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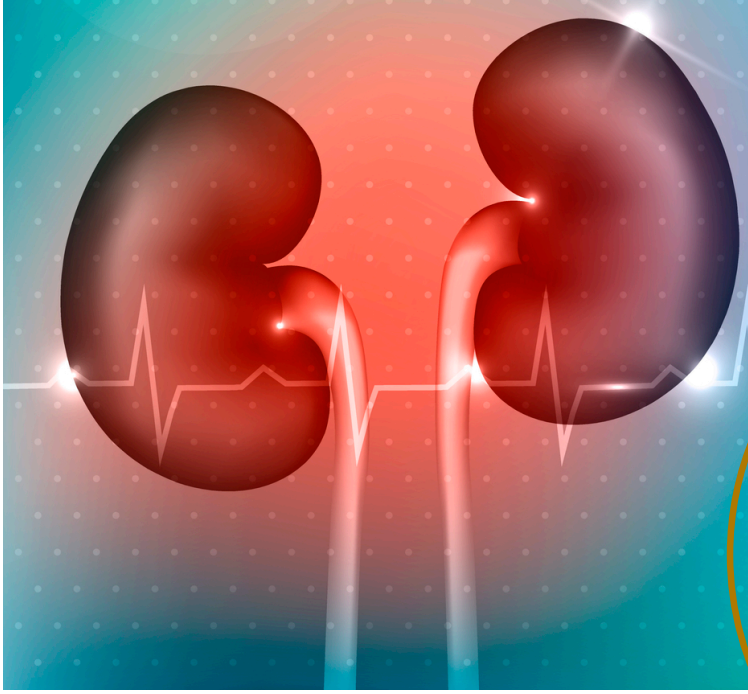

Importance of renal function in cardiac surgery

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The Importance of renal function in cardiac surgery

Abstract

Acute kidney injury (AKI) is a common and serious complication after cardiothoracic surgery and is associated with increased short- and long-term mortality risk. Despite extensive studies in the field, a comprehensive understanding of this syndrome has remained elusive, partly due to divergent definitions of AKI and partly due to the limitations of available routine biomarkers to predict, prevent, and detect AKI. In recent years, much has been done to better define AKI. There is also ongoing work on finding better suited biomarkers for AKI as well as improving treatment of patients at risk or suffering from AKI.

In this work we studied different aspects of renal function after cardiac surgery.

The first paper shows in a retrsospective study of 5261patients, when preoperative estimated glomerular filtration (eGFR) rate by s-creatinine and preoperative hemoglobin is entered into a Cox analysis together with known traditinoal risk factors for decreased long-term survival, blood transfusion did not affect survival significantly. In the subgroups of patients with normal eGFR and hemoglobin, blood transfusions did not have any effect on long-term survival.

In the second paper, incidence of AKI is evaluated in 5746 patients, defined by different measures (i.e creatinine, creatinine clearance and eGFR) and evaluated in relation to long-term mortality. The effect of renal recovery on survival was also described. The Risk, Injury, Failure, Lost and Endstage (RIFLE) system was used to stratify AKI. The study showed that estimated GFR by the modification of diet in renal disease (MDRD) formula had a more robust predictive ability for mortality and that renal recovery in general was associated with better outcome compared with those without renal recovery.

The third paper describes a randomized, double-blind, placebo-controlled trial, where the effect of a single high dose erythropoeitin (EPO) preoperatively, as a protective drug against AKI after cardiac surgery, is evaluated. Seventy five patients were enrolled in the study, AKI was evaluated by the changes of s-cystatin C at the third postoperative day from baseline. No protective effect against AKI by EPO could be shown.

In the fourth paper the predictive value for mortality of s-creatinine and s-cystatin C and their eGFR were evaluated at different time points in patients undergoing cardiac surgery. The prospective study included 1955 patients. Different creatinine and cystatin C eGFR equations were used in the analysis. S-Cystatin C was shown to have a stronger and earlier predictive value for mortality compred with s-creatinine, and the predictive abliltiy of cystatin C was also shown preoperatievly.

Key words Acute kidney injury, eGFR, Transfusion, Mortality, Erythropoietin, Cardiac surgery.

Classification system and/or index terms (if any) RIFLE

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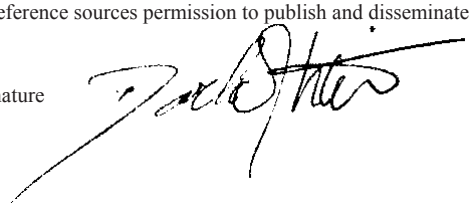
Signature  Date 2014-11-10

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To my beloved
Elisa, Edvin, Emilia & my wife Virpi

Contents

Populärvetenskaplig sammanfattning

Njurfunktion inom hjärtkirurgi

Akut njursvikt (AKI) efter hjärtkirurgi är en allvarlig komplikation som förekommer i 25-35 % av alla patientfall. AKI medför förhöjd risk för allvarliga komplikationer och död efter hjärtkirurgi. Ett stort fokus ligger på detektion, prevention och behandling av AKI och forskningsfältet uppdateras kontinuerligt. En rad olika läkemedel har provats i samband med hjärtkirurgi i syfte att skydda njurfunktionen men hittills har ingen av dessa visat någon säker positiv effekt.

Njurfunktionen har traditionellt mäts med ett blodprov med mätning av kreatinin-nivåer i blodet. Kreatinin är en biprodukt av ämnesomsättningen i kroppens muskler och filtreras via njurarna i urinen. En av njurens viktigaste uppgifter är att filtrera ut små molekyler. Denna filtrationsförmåga kallas *glomerular filtration*, och mäts i en *glomerular filtration rate* - GFR. GFR speglar hur väl njurarna fungerar. Nedsatt njurfunktion leder till minskad filtrationsförmåga och följaktligen stegrade kreatinin-nivåer i blodet. Denna stegring tyder vanligtvis på nedsatt njurfunktion. Vid en normal njurfunktion krävs det dock en 50 % nedsättning av njurfunktion för att kreatinin-nivåerna ska stiga. Detta är en av bristerna med kreatinin som markör för njurfunktion vilket innebär att det tar tid innan njurfunktionsnedsättning kan upptäckas. Kreatinin har även andra brister som njurfunktionsmarkör, varför forskning pågår för att hitta bättre markörer. Det bör dock nämnas att kreatinin i dagsläget, trots allt är den vanligaste njurfunktionsmarkören i kliniskt bruk. För att bl.a. kunna definiera graden av AKI och driva forskningen framåt har det skapats internationell definitioner och klassificeringar för AKI. En av de vanligaste klassificeringarna kallas för RIFLE som är beroende av kreatinin-stegring i blodet eller graden av GFR-sänkning. En annan njurfunktionsmarkör är cystatin C, denna är en kroppsegen substans som upptäckts i Lund för ca 30 år sedan och presenterats som en bättre njurfunktionsmarkör jämfört med kreatinin. Cystatin C har inte de brister som kreatinin har och kan möjligen ge mer tillförlitlig information om njurens funktion.

I kliniskt arbete anser man att det är viktigt att även ange GFR värdet när man utvärderar njurfunktion och inte enbart värdet av njurfunktionsmarkören. Detta är oftast ett uträknade värde genom formler som baseras på kreatinin eller cystatin C-nivåer i blodet. Det finns ett flertal sådana formler. Utöver kostnads- och tillgänglighetsaspekter råder även oenighet om vilken njurfunktionsmarkör och via

vilken formell man bör bedöma njurfunktionen. Genom att optimera njurfunktionsmätningar i tid och precision kan progress av njursjukdomen i samband med hjärtkirurgi begränsas, förebyggas eller behandlas.

Avhandlingens delarbeten

Delarbete 1.

Blodtransfusion har generellt sett ansetts medföra en ökad risk för komplikationer och även öka risken för långtidsmortalitet i samband med hjärtkirurgi. I delarbete I har interaktionen mellan njurfunktion och transfusion med avseende på långtidsmortalitet studerats. I detta arbete kunde vi visa att blodtransfusion efter hjärtkirurgi är associerat till sämre överlevnad. Men när hänsyn togs till patientens njurfunktion och blodvärde före operation minskade risken med blodtransfusioner. När enbart patienter med normal njurfunktion och normalt blodvärde före operation studerades sågs ingen påverkan på överlevnad på sikt av blodtransfusioner jämfört med patienter som inte fått blodtransfusioner.

Delarbete II

I detta delarbete studeras riskerna med akut njursvikt efter kranskärlskirurgi. Kreatinin samt två vanliga formler för beräkning av GFR baserat på kreatinin-nivåer i blodet studerades och jämfördes för över 5000 patienter vid olika tidpunkter före och efter operation. Data kategoriserades enligt det s.k. RIFLE systemet och effekten på återhämtning av njurfunktionen sattes i förhållande till långtids-överlevnad. I denna studie visades att GFR-beräkning (jämfört med kreatinin) kan vara mer informativ ur prognostisk synpunkt samt att den sämsta njurfunktionen är den som starkast är kopplad till minskad överlevnad, samtidigt som återhämtning av njurvärden allmänt sett är positivt ur överlevnadssynpunkt.

Delarbete III

I dagsläget finns inget läkemedel som visats kunna skydda njurarna mot akut njursvikt vid hjärtkirurgi. Erythropoietin (EPO) är ett kroppseget hormon som är känt för att stimulera produktionen av röda blodkroppar. EPO har i många djurstudier samt i några kliniska studier kunnat visas ha skyddande effekt mot syrebrist kroppsorgan.

I detta delarbete studerades effekten av EPO given innan operation i syfte att studera en eventuell skyddande effekt mot akut njursvikt hos patienter som redan innan operation har större risk att utveckla akut njursvikt pga. habituellt nedsatt njurfunktion. Patienterna randomiserades till EPO och placebo. Gruppen som fick EPO uppvisade inga tecken på att ha fått njurskyddande effekter av det administrerade läkemedlet.

Delarbetet IV

Betydelsen av cystatin C inom intensivvård och hjärtkirurgi är inte fullständigt studerat. Cystatin C prognostiska förmåga för att förutsäga komplikationer och död i jämförelse med kreatinin är okänt för hjärtopererade patienter. I detta delarbete har cystatin C och kreatinin samt GFR beräkningar baserad på dessa jämförts inbördes hos närmare 2000 patienter som genomgått hjärtkirurgi och satts i relation till långtidsöverlevnad. I denna studie visas att cystatin C och dess GFR beräkningar har en starkare prognostisk värde jämfört med kreatinin för överlevnad efter hjärtoperation. Det prognostiska värdet kan avläsas i ett tidigare skede jämfört med kreatinin. Ur klinisk synpunkt medför detta att cystatin C och dess GFR beräkningar kan redan före operation indikera vilka patienter som är riskpatienter och som behöver extra tillsyn avseende njurfunktionen efter en hjärtoperation.

List of Publications

I. *Blood transfusion after cardiac surgery: is it the patient or the transfusion that carries the risk?* Dardashti A, Ederoth P, Algotsson L, Brondén B, Lührs C, Bjursten H. *Acta Anaesthesiol Scand. 2011;55:952-61.*

II. *Incidence, dynamics, and prognostic value of acute kidney injury for death after cardiac surgery.* Dardashti A, Algotsson A, Brondén B, Bjursten H. *J Thorac Cardiovasc Surg. 2014;147:800-7.*

III. *Erythropoietin and Protection of Renal Function in Cardiac Surgery (the EPRICS Trial).* Dardashti A, Ederoth P, Algotsson L, Brondén B, Grins E, Larsson M, Nozohoor S, Zinko G, Bjursten H. *Anesthesiology, 2014;121:582-590.*

IV. *The predictive value of different renal function measures for mortality after cardiac surgery based on traditional creatinine and novel cystatin C.* Dardashti A, Nozohoor S, Algotsson L, Ederoth P, Bjursten H. *Submitted.*

Abbreviations

ADQI	Acute Dialysis Quality Initiative
AKI	Acute Kidney Injury
AKIN	Acute Kidney Injury Network
ANOVA	Analysis of variance
BMI	Body Mass Index
CABG	Coronary Artery Bypass Grafting
CAPA	Caucasian Asian Pediatric and Adult subjects
CKD-EPI	Chronic Kidney Disease Epidemiology collaboration
COPD	Chronic Obstructive Pulmonary Disease
CPB	Cardiopulmonary Bypass
CrCl	Creatinine Clearance
CSA-AKI	Cardiac Surgery Associated Acute Kidney Injury
eGFR	Estimated Glomerular Filtration Rate
eCrCl	Estimated Creatinine Clearance
EPO	Erythropoietin
GFR	Glomerular Filtration Rate
Hb	Hemoglobin
IABP	Intra-Aortic Balloon Pump
ICU	Intensive Care Unit
KDIGO	Kidney Disease Improving Global Outcomes work group
LIMA	Left Internal Mammary Artery
LVEF	Left Ventricular Ejection Fraction
NYHA	New York Heart Association
PCI	Percutaneous Coronary Intervention
RCT	Randomized Control Study
RIFLE	Risk, Injury, Failure, Loss and End stage kidney disease
RRT	Renal Replacement Therapy
UO	Urinary Output

Introduction

Acute Kidney Injury in Cardiac surgery

Cardiac surgery has evolved over the last few decades, and significant advances in perioperative and intraoperative care of patients have been achieved. At the present time, increasingly elderly patients are undergoing surgery with cardiopulmonary bypass (CPB), and these patients may subsequently benefit from an improved quality of life and a longer life expectancy. While the mortality rate for cardiac surgery is relatively low, patients are at risk from serious postoperative complications, including poor cardiac function, infection, gastrointestinal dysfunction, acute lung injury, stroke, and postoperative renal dysfunction, now called acute kidney injury (AKI). In the past, AKI in cardiovascular patients was considered to be a relatively minor event managed with conservative treatment in most patients. However, there is much evidence showing that AKI after cardiac surgery is among the most serious complications, whether pre-existing or developing de novo, and it has been shown that postoperative AKI is a major contributor to morbidity as well as short- and long-term mortality [1-3]. In its most severe form *i.e*, requiring renal replacement therapy, mortality rates of more than 50% has been reported [1] [4]. Furthermore, it has also been shown that even milder forms of AKI are associated with an increased risk of mortality [5, 6]. The incidence of postoperative AKI and its associated mortality and morbidity have changed little in the last decade. While some aspects of the pathophysiology of postoperative AKI are understood, much is still not known. Different intraoperative strategies have been developed to provide renal protection in patients undergoing cardiovascular procedures. However, no pharmacological intervention has proven to be renoprotective. Much research on the AKI definition and epidemiology and interventional studies have been based on s-creatinine increase. S-creatinine, however, as a renal biomarker has shortcomings, which together with a lack of a uniform definition of AKI, have brought difficulties in interpreting AKI studies. In recent years the AKI definitions Risk, Injury, Failure, Late and End stage (RIFLE) and the Acute Kidney Injury Network (AKIN) have been introduced, and are undergoing evaluations to provide for a better understanding of the syndrome. There is also extensive ongoing research to find better suited biomarkers to define, detect, treat and ultimately, prevent AKI. Taken together, these data highlight the importance of understanding the pathophysiology of postoperative renal

dysfunction, the identification of the patients at risk, and the implementation of effective renoprotective therapies.

Definition of Acute Kidney Injury

Until a few years ago, there was no universally recognized definition for acute renal dysfunction. In recent years the term AKI has replaced the terms acute renal failure and acute renal dysfunction [7]. The term AKI compromises a broader range of renal function changes reflecting smaller decrements in kidney function. While dysfunction or failure express greater abnormality, which may require support, the concept of injury prompts the need for protection.

Acute kidney injury (AKI) is defined as a sudden and sustained decrease in kidney function with a rapid decrease in glomerular filtration rate (GFR), resulting in disturbances in electrolyte- and acid-base balance, derangement of extra cellular fluid volume, retention of nitrogenous waste products, and often decreased urine output [8]. This is often associated with an increase in s-creatinine and other biomarkers reflecting renal function.

Assessing Kidney Function

Kidney function cannot be measured directly. The most accurate method to approximate the renal function is with a clearance method where an exogenous filtration marker (*e.g.* inulin, iohexol), which is evenly distributed into the extracellular fluid and freely filtered through the glomeruli, is injected into the blood stream. With repeated blood samples, the amount of fluid filtered through the glomeruli per minute is calculated, called the glomerular filtration rate (GFR). GFR is accepted as the best overall measure of kidney function. Normal values, which are related to age, sex, and body size, are approximately 130 ml per minute per 1.73 m^2 in young men and 120 ml per minute per 1.73 m^2 in young women. Mean values decline as persons age [9]. The GFR can also be calculated using endogenous filtration markers (*i.e.* creatinine, urea, cystatin C). Urea concentration is a poor measure of GFR as it does not represent real-time changes in GFR and requires time to accumulate. Reliance on urea can lead to potential delays in diagnosis of acute changes to GFR or detection of AKI [10]. Urinary output is an indirect measure of renal function and is routinely measured in ICU patients. Trends in urine volume can be helpful in that continuous output can be used as a crude dynamic gauge of kidney function. Urine output may also be a more sensitive barometer for changes in renal hemodynamics than biochemical markers of solute clearance. The importance of dynamic changes to urine output has been recognized by having been integrated into the RIFLE classification system for AKI. However, urine output may

be affected by numerous factors and is not a reliable surrogate of renal function and AKI. [11].

Table 1. The stages of chronic kidney disease [12]

Stage	GFR	Description
1	>90	Normal kidney function
2	60-89	Mildly reduced kidney function
3A 3B	45-59 30-44	Moderately reduced kidney function
4	15-29	Severely reduced kidney function
5	<15 or on dialysis	Very severe, or endstage kidney failure

All GFR values are normalized to an average surface area (size) of 1.73m^2. GFR= Glomerular filtration rate.

Creatinine

Creatinine is an amino acid compound derived from the nonenzymatic conversion of creatine to phosphor-creatinine in skeletal muscle and subsequent liver metabolism of creatine through methylation of guanidine aminoacetic acid to form creatinine. Creatinine has a molecular weight of 113 Dalton, is released into the plasma at a relatively constant rate, is freely filtered by the glomerulus, and is not reabsorbed or metabolized by the kidney. The clearance of s-creatinine is the most widely used for estimating GFR, and s-creatinine levels generally have an inverse relationship to GFR [13]. However, there are several limitations to using serum creatinine as a biomarker of AKI. First, its release varies with age, gender, diet, muscle mass, drugs, and vigorous exercise. Second, creatinine clearance exceeds GFR as it is also secreted by proximal tubular cells. Tubular secretion of creatinine varies among and within individual persons, especially in those with a mild-to-moderate reduction in kidney function, and may account for 10-40% of creatinine clearance [14, 15], which could mask a decrease in GFR. Third, the accuracy of s-creatinine assays can be reduced by artifact. Fourth, creatinine becomes abnormal only when more than 50% of GFR is lost and may require up to 24 hours before sufficient increases in blood concentration are detectable [10]. During critical illness, s-creatinine concentrations are subject to large fluctuations due to induced dilution, volume status, the catabolic effects of the critical illness, and septic conditions in the early stages of renal injury. It has, therefore, poor predictive accuracy for renal injury due to the slow increase in the s-creatinine concentrations [16].

Cystatin C

Cystatin C is an endogenous cysteine proteinase inhibitor of low molecular weight (13,000 Da) that holds many ideal features for use as a surrogate marker of kidney function and estimate of GFR. It was initially identified in Lund by Dr. Anders Grubb and his group as an alternative filtration marker in an attempt to overcome known limitations of serum creatinine [17-19]. Similar to creatinine, cystatin C is filtered freely at the glomerulus; it is nearly completely metabolized by proximal renal tubular cells. As a consequence, there is little to no detectable cystatin C present in the urine, so its clearance cannot be calculated. It is synthesized at a relatively constant rate and released into plasma by all nucleated cells in the body. Cystatin C is not secreted or reabsorbed and its concentrations are not affected by dietary protein [20], and the influence of muscle mass on its concentration is substantially smaller compared to s-creatinine [21, 22]. Thus, a reduction in GFR correlates well with a rise in s-cystatin C level, and vice versa. S-cystatin C levels, however, may be affected by older age, elevated C-reactive protein levels, abnormal thyroid function, and use of corticosteroids [23]. The diagnostic value of s-cystatin C as an estimate of GFR has been investigated in multiple clinical studies and has been shown to be superior to s-creatinine in discriminating between normal and impaired kidney function. In addition, cystatin C may be more sensitive to early and mild changes to kidney function compared with creatinine [24-30]. S-cystatin C-based estimates of GFR may also perform superiorly in selected patient populations, in particular, those with lower s-creatinine concentrations, such as elderly patients, children, renal transplant recipients, those with cirrhosis, and those who are malnourished [31-33].

Equations to Estimate Glomerular Filtration Rate

To overcome some of the limitations of using s-creatinine values, the glomerular filtration rate estimates are developed by including variables such as age, sex, race, and body size in addition to s-creatinine GFR estimating equations [9]. Furthermore, reporting eGFR is recommended as it is directly related to a patient's kidney function, and studies on the general population show that a reduced eGFR (less than 60 ml/min per 1.73m^2) is associated with increased risk of adverse outcomes of chronic kidney disease (CKD) [9]. Most equations estimating GFR have been developed in study populations consisting predominantly of patients with chronic kidney disease and reduced GFR. Although estimates of GFR constantly improve, the innate difficulties of estimating GFR have made some researchers question the achievability of producing a single equation that will, with reasonable accuracy, estimate the GFR over the entire GFR spectrum and in all sub-populations [34, 35]. Newer equations have recently been developed using cystatin C, either alone or in

combination with creatinine, and they may be more accurate than creatinine-based equations [36, 37].

Cockcroft Gault formula

The Cockcroft-Gault formula was published in 1976 based on data from 249 adult men with creatinine clearances from 30 to 130 ml per minute [38]. However, the equation has major drawbacks as it systematically overestimates GFR because of the tubular secretion of creatinine, and the values are not adjusted for body-surface area. A comparison with normal values for creatinine clearance requires measurement of height, computation of body-surface area, and adjustment to 1.73 m^2. Given these issues, widespread clinical use of the Cockcroft-Gault equation is not encouraged. However, it is still used in clinical practice. Recommendations on drug dosage are often based on Cockcroft-Gault estimates, as these provide the absolute eGFR which in that specific setting may be of greater interest.

MDRD

The Modification in Diet and Renal Disease equation was presented in 1999 to overcome some of the difficulties with the Cockcroft-Gault equation [39]. The equation was derived using 1628 patients from the Modification of Diet in Renal Disease (MDRD) Study where 1070 patients were randomly selected as the test cohort while 558 patients were used as a validation cohort [39]. The increased bias and imprecision at high eGFR is partly due to the fact that the studied population was not representative of a general population as it exhibited a median measured GFR of 40 mL/min/1.73m^2. Instead, the study represented a population with decreased kidney function [39]. On average, GFR estimates of less than 90 ml per minute per 1.73 m^2 are lower than the directly measured values, which may lead to a false positive diagnosis of chronic kidney disease in individuals who do not have the disease but have a mild reduction in GFR [9]. However, in individuals with measured GFR below 30 mL/min/m^2, the eGFR may be overestimated in the MDRD equation [40, 41].

CKD-EPI

The Chronic Kidney Disease Epidemiology collaboration (CKD-EPI) published the CKD-EPI equation in 2009 [42]. The equation was developed to give more accurate eGFR, compared with the MDRD, even in patients with higher GFR [42]. In 2012, the Chronic Kidney Disease-Epidemiology collaboration published a new cross-sectional analysis after analyzing and validating different GFR equations in several

large patient cohorts including participants both with kidney disease and healthy volunteers and by the new standardized cystatin C calibration method [43]. The authors presented two new equations the 2012 CKD-EPI$_{cystatin\ C}$ and the new combined CKD-EPI$_{cystatin + creatinine}$ [36]. It has been shown that the combination of s-creatinine and s-cystatin C GFR estimates (eGFR$_{cystatin\ C + creatinine}$) may provide a better GFR estimate compared to the eGFR$_{cystatin\ C}$ or eGFR$_{creatinine}$ alone in elderly and patients with chronic kidney disease [44, 45].

However, it is not known if the advantage of the combination formula is applicable for cardiac surgery patients with more rapid changes in renal function.

Caucasian, Asian, Pediatric & Adult formula (CAPA)

CAPA is a simple cystatin C-based equation for estimation of GFR with only two variables (cystatin C concentration and age) compared to the CKD-EPI$_{cystatin\ C}$ equation requiring also sex as a variable. The equation is also biologically oriented, with one term for the theoretical renal clearance of small molecules and one constant for extra-renal clearance of cystatin C [37].

Table 2. eGFR equations [36-39, 46]

Cockroft-Gault	eCrCl (female) = ([140-age]/s-creatinine x weight in kg x 0.85) eCrCl (male) = ([140-age]/s-creatinine x weight in kg)
MDRD	eGFR =186 x p-creatinine$^{-1.154}$ x Age$^{-0.203}$ x [1.210 if black] x [0.742 if female]
CKD-EPI $_{creatinine}$	eGFR = 141 x min(Scr/κ,1)$^{\alpha}$ x max(Scr/κ,1)$^{-1.209}$ x 0.993^{Age} x 1.018 [if female] x 1.159 [if black] If female: κ =0.7, α =0.248 If male: κ =0.9, α =0.207
CKD-EPI $_{CysC+crearinine}$	eGFR = 135 x min(Scr/κ, 1)$^{-\alpha}$ x max(Scr/κ, 1)−0.601 × min(Scys/0.8, 1)$^{-0.375}$ x max(Scys/0.8, 1)$^{-0.711}$ x 0.995Age [x 0.969 if female] [x 1.08 if black]
CKD-EPI$_{Cystatin\ C}$	eGFR = 133 x min(S-CysC/0.8, 1)$^{-0.499}$ x max(S-CysC /0.8, 1)$^{-1.328}$ x 0.996age [x 0.932 if female]
CAPA	eGFR = 130 x cystatin C $^{-1.069}$ x age$^{-0.117}$-7

eGFR unit ml/min/1.73m^2, eCrCl unit is ml/min, p-creatinine in mg/dL

Neutrophil Gelatinase-associated lipocain (NGAL) a renal injury biomarker

NGAL has been presented as a promising early biomarker of AKI. NGAL, a 25 kDa protein, is synthesized in the bone marrow during myelopoiesis and stored in the neutrophil granules. It has bactericide properties and its levels rise in response to

bacterial infections [47]. Although it is expressed in very low levels in several human tissues, including kidneys, colon, liver and lungs, it is markedly induced in injured epithelial cells [48]. NGAL was presented as an early marker of AKI in 2003 [49], and for several years it was considered the 'troponin' of the kidney [48]. It has been investigated across a range of different clinical settings of AKI, such as after cardiac surgery, with conflicting results [50-52]. The reputation of NGAL as a troponin like diagnostic marker for the kidney has fallen as the understanding of the molecule's complex nature has emerged [53]. NGAL levels increase unpredictably during evolving AKI but also during other chronic and acute inflammatory conditions, such as in sepsis, in CKDs, during urinary tract infections, exacerbations of obstructive pulmonary diseases, and after CPB. Therefore, the value of NGAL for evaluation of critically ill patients with or at risk of AKI is limited until assays measuring kidney-specific NGAL are available [54]. Despite these limitations, plasma NGAL has performed decently in cardiac surgical patients with AUC 0.64 [55] to 0.89[50] in predicting early AKI, and has shown to be superior to s-creatinine [56].

AKI classifications, RIFLE, AKIN & KDIGO

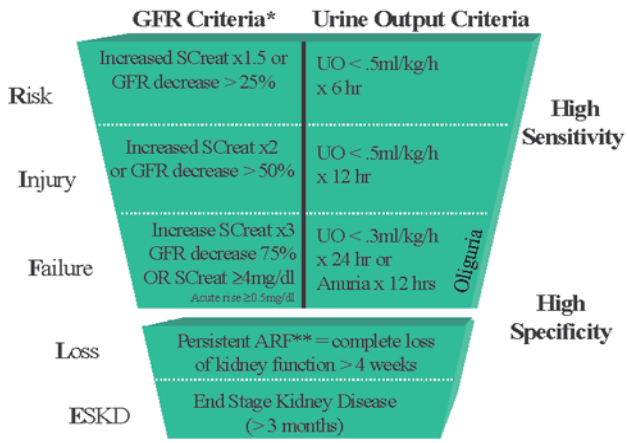
RIFLE

The reported incidence of AKI after cardiac surgery varies widely depending on the definition used. In AKI, in general, the most commonly used definitions are the Risk/Injury/Failure/Loss/End-stage (RIFLE) criteria. The RIFLE classification criteria emerged in May 2002 by the Acute Dialysis Quality Initiative (ADQI) group composed of nephrologists and intensivists, with the purpose of defining AKI [11]. The RIFLE classification (*Figure 1*) is based on serum creatinine and urinary output (UO) determinants, and considers three severity classes of AKI (Risk, Injury and Failure), according to the variations in s-creatinine and/or UO, and two outcome classes (loss of kidney function and end-stage kidney disease). The patient should be classified using the criteria (s-creatinine and/or UO) which leads to the highest classification (maximum RIFLE).

Among strengths of the RIFLE classification is that it has been largely validated in terms of determining the incidence of AKI and its prognostic stratification in several settings of hospitalized patients [57-63]. Although the RIFLE classification system was originally established to standardize the definition and stratification of AKI severity, several studies have evaluated its ability to predict mortality, and it has become an important tool and complement to general scores in predicting patient outcome [58, 61, 64].

Among the RIFLE classification limitations is that it requires a baseline creatinine, which is commonly unknown in the general intensive care setting. This issue is solved by calculating the s-creatinine using the MDRD formula and assuming a baseline GFR of 75 ml/min/m^2 if there is no history of CKD [59, 62, 64-66]. However, in cardiac surgery the baseline s-creatinine commonly is known.

Figure 1- The RIFLE criteria



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AKIN

The Acute Kidney Injury Network (AKIN) classification of AKI was proposed in 2005 by a working group composed of nephrologists and critical care physicians. It is essentially a modified version of the RIFLE criteria but relies on s-creatinine and not on GFR changes. Further, the AKI definition is only considered after adequate hydration is achieved [7, 11] (*Table 3*). Baseline s-creatinine is not necessary in the AKIN classification, and it requires at least two values of s-creatinine obtained within a period of 48 hours. AKI is defined by the sudden decrease (in 48 h) of renal function, defined by an increase s-creatinine of at least 26.5 μmol/L (0.3 mg/dL) or by a percentage increase in s-creatinine ≥50% (1.5× baseline value), or by a decrease in the urine output (documented oliguria <0.5 mL/kg/h for more than 6 h). Stage 1 corresponds to the risk class, but it also considers an absolute increase in s-creatinine ≥26.5 μmol/L (0.3 mg/dL); Stages 2 and 3 correspond to injury and failure classes, respectively. Stage 3 also includes patients requiring renal replacement therapy (RRT) independently of the stage. The two outcome classes (loss of kidney function and end-stage kidney disease) were removed from the classification. These modifications were based on the cumulative evidence that even small increases in

s-creatinine are associated with a poor outcome. Also in the extreme variability of hospital resources and different indications to start RRT in different countries and hospitals [67, 68]. A limitation of AKIN is that it does not allow the identification of AKI when s-creatinine elevation occurs in a time frame higher than 48 h [69]. In a recent study it was shown that 40% of the cases of AKI had occurred beyond the second postoperative day [70].

Epidemiologic studies collectively enrolling more than 500 000 patients confirmed that the RIFLE and/or AKIN criteria were valid tools to diagnose and stage AKI [70]. The AKIN classification could theoretically improve the RIFLE criteria sensitivity and specificity. However, the AKIN classification compared with the RIFLE classification did not exhibit a prognostic improvement in terms of in-hospital mortality, although it enabled the identification of more AKI patients [65, 71-76].

Table 3. The AKIN criteria [7]

Stage	GFR	Urine output
1	1.5-1.9 times baseline s-creatinine OR ≥ 26.5 µmol/L increase in	<0.5 mL/kg/h for 6 -12h
2	2.0-2.9 times baseline s-creatinine	<0.5 mL/kg/h for ≥ 12h
3	3.0 times baseline s-creatinine OR Initiation of renal replacement therapy OR In patients <18 years dedcrease in eGFR to <35 l/min 1.73m^2	<0.3 mL/kg/h for ≥ 24 h OR Anuria for ≥ 12 h

KDIGO

The Kidney Disease Improving Global Outcomes work group recently combined the RIFLE and AKIN classifications in order to establish one classification of AKI for practice, research, and public health [77]. However, the role of the KDIGO needs to be explored.

Cardiac surgery-associated AKI (CSA-AKI)

Incidence and pathophysiology of AKI after cardiac surgery

CSA-AKI is the second most common cause of AKI in the intensive care unit [78]. The incidence of CSA-AKI based on the RIFLE and AKIN criteria has been reported from 9-39% [75, 79-81]. CSA-AKI may not be detected in the first 24-48 h using conventional monitoring by s-creatinine levels because of the shortcomings of s-creatinine as a biomarker and the dilutional effects of the CPB pump prime [10, 14] [82]. The etiology of CSA-AKI is complex and not fully understood; the concept and data available are mostly based on experimental studies [83]. Although hypoperfusion and ischemia is considered a major cause of AKI [84, 85], the kidney can generally tolerate isolated insults such as hypoperfusion; for kidney injury to occur, a combination of several insults or risk factors, or multiple hits, is thought to be necessary. CSA-AKI has frequently been associated with the use of CPB and especially the duration of CPB [86-88], however, the etiology is multifactorial. In addition to patient risk factors, other causative factors related to CSA-AKI include pharmacologic therapy exogenous and endogenous toxins, metabolic abnormalities, ischemia and reperfusion injury, neurohormonal activation, inflammation, and oxidative stress related to the intra- and postoperative management [89] [82].

Patient-related risk factors for CSA-AKI

Well-validated risk factors associated with the development of AKI post-CPB include female gender, reduced left ventricular function or the presence of congestive heart failure, diabetes mellitus, peripheral vascular disease, preoperative use of an aortic balloon pump (IABP), chronic obstructive pulmonary disease, the need for emergent surgery, and an elevated preoperative serum creatinine. Other risk factors indicated recently as contributing to the increased risk of cardiac surgery-associated AKI are preoperative anemia and transfusion [90, 91].

Prevention

Among preventive measures advocated to protect patients' kidneys during the perioperative, and immediate postoperative period, the most frequently proposed include optimization of renal perfusion (*i.e.* avoidance of hypotension and hypovolemia adequate volume resuscitation), avoidance of potentially nephrotoxic agents (*e.g.* aminoglycoside antibiotics, angiotensin converting enzyme inhibitors, and radiologic contrast agents) and optimal hemodynamic monitoring [89]. Several

pharmacological interventions have been attempted in cardiac surgery, however, no reliable evidence from available literature suggests that interventions during surgery can protect the kidneys from damage.

Attempts have also been made to protect the kidneys both during surgery and in the immediate postoperative period. Various regimens, such as *N*-acetyl cysteine, diuretics, calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, and N-acetyl cysteine did not demonstrate any benefit in terms of mortality, need for renal replacement therapy, or incidence of AKI [82, 92]. Statins by their pleiotropic effects were proposed as potentially renoprotective in cardiac surgical patients. They prevented kidney injury after cardiac surgery measured by lower levels of kidney injury biomarkers or earlier recovery [93, 94] [95] although larger studies and randomized controlled trials (RCT) have not shown a reduction in incidence of CSA-AKI [96, 97]. Sodium-bicarbonate showed promise in cardiac surgery initially [98], however, subsequent multicenter studies failed to confirm the renoprotective effect. [99, 100]. Fenoldopam, a dopamine agonist, has also been the subject of clinical trials as it may improve the quality of perfusion during CPB in patients receiving catecholamines [101, 102], although the results have not been reproducible [103]. Atrial natriuretic peptide (ANP) is suggested as a renoprotective drug during the CPB and there are ongoing studies to evaluate its potential renoprotective effects. Considerable enthusiasm has surrounded the potential for erythropoietin (EPO) to provide some renal protection [104, 105]; EPO has shown prophylactic effects against AKI [106, 107], however, conflicting results has been reported even for EPO [108, 109].

The criteria used to diagnose acute renal damage varies in many older studies, many of which suffered from poor methodological quality such as insufficient participant numbers and poor definitions of end points such as acute renal failure and acute renal injury. Recent methods of detecting AKI such as the use of specific biomarkers and better-defined criteria for identifying renal damage (i.e. RIFLE, AKIN or KDIGO) will likely facilitate advances in the interventions used to protect the kidneys during the perioperative period [92, 110].

Erythropoietin (EPO)

Erythropoietin is an endogenous hormone and the primary regulator of erythropoiesis. It is mainly produced in the kidneys with its production controlled through the hypoxic inducing factor system [111]. In recent years, additional tissue/organ protective properties of EPO against ischemia and reperfusion injury have become apparent [112]. The cytoprotective, preconditioning, and anti-apoptotic effects of EPO on kidneys have been shown in both experimental [105, 113-118] and a few human studies [106, 107, 119]. Available evidence suggests that these pleiotropic effects of EPO are mediated by a tissue protective receptor (TPR)

that is distinct from the receptor responsible for erythropoiesis [120]. Activation of the TPR requires a higher concentration of EPO than is needed for maximal erythropoiesis [120]. Doses between 100U/kg and 500U/kg have been used in human studies to attain the pleiotropic effects of EPO, with no adverse events reported [106, 108, 109, 121-123]. However, in a study by Ehrenreich [124] in using erythropoietin for treatment of ischemic stroke, increased mortality was reported in the erythropoietin treated group. Several RCTs [106-108, 119, 125] have also been performed in adult patients undergoing cardiac surgery, with controversial results.

Aims

Study I

The aim of this study was to investigate the interaction between preoperative renal function, preoperative hemoglobin and the risk of blood transfusions with long-term mortality as the primary outcome in patients undergoing coronary artery bypass grafting (CABG) where transfusions are not numerous and presumably not life-saving.

Study II

The primary aim was to assess the importance of time-point for measuring, and consequently, the dynamics of renal recovery in relation to long-term mortality in patients undergoing CABG. The secondary aim was to compare three different plasma creatinine-based assessments of renal function (*i.e.* absolute p-creatinine concentrations, eCrCl using the Cockroft–Gault formula [38], and eGFR according to the MDRD formula [39] employing the RIFLE criteria [11] in order to find the best method for renal assessment following surgery.

Study III

The aim of this randomized, double blind, placebo-controlled clinical trial was to study if a single high-dose (400U/kg) EPO administered preoperatively in patients with pre-existing impaired renal function undergoing CABG has a reno-protective effect as evaluated by postoperative changes in p-cystatin C in the third postoperative day.

Study IV

In order to investigate the usage of s-cystatin C in identifying those at increased risk of postoperative mortality we aimed to evaluate and compare s-creatinine and s-cystatin C, their corresponding eGFR by different equations (*i.e.* according to

MDRD eGFR [39], the 2009 s-creatinine eGFR [42], the 2012 s-cystatin C CKD-EPI eGFR [36], the cystatin C CAPA eGFR [37] formulas, and the combined s-creatinine and s-cystatin C eGFR 2012 CKD-EPI [36] formula), and to study at which time point they carry the highest risk for mid-term mortality in a large cohort of patients undergoing elective CABG.

Methods

Material and design in study I

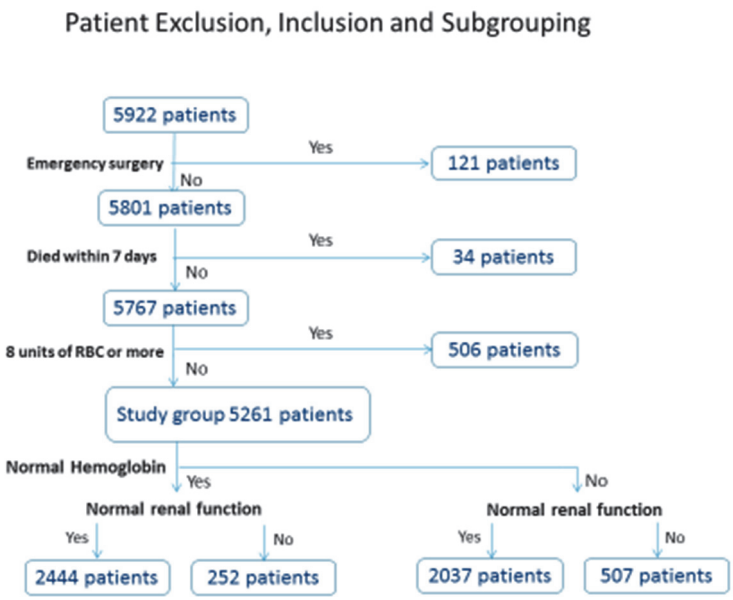
Study design

The patients included in this study underwent cardiac surgery at the Cardiothoracic Department at the University Hospital in Lund, Sweden, from 1 January 2002 to 31 December 2008. Data were collected from four principal sources. The clinical data were retrieved from the in-house quality database, which continuously collects relevant clinical information from the perioperative care during the patients' hospital stay. Extracts from the databases of the hospital clinical chemistry laboratory and hospital blood bank served as the second and third source of data. The survival and time of death for each patient was checked against the national tax registry in May 2009 defining the follow-up period from 0.5 to 7.5 years. Where data were missing or extreme outliers were identified, patient records were read to complete the database. In any case of data mismatch between different data sources, the data were manually controlled by assessing the patient's records.

Patient inclusion and exclusion

All patients who underwent CABG as their sole cardiac procedure were included in the study (n=5922). Patients who underwent emergent surgery, defined as surgery within 1 h of the decision to operate, were excluded (n=121). Patients who died during the first 7 days were also excluded (n=534). Patients who received 8 or more units of blood (n=506) were excluded. The cut-off of 8U was chosen as 8U of blood clinically represents, together with plasma, more than half the blood volume in most patients and indicates a massive bleeding where the transfusion is lifesaving. A total of 5261 patients were finally included in the analysis.

Figure 2



Selection of the study group and subgroup creation

Database management

The preoperative characteristics and intraoperative information (*Table 5*) were entered in the in-house quality database by each surgeon, with a 100% completion rate. Survival data were based on the tax registry, which has no missing data. Laboratory test results were extracted from the hospital laboratory and, therefore, all blood samples analyzed were entered into the database. One hundred and four patients had missing preoperative creatinine values. For these patients, the preoperative creatinine was estimated using an imputation technique based on the first postoperative day creatinine value [126]. The formula used (preoperative creatinine=15.0 + 0.86 x creatinine day 1) was calculated using regression analysis for the relation between preoperative creatinine and creatinine day 1 [*The correlation for this estimate was r=0.89, and bias and precision (SE) according to Bland Altman were 1.3 and 0.25, respectively [127]*]. Twenty one patients did not have a preoperative Hb concentration, and mean value substitution was used by the statistical program. Post-operatively, renal function for the patients was categorized using RIFLE criteria based on preoperative creatinine and the maximum creatinine during the hospital stay [128].

The study cohort was divided into four subgroups based on a dichotomization of preoperative Hb and renal function (high Hb/normal eGFR, low Hb/normal eGFR,

high Hb/low eGFR, and low Hb/low eGFR, respectively, *Figure 2*). The cut-off for normal Hb was defined as Hb>136 g/l (8.44mmol/l) for males and Hb>125 g/l (7.58mmol/l) for females, which represent the median values for each sex in the study population. The cut-off for renal function was a normal or a mild decrease in the preoperative eGFR, defined as eGFR>60 mg/ml/1.73m^2, calculated according to the MDRD formula *(eGFR=32,788 x Serum creatinine$^{-1.154}$ x Age$^{-0.203}$ x [0.704 if female])*.

Material and design in study II

Study design

The study protocol was approved by the local ethics committee. All patients who had undergone cardiac surgery at the Department of Cardiothoracic Surgery at the University Hospital in Lund, Sweden, from January 1st 2002 to December 31st 2008 were included.

Database management

Data were mainly collected from the hospital's quality control database, in which information is continuously collected on perioperative care during patients' hospital stay. Other sources of data were the blood bank and clinical chemistry databases at the hospital. Survival or time of death for each patient was determined from the national tax registry in May 2011, defining the follow-up period from 2.5 to 9.5 years. Where data were missing or extreme outliers were identified, patient records were read to complete the database. In cases of data mismatch between different data sources, the data were manually checked by consulting the patient's records.

After completing missing data from patient records, preoperative p-creatinine values were still missing in 104 patients. For these patients, the preoperative p-creatinine level was estimated using an imputation technique based on the p-creatinine level one day after surgery. The formula used was: Creatinine day 0 = 15.0 + (0.86 x Creatinine day 1), calculated by applying regression analysis to the relation between the preoperative p-creatinine level (on day 0) and the p-creatinine one day after surgery (day 1) [126, 129].

Patient inclusion and exclusion

All patients who had undergone CABG as the sole surgical intervention between January 1st 2002 and December 31st 2008 were included. The following predetermined exclusion criteria were used: emergency operation (defined as operation within one hour of the decision to operate) (n=125, 2.1%), preoperative eGFR (MDRD) < 15 ml/min (n=20, 0.3%) and death within 7 days of operation (n=51, 0.9%) as we aimed to study long-term outcomes, and patients that die early most often suffer major cerebral or cardiac insult, and very seldom die from AKI due to available renal replacement therapies. Of a total of 5943 patients, 5746 constituted the study group after application of the exclusion criteria.

Measurements of renal function

Kidney function was based on p-creatinine levels, either the absolute p-creatinine value, eGFR [39], or the eCrCl [38]. Changes in p-creatinine, eGFR and eCrCl during the hospital stay were compared with the preoperative values (baseline value) to assess changes in kidney function. The commonly used MDRD formula used is:

eGFR ml/min/1.73m^2=32788 x p-creatinine$^{-1.154}$ x Age$^{-0.203}$ x [1.210 if black] x [0.742 if female] [39].

In our population, the number of black patients is well below one percent and not recorded in journals. Therefore, we used the original formula with only that adjustment: eGFR=32788 x p-creatinine$^{-1.154}$ x Age$^{-0.203}$ x [0.742 if female]. The Cockcroft–Gault formula used was:

eCrCl (female) ml/min= ([140-age] x weight in kg x 1.04)/ (p-creatinine in µmol/l) or

eCrCl (male) ml/min= ([140-age] x weight in kg x 1.23)/ (p-creatinine in µmol/l) [38]. eGFR and eCrCl values calculated on the highest concentration of post-operative p-creatinine (p-creatinine$_{peak}$) are denoted eGFR$_{peak}$ and eCrCl$_{peak}$, respectively. In line with this, eGFR and eCrCl values calculated on the level of p-creatinine closest to the day of discharge from hospital (p-creatinine$_{discharge}$) are denoted eGFR$_{discharge}$ and eCrCl$_{discharge}$, respectively.

We assumed that the "peak" values corresponded to the poorest kidney function and "discharge" values to the level of kidney function at the time of discharge from hospital. All p-creatinine concentrations were determined with enzymatic colorimetric method [130] with a 1.4-1.7% co-efficient of variance. The reference method for determination of the calibrator used was ID/MS (isotope dilution / Mass spectrometry).

Definition of acute kidney injury

AKI was classified utilizing the RIFLE scale [11]. The RIFLE-staging was made according to changes in levels of kidney function measured by p-creatinine, the eGFR and eCrCl (*Table 4*). Urinary output data were not available, so we used only the p-creatinine-based RIFLE classifications.

Table 4.

Classification of acute kidney injury (AKI) using the RIFLE classification system

	Non-AKI	Risk	Injury	Failure
Creatinine	<50% creatinine increase	50-100% creatinine increase	100-150% creatinine increase	>150% creatinine increase or p-creatinine >350 µmol/l
MDRD eGFR	<25% eGFR decrease	25-50% eGFR decrease	50-75% eGFR decrease	>75% eGFR decrease or preop. p-creatinine >350 µmol/l
Cockroft–Gault eCrCl	<25% eCrCl decrease	25-50% eCrCl decrease	50-75% eCrCl decrease	>75% eCrCl decrease or preop. p-creatinine >350 µmol/l

MDRD = modification of diet in renal disease. eGFR = estimated glomerular filtration rate, eCrCl = estimated creatinine clearance.

Material and design in study III

Endpoints

The primary endpoint of this study was renal function measured by p-cystatin C level changes measured at the third postoperative day compared to the preoperative p-cystatin C level.

The secondary endpoints were other renal biomarkers including the changes in plasma-Neutrophil gelatinase-associated lipocain (p-NGAL), p-creatinine, p-urea and incidence of AKI according to the Risk Injury Failure Loss End-stage (RIFLE) criteria [11] and based on eGFR calculated by the Modification of Diet in Renal Disease (MDRD) formula [39].

Additional secondary endpoints were cardiac and cerebral organ injury assessed by p-troponin-I, p-CKMB, p-BNP, p-S100B, time in ventilator, time in cardiothoracic intensive care unit (ICU), postoperative bleeding, transfusion requirement during days 0-4, and overall outcome after surgery.

Patient selection

Patients scheduled for surgery were screened for the study, and if determined to be suitable, they were informed about the study and asked to participate (usually 1-3 days before the operation) by a physician not responsible for their immediate care. Inclusion criteria were non-emergent CABG, preoperative estimated glomerular filtration rate (eGFR) < 60 ml/min and ≥15ml/min (based on p-Cystatin C), and written and oral consent. Exclusion criteria were uncontrolled hypertension (defined as previously undetected hypertension with no anti-hypertensive therapy), hypersensitivity to the active drug, pregnancy, fertile women (<50 years old), treatment with erythropoietin up to 4 weeks prior to the surgery, on-going dialysis, planned off-pump CABG surgery, known malignancy, inclusion in other on-going clinical trial, or clinical judgment by the investigators that the patient could not participate in the study due to inability to assimilate information such as linguistic barriers.

Material and design in study IV

Study design

This was a prospective study where s-cystatin C was sampled for all patients undergoing cardiac surgery at the Department of Cardiothoracic Surgery at Skane University Hospital in Lund, Sweden, from January 1, 2010 to December 31, 2012. The study design and protocol was approved by the local ethics committee. All patients who had undergone elective CABG as their sole intervention were included in this analysis. Death within 7 days of operation (n=9, 0.05%) was a predetermined exclusion criteria as patients that die early most often suffer major cerebral or cardiac insult, and very seldom die from renal causes due to available renal replacement therapies. The final study cohort included 1995 patients.

Database Management

Data was mainly collected from the internal quality database, which is continuously updated with data on pre-, peri-, and postoperative patient care. The second source

of data was collected from the blood bank and the clinical chemistry databases in the hospital. Survival and time of death for each patient was checked against the national tax registry in December 2013 defining the follow-up period from a minimum of 1 year to a maximum of 4 years. One hundred and forty seven patients had missing preoperative s-creatinine values and 255 patients had missing preoperative s-cystatin C values. For those patients, the preoperative missing values were estimated using an imputation technique based on the value on day 1 as previously described [126]. The formula used for estimating missing preoperative s-creatinine values *(preoperative creatinine = 14.648 + 0.889*creatinine day 1)* was calculated by applying regression analysis for the relation between preoperative s-creatinine level and s-creatinine level at day 1 *(r = 0.927*) for patients with complete data. The formula used for estimating the preoperative s-cystatin C *(pre-op cystatin C= 0.178 + 0.901* cystatin C day 1)* was calculated by applying regression analysis on the relation between preoperative s-creatinine level and s-creatinine level at day 1 *(r = 0.918*) for patients with complete data.

Measurements of renal function

The assessment of renal function was based on the following measurements and formulas: (1) absolute s-creatinine and by two different equations ($eGFR_{creatinine}$ according to the MDRD [39] and the Chronic Kidney Disease Epidemiology [CKD-EPI] formula [42]); (2) absolute s-cystatin C and by two different $eGFR_{cystatin\ C}$ equations (the 2012 CKD-EPI as presented by Inker *et al*. [36] and the Caucasian and Asian Pediatric and Adult subjects (CAPA) as presented recently by Grubb *et al*. [37]); (3) the combined GFR estimates of s-creatinine and s-cystatin C according to the formula presented by Inker *et al* [36]. The preoperative renal function was referred to as baseline for assessing changes in renal function postoperatively.

All s-creatinine concentrations were determined using an enzymatic colorimetric method with a 1.4-1.7% coefficient of variation. The reference method for determining the calibrator used was ID/MS (Isotope Dilution / Mass spectrometry) [130]. S-cystatin C levels were measured by particle-enhanced turbidimetric immunoassay (Tina-quant®) with a 1.0-4.1% coefficient of variation (Roche Diagnostics Ltd. Rotkreuz, Switzerland).

Surgery and postoperative care

Surgery was performed in a standardized manner. All patients underwent on-pump surgery with either cold St. Thomas crystalloid cardioplegia or cold blood cardioplegia administered in the aortic root. Postoperatively, patient circulation was monitored invasively and urinary output was monitored during patient care in the cardiothoracic intensive care unit. The need of diuretics, volume substitute, and

transfusion therapy was evaluated in each patient by the physician in charge at the cardiothoracic intensive care unit.

Statistics

Statistical analysis was performed with Statistica software, version 9.0 in studies I-III & version 12.0 in study IV (Stat Soft, Inc. Tulsa, OK, USA). The R project software was also used in studies I&II (version 1.13.0 in study I and version 2.10.1 in study II). Results are presented as mean ± 1SD, median (interquartile ranges [25th to 75th]), or number of patients (percentages). A p-value of 0.05 was considered as statistically significant.

The Chi-square, adjusted Hazard Ratio (HR) and 95% confidence intervals (95% CI) were calculated for each predictor. P values <0.05 (two-tailed) were considered statistically significant.

Student's t test

Student's t-test was used for group comparisons, where numbers were large and not strongly skewed.

Wilcoxon-Mann-Whitney test

The Wilcoxon-Mann-Whitney test was performed for group comparisons where numbers were small or skewed.

Cox analysis

The Cox proportional hazard model has been used as the statistical instrument in studies I, II, & IV to determine which factors had an adverse predictive impact on long-term mortality. Wald statistics was used to determine the strength of relations in study I & II. In study IV, the chi-square has been used to determine the strength of a relation due to the newer version of the Statistica® program. The increased risk for mortality during the study period expressed as adjusted hazard ratio (HR) was obtained from the multivariate analysis. The Cox model in study I was based on predictors for decreased survival found in previous studies focusing on mortality, renal function, or blood transfusion. In addition, a stepwise removal of non-significant variables was performed separately. In study II, the covariates for the final model were selected from a stepwise elimination of non-significant variables.

In study IV, all known risk factors for increased risk of mortality on the subject were initially entered into the model, and a stepwise backward elimination method was used to determine the final model.

In studies I & II, to test the proportional hazard assumption for a Cox regression model fit, Schoenfeld residuals were used with R-project (version 1.13.0). In study IV, proportional hazard assumption was tested by using scaled schoenfeld residuals.

Kaplan-Meier

Unadjusted Kaplan-Meier curves were used to show differences in survival between groups in study II and between the quintiles in study IV. The log-rank test was used to compare statistical significance of differences between groups in study IV.

ANOVA model

Continuous repeated measures in study III were compared using a two-way repeated measures ANOVA model (group x time and group). The tests were two tailed.

Results

Results study I

Study population

In the study group, 2618 patients (49.8%) received a blood transfusion. These patients had a higher preoperative morbidity (*Table 5*) and their postoperative outcome was also worse compared with patients not receiving a blood transfusion (*Figure 3*). The survival analysis was divided into two parts, where the first used a Cox analysis on the entire cohort excluding or including preoperative Hb and renal function. The second analysis divided the study cohort into four groups depending on their preoperative level of anemia and renal function (*Table 6*).

The Cox proportional hazard ratio analysis without preoperative Hb and eGFR showed a hazard ratio 1.097 [95% confidence interval (CI) 1.05–1.15, P<0.0001] per unit of transfused blood (*Table 7*).

Age, a history of COPD, diabetes, history of cerebrovascular disease, and perfusion time were all associated with worse outcome in the analysis. Female gender and use of CPB were associated with better outcome. When preoperative Hb and eGFR were added to the Cox proportional hazard analysis, the hazard ratio for blood transfusion became 1.046 (95% CI 1.00–1.10, P=0.0650) per unit transfused (*Figure 3, Table 7*).

Table 5 Preoperative patient characteristics

Variable		Entire study group					Subgroup			
			Transfused with RBC				Transfused with RBC			
			No	Yes				No	Yes	
	n=5261	Mean	n=2643	n=2618	p	N=2444	Mean	n=1562	N=882	p
Age	5261	67 (9.7)	65.1 (9.4)	68.9 (9.6)	n.s.	2444	64.4 (9.3)	63.5 (9)	65.9 (9.7)	<0.0001
Female gender	5261	1155 (22%)	344 (13%)	811 (31%)	<0.0001	2444	471 (19%)	203 (13%)	268 (30.4%)	=0.013
Body Mass Index (BMI)	5256	27.2 (4)	27.6 (3.8)	26.7 (4.1)	0.0017	2441	27.5 (3.9)	27.9 (3.8)	26.7 (4)	<0.0001
Diabetes	5261	1180 (22%)	573 (21.7%)	607 (23.2%)	n.s	2444	473 (19%)	311 (19.9%)	162 (18.4%)	n.s
COPD	5261	483 (9.2%)	209 (7.9%)	274 (10.5%)	<0.0001	2444	172 (7%)	110 (7%)	62 (7%)	n.s
Peripheral vascular disease	5261	483 (9.2%)	303 (11.5%)	438 (16.7%)	<0.0001	2444	273 (11.2%)	164 (10.5%)	109 (12.4%)	n.s
Neurological dysfunction	5261	88 (1.7%)	37 (1.4%)	51 (1.9%)	<0.0001	2444	40 (1.6%)	25 (1.6%)	15 (1.7%)	=0.016
History of Cerebrovscualar	5261	460 (8.7%)	194 (7.3%)	266 (10.2%)	<0.0001	2444	179 (7.3%)	98 (6.3%)	81 (9.2%)	n.s
Previous vascular surgery	5261	229 (4.4%)	88 (3.3%)	141 (5.4%)	<0.0001	2444	77 (3.2%)	47 (3%)	30 (3.4%)	<0.0001
Critical Pre-operative state	5261	160 (3%)	32 (1.2%)	128 (4.9%)	<0.0001	2444	42 (1.7%)	9 (0.6%)	33 (3.7%)	=0.044
LVEF 30-50%	5261	1435 (27.3%)	637 (24.1%)	798 (30.5%)	=0.0002	2444	552 (22.6%)	335 (21.4%)	217 (24.6%)	<0.0001
LVEF<30%	5261	309 (5.9%)	112 (4.2%)	197 (7.5%)	<0.0001	2444	100 (4.1%)	58 (3.7%)	42 (4.8%)	n.s
Euroscore	5261	4.6 (3.1)	3.7 (2.7)	5.5 (3.3)	<0.0001	2444	3.6 (2.8)	3.1 (2.5)	4.4 (3)	=0.0001
NYHA I	5261	1294 (24.6%)	732 (27.7%)	562 (21.5%)	<0.0001	2444	682 (27.9%)	458 (29.3%)	224 (25.4%)	<0.0001
II	5261	1764 (33.5%)	957 (36.2%)	807 (30.8%)	=0.040	2444	881 (36%)	594 (38%)	287 (32.5%)	n.s
III	5261	1620 (30.8%)	742 (28.1%)	878 (33.5%)	=0.011	2444	680 (27.8%)	408 (26.1%)	272 (30.8%)	n.s
IV	5261	582 (11.1%)	212 (8%)	370 (14.1%)	<0.0001	2444	200 (8.2%)	102 (6.5%)	98 (11.1%)	n.s
CCS I	5261	203 (3.9%)	122 (4.6%)	81 (3.1%)	<0.0001	2444	105 (4.3%)	79 (5.1%)	26 (2.9%)	<0.0001

	II	5261	1786 (33.9%)	1012 (38.3%)	774 (29.6%)	=0.0012	2444	908 (37.2%)	620 (39.7%)	288 (32.7%)	<0.0001
	III	5261	2265 (43.1%)	1141 (43.2%)	1124 (42.9%)	n.s	2444	1070 (43.8%)	684 (43.8%)	386 (43.8%)	n.s
	IV	5261	1003 (19.1%)	366 (13.8%)	637 (24.3%)	<0.0001	2444	359 (14.7%)	178 (11.4%)	181 (20.5%)	n.s
Urgency	Elective (>1 week)	5261	3403 (64.7%)	1844 (69.8%)	1559 (59.5%)	=0.0007	2444	1720 (70.4%)	1167 (74.7%)	553 (62.7%)	<0.0001
	Prioritized (<1 week)	5261	1509 (28.7%)	688 (26%)	821 (31.4%)	=0.0043	2444	610 (25%)	346 (22.2%)	264 (29.9%)	=0.0003
	Urgent (<24 hours)	5261	349 (6.6%)	111 (4.2%)	238 (9.1%)	<0.0001	2444	114 (4.7%)	49 (3.1%)	65 (7.4%)	=0.0009
	Emergent (<1 hour)	5261	0 (0%)	0 (0%)	0 (0%)	<0.0001	2444	0 (0%)	0 (0%)	0 (0%)	<0.0001
Use of CPB		5261	5066 (96.3%)	2531 (95.8%)	2535 (96.8%)	<0.0001	2444	2351 (96.2%)	1498 (95.9%)	853 (96.7%)	<0.0001
Perfusion time (min)		5066	79.5 (24.8)	74.4 (22.2)	84.6 (26.2)	<0.0001	2351	78.2 (24.9)	74.8 (22.3)	84.1 (28.1)	=0.0005
Crossclamp time (min)		5066	47.4 (16.7)	45.1 (15.8)	49.7 (17.2)	<0.0001	2351	46.9 (16.7)	45.5 (15.4)	49.3 (18.5)	<0.0001
IABP before surgery		5261	131 (2.5%)	25 (0.9%)	106 (4%)	<0.0001	2444	38 (1.6%)	9 (0.6%)	29 (3.3%)	<0.0001
Previous CABG		5261	98 (1.9%)	34 (1.3%)	64 (2.4%)	<0.0001	2444	50 (2%)	24 (1.5%)	26 (2.9%)	<0.0001
Previous PCI		5261	636 (12.1%)	312 (11.8%)	324 (12.4%)	n.s	2444	305 (12.5%)	195 (12.5%)	110 (12.5%)	<0.0001
Pre-op Hemoglobin (g/L)		5241	133.2 (15.5)	138.2 (14.2)	128 (15.1)	=0.001	2444	144.8 (9)	146.4 (8.9)	142.1 (8.7)	n.s
Pre-op Creatinine (µmol/L)		5260	86.5 (36.1)	83.1 (23.6)	90 (45.1)	<0.0001	2444	77.6 (14)	78.2 (13.3)	76.5 (15.1)	n.s
Pre-op eGFR (mL/min/1,73 m2)		5260	84.3 (23.6)	88.3 (21.8)	80.1 (24.7)	<0.0001	2444	91.3 (18.1)	92.1 (17.9)	89.8 (18.5)	<0.0001
30-day mortality (study population)		5261	20 (0.4%)	2 (0.08%)	18 (0.7%)	=0.0002	2444	2 (0.1%)	0 (0%)	2 (0.2%)	n.s
30-day mortality (all)*		5942	116 (2.0%)	8 (0.3%)	108 (3.3%)	<0.0001	2625	24 (1%)	2 (0.13%)	22 (2.0%)	<0.0001

Pre-op characteristics of the study population presented as either mean (standard deviation) for continuous variables or number (percent) for dichotomous variables. The subgroup is defined as patients with no anemia and normal or a mild decrease in renal function preoperatively.Cox analysis on the entire cohort

Table 6

Post-operative outcome.

Variable		All (mean/median)	Entire study group		P	Mean/median	Subgroup (normal Hb and eGFR)		P
			Transfused with RBCs				Transfused with RBCs		
			No (n = 2643)	Yes (n = 2618)			No (n = 1562)	Yes (n = 882)	
Time in ICU (h)	5152	22 (19–24)*	22 (18–23)*	23 (20–30)*	0.0007	22 (19–24)*	22 (18–23)*	22 (19–26)*	0.1849
Time on ventilator (min)	5161	360 (255–540)*	320 (230–450)*	420 (300–675)*	<0.0001	355 (240–505)*	310 (220–430)*	425 (300–695)*	<0.0001
Reoperation for bleeding	5258	123 (2.3%)	7 (0.3%)	116 (4.4%)	<0.0001	72 (2.9%)	7 (0.4%)	65 (7.4%)	<0.0001
Post-operative myocardial infarction	4672	70 (1.5%)	20 (0.8%)	50 (2.2%)	0.0002	26 (1.2%)	6 (0.4%)	20 (2.6%)	<0.0001
Post-operative permanent stroke	4672	23 (0.5%)	10 (0.4%)	13 (0.6%)	0.4975	6 (0.3%)	4 (0.3%)	2 (0.3%)	0.8964
Post-operative transient cerebral ischemia	4672	28 (0.6%)	11 (0.5%)	17 (0.7%)	0.2325	7 (0.3%)	6 (0.4%)	1 (0.1%)	0.2330
Post-operative sepsis	4672	38 (0.8%)	7 (0.3%)	31 (1.3%)	0.0001	17 (0.8%)	4 (0.3%)	13 (1.7%)	0.0005
Post-operative atrial fibrillation	4672	1139 (24.4%)	498 (21%)	641 (28%)	<0.0001	482 (22.2%)	281 (20%)	201 (26%)	0.0025
Post-operative mediastinitis	5258	56 (1.1%)	22 (0.8%)	34 (1.3%)	0.1003	20 (0.8%)	13 (0.8%)	7 (0.8%)	0.9157
RIFLE – risk at discharge	5228	500 (9.6%)	210 (8%)	290 (11%)	0.0002	192 (7.9%)	120 (8%)	72 (8%)	0.7120
RIFLE – injury at discharge	5228	117 (2.2%)	58 (2.2%)	59 (2.3%)	0.9222	48 (2%)	28 (1.8%)	20 (2.3%)	0.4324
RIFLE – failure at discharge	5228	95 (1.8%)	26 (1%)	69 (2.6%)	<0.0001	23 (0.9%)	12 (0.8%)	11 (1.2%)	0.2470
Received RBCs	5261	2618 (49.8%)	0 (0%)	2618 (100%)	<0.0001	882 (36.1%)	0 (0%)	882 (100%)	<0.0001
Units of RBCs transfused	5261	1.6 (1.9)	0 (0)	3.1 (1.6)	<0.0001	1.1 (7)	0 (0)	3 (1.5)	<0.0001
Received plasma	5261	1533 (29.1%)	296 (11%)	1237 (47%)	<0.0001	635 (26%)	176 (11%)	459 (52%)	<0.0001

Units of plasma transfused	5261	1.2 (2.9)	0.4 (1.3)	2.1 (3.7)	<0.0001	1.1 (45)	0.4 (1.4)	2.5 (4)	<0.0001
Received platelets	5261	418 (7.9%)	51 (2%)	367 (14%)	<0.0001	195 (8%)	31 (2%)	164 (19%)	<0.0001
Units of platelets transfused	5261	0.2 (0.7)	0 (0.4)	0.3 (0.8)	<0.0001	0.2 (8)	0 (0.4)	0.4 (0.9)	<0.0001
Hemoglobin at discharge	5190	103 (11.2)	103.4 (11.6)	102.5 (10.8)	0.0078	104.6 (153)	105.4 (12)	103.1 (10.3)	<0.0001
30-day mortality (study population)	5261	20 (0.4%)	2 (0.08%)	18 (0.7%)	=0.0002	2 (0.1%)	0 (0%)	2 (0.2%)	0.1786
30-day mortality (all)*	5942	116 (2.0%)	8 (0.3%)	108 (3.3%)	<0.0001	24 (1%)	2 (0.13%)	22 (2.0%)	<0.0001

Post-operative outcome in the entire cohort and in the subgroup with no anemia and normal or a mild decrease in renal function. Significant levels in italics. *Outcome variables that are strongly skewed and are presented as median with a range between the first and the third quartile. *30-day mortality without exclusion criteria (emergent surgery, 48 RBCs transfused, and dead within 7 days), RBC=Red blood cell

Table 7 Cox Proportional hazard model

	All without Hb/GFR				All with Hb/eGFR				Subgroup normal Hb and eGFR			
	HR	95% C.I.	Wald	p	HR	95% C.I.	Wald	p	HR	95% C.I.	Wald	p
Age (per year)	1.063	1.05-1.07	115.7	<0.0001	1.049	1.04-1.06	65.1	<0.0001	1.074	1.05-1.10	38.9	<0.0001
COPD	1.966	1.57-2.46	34.8	<0.0001	1.855	1.48-2.33	28.8	<0.0001	2.240	1.41-3.56	11.6	=0.001
Diabetes	1.668	1.37-2.02	26.7	<0.0001	1.577	1.30-1.92	21.0	<0.0001	1.296	0.87-1.93	1.6	n.s.
History of Cerebrovscualar	1.394	1.09-1.79	6.8	=0.009	1.365	1.06-1.75	5.9	=0.015	1.278	0.77-2.13	0.9	n.s.
Peripheral vascular disease	1.751	1.43-2.15	28.5	<0.0001	1.608	1.30-1.98	19.8	<0.0001	1.896	1.24-2.91	8.6	=0.003
Female gender	0.709	0.57-0.88	9.6	=0.002	0.626	0.50-0.78	17.4	<0.0001	0.818	0.49-1.36	0.6	n.s.
Previous CABG	1.445	0.90-2.31	2.4	n.s.	1.446	0.91-2.31	2.4	n.s.	1.784	0.84-3.79	2.3	n.s.
Use of CPB	0.671	0.49-0.92	6.1	=0.014	0.766	0.56-1.06	2.6	n.s.	0.564	0.31-1.04	3.4	n.s.
Perfusion time (per min)	1.006	1.00-1.01	9.6	=0.002	1.006	1.00-1.01	10.5	=0.001	1.012	1.01-1.02	15.6	<0.0001
IABP after surgery	1.363	0.87-2.15	1.8	n.s.	1.510	0.96-2.38	3.1	n.s.	1.317	0.41-4.22	0.2	n.s.
Body Mass Index (BMI)	0.990	0.97-1.01	0.6	n.s.	0.989	0.96-1.01	0.8	n.s.	0.997	0.95-1.05	0.0	n.s.
Reoperated for bleeding	0.542	0.25-1.16	2.5	n.s.	0.653	0.30-1.40	1.2	n.s.	0.265	0.04-1.97	1.7	n.s.
Pre-operative Hemoglobin (g/l)	n/a	n/a	n/a	n/a	0.986	0.98-0.99	19.7	<0.0001	1.008	0.99-1.03	0.5	n.s.
Pre-operative eGFR mL/min/1.73m2	n/a	n/a	n/a	n/a	0.990	0.99-0.99	23.8	<0.0001	1.005	0.99-1.02	0.7	n.s.
Transfusion of RBC (per unit)	1.097	1.05-1.15	16.1	<0.0001	1.046	1.00-1.10	3.4	n.s.	1.015	0.91-1.13	0.1	n.s.

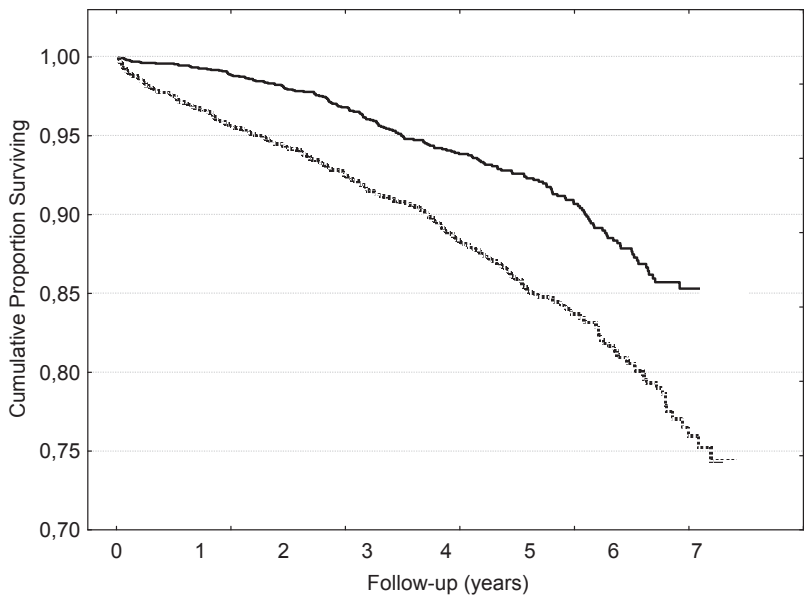
Cox proportional hazard analysis of two groups. The entire cohort with and without pre-operative hemoglobin. Subgroup with normal, or near normal, eGFR and normal hemoglobin. COPD=Chronic Obstructive Pulmonary Disease. CABG = Coronary Artery Bypass grafting. CPB = Cardiopulmonary Bypass. IABP=Intraaortic Balloon Pump. eGFR= estimated Glomerular Filtration rate. RBC=Red blood Cells. n/a = not applicable, n.s. = not significant

Subgroup analysis

By dichotomizing preoperative hemoglobin (Hb) and eGFR, four subgroups were created. (*Figure 2*) There were 2444 patients who had a preoperative Hb level higher than the median for their sex and an eGFR >60 ml/min/ 1.73m2. Of these, 882 (36.1%) received a transfusion of blood.

The patients receiving blood transfusions had more preoperative comorbidities and worse post-operative outcome (*Tables 6 & 7*). Cox proportional hazard analysis yielded a hazard ratio of 1.015 (95% CI 0.91–1.13, P=0.7873.) per unit blood transfused (*Table 8, Figure 4*) in patients with Hb levels lower than the median for their sex and an eGFR <60 ml/min/1.73m2. Of these, 383 (75.5%) received a transfusion of blood. Cox proportional hazard analysis yielded a hazard ratio of 1.016 (95% CI 0.92–1.12, P=0.7503) per unit transfused (*Table 8*). The hazard ratio in a survival analysis did not reach significance for the other two groups, patients with normal Hb/low eGFR and low Hb/normal eGFR, respectively.

Figure 3



Patients at risk								
Not transfused	2643	2429	2011	1634	1243	888	539	158
Transfused	2518	2361	2023	1623	1285	862	448	105

Unadjusted Kaplan–Meier plot for the entire study group, where the group is divided between patients receiving a transfusion (broken red line) and patients not receiving red blood cell transfusion (solid blue line).

Table 8

Cox proportional hazard model – subgroups.

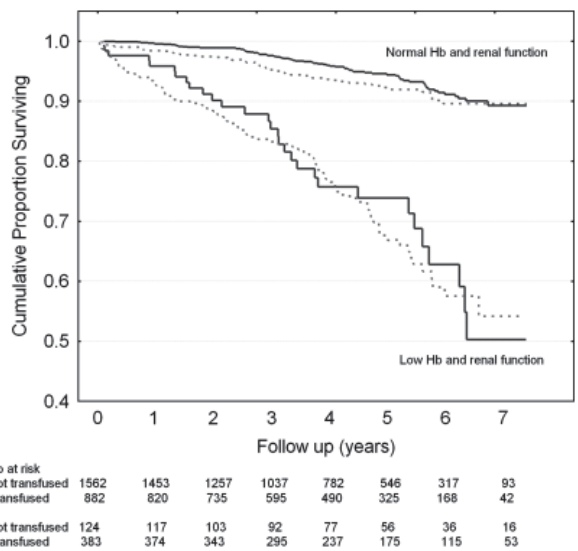
	Subgroup with normal Hb and eGFR				Subgroup with low Hb and eGFR			
	HR	95% CI	Wald	*P*	OR	95% CI	Wald	*P*
Age (/year)	1.074	1.05–1.10	38.9	*<0.0001*	1.031	1.01–1.06	6.1	*0.0132*
COPD	2.240	1.41–3.56	11.6	*0.0007*	1.885	1.20–2.96	7.6	*0.0058*
Diabetes	1.296	0.87–1.93	1.6	0.2047	1.551	1.05–2.28	5.0	*0.0259*
History of cerebrovascular	1.278	0.77–2.13	0.9	0.3472	1.179	0.73–1.91	0.5	0.5022
Peripheral vascular disease	1.896	1.24–2.91	8.6	*0.0034*	1.854	1.25–2.75	9.4	0.0022
Female gender	0.818	0.49–1.36	0.6	0.4368	0.985	0.65–1.49	0.0	0.9429
Previous CABG	1.784	0.84–3.79	2.3	0.1325	0.717	0.22–2.33	0.3	0.5796
Use of CPB	0.564	0.31–1.04	3.4	0.0661	1.051	0.56–1.96	0.0	0.8751
Perfusion time (/min)	1.012	1.01–1.02	15.6	*<0.0001*	1.005	1.00–1.01	1.8	0.1850
IABP after surgery	1.317	0.41–4.22	0.2	0.6431	4.181	1.88–9.28	12.4	*0.0004*
BMI (kg/m^2)	0.997	0.95–1.05	0.0	0.8953	0.973	0.93–1.02	1.2	0.2719
Reoperated for bleeding	0.265	0.04–1.97	1.7	0.1946	0.376	0.05–2.83	0.9	0.3417
Pre-operative hemoglobin (g/l)	1.008	0.99–1.03	0.5	0.4759	1.001	0.98–1.02	0.0	0.8773
Pre-operative eGFR ml/min/1.73 m^2	1.005	0.99–1.02	0.7	0.4083	0.982	0.97–1.00	5.6	0.0178
Transfusion of RBCs (/U)	1.015	0.91–1.13	0.1	0.7873	1.016	0.92–1.12	0.1	0.7503

Cox proportional hazard analysis of two groups. First, subgroup with normal, or near normal, eGFR and normal Hb (2444 patients total with 133 deaths) and thereafter the subgroup with decreased eGFR and low hemoglobin (507 with 124 deaths).

Significant *p*-levels in italics. A stepwise elimination with transfusion forced to remain in the model (normal Hb and eGFR), gave the following results (hazard ratio [confidence interval], significance level): Age 1.070 [1.06–1.09], P<0.0001, COPD 2.332 [1.47–3.70] P<0.0001, peripheral vascular disease 2.022 [1.34–3.04], P<0.0001, use of CPB 0.529 [0.29–0.96], P = 0.0372, perfusion time 1.014 [1.01–1.02], P<0.0001, and transfusion of RBCs 0.978 [0.89–1.08], P = 0.647.

A stepwise elimination with transfusion forced to remain in the model (low Hb and eGFR), gave the following results: Age 1.03 [1.01–1.06], P=0.0087, COPD 1.761 [1.14–2.72] P=0.0109, diabetes 1.498 [1.03–2.18] P=0.0360, peripheral vascular disease 1.982 [1.38–2.86], P<0.0001, IABP post-operatively 3.782 [1.69–8.46], P= 0.0011, pre-op eGFR 0.982 [0.97–1.00], P= 0.0106, & transfusion of RBCs 1.020 [0.93–1.11], P=0.659.

Figure 4



Unadjusted Kaplan-Meier plot for two of the subgroups of patients depending on the preoperative renal function and hemo-globin levels. The groups are divided into patients receiving red blood cell (RBC) transfusion (broken red line) and patients not receiving an RBC transfusion (solid blue line).

Results study II

A total of 5746 patient were analyzed over a median follow-up time of 6.0 years (range 2.5-9.5 years). The incidence of postoperative AKI (RIFLE classes R, I and F) varied depending on the method used to determine renal function. The number of patients classified as non-AKI differed depending on time point (*i.e.* peak or discharge) and the method of measuring renal function (*i.e.* p-creatinine, eCrCl or eGFR). The lowest number was found using eGFR, and the highest using p-creatinine as presented in *Tables 9* and *10*.

Table 9.

Relative risk of mortality (expressed as adjusted hazard ratio) after CABG surgery depending on RIFLE class at poorest measured renal function ($RIFLE_{peak}$) during hospital stay. The three methods used to measure renal function were: 1) p-creatinine, 2) the Cockroft-Gault formula for creatinine clearance and 3) Modification of Diet in Renal Disease (MDRD) formulae. P-values refer to comparison with non-AKI.

		Non-AKI	R	I	F
1	P-creatinine n=5742	5092 (88.7%)	1.57 (1.31-1.89) [p<0.000001] 463 (8.1%)	1.62 (1.13-2.33) [p=0.009435] 103 (1.8%)	1.90 (1.32-2.74) [p=0.000600] 84 (1.5%)
2	Cockroft-Gault eCrCl n=5735	4147 (72.3%)	1.32 (1.13-1.56) [p= 0.000716] 942 (16.4%)	1.53 (1.23-1.90) [p=0.000145] 327 (5.7%)	2.03 (2.03-2.53) [p<0.000001] 319 (5.6%)
3	MDRD eGFR n=5742	3855 (67.1%)	1.29 (1.09-1.51) [p=0.002536] 1051 (18.3%)	1.85 (1.51-2.27) [p<0.000001] 400 (7.0%)	1.85 (1.85-2.26) [p<0.000001] 436 (7.6%)

Table 10.

Relative risk of mortality (expressed as hazard ratio) during follow-up depending on RIFLE class at discharge ($RIFLE_{discharge}$). The three methods used to measure renal function were: 1) p-creatinine, 2) the Cockroft–Gault formula for creatinine clearance, and 3) MDRD eGFR.

		Non-AKI	R	I	F
1	P-creatinine n=5742	5579 (97.2%)	1.90 (1.39-2.87) [p=0.000050] 123 (2.1%)	2.07 (1.09-3.92) [p=0.025779] 24 (0.4%)	1.15 (0.57- 2.33) [p=0.693115] 16 (0.3%)
2	Cockroft-Gault eCrCl n=5735	5116 (89.2%)	1.37 (1.13-1.67) [p=0.001363] 442 (7.7%)	2.00 (1.40-2.86) [p=0.000160] 89 (1.6%)	1.60 (1.12-2.28) [p=0.010398] 88 (1.5%)
3	MDRD eGFR n=5742	4920 (85.7%)	1.45 (1.21-1.74) [p=0.000045] 561 (9.8%)	1.75 (1.30-2.37) [p=0.000275] 143 (2.5%)	1.94 (1.43-2.63) [p=0.000018] 118 (2.0%)

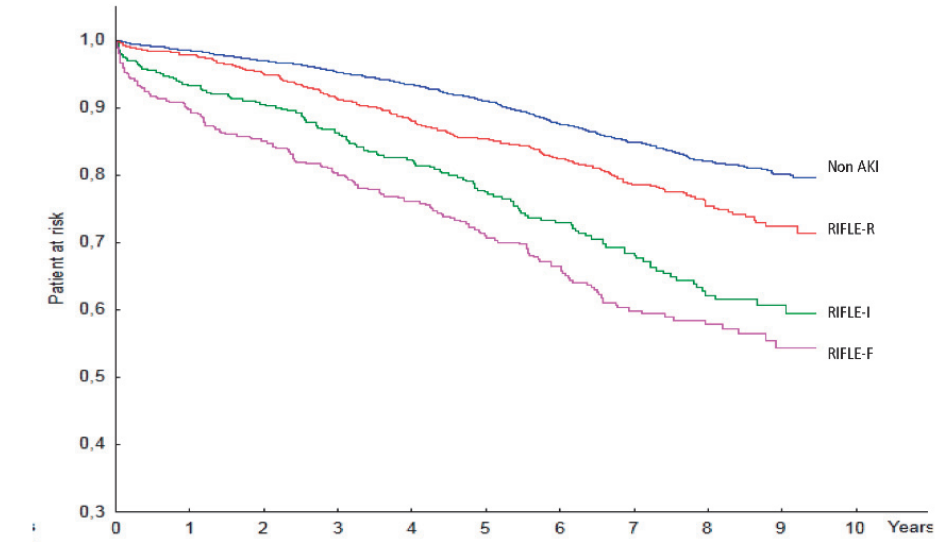
RIFLE classification based on poorest renal function

Comparing the three methods of renal function measuring, the $eGFR_{peak}$ method yielded the highest number of patients classified as AKI while the lowest number of patients classified as AKI was found in the p-$creatinine_{peak}$ group. The risk of mortality during the study period, expressed as HR, increased significantly in all RIFLE classes as compared to non-AKI for all three expressions of renal function (*Table 9 and Figure 5*). The HR increased with more severe RIFLE class, *i.e.* HR for RIFLE class F>I>R>non-AKI, with $eGFR_{peak}$ F as the only exception. Using the $eGFR_{peak}$ and $eCrCl_{peak}$ led to more patients being classified as RIFLE classes R, I and F, than when p-$creatinine_{peak}$ was used.

RIFLE classification based on renal function at discharge

More patients were classified in RIFLE classes R, I, and F when using $eCrCl_{discharge}$ or $eGFR_{discharge}$ than when using p-$creatinine_{discharge}$. In addition, $eGFR_{discharge}$ led to more patients being classified as AKI than eCrCl and p-creatinine (*Table 10*). HR increased progressively with more serious RIFLE class only in the $eGFR_{discharge}$ measures. Classification using p-$creatinine_{discharge}$ and $eCrCl_{discharge}$ showed a progressive increase in hazard ratio compared to non-AKI in RIFLE classes R and I, but the value of HR decreased in RIFLE class F. With p-$creatinine_{discharge}$ RIFLE class F as only exception, all hazard ratios in RIFLE classes R, I, and F for all three methods were significantly higher as compared to non-AKI, respectively.

Figure 5



Non AKI	3855	3511	3031	2520	1955	1392	826	278	118	60
RIFLE R	1051	948	784	642	497	383	247	109	52	23
RIFLE I	400	348	295	241	209	162	120	60	38	27
RIFLE F	436	390	345	295	245	202	148	96	65	45
Total	5742	5197	4455	3698	2906	2139	1341	543	273	155

Unadjusted Kaplan-Meier plot depicting the survival of patients depending on level of AKI stratified by the RIFLE classification based on MDRD $eGFR_{peak}$ calculations.

Dynamics of renal recovery and its impact on survival

A two-dimensional matrix in which one dimension was $RIFLE_{peak}$ and the other was $RIFLE_{discharge,}$ both based on eGFR, was constructed to demonstrate the effect of recovery (*Table 11*). The HR for long-term mortality for all postoperative AKI (RIFLE classes R, I and F) with or without recovery was 1.56 (CI 1.37-1.77, $p<0.001$). The hazard ratio for patients with any degree of AKI who showed complete recovery to non-AKI was 1.42 (CI 1.21-1.66, $p<0.001$). The hazard ratio for patients with any degree of remaining AKI at discharge was 1.69 (CI 1.44-1.99, $p<0.001$). Long-term mortality HR for patients who recovered to non-AKI at discharge was not increased if $eGFR_{peak}$ had been RIFLE R. However, if $eGFR_{peak}$ classified the patients as RIFLE I or F, it resulted in an increased HR, where $RIFLE_{peak}$ I had an HR 1.58 (CI 1.18-2.10, $p=0.002$), and those classified as $RIFLE_{peak}$ F had an HR 2.53 (CI 1.84-3.48, $p<0.001$).

Table 11.

Relative risk of mortality (expressed as hazard ratio) during follow-up depending on the combination of RIFLE class at discharge ($RIFLE_{discharge}$) and poorest measured renal function ($RIFLE_{peak}$). Renal function was measured by MDRD eGFR.

	Non-AKI peak	R peak	I peak	F peak
Non-AKI at discharge	3855 (67.1%)	1.18 (0.98-1.43) P=0.085694 723 (12.6%)	1.58 (1.18-2.10) P=0.001975 204 (3.6%)	2.53 (1.84- 3.48) P<0.00001 139 (2.4%)
R at discharge		1.49 (1.16-1.91) P=0.001891 328 (5.7%)	2.17 (1.59-2.96) P<0.00001 125 (2.2%)	1.19 (0.80-1.76) P=0.382464 107 (1.9%)
I at discharge			1.95 (1.27-3.00) P=0.002285 71 (1.2%)	1.82 (1.18-2.81) P=0.007228 72 (1.3%)
F at discharge				2.07 (1.50-2.85) P=0.000008 118 (2.1%)

Relative importance of renal function at different time points

In a separate survival analysis where the three values of eGFR—preoperative ($eGFR_{Preoperative}$) levels, levels at poorest renal function ($eGFR_{peak}$), and levels at discharge ($eGFR_{discharge}$)—were entered separately, the $eGFR_{peak}$ levels contributed the most to the model and were thus the most predictive for outcome. The HR for $eGFR_{Preoperative}$ was 0.99 ($p<0.0001$, Wald=32.91), the HR for $eGFR_{discharge}$ was 0.98 ($p<0.0001$, Wald=44.98), and the HR for $eGFR_{peak}$ was 0.98 ($p<0.0001$, Wald=80.16).

To further clarify the most important time point for renal measurement, all three variables ($eGFR_{preoperativ}$, $eGFR_{discharge}$, and $eGFR_{peak}$) were forced into a separate Cox analysis, and only the $eGFR_{peak}$ values remained significant with a HR 0.98 ($p<0.0001$, Wald=41.4). The values for $eGFR_{discharge}$ changed to HR 1.0 ($p=0.3$, Wald=1.1), and the values for preoperative eGFR changed to HR1.0 ($p=0.6$, Wald=0.25).

Results study III

A total of 633 patients were screened in the study. Non eligibility was mainly due to an eGFR greater than 59 ml/min. A total of seventy-five patients met the inclusion criteria and were enrolled. Five patients were withdrawn due to protocol violation, and the remaining 70 patients completed the study per protocol with 35 patients randomized to receiving EPO and 35 patients to receiving saline (*Table 12*). In a two-way repeated measures ANOVA (group and group x time), there were no significant differences in the primary outcome or other renal outcome (*i.e.* p-Cystatin C, p-NGAL, p-Creatinine, p-Urea, and MDRD eGFR), between the two groups (*Table 13 & Figure 6*). The relative p-Cystatin C level changes from baseline between the groups were 132% ± 43% (mean ± SD) for the study group and 122% ± 23 % for the control group on day 2, 131%± 31% and 125% ± 24 % on day 3, and 124%± 35% and 113% ± 21 % on day 4 (*Figure 6*). In addition, there was no difference in incidence of postoperative AKI according to RIFLE criteria. All data was complete to 100% except for one p-Cystatin C value on the second postoperative day in the placebo group and one p-Cystatin C value on the fourth postoperative day in the EPO group.

Figure 6

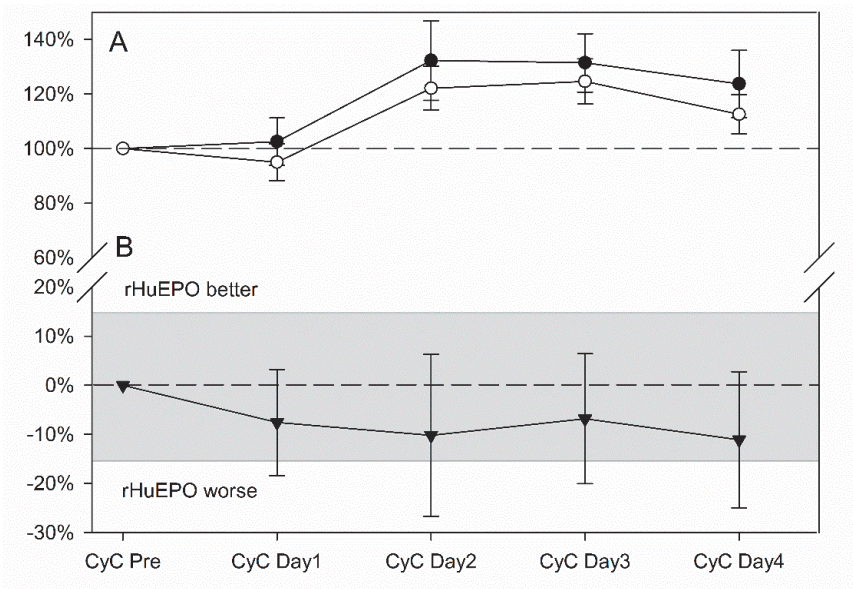


Figure 6A shows Cystatin-C changes, expressed as percent of baseline, for the EPO group (solid dots) and placebo (open dots) with 95% confidence interval. Figure 6B shows relative differences between Cystatin C levels for the groups (triangles) with 95% confidence interval. Grey area in figure 6B reflects a ±15% change in the primary end-point. CyC= Cystatin C; Pre= Preoperative; EPO= recombinant Human Erythropoietin. Day1-Day4 denote days after surgery.

Table 12. Preoperative characteristics

Variable	EPO	Placebo
Female	7(20%)	8(23%)
Age	72.4 ± 8.1	72.5 ± 10.5
Weight	82.9 ± 14.2	82.8 ± 15.6
Height	174.3 ± 10.1	174.6 ± 8.0
Systolic BP	142.7 ± 19.7	135.1 ± 18.9
Diastolic BP	73.7 ± 7.9	75.5 ± 12.8
Hypertension	29(83%)	30(86%)
Chronic heart failure	15(43%)	15(43)%
LVEF<30%	4(11%)	4(11%)
LVEF30-50%	11(31%)	11(31%)
LVEF>50%	20(57%)	20(57%)
COPD	1(3%)	2(6%)
Diabetes	12(34%)	15(43%)
PVD	4(11%)	4(11%)
Previous CVI	6(17%)	5(14%)
Thyroid disease	4(11%)	8(23%)
CAF	1(3%)	1(3%)
PAF	5(14%)	2(6%)
Previous PCI	6(17%)	5(15%)
Preoperative IABP	0	0
Diuretics	17(49%)	18(51%)
ACE-i/ARB	24(69%)	22(86%)
ß-blocker	29(80%)	33(94%)
Statins	32(91%)	32(91%)
Vitamin-K antagonist	6(17%)	1(3%)
ASA	32(91%)	32(91%)
Other antithrombotic drug	10(29%)	7(20%)

ACE-i = angiotensin converting enzyme-inhibitor; ARB = angiotensin receptor blocker; ASA = acetyl salicylic acid; CAF = chronic atrial fibrillation; COPD = chronic obstructive pulmonary disease; CVI = cerebrovascular insult; IABP = intraaortic balloon pump; LVEF = left ventricular ejection fraction; PAF = paroxysmal atrial fibrillation; PCI = percutaneous coronary intervention; PVD = peripheral vascular disease; rHuEPO = recombinant human erythropoietin.

No patient in either group required renal replacement therapy postoperatively. The overall incidence of AKI was 31% (n=22/70), as defined by the RIFLE classification based on p-Creatinine and the MDRD formula, where 24% (17/70) were classified as RIFLE Risk, 6% (4/70) classified as RIFLE Injury, and 1% (1/70) classified as RIFLE Failure. The overall postoperative outcomes measures were also similar with no statistically significant difference between the two groups. (*Table 14*). Transfusions of erythrocyte, plasma, and platelets did not differ during the perioperative period and four days after surgery (*Table 14*). There were no

statistically significant differences in the other secondary end-points between the two groups (*Table 14*). BNP on day 1 was 305 ± 215 for the placebo group and 346 ± 318 for the EPO group (p=0.48). The CKMB levels for the placebo group at the first postoperative day was 14.0 ± 15.0 for the placebo group and 20.1 ± 28.4 for the EPO group (p=0.46). S100B levels on the second postoperative day were 0.13 ± 0.06 for the placebo group and 0.13 ± 0.06 for the EPO group (p=0.94). There was no statistically significant difference in the incidence of pre-defined adverse events, (i.e. postoperative mediastinitis, atrial fibrillation, peri-procedural acute myocardial infarction, chest tube drainage, and reoperation for bleeding) or other adverse events. One patient in the control group suffered a postoperative stroke. The total number of reported adverse events was 24, with 13 events in the placebo group and 11 events in the EPO group (p=0.62), none of which were deemed to be associated with the drug.

Table 13. Renal outcome

	EPO n=35	**Placebo n=35**	**p**
Preop CyC	1.6 ± 0.4	1.5 ± 0.2	
CyC Day 1	1.6 ± 0.6	1.4 ± 0.5	
CyC Day 2	2.1 ± 0.8	1.8 ± 0.5	0.35*
CyC Day 3	2.1 ± 0.8	1.9 ± 0.5	
CyC Day 4	1.9 ± 0.8	1.7 ± 0.5	
Preop Creatinine	119.2 ± 33.6	115.4 ± 34.7	
Creatinine Day 1	121.2 ± 47.8	108.4 ± 45.5	
Creatinine Day 2	150.5 ± 75.0	131.7 ± 60.2	0.38*
Creatinine Day 3	149.6 ± 75.6	132.7 ± 61.0	
Creatinine Day 4	139.0 ± 66.5	123.7 ± 55.0	
MDRD eGFR preop	56.3 ± 14,4	58.0 ± 14,5	
MDRD eGFR day3	49.4 ± 20.5	53.8 ± 19.0	0.60*
RIFLE 0 MDRD eGFR	24(66%)	25(71%)	0.61
RIFLE R MDRD eGFR	8(23%)	9(26%)	0.78
RIFLE I MDRD eGFR	3(9%)	1(3%)	0.31
RIFLE F MDRD eGFR	1(3%)	0	0.32
Preop NGAL	105.7 ± 32.7	94.5 ± 12.7	0.51*
Postop NGAL	165.5 ± 98.7	167.7 ± 105.0	

Preop=Preoperative; Postop= Postoperative; CyC= Plasma-Cystatin-C; Creatinine= Plasma-Creatinine; eGFR= estimated Glomerular Filtration Rate; and p- NGAL= Plasma Neutrophil Gelatinase-associated Lipocain. MDRD eGFR represents GFR estimated by Modification of Diet in Renal Disease formula. All eGFR is standardized to 1.73m2 body surface. P-Cystatin C unit is mg/L; P-Creatinine unit is µg/L, and P-NGAL unit is ng/ml. RIFLE categorization is based on the third postoperative eGFR according to the MDRD formula. Day 1-4 represents the first to fourth day after surgery. Figures are presented as mean±SD or number (percent). * Denotes that statistical testing was performed with repeated measures ANOVA (p-value for group x time), otherwise a Student's t-test was performed.

Table 14. General Outcome

Variable	EPO n = 35	Placebo n = 35	*P*
ICU time (hours) †	23 (22 - 48)	25 (21 - 46)	0.67
Ventilator time (minutes) †	300 (215 - 420)	255 (210 - 400)	0.30
Fluid balance day 1 (L) †	2.9 (2.2 - 3.7)	3.0 (2.3 - 3.9)	0.93
Diuresis 12h postop (ml)	1715 ± 732	1725 ± 667	0.96
Bleeding 12 hours postop (ml) †	450 (340 - 600)	450 (360 - 620)	0.95
Total bleeding (ml) †	650 (500 - 980)	450 (500 - 940)	0.54
Weight postop day 3 (Kg)	84.4 ± 15.4	84.3 ± 13.8	0.96
Reoperation for bleeding	2 (6%)	1 (3%)	0.56
ECC time (min) †	64 (49 - 82)	71 (42 - 90)	0.92
Postoperative mediastinitis	1 (3%)	1 (3%)	1.00
Postoperative atrial fibrillation	10 (29%)	11 (31%)	0.80
Peri procedural myocardial damage	2 (6%)	0	0.16
Postoperative heart failure	0	2 (6%)	0.16
Reoperation for bleeding	0	2 (6%)	0.15
Postoperative transient cerebral insult	1 %)	0	0.33
Postoperative permanent stroke	0	0	
RRT/dialysis	0	0	
Erythrocyte transfusion	22 (63%)	16 (45%)	0.15
Plasma transfusion	3 (9%)	4 (11%)	0.40
Platelet transfusion	2 (6%)	4 (11%)	0.70
Hb preoperative	129.1 ± 14.6	133.6 ± 14.8	
Hb Day 1	108.8 ± 13.2	112.0 ± 13.2	
Hb Day 2	98.9 ± 14.4	101.8 ± 12.3	0,09
Hb Day 3	98.1 ± 15.2	99.1 ± 8.3	
Hb Day 4	103.1 ± 12.4	100.3 ± 10.9	

Figures are presented as mean ±SD or number of patients (percent). * Denotes that statistical testing was performed with repeated measures ANOVA (p-value for group x time), otherwise a Student's t-test was performed. Outcome variables that are skewed (marked †) are presented as median (interquartile range) and tested with Mann-Whitney test. rHuEPO= Recombinant Human Erythropoietin; RRT= Renal Replacement Therapy.

Results study IV

Survival

A total of 1955 patients were analyzed over a median follow-up time of 2.5 years (range 1.0-4.0 years). Follow up was 100% complete. Overall survival was 97.5 ±0.4% and 93.0 ±0.8% at 1 and 4 years, respectively.

Table 15

Variable	Mean (± SD)
Age (year)	67.3 (± 9.8)
Female gender	405 (21 %)
Body Mass Index (BMI)	27.5 (± 3.8)
Diabetes	493 (25 %)
COPD	232 (12 %)
Anemia	181 (9 %)
Previous vascular surgery	100 (5 %)
Peripheral vascular disease	257 (13 %)
History of cerebrovascular injury	181 (9 %)
LVEF 30-50%	446 (23 %)
LVEF<30%	149 (8 %)
Previous myocardial infarction	1022 (52 %)
Euro score - additive	4.7 (±3.4)
NYHA class	
I	629 (32 %)
II	591 (30 %)
III	549 (28 %)
IV	186 (10 %)
CCS class	
I	232 (12 %)
II	833 (43 %)
III	626 (32%)
IV	264 (14 %)
IABP before surgery	62 (3 %)
Preoperative Hemoglobin (g/L)	135.9 (± 14.5)
Preoperative dialysis	70 (3.6 %)

Preoperative characteristics of the study cohort (No. 1955). Presented as either mean (± standard deviation) for continuous variables or number (percent) for dichotomous variables. CABG = coronary artery bypass grafting; CCS = Canadian Cardiovascular Society; COPD = chronic obstructive pulmonary disease; IABP = intra-aortic balloon pump: LVEF= left ventricular ejection fraction; NYHA= New York Heart Association

Baseline and outcome values

The general characteristics and outcome of the cohort are presented in *Tables 15* and *16*. The preoperative, first, second, and fourth postoperative days as well as the maximum values of s-creatinine and s-cystatin C registered together with the different GFR estimates are presented in *Table 17.*

Table 16

Use of CBP	1950 (99.7%)
Perfusion time (min)	77.2 (± 26.5)
Cross clamp time (min)	46.1 (± 17.6)
IABP after surgery	63 (3.2 %)
Time in the ICU (h)	37.5 (21-27)*
Sepsis	12 (0.6 %)
Myocardial infarction	22(1.1%)
Permanent stroke	9(0.5)%
Pneumonia	31(1.6%)
RRT (n=1706)	20 (1.0%)
Atrial fibrillation	465 (23.9%)
Time in ventilator (min)	305 (215-455)*
Re-operated for bleeding	44 (2.3%)
Re-operated for mediastinitis	17 (0.9%)
Blood transfusion (units)	1.3 (± 2.8)
Plasma transfused (units)	0.61 (± 2.5)
Platelets transfused (units)	0.28 (± 0.98)

Postoperative characteristics of the study cohort (No. 1955). Presented as either mean (± standard deviation) for continuous variables, number (percent) for dichotomous variables, or as median (interquartile range) for outcome variables that are skewed*. CBP = cardiopulmonary bypass; CCS = Canadian Cardiovascular Society; COPD = chronic obstructive pulmonary disease; IABP = intra-aortic balloon pump; LVEF = left ventricular ejection fraction; NYHA= New York Heart Association; PCI = percutaneous coronary intervention; Post-op = Postoperative; RRT = renal replacement therapy.

Different GFR estimates

All s-creatinine-based eGFR formulas resulted in statistically significant higher mean eGFR values compared to the s-cystatin C-based formulas with the closest estimates between the first postoperative day eGFR values according to the 2009 CKD-EPI$_{creatinine}$ formula and the 2012 CKD-EPI$_{cystatin\ C\ formula}$, (81.8±22.3 *vs.* 73.5± 26.3, p<0.001) (*Table 17 panels D&F*). The MDRD eGFR formula yielded statistically significant higher eGFR values compared to the 2009 CKD-EPI$_{creatinine}$ formula at all time-points with the largest difference preoperatively (82.3±24.0 for the MDRD formula *vs*. 66.9±20.6 for 2009 CKD-EPI$_{creatinine}$ formula, p<0.001). However, the difference between these two estimates was smaller postoperatively with the closest GFR estimates calculated when the renal function was measured at its lowest level (68.6± 24.6 for the 2009 CKD EPIcreatinine and 71.0±26.5 for the MDRD formula, p<0.001) (*Tables 17 panels C&D*). The 2012 CKD-EPI$_{cystatin\ C}$ formula yielded slightly higher eGFR estimates preoperatively (66.8± 22.6 compared to 65.4± 20.2 for the CAPA eGFR$_{cystatin\ C}$ formula, p=0.03). The eGFR by

these two formulas were similar for all postoperative GFR estimates with no statistically significant differences (*Table 17 panels F&G*).

Table 17

		No	Mean	Median	min	max	LQ	UQ	SD
A	*S-cystatin C, mg/ml*								
	Pre-op	1955	1.22	1.08	0.64	7.16	0.94	1.28	0.61
	Day 1 postop	1858	1.16	0.99	0.43	6.36	0.85	1.24	0.63
	Day 2 postop	1733	1.49	1.27	0.61	7.23	1.08	1.62	0.74
	Day 4 postop	1608	1.40	1.18	0.60	7.34	1.00	1.50	0.76
	Max measured value	1950	1.54	1.29	0.61	7.61	1.09	1.66	0.82
B	*S-creatinine, µmol/l*								
p	Pre-op	1955	92.7	82.0	34.0	949.0	71.0	96.0	61.1
	Day 1 postop	1951	87.4	75.0	28.0	806.0	63.0	90.0	63.5
	Day 2 postop	1934	105.7	89.0	34.0	719.0	74.0	114.0	61.9
	Day 4 postop	1830	99.0	82.0	37.0	671.0	70.0	103.0	62.4
	Max measured value	1955	112.4	92.0	34.0	992.0	76.0	119.0	77.6
C	*MDRD eGFR, ml/min/1.73m²*								
	Pre-op	1955	82	83	5	171	68	98	24
	Day 1 postop	1951	92	92	6	216	73	111	31
	Day 2 postop	1934	74	74	7	183	56	93	28
	Day 4 postop	1830	81	82	8	180	62	101	29
	Poorest measured value	1955	71	72	5	183	53	90	27
D	*CKD-EPIcreatinine ml/min/1.73m²*								
	Pre-op	1955	77	81	4	121	66	92	20
	Day 1 postop	1955	82	87	5	134	70	97	22
	Day 2 postop	1955	70	73	7	126	53	91	25
	Day 4 postop	1830	75	81	7	123	60	93	24
	Poorest measured value	1955	68	70	5	123	51	88	25
E	*CKD-EPIcystatin C creatinine ml/min/1.73m²*								
	Pre-op	1955	72	74	5	124	59	87	21
	Day 1 postop	1858	78	81	6	144	63	97	25
	Day 2 postop	1733	61	62	6	123	46	78	23
	Day 4 postop	1607	67	70	6	129	51	84	24
	Poorest measured value	1950	59	61	5	123	43	77	23
F	*CKD-EPI cystatin C ml/min/1.73m²*								
w	Pre-op	1955	67	67	6	124	52	83	23
	Day 1 postop	1858	74	75	7	138	55	94	26
	Day 2 postop	1733	54	54	5	126	38	69	22
	Day 4 postop	1608	59	60	5	127	42	76	24
	poorest measured value	1950	53	52	5	126	37	67	22
G	*CAPAcystatin C ml/min/1.73m²*								
	Pre-op	1955	65	66	3	124	54	79	20
	Day 1 postop	1858	72	73	4	191	56	89	25
	Day 2 postop	1733	54	54	2	134	40	67	20
	Day 4 postop	1608	58	60	2	136	44	73	22
	poorest measured value	1950	52	53	2	134	39	66	21

Distribution of renal measures with mean, median, min, max, upper quartile (UQ), lower quartile (LQ) and standard deviation (SD). S-cystatin C unit is mg/L; P-Creatinine unit is µmol/L; all eGFR is standardized to ml/min/1.73m^2 body surface. Day 1 = first postoperative day; s-cystatin C max = the maximum value of s-cystatin C registered postoperatively; s-creatinine max = the maximum value of creatinine registered postoperatively; pre-op=preoperative. MDRD = Modification of Diet in Renal Disease formula; eGFR = estimated glomerular filtration rate; CKD-EPI = Chronic Kidney Disease-Epidemiology; CKD-EPI $eGFR_{creatinine}$ eGFR based on creatinine according to CKD-EPI 2009; CAPA= Caucasian and Asian Pediatric and Adult subjects; CAPA $eGFR_{cystatin\ C}$ eGFR based on the revised formula cystatin C; CKD-EPI $eGFR_{cystatin\ C\ creatinine}$ = eGFR based on the combination of cystatin C and creatinine eGFR according to CKD-EPI 2012.

Predictors of mortality

The risk factors used in the cox model had the following HR for mortality: Age HR 1.06 (95% CI 1.03-1.08, $p<0.001$), COPD HR 1.70 (95% CI 1.05-2.76, $p=0.031$), EF 30-50% HR 1.76 (95% CI 1.10-2.81, $p=0.019$) EF<30% HR 2.73 (95% CI 1.55-4.82, $p<0.001$), ventilator time HR 1.31 (95% CI 1.02-1.68, $p=0.032$), sepsis HR 3.49 (95% CI 1.17-10.41, $p= 0.025$), preoperative hemoglobin HR 0.97 (95%CI 0.96-0.98, $p< 0.001$), plasma transfusion HR 1.41 (95% CI 1.04-1.90, $p=0.027$).

S-cystatin C and eGFR based on s-cystatin C showed a higher hazard ratio for mortality compared to s-creatinine and s-creatinine-based eGFR regardless of time point or method of measurement. For s-creatinine-based estimates, the CKD-$EPI_{creatinine}$ max (*i.e.* the maximum measured s-creatinine level during hospital stay) showed the highest predictive risk value (*Table 18 panel B*). The relative changes in GFR estimates (preoperative to maximum) were not significant predictors in multivariate analysis whereas relative changes in absolute s-creatinine and s-cystatin C (preoperative to maximum) independently predicted mortality (*Table 18 panel D*). S-cystatin C and $eGFR_{cystatin\ C}$ had higher predictive risk values preoperatively compared to s-cystatin C max and $eGFR_{cystatin\ C}$ max (*Table 18 panel A&B*).

The association between preoperative s-cystatin C stratified in quintiles with overall mortality is presented in *Figure 7*. Patients with preoperative s-cystatin C in the fourth and fifth quintiles had significantly higher mortality compared to those with preoperative s-cystatin C in the first three quintiles (quintile 4 vs. quintile 1-3, log rank $p=0.01$; quintile 5 vs. quintile 1-3, log rank $p<0.01$; quintile 4 vs. quintile 5, log rank $p<0.01$).

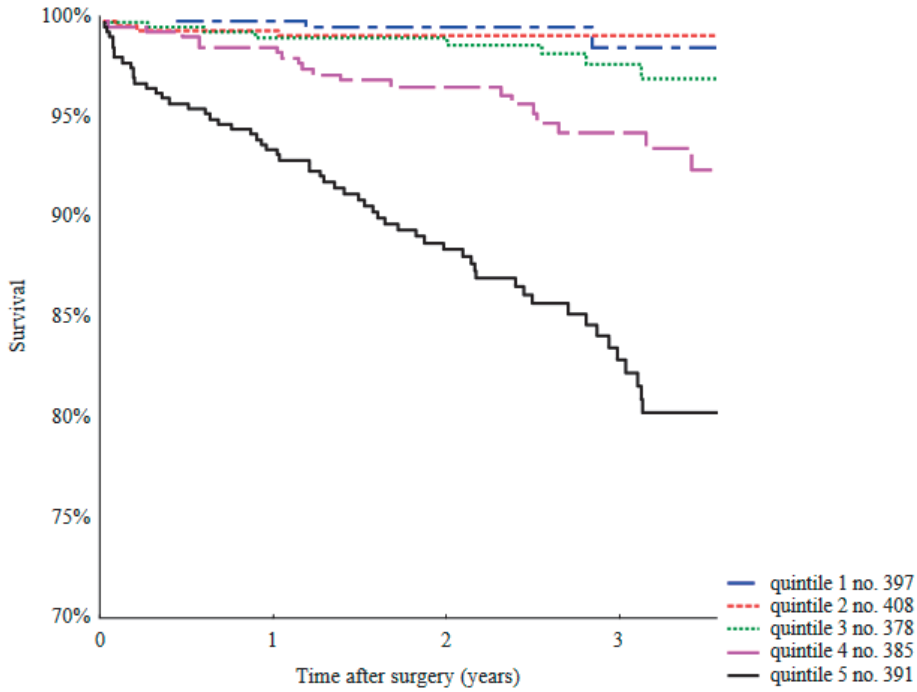
Table 18

	Variable	Chi 2	P	HR	95% CI
A	*Preoperative measures*				
	S-creatinine (µmol/L)	2.56	0.1093	1.00	1.00-1.00
	S-cystatin C (mg/L)	6.97	0.0083	1.30	1.07-1.58
	MDRD eGFR	4.19	0.0406	0.99	0.98-1.00
	CKD EPIcreatinine eGFR	4.07	0.0436	0.99	0.98-1.00
	CKD EPIcystatin C eGFR	22.84	0.0000	0.97	0.96-0.98
	CKD-EPIcystatin C + creatinine eGFR	14.45	0.0001	0.98	0.97-0.99
	CAPAcystatin C eGFR	21.04	0.0000	0.97	0.96-0.98
B	*Poorest renal function measures*				
	S-creatinine (µmol/L)	4.90	0.0269	1.00	1.00-1.00
	S-cystatin C (mg/L)	15.22	0.0001	1.41	1.19-1.68
	MDRD eGFR	5.69	0.0170	0.99	0.98-1.00
	CKD EPIcreatinine	6.63	0.0100	0.99	0.98-1.00
	CKD EPI cystatin C	20.57	0.0000	0.97	0.96-0.98
	CKD-EPI cystatin Creatinine	14.37	0.0002	0.98	0.97-0.99
	CAPA cystatin C	20.43	0.0000	0.97	0.96-0.98
C	*Day 1 measures*				
	s-creatinine (µmol/L)	3.63	0.0567	1.00	1.00-1.00
	s-cystatin C (mg/L)	13.12	0.0003	1.44	1.18-1.75
	MDRDcreatinine	5.55	0.0185	0.99	0.98-1.00
	CKD EPIcreatinine	6.02	0.0141	0.99	0.98-1.00
	CKD EPIcystatin C	26.20	0.0000	0.97	0.96-0.98
	CKD-EPIcystatin C creatinine	19.43	0.0000	0.98	0.97-0.99
	CAPAcystatin C	25.32	0.0000	0.97	0.96-0.98
D	*Changes from preoperative to poorest renal function*				
	s-creatinine	5.35	0.0208	1.64	1.08-2.49
	s-cystatin C	6.81	0.0091	2.05	1.20-3.51
	MDRD	1.71	0.1913	1.97	0.71-5.42
	CKD-EPIcreatinine	2.89	0.0892	2.39	0.87-6.55
	CKD-EPIcystatin C	0.65	0.4202	1.58	0.52-4.76
	CKD-EPIcystatin creatinine	1.68	0.1953	2.08	0.69-6.31
	CAPAcystatin C	1.14	0.2846	1.80	0.61-5.29

Methods of renal measurements as predictors for mortality: (**A**) preoperative; (**B**) maximum s-cystatin C and s-creatinine values; (**C**) at the first postoperative day; (**D**) the relative changes in renal function from baseline to maximum.

HR = hazard ratio; CI = confidence interval; Day 1=first postoperative day; s-cystatin C max = the maximum value of s-cystatin C registered postoperatively; s-creatinine max = the maximum value of creatinine registered postoperatively; pre-op = preoperative; COPD = chronic obstructive pulmonary disease. MDRD = Modification of Diet in Renal Disease formula; eGFR = estimated Glomerular Filtration Rate; CKD-EPI = Chronic Kidney Disease-Epidemiology; CKD-EPI $eGFR_{creatinine}$ = eGFR based on creatinine according to CKD-EPI 2009; CAPA= Caucasian and Asian Pediatric and Adult subjects; CAPA $eGFR_{cystatin\ C}$ = eGFR based on the revised formula cystatin C; CKD-EPI $eGFR_{cystatin\ C\ creatinine}$ = eGFR based on the combination of cystatin C and creatinine eGFR according to CKD-EPI 2012.

Figure 7



Preoperative s-cystatin C quintiles and all-cause mortality. Cut-off points for s-cystatin C quintiles are <0.91, 0.91 to 1.02, 1.02 to 1.15, 1.15 to 1.35, and >1.35 mg/L. For comparison between quintiles there were no statistical significant difference between the quintiles 1-3. There were statistical significant difference between quintiles 1-3 and 4 ($p < 0.001$) and between quintiles 1-3 and 5 ($p< 0.001$) and also between quintiles 4 and 5 ($p<0.001$).

Discussion

Acute kidney injury is a common and serious complication after cardiothoracic surgery and is associated with increased risk of short- and long-term mortality. Despite extensive studies in the field, it has been difficult to come to an understanding of this syndrome partly due to divergent definitions of AKI and partly due to the limitations of available routine biomarkers to predict, prevent, and detect AKI. Much has been done recently to better define AKI. There is also ongoing work to find better suited biomarkers for AKI as well as to improve treatment of patients at risk or suffering from AKI.

In general, the aim of this work has been to contribute information on some aspects of AKI including *a*. the risk of blood transfusion when other comorbidities including renal function are taken into account; *b.* the epidemiology of AKI; *c*. the value of a routine available biomarker of kidney function (*i.e.* creatinine) in predicting mortality and in classifying AKI based on the RIFLE classification; *d.* the impact of renal recovery on long-term mortality; *e*. the renoprotective effect of erythropoietin in patients with high risk of developing AKI; *f.* evaluation of s-cystatin C as a renal biomarker and predictor of mortality in a patients undergoing cardiac surgery.

Paper I.

In the first paper, in a large cohort of patients, the effects of preoperative renal function, preoperative hemoglobin, and blood transfusion were studied to determine long-term survival. The study showed that by excluding patients with preoperative CKD, anemia, and those requiring life-saving blood transfusions (>8U), the blood transfusion itself did not increase the risk for long-term mortality. Thus, by excluding severely ill patients, blood transfusion did not exhibit the risk that otherwise has been associated with it [131-135].

The analysis in this study was performed in two parts in order to test the hypothesis methodically. The first step analyzed the relation between classical risk factors and survival, and a significant hazard ratio of 1.097 for each unit of blood transfused was found. When preoperative Hb level and eGFR was entered into the analysis, the hazard ratio was reduced with a half to 1.046 and was no longer significant (*Tabel 7*). This first analysis suggests that the risk of increased long-term mortality after blood transfusion is a covariate to the risk of having preoperative renal dysfunction

and anemia. In order to further elucidate the impact of preoperative Hb level and eGFR on survival, patients were divided into subgroups (*Figure 2*). The variables selected for the Cox survival analysis in paper I were predetermined based on previous reports in an effort to apply a more universal explanation model for the study material. The selection of exclusion criteria was primarily made to create a cohort that represents patients where the risks of transfusion are considered, so as to inform and guide the clinical decision process.

Although this paper challenges previous reports that link blood transfusion to increased long-term mortality [131-135]. The main finding is that blood transfusion may be a marker of other factors (i.e., renal dysfunction and anemia) that increase long-term mortality. These co-morbidities may act as markers of underlying disease, which may lead to a transfusion, and are ultimately the true reason—instead of the transfusion—that the patient has a worse prognosis. Thus, this study further points to the inherent risks of preoperative comorbidities for patients undergoing cardiac surgery.

Paper II

In the second paper, several aspects of postoperative renal function and AKI are studied in a retrospective large cohort of patients who have undergone non-emergent CABG. The aim was assessing different methods for evaluation of postoperative AKI (*i.e.* s-creatinine, MDRD eGFR, and CrCl) with the RIFLE system and correlating them to long-term mortality, as well as to study the effect of changes in renal function (*i.e.* recovery) on long-term mortality.

Method of measurement (s-creatinine, CrCl & MDRD eGFR)

The use of eGFR and eCrCl in conjunction with AKI has been questioned, and caution for the use of these estimates in the context of AKI has been advocated [69, 136, 137] given that these estimations have been developed for patients with chronic kidney disease and a steady p-creatinine, which is not the case in an AKI scenario. It has been shown that GFR equations perform poorly in critically ill patients [138]. However, these estimates have previously been used in conjunction with CSA-AKI [139-141]. Furthermore, when different estimates of renal function versus iohexol clearance in cardiac surgery were evaluated, it was found that MDRD eGFR was better than Cockcroft Gault eCrCl in estimating clearance in postoperative patients [33]. Nonetheless, the method of measurement showed to be important in this paper as eGFR based on the MDRD formula performed better compared to s-creatinine and eCrCl. Taken together, MDRD eGFR seems to be a better biomarker for GFR in cardiac surgery patients among the available routine biomarkers and under non

steady state conditions. In addition, this study supports that both eCrCl and eGFR were superior to crude p-creatinine in identifying patients with AKI after CABG surgery, and eGFR was slightly superior to eCrCl (*Tabel 9 & 10*).

Time point of measuring renal function (Pre-op, peak or discharge)

The findings in this study regarding the association between renal function and long-term mortality are in concordance with previous studies [142, 143]. Regarding the time point of measuring renal function, an analysis was performed where eGFR from three different time points (preoperative, peak and discharge) are entered separately into the Cox regression model, followed by another analysis where all eGFR measures are entered at the same time. The results are the same in both analyses, where $eGFR_{peak}$ is indisputably the strongest predictor (HR 0.98, $p<0.0001$). This corresponds to a risk increase of 2% for every ml/min decrease in $eGFR_{peak}$. The results of the survival analysis based on the renal function estimated from the highest postoperative level of p-creatinine (p-$creatinine_{peak}$) were similar to those reported in previous studies [140, 144, 145], where increasing renal dysfunction/RIFLE class resulted in increasing risk of long-term mortality.

The two dimensional matrix, studying the effect of recovery from AKI

To study the effect of renal recovery during hospital stay and its effect on long-term mortality, the poorest postoperative renal function and the renal function at discharge from hospital are combined to assess the impact of these changes in renal function on survival. RIFLE classes based on MDRD eGFR were used to create a two-dimensional matrix with peak and discharge RIFLE classes. In this analysis, recovery in general shows a beneficial effect in terms of survival with the exception of the group with a higher degree of AKI (i.e. RIFLE F). Nonetheless, once a patient has had an AKI, regardless of the level of recovery, a remaining increased risk of long-term mortality is shown in this analysis with the mortality risk proportional to the RIFLE class at discharge (*Table 11*). These findings are also in accordance with previous studies [81, 140, 142, 145-148].

Study design, strengths & limitations

One of the strengths of this paper is the database, which contains information on many perioperative variables such as laboratory results and transfusion data. In addition, many variables had a high completion rate, with more than 99% complete data follow up. Moreover, the present study includes only CABG patients, whereas

several other studies have included a mixed population [65, 146]. Although the study comprises more than 5000 individuals, the numbers of patients in the more serious classes of renal dysfunction are relatively small, especially in the two-dimensional matrix. This could lead to an over parameterization, which subsequently will lead to a risk of over- or underestimation of the HRs in these small groups. Other limitations are the retrospective nature of the study, lack of data on urinary output, and the inherent weaknesses of the MDRD and CG formula. A more accurate measure of GFR would further strengthen the results of a study of this type.

Paper III

The third paper describes a randomized clinical trial whose aim was to test EPO, an endogenous hormone with ertythropoetic and pleitotropic properties as a protective drug against AKI. It has shown promising results in animal and several human studies and therefore made it a good candidate. This trial included a group of patients with pre-existing impaired renal function who were at high risk for developing postoperative AKI [149]. This grouping of patients were assumed to be those most in need of a prophylactic drug.

Several renal outcome measures were used, and as primary end-point s-cystatin C was chosen since it is considered a highly accurate surrogate for GFR [33, 50, 150-152]. The salient finding of this study is that EPO has no renoprotective effects, either measured by p-cystatin C, p-Creatinine, p-NGAL, or incidence of AKI according to the RIFLE criteria (*Figure 6, Tabel 13*).

The results in this paper contrast with two recent human studies, where renoprotective effects of EPO in conjunction with cardiac surgery was found [106, 107]. However, other studies on cardiac surgery patients have failed to show this renoprotective effect [108, 109, 122]. The diverging results may be due to patient selection, time point, and dose of administered EPO. Notably, the two trials where EPO was reported to have a renoprotective effect included patients with low risk of developing AKI, in contrast to the trials finding no renoprotective effect, which included patients with high risk of developing AKI.

In summary, given the frequency and severity of AKI after cardiac surgery, there is an undisputed need for an efficient prophylactic treatment. Both animal and human data have indicated EPO as a promising and inexpensive candidate that could meet this demand. However, in this study including patients with the largest risk of developing AKI after CABG surgery, no renoprotective effect was found.

Adverse effects of EPO

This study was a phase II equivalent and was conducted as a pilot trial in a larger program of AKI prevention. The safety aspects of a single high-dose EPO was also considered given that in a study by Ehrenreich [124] using EPO for treatment of ischemic stroke, increased mortality in the EPO group was reported. We could not observe statistically significant difference or any trends between the two groups regarding the adverse effects of the drug, however, the trial was not designed and powered to study the safety aspects of EPO in this context.

Study design, strengths & limitations

Two important strengths in this paper are a homogenous patient population undergoing only CABG with CPB and that all patients had preoperative renal dysfunction, which is considered one of the strongest predictors for developing AKI after cardiac surgery [149]. This makes the study more relevant for clinical implementation. Another strength is that anaesthetic drugs during surgery were standardized to clinic routine, with no use of volatile agents or remifentanil, both shown to have preconditioning effects [153, 154] [155]. The fact that this was a single center study should be viewed as a strength as it contributes to limiting heterogeneity and confounding factors. A few limitations could be discussed. First, the absence of long-term follow up. Secondly, patients were not selected on the underlying mechanism of renal dysfunction, and, therefore, there was heterogeneity in this aspect. Another limitation is that only a single dosage of EPO was evaluated in this study.

Paper IV

In the fourth paper s-creatinine and s-cystatin C and their GFR estimates were compared regarding their ability to predict mortality in a single-center cohort of 1955 patients undergoing coronary artery bypass surgery. The findings show that the strongest predictors of mortality 1-4 years after cardiac surgery are eGFR based on p-cystatin C measured preoperatively and on the first postoperative day (*Table 18 & Figure 7*).

S-creatinine-based measures

The results of this paper show that preoperative absolute s-creatinine does not independently predict postoperative mortality. The maximum postoperative s-creatinine levels have a better predictive ability compared to preoperative s-

creatinine. The $eGFR_{creatinine}$ based on the MDRD and the 2009 CKD-EPI formula performs similarly in predicting mortality measured on the first postoperative day as well as when s-creatinine is at its maximum (*Table 18 panel C&D*). Interestingly both log s-creatinine and the two different $eGFR_{creatinine}$ (CKD-EPI & MDRD) at the first postoperative day show similar predictive ability to the corresponding values when renal function is at its lowest measured level. Thus it seems that the risk assessed by s-creatinine measured after the first postoperative day remains unchanged regardless of the further rises in s-creatinine beyond the first postoperative day [73]. This finding is also strengthened by the results presented in *Table18 panel D* where there is no increase in the risk for mortality when the relative changes of $eGFR_{creatinine}$ from baseline to the maximum level of s-creatinine postoperatively are evaluated in the Cox model. This finding is important since most of the previous studies focused on renal function and mortality have evaluated the peak levels of s-creatinine or the effect of recovery at discharge [140, 144, 145, 148, 156].

Thus the first postoperative s-creatinine and $eGFR_{creatinine}$ have similar predictive strength to the maximum postoperative values regardless of method used, *i.e.*, MDRD or CKD-EPI. This finding is important from a clinical point of view as the clinician may have the possibility to optimize patients' treatment in an earlier phase.

The cystatin C-based measures (s-cystatin C, CAPA & CKD-EPI equations)

Ledoux *et al*. [157] have previously shown that preoperative $eGFR_{cystatin\ C}$ is a better risk assessment tool for prediction of one-year mortality compared to $eGFR_{creatinine}$ after cardiac surgery including complex procedures [157]. However there is no data available at which time point cystatin C carries the most predictive value for mortality in patients undergoing cardiac surgery. Recently, two equations have been presented according to the new standardized calibration method [43], and validated in larger cohorts with better performance in GFR estimation compared with previous cystatin C-based GFR estimates, the 2012 CKD-EPI $eGFR_{cystatin\ C}$ presented by Inker *et al.* [36] and the CAPA formula presented by Grubb *et al*.[37]. These equations have not been evaluated in a cardiac surgical population.

In this paper the preoperative absolute s-cystatin C value, in contrast to s-creatinine, has shown to be a strong predictor of mortality. The strongest predictor for mortality is found when the GFR estimates of s-cystatin C are entered into the Cox analysis rather than absolute levels at all time-points measured. The cystatin C eGFR are calculated both by the 2012 CKD-EPI and the CAPA formulas, and they have performed similarly.

The relation between time point of s-cystatin C measures and mortality differs compared to s-creatinine. The first postoperative s-cystatin C measurement is a strong predictor, with a hazard ratio in level with the maximum s-cystatin C (*Table 18 panel B&C*). Furthermore, the preoperative $eGFR_{cystatin\ C}$ has a similar hazard ratio compared to that of maximum $eGFR_{cystatin\ C}$ (*Table 18 panels A&B*). We could also see that the relative changes of eGFR from baseline to maximum were not independent predictors of mortality when entered in the Cox proportional hazards model unless the crude value of s-cystatin C was used, and these relative changes resulted in a weak predictive power (*Table 18 panel D*). Thus, the preoperative $eGFR_{cystatin\ C}$ shows a hazard ratio for mortality similar to renal function when It was at its lowest level (*Figure 7*). In a study by Bell *et al*. [158] performed on general ICU patients, s-cystatin C was found to be a predictor of mortality independent of renal function measured by s-creatinine. As one explanation for their results, the authors suggest it could be due to subclinical renal impairment not being detected by s-creatinine. This may be an applicable explanation for the findings reported in this paper as well. It has also been shown that AKI independently predicts mortality after cardiac surgery [1]. Therefore, it can be assumed that s-cystatin C predicts the risk of an upcoming AKI better than s-creatinine. This is difficult to assess as AKI is challenging to use as an endpoint due to varying definitions and the dichotomous nature of the variable. However, in a study by Shlipak *et al*. [159], a strong association between preoperative s-cystatin C and postoperative AKI defined by the AKIN criteria was found.

The results of this study indicate that $eGFR_{cystatin\ C}$ is a powerful independent preoperative predictor of mortality, which can help clinicians make a better risk stratification and optimization of patients pre- and perioperatively.

Combination of s-cystatin C and s-creatinine GFR estimates

It has previously been shown that the combination of s-creatinine and s-cystatin C may produce a more accurate eGFR in the elderly, in patients with chronic kidney disease, in patients treated with corticosteroids and those with thyroid disease [44, 45, 160, 161]. However, other studies, reporting measures of outcome, have shown that GFR estimates based on cystatin C alone is superior to the combination formula and a strong predictor of dialysis and impaired survival.

The $eGFR_{cystatin\ C\ +creatinine}$ at different time points were calculated according to the CKD-EPI 2012 presented by Inker *et al*. [36]. The predictive power for mortality based on this combination was, as expected, stronger than for s-creatinine, but weaker than that for s-cystatin C.

Thus this study shows, the predictive ability of eGFR based on s-cystatin C alone for mortality in the cardiac surgical setting seems to be stronger than the eGFR based

on the combination of s-cystatin C and s-creatinine which is in line with previous studies reporting measures of outcome [162, 163].

Study design, strengths & limitations.

Two major strengths of this study is the relatively large number of patients with prospectively collected data and the homogenous patient population undergoing CABG as the sole intervention. The single center design of the study should be considered an additional strength as it contributes to limiting heterogeneity and confounding factors such as different care of patients and sampling point's methods for measuring s-creatinine and s-cystatin C. One limitation of this study is that, despite the large number of patients included, there were only 94 deaths during the follow-up period, which may be explained by the follow-up period of 1 to 4 years. This number was nonetheless enough to create statistically robust Cox proportional hazard survival models. Another limitation is that there were 255 patients that had missing pre-operative s-cystatin C, that we imputated from day1. However, in a post-hoc analysis we performed the same analysis as presented but with these patients excluded, and the results were unchanged.

Conclusions

- When preoperative hemoglobin levels and renal function are taken into account, moderate transfusions of RBC after CABG surgery do not seem to be associated with reduced long-term survival.

- The poorest measured postoperative renal function is the most predictive for mortality; recovery of renal function also seems to be beneficial.

- Until better biomarkers for renal function are found and used routinely, eGFR based on the MDRD formula seems to yield the most reliable results among the traditional biomarkers being used today to identify patients with AKI and risk assessment from long-term mortality after CABG.

- Intravenous administration of a single high-dose (400IU/kg) EPO did not have a renal protective effect in patients with reduced kidney function undergoing coronary artery bypass surgery.

- Preoperative eGFR based on cystatin C alone has a good predictive value for mortality risk prediction in cardiac surgery patients.

- Estimated glomerular filtration rate based on the CKD-EPI and CAPA formula have similar performance in predicting long-term mortality in cardiac surgery patients.

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