.8	3.55-9.47	<0.001	5.15	2.69-9.83	<0.001
7-12 units	7.78	4.3-14.1	<0.001	7.56	3.29-17.3	<0.001
>12 units	13.7	6.34-29.6	<0.001	11.3	3.41-37.2	<0.001
HAES	0.832	0.427-1.62	0.59			
Intraoperative fluid balance	1.25	1.16-1.36	<0.001	1.04	0.954-1.13	0.374
Other induction immunosuppression then ATG	3.14	1.94-5.09	<0.001	2.91	1.64-5.15	<0.001
Perioperative antibiotics:						
Cefotaxim/other cephalosporin			<0.001			0.3
Other	1.3	0.772-2.19	0.324	0.571	0.278-1.17	0.127
Carbapenem	2.43	1.66-3.55	<0.001	0.75	0.327-1.72	0.496
Inotropic score	1.01	1-1.02	0.0454	1	0.989-1.01	0.981
Use of inhaled nitric oxide	1.05	0.669-1.63	0.845			

Abbreviations: ECMO = extracorporeal membrane oxygenation, HAES = hydroxyethyl starch solution, ATG = antithymocyte globuline.

1-year Mortality and AKI (Fig. V. 5)

The 1-year mortality was significantly increased in patients with grade 3 AKI as compared to no AKI patients (p<0.0001)
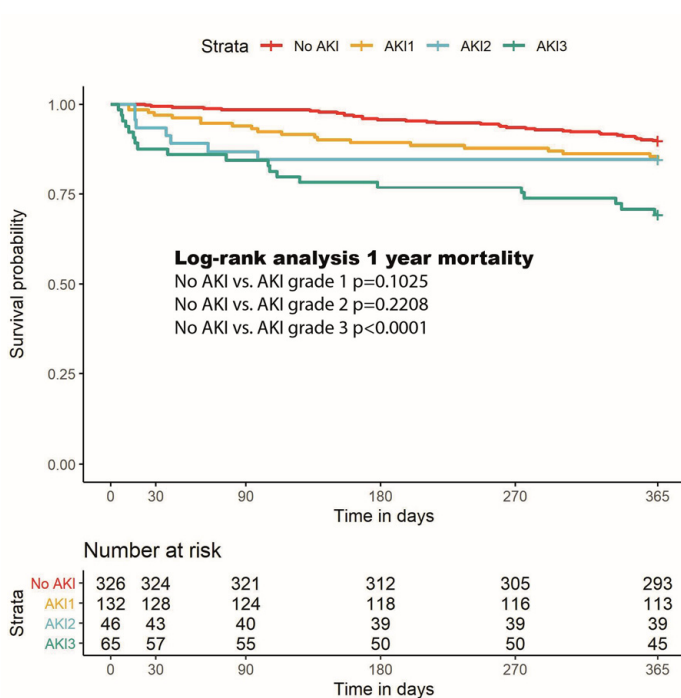

Figure 5. Kaplan-Meier analysis of survival at 30 days and 1 year and log-rank test for 1 year mortality.
Log-rank analysis for 1-year mortality. No AKI vs. AKI grade 1 p=0.1025; no AKI vs. AKI grade 2 p=0.2208; no AKI vs. AKI grade 3 p<0.0001.

Discussion

In the five studies included in this doctoral thesis, we investigated aspects of AKI in elective CABG, ECMO-treated, and lung-transplanted patients. A common denominator for the above-mentioned procedures is the use of ECC.

The study protocol (paper I) was important background research in preparation for the main CiPRICS study (paper II) and the sub-study paper III, not only because of methodological aspects of the study, but also because of safety considerations. Cyclosporine is a well-known immunosuppressive drug with a potential risk to increase postoperative infections and kidney dysfunction, when used chronically.

We hypothesized that cyclosporine administered before CABG might reduce postoperative kidney dysfunction. This assumption was based on experimental and clinical studies showing cyclosporine's renoprotective effect in experimental studies and myocardial protective effect in cardiac surgery patients. Also, one clinical study showed a trend towards renoprotection (our interpretation) further encouraging our hypothesis[3].

Although previous studies using the same dose of cyclosporine (2.5 mg/kg), failed to show any impact on renal function, in our study, pretreatment with cyclosporine resulted in a decrease in renal function in the immediate postoperative period. Thus, instead of protecting kidneys from ischemia-reperfusion injury, we actually inflicted a worse kidney function on patients treated with cyclosporine. In the ad-hoc follow-up investigation one to six months after the surgery, all controlled patient's kidney functions had returned to baseline. However, this immediate harmful renal effect of cyclosporine has not previously been demonstrated.

Cyclosporine is known to induce renal vasoconstriction, raising the question of whether a postoperative decrease in eGFR in the cyclosporine-treated cohort represents a nephrotoxic effect or a temporal decrease in eGFR due to vasoconstriction. We found no effect of cyclosporine treatment on albumin/creatinine ratio (Ederoth et al., unpublished data) or two urinary biomarkers of AKI: tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor binding protein-7 (IGFBP7).

So, the question of whether postoperative increase in cystatin C and SCr represents kidney injury or temporal decrease in eGFR is not completely answered.

The main conclusion of the CiPRICS (paper II) study is that cyclosporine pre-treatment is not renoprotective in elective CABG patients but instead causes a decrease in renal function that resolved after one month. Thus, our findings contrast with previous reports, where preoperative administration of 2,5 mg of cyclosporine was cardioprotective and without reported renal side effects[3, 52]. However, we consider our finding robust, representing a prospective, blinded, randomized, placebo-controlled study with renal function as a primary outcome.

The augmented inflammatory response has been identified as a risk factor for the development of AKI, and a correlation between individual cytokines and AKI in ECC-treated patients has been reported previously. In cardiac surgery, increased cytokine production, particularly of IL-6 and IL-10, has been reported to an early indicator for AKI in children and adult patients[73-75]. At the same time, other research groups did not find any association between IL-10, IL-6, and AKI[77].

In paper III, we investigated cyclosporine's potential role in decreasing the perioperative inflammatory response in elective CABG patients, as CsA has been reported to exert an inhibitory effect on cytokine release. The main finding in this study is that perioperative cytokine production is not affected by CsA pretreatment. Thus, CsA cannot be used to modify the inflammatory response in elective CABG patients. Furthermore, the overall cytokine concentrations in our study were low, and neither IL-6 nor IL-10 levels correlated to postoperative AKI.

The low postoperative cytokine concentrations might explain the lack of correlation between cytokine levels and AKI since we only included elective uncomplicated CABG patients[94, 95]. Another plausible explanation might be the routine use of tranexamic acid in all CABG patients which has been shown to reduce plasma cytokine levels[96, 97]. However, despite earlier reports of high cytokine release during ECC, a valid interpretation of our results may be is that the uncomplicated use of ECC in routine CABG surgery does not induce much cytokine releases.

Since neither IL-10 nor IL-6 significantly correlates with postoperative kidney dysfunction, our data contribute to the existing controversy regarding postoperative cytokine levels and AKI in cardiac surgery patients. Furthermore, in our study, the correlation between cytokine levels and AKI was not a primary outcome; therefore, the results can only be considered exploratory.

In papers I, II, and III, we could prove that, despite promising experimental data, CsA pretreatment does not protect kidneys from ischemia-reperfusion injury and does not affect perioperative cytokine production in response to CABG surgery and ECC.

While we did not find a significant correlation between cytokine concentrations and AKI in elective CABG surgery (paper III), in our study of 100 V-A ECMO-treated patients (paper IV), the levels of IL-10 were significantly increased in patients who later developed AKI. The difference between the studies' results might be explained

by the patient populations since, in contrast to CABG patients, the VA ECMO patients were severely hemodynamically compromised. Furthermore, the timing of cytokine sampling was different in these two studies.

We found that patients who developed AKI on the VA ECMO circuit had more than four times higher pre-cannulation levels of IL-10. Interestingly, the levels of IL-10 normalized within 48 hours after the initiation of hemodynamic support.

The association between increased levels of IL-10 and AKI in critically ill patients has been reported earlier. For example, in a study of severely ill, ECMO treated ARDS patients, an early increase in IL-10 was associated with a poor prognosis[80]. Furthermore, in septic patients, increased levels of IL-10 at admission, were associated with both AKI and mortality[79]. Thus, our data in paper IV are in line with earlier published studies and support the use of IL-10 as a biomarker for AKI in critically ill patients.

Most studies reporting long-term survival in ECMO-treated patients who developed AKI report in-hospital, 30-day, or 90-day mortality, with limited data on long-term survival[98, 99]. Also, the patient populations reported include pediatric and adult patients, and different types of ECMO treatment: cardiac, respiratory, or mixed.

In our cohort of 100 VA ECMO-treated patients, the highest mortality was observed within the first 30 days of treatment; however, the survival at one year was not different in AKI patients compared to non-AKI patients, regardless of kidney function at cannulation. This poses a very interesting question: is kidney function before the start of ECMO treatment of any interest? When comparing patients with eGFR$\geq$ 45ml/min to those with eGFR < 45ml/min, and patients who develop AKI during VA ECMO treatment, we found a significant survival benefit at 30 days for patients with better kidney function at the start. This emphasizes the importance of timely initiation of hemodynamic support, avoiding hypoperfusion and factors contributing to AKI, such as infectious complications, circuit-related hemolysis, and nephrotoxic medications.

The development of AKI is especially problematic in LTx patients considering lifelong treatment with calcineurin inhibitors, known to induce renal insufficiency when used chronically. Therefore, searching for pre- and intraoperative risk factors is of clinical importance.

In our nationwide retrospective study of 569 LTx patients (paper V), the incidence of AKI was 43%, which is in the lower range of that previously reported[2]. Moreover, most AKI (55%) patients developed mild, grade 1 AKI. However, the importance of preventing AKI is further emphasized by increased 30-day and 1-year mortality in AKI grade 3 patients compared to patients without AKI, especially when CRRT was utilized. CRRT treatment is widely used for critically ill patients with AKI, and recent studies have reported the mortality at around 64% and the worst short- and long-term prognosis[100]. In our study, the numbers of CRRT-treated patients are few,

and we cannot elucidate the contributing factors to increased mortality. However, our clinical experience is that multi-organ failure is more common in patients requiring CRRT.

In terms of preoperative risk factors, we found tricuspid regurgitation (TR) to be associated with postoperative AKI, which has not been described previously. TR might be an indicator of increased pulmonary vascular resistance resulting in venous congestion and increased central venous pressure (CVP), which is associated with AKI in cardiac surgery and intensive care patients[101, 102].

In contrast to our findings in paper II, preoperative CNI treatment did not increase the risk of AKI in paper V. However, preoperative CNI treatment was given to only 22% of the LTx patients, and the extent of surgical trauma is more extensive in LTx surgery compared to elective CABG. We also found that a longer time on the waiting list and retransplantation surgery were associated with AKI, probably reflecting a high burden of pulmonary disease.

The analysis and interpretation of data in a large retrospective study is a vast statistical challenge. We used univariate analysis to interpret the distribution of the values and a multivariate regression model to understand the relationship between variables. However, mathematical modeling of complex biological relationships has limitations as inclusion or exclusion of only one variable might change the outcome and thus conclusions. An understanding of the pros and cons of different study designs and the meaning of p values are essential for the correct interpretation of results. The term "statistical significance" should not be misinterpreted as "clinically important"[103].

For example, in our study of LTx patients, the intraoperative use of CPB did not reach significance in the multivariable analysis; however, there was a trend toward significance (p=0.052). Interpreting p-values close to the threshold (0.05) for statistical significance as a strict yes or no is challenging. The p-value should not be used as a substitute for scientific reasoning, as the interpretation of results as statistically significant may give a false sense of confidence that the finding is true, while statistical rejecting of a hypothesis may give a false sense that the finding does not exist[104]. Though, the p value should be reported as an exact value and interpreted as a continuous variable together with the context and the validity of the study. This is further emphasized by the fact that in some studies, results and conclusions can be changed by changing statistical methods[15, 105].

It is important to emphasize that retrospective studies should be considered hypothesis-generating, and findings confirmed in a prospective study.

One confounding factor, not included as a variable in our or other renal-outcome studies, is the kidney's capacity to increase filtration in response to stimuli. In clinical studies, patients with equal creatinine or eGFR are considered to have equal renal function. However, this is not always the case, as commonly used renal

function markers, such as creatinine, may remain within a normal range until 50% of nephrons are lost[106]. Thus, the calculated baseline eGFR will not reveal the kidney's capacity to increase the filtration in response to physiological or pathological stimuli, the so-called renal functional reserve (RFR). Consequently, two patients with equal baseline eGFR could have different capacities to cope with pathological stress, such as cardiac surgery, ECMO treatment, or lung transplantation[107, 108]. In clinical praxis, RFR is challenging to assess, especially in acute settings; however, in future studies, it could be used as a marker to assess the susceptibility to renal injury[109].

Strength and limitations

The main strength of the studies in this thesis is that they represent true clinical studies conducted on patients in cardiac surgery, lung transplantation, and intensive care and therefore represent real-world clinical data on aspects of AKI in these patient populations. However, the design of the studies varies from a prospective, placebo-controlled study to an observational retrospective study with the limitations of the retrospective design.

The strength of papers I and II is that they can provide strong evidence against using cyclosporine to prevent a postoperative decrease in kidney function in elective CABG surgery. This is due to using a control group, a true placebo solution, and double-blinded random assignment of subjects to reduce the impact of bias and confounding variables on study results. A limitation of this study is that it may not be generalizable to wider patient populations, as it was conducted on elective CABG patients and may not apply to all clinical situations of ischemia-reperfusion.

Paper III is a sub-study of larger clinical study and is limited by its size. It is, however, the first study to report on cyclosporine's effect on the inflammatory response in elective CABG surgery, which is a strength of the study.

ECMO-treated patients are a complex population to study due to the different underlying causes leading to ECMO and the different modalities of ECC used. The strength of paper IV is that it is the first study to report on IL-10 as a potential biomarker preceding the development of AKI in VA ECMO-treated patients. Moreover, in our study, we present long-term survival data in ECMO-treated patients and the impact of AKI on survival at one year.

Paper V is, by its size, the most extensive study in this thesis, as it comprises 569 lung-transplanted patients. Because of the study's retrospective design, we cannot draw any strong conclusions regarding causality. However, only a few studies of this size on AKI incidence in lung-transplanted patients have been published, and a prospective study of this size would require another ten years of research.

Conclusions

Papers I and II

In papers I and II, we found that, in contrast to our hypothesis, in elective CABG surgery with ECC, cyclosporine pre-treatment was not protective to the kidneys but caused a decrease in postoperative renal function that resolved after one month.

The results of this study are only applicable to elective CABG patients, as our findings do not exclude that cyclosporine might prevent ischemia-reperfusion injury in other clinical scenarios.

Paper III

In paper III, we could demonstrate that in elective CABG surgery with ECC, perioperative cytokine production is not affected by the preoperative administration of cyclosporine. Thus, cyclosporine cannot be used to ameliorate the perioperative inflammatory response. We also found that changes in cytokine concentrations do not correlate with kidney function postoperatively, meaning that cytokines cannot be used as early biomarkers for AKI in elective CABG surgery patients.

Paper IV

In paper IV, we could identify IL-10 as an early pre-cannulation marker that precedes the AKI development in patients receiving VA ECMO support. We also found that AKI development is associated with increased mortality compared to no-AKI patients with better renal function before the start of ECMO treatment. In our study, AKI patients who survived the first 30 days have similar one-year survival to those without AKI.

Paper V

In our study of 569 lung-transplanted patients, AKI was a common complication affecting 43% of the patients and most AKI patients developing grade 1 AKI. In addition, we found that preoperative tricuspid regurgitation, intraoperative blood transfusions, and low diuresis were associated with early postoperative AKI development.

Acknowledgements

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Paper I

BMJ Open

Ciclosporin to Protect Renal function In Cardiac Surgery (CiPRICS): a study protocol for a double-blind, randomised, placebo-controlled, proof-of-concept study

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ABSTRACT

Introduction: Acute kidney injury (AKI) after cardiac surgery is common and results in increased morbidity and mortality. One possible mechanism for AKI is ischaemia–reperfusion injury caused by the extracorporeal circulation (ECC), resulting in an opening of the mitochondrial permeability transition pore (mPTP) in the kidneys, which can lead to cell injury or cell death. Ciclosporin may block the opening of mPTP if administered before the ischaemia–reperfusion injury. We hypothesised that ciclosporin given before the start of ECC in cardiac surgery can decrease the degree of AKI.

Methods and analysis: Ciclosporin to Protect Renal function In Cardiac Surgery (CiPRICS) study is an investigator-initiated double-blind, randomised, placebo-controlled, parallel design, single-centre study performed at a tertiary university hospital. The primary objective is to assess the safety and efficacy of ciclosporin to limit the degree of AKI in patients undergoing coronary artery bypass grafting surgery. We aim to evaluate 150 patients with a preoperative estimated glomerular filtration rate of 15–90 mL/min/ 1.73 m^2. Study patients are randomised in a 1:1 ratio to receive study drug 2.5 mg/kg ciclosporin or placebo as an intravenous injection after anaesthesia induction but before start of surgery. The primary end point consists of relative P-cystatin C changes from the preoperative day to postoperative day 3. The primary variable will be tested using an analysis of covariance method. Secondary end points include evaluation of P-creatinine and biomarkers of kidney, heart and brain injury.

Ethics and dissemination: The trial is conducted in compliance with the current version of the Declaration of Helsinki and the International Council for Harmonisation (ICH) Good Clinical Practice guidelines E6 (R1) and was approved by the Regional Ethical Review Board, Lund and the Swedish Medical Products Agency (MPA). Written and oral informed consent is obtained before enrolment into the study.

Strengths and limitations of this study

- Randomised, controlled, double-blind, prospective clinical trial.
- Two Drug Safety Monitoring Board (DSMB) meetings have recommended that the study be continued.
- Strictly standardised study population.
- Possible selection bias because all possible patients may not be entered in the study.

INTRODUCTION

Acute kidney injury (AKI) is a common complication after cardiac surgery, with an incidence between 5% and 40% depending on the definition.[1–5] A decreased renal function after cardiac surgery is associated with decreased long-term survival.[2–5] Despite trials investigating several pharmacological agents,[6–9] no effective prophylactic treatments have so far been found.

Cardiac surgery with extracorporeal circulation (ECC) may result in renal ischaemia–reperfusion injury, especially in the poorly oxygenated and metabolic active outer medulla. Thus, ECC-induced renal ischaemia–reperfusion injury is claimed to play a role in the resulting AKI.[10]

The proposed mechanism for AKI induced by renal ischaemia–reperfusion injury is opening of channels called the mitochondrial permeability transition pore (mPTP) during reperfusion. This can amplify or accelerate cell death, resulting in reperfusion-induced necrosis.[11–17]

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The inner mitochondrial membrane is normally impermeable to most solutes, enabling efficient ATP production through oxidative phosphorylation. Under conditions of elevated Ca^{2+} levels and oxidative stress triggered by reperfusion after ischaemia, the mPTP in the inner mitochondrial membrane opens. On the mPTP opening, energy production is immediately halted and molecules smaller than ~1500 Da equilibrate over the membrane. The osmotic force of matrix proteins results in matrix swelling, leading to rupture of the outer membrane and release into the cytosol of proapoptotic factors such as cytochrome C, further pushing the cell towards death.[13 18 19]

Cyclophilin-D is a key regulator of the mPTP, which has been confirmed in several independent cyclophilin-D knock-out studies. Ca^{2+} causes a conformational change in the mPTP from a closed to an open state.[20–22] The opening of the mPTP can be inhibited pharmacologically by the immunosuppressive agent ciclosporin via inhibition of cyclophilin-D,[23 24] and several reports in animals have indicated that it may limit ischaemia–reperfusion injury in various organs, including the kidneys.[25–29] A cyclophilin-D activated mPTP has also been demonstrated in human mitochondria.[30–33]

Further, there are a number of animal studies showing cytoprotective preconditioning, antinecrotic and also antiapoptotic effects of ciclosporin against ischaemia–reperfusion injury in the kidneys.[34–38] Importantly, ciclosporin has been administered before the kidney is exposed to ischaemia and subsequent reperfusion in these studies.

To the best of our knowledge, no clinical studies with the specific aim of investigating if ciclosporin has renoprotective effects if administered before the ischaemia–reperfusion episode have previously been performed. In contrast, ciclosporin is known to cause renal failure following high and/or long-term exposure. However, this side effect is caused by other mechanisms, discussed under 'Safety considerations' section.

Hypothesis
On the basis of the aforementioned, we raised the hypothesis that ciclosporin, administered as a single intravenous bolus dose preoperatively in cardiac surgery with ECC, will reduce the level of renal dysfunction associated with this type of surgery.

METHODS AND ANALYSIS
Study design
We designed an investigator-initiated, clinical, double-blind, randomised, placebo-controlled, parallel design clinical trial based on a single centre aiming to detect if there is a role for ciclosporin in renal protection. Study patients will be randomised in a 1:1 ratio. We plan to have a total of 150 consecutive evaluable study patients with ~75 patients in each arm. Approximately 170 patients are estimated to be enrolled, in order to have 150 evaluable patients for the two groups.

Definitions
Day numbering: Day 0 will be the surgery and study drug administration day. Day −1 is the day the study patients are included and is normally the day before surgery and day 1 the first day after surgery, etc. On day 0, the end of ECC is defined as time zero.

Data collection
Efficacy data will be collected on day −1 until, and including, day 4 as long as the patient is present at the hospital. Safety data will be collected from the time for distribution of study drug until, and including, day 4 as long as the patient is present at the hospital. From day 4 until day 30, serious adverse events (SAEs) and serious unexpected adverse reactions (SUSARs) will be reported.

For details, see table 1 and figure 1.

Study drug
The study drug is CicloMulsion, a 5 mg/mL ready-to-use lipid emulsion of ciclosporin (NeuroVive Pharmaceutical AB, Lund, Sweden) or its matching placebo, given as a single intravenous bolus dose of 0.5 mL/kg. The qualitative composition of CicloMulsion and its placebo only differ in the presence or absence of ciclosporin, so the final emulsions will be visually indistinguishable.

Eligibility criteria
This study will be eligible for patients planned for coronary artery bypass grafting (CABG), a standardised operation including the use of ECC.

Inclusion criteria
1. The study patient is scheduled for non-emergent (decision to operate more than 1 hour before start of surgery) CABG surgery.
2. Preoperative cystatin C estimated glomerular filtration rate (eGFR) or the Modification of Diet for Renal Disease (MDRD) eGFR is 15–90 mL/min/$1.73/m^2$. eGFR will be calculated using both creatinine based on the MDRD[39] and the cystatin C based formula on the Chronic Kidney Disease Epidemiological collaboration (CKD-EPI).[40] The lowest eGFR value will be used as inclusion criteria.
3. The patient has given his/her written consent to participate.

Exclusion criteria
Patients are excluded if they meet one or more of the following criteria:
1. The patient has an uncontrolled hypertension.
2. Hypersensitivity to the active drug or vehicle, including egg protein, soya protein or peanut protein.
3. The patient is pregnant or is a fertile woman.

Table 1 Participant timeline

Day number in relation to surgery day	−1	0	1	2	3	4	1 month telephone call
Informed consent	X						
Inclusion/exclusion criteria	X	X					
Randomisation		X					
Study drug		X					
AE and SAE registration and report		X	X	X	X	X	X
Blood ciclosporin concentration		X	X				
Blood tests efficacy: P-cystatin C, P-creatinine	X		X	X	X	X	
Analysis U-TIMP-2, U-IGFBP7, U-albumin/creatinine		X	X				
Blood tests safety: Mg, K, urea, myoglobin, ASAT, ALAT, bilirubin, ALP, GT, leucocytes, CRP, CK, Hb, Trc	X		X	X	X	X	
Exploratory immunological tests		X	X				
Blood tests cardiac: troponin T, CK MB	X	X	X	X	X		
Blood test cerebral: S-S100B	X		X	X			
Documentation of: hourly diuresis, bleeding at 12 hours and total, time to extubation, time in ICU, fluid balance		X	X				
Temperature, blood pressure	X		X	X	X	X	
Scoring leg wound infection.							X

Day −1 illustrates the day before surgery, usually the same as admission day, day 0 surgery day, day 1 the day after surgery, etc.
AE, adverse event; ALAT, alanine aminotransferase; ALP, alkaline phosphatase; ASAT, aspartate aminotransferase; CK, creatine kinase; CRP, C reactive protein; GT, γ-glutamyl transferase; Hb, haemoglobin; ICU, intensive care unit; IGFBP7, insulin-like growth factor binding protein 7; K, potassium; Mg, magnesium; SAE, serious adverse event; TIMP-2, tissue inhibitor of metalloproteinase 2; Trc, thrombocytes.

4. The patient has been treated with ciclosporin within 4 weeks prior to the surgery.
5. The patient has a known ongoing malignancy.
6. The patient has ongoing immunosuppressive treatment.
7. The patient has severe hepatic dysfunction.
8. The patient is treated with dialysis.
9. The patient has preoperatively ongoing and/or increasing clinical infection with C reactive protein (CRP) levels of >50 mg/L. Clinical signs of infection may or may not be present. Increase in CRP due to signs of cardiac origin,[41] according to the investigator, should not be considered as exclusion criteria.
10. The patient has a severe ongoing viral infection, including HIV, hepatitis C, current or history of hepatitis B.
11. For non-allowed and restricted ongoing and concomitant medications, see Protocol section 12.2.
12. The patient is planned for off-pump CABG surgery.
13. The patient is included in other ongoing clinical trials.
14. For any other reason, the patient is unsuitable to participate in the study, according to the investigator.

Recruitment and stratification

Potential participants are identified from patients planned for elective CABG and included in a screening log. Patients are checked against the inclusion/exclusion criteria, and thereafter oral and written information is given to the patient. When written informed consent is obtained by an investigator, the patient is enrolled in the study.

At enrolment, the investigator also stratifies the patient to a predefined subgroup with reference to preoperative eGFR (15–59 or 60–90 mL/min/1.73 m^2). When several patients fulfil the inclusion criteria, those with preoperative eGFR 15–59 are selected over those with 60–90 mL/min/1.73 m^2 with the aim of including ~50 patients from the group with eGFR 15–59 mL/min/1.73 m^2. The reason for this is that preoperative renal function impairment is an important risk factor for developing postoperative AKI,[42] and we know that without stratification there will only be a few patients enrolled with eGFR 15–59 mL/min/1.73 m^2. Thus, we also investigate if treatment effects are similar in patients with preoperative milder and more severe renal impairment. Also, within each eGFR group, the study drug and placebo will be stratified in a 1:1 ratio. In summary, the stratification will increase the proportion of patients with a higher risk of developing AKI.

Randomisation

For randomisation to study drug or placebo treatment, a blinded randomisation list was pregenerated by a statistician not included in the study group (FoU-centrum Skane, Skane University Hospital, Lund, Sweden), to assign a unique sequential treatment number to each bottle of the study drug/placebo. The unique treatment number was printed on the study medication bottle, where also a sticker for the case report form (CSF) was positioned. The bottles with the study drug/placebo were packed in boxes marked with eGFR 15–59 or 60–90 mL/min/1.73 m^2 according to the stratification aforementioned. Study drug/placebo is kept in two separate sets, one set for patients with eGFR 15–59 mL/min/
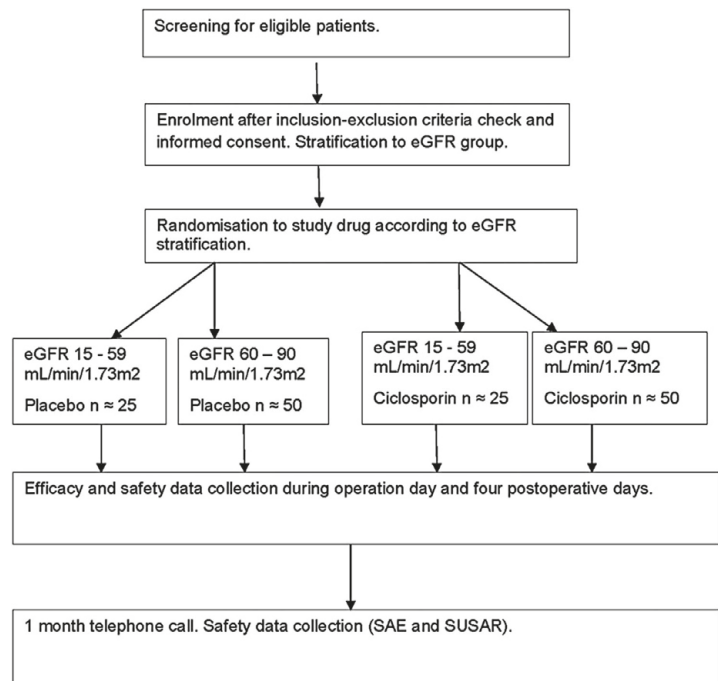
1.73 m^2 and one set for patients with eGFR 60–90 mL/min/1.73 m^2.

Following completion of the assessments listed above, eligible patients will be allocated to one of the two treatment groups in a 1:1 ratio, to receive either a single intravenous bolus injection of ciclosporin or matching placebo by the following action.

Once the study patient has been admitted to the operation ward, a box with the allocated study drug/placebo is taken by the study nurse and the treatment number from that box and study drug/placebo bottle is attached in the CRF. The allocation to the study drug/placebo following the generated randomisation list will thus take place when the study nurse takes the next study medicine bottle, as described here, and prepares the syringe with the study drug/placebo.

Thus, the study treatment is blinded for all involved staff. Closed envelopes with unblinding information is available at the operating ward in case of emergency situations.

Interventions

After randomisation, the study drug/placebo will be administered as an intravenous single bolus dose injection, 0.5 mL/kg body weight (corresponding to 2.5 mg/kg ciclosporin), after anaesthetic induction has been performed and the patient is in a stable circulatory state before the surgery starts. The study drug/placebo will be given in a central venous catheter as an injection during 10 min, without concomitant administration of other drugs in this line. Anaesthesia is standardised using propofol, fentanyl and rocuronium. Anaesthetic gas is prohibited medicine in this study.

Sample size calculations

A total of 150–170 patients will be included to obtain a total of 150 evaluable study patients with ∼75 patients in each arm. The relative difference between groups in change from baseline P-cystatin C to the third postoperative day will serve as the primary end point. In a previous study at our department,[7] the response within each participant group was normally distributed with an SD of 27%. The primary efficacy analysis will compare the ciclosporin group to placebo at a two-sided 5% level. A sample size equal to 75 in each arm will provide at least 80% power to detect a 13% units (0.5 SD) difference from placebo. The study will continue until the planned number of study patients have finalised the protocol.

Outcomes
Primary end point
Relative P-cystatin C change from day −1 to day 3.

Secondary end points

- Secondary end points to evaluate ciclosporin's effect on renal function include, but are not limited to, P-cystatin C day −1 versus days 1, 2 and 4, P-cystatin C area under curve day −1 to day 4, P-cystatin C and P-creatinine eGFR according to CKD-EPI, P-creatinine, MDRD eGFR. Biomarker for renal damage based on tissue inhibitor of metalloproteinases-2 (U-TIMP-2) and urine insulin-like growth factor binding protein 7 (U-IGFBP7) levels.[43] U-albumin/creatinine. Incidence of AKI according to predefined eGFR stratification (15–59 and 60–90 mL/min/1.73/m^2) and Risk, Injury, Failure, Loss and End-stage renal failure (RIFLE) criteria based on P-creatinine and/or eGFR.[44]
- To evaluate ciclosporin's possible effect on myocardial injury,[45] plasma creatine kinase isoenzyme MB (P CK MB) and P-troponin T will be measured.
- Ciclosporin also has a possible cerebral protective effect,[46] and therefore S-S100B is evaluated from day −1 throughout day 2.
- Clinical and procedural secondary variables include ECC time, operation time, respiratory time, blood pressure, temperature, etc.
- B-ciclosporin will be measured after injection, after end of ECC and on the morning of day 1.
- In addition, a number of immunological parameters will be measured on an exploratory basis; serum will be screened for cytokines related to the inflammatory cell pattern aiming for tissue aggressive inflammatory reaction (tumour necrosis factor-α, interleukin (IL)-1β, IL-6, IL-7, IL-12, IL-17, interferon-γ), regulatory/immune-suppressive function (IL-2, IL-10), neutrophil activation (IL-8) and tissue-lenient inflammatory responses (IL-2, IL-4, IL-13). Natural killer cells, T-cell populations (Th1, Th2, Th17) as well as B cells will be evaluated as well.

Monitoring

The study is monitored by the clinical research organisation at Skane University Hospital, Lund (FoU-centrum Skane, Skane university hospital, Lund, Sweden). This is an independent in-house Contract Research Organisation (CRO).

Data analysis plan

The statistical analysis plan (SAP) will be signed before database lock. Analyses not described here or in the SAP will be considered exploratory/post hoc analyses.

In general, descriptive statistics will be presented for all efficacy and safety variables, as appropriate. Continuous variables will be summarised by descriptive statistics (sample size (n), mean, SD, minimum, median and maximum values). Categorical data will be summarised in frequency tables showing the number of study patients, frequency and percentage of occurrence.

All statistical tests will be conducted at the two-sided 5% level unless otherwise specified. Where appropriate, model-based point estimates, together with their 95% CIs, will be presented along with the two-sided p value for the test.

The primary comparison will be to investigate difference in P-cystatin C change from baseline to the third postoperative day between ciclosporin and placebo. The treatment difference in the secondary and exploratory end points described earlier will also be tested.

The primary end point, P-cystatin C relative change from baseline to the third postoperative day, will be analysed using analysis of covariance (ANCOVA). The model will include treatment and baseline P-cystatin C as explanatory variables.

Secondary end points will mainly be analysed by using ANCOVA, following the same conventions as in the analysis of the primary end point.

Exploratory analyses will be performed of several quality indices including, for example, time on mechanical ventilator, time on intensive care unit, extent of bleeding, incidence of atrial fibrillation, time on ECC, immunological parameters, etc.

If assumptions for parametric analysis are clearly violated, data transformations or a non-parametric approach will be applied.

Organisation and time line

The study team involves at the moment 10 investigators and 2 research nurses. It is a single-centre study performed in a tertiary hospital (Department of Cardiothoracic Surgery, Anaesthesia and Intensive Care, Skane University Hospital, Lund, Sweden). The first patient was included on 6 April 2015. The last patient is expected to be included during 2016.

ETHICS AND DISSEMINATION

Ethics

The trial is conducted in compliance with the current version of the Declaration of Helsinki and the ICH Good Clinical Practice guidelines E6 (R1). The trial has been registered under NCT02397213 and EudraCT: 2014-004610-29, Sponsor's Protocol Code Number 2014.001.

Safety considerations

The reporting of adverse events (AE) will begin after the start of study medication and last until the follow-up phone call is made 1 month after day of operation. AEs also include SAE and SUSARs reporting. This is performed according to the Swedish Medical Products Agency's (MPA) regulations.

AEs, safety blood chemistry (table 1) and clinical data are collected daily including day 4. A telephone call on day 30 will assess and register possible SAE and signs of infection.

Drug Safety Monitoring Board

An independent Drug Safety Monitoring Board (DSMB) including three experts in clinical testing and/or renal function and one biostatistician will assess safety, including acute renal failure, when the study drug has been administered to 50 and 100 patients. The DSMB will recommend the study team to continue the study or not based on these safety data. A first meeting after 50 patients, in October 2015, and a second meeting in March 2016 after 100 patients were enrolled, resulted in the recommendation to continue the study without any changes in the protocol.

Specific safety considerations

The risks associated with a single 2.5 mg/kg intravenous dose of ciclosporin are considered small. Clinically relevant ciclosporin-associated side effects according to the Investigator Brochure (IB) for CicloMulsion are adverse allergic responses (anaphylactoid reactions) that may lead to anaphylactic shock, acute renal failure, hypertension or infections. These last three AEs are dose-dependent and usually occur during repeated administration.

Anaphylactoid reactions

Importantly, anaphylactoid reaction observed with the commercially available form of ciclosporin (Sandimmun) has been attributed to the presence of Kolliphor EL (Cremophor EL) in the product. In this study, a cremophor-free ciclosporin (CicloMulsion) and placebo will be used. However, this does not fully exclude the risk for allergic reactions to the study drug.

Renal failure

Calcineurin inhibitors such as ciclosporin are associated with induction of renal failure. The proposed mechanism for this is induction of vasoconstriction of the afferent arteriole by causing an imbalance between vasoconstricting[47–49] and vasodilating[50 51] agents. This results in an acute reversible[52 53] impairment of renal function with reduction in glomerular filtration rate[54] and tubular dysfunction.[55] Renal failure as an AE is associated with long-term use and higher doses of ciclosporin than will be used in our study.

Induction of acute reversible impairment of renal failure as described above cannot be excluded in our study. However, we consider it as unlikely. Three clinical trials in a similar population administered ciclosporin in the same dose, but different formulations, as in our study.[45 56 57] No renal side effects were reported. A recent large trial administering CicloMulsion in the same dose and in a similar population as in our study did not find any renal side effects.[58] These four studies excluded patients with severe renal impairment and the CicloMulsion IB reports known creatinine clearance $<30 \text{ mL/min/1.73 m}^2$ as a contraindication. Preoperative impairment of renal function is a strong risk factor for developing AKI after cardiac surgery.[42] To investigate if patients with the lowest renal function might also benefit from ciclosporin preoperatively, we include patients with eGFR 15–90 mL/min/1.73/m^2, that is, with worse renal function. In addition, we also obtain safety data on these patients.

Hypertension

Blood pressure is included as a safety parameter.

Infections

We follow infection parameters such as CRP, leucocytes and body temperature daily. The leg wound infection rate is assessed on D4 using a standardised method.[59] Also, we study immunological parameters as secondary variables on an exploratory base.

Dissemination plan

Results are going to be presented in a peer-reviewed medical journal. The study is investigator initiated and the protocol is written without any external influence. The study group will have the freedom to publish results regardless of the outcome. A clinical study report including study results will also be sent to the Swedish MPA and to the Regional Ethical Review Board. Archived study documents and source data will be filed at least 10 years after the study report has been finalised and submitted to the MPA. All processed data will be stored on the hospitals data servers with the same level of security as patient electronic records.

DISCUSSION

Ciclosporin to Protect Renal function In Cardiac Surgery (CiPRICS) is designed to investigate if ciclosporin has renoprotective effects if administered as a single intravenous injection before start of ECC in CABG surgery. It is, to the best of our knowledge, the first study to test this hypothesis and therefore a proof-of-concept study.

We consider the experimental studies where ciclosporin is demonstrated to inhibit a cyclophilin D-associated opening of the mPTP and thus protect the mitochondria from deterioration after ischaemia–reperfusion injury as a good scientific rationale to raise and test our hypothesis.

Earlier clinical trials administering ciclosporin in the same way, to a similar population, have not reported a difference in creatinine between active study drug and placebo. However, renal function was only evaluated through P-creatinine and only as a safety variable. Although it is by far the most used biomarker for AKI, we consider P-creatinine to have limitations. Consensus does not exist in the literature on how renal function could best be evaluated. In our opinion, cystatin C has a higher precision and less confounding factors than creatinine,[60 61] especially in the milder degrees of renal impairment. Other interesting biomarkers are U-TIMP-2 and U-IGFBP7[62] and also U-albumin/creatinine. We believe that the probability for

finding a true renal effect is higher with these biomarkers as compared with fluctuations in P-creatinine only.

Since existing impairment in renal function already preoperatively is a considerable risk factor for developing AKI after cardiac surgery, we only include patients with eGFR 15–90 mL/min/1.73/m^2. Thus, we believe that we study the population most prone to postoperative AKI. Since we do not know if it is the patients with the worst or the best renal function who might benefit the most from pretreatment with ciclosporin, if the hypothesis should be confirmed, we will stratify the patients into groups with milder (eGFR 60–90 mL/min/1.73/m^2) and more severe (eGFR 15–59 mL/min/1.73/m^2) impairment of renal function preoperatively. Based on the prevalence in patients with CABG, it can be difficult to enrol a sufficient number of patients in the lower eGFR range. We therefore predefined that this group should enclose ~50 patients. Importantly, we also needed to evaluate the eGFR 15–59 mL/min/1.73/m^2 group from a safety perspective, which also motivated a stratification. The primary objective, however, is to examine the whole study population with eGFR 15–90 mL/min/1.73/m^2. In summary, we consider our design to reflect a good balance between safety and efficacy where the proof-of-concept can be tested.

As presented above, the cellular mechanisms for our hypothesis concerning ciclosporin's renoprotective effect and its documented nephrotoxic effects are different. Approximately 580 patients have been exposed to ciclosporin in an identical dose as in our study.[45 56–58] The safety data collected in these four clinical studies in cardiac patients is of high quality and renal side effects are reported equally in placebo and ciclosporin-treated patients. However, CicloMulsion was used only in the CIRCUS study, the largest of the studies.[58] Other formulations were used in the other three. Taken together, the safety profile in our study is, in our view, reasonable.

Possible weaknesses derive from the fact that we cannot include all patients suitable for enrolment because of an uneven distribution in the operation programme over the weekdays. Thus, on some days when several patients are available for enrolment, there could be a bias introduced in the selection of patients. Also, the eGFR stratification might infer a bias in the sense that a larger proportion of the study population might be shifted towards higher stages of chronic kidney disease compared with the general CABG population. Moreover, if the hypothesis is true, we do not know the optimal dose for ciclosporin to exert its renoprotective effect. The choice of 2.5 mg/kg is considered to give the best efficacy combined with good safety. However, the measurement of ciclosporin concentration in blood during the day of operation will help us to evaluate this issue.

In summary, the CiPRICS study investigates the possibility of ciclosporin as a novel preventive pharmacological treatment to attenuate the AKI associated with CABG surgery. The study aims to be completed during the second half of 2016.

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Contributors The authors are exclusively responsible for the design and conduct of this study. All study analyses, the drafting and editing of the final manuscript, and its final contents are planned to include all authors. PE is the co-principal investigator and was involved in writing of study protocol, writing and review of study protocol article, conducting of study including enrolment of patients, collection of study data. EG is the investigator and was involved in writing of study protocol, writing and review of study protocol article, conducting of study including enrolment of patients, collection of study data, performing analysis for immunological exploratory parameters. AD, BB, CM, AE, SN and AM are investigators and were involved in writing of study protocol, writing and review of study protocol article, conducting of study including enrolment of patients, collection of study data. MJH, EE and LA are investigators and were involved in writing of study protocol, writing and review of study protocol article. SJ was involved in providing laboratory and performing analysis for immunological exploratory parameters, writing of study protocol, writing and review of study protocol article. HB is the sponsor and primary investigator and was involved in writing of study protocol, writing and review of study protocol article, conducting of study including enrolment of patients, collection of study data.

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Paper II

Cyclosporine before Coronary Artery Bypass Grafting Does Not Prevent Postoperative Decreases in Renal Function

A Randomized Clinical Trial

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ABSTRACT

Background: Acute kidney injury is a common complication after cardiac surgery, leading to increased morbidity and mortality. One suggested cause for acute kidney injury is extracorporeal circulation–induced ischemia–reperfusion injury. In animal studies, cyclosporine has been shown to reduce ischemia–reperfusion injury in the kidneys. We hypothesized that administering cyclosporine before extracorporeal circulation could protect the kidneys in patients undergoing cardiac surgery.

Methods: The Cyclosporine to Protect Renal Function in Cardiac Surgery (CiPRICS) study was an investigator-initiated, double-blind, randomized, placebo-controlled, single-center study. The primary objective was to assess if cyclosporine could reduce acute kidney injury in patients undergoing coronary artery bypass grafting surgery with extracorporeal circulation. In the study, 154 patients with an estimated glomerular filtration rate of 15 to 90 ml · min^{-1} · 1.73 m^{-2} were enrolled. Study patients were randomized to receive 2.5 mg/kg cyclosporine or placebo intravenously before surgery. The primary endpoint was relative plasma cystatin C changes from the preoperative day to postoperative day 3. Secondary endpoints included biomarkers of kidney, heart, and brain injury.

Results: All enrolled patients were analyzed. The cyclosporine group (136.4±35.6%) showed a more pronounced increase from baseline plasma cystatin C to day 3 compared to placebo (115.9±30.8%), difference, 20.6% (95% CI, 10.2 to 31.2%, $P < 0.001$). The same pattern was observed for the other renal markers. The cyclosporine group had more patients in Risk Injury Failure Loss End-stage (RIFLE) groups R (risk), I (injury), or F (failure; 31% *vs.* 8%, $P < 0.001$). There were no differences in safety parameter distribution between groups.

Conclusions: Administration of cyclosporine did not protect coronary artery bypass grafting patients from acute kidney injury. Instead, cyclosporine caused a decrease in renal function compared to placebo that resolved after 1 month. **(Anesthesiology 2018; 128:710-7)**

A CUTE kidney injury (AKI) after coronary artery bypass grafting (CABG) with extracorporeal circulation (ECC) occurs in approximately one third of patients in most institutions, including our own,[1] and leads to increased long- and short-term morbidity and mortality.[1] The source for AKI in CABG is multifactorial, but renal ischemia–reperfusion injury induced by the use of ECC is at least part of the cause,[2,3] especially in the poorly oxygenated and metabolic active outer medulla. A suggested mechanism is induced mitochondrial damage through the opening of the mitochondrial permeability transition pore (mPTP) during reperfusion, leading to cell injury or death.[4,5] Animal

What We Already Know about This Topic

- Acute kidney injury is common after cardiac surgery with cardiopulmonary bypass
- Animal studies suggest that cyclosporine may be protective

What This Article Tells Us That Is New

- In a double-blind trial, 154 cardiac surgical patients were randomly assigned to 2.5 mg/kg cyclosporine or placebo
- Plasma cystatin C, a marker of renal injury, increased more in patients given cyclosporine
- Cyclosporine does not reduce the risk of acute renal injury after cardiac surgery

studies demonstrate that mPTP opening may be inhibited in cyclophilin D knockout animals[6] and by the cyclophilin inhibitor cyclosporine[7] administered before the ischemic event, resulting in decreased ischemia–reperfusion injury in the kidneys,[8] heart,[9] and brain.[10]

Several clinical studies of cardiac patients have investigated cyclosporine's cytoprotective effects against ischemia–reperfusion injury in the heart.[11–16] The collection of safety data was structured, and none of these studies reported renal side effects. All these studies administered cyclosporine in the same dose, 2.5 mg/kg, as an intravenous bolus injection.

At the same time, cyclosporine is known for inducing renal insufficiency[17] when used as a continuous medication, caused by an imbalance of the vascular tone in the efferent and afferent arterioles.[18,19] This impairment is reported to be reversible after 3 months of continuous medication.[20–22] Importantly, this is a different pathway than the proposed renoprotective mechanism of cyclosporine.

In summary, a single pretreatment dose of cyclosporine has been demonstrated to have renoprotective effects against ischemia–reperfusion injury in the experimental setting. Clinical studies with cyclosporine in patients with cardiac disease, including cardiac surgery, have not shown any adverse renal effects.[11–16] Therefore, we raised the hypothesis that cyclosporine, administered as a single dose intravenously before CABG, may reduce the level of postoperative renal injury.

Materials and Methods

Trial Design
The Cyclosporine to Protect Renal Function in Cardiac Surgery (CiPRICS) trial was an investigator-initiated, clinical, double-blind, randomized, placebo-controlled, parallel-design, single-center clinical trial and was performed at Skåne University Hospital in Lund, Sweden.

The trial was performed according to the 1964 Declaration of Helsinki and its later amendments and the European Guidelines for Good Clinical Practice, and in accordance with Swedish laws and regulations. Informed consent was obtained from all individual participants included in the study. Permits were obtained from the local ethics committee (LU 2014/777) and the Swedish Medical Products Agency (Uppsala, Sweden). The trial was registered under EudraCT No. 2014-004610-29 and at ClinicalTrials.gov (NCT02397213). The rationale for and the design of the study have been published previously.[23]

Study Population
Men and women scheduled for nonemergent CABG as their sole procedure at Skåne University Hospital with a preoperative estimated glomerular filtration rate (eGFR) between 15 and 90 ml · min⁻¹ · 1.73 m⁻² were eligible for the study. Inclusion and exclusion criteria have been published in the protocol.[23]

The study dictated two predefined strata based on renal function with the aim to cover a sizeable number of patients with decreased renal function in the study. The two strata were preoperative eGFR 15 to 59 or 60 to 90 ml · min⁻¹ · 1.73 m⁻².

Anesthesia and Surgery
Anesthesia was standardized using propofol, fentanyl, and rocuronium. Inhalation anesthetic agents were prohibited.

All patients underwent CABG with ECC, with blood cardioplegia or St. Thomas crystalloid cardioplegia using a single cross-clamp technique. ECC was performed with a pump flow of 2.2 l/m² and mean arterial pressure at 50 to 70 mmHg in mild hypothermia or normothermia and a nadir hematocrit at 25%. The left internal mammary artery was used in a majority of cases, and saphenous vein graft (open harvesting technique) as the other bypass grafts.

Experimental Protocol
A block randomization was performed (block size of four) in a 1:1 ratio by prepacking the drug vials with placebo or active substance in two batches (one for each stratum). Once the patient arrived at the operating ward, the next vial in line (in the correct stratum) was taken, thereby allocating the patient to a group. The investigational drug was a lipid emulsion of cyclosporine,[24] CicloMulsion 5 mg/ml (NeuroVive Pharmaceutical AB, Sweden). As placebo, a lipid emulsion provided by the same manufacturer was used. The only difference between the placebo and active drug formulation was the content of cyclosporine. After anesthetic induction and before surgery, the study drug/placebo was administered at 0.5 ml/kg, corresponding to a dose of 2.5 mg/kg cyclosporine, in a central venous catheter as a 10-min infusion.

Efficacy data were collected preoperatively and daily until postoperative day 4. The study was terminated after a follow-up phone call after day 30.

Endpoints
The primary endpoint was relative plasma cystatin C concentration change from preoperative concentrations to day 3 after surgery. Secondary endpoints to evaluate renal function were plasma concentrations of cystatin C, creatinine, urea, and eGFR according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula[25] during the first 4 days. Incidence of AKI was assessed by the Risk Injury Failure Loss End-stage (RIFLE) criteria based on changes in plasma creatinine and without urine output criteria.[26] Blood cyclosporine concentrations were followed.[23] To evaluate the possible protective effect on the heart and brain, plasma creatinine kinase-MB, troponin T, and serum S100B[27] were followed.

Safety Measurements
According to protocol, an independent Drug Safety Monitoring Board (DSMB; Lund, Sweden) assessed the safety of

the study after 50 and 100 patients.[23] Adverse event (AE) and serious adverse event (SAE) data were collected daily from drug administration to postoperative day 4. The leg wound after the saphenous vein harvesting was assessed on day 4 using a standardized method.[28] A follow-up telephone call 30 days after surgery was made to determine if any new SAE had occurred, and to follow up ongoing AE/SAE. Events that could normally be attributed to the operation (bleeding, myocardial infarction, deep sternal wound infection, and atrial fibrillation) were not reported as AE/SAE. SAE and suspected unexpected serious adverse reaction data were reported according to the Swedish Medical Products Agency's instructions.

Statistical Analysis

The power calculation was based on a previous study in our department, in which we found cystatin C on day 3 to be 1.98 ± 0.67 mg/l.[29] To detect half a SD change (13%) in plasma cystatin C on day 3 with 80% power and an alpha of 5%, we estimated a sample size of 75 patients in each arm.

Noncompliance after enrollment depended primarily on rescheduled surgery (fig. 1). Therefore, the analysis was performed as an all-patients-treated/modified-intention-to-treat analysis.[30]

A linear mixed model with stratification according to preoperative eGFR as the covariate was used for testing of the primary endpoint and secondary endpoints. If the linear mixed model gave a significant result, individual testing was performed, and a Bonferroni–Holm correction was applied. A log-transformation was performed for skewed distributions (cystatin C, creatinine, and urea) before analysis. For

testing of single measurements, Student's t test or the Mann–Whitney U test was performed depending on the distribution of data. Data are presented as mean ± SD, number (%), or median with interquartile range. A P value less than 0.05 was considered statistically significant.

All statistical analyses were performed according to a predefined statistical analysis plan by an independent statistician (Clinical Studies Sweden, Forum South, Unit for Medical Statistics and Epidemiology, Skåne University Hospital) with SAS Enterprise Guide 6.1 (SAS Institute Inc., USA) and SPSS Statistics 22 (IBM Corp., USA).

Results

Patient Characteristics

From April 2015 through June 2016, we assessed 456 patients for eligibility and enrolled 154 patients, with 75 patients assigned to the cyclosporine group and 79 to the placebo group (fig. 1). One patient met a predefined withdrawal criterion (decision to perform operation other than CABG during surgery) and was excluded from the modified-intention-to-treat group. One patient in the placebo group had a missing value for cystatin C on day 3 and was excluded from the analysis of the primary variable but was used in all other analyses.

Baseline characteristics were well balanced between the groups. The preoperative eGFR were similar, both in the entire group and in the two strata (table 1).

Primary Outcome

The cyclosporine group ($136.4 \pm 35.6\%$) had a more pronounced increase from baseline plasma cystatin C to day 3 compared to
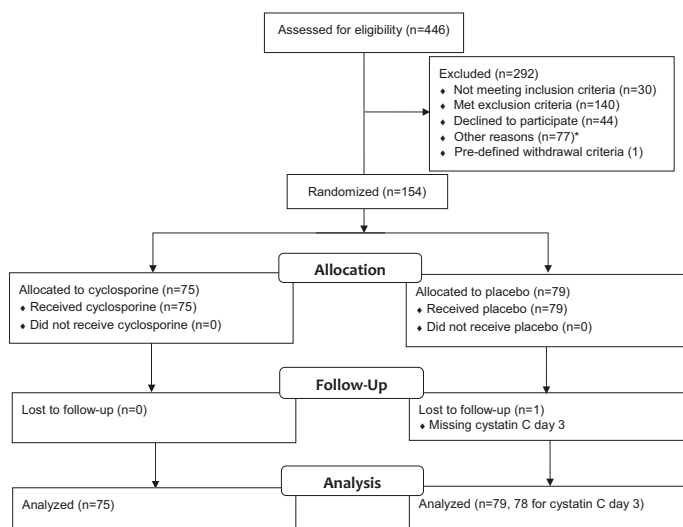

Fig. 1. Consolidated Standards of Reporting Trials flow chart for the Cyclosporine to Protect Renal Function in Cardiac Surgery study. *Mostly due to rescheduled surgery.

Table 1. Baseline Characteristics

Baseline Characteristics	Placebo (N = 79)	Cyclosporine (N = 75)
Male sex, No. (%)	68 (86.1)	62 (82.7)
Age (yr)	69 ± 8	69 ± 8
Height (cm)	175 ± 8	174 ± 9
Weight (kg)	86 ± 14	82 ± 13
Systolic blood pressure (mmHg)	137 ± 18	134 ± 17
Diastolic blood pressure (mmHg)	74 ± 8	72 ± 9
Medical history, No. (%)		
Hypertension	62 (78.5)	54 (72.0)
Congestive heart failure	15 (19.0)	10 (13.3)
LVEF < 30%	3 (3.8)	2 (2.7)
LVEF 30–50%	14 (17.7)	9 (12.0)
LVEF > 50%	59 (74.7)	62 (82.7)
COPD	0 (0)	4 (5.3)
Diabetes	26 (32.9)	14 (18.7)
Peripheral vascular disease	5 (6.3)	4 (5.3)
Previous CVI	5 (6.3)	6 (8.0)
Thyroid disease	8 (10.1)	3 (4.0)
Chronic AF	4 (5.1)	1 (1.3)
Paroxysmal AF	6 (7.6)	5 (6.7)
Medication use, No. (%)		
Diuretics	23 (29.1)	10 (13.3)
ACE/ARB	59 (76.0)	60 (78.7)
β-Blocker	64 (82.7)	62 (81.1)
Statins	76 (96.2)	69 (92.0)
Warfarin	2 (2.5)	1 (1.3)
ASA	73 (92.4)	68 (90.7)
Clopidrogel/prasurgel	3 (3.8)	5 (6.7)
Antithrombotic treatment	20 (25.3)	16 (21.3)
Antibiotics	4 (5.1)	1 (1.3)
Preop eGFR CKD-EPI (ml · min^{-1} · 1.73 m^{-2})		
All patients	65.1 ± 18.9	69.0 ± 20.0
Subgroup eGFR 15–59 (ml · min^{-1} · 1.73 m^{-2})	51.1 ± 11.2	54.4 ± 11.9
Subgroup eGFR 60–90 (ml · min^{-1} · 1.73 m^{-2})	79.9 ± 10.0	81.5 ± 10.1

Values are presented as mean ± SD or No (%). ACE = angiotensin conversion enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin receptor blocker; ASA = acetylsalicylic acid; CKD-EPI = Chronic Kidney Disease-Epidemiology Collaborative Group; COPD = chronic obstructive pulmonary disease; CVI = cerebrovascular incident; eGFR = estimated glomerular filtration rate; LVEF = left ventricular ejection fraction.

the placebo group (115.9 ± 30.8%). The difference between groups was 20.6% (95% CI, 10.2 to 31.2%, $P < 0.001$; fig. 2).

Secondary Outcome

The secondary renal outcomes, relative difference in plasma creatinine, and absolute values for plasma cystatin C and plasma creatinine were also significantly higher in the cyclosporine group (table 2; fig. 3). The classification according to RIFLE on postoperative day 3 also differed, as 7 patients (9%) in the placebo group were classified in RIFLE group R (risk), I (injury), or F (failure) compared to 23 (31%, $P < 0.001$) in the cyclosporine group (table 2), and 3 patients (4%) in the placebo group were classified as RIFLE R compared to 15 (20%, $P < 0.001$) in the cyclosporine group.

Because of the results in the primary variable, a *post hoc* investigation, not included in the study protocol, of plasma creatinine 1 to 3 months and 3 to 6 months after the end of study was performed by retrieving plasma creatinine from the patients' electronic medical records. We were able to obtain measurements from 86% of all study patients, revealing that plasma creatinine was normalized in both groups at both time intervals (fig. 3). There were no differences between the groups for troponin T, creatinine kinase-MB, or S100B (tables S1 and S2, Supplemental Digital Content, http://links.lww.com/ALN/B623, listing the result of cardiac injury markers and S100B, respectively, in this study).

Predefined Subgroups

In the subgroup with eGFR 15 to 59 ml · min^{-1} · 1.73 m^{-2}, the relative increase in plasma cystatin from preoperative to day 3 was 1.39 ± 0.35 (mean ± SD) for cyclosporine *versus* 1.11 ± 0.24 for placebo ($P < 0.001$). The corresponding figures for the subgroup with an eGFR 60 to 90 were 1.34 ± 0.36 (mean ± SD) for the cyclosporine group *versus* 1.18 ± 0.34 for the placebo group ($P = 0.011$). The stratification variable was included in the primary analyses, and there was no difference between groups ($P = 0.858$). The same pattern was observed for the dynamics of eGFR during the study days (fig. 4, depicting the dynamic of eGFR in the two strata in this study).

Pharmacokinetics

Mean blood cyclosporine concentrations were 4423 ± 887 ng/ml 5 min after end of infusion, 775 ± 180 ng/ml at the end of ECC, and 106 ± 32 ng/ml the next morning. No clear relationships were observed between the cyclosporine exposure and change from baseline in plasma cystatin C or creatinine.

Safety

A total number of 31 AE were reported, with 16 in the cyclosporine group and 15 in the placebo group. A total number of 26 SAE were reported, with 12 in the cyclosporine group and 14 in the placebo group (table S3, Supplemental Digital Content, http://links.lww.com/ALN/B623, listing the SAE in this study). All AE/SAE were resolved. Two patients, both in the placebo group, suffered a stroke. One of these patients died during the study period, which was the only death in the study. Safety biochemistry in this study is presented in table S3, Supplemental Digital Content (http://links.lww.com/ALN/B623). The cyclosporine group had both higher C-reactive protein concentrations on postoperative day 2 to 4 and higher leukocyte count on all 4 postoperative days compared with placebo. Also, plasma potassium was higher on day 2, thrombocytes were lower on days 3 and 4 and hemoglobin was lower on day 3 in the cyclosporine group as listed in table S4, Supplemental Digital Content (http://links.lww.com/ALN/B623). Two DSMB meetings after 50 and 100 patients recommended continuation of the study according to study protocol.
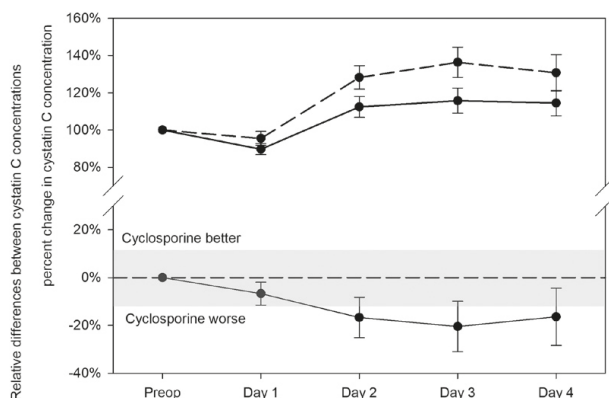
Fig. 2. Cystatin C changes expressed as percentage of baseline for the cyclosporine group (*dashed line*) and the placebo group (*solid line*) with 95% CI. Relative differences between cystatin C concentrations for the groups with 95% CI. *Gray area* reflects a ±13% change (number used in power calculations) in the primary endpoint. Preop = preoperative.

Clinical Outcome

There were few differences in clinical outcome between the groups. The cyclosporine group had more a positive fluid balance and received more diuretics on postoperative days 2 to 4 (table 3; table S5, Supplemental Digital Content, http://links.lww.com/ALN/B623, listing the use of diuretics in this study). The cyclosporine group had a shorter time until extubation, but time in the intensive care unit did not differ. No patient in either group was treated with continues renal replacement therapy. There were no differences in leg scoring on day 4 (table S5, Supplemental Digital Content, http://links.lww.com/ALN/B623, listing the leg scoring in this study).

Discussion

In this study, administration of 2.5 mg/kg cyclosporine as an intravenous bolus before CABG surgery with ECC resulted in decreased renal function postoperatively according to all measured renal parameters, compared with placebo. No protective effects were found.

The *post hoc* review found plasma creatinine for 86% of patients, while there were very few plasma cystatin C values. We found that plasma creatinine had normalized in both groups, and there was no difference between the groups, as depicted in figure 2. This supports previous findings that renal impairment induced by cyclosporine can be reversible.[20–22]

One explanation for our findings might be that the AKI induced by CABG surgery with ECC is not linked to ischemia–reperfusion injury with mPTP-mediated dysfunction. However, Lannemyr *et al.*[3] recently demonstrated reduced renal oxygenation both during and after ECC, in combination with signs of tubular injury, implying hypoxia is an important factor for AKI. In contrast to the clinical situation, animal models evaluating cytoprotective compounds typically use models in which the renal injury results in massive necrotic cell death,[31] perhaps not representative of a milder hypoxia during ECC.

Anesthesia may influence the results. Propofol[32] and anesthetic gases[33] may have renoprotective effects. We used a standardized protocol in which propofol was allowed, while use of anesthetic gas was prohibited. No protocol violations were reported.

In addition to the renal findings, we were unable to demonstrate any cytoprotective effects on the heart. This contrasts with the results of Chiari *et al.*[11] and Hausenloy *et al.*,[14] who both found improved myocardial protection, with the caveat that Hausenloy *et al.* observed this effect only in patients with longer cross-clamp times. The different results may be explained by the fact that our study had shorter perfusion times, did not use intermittent cross-clamp fibrillation, and had lower biomarkers for myocardial injury. In addition, Chiari *et al.*[11] studied patients with aortic valve stenosis who had left ventricle hypertrophy, which differs from our study. Our results also suggest that the two previous cardiac surgery studies were not statistically powered to detect renal side effects. In our study, the DSMB recommended continuing the study after 50 and 100 patients, but the final analysis revealed a clear negative renal effect, emphasizing the strength of prospective testing with adequate statistical power.

We also found a higher inflammatory response, measured with C-reactive protein and leukocyte count, in the cyclosporine group. An increase in leukocyte count was also found by Mazzeo *et al.*[34] in a traumatic brain injury study. Despite this, the AE/SAE did not imply an increased infection rate in the cyclosporine group.

The study was designed using two strata and prespecified subgroups according to preoperative eGFR in order to evaluate whether the potential protective effect or safety profile differed at lower or higher GFRs.[23] No clear differences in change in the fraction of plasma cystatin C or plasma

Table 2. Clinical Chemistry Renal Function

Clinical Chemistry Renal Function	Placebo (N = 79)	Cyclosporine (N = 75)	P Value
Plasma cystatin C (mg/l)			0.001*
Day −1	1.18 (0.31)	1.13 (0.30)	
Day 1	1.06 (0.38)	1.08 (0.36)	0.007†
Day 2	1.33 (0.48)‡	1.48 (0.64)	< 0.001†
Day 3	1.37 (0.51)‡	1.57 (0.69)	< 0.001†
Day 4	1.32 (0.45)‡	1.51 (0.79)	0.001†
Plasma creatinine (μmol/l)			< 0.001*
Day −1	91.9 (19.1)	89.3 (19.4)	
Day 1	88.6 (23.2)	91.8 (23.6)	0.009†
Day 2	107.9 (40.9)	122.0 (48.1)	0.001†
Day 3	106.2 (49.3)	123.9 (55.9)	< 0.001†
Day 4	102.1 (48.1)	112.5 (56.7)	0.019
1–3 months	93.4 (33.0)	89.9 (22.0)	0.498§
3–6 months	94.5 (29.0)	91.7 (24.1)	0.643§
Plasma urea (mmol/l)			0.006*
Day −1	6.9 (2.3)	6.4 (2.2)	
Day 1	5.3 (1.9)	5.4 (2.1)	0.026†
Day 2	6.4 (2.8)	7.1 (2.6)	< 0.001†
Day 3	7.1 (3.2)	8.6 (3.9)	< 0.001†
Day 4	7.4 (3.5)	8.8 (4.8)	0.002†
eGFR P-CystatinC/P-Creatinine (ml · min⁻¹ · 1.73 m⁻²)			0.001*
Day −1	69.0 (17.5)	71.4 (17.0)	
Day 1	77.0 (22.3)	74.0 (21.0)	0.003†
Day 2	61.9 (21.4)	55.1 (21.1)	< 0.001†
Day 3	62.0 (21.8)	53.7 (22.0)	< 0.001†
Day 4	63.7 (21.5)	59.0 (22.6)	0.002†
RIFLE-creatinine classification, postoperative day 3 (%)			
No damage	72 (91.1)	52 (69.3)	0.001†‖
R	3 (3.8)	15 (20.0)	0.001†‖
I	2 (2.5)	5 (6.7)	0.192‖
F	2 (2.5)	3 (4.0)	0.522‖

Outcome in terms of clinical chemistry, renal function. eGFR (estimated glomerular filtration rate) calculated based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula for creatinine and cystatin C. Values are presented as mean (SD) or No. (%). *Linear mixed model. †Statistical significance (*P* < 0.05) after Bonferroni–Holm correction. ‡N = 78. §Data retrieved *post hoc* and not included in linear mixed model, but tested with *t* test. ‖Mann–Whitney test. Day −1 = preoperative measurement, usually the admission day; Day 1 = the first day after surgery, and so forth; F = failure; I = injury; R = risk; RIFLE = Risk Injury Failure Loss End-stage.

Fig. 3. Mean values with 95% CI for plasma creatinine in the cyclosporine (*dashed line*) and placebo (*solid line*) groups. The *broken axis* denotes that a *post hoc* analysis was performed in the period 1 to 6 months after operation. Preop = preoperative sampling, usually the day of admission. Days 1 to 4 = days after surgery.

Fig. 4. The dynamics of eGFR (estimated glomerular filtration rate; based on cystatin C and creatinine according to Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI]) during the postoperative period for the two subgroups (eGFR 15 to 59 and 60 to 90 ml · min⁻¹ · 1.73 m⁻²) expressed as mean ± SD. The stratification variable is included in primary the analyses and there are no difference between the stratification groups (*p* = 0.858). *Dashed line* denotes cyclosporine and *solid line,* placebo. Preop = preoperative.

creatinine from baseline between the two eGFR groups were observed. We chose to include patients with an eGFR as low as 15 ml · min⁻¹ · 1.73 m⁻². On the other hand, we excluded patients with normal renal function (eGFR greater than 90 ml · min⁻¹ · 1.73 m⁻²), which is reflected in a higher baseline mean plasma creatinine compared to the other studies. In conclusion, we could not discern any difference in results for cyclosporine relating to preoperative renal function.

A limitation of the current study is the single-center design. However, the consistency of the results under the well-controlled study settings suggests that the main findings would likely be generalizable. Other doses, repeated doses, or different timings may have yielded other results. However, the tested single administration of 2.5 mg/kg is the same as in
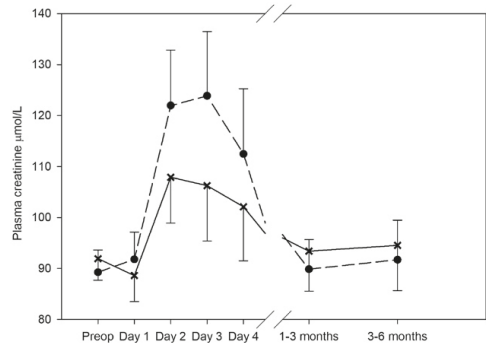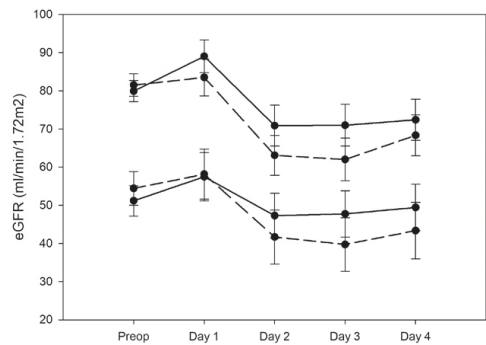
previous studies reporting positive outcomes. Furthermore, we did not observe any exposure-dependent effects in a *post hoc* pharmacokinetic–pharmacodynamic analysis. In contrast, the negative effects on renal function were consistent among all the measured endpoints, which emphasizes the strengths of a sufficiently powered, prospective, randomized, double-blinded trial in a well-defined study population.

In conclusion, despite promising animal data, pretreatment of patients with cyclosporine intravenously before CABG with ECC resulted in decreased renal function in the immediate postoperative period compared with placebo. Further studies on cyclosporine should take these findings into account when assessing the safety of the drug.

Table 3. General Outcome

General Outcome	Placebo (N = 79)	Ciclosporin (N = 75)	P Value
Surgical procedure			
Perfusion time, ECC (min)	74 (27)	77 (30)	0.423*
Aortic cross-clamp duration (min)	46 (16)	47 (20)	0.849*
Number of distal coronary grafts (n)	3.4 (0.9)	3.2 (0.9)	0.298*
Diuresis and fluid balance			
Diuresis before ECC (ml)	182 (198)	163 (142)	0.930*
Diuresis during ECC (ml)	168 (121)	165 (106)	0.723*
Diuresis 12 h (ml)	1723 (579)	1575 (581)	0.067*
Fluid balance during surgery	1933 (737)	2071 (709)	0.240†
Fluid balance until first morning after surgery (ml)	2540 (1157)	291 (1171)	0.050†
Postoperative outcome			
Time to extubation (min)	467 (794)	426 (214)	0.015*
ICU time (h)	29 (25)	32 (32)	0.261*
Bleeding 12 h (ml)	477 (244)	536 (310)	0.392*
Complications, No. (%)			
Reoperation for bleeding	3 (4.0)	2 (2.5)	0.675‡
Postoperative DSWI	1 (1.3)	0 (0.0)	1.000‡
Postoperative myocardial damage	0 (0)	2 (2.7)	0.235‡
Postoperative stroke	2 (2.5)	0 (0)	0.497‡
Postoperative heart failure	3 (3.8)	4 (5.3)	0.714‡
Postoperative atrial fibrillation	29 (36.7)	32 (42.7)	0.511‡
Leg scoring	4.9 (2.8)	5.6 (2.4)	0.057*
30-day mortality, No. (%)	1 (1.3)	0 (0)	1.000‡

Testing was done with *Mann–Whitney test, †t test, or ‡Fisher exact test. DSWI = deep sternal wound infection; ECC = extracorporeal circulation; ICU = intensive care unit.

Research Support

Competing Interests

Reproducible Science

Full protocol available at: henrik.bjursten@med.lu.se. Raw data available at: henrik.bjursten@med.lu.se.

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Paper III

Original Article

Effect of Cyclosporine on Cytokine Production in Elective Coronary Artery Bypass Grafting: A Sub-Analysis of the CiPRICS (Cyclosporine to Protect Renal Function in Cardiac Surgery) Study

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Objectives: The augmented inflammatory response to cardiac surgery is a recognized cause of postoperative acute kidney injury. The present study aimed to investigate the effects of preoperative cyclosporine treatment on cytokine production and delineate factors associated with postoperative kidney impairment.
Design: A randomized, double-blind, placebo-controlled, single-center study.
Setting: At a tertiary care, university hospital.
Participants: Patients eligible for elective coronary artery bypass grafting surgery; 67 patients were enrolled.
Interventions: Patients were randomized to receive 2.5 mg/kg cyclosporine or placebo before surgery. Cytokine levels were measured after the induction of anesthesia and 4 hours after the end of cardiopulmonary bypass.
Measurements and Main Results: Tissue-aggressive (interleukin [IL]-1β, macrophage inflammatory protein [MIP]-1β, granulocyte colony-stimulating factor [G-CSF], IL-6, IL-8, IL-17, MCP-1), as well tissue-lenient (IL-4) cytokines, were significantly elevated in response to surgery. Changes in cytokine levels were not affected by cyclosporine pretreatment.

Conclusions: Elective coronary artery bypass grafting surgery with cardiopulmonary bypass triggers cytokine activation. This activation was not impacted by preoperative cyclosporine treatment.

ACUTE KIDNEY INJURY (AKI) after cardiac surgery is a recognized cause of perioperative morbidity and mortality.[1] It affects one-third of the patients, and AKIs long-term consequences include reduced lifespan,[2] chronic renal failure, and dialysis.[3] In cardiac surgery with extracorporeal circulation (ECC), the perioperative causes of kidney injury are multifactorial, including ischemia-reperfusion, augmented inflammatory response, renal vasoconstriction, and hemolysis.[4,5]

One suggested mechanism behind cell injury is through the ischemia-reperfusion−induced opening of the mitochondrial permeability transition pore (mPTP), leading to mitochondrial damage and apoptosis.[6] A key component in the opening of mPTP is cyclophilin-D. It was discovered that the opening of mPTP could be inhibited pharmacologically by the calcineurin inhibitor cyclosporine[7] through its binding to cyclophilin-D. In animal studies, kidney injury could be reduced by the administration of cyclosporine before the ischemia-reperfusion injury,[8,9] thus promoting the idea of also preventing AKI with cyclosporine administered before surgery in humans.

The administration of cyclosporine to prevent renal failure in cardiac surgery is controversial; long-term cyclosporine treatment induces renal insufficiency through vasoconstriction, vascular inflammation, and endothelial activation. At the same time, cyclosporine's protective effect against ischemia-reperfusion−induced myocardial injury has been tested in several human studies without any reported renal side effects.[10]

The current report was based on the previously published Cyclosporine to Protect Renal function In Cardiac Surgery (CiPRICS) study conducted at the authors' institution, in which cyclosporin's ability to protect the kidneys from ischemia-reperfusion injury was tested in a randomized, double-blinded, placebo-controlled trial.[11] The preoperative administration of cyclosporine in coronary artery bypass graft (CABG) surgery was not protective to the kidneys, but instead associated with a decrease in postoperative renal function. These findings were in contrast to the authors' hypothesis of a treatment benefit for patients in the cyclosporine group. However, after the observed decline in kidney function from cyclosporine treatment, the kidney function normalized in all cases. The current substudy investigated cyclosporine's effects on the perioperative inflammatory response in the same patient population.

The role of the inflammatory response in AKIs development after cardiac surgery with ECC is well- established, and increased cytokine production, particularly interleukin-6 (IL-6) and IL-10, has been linked to postoperative AKI.[12] The mechanism behind cyclosporine's cytoprotective effect has been ascribed solely to the inhibition of the mPTP. Cyclosporine's effect on early inflammatory response in cardiac surgery has not been studied earlier. The current study was a predefined analysis of cyclosporine's effect on cytokine production and its possible relation to postoperative kidney dysfunction in a subpopulation of CiPRICS patients.[13]

In this study, the authors hypothesized that the preoperative administration of cyclosporine alters the early inflammatory response in cyclosporine-treated patients compared to placebo and that changes in cytokine production correlate with postoperative kidney function.

Material and Methods

Trial Design

This was a substudy of 67 randomly selected patients from the original CiPRICS trial.[11,13] A local ethics committee approval was received, and written informed patient consent was obtained. The trial was registered under EudraCT number 2014-004610-29 and at ClinicalTrials.gov (NCT02397213).

Study Population

Men and women scheduled for elective CABG procedures with preoperative kidney function, estimated by the estimated glomerular filtration rate between 15 and 90 mL/min/1.73/m^2 were included. The study inclusion and exclusion criteria previously were published in the original CiPRICS study protocol.[13] The cohort analyzed in this study consisted of 36 patients treated with a placebo and 31 patients given a single dose of cyclosporine.

Study Endpoints

The endpoint was the plasma cytokine concentration change from preoperative to 4 hours after the end of ECC. In addition, an ad hoc analysis was performed for factors associated with a 30% increase in cystatin C on postoperative day 3.

Anesthesia and Surgery

General anesthesia was used in all patients according to the standard clinical routine, using the anesthetics propofol, fentanyl, and rocuronium. Inhalation anesthetics were prohibited.

All cases were elective, using ECC and normothermia. During ECC, pump flow was maintained at 2.2 L/m^2, mean arterial pressure was kept between 50 and 70 mmHg, and target hematocrit was greater than 25%. All patients received 2-to-4 g of tranexamic acid, either as a single dose or as 2 doses before and after ECC.

Experimental Protocol

Randomization was performed in a 1:1 ratio. After the induction of anesthesia, patients allocated to the treatment group received 1 dose of 2.5 mg/kg cyclosporin A—CicloMulsion 5 mg/mL (Abliva AB formerly NeuroVive Pharmaceutical AB, Lund, Sweden). Patients allocated to the placebo group received a lipid emulsion provided by the same manufacturer. The content of cyclosporine was the only difference between the active drug and the placebo.

Blood samples for cytokine analysis were drawn from the arterial line before the induction of anesthesia and 4 hours after the end of cardiopulmonary bypass. Kidney function was assessed by measuring daily plasma creatinine and cystatin C levels until postoperative day 4.

Definition of Renal Dysfunction

In the main CiPRICS study, the authors used P-cystatin C concentrations to monitor postoperative renal function, as it has been shown to be a more accurate surrogate marker for the glomerular filtration rate in cardiac surgery patients than P-creatinine.[14,15] They also defined AKI according to the Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease (RIFLE) classification. The primary endpoint was a 30% increase in P-cystatin C levels on day 3. The authors used the same primary endpoint in the current study. For comparison with other studies, they also presented plasma creatinine (μmol/L) levels and AKI classification only according to the Kidney Disease: Improving Global Outcomes (KDIGO) classification. Because urinary output is controlled with the administration of diuretics at the authors' institution, the parameter urinary output was not included in the KDIGO calculation.

Cytokine Analysis

Aliquots of clarified plasma from patient samples were stored at −80°C; until cytokine analysis could be performed, thawing and refreezing were not allowed. Plasma samples were assayed according to the manufacturer's protocol for the Bio-Plex Pro Human Cytokine 17-plex Assay kit, which quantifies the following multiplexed cytokines: granulocyte-macrophage colony-stimulating factor (GM-CSF), G-CSF, interferon-gamma (IFN-γ), IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein-1 beta (MIP-1β), tumor necrosis factor-alpha (TNF-α). The kit profiles cytokine expression using the Luminex xMAP fluorescent bead-based technology in a capture/detection sandwich immunoassay format. Plasma from each patient sample was diluted 1:4 in sample diluent, and 50 μL of diluted plasma were loaded into each well of the 96-well plate provided and incubated with antibody-coupled capture beads. Typically, 5 patient samples were loaded in triplicate wells, and the remaining 28 samples were loaded as duplicates. Cytokine concentrations were calculated using an 8-point standard curve generated by a 4-fold series dilution of the reconstituted reference cytokine standards supplied in the kit. Fifty μL of each dilution for the standard curve were added to each well in duplicate, and blanks were assayed in triplicate. A biotinylated detection antibody then was added and incubated with the beads, followed by the addition of a streptavidin-phycoerythrin reporter dye used for detection. Additionally, normalization between plates was done using standards purchased from the National Institute for Biological Standards and Control for each of 16 cytokines plus MIP-1β, which was purchased from Prospec Bio (East Brunswick, NJ). Each of these was reconstituted in a standard diluent and added to a single mixture containing all 17 multiplexed cytokines, for final concentrations of 200 pg/mL and 20 pg/mL and aliquoted to be used without a freeze/thaw cycle. Fifty μL of each of these mixtures for both concentrations were added to each well of the 96-well plate in triplicate. Samples and controls were measured immediately following the addition of an assay buffer and read on the Bio-Plex 200 system at a low RP1 target setting, using Bio-Plex Manager software (v.6.1).

Statistical Analysis

Normally distributed continuous data were compared between groups in an unpaired Student's *t*-test, while data with nonnormal distribution were analyzed with Wilcoxon's test. The categorical data were compared between groups with the Fisher exact test. Paired comparisons were performed before and after ECC, and unpaired proportions were compared with the Fisher exact test. Normally distributed data were described as mean ± standard deviation, and nonnormally distributed data as median with interquartile range. The model predicting renal function based on cystatin C was created by the step-by-step exclusion of predictor variables in a linear multivariate regression model, which was initiated with a univariate analysis to harvest unknown predictors. The criteria for the selection of variables in the univariate analysis was set at $p < 0.2$. All p values < 0.05 were considered as significant.

Results

Patient Characteristics

There were no significant differences in the patient baseline characteristics between the study cohorts (Table 1).

CABG-Induced Cytokine Activation is Not Affected by Cyclosporine Pretreatment

Postoperative tissue-aggressive (IL-1β, MIP-1β, G-CSF, IL-6, IL-8, IL-17, MCP-1) as well tissue-lenient (IL-4) cytokines, were significantly elevated. Changes in cytokine levels were not affected by cyclosporine pretreatment (Fig. 1 and 2).

Table 1
Baseline Demographics and Characteristics of the Patients

Baseline Characteristics	Placebo (N = 36)	Cyclosporin (N = 31)	p Value
Male sex, n (%)	31 (86.1)	28 (90.3)	0.716
Age, y	67.9 ± 6.7	70.9 ± 8	0.112
Height, cm	175.8 ± 9.	174.74 ± 7.9	0.579
Weight, kg	86 ± 13.5	81.23 ± 10.9	0.062
Systolic blood pressure, mmHg	135 ± 19.2	135 ± 14.4	0.887
Diastolic blood pressure, mmHg	75.2 ± 8.4	72 ± 7.7	0.332
Medical history, n (%)			
Hypertension	28 (77.7)	24 (77.4)	0.972
Congestive heart failure	8 (22.2)	7 (22.6)	0.972
LVEF <30%	1 (2.7)	1 (3.2)	0.914
LVEF 30%-50%	8 (22.2)	7 (22.5)	0.972
LVEF >50%	26 (72.2)	23 (74.1)	0.856
COPD	0	1 (3.23)	0.463
Diabetes	10 (27.7)	3 (9.6)	0.072
Peripheral vascular disease	2 (5.5)	1 (3.2)	0.645
Previous CVI	3 (8.3)	2 (6.4)	0.770
Thyroid disease	2 (5.5)	0	0.495
Chronic AF	1 (2.7)	0	0.349
Paroxysmal AF	4 (11.1)	4 (12.9)	0.821
Medication use, n (%)			
Diuretics	9 (25)	4 (12.9)	0.236
ACE/ARB	26 (72.2)	26 (83.8)	0.397
Beta-blocker	27 (75)	24 (77.4)	0.817
Statins	33 (91.6)	29 (93.5)	0.770
Warfarin	1 (2.7)	1 (3.2)	0.914
ASA	33 (91.6)	27 (87.1)	0.696
Clopidrogel/prasugrel	2 (5.5)	1 (3.2)	0.645
Antithrombotic treatment	9 (25)	8 (25.8)	0.939
Antibiotics	1 (2.7)	1 (3.2)	0.914
Pre-op eGFR CKD-EPI, mL/min/ 1.73m^2			
All patients	64.3 ± 17.1	65.96 ± 21.2	0.73
Perfusion time, ECC, min	70.14 ± 22.73	73.81 ± 26.87	0.477
Aortic cross-clamp duration, min	44.81 ± 14.95	45.48 ± 16.58	0.861

Abbreviations: ASA, acetylsalicylic acid; ACE, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin-receptor blockers; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; COPD, chronic obstructive pulmonary disease; CVI, cerebrovascular incident; ECC, extracorporeal circulation; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; SD, standard deviation.
* n (%); plus-minus values are means ± SD for all other variables.

Independent Predictors of Postoperative Decrease in Kidney Function

In a linear regression model with 30% increase in cystatin C as the outcome, age (estimate ±, p = 0.004) and total perioperative norepinephrine dose (estimate ±, p < 0.001; Table 2) proved to be independent predictors.

Postoperative IL-6 Levels and Kidney Function

There was no correlation between investigated cytokines and postoperative increase in cystatin C at day 3 (r = 0.29 [0.03, 0.56]; p = 0.09) and day 4 (r = 0.3 [−0.04 to 0.57]; p = 0.08) (Fig 3).
A significant correlation (p < 0.05) between IL-6 and cystatin C at days 3 and 4 in the placebo group was found; however, this correlation depended solely on 1 outlier, and when removed the correlation became insignificant (r = 0.29 [0.03,

0.56]; p < 0.09) and day 4 (r = 0.3 ([−0.04, 0.57]; p < 0.08) (Supplementary Figure 6, A and B).

Perioperative Dose of Norepinephrine and Kidney Function on Day 3

The total perioperative dose of norepinephrine used correlated with a postoperative rise in cystatin C levels (Fig 4) at day 3 in the placebo group (r = 0.45 [0.14, 0.68]; p < 0.01), as well as in the cyclosporine group (r = 0.75 [0.51, 0.86]; p < 0.01).

Perioperative Use of Norepinephrine Does Not Correlate With Postoperative IL-6 Levels

A postoperative rise in IL-6 was not associated with the concurrent increase in perioperative use of norepinephrine (Fig 5), neither in the cyclosporine (r = 0.16 [0.24, 0.52];
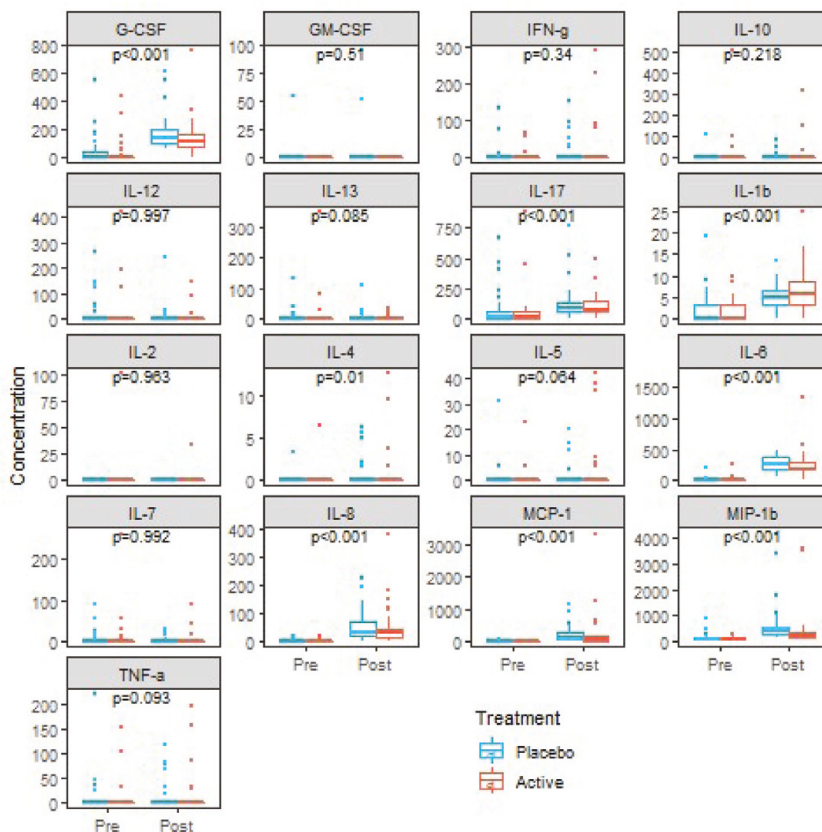
Fig 1. A comparison of plasma cytokine levels before and after CABG surgery in cyclosporine- and placebo-treated patients. Shown are plasma cytokine levels preoperatively and postoperatively after CABG surgery in the placebo- and cyclosporine-treated patients. Although there were significant differences between preoperative and postoperative levels of G-CSF, IL-1β, IL-4, IL-6, IL-8, IL-17, and MCP-1, there were no statistically significant differences in cytokine levels in cyclosporine-treated patients versus placebo. The presented p values (Wilcoxon's test) represent differences preoperatively and postoperatively when placebo- and cyclosporine- treated patients were pooled together. CABG, coronary artery bypass grafting; G-CSF, granulocyte colony-stimulating factor; IL, interleukin; MCP-1, monocyte chemoattractant protein-1.

$p = 0.28$) nor the placebo group ($r = 0.2$ [0.16, 0.51]; $p = 0.28$).

Comparison of Different Kidney Outcome Parameters

In Table 3, concentrations of P-cystatin C and P-creatinine from day 0 to day 4, together with ratios between preoperative and postoperative concentrations expressed in percentage, are presented. Table 4 presents the maximal stage of AKI days 1 to 4 according to the KDIGO classification. Table 5 (supplementary material) presents the daily stage of AKI day 1 to 4 according to KDIGO.

No patient was treated with renal replacement therapy.

Discussion

Here the authors present their findings of cyclosporine's effect on early inflammatory response and kidney function in an elective patient population exposed to CABG surgery with ECC. Firstly, preoperative administration of cyclosporine did not affect cytokine production in response to surgery and ECC (Fig 1). Secondly, changes in perioperative cytokine concentrations did not correlate with postoperative kidney impairment, neither in cyclosporine-treated patients nor in the placebo group.

Ischemia-reperfusion and the augmented inflammatory response have been identified as factors in the development of AKI in patients undergoing cardiac surgery. As a consequence,
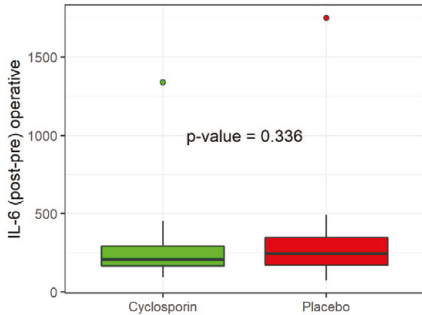
Fig 2. A comparison of the postoperative rise in IL-6 levels. Postoperative increase in IL-6 concentrations in the cyclosporine and placebo-treated patients after CABG surgery, p > 0.336 (Wilcoxon's test). CABG, coronary artery bypass grafting; IL, interleukin.

Table 2
Linear Regression Analysis, Outcome 30% Increase of P-Cystatin C Postoperative Day 3 From Preoperatively

Variable	Estimate	Standard Error	t Value	p Value
Intercept	−0.439	0.553	−0.794	0.43
Age, y	0.024	0.008	2.95	0.004
Norepinephrine, day 0	0.072	0.012	5.852	<0.001

NOTE. Age and total perioperative dose of norepinephrine (mg) on the day of CABG surgery with ECC as significant variables (p < 0.05) associated with a postoperative increase of P-cystatin C.

intriguing opportunities for new treatments targeting details of ischemia-reperfusion and the inflammatory response open up. Cyclosporine initially emerged as an interesting candidate in 2 ways. Firstly, from studies in mice, cyclosporine has been shown to prevent ischemia-reperfusion injury in the kidneys when administered before an ischemic event,[8] an effect attributed to cyclosporine's inhibitory effect on mitochondrial permeability by preventing the mPTP from opening.[6] However, the CiPRICS study showed increased renal injury in CABG-operated patients treated with cyclosporine before surgery.[11] Secondly, cyclosporine is also an immunosuppressive substance, a recognized T-cell inhibitor, and potentially could decrease the inflammatory response to surgery and ECC.[16] This is of particular interest in organ transplantation surgery, in which the exact timing of the introduction of calcineurin inhibitors is under discussion.[17]

The cytokine profile changes after ECC in this study corresponded with previously reported.[18,19] Four hours after the end of ECC, there was a significant increase in proinflammatory cytokines: IL-6, MIP-1β, MCP-1, IL-1β, IL-8, G-CSF, GM-CSF, and antiinflammatory cytokine IL-4. However, postoperative cytokine levels were less prominent in the authors' study than previously reported,[18] which supported the notion that uncomplicated elective cardiac surgery with ECC might render a less inflammatory response than under more complicated procedures because previous studies have included different types of cardiac surgery.[18,19] Another plausible explanation might be the routine use of tranexamic acid before and after ECC in CABG patients at the authors' institution, which has
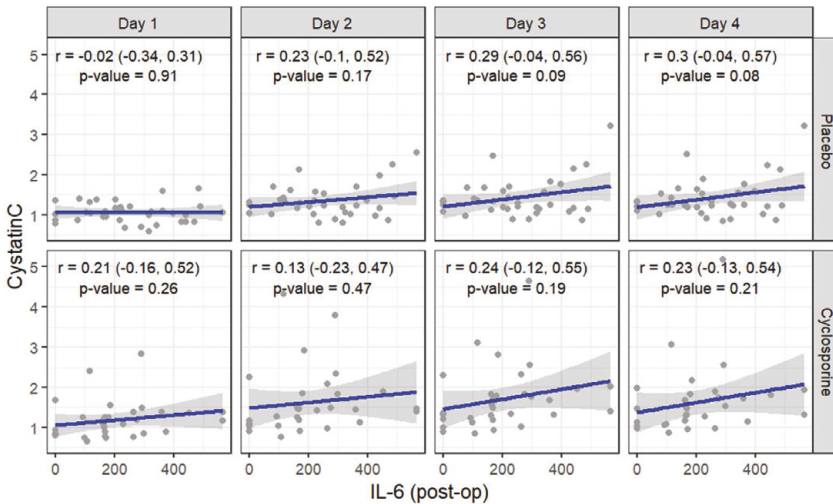
Fig 3. Cystatin C levels as function of postoperative IL-6 levels. Outliers in the placebo and cyclosporine groups are removed from the statistical analysis in this figure. For results including outliers, see Supplementary Figure 6. Postoperative IL-6 levels did not correlate with rise in P-cystatin C from day 1 to day 4. The correlation between postoperative IL-6 levels and cystatin C at day 3 (r = 0.29 [0.03, 0.56]; p = 0.09) and day 4 (r = 0.3 [−0.04 to 0.57]; p = 0.08). Days 1 to 4 = days after surgery. IL, interleukin.

Fig 4. Cystatin C levels at day 3 as a function of perioperative use of norepinephrine.
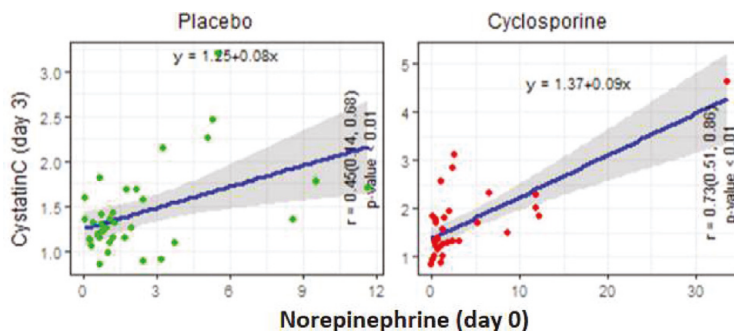
The correlation between total perioperative dose of norepinephrine and cystatin C level at day 3 in the placebo (r = 0.45; p < 0.001) and cyclosporine (r = 0.73; p < 0.001) groups. Elimination of the outliers in the placebo and cyclosporine groups did not change the result (Supplementary Figure 7).
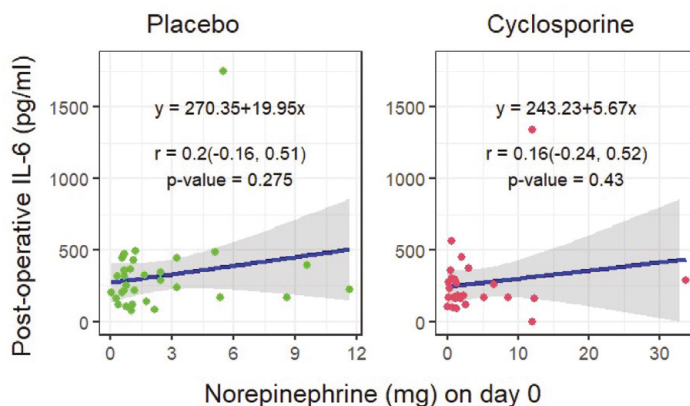

Fig 5. IL-6 levels as a function of perioperative use of norepinephrine. There were no correlations between postoperative levels of IL-6 4 hours after ECC and perioperative dose of norepinephrine (mg) on the day of surgery after CABG with ECC in the placebo-treated group (r = 0.20 [16, 0.51]; p = 0.28) and the cyclosporine-treated group (r = 0.16 [0.24, 0.52]; p = 0.43). Day 0 = day of surgery. CABG, coronary artery bypass grafting; ECC, extracorporeal circulation.

been shown to significantly reduce the inflammatory response measured both as inflammatory gene expression and cytokine levels in plasma.[20,21]

As cyclosporine has been described as a T-cell inhibitor that represses T-cell transcription,[16] the authors expected to see lower postoperative levels of IL-2. However, the T-cell inhibition by cyclosporine did not result in lower IL-2 levels. This could be because T-cells are not the only source of IL-2; dendritic and thymic cells also have been identified as sources of IL-2.[22]

In the ad hoc analysis of this study, the authors investigated factors associated with a postoperative decrease in renal function. Plasma concentrations of cystatin C were chosen to monitor renal function because it is considered a more accurate surrogate marker for the glomerular filtration rate in cardiac surgery[14,15] than P-creatinine. Cystatin C is produced at a constant rate by all nucleated cells and not secreted by renal tubules, and, in contrast to creatinine, cystatin C levels are not affected by sex, race, or muscle mass. The life cycle of serum cystatin C is merely half of that of creatinine (1.5-2 hours v 4 hours). Thus, cystatin C levels change earlier once the renal function is affected.[23]

IL-6 and IL-10 are of particular interest since increased postoperative plasma concentrations have been reported to be early biomarkers for AKI in both children and adult patients[12,24,25] after cardiac surgery. At the same time, other groups did not find any association among IL-10, IL-6, and AKI.[26] This study found no increase in IL-10 at all.

IL-6 levels increased significantly in the authors' study without any difference between placebo and cyclosporine groups. They found no correlation between IL-6 and cystatin C in their patient population, neither in the cyclosporine treated patients nor in the placebo group. The initially statistically significant correlation between IL-6 and cystatin C in the

Table 3

Treatment group	Cyclosporine		Placebo			
Variable	N	Mean ± SD	N	Mean ± SD	p Value	Test
Cystatin C on day 0	31	1.19 ± 0.36	36	1.19 ± 0.25	0.546	Wilc
Cystatin C on day 1	31	1.18 ± 0.48	36	1.06 ± 0.25	0.584	Wilc
Cystatin C on day 2	31	1.62 ± 0.81	36	1.35 ± 0.4	0.193	Wilc
Cystatin C on day 3	31	1.7 ± 0.78	36	1.43 ± 0.48	0.125	Wilc
Cystatin C on day 4	31	1.62 ± 0.83	36	1.42 ± 0.49	0.379	Wilc
Creatinine on day 0	31	95.77 ± 22.33	36	93.06 ± 16.86	0.905	Wilc
Creatinine on day 1	31	99.13 ± 24.19	36	87.72 ± 19.63	0.037	t-test
Creatinine on day 2	31	135.52 ± 55.69	36	112.39 ± 49.5	0.016	Wilc
Creatinine on day 3	31	136.55 ± 59.96	36	115.56 ± 64.66	0.015	Wilc
Creatinine on day 4	31	123.84 ± 62.28	36	112.53 ± 63.48	0.101	Wilc
% Cystatin change day 1	31	−2.58 ± 17.05	36	−10.42 ± 12.48	0.039	t-test
% Cystatin change day 2	31	31.8 ± 31.03	36	14.77 ± 31.67	0.001	Wilc
% Cystatin change day 3	31	40.05 ± 34.49	36	22.03 ± 40.66	0.001	Wilc
% Cystatin change day 4	31	32.93 ± 35.75	36	21.46 ± 41.78	0.012	Wilc
% Creatinine change day 1	31	4.11 ± 16.16	36	−5.81 ± 12.28	0.006	t-test
% Creatinine change day 2	31	40.56 ± 38.62	36	21.68 ± 52.12	0.003	Wilc
% Creatinine change day 3	31	41.59 ± 42.48	36	25.12 ± 69.89	0.001	Wilc
% Creatinine change day 4	31	27.72 ± 39.73	36	22.03 ± 69.26	0.026	Wilc

NOTE. Presented are changes in daily P-cystatin C and P-creatinine concentrations from day 0 to day 4 in the cyclosporine and the placebo groups. P-cystatin c (mg/L), P-creatinine (μmol/L). % Cystatin change = change in P-cystatin C expressed in % from day 0 (preoperatively) to postoperative day 1-4. Percent creatinine change = change in P-creatinine expressed in % from day 0 (preoperatively) to postoperative day 1-4.
Abbreviations: SD, standard deviation; t-test, Student t-test; Wilc, Wilcoxon test.

Table 4
Acute Kidney Injury by KDIGO Classification

Treatment group, (n)	AKI, n (%)	Stage 1, n (%)	Stage 2, n (%)	Stage 3, n (%)
Cyclosporine (31)	15 (48)	10 (32)	5 (16)	0
Placebo (36)	8 (22)	5 (14)	1 (3)	2 (6)
p value (t-test)	0.008	0.007	0.005	0.1

NOTE. Presented are n (%) of patients developing AKI and stage of AKI by KDIGO classification from postoperative days 1 to day 4 in the cyclosporine- and the placebo-treated groups after CABG surgery with ECC.
Abbreviations: AKI, acute kidney injury; CABG, coronary artery bypass grafting; ECC, extracorporeal circulation; KDIGO, Kidney Disease: Improving Global Outcomes.

placebo group became nonsignificant (p = 0.09) when the outlier was removed (Fig 3). Larger sample size will be needed to determine if the loss of correlation is due to the type II error.

One study reported that cyclosporine had an inhibitory effect on IL-6 release in patients with autoimmune diseases.[27] However, in the authors' setting, IL-6 release was not affected by cyclosporine treatment (Fig 2.). A possible explanation for this finding was that in their population of patients, IL-6 might be produced by a wide variety of cells and not only by T-cells.

The relation between increased levels of IL-6 and AKI is complex. Animal studies have established a critical role of IL-6 in the development and resolution of AKI, in which IL-6 simultaneously promotes an injurious inflammatory response and protects the kidneys from further injury.[28] In addition, human studies have linked higher serum levels of IL-6 to AKI in different patient populations. Thus, higher post-ECC IL-6 levels in patients with postoperative renal dysfunction could mean increased production as a response to a higher degree of perioperative insult or reflect early kidney dysfunction, due to

the decreased renal capacity to eliminate IL-6.[29] IL-6 is partially eliminated by the kidneys through filtration in the glomeruli, excreted as an intact cytokine, and metabolized by the proximal tubule. Therefore, proximal tubular injury—a hallmark of AKI—might contribute to increased serum IL-6 concentrations in patients with AKI.[30] As the treatment with cyclosporine came with a significant reduction of kidney function (which was reversible) without affecting the IL-6 levels, the authors could speculate that either an inhibited release of IL-6 is counteracted by a decreased elimination of IL-6 by the kidney or that IL-6 release from inflammatory cells is not affected by cyclosporine treatment.

This substudy was neither designed nor powered to establish the role of IL-6 as a renal biomarker in elective cardiac surgery patients. However, the authors' data indicated that the postoperative decrease in renal function in patients undergoing elective CABG surgery was not associated with a concomitant increase in IL-6, independently of treatment with cyclosporine. This raises the question of IL-

Table 5

Treatment group	Cyclosporine		Placebo			
Variable	N	AKI, n (%)	N	AKI, n (%)	p Value	Test
AKI by KDIGO on day 1	31	3 (10)	36	0	0.05	t-test
Stage 1 by KDIGO day 1	31	3 (10)	36	0	0.05	t-test
Stage 2 by KDIGO day1	31	0	36	0		
Stage 3 by KDIGO day 1	31	0	36	0		
AKI by KDIGO on day 2	31	14 (45)	36	4 (11)	0.001	t-test
Stage 1 by KDIGO on day 2	31	9 (29)	36	1 (3)	0.002	t-test
Stage 2 by KDIGO on day 2	31	5 (16)	36	2 (6)	0.16	t-test
Stage 3 by KDIGO on day 2	31	0	36	1 (3)	0.32	t-test
AKI by KDIGO on day 3	31	14 (45)	36	4 (11)	0.001	t-test
Stage 1 by KDIGO on day 3	31	9 (29)	36	1 (3)	0.002	t-test
Stage 2 by KDIGO on day 3	31	5 (16)	36	2 (6)	0.16	t-test
Stage 3 by KDIGO on day 3	31	0	36	1 (3)	0.32	t-test
AKI by KDIGO on day 4	31	14 (45)	36	4 (11)	0.001	t-test
Stage 1 by KDIGO on day 4	31	9 (29)	36	1 (3)	0.002	t-test
Stage 2 by KDIGO on day 4	31	5 (16)	36	2 (6)	0.16	t-test
Stage 3 by KDIGO on day 4	31	0	36	1 (3)	0.32	t-test

NOTE. Presented are n (%) of patients developing AKI and stage of AKI by KDIGO classification day by day from post-operative day 1 to 4 in the cyclosporine- and the placebo-treated groups after CABG surgery with ECC. P-cystatin c (mg/L), P-creatinine (μmol/L). Percent cystatin change = change in P-cystatin C expressed in % from day 0 (preoperatively) to postoperative days 1-4.
Abbreviations: AKI, acute kidney injury; CABG, coronary artery bypass grafting; ECC, extracorporeal circulation; KDIGO, Kidney Disease: Improving Global Outcomes.

6 as an early biomarker of kidney injury in elective CABG surgery.

Systemic vasodilatation, due to cytokine release or not, is common in cardiac surgery, and norepinephrine is considered a drug of choice. The study authors here found no correlation between the dosing of norepinephrine and the release of IL-6 (Fig 5); however, when norepinephrine was used, there was a correlation among the norepinephrine dose, age, and P-cystatin C increase >30% on postoperative day 3 (Fig 4; Table 2). The perioperative dosing of norepinephrine can be affected by multiple factors, such as preoperative treatment with antihypertensive medications, degree of postoperative stunning, fluid responsiveness, etc. As norepinephrine was used to maintain the mean arterial pressure, and vascular resistance was not calculated, the authors cannot exclude that the patient cohort treated with higher doses of norepinephrine represented patients with lower cardiac output than with vasodilatation. This study was too small to permit any conclusions for this interesting finding.

This study had both strengths and limitations. To the authors' knowledge, this was the first study to assess the early effects of preoperative cyclosporine treatment on the inflammatory response in patients exposed to ECC and cardiac surgery or in humans at all. Together with the conclusion in the main CiPRICS study, the study was clinically relevant, as cyclosporine preoperatively is used routinely in thoracic organ-transplant surgery. The prospective, randomized, and double-blinded design with a clearly defined study population, the uses of a true placebo solution, and the standardized anesthesia, are all examples of study strengths. Among the limitations were the single-center design, limited sample size, and that this study was a substudy of a larger clinical study.

In conclusion, in a patient population exposed to elective CABG surgery and ECC, the perioperative cytokine production was not affected by preoperative cyclosporine treatment. Changes in perioperative cytokine concentrations do not correlate with postoperative kidney impairment, and the postoperative decline in renal function is not associated with a concomitant rise in IL-6 independently of treatment with cyclosporine.

Conflict of Interest

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1053/j.jvca.2021.11.026.

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