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Tone Bull Enger

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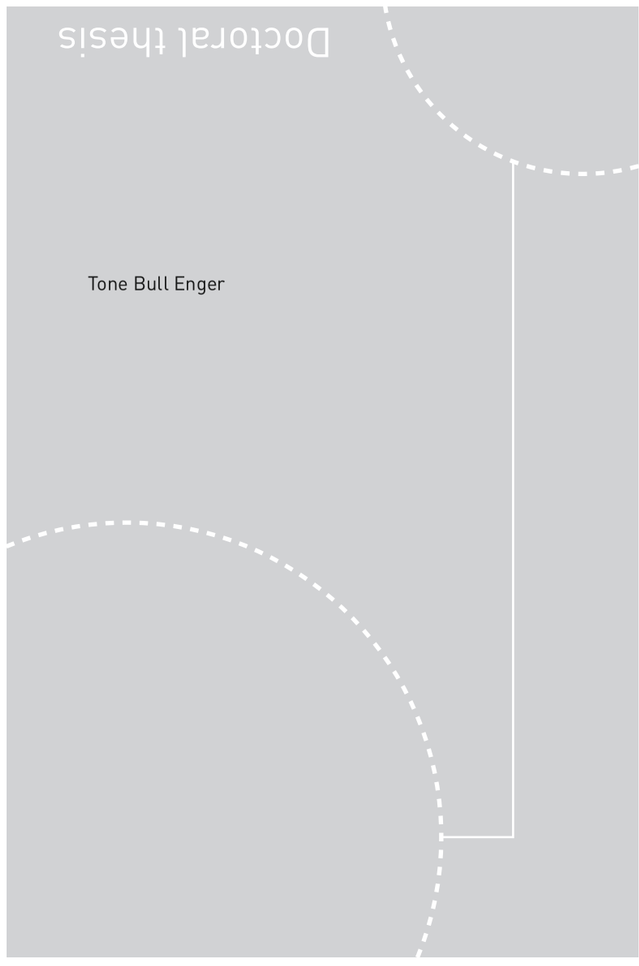

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Thesis for the Degree of Philosophiae Doctor

Trondheim, March 2017

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Risikofaktorer for kort- og langtidskomplikasjoner etter åpen hjertekirurgi hos voksne

Informasjon om forskjeller i signalveier relatert til inflammasjon (betennelse) og/eller endret sirkulasjon kan bidra i å identifisere pasienter med økt risiko for komplikasjoner etter hjerteoperasjon. Vi studerte sammenhengen mellom genetiske variasjoner og risikoen for økt væskeoppsamling under og etter operasjonen. En genetisk risikoskår viste at risikoen for væskeoverskudd økte lineært med antall genetiske risikovarianter. Samtidig analyse av fem markører (stoffer) i blod tilknyttet ulike potensielle årsaksmekanismer bedret evnen til å forutsi risikoen for akutt nyresvikt etter operasjonen.

Våre funn understreker hvordan komplikasjoner etter åpen hjertekirurgi ofte kan være sammensatte og skyldes flere faktorer. I mange tilfeller er det vanskelig å forutsi utfallet etter operasjonen ut fra tradisjonelle kliniske risikofaktorer. Den underliggende sårbarheten kan ha sammenheng med endret organfunksjon og/eller redusert reservekapasitet som kan fanges opp med markører i form av genetiske variasjoner eller stoffer i blod. Markørene kan avspeile en økt risiko for komplikasjoner uavhengig av tradisjonelle kliniske risikofaktorer. Ulike årsakssammenhenger kan være forskjellig vektet fra pasient til pasient, og derfor er det viktig å ta hensyn til mange faktorer samtidig når man skal vurdere pasientens totale risiko før operasjonen.

Vi fant at kort- og langtidsdødeligheten etter hjerteoperasjon har holdt seg uendret fra år 2000 til 2014, til tross for at hjertekirurgiske pasienter gjennomgår stadig mer kompliserte inngrep og i økende grad har flere preoperative risikofaktorer. Totalt sett hadde pasientene sammenliknbar overlevelse med den norske befolkningen de første syv årene etter operasjonen. Deretter falt overlevelsen gradvis. Nærmere analyse viste at overlevelsen til enkelte pasientgrupper var lavere enn den forventede levealderen ut ifra kjønn og alder. Dette gjaldt yngre pasienter, kvinner og pasienter som gjennomgikk andre inngrep enn bypassoperasjon.

Metodikk: Studiene har tatt utgangspunkt i pasienter som gjennomgikk åpen hjertekirurgi ved St. Olavs Hospital, Trondheim mellom 2000-2014. Studiene som ser på genetiske variasjoner og markører i blod er basert på data fra omtrent 1000 pasienter som ble operert i årene 2008-2010. Logistisk regresjon ble brukt til å studere sammenhengen mellom genetiske risikofaktorer og nivå av ulike stoffer i blod med risikoen for henholdsvis et væskeoverskudd og akutt nyresvikt etter operasjonen. Langtidsoverlevelse og -dødelighet ble fulgt opp hos ca. 8,500 voksne pasienter operert mellom 2000 og 2014. Data om død frem til 31.12.2014 ble utlevert fra Dødsårsaksregisteret. Observert langtidsoverlevelse og -dødelighet ble sammenliknet med data fra den generelle norske befolkningen, matchet på kjønn, alder og kalenderår.

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"The journey of a thousand miles begins with one step."

Lao Tzu

Contents

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Tone Bull Enger
Trondheim, 2016

List of publications

Paper 1

Enger TB, Pleym H, Stenseth R, Wahba A, Videm V: Genetic and clinical risk factors for fluid overload following open-heart surgery. *Acta Anaesthesiol Scand.* 2014, 58:539-548.

Paper 2

Enger TB, Pleym H, Stenseth R, Greiff G, Wahba A, Videm V (in press): A preoperative multimarker approach to evaluate acute kidney injury following cardiac surgery. *J Cardiothorac Vasc Anesth*. doi: 10.1053/j.jvca.2016.10.005

Paper 3

Enger TB, Pleym H, Stenseth R, Greiff G, Wahba A, Videm V. Reduced long-term relative survival in females and younger adults undergoing cardiac surgery: A prospective cohort study. *PloS One*. 2016;11(9):e0163754.

Summary

Background
Cardiac surgery is performed in order to treat complications of ischemic heart disease, correct congenital heart disease, or treat valvular heart disease from various causes, including endocarditis, rheumatic heart disease and atherosclerosis. Increased attention on continuous quality monitoring as well as improvements in surgical techniques, anaesthesia, and perioperative treatment and care have played vital roles in improving the safety of cardiac surgery and contributed to a decline in operative mortality. Nevertheless, cardiac surgery still encompasses a risk for complications in which several organs systems can be afflicted. Despite many efforts at designing preoperative risk scoring algorithms to predict postoperative outcomes, identification of patients at-risk remains difficult. The substantial morbidity still suffered by cardiac surgery patients, the discrepancy between observed and predicted outcomes, as well as lack of effective preventive and therapeutic interventions indicate that underlying mechanisms are only partially understood.

Aim
To investigate risk factors for short- and long-term complications following open-heart surgery. More specifically, we aimed to elaborate on possible mechanisms behind postoperative fluid overload, cardiac surgery-associated acute kidney injury (CSA-AKI) and long-term mortality.

Method
Based on 1,179 adult patients undergoing cardiac surgery between 2008 and 2010 at St. Olavs University Hospital, Trondheim, we investigated the associations of genetic and plasma biomarkers with the risk of postoperative fluid overload and CSA-AKI, respectively. Biomarkers were selected based on causal hypotheses, having been related to hemodynamic, inflammatory or renal signalling pathways. For each binary end-point, logistic regression modelling was applied to (1) examine the associations of the biomarkers, separately and in a multimarker approach; (2) derive a clinical model allowing appropriate adjustment for clinical variables; and (3) evaluate the biomarkers as independent predictors.

In order to provide an update on long-term survival following cardiac surgery, all 8,564 adult patients undergoing cardiac surgery at St. Olavs University Hospital, Trondheim, between 2000 and 2014 were prospectively followed until linkage with the Norwegian Cause of Death Registry 31.12.2014. Observed long-term mortality following surgery was compared to the expected mortality in the Norwegian population, matched on gender, age and calendar year. This enabled assessment of relative survival (observed/expected survival rates) and relative mortality (observed/expected deaths). After exclusion of patients not surviving the first 30 days postoperatively, we explored predictors of observed and relative mortality, with special focus on the effects of gender, age and surgical procedure.

Results
Advanced age, longer duration of cardiopulmonary bypass and use of intraoperative red cell transfusion emerged as clinical risk factors for postoperative fluid overload following cardiac surgery. A single-nucleotide polymorphism (SNP) in the *UMOD* gene possibly related to altered renal fluid handling, as well as a genetic risk score based on 14 selected SNPs related to alterations in inflammatory and/or vascular pathways, were identified as independent risk factors for postoperative fluid overload.

Neopterin and N-terminal pro-brain natriuretic peptide emerged as independent preoperative predictors of CSA-AKI. Higher baseline lactoferrin concentrations may exert a protective effect on CSA-AKI, but further investigation is warranted. Inclusion of the biomarkers into a parsimonious clinical prediction model with age, gender, obesity, surgical category and preoperative renal function provided a significant increment in predictive utility for CSA-AKI. Improved prediction was especially seen in patients with intermediate risk.

Short- and long-term observed mortality rates remained constant throughout the study period from 2000 through 2014. When compared to data from the general population, patients undergoing cardiac surgery showed excellent survival throughout the first seven years of follow-up. Subsequently, there was a modest reduction in overall annual relative survival, which was more pronounced in females as well as patients undergoing other procedures than isolated coronary artery bypass grafting (CABG). The ratio of observed and expected deaths was higher for females, in younger age groups and in patients undergoing aortic valve replacement (AVR). Increasing observed mortality with ageing was therefore due to population risk, and the female survival advantage in the general population was lost in those who had undergone cardiac surgery.

Conclusions

The independent effects of novel biomarkers and clinical variables add evidence to the hypothesis that some patients have a subclinical risk of adverse outcome that may not be conveyed by clinical risk factors alone. Our findings underscore the importance of combining markers of different pathways in the pathophysiology of postoperative fluid overload and CSA-AKI in order to identify patients at-risk.

The excellent results up to seven years postoperatively underline the benefits of cardiac surgery in appropriately selected patients. The beneficial effect lasted shorter in younger patients, females and patients undergoing AVR or other procedures than isolated CABG. Thus, the study identified three groups that need increased attention for further improvement of outcomes.

Sammendrag (Summary in Norwegian)

Bakgrunn
Åpen hjertekirurgi utføres for å behandle komplikasjoner av ischemisk hjertesykdom, korrigere medfødte hjertefeil eller behandle klaffesykdommer av ulike årsaker, inkludert endokarditt, revmatisk hjertesykdom og aterosklerose. Økt fokus på kvalitetskontroll og forbedringer innen kirurgi, anestesi, samt perioperativ behandling og oppfølging har økt sikkerheten og redusert operasjonsdødeligheten assosiert med hjertekirurgi. Allikevel innebærer åpen hjerteoperasjon en risiko for postoperative komplikasjoner som kan affisere flere organsystem. Det har vært gjort flere forsøk på å utvikle preoperative skåringsalgoritmer for å hjelpe klinikere med å identifisere pasienter med økt risiko. Forskjellene mellom antatte og observerte utfall samt mangelen på effektive forebyggende og terapeutiske behandlingsmetoder indikerer allikevel at underliggende mekanismer bare er delvis forstått.

Formål
Å studere risikofaktorer for kort- og langtidskomplikasjoner etter åpen hjertekirurgi, med fokus på forståelse av mulige underliggende biologiske mekanismer i utviklingen av postoperativt væskeoverskudd, akutt nyresvikt og langtidsdødelighet.

Metode
Basert på 1,179 voksne hjertekirurgipasienter operert ved St. Olavs Hospital, Trondheim, mellom 2008 og 2010, studerte vi assosiasjonene mellom genetiske og plasma biomarkører med risikoen for henholdsvis postoperativt væskeoverskudd og akutt nyresvikt. Biomarkører ble valgt ut basert på hypoteser om potensielle årsakssammenhenger relatert til hemodynamiske, inflammatoriske eller renale signalveier. For hvert endepunkt brukte vi logistiske regresjons-analyser for å (1) studere assosiasjonene med biomarkørene, enkeltvis og som en multimarkør-analyse; (2) finne relevante kliniske risikofaktorer for å justere biomarkør-analysene; og (3) evaluere biomarkørene som uavhengige prediktorer.

For å gi oppdaterte data for langtidsoverlevelse etter åpen hjertekirurgi, ble alle 8,564 voksne hjertekirurgipasienter operert ved St. Olavs Hospital, Trondheim, mellom 2000 og 2014 fulgt prospektivt inntil kobling til Dødsårsaksregisteret den 31.12.2014. Observert langtidsdødelighet etter hjertekirurgi ble sammenliknet med den forventede dødeligheten i den norske befolkningen matchet på alder, kjønn og kalenderår. Dette muliggjorde utregning av relativ overlevelse (observert/forventet overlevelsesrate) og relativ dødelighet (observert/forventet antall dødsfall). Risikofaktorer for observert og relativ dødelighet ble studert blant pasienter som overlevde de første 30 dagene etter operasjon, med spesiell vekt på effekten av kjønn, alder og kirurgisk prosedyre.

Resultater
Høyere alder, lengre tid på hjerte-lunge maskinen og behov for transfusjon av røde blodlegemer under operasjonen fremsto som uavhengige risikofaktorer for postoperativt væskeoverskudd etter hjertekirurgi. En enkelt-nukleotid polymorfisme i *UMOD*-genet, muligens relatert til endret væskehåndtering i nyrene, så vel som en genetisk risikoskår bestående av 14 selekterte enkelt-nukleotid polymorfismer forbundet med endringer i inflammatoriske og/eller vaskulære signalveier, var assosiert med økt risiko for postoperativt væskeoverskudd, uavhengig av kliniske risikofaktorer.

Neopterin og N-terminal del av pro-B-type natriuretisk peptid var uavhengige preoperative risikofaktorer for akutt nyresvikt etter hjertekirurgi. Høyere preoperative laktoferrinkonsentrasjoner hadde muligens en beskyttende effekt på risikoen for postoperativ nyresvikt, men dette må undersøkes i en større studiepopulasjon. Inklusjon av biomarkørene i en klinisk prediksjonsmodell bestående av alder, kjønn, overvekt, kirurgisk prosedyre og preoperativ nyrefunksjon bidro til en signifikant bedre prediksjonsevne. Dette gjaldt særlig hos pasienter med intermediær risiko for postoperativ akutt nyresvikt.

Den observerte kort- og langtidsdødeligheten blant voksne hjertekirurgipasienter har holdt seg konstant gjennom studieperioden mellom 2000 og 2014. Sammenliknet med data fra den generelle befolkningen, viste de hjertekirurgiske pasientene en høyere eller tilsvarende overlevelse i de første syv årene etter operasjonen. Deretter ble den relative overlevelsen redusert; mer uttalt blant kvinner og pasienter som gjennomgikk andre prosedyrer enn isolert koronar bypassoperasjon (CABG). Relativ dødelighet var høyere blant yngre pasientgrupper, hos kvinner og pasienter som gjennomgikk aortaklaffekirurgi (AVR). Sammenlikning av observert og relativ dødelighet viste at den kvinnelige overlevelsesfordelen i befolkningen var opphevet hos hjertekirurgiske pasienter, og at den økte observerte dødeligheten hos de eldre pasientene tilsvarte dødeligheten ved aldring i den generelle befolkningen. Derimot var ung alder en uavhengig risikofaktor for økt relativ dødelighet.

Konklusjoner
Den forbedrede prediksjonsevnen sett ved å kombinere informasjon om biomarkører og kliniske risikofaktorer styrker hypotesen om at enkelte pasienter har en subklinisk risiko for postoperative komplikasjoner som ikke kan forutses med kliniske variabler alene. Aktuelle funn understreker viktigheten av å kombinere markører fra ulike patofysiologiske signalveier for å bedre identifikasjon av pasienter med økt risiko for postoperativt væskeoverskudd og postoperativ akutt nyresvikt.

Hjertekirurgiske pasienter hadde sammenliknbar overlevelse med den norske befolkningen. Den gunstige effekten varte allikevel kortere hos yngre pasienter, kvinner og pasienter som gjennomgikk AVR eller andre prosedyrer enn isolert CABG. Disse gruppene fremstår som målgrupper for ytterligere forbedring av resultatene etter åpen hjertekirurgi.

List of abbreviations

ADQI	Acute Dialysis Quality Initiative
AIC	Akaike information criterion
AKIN	Acute Kidney Injury Network
ASA	Acetylsalicylic acid
AUC	Area under the receiver operating characteristics curve
AVR	Aortic valve replacement
BIC	Bayesian information criterion
BNP	Brain natriuretic peptide
CABG	Coronary artery bypass grafting
CI	Confidence interval
CIGENE	Centre for Integrative Genetics
Cox PH	Cox proportional hazards
CPB	Cardiopulmonary bypass
CRP	C-reactive protein
CSA-AKI	Cardiac-surgery associated acute kidney injury
EF	Ejection fraction
EuroSCORE	European System for Cardiac Operative Risk Evaluation
GDT	Goal-directed fluid therapy
GFR	Glomerular filtration rate
GWAS	Genome-wide association study
HBC	Heparin-bonded circuit
HL test	Hosmer-Lemeshow test
HR	Hazard ratio
I/R	Ischemia/reperfusion
ICU	Intensive care unit
IDI	Integrated discrimination improvement
KDIGO	Kidney Disease: Improving Global Outcomes
LF	Lactoferrin
LR test	Likelihood ratio test
LVEF	Left ventricular ejection fraction
NRI	Net reclassification improvement
NT-proBNP	N-terminal pro-brain natriuretic peptide
NYHA	The New York Heart Association Functional Classification (I-IV)
OR	Odds ratio
PCI	Percutaneous coronary intervention
RIFLE	Risk/Injury/Failure/Loss/End-stage
ROS	Reactive oxygen species
SIRS	Systemic inflammatory response syndrome
SNP	Single-nucleotide polymorphism
STS	Society of Thoracic Surgeons
TAVI	Transcatheter aortic valve insertion

1. Introduction

1.1 The development of cardiac surgery

When John H. Gibbon completed the first successful open-heart operation with the use of cardiopulmonary bypass (CPB) in 1953 (1), he revolutionized cardiac surgery. CPB allowed surgeons to work on a non-beating heart and in a bloodless field. Since then, generations of cardiac surgeons have been able to operate on millions of human hearts with efficiency and accuracy to correct congenital and acquired heart dysfunctions. Today, cardiac surgery is one of the most common and costly surgical interventions.

Nevertheless, cardiac surgery is changing: First, in many patients, the need for surgical revascularization has been delayed, both as a result of an increased focus on cardiovascular health and a healthy lifestyle, as well as advances in medical treatment and non-surgical techniques. Uncomplicated cases with low-risk profiles are primarily referred to percutaneous coronary intervention (PCI), with a resulting reduction in coronary artery bypass graftings (CABG). On the other hand, the reduction in isolated CABG has been partially compensated by an increase in valvular surgery. Reasons underlying this increase may include earlier identification and surgery of patients with valvular disease, an increasingly elderly population, as well as acceptance of older patients for surgery (2). These trends with a changing case mix are also confirmed in reports from Norwegian cardiac surgery centres (3), as summarized in Figure 1.

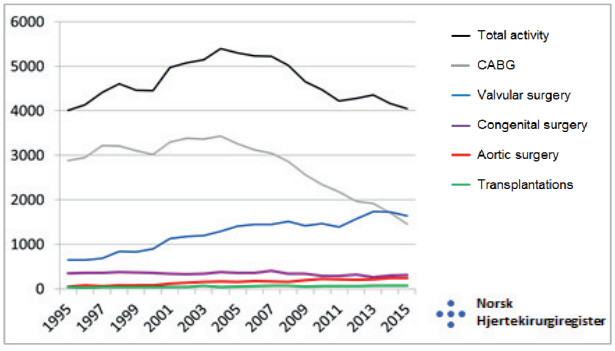

Figure 1: Trends in activity of cardiac surgery in Norway between 1995 and 2013. Reprinted with permission from the Norwegian Register for Cardiac Surgery (3).

Second, both as a result of the delayed need for surgery and changing population demographics, the referral pattern in surgery has changed. Patients tend to be older and have more preoperative comorbidities. A temporal comparison of patient characteristics in the Society of Thoracic Surgeons (STS) database from USA showed that more patients presented with diabetes, dialysis-dependent renal failure and cardiovascular risk factors such as hypertension and hypercholesterolemia in 2009 compared to 2000 (4). Data from the Norwegian Register for Cardiac Surgery affirm that comparable trends can be seen in Norwegian cardiac surgery patients (Table 1). Thus, cardiac surgeons today more often operate on patients with high-risk profiles and complex cardiovascular disease (5-7).

Table 1. Comparison of preoperative risk factors, postoperative complications and 30-day mortality rates in Norwegian cardiac surgery in the years 2004, 2009 and 2013.

	2004	2009	2013
Preoperative risk factors			
Diabetes	11.9%	9.9%	16.5%
Stroke/Transient ischemic attack	4.6%	3.8%	5.4%
Renal failure	2.1%	1.5%	3.3%
EuroSCORE	5.3%	5.4%	5.4%
Postoperative complications			
Respiratory failure	4.2%	4.3%	5.4%
Mechanical support	2.2%	2.5%	2.4%
Myocardial infarction	1.5%	0.8%	---
Renal failure	1.8%	2.2%	2.2%
Stroke	1.3%	1.1%	1.6%
Reoperation for bleeding	4.2%	3.5%	4.2%
Sepsis	0.7%	0.6%	---
Deep sternal infection	0.7%	0.8%	0.9%
30-day mortality			
Coronary artery bypass grafting (CABG)	1.1%	0.9%	1.0%
Isolated valvular surgery	3.3%	3.7%	2.2%
Combined valvular and CABG	4.8%	4.2%	2.5%
Total for all procedures	2.6%	2.6%	2.2%

Data obtained through the Norwegian Register for Cardiac Surgery (8). --- Statistics were not available. Abbreviations: EuroSCORE; European System for Cardiac Operative Risk Evaluation.

1.2 The risk paradox in cardiac surgery

Despite changes in case-mix and patient risk factors, and subsequently an increase in the number of high-risk patients and procedures, there has been a significant reduction in observed short-term mortality following cardiac surgery (4, 9, 10). In the STS database, 30-day mortality following CABG was reduced from 2.4% in 2000 to 1.9% in 2009 (4). The overall present 30-day mortality rate of cardiac surgery varies between 1.5-4% (11-13), depending on the surgical intervention. Data from the Norwegian Register for Cardiac Surgery compare favourably with these international results, as summarized in Table 1.

Continuous quality monitoring as well as improvements in training, anaesthesia, critical care and surgical techniques have played vital roles in improving the safety of cardiac surgery and contributed to the decline in observed mortality. Internationally, the concomitant increase in risk and decrease in mortality has been denoted the "risk paradox" (14).

1.3 Postoperative complications

1.3.1 Pathophysiology

Despite improvements in operative mortality, postoperative morbidity suffered by cardiac surgery patients still remains substantial (15). Major complications include cardiac, respiratory and renal dysfunction, as well as bleeding and neurological complications. The pathogenesis behind postoperative complications involves hemodynamic and inflammatory signalling pathways, which are activated in response to cardiac arrest, artificial and non-pulsatile circulation, hypothermia, blood contact with the artificial surface of CPB and the surgical insult with skin incision and sternotomy.

1.3.1.1 Hemodynamic challenges

During open-heart surgery, aortic cross-clamping and non-pulsatile perfusion using CPB expose tissues to a greater risk of ischemic events. Perioperative inflammation with vasodilation and increased capillary leakage may also contribute, as well as post-CPB myocardial stunning causing low cardiac output. Additionally, patient-related factors, such as compromised cardio-renal function and reduced functional reserves, may render some patients more vulnerable to

ischemic insults. Thus, maintaining adequate organ perfusion during and after cardiac surgery requires consideration of multiple factors.

Although restoration of blood flow to an ischaemic organ is essential to prevent irreversible tissue injury, reperfusion may also result in a local and systemic inflammatory response including generation of reactive oxygen species (ROS), cytokine release and complement activation. This may lead to microvascular dysfunction and extended tissue injury, thus causing greater harm than that produced by ischaemia alone. Cellular damage after reperfusion of previously viable ischaemic tissues is defined as ischaemia–reperfusion (I/R) injury (16).

Oxidative stress has been pointed out as a central mechanism in I/R injury (17). In addition to I/R, inflammation with activation of neutrophils, the lysis of red blood cells due to the shear forces of the CPB, and blood transfusion contribute to increased oxidative stress. Furthermore, patients undergoing cardiac surgery tend to have other coexisting comorbidities such as diabetes, renal and lung diseases, which are associated with abnormal tissue perfusion and increased oxidative stress. Oxidative stress has been associated with postoperative outcomes, especially the risk of cardiac dysfunction (18) and acute kidney injury (19).

1.3.1.2 Inflammation

In addition to the inflammatory response generated locally in afflicted tissues and due to tissue reperfusion injury, a significant systemic inflammatory response is triggered during open-heart surgery. The combination of anaesthesia, surgical stress and CPB triggers an extensive activation of platelets, neutrophils, complement and the fibrinolytic system, as well as release of cytokines, evolving into a complex and intensive inflammatory response (Figure 2).

The inflammatory response triggered by cardiac surgery with CPB can be divided into an early and late phase (20). The early phase is characterized by the contact of blood with the artificial surface of the CPB, with subsequent activation of the four plasma protein systems: The contact, coagulation, complement and fibrinolytic cascades. Furthermore, there is an activation of the vascular endothelium and cellular defence, with leukocytes and platelets. The late phase is characterized by I/R and the release of endotoxins from the intestinal microflora.

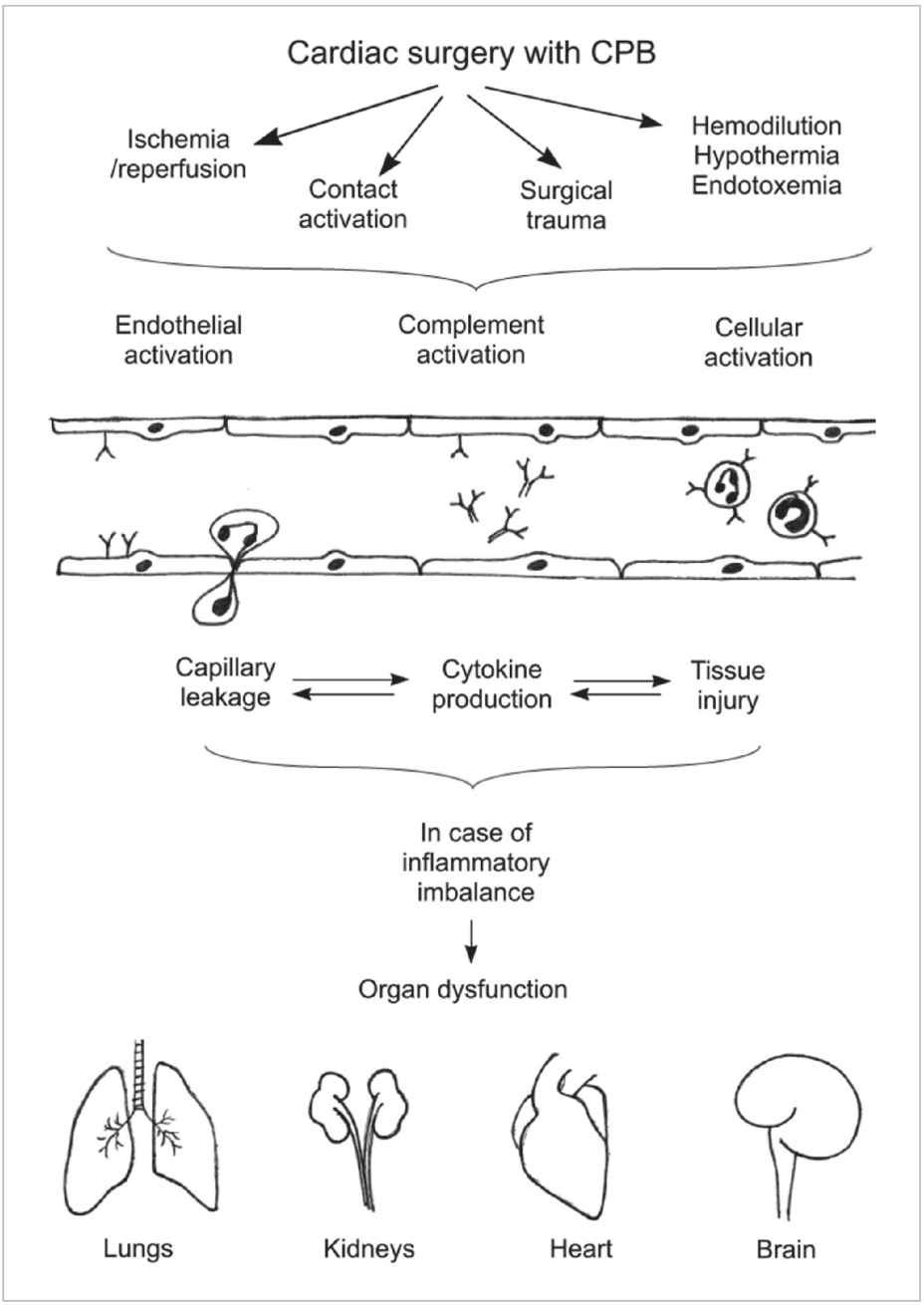

Figure 2: **Important mediators in the inflammatory response to cardiac surgery with cardiopulmonary bypass (CPB).** The different processes have a synergistic effect and may lead to organ dysfunction. (Based on a figure from Brix-Christensen V. The systemic inflammatory response after cardiac surgery with cardiopulmonary bypass in children. Acta Anaesthesiol Scand (2001; 45(6):671-9). With permission from Wiley.)

Whereas in most patients the inflammatory response is short-lived and without residual defects, some patients demonstrate a marked and protracted activation of several molecular pathways indicating inflammatory and haemostatic dysregulation, increased oxidative stress and endothelial dysfunction (21). In this subset of cardiac surgery patients, the systemic inflammatory response may progress into tissue oedema, coagulopathy, and organ dysfunction.

Some clinicians and researchers have used the criteria for the systemic inflammatory response syndrome (SIRS)* in order to identify patients at risk. SIRS was originally defined in order to assist clinicians and researchers who treat patients with sepsis and its sequelae (22). It represents a sensitive but unspecific indicator for injury. A recent study showed that two or more SIRS criteria was met by almost 60% of patients in the first hour after cardiac surgery and by >95% within the first day (23). Therefore, modifications of these criteria for use in cardiac surgery patients are currently being discussed, with the aim of increasing their positive predictive value (23, 24).

Nevertheless, patients with SIRS show higher levels of inflammatory mediators (25, 26). Furthermore, SIRS following cardiac surgery has been associated with the development of adverse postoperative outcomes, including renal, pulmonary and neurological complications, bleeding and multiple organ dysfunction (27). Intertwining these observations, in a study by Holmes and colleagues, higher plasma levels of interleukin-6, interleukin-8 and C3a were independently associated with adverse outcomes, indicating that patients who experienced relatively greater degrees of inflammation suffered worse clinical outcomes (28). Similarly, markers of increased oxidative stress (19, 29) and also more general inflammatory markers such as procalcitonin (30, 31) have been associated with an increased incidence of postoperative complications. Thus, postoperative complications are generally understood as a result of the conversion from a self-limiting, tightly controlled physiologic response to surgery and perioperative events, to an uncontrolled destructive process (32, 33).

*The SIRS-diagnosis requires presence of two or more of the following: Temperature >38°C or <36°C; Heart rate >90 beats/min; Respiratory rate >20 breaths/min or $PaCO_2$ <4.3 kPa; Leukocytes >12,000/µl, <4,000/µl or >10% immature (band) forms.

1.3.2 Preventive strategies

Hemodynamic instability and systemic inflammation play pivotal roles in the development of complications following cardiac surgery with CPB (Figure 3). Each related pathogenic pathway may offer a potential target for intervention. Thus, with the aim of reducing postoperative morbidity and mortality, considerable efforts have been put into the search for preventive drug and treatment strategies. Examples of the most promising targets will be briefly discussed.

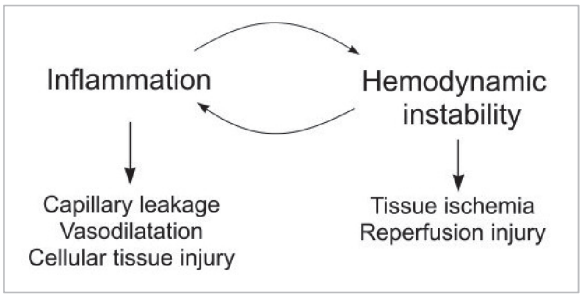

Figure 3. Inflammatory and hemodynamic pathways play important roles in the pathogenesis behind postoperative complications following cardiac surgery with CPB. They also influence each other, where inflammation challenges hemodynamic stability and hemodynamic instability increases inflammation.

1.3.2.1 Limiting I/R injury

Ischaemic preconditioning, controlled reperfusion, and anti-oxidant, complement or neutrophil therapy have been proposed as therapeutic strategies that may prevent or limit I/R-induced injury in humans. Although some strategies have been effective in controlled experimental models, most of these have yielded equivocal results in clinical practice or have yet to be confirmed effective in human clinical trials.

Levosimendan is a calcium-sensitising inotropic agent and a vasodilator used in the treatment of heart failure. Levosimendan has been claimed to increase myocardial contractility without increasing myocardial oxygen use (34), and thus may protect against I/R myocardial injury. It has been demonstrated that preoperative administration of levosimendan protects against the development of low output syndrome and reduces mortality in cardiac surgery patients (35, 36). However, routinely prophylactic infusion of levosimendan before weaning from CPB did not lead to significant hemodynamic improvement in patients with preoperatively impaired left

ventricular function (ejection fraction (EF) < 30%) undergoing cardiac surgery in Trondheim (37). Despite the conflicting evidence regarding the clinical impact of levosimendan in cardiac surgery, a panel of European experts recently recommended preoperative use of levosimendan in high-risk patients with preoperative compromised myocardial function (38). Meanwhile, we are awaiting the results of an ongoing trial (LEVO-CTS NCT02025621), in which 760 patients with preoperative EF < 35% have been randomized to levosimendan vs. placebo treatment. Furthermore, another ongoing multicentre clinical trial (CHEETAH NCT00994825) is comparing the effect of levosimendan on survival in patients with postoperative myocardial dysfunction.

As reviewed by Kunst and Klein, extensive research from experimental studies have suggested that **volatile anaesthetics** (such as sevoflurane, desflurane and isoflurane) may protect against ischemic myocardial injury (39). By inducing a dose-dependent decrease in myocardial contractility and cardiac loading conditions, myocardial oxygen demand is reduced. Thus, volatile anaesthetics seem to a have a beneficial effect on myocardial oxygen balance during myocardial ischemia compared to total intravenous anaesthesia. A handful of meta-analyses have supported the experimental results on clinical outcomes (40-43), but inconclusively. A recent randomized clinical trial on the use of sevoflurane vs. propofol, with 100 patients undergoing high risk cardiac surgery (combined CABG and valvular procedures) in each intervention arm, was not able to demonstrate any beneficial effect of sevoflurane, neither on intensive care unit stay nor mortality (44). The translation of evidence from experimental studies into the clinical setting has been hampered by study weaknesses including small sample sizes and use of surrogate endpoints for hard clinical outcomes. There are still large variations in anaesthetic techniques, with different administration patterns, including volatile anaesthetics and/or propofol. Larger pragmatic, multicentre trials have been called for. As a result, an ongoing large multicentre trial (MYRIAD NCT02105610) is investigating whether use of volatile anesthetics over total intravenous anaesthesia can translate into a reduced 1-year mortality rate in CABG patients, and is expected to be completed by December 2016.

In a local study by Berg and coworkers, it was shown that continuation of routine treatment with **acetylsalicylic acid** (ASA) until the time of surgery, as opposed to withdrawal 7 days preoperatively, reduced oxidative stress and inflammation during and after cardiac surgery (45).

There were no differences in bleeding complications within the first 18 hours between the groups. This implied a protective role of continued ASA-treatment until the time of surgery.

The mentioned study was included in the subsequent systematic review and meta-analysis by Hastings and colleagues (46), which concluded with minimal adverse effects of ASA on bleeding complications and a likely reduction in the incidence of perioperative myocardial infarction. In a retrospective cohort study, Yao and coworkers showed that preoperative ASA treatment in patients with chronic kidney disease was associated with renal protection and mortality decline (47). The magnitude of the survival benefit was greater in patients with chronic kidney disease than normal kidney function. However, the recent multicentre, double-blind, randomized ATACAS trial found no differences neither in death, thrombotic or bleeding complications in at-risk patients undergoing cardiac surgery (48).

Much attention has also been given to **N-acetylcystein** in reducing postoperative complications following cardiac surgery. N-acetylcystein acts as an antioxidant and anti-inflammatory agent, and reduces cellular oxidative damage and systematic inflammation during cardiac surgery (49-51). However, neither of two recent meta-analysis were able to demonstrate a benefit on clinically important outcomes (52, 53). Whereas lacking association to clinical outcomes may suggest that oxidative stress induced during cardiac surgery might be benign, it must be noted that most studies were conducted in a small number of low-risk patients with minimal increases in oxidative stress, which renders detection of significant differences in clinical outcomes difficult (54). High-risk patients may experience increased oxidative stress as a consequence of less tolerance to ischemia and thus benefit more from preventive treatment.

1.3.2.2 Limiting systemic inflammation

There has been a great interest in efforts of attenuating the inflammatory response in cardiac surgery patients. In general, strategies to hamper the harmful side-effects of inflammation can be summarized into 3 categories: (1) technical strategies, (2) pharmaceutical strategies or (3) avoiding CPB (55). Some of the most promising interventions include heparin-coated circuits, perioperative corticosteroid or aprotinin therapy, and off-pump surgery.

Technical strategies

Technical strategies include modification of the bio-incompatible CPB circuit (heparin-bonded circuits), filtrations techniques (ultrafiltration, leukocyte depletion), and blood conservation.

Heparin-bonded circuits (HBCs) have been designed in order to improve the biocompatibility of CPB, as heparin plays multiple roles in attenuating the systemic inflammatory response (56). HBCs have been widely adopted as the standard CPB today. Mangoush and coworkers performed a systematic review and meta-analysis, addressing the clinical impact of HBCs following cardiac operations (57). Although HBCs showed a positive effect on some of the clinical outcomes, including the need for blood transfusion, prolonged ventilation and re-sternotomy, only marginal differences were demonstrated for other outcomes. As also discussed by Laffey *et al.* (27), despite lacking overall clinical benefit, a beneficial effect may be confined to high-risk patients (58).

In **hemofiltration**, the patient's blood is passed through a semi-permeable membrane where waste products and excess water are removed. Hemofiltration during CPB is recommended in patients with impaired renal function, excessive and positive fluid balance, reduced response to diuretics or prolonged bypass time more than 2 hours (59). Whereas the benefits of hemofiltration in paediatric cardiac surgery has been well described, the potential benefits in adult CPB patients remain controversial. Hemofiltration increases haematocrit, reduces some inflammatory markers, reduces post-operative blood transfusion, and possibly improves hemodynamics immediately after hemofiltration (60). However, a difference in morbidity or mortality has never been successfully demonstrated.

Pharmaceutical strategies

These comprise anti-inflammatory and anti-oxidant agents, including glucocorticoids, antioxidants, serine protease inhibitors, complement inhibitors and other direct anti-mediator therapies that are directed at key effector molecules and central pathogenic pathways of the inflammatory response.

One of the most discussed treatment strategies is the use of **corticosteroid prophylaxis**. Steroids have pleiotropic effects which act to attenuate the inflammatory response. However, it is important to preserve the ability of the patient to mount an appropriate defence to the

physiological threats during the perioperative period. Multiple randomized clinical trials have been performed, which have been pooled in a series of 5 meta-analyses (61-65). Overall, they suggested that steroid prophylaxis in adult cardiac surgery with CPB reduces perioperative morbidity with minimal risk of harm. In conclusion, they encouraged adequately powered multicentre trials to assess this intervention with regards to clinical outcomes. Subsequently, two randomized controlled trials have recently been published. However, neither demonstrated a significant effect on major morbidity or mortality (66, 67). Thus, routine use of steroids for patients undergoing CPB was not supported.

Aprotinin is a serine protease inhibitor with anti-inflammatory, anti-fibrinolytic and antithrombotic effects. It was adopted into clinical practice in the early 2000s after it was shown effective in reducing bleeding and the need for blood transfusions after cardiac surgery with CPB. However, its licence was suspended in 2007 after concerns were made about its safety. New evidence was put forward that associated aprotinin use with an increased risk of renal failure, myocardial infarction, heart failure, stroke, encephalopathy and mortality (68-71). However, weaknesses were pointed out in these studies, which questioned their reliability for assessing the benefit-risk balance of aprotinin (72, 73). Aprotinin was therefore re-allowed in Canada and Europe in 2011. The use of aprotinin in cardiac surgery remains controversial.

Alternatives to conventional CPB

CPB is a major trigger of the inflammatory response, where use of **off-pump surgery** (74, 75) and **miniaturized CPB** (76) have been associated with reduced activation of inflammatory mediators. However, so far, trials exploring these novel treatment strategies have failed to demonstrate improvements in end-organ injury and true clinical benefits in patients. Parissis *et al.* recently published a summary of available literature on off-pump cardiac surgery (77). The lack of robustness in available evidence makes off-pump surgery controversial with regards to its effectiveness and indication. Both on- and off-pump surgery show similar graft patency and clinical outcomes up to 1 year. However, there is some evidence that off-pump surgery reduces the incidence of postoperative atrial fibrillation and stroke, postoperative complications in octogenarians, as well as morbidity and mortality in high-risk patients (reduced EF or severe lung, renal or vascular dysfunction). Thus, off-pump surgery may be superior in certain subgroups with increased risk.

1.3.2.3 Current practice

The proposed strategies to inhibit or reduce the oxidative injury and inflammatory response in order to improve outcomes following cardiac surgery have been theoretically justified and may even have been experimentally proved. However, their impact on clinical outcomes remain debated. An evaluation of current evidence revealed that the development in the equipment and techniques of CPB, including heparin-coating of the CPB, use of centrifugal blood pumps over roller pumps and open venous reservoirs, are grounded on empiricism rather than evidence-based medicine (78). By this time, no pharmacological interventions designed to prevent postoperative complications have been routinely implemented. The reported use of off-pump surgery varies substantially, and in the absence of solid data to guide selection, local practice currently depends on the attitude, experience, prediction, and biases of the surgical group (79). Thus, the management of cardiac surgery patients varies substantially by institution.

One possible reason contributing to the lack of distinct clinical benefits may be large patient heterogeneity or studies limited to low-risk patients. Large-scale multicentre trials in selected high-risk patients may strengthen the power to detect meaningful differences (27).

Furthermore, the substantial intra-individual variations among different patients in their postoperative response imply that there is a complex interaction between several pathogenic pathways (55). Whereas each identified pathway offers a potential target for intervention, the diversity and interactive effect of multiple pathways makes it rather unlikely that any single drug will prevent postoperative complications. In addition, despite the reduced inflammatory response demonstrated with many interventions, there may not always be a direct correlation between different inflammatory markers and physiologic consequences (80). Absolute levels of inflammatory markers may be less important than the balance and interactive effects between pro- and anti-inflammatory cytokines as well as with other mediators (81). The causative links between the inflammatory response and adverse clinical sequela remain poorly understood (31).

Another common aspect for many studies, is that they study the clinical impact on death or composite outcomes. However, different outcomes may have both distinct and overlapping underlying pathogenic pathways, thus different risk factors and different weighting amongst them. Even though an attenuation of the inflammatory response has been demonstrated, the lack of clinical implications and an overall reduction of postoperative morbidities may be an overly

ambitious attempt, whereas a more thorough investigation of separate outcomes might be more appropriate.

In summary, our understanding of underlying mechanisms contributing to adverse outcomes following cardiac surgery remains incomplete. A better understanding may be a key to the development of successful strategies in order to further improve patient outcomes. This thesis explores three different outcomes following adult cardiac surgery, namely the risk of postoperative fluid overload, acute kidney injury (AKI) and long-term mortality.

1.3.3 Postoperative fluid overload

Under 'normal', healthy conditions, the heart and kidneys collaborate in an intricate relationship to regulate cardiac output, volume status and vascular tone, assuring hemodynamic stability and end-organ perfusion (82). However, during cardiac surgery, altered cardio-renal function as well as changes related to the inflammatory response may disrupt hemodynamic balance.

Fluid balance represents the sum of fluid intake and output. During surgery, fluid is administered for volume control (mainly before CPB), as priming volume for the CPB and cardioplegia, in addition to transfusion of red blood cells or plasma, if indicated. The fluid balance sheet is followed regularly in context with urine output and hemodynamic parameters such as blood pressure, pulse and clinical signs of organ function.

Whereas the traditional clinical focus has been to assure adequate organ perfusion in order to prevent ischemic injury, increasing attention has been put on the hazards of excessive fluid administration. Kamphambati and coworkers showed that fluid administration was not clearly associated with any identifiable indications during surgery, such as hypotension or blood loss, suggesting that most of the fluid administration was protocol-driven (83). However, perioperative fluid accumulation following cardiac surgery has been related to higher incidences of AKI (83) and cardiorespiratory dysfunction (84), as well as increased duration of hospital stay (84) and mortality (85). Thus, protocol-driven fluid administration might be excessive for some patients, and identification of high-risk patients who should be followed with heightened vigilance and close monitoring of hemodynamic function is important in order to reduce both patient morbidity and hospital costs.

Patients with reduced baseline cardiac and/or renal function may be more vulnerable to perioperative fluid accumulation. Reduced cardiac function may cause venous congestion with increased hydrostatic pressure and increased risk of fluid extravasation. Patients with preoperative reduced renal function are more vulnerable to AKI, where a rapid decline in glomerular filtration rate (GFR) becomes clinical manifest by increases in serum creatinine, reduced urine output, and fluid and solute retention with subsequent oedema formation. Fluid overload following cardiac surgery may therefore be a consequence of compromised cardiac and/or renal function.

However, fluid overload is not solely a surrogate marker for cardio-renal dysfunction. Identification of these patient groups is not sufficient for identification of patients who develop postoperative fluid overload. Another important contributor that may help to explain the risk of perioperative fluid accumulation is inter-individual variation in the perioperative systemic inflammatory response.

The inflammatory response to surgery is associated with endothelial injury and increased microvascular permeability. In some patients, a situation arises where more fluid administration is required to maintain circulatory volume and adequate organ perfusion, whilst "third space" tissues are being increasingly waterlogged (86). This has led to the definition of a capillary leak syndrome; defined as a pathologic shift of fluid and protein from the intravascular to the interstitial space, with the subsequent threat of hypovolemia (87).

It has been demonstrated that the capillary leakage, as measured through weight gain, increases with the magnitude of the inflammatory response (28). The capillary leak syndrome has been of greater interest in paediatric cardiac surgery, where preoperative levels of inflammatory mediators, including C3 and C5 complement components, tumor necrosis factor-alpha, neutrophil proportions and leukocyte counts, have been associated with clinical signs of capillary leak syndrome (88, 89). These findings suggest that there exist preoperative differences in the immune system and capillary permeability status that render some patients at a higher risk of perioperative fluid accumulation.

Thus, development of postoperative fluid overload seems to result from an interplay between both cardio-renal function and the inflammatory response following cardiac surgery with CPB. A thorough understanding of their role and a comprehensive consideration of different pathways involved might improve our ability to ascertain the group of patients who need heightened vigilance during and after surgery.

1.3.4 Acute kidney injury

Cardiac-surgery associated AKI (CSA-AKI) is a significant clinical problem associated with increased postoperative morbidity, short- and long-term mortality (90, 91). Whereas the incidence of dialysis-dependent renal failure is estimated to 1.1-5.0% (92), the incidence increases up to 30% when based on increased postoperative serum creatinine concentrations (93). There is evidence that the incidence of CSA-AKI is increasing (94).

The pathogenesis underlying CSA-AKI involves a complex interplay between renal hemodynamics, tubular and endothelial cell injury, and inflammatory processes. The multifactorial mechanisms include exogenous and endogenous toxins, metabolic factors, ischemia and reperfusion, neurohormonal activation, inflammatory mediators and oxidative stress (95). These factors are likely to be active at different times with different intensities, are interrelated and probably synergistic.

It has been largely assumed that the CSA-AKI is a form of acute ischemic tubular necrosis (93). Sutton and colleagues have defined different clinical phases of ischemic acute renal failure (96). In the initiation phase, altered renal vascular function, especially at the microvascular level, initiate and subsequently extend the initial tubular injury, with a progressive decline in GFR. Vascular and inflammatory processes that contribute to further cell injury and a further decline in GFR lead into the proposed extension phase. The extension phase of ischemic acute renal failure involves continued reduction in renal perfusion, ongoing hypoxia, and inflammatory processes that occur during reperfusion. Vascular endothelial cell injury and dysfunction play central roles in this extension phase. With injury, the endothelial cell loses its ability to regulate vascular tone, perfusion, permeability, and inflammation/adhesion. This loss of regulatory function has a detrimental impact upon renal function.

Preoperative risk factors for CSA-AKI can be related to impaired renal perfusion, decreased renal functional reserves or the establishment of a pro-inflammatory milieu (93). Preoperative renal function, as indicated by preoperative dialysis or estimated by serum creatinine, creatinine clearance or estimated GFR, consistently emerges as the most important predictor (93, 97). Otherwise, the presence of diabetes, chronic pulmonary disease and congestive heart failure have been repeatedly associated with increased AKI risk (93, 97). Previous findings also indicate that female patients are predisposed to postoperative AKI.

Furthermore, exposure to anaesthesia and CPB during cardiac surgery leads to dramatic hemodynamic alterations as well as activation of inflammatory pathways which can initiate or extend renal injury. Thus, as summarized in the review by Rosner and Okusa, intraoperative factors including the surgical procedure, durations of aortic cross-clamping and CPB, pulsatile versus non-pulsatile bypass flow, and temperature during surgery may be important (93).

Several risk scoring algorithms have been developed aiming to identify patients at increased risk of CSA-AKI (97). These can be used to identify high-risk patients and facilitate the initiation of preventive and therapeutic treatment before irreversible kidney damage takes place (Figure 4). However, up to today, none of the risk algorithms have been implemented in clinical practice. A necessity for clinical use is that preoperative prediction has clear clinical implications. However, despite an increasing understanding of the prognostic impact of postoperative AKI, our ability to prevent and treat CSA-AKI remains limited.

In addition to interventions thought to reduce postoperative complications in general (section 1.3.2), a variety of different agents for specific prevention and treatment of CSA-AKI have been studied. These include hemofiltration and prophylactic haemodialysis, as well as pharmacological interventions that aim to either increase renal blood flow, induce natriuresis or block inflammation (93). Most studies have, however, been disappointing (98, 99). Urine alkalinisation with sodium bicarbonate was long thought to have renal-protective effects and was conventionally administered in many institutions. However, a recent meta-analysis of relevant randomized controlled trials was not able to demonstrate a reduced incidence of AKI (100). The usefulness of the different strategies therefore remain controversial and further research is required to prove their effectiveness. Until then, supportive measures, including avoidance of nephrotoxic drugs, tight perioperative glucose control, as well as altered

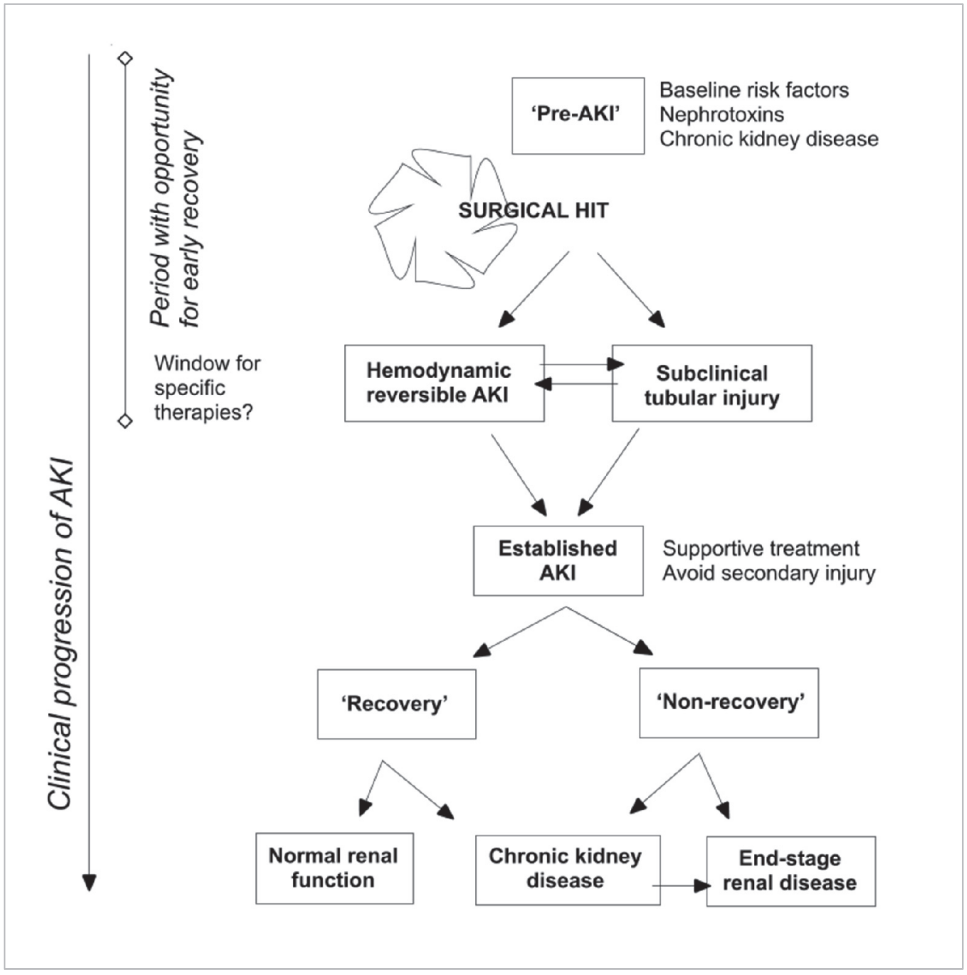

Figure 4: Clinical course of acute kidney injury (AKI). Initiation of AKI can involve hemodynamic changes in glomerular filtration rate, sub-clinical tubular injury, or both processes occurring simultaneously. A short time window may exist where specific therapy might reverse AKI; however, this treatment may need to be tailored to the nature of the injury and risk profile of the patient. Established AKI requires days to weeks for recovery, and the emphasis during this period should be on supportive therapy and the avoidance of secondary renal injury that may result in non-recovery of renal function or chronic kidney disease. These remain the main strategies in this patient group to date. (Figure and legend reproduced with modifications from Prowle JR. Acute kidney injury: an intensivist's perspective. Pediatr Nephrol (2014; 29:13-21.). With permission from Springer.)

management strategies of CPB, attentive hemodynamic monitoring and optimization of renal perfusion, remain the main preventive and treatment strategy for high-risk patients (101).

1.3.5 Long-term mortality

In-hospital mortality and postoperative morbidities have been dominant cardiac surgery outcome metrics. However, as short-term outcomes improve, it is increasingly important to better understand the determinants for long-term survival. Data on long-term outcome may enhance shared decision making, individualization in the care and follow-up of patients, the study of long-term efficacy, and is essential for comparing alternative treatment strategies.

Comparable to other industrialized countries, cardiovascular diseases, with ischemic heart disease in the front, constitute the number one cause of death in the Norwegian population (102). Cardiovascular risk factors are thus prevalent in the general population, where cardiac surgery patients may represent the severely affected cases with manifest disease. On the other hand, as shown in the Norwegian as well as Swedish populations, a substantial portion of cardiovascular deaths occur out-of-hospital (103, 104). This might imply that cardiac surgery patients represent the fortunate proportion that are offered treatment in time. With this in mind, it would be of interest to assess long-term mortality in cardiac surgery patients compared to the general population.

Cardiac surgery is seldom curative, but aims to improve life quality and survival. Reports from Stahle and colleagues, using data from Swedish cardiac surgery patients operated in the 1970-90s, compared long-term survival following CABG with the expected mortality in the general population matched on age, gender and calendar year (105, 106). They showed that patients undergoing CABG had a slight excess mortality compared to the general Swedish population during the first year, subsequently an excess mortality close or even lower than zero, before a rapid increase in death risk from year 7-10. Described in a related study by Kvidal *et al*., patients undergoing surgical aortic valve replacement (AVR) showed increased excess mortality after the fourth year of follow-up (107). In a more recent study by Lassnigg and coworkers, Austrian patients undergoing AVR between 1997 and 2008 were followed up until censoring at the end of 2011, where patients surviving the first postoperative year demonstrated similar survival to the matched general population.

The majority of previous studies on long-term mortality following cardiac surgery have considered trends and risk factors for all-cause mortality. Amongst CABG patients, age consistently emerged as the most important risk factor for increased mortality (108-115). Moreover, based on data from the STS database, Shahian and colleagues showed that late outcomes for patients who initially survive CABG surgery were less affected by traditional predictors of early mortality, such as emergency status, shock, and reoperation (112). On the other hand, late mortality was associated with chronic comorbid diseases such as insulin-dependent diabetes mellitus and dialysis-dependent renal failure, and behaviours such as smoking (112). Similarly, in a recent study on long-term outcomes following AVR, baseline chronic kidney disease, severely impaired left ventricular function and current smoking was associated with poor long-term prognosis (116).

However, patients undergoing cardiac surgery are often old and have several comorbidities, which may relate to a higher baseline risk for mortality in general. Old reports show that when adjusting for the expected mortality in the matched general population, increased age was no longer associated with increased long-term mortality (105-107, 117). Thus, the association of ageing with increased long-term all-cause mortality may have been due to population risk.

Over the past decades, changes in case mix and patient risk factors, together with advances in technology and pharmaceutical treatment options may have influenced long-term outcomes following cardiac surgery. Today, patients and clinicians are faced with the decision between surgery, endovascular intervention or continued medical treatment. Whereas uncomplicated patients with coronary heart disease are primarily treated medically or referred to PCI, CABG is the recommended treatment strategy for patients with complex vessel disease, significant left main stem stenosis and multi-vessel disease (118). In patients with multi-vessel disease, open-heart surgery was associated with a reduced incidence of future myocardial infarctions, a reduced need for re-interventions and an overall survival benefit compared to coronary angioplasty (119).

Similarly, transcatheter aortic (TAVI) and mitral valve implantation have emerged as alternatives for surgical aortic and mitral valve replacement, respectively (120). Both have shown to be superior to medical treatment (121-123). However, until now, these catheter-based

procedures have been restricted to inoperable or high-risk patients, hampering the direct comparison with conventional surgical valve replacement. Furthermore, despite the beneficial effects observed in selected patients since the implementation of these methods from 2009, data on long-term outcomes for these new interventions are limited. Increasing data on performance in intermediate risk patients (124) and over a longer follow-up period may enable such comparisons.

There have also been advances in medical treatment of cardiovascular diseases. Patients undergoing cardiac surgery often have several co-existing cardiovascular risk factors, including diabetes, overweight, hypercholesterolemia and smoking. Today, statins and antiplatelet therapy are standard for all CABG patients postoperatively. Optimizing treatment of diabetes and hypertension is also important, whereas patients with reduced cardiac function may benefit from recent advances in the treatment of heart failure. Together with lifestyle intervention such as smoking cessation, regular exercise and weight reduction, these measures aim to delay the progression of graft atherosclerosis, reduce the need for repeat revascularization and improve long-term prognosis.

Due to the continuous evolvement in the indications and techniques of open-heart surgery, as well as postoperative medical treatment and follow-up, it would be of interest to provide an update on long-term outcomes.

1.4 Prediction of outcome

Risk stratification plays an important role in cardiac surgery. Several risk stratification tools have been developed, combining key clinical predictors in order to characterize patients who are at a higher risk of an unfavourable outcome. In-hospital mortality has been the endpoint for many proposed models (125), defined as death within 30 days of operation or within the same hospital admission. The multinational European System for Cardiac Operative Risk Evaluation (EuroSCORE) (126) is the most employed risk prediction model world-wide, and has been made easily available at the EuroSCORE website (127). The original EuroSCORE dataset included 19,030 adult patients from 128 European cardiac centres across 8 countries. The investigators' primary goal was to provide a benchmarking risk algorithm that adjusts for the patients' risk profiles when comparing surgical outcomes across different institutions and

cardiac surgeons. Today, EuroSCORE also facilitates clinicians during clinical decision making, when providing patients and their relatives with information about the risks of surgery, as well as in planning the operational program and allocation of hospital resources.

EuroSCORE has been widely validated. However, during the 2000s, several investigators provided reports showing that the original EuroSCORE overestimated the risk of cardiac surgery procedures. The model showed poor calibration (i.e. agreement between observed outcomes and predictions) and discrimination (i.e. ability to separate between low and high risk patients). Reasons underlying the drift in model calibration include the risk paradox. The changes in patient risk profiles parallel to improved clinical outcomes may also have influenced the relation between prognostic factors and outcomes (128). The original EuroSCORE was therefore recently updated into EuroSCORE II, resulting in improved predictive performance (14).

The focus on risk prediction tools and monitoring quality of care has led to increased surgical safety and identification and elimination of system weaknesses, contributing to reduced mortality rates. However, as even the sickest patients increasingly survive surgery and the immediate postoperative period, the burden of postoperative complications may increase. Thus, in order to further improve patient outcomes, alternative outcomes measures such as postoperative comorbidities and long-term mortality have gained increasing attention.

EuroSCORE has been validated for other outcome measures including renal failure, respiratory failure, sepsis and/or endocarditis, heart failure, pneumonia and mediastinitis (129, 130), as well as length of stay in the intensive care unit (ICU) and hospital costs (129, 131-133). EuroSCORE shows good discrimination, however calibration remains poor. Even though there is some overlap between patients suffering postoperative complications and those who die, the incidence of complications and their underlying mechanisms may be different. A common model for several different endpoints may therefore not be appropriate (134, 135).

Consequently, many attempts have been made at developing preoperative risks scoring algorithms for separate outcomes including postoperative kidney dysfunction (97), cardiac dysfunction (136), respiratory dysfunction (134), neurological complications (137, 138), bleeding complications (139, 140), prolonged stay in the intensive care unit (141) and long-

term mortality (110, 112, 114). Suggested prediction models show moderate performance, but their performance remains limited by as yet unknown predictors, difficulties in measuring and representing certain clinical states, and random, unforeseen events which may be important in individual patients but rare in general (142). Presently known patient and surgical factors only account for a small portion of the variations in the perioperative response to surgery, and substantial intra-individual variability to similar stimuli and the subsequent clinical complications persist. The substantial morbidity still suffered by cardiac surgery patients, the discrepancy between observed and predicted outcomes, as well as lack of success of specific treatment and preventive strategies indicate that "we have more to learn than we have mastered" (15, 27, 143).

1.5 Novel biomarkers

Predictive models have improved our understanding of the most relevant clinically observable risk factors. Nevertheless, there persists inter-individual variability in the preoperative risk profiles and the observed outcomes. Some of the persisting inter-individual variability may be elucidated by the emerging field of novel biomarkers.

A biomarker can be defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention (144). Biomarkers may help to identify patients that have an underlying susceptibility to an abnormal response to cardiac surgery with CPB, which is not captured by observable clinical risk factors. They may provide insight into pathogenic aspects of postoperative complications and potentially enhance outcome prediction by providing important additional knowledge on unmeasured risk. In the following thesis, two types of preoperative biomarkers were explored: Genetic variations and circulating levels of plasma proteins.

1.5.1 Genetic biomarkers

The effect of genetic sequence variants on postoperative complications has been increasingly investigated (145-150). High-risk genetic variants may be associated to adverse outcomes through gene products that contribute to a pro-inflammatory state, modulate the response to

oxidative stress or alter vascular responsiveness. Parolari and coworkers recently published a useful overview over studies investigating the effect of single nucleotide polymorphisms (SNPs) in candidate genes regulating the inflammatory response, haemostasis, oxidative stress, renin-angiotensin system or cell damage, with postoperative complications (21). These studies have hypothesized that there exists a genetic predisposition related to alterations in signalling pathways, which contribute to explain the inter-individual variations in the postoperative response to surgery. Some genetic polymorphisms have been associated with plasma levels of corresponding biomarkers. However, studies relating genetic variants and clinical outcomes remain inconsistent. Thus, as of now, results have been inconclusive. Reasons may include that the biological processes related to postoperative complications are incompletely understood, only few SNPs have been chosen for analysis, inconsistent endpoint definitions, or heterogeneous or small study populations, resulting in inadequate power to detect true associations.

1.5.2 Plasma biomarkers

In addition to genetically determined variations in preoperative plasma biomarkers, individual variations may also indicate alterations in baseline organ function or abnormal ongoing processes, such as a primed immune system with increased baseline inflammation. Thus, preoperative plasma markers may be more sensitive in the identification of patients with a subclinical susceptibility to adverse clinical outcomes. This vulnerability may become of clinical importance when patients are exposed to the insults of an operation. Furthermore, preoperative plasma markers may also represent more objective markers of clinical states and thus improve predictive performance compared to conventional variables.

Previous studies have associated preoperative white blood cell counts (151, 152), troponin T (153, 154), high-sensitivity C-reactive protein (CRP) (155, 156) and brain natriuretic peptide (BNP) or N-terminal pro-BNP (NT-proBNP) (157-162) with adverse outcomes following cardiac surgery. However, the predictive utility and thus the role of preoperative plasma biomarkers in outcome prediction remains controversial. Whereas the abovementioned studies reported associations between biomarkers and the postoperative outcome independent of conventional risk factors, others have not been able to reproduce this (163).

A possible explanation for this, is that the biomarkers only improve prediction if clinical data on corresponding factors are missing. Moreover, many novel markers are correlated with traditional risk factors and therefore may not have sufficiently high independent risk estimates to statistically improve traditional risk models. However, within subgroups of patients predicted at intermediate risk by traditional models, the addition of novel biomarkers may help to reclassify some individuals (164).

Furthermore, considering the complex and multifactorial pathogenesis of postoperative complications, it is less likely that any single marker will be of substantial effect in a heterogeneous study population such as cardiac surgery patients. Thus, a multi-marker approach assessing biomarkers associated with different potential pathogenic pathways may be more likely to improve predictive ability. This new strategy has shown promising results in prediction of heart failure (165), stroke/transient ischemic attack (166) and chronic kidney disease (167) in the non-surgical population. Nevertheless, Brown and coworkers were not able to demonstrate the usefulness of a multi-marker assessment in preoperative prediction of all-cause, in-hospital mortality in patients undergoing CABG (163). However, all-cause mortality is an end-point with potentially many underlying causes, and thus a narrowing of the outcome measure may be necessary. A multi-marker approach in the prediction of postoperative AKI has been warranted (158).

2. Study hypotheses

In this thesis regarding short- and long-term postoperative complications in cardiac surgery patients, we hypothesized that poor outcomes are closely associated with patient-related risk factors.

In Papers 1 and 2, we hypothesized that preoperative prediction of postoperative fluid overload and AKI, respectively, could be enhanced by inclusion of novel biomarkers. Biomarkers related to baseline cardiac and renal function as well as inflammatory and vascular signalling pathways may be of incremental value to traditional clinical risk factors.

In Paper 3, we hypothesized that observed long-term mortality in cardiac surgery patients has remained unchanged over the last couple of decades. Despite the increasing risk seen in patients referred to cardiac surgery, surgical safety, perioperative as well as follow-up care have improved. Risk factors for reduced survival may be related to the chronic and progressive cardiovascular disease these patients suffer. However, considering the advances in secondary prevention and subsequent improvements in cardiovascular mortality, we hypothesized that patients undergoing cardiac surgery show similar long-term survival to the general population.

The specific hypotheses to be tested in the thesis were:

1) Clinical and genetic risk factors related to inflammatory and haemodynamic signalling pathways act independently on the risk of perioperative fluid accumulation.
2) A multi-marker approach including preoperative plasma biomarkers related to inflammatory and haemodynamic signalling pathways enhances prediction of CSA-AKI.
3) Despite a trend of increased preoperative risk, observed long-term mortality in cardiac surgery patients has remained unchanged over time. However, compared to data from the general population, they show similar long-term survival.
4) Predictors of reduced survival are related to cardiovascular risk factors and co-existing comorbidities.

3. Aims

The main aim of this thesis was to investigate risk factors for short- and long-term complications following open-heart surgery. We primarily adopted an explanatory approach, applying statistical modelling strategies in order to delineate possible mechanisms behind postoperative fluid overload, acute kidney injury and long-term mortality in patients undergoing cardiac surgery. The specific aims of each paper are outlined more explicitly below.

Paper 1

Perioperative fluid administration is a debated topic. Standardized protocols may lead to excessive fluid administration in some patients, which has been associated with worse clinical outcomes. Despite consideration to relevant clinical risk factors such as myocardial and renal dysfunction, as well as procedure-related factors such as complicated, prolonged surgery and sedation, identification of patients who are more likely to accumulate fluid and should receive an adjusted fluid therapy regimen is difficult. The aims of Paper 1 were:

1) to investigate the association between 31 single-nucleotide polymorphisms (SNPs) related to inflammatory and/or vascular pathways and the occurrence of postoperative fluid overload following cardiac surgery
2) to evaluate whether genetic risk variants were cumulatively associated with postoperative fluid overload
3) to identify clinical risk factors associated with the risk of postoperative fluid overload
4) to evaluate the relationship between clinical and genetic risk factors in the prediction of postoperative fluid overload

Paper 2

Despite an increasing understanding of the pathogenesis and consequences of CSA-AKI, the incidence remains unchanged or even tends to increase. Moreover, the morbidity and mortality in these patients remain high. Several potential preventive and therapeutic strategies have been proposed, albeit none have shown clinical significance. This might indicate that our understanding of the underlying mechanisms still remains insufficient. Thus, the aims of Paper 2 were:

5) to investigate the association of a panel of preoperative plasma biomarkers, related to different potential pathologic pathways, with CSA-AKI
6) to find a set of relevant clinical variables that accurately predicts CSA-AKI, enabling adjusted analysis of novel biomarkers
7) to assess whether the identified biomarkers were independently associated with CSA-AKI and estimate the incremental value they provided above traditional clinical risk factors

Paper 3

Short-term mortality has been reduced in parallel with technological advances and improvements in surgical safety. However, despite surviving the immediate postoperative period, patients undergoing cardiac surgery often suffer severe cardiovascular disease with several risk factors and comorbidities. The aims of Paper 3 were therefore:

8) to assess long-term observed and relative long-term survival in patients undergoing cardiac surgery
9) to explore potential prognostic factors for long-term mortality, with special focus on the effects of age, gender and the surgical procedure

4. Patients and methods

4.1 The Trondheim Heart Surgery Database

The present study was part of a larger project investigating clinical and genetic risk factors for different complications following cardiac surgery (Cardiac Surgery Outcome Study – CaSOS). Study approval was given by The Norwegian Data Inspectorate and The Regional Research Ethics Committee in Medicine (Project number 4.2007.1528), Trondheim, Norway (Chairperson Arne Sandvik) on 27 June 2007. Consecutive adult patients undergoing elective cardiac surgery at St. Olavs University Hospital in Trondheim, Norway, January 2000 through December 2014 were included (n=8,759). Preoperative and perioperative patient characteristics were prospectively recorded in a local database. All patients were followed to hospital discharge. Registry input was systematically revised at several occasions during their hospital stay and in the end quality assured by a senior anaesthesiologist. Thus, problems of missing data and computation errors were generally small. Data on cause and date of death through December 2014 were obtained through linkage to the Norwegian Cause of Death Registry. Causes of death were provided according to the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10) (168).

As of April 2008, preoperative blood samples were collected from all operative patients. Samples were centrifuged, separated and kept at -80˚C for later analysis. The study cohort for biomarker analyses (Papers 1 and 2) included patients undergoing cardiac surgery from April 2008 to April 2010 (n=1,179). Patients who did/could not consent (n=60) and whose blood samples were missing/infectious (n=58) were excluded. In Paper 1, patients undergoing cardiac surgery from January 2006 through December 2007 (n=1,110) were used as a validation cohort for the clinical predictors associated with postoperative fluid overload. No preoperative blood samples were available from these patients.

4.2 Endpoint definitions and study population

Paper 1

Postoperative fluid balance was recorded as the cumulative net fluid balance from induction of anaesthesia until the first postoperative morning. Operations for all patients were completed

before 5 pm, thus resulting in an observational period of 16 (range 13-19) hours. Patients with preoperative endocarditis and who underwent off-pump surgery were excluded from the study. Intraoperative hemofiltration may affect postoperative fluid balance directly through removal of excess fluid as well as indirectly by modifying the inflammatory response. Therefore, in a supplementary analysis of clinical predictor variables, patients who were subjected to intraoperative hemofiltration (n=57) were excluded. The cut-off for an increased postoperative fluid balance, denoted postoperative fluid overload, was the 90th percentile of the postoperative fluid balance per kilogram (kg) body weight in the study cohort, corresponding to 80.40 ml/kg. With five patients missing records of postoperative fluid balance, there were 102 cases with postoperative fluid overload and 919 controls (n=1021). In the validation cohort, 147 patients (13.2%) developed postoperative fluid overload.

Paper 2

AKI following cardiac surgery was defined as an increase from the baseline serum creatinine concentration ≥ 50% using the maximum postoperative concentration, an absolute increase ≥ 26 μmol/l or a new requirement for dialysis postoperatively, including all severity stages of AKI according to the *Kidney Disease: Improving Global Outcomes* (KDIGO)-guidelines (169). A slight modification from the original definition was used, as we allowed a longer time span than 48 hours postoperatively for increases in serum creatinine to occur. Furthermore, data on urine output were not available. After exclusion of seven patients on preoperative dialysis, two with missing data on preoperative serum creatinine concentrations, and seven with an identification error preventing coupling with clinical data, 1,015 patients remained for analysis. 100 patients (9.9%) fulfilled the criteria for postoperative AKI.

Paper 3

The primary endpoint was all-cause mortality, referred to as observed mortality in this thesis. Second, we attempted to explore mortality specifically seen in cardiac surgery patients by adjusting for the expected mortality in the Norwegian population matched on gender, age and calendar year. As a sensitivity analysis, calculations were also repeated when using cardiovascular death (ICD-10 chapter IX, block I00-I99) as the outcome variable.

Patients undergoing off-pump coronary artery bypass (n=130), TAVI (n=109) and surgery for a thoraco-abdominal aortic aneurysm (n=22) were excluded. Only patients' first entry into the data registry during the study period were included (n=8,564). Nevertheless, as eligible cardiac surgery patients still comprise a heterogeneous group, a subgroup analysis was performed where patients undergoing isolated CABG (n=5,648), isolated AVR (n=726) or combined AVR and CABG (n=829) were compared.

4.3 Selection of genetic biomarkers and genotyping

For the study population of Paper 1, isolation of genomic DNA was performed using a commercial kit (E.Z.N.A. Blood DNA Kit, Omega Bio-Tek, Georgia, USA). A wide selection of genes was included to evaluate the combined effect of several SNPs that may predispose to perioperative fluid accumulation. The included 31 SNPs (Table 2) had either been (1) described as functional in mediators central to inflammatory and vascular responses, (2) previously associated with adverse outcomes following cardiac surgery, or (3) identified in genome-wide association studies (GWAS) of adverse outcomes following cardiac surgery. All SNPs were found in Ensemble release 74. Genotyping for the *LTF* gene was carried out by Sanger sequencing (170). The remaining SNPs were analysed by Centre for Integrative Genetics (CIGENE, Norwegian University of Life Sciences, Ås, Norway) using Sequenom MassArray technology (Sequenom, San Diego, CA, USA).

Table 2. Selection of SNPs and selection rationale

SNP ID	Gene	Gene selection rationale	SNP selection rationale			References
			Identified by previous association studies	Identified by genome wide association studies (GWAS)	Identified through pathway analysis	
rs2115763	***BCO2***	Proinflammatory		X	X	(171)
rs353625	***CD44***	Proinflammatory	X		X	(172)
rs13038305	***CST2***	Immunomodulatory		X	X	(173-175)
rs7933007	*CXCR5*	Proinflammatory	X		X	(172, 176)
rs1799983	*eNOS*	Released under oxidative stress; involved in vasomotor regulation	X		X	(177)
rs17609240	*GSDMA/ORMDL3*	Proinflammatory		X	X	(178)
rs17379472	*HSP-A1L*	Chaperone activity; protects against inflammation and oxidative stress			X	(179, 180)
rs5498	***ICAM 1***	Proinflammatory	X		X	(148, 181)
rs1861494	*IFN-γ*	Proinflammatory			X	(182)
rs1800872	*IL-10*	Antiinflammatory	X		X	(177, 181)
rs1800896	***IL-10***	Antiinflammatory	X		X	(177, 181)
rs1800871	***IL-10***	Antiinflammatory	X		X	(177, 181)
rs1834481	***IL-18***	Proinflammatory		X	X	(171)
rs11209026	*IL23-R*	Proinflammatory and immunomodulatory			X	(183)
rs1800795	***IL-6***	Proinflammatory	X		X	(177, 181)
rs4073	*IL-8/CXCL8*	Proinflammatory			X	(181)
rs2227306	*IL-8/CXCL8*	Proinflammatory			X	(181)
rs10662431	***LTF***	Antimicrobial, antiinflammatory and immunomodulatory			X	(184-186)
rs1126478	***LTF***	Antimicrobial, antiinflammatory and immunomodulatory			X	(184-186)
rs1126477	*LTF*	Antimicrobial, antiinflammatory and immunomodulatory			X	(184-186)
rs17078878	*LTF*	Antimicrobial, antiinflammatory and immunomodulatory			X	(184-186)
rs243865	*MMP-2*	Immunomodulatory	X		X	(187)
rs12119788	*MRP-14 =S100A9*	Proinflammatory		X	X	(188, 189)
rs4673	***p22phox***	Superoxide production under oxidative stress and respiratory burst; proinflammatory	X		X	(177)
rs2107538	***RANTES/CCL5***	Proinflammatory			X	(181)
rs1805193	***SELE***	Proinflammatory	X		X	(148)
rs12917707	***UMOD***	Potential facilitator of inflammation		X	X	(173, 190)
rs699947	*VEGF-A*	Increases vascular permeability and leakage	X		X	(173, 177, 191)
rs2010963	*VEGF-A*	Increases vascular permeability and leakage	X		X	(173, 177, 191)
rs3025039	*VEGF-A*	Increases vascular permeability and leakage	X		X	(173, 177, 191)
rs833061	*VEGF-A*	Increases vascular permeability and leakage	X		X	(173, 177, 191)

SNPs given in bold were included in the genetic risk score for postoperative fluid overload.

4.4 Selection of plasma biomarkers and measurement protocols

To investigate preoperative plasma biomarkers that may be associated with an increased risk of CSA-AKI in Paper 2, plasma concentrations of CRP, neopterin, terminal complement complex (C5b-9), lactoferrin, cystatin C and NT-proBNP were analysed using enzyme immunoassays as described in their corresponding commercial kits (please refer to original article). Biomarker selection was based on causal hypotheses, incorporating different aspects of the multifactorial pathogenesis behind CSA-AKI (Table 3). The selected biomarkers have been related to either inflammatory, hemodynamic or renal signalling pathways.

Table 3. Overview over the origin, function and application of analysed biomarkers

Abbreviations: NT-proBNP; N-terminal pro-brain natriuretic peptide.

Biomarker	Origin	Indicator of	Current clinical application
C-reactive protein	Produced by hepatocytes in response to circulating interleukin-6	Inflammation; acute-phase-reactant	Diagnosis and monitoring of inflammatory and infectious diseases, prediction of cardiovascular disease (192)
Neopterin	Produced by activated macrophages and monocytes	Inflammation; cellular immune response	Novel biomarker; suggested in monitoring of inflammatory and infectious diseases (193)
Terminal complement complex	End product of the complement cascade; assembled by its five late components; C5b-9	Inflammation; complement activation	Novel biomarker; suggested in evaluation of biocompatibility of biomaterials such as CPB (194)
Lactoferrin	Produced and released by neutrophils	Inflammation; neutrophil activation	Novel biomarker; suggested in monitoring of inflammatory and infectious diseases (195)
NT-proBNP	Released from ventricular myocytes in response to myocardial stretch	Hemodynamic function; diastolic heart dysfunction and volume overload	Diagnostics of acute dyspnoea (196) and monitoring of heart failure (197)
Cystatin C	Produced by all nucleated cells at a constant rate	Renal function; estimated glomerular filtration rate	Estimation of kidney function and diagnosis of chronic kidney disease (198, 199)

4.5 Statistical analysis

Statistical analyses were performed using SPSS (version 20.0, SPSS Inc., Chicago, IL, USA), Stata (version 13.1, StataCorp, College Station, TX, USA), SigmaPlot (version 12.0, Systat Software, San Jose, CA, USA), Minitab (version 16.2.3, Minitab, State College, PA, USA) and *R* statistical software (version 3.0.0, Foundation for Statistical Computing, Vienna, Austria). For the simultaneous analysis of many SNPs in Paper 1, we applied HyperLasso (The European Bioinformatics Institute, http://www.ebi.ac.uk/projects/BARGEN) (200).

4.5.1 Binary outcome data

In Papers 1 and 2, binary endpoint-definitions (no/yes) were defined. Baseline patient characteristics across cases and controls were compared using the Mann-Whitney U-test or Chi-squared test for continuous and categorical data, respectively. Logistic regression modelling was the cornerstone of the analysis, where the association of novel biomarkers on perioperative fluid accumulation and CSA-AKI, respectively, followed a 3-step procedure:

4.5.1.1 Analysis of novel biomarkers

Identification of genetic determinants associated with postoperative fluid overload

The quality of genotyping for all SNPs was checked by evaluating missingness and whether the genotype frequencies were in Hardy-Weinberg equilibrium. In order to identify genetic risk variants possibly related to postoperative fluid overload, we applied penalized maximum likelihood-based logistic regression with HyperLasso. HyperLasso enables a simultaneous comparison of multiple SNPs, thus testing the joint influence of several SNPs. Thereby, the risk of false positives is reduced and the ability to find true associations is strengthened (200). The total type I error was set to 0.05 in order to reduce the family-wise error rate and adjust for multiple comparisons.

Identification of preoperative plasma biomarkers associated with postoperative AKI

The linearity assumption was tested by plots and splines. Plasma biomarkers were natural log-transformed when appropriate. NT-proBNP was dichotomized with a cut-off value >125 pg/ml, as recommended for preoperative screening in moderate-to-high risk patients (201, 202).

Biomarkers with p-value <0.10 were included into a simultaneous testing of all plasma biomarkers.

4.5.1.2 Identification of clinical adjustment variables

Clinical prediction modelling was applied in Papers 1 and 2 in order to appropriately adjust for clinical variables when searching for novel biomarkers. The selection of clinical covariates potentially related to the endpoint were derived based on literature review, clinical experience and hypotheses of potential influence, a method recommended to avoid overfitting and confounding as found with selection based on univariate analyses (203, 204). The models with all potentially related variables were analysed with multivariate logistic regression. They were checked for deviations from the linearity assumption, predefined interactions and overly-influential observations. Backward limited step-down was applied in order to derive parsimonious models. Final predictor coefficients and their respective CI were calculated using bootstrap methods (400 runs).

Model performance was assessed through discrimination and calibration statistics: Model discrimination is defined as the model's ability to separate between patients with lower and higher risk, and was assessed by calculation of the area under the receiver operating characteristics curve (AUC). An AUC of 0.5 indicates no discrimination, between 0.7-0.8 acceptable discrimination and >0.80 good discrimination. Model calibration evaluates the agreement between observed and predicted outcomes and was evaluated graphically by a calibration plot. Perfect fit would produce a graph line of 45° between observed and predicted probabilities. Additionally, calibration was formally tested with a Hosmer-Lemeshow (HL) test, in which observed risk is compared against deciles of predicted risk. A *P*-value > 0.05 indicates non-significant differences between predicted and observed risks.

Internal validation of the models was performed using bootstrapping. This is preferred over data-splitting methods due to more unstable estimates yielded from smaller study cohorts (203). The risk of overfitting was assessed by calculation of the shrinkage factor. If the shrinkage falls below 0.85, there is a reason for concern. Bias-corrected estimates after bootstrapping were compared with the original estimates.

4.5.1.3 Multivariate, adjusted analysis of biomarkers

In the final step, we investigated whether the novel biomarkers added incremental information to clinical variables in predicting the outcome. Models with and without biomarkers were compared using likelihood ratio tests.

4.5.2 Survival time data

In Paper 3, we explored observed and relative long-term survival. Temporal trends were analysed across year of surgery continuously as well as categorized into 3 prespecified time periods (2000-2004, 2005-2009 and 2010-2014). Differences in patient characteristics across time periods were tested using the Chi-squared and Kruskal-Wallis tests for categorical and continuous data, respectively. Changes in mortality rates during the study period were assessed with a Chi-squared test for departure from the trend line (205).

4.5.2.1 Observed and relative survival and mortality

Observed survival and cumulative hazard rates were calculated using the Kaplan-Meier and Nelson-Aalen estimators, respectively. Furthermore, we compared long-term survival in cardiac surgery patients with that of the general population (=expected survival). Survival data from the Norwegian population matched on gender, age and calendar year were retrieved from the Human Mortality Database (206). Relative survival was calculated as the ratio between the observed and expected survival rates and presented graphically across follow-up time. Calculated observed and relative survival in the present study cohort was also compared with data retrieved from older reports from Sweden based on data from 1970s through the 1990s (106, 207).

For the complete follow-up period, relative mortality was calculated as the ratio between the observed and expected number of deaths (multiplicative hazard model), providing a direct comparison between the observed mortality in the cohort and the expected mortality based on data from the general population. Results are presented as standardized mortality ratios (SMR). Relative mortality was compared across age categorized into < 60 years, 60-69 years, 70-79 years and ≥ 80 years.

4.5.2.2 Predictors of long-term mortality

Predictors of observed long-term mortality were investigated with Cox proportional hazards (PH) modelling. Deviations from the proportionality assumption were assessed graphically and by inclusion of interaction terms between the predictors and time. Separate parameter estimates for pre-specified time periods (<1, 1-5 and >5 years) were compared in order to assess time-dependent effects.

In order to evaluate factors associated with long-term relative mortality, we applied multiplicative modelling of relative mortality as described by Pohar *et al*. (208, 209). Differences in relative mortality between patients with different covariate levels are expressed as relative mortality ratios (RMR).

4.5.3 Further details on the statistical analysis

4.5.3.1 Paper 1

Two different approaches to the genetic analyses were taken: First, we investigated single SNPs strongly associated with postoperative fluid overload as described above. Second, we explored the hypothesis that genetic risk variants may exert a cumulative effect on perioperative fluid accumulation, and tested the significance of a genetic risk score on the incidence of postoperative fluid overload.

In order to evaluate the cumulative effect of multiple genetic sequence variants, the criteria in HyperLasso was relaxed (total type I error of 0.1) in order to identify all potentially relevant SNPs. Both genetic analyses were first performed on the original dataset. Subsequently, the analyses were repeated in bootstrapped datasets (100 runs). SNPs that were significant in more than 20% of the datasets were included in the genetic risk score. The risk score was calculated by adding the number of risk alleles carried by each study participant and had a theoretical range from 0 (no risk alleles) to twice the number of SNPs included in the score. The associated SNPs and the genetic risk score were investigated further with logistic regression analyses.

Subsequently, we explored clinical predictors of perioperative fluid accumulation. The final predictors were validated in the validation cohort to evaluate the presence of overfitting. The

genetic risk variants and risk score were then evaluated as independent predictors of postoperative fluid overload by adding the identified predictors from the clinical model into the logistic regression analysis.

4.5.3.2 Paper 2

A multi-marker analysis of preoperative plasma biomarkers was performed as described. Furthermore, we explored different approaches of selecting relevant clinical risk factors of CSA-AKI for the adjustment analysis. There have been many attempts to develop robust clinical prediction models of CSA-AKI. We evaluated the performance of the most relevant scoring tools including:

1) *The CaSOS' AKI risk score*; a local prediction model which was previously constructed based on patients who underwent cardiac surgery in Trondheim from 2000 through 2007 (210). The model has not been externally validated yet.
2) *The Cleveland clinical risk score*; a risk score developed based on 15,838 patients who underwent open-heart surgery at the Cleveland Clinic Foundation from 1993 through 2002 (211). It has performed well in some external cohorts and several comparison studies have found that the Cleveland Clinic score had the highest discriminative power (212-216). The risk score was originally constructed for dialysis-dependent renal failure, however, it has been of predictive utility for different severity levels of CSA-AKI (212, 214, 216). The risk score has been made available as an online risk calculator (217).
3) *The UK any-stage AKI risk score*; a more recent risk score aiming to predict the risk of any-stage AKI in accordance with the definitions of the KDIGO guidelines (218). The model was developed in a multi-centre study from three UK hospitals in patients undergoing cardiac surgery between 1996 and 2010 (n=20,995). The risk score has not been externally validated yet.

The variables included in each risk score are outlined in Table 4.

Table 4. Variables included in pre-existing clinical risk models for acute kidney injury

CaSOS' CSA-AKI risk model	Cleveland clinical risk score	UK any-stage AKI risk calculator	
1 Age	1 Female gender	1 Age	10 GFR (mL/min per 1.73 m^2)
2 BMI >30 kg/m^2	2 Left ventricular ejection fraction <35%	- < 60 years†	- <30.0
3 Lipid-lowering treatment	3 Preoperative use of IABP	- 60-74 years	- 30.0-59.9
4 Hypertension	4 Chronic obstructive pulmonary disease[a] (medically treated)	- ≥ 75 years	- 60.0-89.9†
5 Peripheral vascular disease	5 Insulin-dependent diabetes	2 Female gender	- ≥ 90.0
6 Chronic pulmonary disease	6 Previous cardiac surgery	3 BMI (kg/m^2)	11 PCI prior to surgery[b]
7 Haemoglobin concentration	7 Emergency surgery	- <20.0	12 Triple vessel disease[b]
8 Preoperative creatinine	8 Surgery type	- 20-0-24.9	13 Ejection fraction
- Below 100 µmol/l†	- CABG only†	- 25.0-29.9†	- Good (≥ 50%)†
- 100 to 140 µmol/l	- Valve only	- 30.0-34.9	- Fair (30-49%)
- Above 140 µmol/l	- CABG + Valve	- ≥ 35.0	- Poor (<30%)
9 Previous cardiac surgery	- Other cardiac surgeries	4 Smoking	14 Operative priority
10 Emergency surgery	9 Preoperative creatinine*	- Never smoked†	- Elective†
11 Surgery type	- < 1.2 mg/dl†	- Ex-smoker	- Urgent
- CABG and ASD†	- 1.2-2.1 mg/dl	- Current smoker	- Emergency surgery
- AVR, AVR and CABG, non-ischaemic mitral valve surgery, aneurysm of ascending aorta	- ≥ 2.1 mg/dl	5 Dyspnoea	15 Surgery type
- Dissection of ascending aorta, rupture of the ventricular septum		- NYHA class I†	- CABG only†
- Miscellaneous		- NYHA class II	- Valve only
		- NYHA class III	- CABG + Valve
		- NYHA class IV	16 Other/multiple cardiac surgeries
		6 Diabetes	
		7 Peripheral vascular disease	
		8 Hypertension	
		9 Haemoglobin (g/dl)	
		- <10.0	
		- 12.0-11.9	
		- ≥ 12.0†	

†Defined as reference categories. *Categories correspond to <106.1 µmol/l, 106.1 µmol/l-185.64 µmol/l and >185.64 µmol/l. Conversion factor from mg/dl to µmol/l; x 88.4. [a]Due to incomplete registration of medical treatment for chronic obstructive lung disease, we included all patients registered with chronic obstructive lung disease. [b]Information about PCI prior to surgery and triple vessel disease was not available, and were therefore excluded from the calculations. Abbreviations: ASD, atrium septum defect; AVR, aortic valve replacement; BMI, body mass index; CABG, coronary artery bypass grafting; CSA-AKI, cardiac surgery-associated acute kidney injury; IABP, intra-aortic balloon pump; GFR, glomerular filtration rate; NYHA, The New York Heart Association Functional Classification (I-IV); PCI, percutaneous coronary intervention; UK, United Kingdom.

The performance of these pre-existing CSA-AKI prediction tools was compared with a novel, parsimonious model. As a sensitivity analysis, alternative models with different indicators of pre-existing renal dysfunction (serum creatinine, creatinine clearance, or estimated GFR based on creatinine or creatinine and cystatin C combined (199)) and heart function (ejection fraction, diagnosis of chronic heart failure or New York Heart Association (NYHA) Functional Classification) were tested.

Subsequently, significant plasma markers from the multimarker assessment were explored in a nested multivariate analysis together with the final clinical predictors. The incremental value of the added biomarkers was tested with the likelihood ratio test. Their clinical usefulness was further evaluated with comparison of the AUC, integrated discrimination improvement (IDI) and net reclassification improvement (NRI) (219). In order to evaluate the effects of biomarker inclusion on AKI risk classification, reclassification with the combined model was analysed in low (<10%), intermediate (10-20%) and high risk groups (>20%).

4.5.3.3 Paper 3

In the investigation of clinical predictors of long-term mortality, general demographics (age, gender, body mass index), procedure-related factors (surgical procedure, redo-operation, emergency level), comorbidity and smoking (never/former vs. current smoker) were included into the models block-wise. Surgical procedures were categorized in accordance with EuroScore 2's definition into isolated CABG, 1 non-CABG procedure, 2 surgical procedures or ≥ 3 surgical procedures, where isolated CABG was defined as the reference category (14). As cardiac surgery patients still constitute a heterogeneous group, a sensitivity analysis was performed by including patients only undergoing CABG and/or AVR.

A secondary analysis was performed to further investigate female gender as a risk factor for long-term mortality. Gender differences in preoperative risk factors were compared with the Mann-Whitney U-test or Chi-squared test for continuous and categorical variables, respectively. Thereafter, a balancing propensity score was developed using logistic regression with gender as the outcome, including the following explanatory variables: Age, body mass index, smoking status, diabetes, hypertension, preoperative history of atrial fibrillation, peripheral vascular disease, chronic pulmonary disease, previous myocardial infarction hypertension, left ventricular hypertrophy, NYHA functional class, diagnosis of chronic heart

failure, kidney disease, preoperative serum creatinine, use of beta-antagonists, statins or diuretics before scheduled for surgery, previous cardiac surgery, emergency level of operation, acute preoperative heart failure, and type of surgical procedure. We used 1:1 greedy matching with a caliper width 0.25*the standard deviation of the propensity score to form female-male pairs. Covariate balance was evaluated using standardized differences, where an absolute standardized difference in the covariate mean for a variable ≤ 10% indicated acceptable balance. Analyses were performed using the boost (220) and psmatch2 (221) programs in Stata. Following adequate balance of preoperative risk factors, Cox PH modelling for all-cause and cardiovascular mortality was repeated in the matched dataset.

In order to assess the fit of a model and compare competing models, we calculated the log likelihood and the Bayesian and Akaike information criterions (BIC and AIC, respectively). Whereas adding more variables will often improve fit and log likelihood, the information measures have penalties for including variables that do not significantly improve fit. Thus, they facilitate the selection of more parsimonious but adequate models and supplemented final model selection in addition to the likelihood ratio test.

4.5.4 Joint findings from all papers

It may be argued that fluid overload represents a surrogate marker for other outcomes, such as renal and cardiac dysfunction. In order to explore this hypothesis, supplementary analyses were performed. Based on all patients undergoing cardiac surgery in Trondheim between 2000 through 2014, we analysed postoperative fluid overload, AKI and cardiac dysfunction as distinct and overlapping syndromes. Postoperative fluid overload and AKI was defined as described for Paper 1 and 2, respectively. Postoperative cardiac dysfunction was defined as the need for more than one inotropic agent, an intra-aortic balloon pump or extracorporeal membrane oxygenation occurring after the operation and until the patient was discharged from the department. The same end-point definition was used in two previously published papers from our group (136, 222).

Furthermore, we applied multivariate logistic regression analysis and Cox PH modelling in order to assess their independent effect on short- and long-term mortality, respectively.

5. Summary of results

5.1 Patient characteristics

A summary of patient characteristics for the complete study cohort (2000-2014), as well as comparisons amongst cases and controls in Papers 1 and 2 (2008-2010) are given in Table 5. For a more comprehensive comparison of patient characteristics, see the original papers as provided in the appendices.

In general, patients suffering fluid overload or CSA-AKI were older, more often had a history of previous cardiac surgery, underwent more complex surgical procedures and spent longer time on CPB. Patients experiencing postoperative fluid overload were more often female ($p<0.001$), and they more often presented with chronic cardiac insufficiency ($p=0.04$), left ventricular hypertrophy ($p=0.004$) and peripheral vascular disease ($p=0.02$). However, co-existing comorbidities were more prominent in patients suffering CSA-AKI, also including diabetes ($p=0.03$), hypertension ($p=0.006$), chronic pulmonary disease ($p<0.001$) and pre-existing renal disease ($p<0.001$).

5.2 Papers 1 and 2

5.2.1 Clinical adjustment variables

In Paper 1, longer CPB time, increasing age and need for intraoperative red cell transfusion were associated with an increased risk of postoperative fluid overload (Table 6). These variables constituted the clinical risk model for postoperative fluid overload, and showed good calibration (HL test, $p=0.85$) and discrimination (AUC 0.797 (0.746-0.847)). Addition of variables related to reduced preoperative renal or cardiac function (chronic cardiac insufficiency, left ventricular hypertrophy) did not improve model performance. Moreover, exclusion of hemofiltrated patients ($n=57$) neither altered the clinical model nor improved model performance. Thus, intraoperative hemofiltration was not considered a relevant exclusion criterion and further analyses included the full study cohort ($n=1021$). The clinical model performed well in the validation cohort, in which the calibration plot showed good prediction and the AUC did not differ significantly from that of the study cohort (0.785 (0.742-0.829)).

Table 5. Patient characteristics

	2000-2014	2008-2010			
	All patients	Fluid overload		Acute kidney injury	
	(n=8,759)	Yes (n=102)	No (n=919)	Yes (n=100)	No (n=915)
Demographics					
Age (years)	67 (66.5-67.0)	74 (72.0-75.5)***	67 (66.0-67.5)	74 (71-76)***	67 (66-68)
Female gender	2,248 (25.7%)	47 (46.1%)***	231 (25.1%)	28 (28.0%)	254 (27.8%)
Body mass index (kg/m^2)	26.6 (26.5-26.7)	23.8 (23.0-24.6)***	27.4 (27.1-27.6)	28.3 (27.3-29.4)**	26.9 (26.6-27.2)
Ever smoker	4,712 (53.8%)	47 (47.0%)	514 (56.3%)	45 (45.0%)*	513 (56.1%)
Comorbidities					
Diabetes mellitus	1,217 (13.9%)	9 (8.8%)	133 (14.5%)	21 (21.0%)*	121 (13.2%)
Peripheral vascular disease	939 (10.7%)	23 (22.6%)*	130 (14.2%)	22 (22.0%)*	131 (14.3%)
Previous myocardial infarction	3,946 (45.1%)				
Chronic pulmonary disease	1,376 (15.7%)	18 (17.7%)	123 (13.4%)	27 (27.0%)***	115 (12.6%)
Chronic heart failure	1,387 (15.8%)	18 (17.7%)*	100 (10.9%)	20 (20.0%)**	101 (11.0%)
Kidney dysfunction	405 (4.6%)	5 (5.0%)	35 (3.8%)	15 (15.0%)***	20 (2.2%)
Surgical characterstics					
Acute preoperative heart failure	91 (1.0%)	---	---	---	---
Acute surgery (<24 hours)	485 (5.5%)	---	---	---	---
Urgent surgery (<2 weeks)	3,630 (41.5%)	50 (49.0%)	409 (44.5%)	51 (51.0%)	400 (43.7%)
Redo operation	488 (5.6%)	11 (10.8%)***	32 (3.5%)	11 (11.0%)***	34 (3.7%)
Surgical procedure:		***		***	
1) Isolated CABG	5,703 (65.1%)	30 (29.7%)	621 (67.6%)	35 (35.0%)	609 (66.6%)
2) 1 non-CABG procedure	1,145 (13.1%)	26 (25.7%)	96 (10.5%)	13 (13.0%)	114 (12.5%)
3) 2 surgical procedures	1,676 (19.1%)	40 (39.6%)	173 (18.8%)	44 (44.0%)	168 (18.4%)
4) ≥ 3 surgical procedures	235 (2.7%)	5 (5.0%)	29 (3.2%)	8 (8.0%)	24 (2.6%)
CPB time (min)	80 (79-80)	109 (99-120)***	76 (74-78)	117 (106-129)***	76 (74-79)

Categorical variables are given in n (%), continuous variables in median (95% confidence interval). For the biomarker studies (Paper 1 and 2) there were no patients with acute preoperative heart failure or acute surgery. *p<0.05, **p<0.01, ***p<0.001. Abbreviations: CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass.

In Paper 2, the final parsimonious clinical model used to adjust for plasma biomarkers in the multivariate analysis of CSA-AKI comprised age, body mass index ≥ 30 kg/m^2, female gender, multiple surgical procedures and preoperative serum creatinine level (Table 6). By overall judgement, this model provided better fit than any of the previously published prediction models for CSA-AKI: Besides being simpler, it showed lower AIC, intermediate BIC, high discrimination (AUC 0.800 (95% CI 0.758-0.842)) and excellent goodness-of-fit (HL test, p=0.47). Alternative models from the sensitivity testing including data on chronic heart failure, NYHA class, diabetes, pulmonary disease or urgency level of operation did not improve model performance (data not shown).

Table 6. Clinical adjustment variables for biomarker analysis

	Odds ratio (95% CI)	P-value
Postoperative fluid overload (n=1,021)		
Age (per 5 years)	1.33 (1.14-1.54)	<0.001
CPB time (per 10 min)	1.15 (1.09-1.21)	<0.001
Intraoperative red cell transfusion	3.85 (2.43-6.11)	<0.001
Postoperative acute kidney injury (n=1,015)		
Age (per 5 years)	1.29 (1.13-1.46)	<0.001
Female gender	0.82 (0.48-1.38)	0.45
Body mass index >30 kg/m^2	2.33 (1.44-3.77)	0.001
Multiple surgical procedures	3.94 (2.50-6.19)	<0.001
Preoperative serum creatinine (per 10 µmol/L)	1.19 (1.11-1.28)	<0.001

Abbreviations: CI, confidence interval; CPB, cardiopulmonary bypass.

5.2.2 Genetic markers associated with postoperative fluid overload

Two SNPs were identified; rs12917707, G>T in the locus of the *UMOD* gene and rs353625, A>G in the *CD44* gene, as significant predictors of postoperative fluid overload (p<0.05) with the rare alleles being protective. When adjusting for the clinical covariates, only rs12917707 remained significant. Due to few homozygous cases for the rare allele, both SNPs were recoded into recessive traits for the common allele associated with an increased risk. Further analysis with ordinary logistic regression confirmed rs12917707 as an independent predictor, where homozygous carriers of the G allele had a 2.24 times greater risk of postoperative fluid overload (Table 7).

Table 7. Logistic regression analyses for association of rs12917707 (UMOD) and rs353625 (CD44) with fluid overload following open-heart surgery

SNP and genotype	Genotype frequency		Logistic regression[1]		Adjusted logistic regression[1,2]	
	Controls	Cases	Odd ratio (95% CI)	*P*-value	Odd ratio (95% CI)	*P*-value
rs12917707 (G>T)						
TT/TG	285 (31.2%)	20 (19.6%)	1.00 [reference]	-	1.00 [reference]	-
GG	628 (68.8%)	82 (80.4%)	1.86 (1.12-3.09)	0.02	2.24 (1.27-3.94)	0.005
rs353625 (A>G)						
GG/GA	429 (47.5%)	39 (38.6%)	1.00 [reference]	-	1.00 [reference]	-
AA	474 (52.5%)	62 (61.4%)	1.44 (0.94-2.19)	0.09	1.43 (0.91-2.27)	0.12

[1]After exclusion of incomplete cases, the models for rs12917707 and rs353625 were based on 1015 and 1004 participants, respectively.

[2]Adjusted for CPB time, age and use of intraoperative red cell transfusion

Abbreviations: CI, confidence interval; SNP, single-nucleotide polymorphism.

The genetic risk score was constructed based on a subset of 14 SNPs (Table 2, section 4.3). The observed score was 7-24 (theoretical range: 0-28) and was linearly associated with the frequency of postoperative fluid overload (Figure 5). In the unadjusted logistic regression analysis, every additional risk genotype was associated with a 12% increased risk of developing postoperative fluid overload (OR 1.119 (1.037-1.207), p=0.004). The genetic risk score remained a significant predictor of postoperative fluid overload when adjusting for clinical variables (OR 1.153 (1.056-1.258), p=0.001).

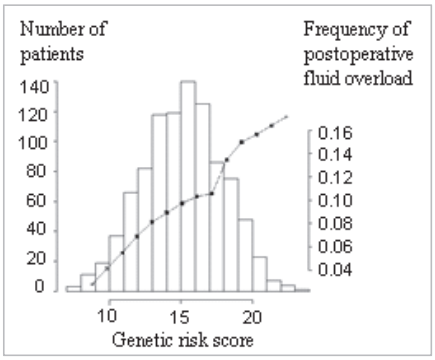

Figure 5: Genetic risk scores in patients with postoperative fluid overload. Left y-axis represents distribution of the genetic risk scores (histogram). Right y-axis shows proportion of patients with a specific risk score suffering from postoperative fluid overload (line graph).

5.2.3 Plasma biomarkers associated with CSA-AKI

Baseline concentrations of fluid-phase markers are given in Table 8. Neopterin, CRP, cystatin C and NT-proBNP concentrations were higher ($p<0.001$ for all) in patients developing CSA-AKI. Lactoferrin concentrations were lower in AKI cases, but this difference did not reach statistical significance ($p=0.05$). Neopterin, lactoferrin, NT-proBNP and cystatin C remained significant in a simultaneous test of all biomarkers.

Neopterin, NT-proBNP and lactoferrin emerged as independent predictors of CSA-AKI (Table 8). Cystatin C was moderately correlated with serum creatinine levels ($R=0.48$), but did not provide significant information above that of preoperative creatinine concentrations ($p=0.15$). Substitution of creatinine with cystatin C did not improve model performance. However, when estimating GFR using the combined creatinine-cystatin C equation, the model showed somewhat better fit. Thus, in the final model, estimated GFR based on serum cystatin C and creatinine concentrations substituted serum creatinine concentration alone (Table 9).

Neopterin was correlated with serum creatinine ($R=0.56$), however neopterin remained significant also after adjusting for kidney function (neopterin/creatinine ratio). This adjustment did not alter any results, thus the parsimonious model without adjustment was kept. In well-calibrated clinical models, NT-proBNP and neopterin consistently emerged as independent predictors of CSA-AKI. The association of serum lactoferrin concentrations with CSA-AKI was no longer significant after bootstrapping the estimates in the final model (Table 9, $p=0.08$).

Table 8. Preoperative plasma concentrations of biomarkers and logistic regression results[a]

Biomarker	Baseline serum concentrations		Univariate analysis	Multimarker analysis	Adjusted multimarker analysis[d]
	AKI-cases (n=100)	Non-AKI cases (n=905)			
Lactoferrin (µg/l)[b]	125 (111-149)	149 (142-158)	0.05	0.04	0.05
Neopterin (nmol/l)[b]	10.5 (9.4-11.9)	7.4 (7.2-7.6)	<0.001	0.001	0.03
C-reactive protein (mg/l)[b]	5.8 (4.4-7.7)	2.5 (2.3-3.0)	<0.001	0.70	0.40
Terminal complement complex (ng/l)	188 (168-208)	174 (167-181)	0.47	---	---
Cystatin C (mg/l)[b]	1.07 (0.98-1.18)	0.85 (0.83-0.87)	<0.001	0.001	0.15
NT-proBNP (pg/ml)[c]	313 (230-488)	108 (99-119)	<0.001	<0.001	0.001

Two overly-influential cases were excluded. Multivariate analyses were performed on cases with complete data on all biomarkers (n=1,005). [a]P-values for the logistic regression analysis. [b]Natural log-transformed. [c]Dichotomized at a cut-off value of >125 pg/ml. [d]With adjustment for gender, age, body mass index ≥ 30 kg/m^2, surgical category and preoperative serum creatinine.

Table 9. Nested logistic regression analysis comparing the clinical model without and with biomarkers

	Clinical model			Clinical model with biomarkers		
Predictor parameters	OR	95% CI	P-value	OR	95% CI	P-value
Age (per 5 years)	1.19	1.02-1.39	0.03	1.09	0.93-1.27	0.30
Female gender	0.56	0.33-0.96	0.04	0.54	0.31-0.96	0.03
Body mass index >30 kg/m^2	2.12	1.25-3.61	0.006	2.51	1.47-4.28	0.001
Multiple surgical procedures	3.63	2.30-5.73	<0.001	3.22	1.87-5.54	<0.001
Estimated GFR[a] (per 5 ml/min per 1.73m^2)	0.84	0.79-0.89	<0.001	0.91	0.85-0.97	0.006
NT-proBNP >125 pg/ml	---	---	---	2.86	1.63-5.01	<0.001
Neopterin[b]	---	---	---	2.70	1.45-5.02	0.002
Lactoferrin[b]	---	---	---	0.70	0.47-1.04	0.08
Model evaluation parameters						
AUC (95% CI)	0.806 (0.764-0.847)			0.832 (0.791-0.873)		
Akaike information criterion	538.7			510.4		
Bayes information criterion	568.1			554.6		

Final model parameters with bootstrapped confidence intervals (200 runs). The nested analysis was performed on cases with complete data for all biomarkers (n=1,005)..

[a]Calculations were based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine-cystatin C single equation (2012): $135\times\min(Scr/\kappa, 1)^{\alpha}\times\max(Scr/\kappa, 1)^{-0.601}\times\min(Scys/0.8, 1)^{-0.375}\times\max(Scys/0.8, 1)^{-0.711}\times0.995^{Age}$ [×0.969 if female][×1.08 if black], where Scr is serum creatinine, Scys is serum cystatin C, κ is 0.7 for females and 0.9 for males, α is −0.248 for females and −0.207 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1 (199).

[b]Natural log-transformed.

Abbreviations: AUC, area under the receiver-operating characteristic curve; CI, confidence interval; GFR, glomerular filtration rate; NT-proBNP, N-terminal pro-brain natriuretic peptide; OR, odds ratio

5.2.4 Incremental value of biomarkers when added to clinical variables

For both postoperative fluid overload and CSA-AKI, model performance was significantly enhanced by inclusion of biomarker data to the models with clinical variables only (LR test, $p<0.001$ for both).

For all levels of clinical risk, an increasing number of genetic risk variants gave a non-linear increase in probability of fluid overload (Figure 6).

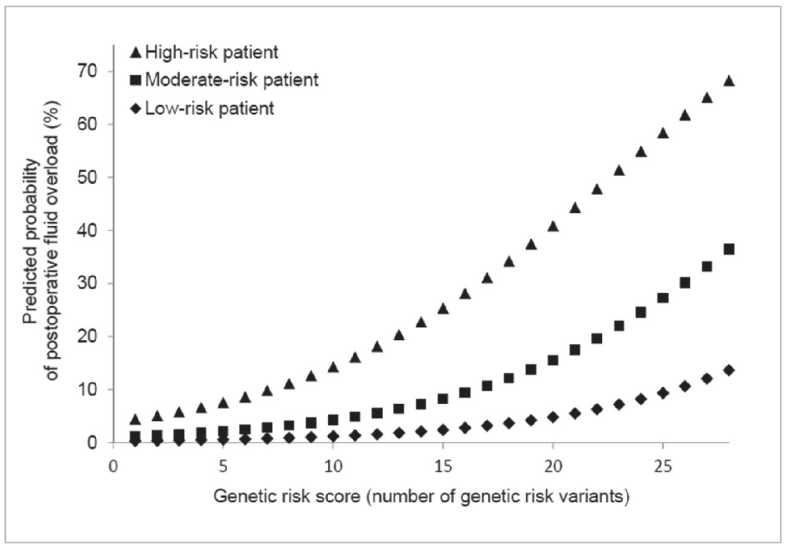

Figure 6: Probability of postoperative fluid overload according to patient risk profile. Examples of influence of genetic risk score on predicted probability of postoperative fluid overload according to clinical risk profile. An increasing number of risk variants gives a non-linear increase in probability of fluid overload. *Low-risk patient:* 65 years old, CPB time 45 minutes, no intraoperative red cell transfusion. *Moderate-risk patient:* 75 years old, CPB time 98 minutes, no intraoperative red cell transfusion. *High-risk patient:* 75 years old, CPB time 98 minutes, received intraoperative red cell transfusion.

With regards to CSA-AKI, the addition of a panel of plasma biomarkers (baseline NT-proBNP, lactoferrin and neopterin levels) resulted in excellent agreement between predicted and observed risks (HL test p=0.87). There was an incremental increase in the AUC from 0.806 (95% CI 0.764-0.847) with clinical variables only, to 0.832 (95% CI 0.791-0.873, p=0.05, Figure 7) when including biomarkers.

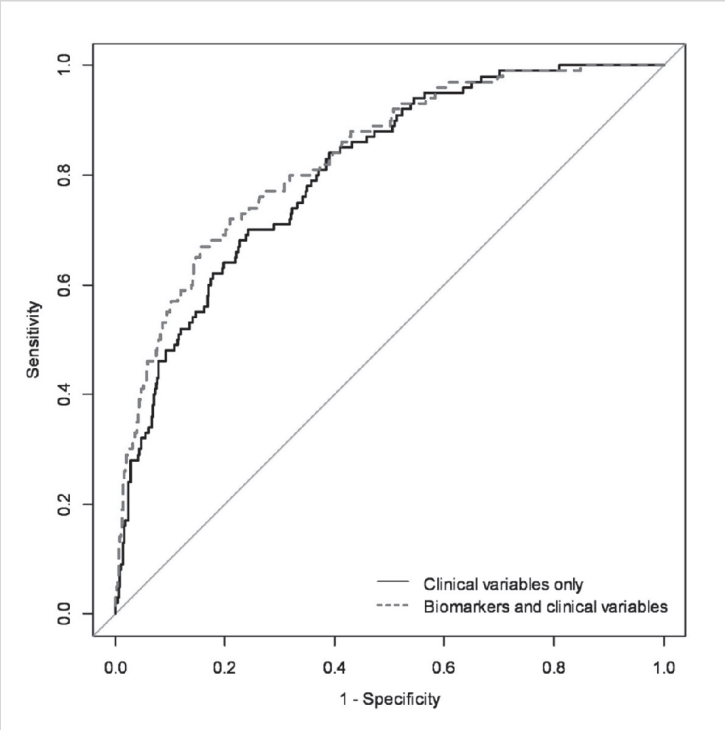

Figure 7. Comparison of the area under the receiver-operating characteristic curve (AUC) of the clinical model without biomarkers, with the model including N-terminal pro-brain natriuretic peptide, lactoferrin and neopterin.

When comparing the effect of including plasma biomarkers on AKI risk categories, a net 12% of all patients were reclassified correctly when combining biomarkers and clinical variables (Table 10). 10% were due to AKI-patients being correctly reclassified to higher risk groups. A subgroup analysis was performed in the intermediate risk group (predicted risk 10-20%, n=175 (17%)). Among AKI cases (n=22), 11 patients were correctly upgraded in risk category, whereas 5 incorrectly downgraded, yielding a net correct reclassification in 6 out of 22 AKI-patients (27%). Correspondingly, among non-AKI cases (n=153), 71 patients were correctly downgraded, whereas 26 incorrectly put into a higher risk category, yielding a correct net reclassification of 45 (29%) non-AKI cases. The overall NRI in the intermediate group was therefore 56%. The improvement in prediction seen in the intermediate risk groups was confirmed by the AUC graph (Figure 7).

Table 10. Comparison of risk classification for AKI based on clinical variables only and combined biomarkers and clinical variables.

		Risk classification with biomarkers and clinical variables			
	Risk classification with clinical variables only	< 10% risk	10-20% risk	> 20% risk	Total
AKI cases	< 10% risk	20	**8**	**2**	*30*
	10-20% risk	*5*	6	**11**	*22*
	> 20% risk	*2*	*4*	42	*48*
	Total	27	18	55	100
Non-AKI cases	< 10% risk	614	*52*	*4*	670
	10-20% risk	**71**	56	*26*	153
	> 20% risk	**5**	**20**	59	84
	Total	690	128	89	907

Reclassification tables stratified on low (<10%), intermediate (10-20%) and high (>20%) risk predicted AKI risk. Values represent number of patients (n). Correct reclassification is indicated with bold; incorrect reclassification in italics. AKI, acute kidney injury.

5.3 Paper 3

5.3.1 Temporal trends

The number of cardiac surgeries performed in Trondheim declined annually from 631 in year 2000, to 506 in 2014. The overall reduction was dominated by a steady drop in the number of isolated CABG performed, from 470 (74.5% of yearly procedures) in 2000 to 294 (58.1%) in 2014.

A temporal assessment of patient characteristics showed that median age, the proportion of females and smokers remained constant during the study period (Table 11). Patients admitted to cardiac surgery during more recent years presented with more comorbid diseases, such as diabetes and chronic obstructive lung disease. More patients presented with acute cardiac insufficiency requiring either inotropic therapy or intra-aortic balloon pump before surgery. Whereas the rate of acute surgeries remained constant, there was an increasing proportion of patients scheduled for urgent surgery (within 2 weeks) during more recent years.

Table 11. Comparison of patient characteristics across time.

	Time period			P-value
	2000-2004 (n=3,122)	2005-2009 (n=2,875)	2010-2014 (n=2,567)	
Preoperative characteristics				
Age (years)	67 (66.5-67.0)	67 (67.0-67.5)	67 (66.5-67.0)	0.22
Female gender	796 (25.5%)	774 (26.9%)	641 (24.9%)	0.23
Body mass index (kg/m^2)	26.3 (26.2-26.4)	26.8 (26.7-26.9)	26.8 (26.6-27.0)	<0.001
Ever smoker	1,686 (54.0%)	1,525 (53.0%)	1,406 (54.8%)	0.44
Diabetes mellitus	371 (11.9%)	426 (14.8%)	393 (15.3%)	<0.001
History of atrial fibrillation	1,517 (48.6%)	1,314 (45.7%)	1,084 (42.2%)	<0.001
Peripheral vascular disease	353 (11.3%)	289 (10.1%)	270 (10.5%)	0.28
Previous myocardial infarction	1,437 (46.0%)	1,298 (45.2%)	1,132 (44.1%)	0.35
Chronic pulmonary disease	443 (14.2%)	384 (13.4%)	493 (19.2%)	<0.001
Chronic heart failure	505 (16.2%)	402 (14.0%)	419 (16.3%)	0.024
Kidney dysfunction	172 (5.5%)	111 (3.9%)	103 (4.0%)	0.003
Acute preoperative heart failure	19 (0.6%)	26 (0.9%)	40 (1.6%)	0.001
Acute surgery (<24 hours)	161 (5.2%)	155 (5.4%)	141 (5.5%)	0.84
Urgent surgery (<2 weeks)	1,240 (39.7%)	1,183 (41.2%)	1,112 (43.3%)	0.02
Redo operation	155 (5.0%)	119 (4.1%)	63 (2.5%)	<0.001
Surgical category:				<0.001
1) Isolated CABG	2,240 (71.2%)	1,824 (63.4%)	1,584 (61.7%)	
2) 1 non-CABG procedure	321 (10.3%)	348 (12.1%)	402 (15.7%)	
3) 2 surgical procedures	493 (15.8%)	602 (20.9%)	522 (20.3%)	
4) ≥ 3 surgical procedures	68 (2.2%)	101 (3.5%)	59 (2.3%)	
Serum creatinine (µmol/l)	95 (95-96)	82 (81-82)	81 (80-82)	<0.001
Creatinine clearance* (ml/min)	73.2 (72.3-74.1)	87.3 (86.1-88.5)	89.8 (88.5-91.0)	<0.001
Hemoglobin (g/dl)	13.8 (13.7-13.8)	13.8 (13.8-13.9)	14.0 (13.9-14.0)	<0.001
Preoperative medications				
Antiarrythmics	42 (1.4%)	70 (2.4%)	62 (2.4%)	0.003
Beta-blockers	2,512 (80.5%)	2,168 (75.5%)	1,699 (66.2%)	<0.001
Diuretics	766 (24.6%)	769 (26.8%)	810 (31.6%)	<0.001
Statins	2,189 (70.1%)	2,264 (78.9%)	1,978 (77.1%)	<0.001
Intraoperative characteristics				
CPB time (min)	72 (71-73)	79 (78-81)	85 (84-87)	<0.001
Intraoperative red cell transfusion (no/yes)	433 (13.9%)	539 (18.8%)	613 (23.9%)	<0.001
Use of inotropic support (no/yes)	759 (24.3%)	676 (23.5%)	776 (30.2%)	<0.001
Use of vasoconstrictors (no/yes)	2,115 (67.8%)	2,667 (92.8%)	2,501 (97.4%)	<0.001
Postoperative factors				
Postoperative hospital stay (days)	6.5 (6.5-6.5)	6 (6-6)	5.5 (5-5.5)	<0.001
Pneumothorax	92 (3.0%)	90 (3.1%)	104 (4.1%)	0.05
Myocardial infarction	203 (6.5%)	160 (5.6%)	146 (5.9%)	0.25
Acute kidney injury	386 (12.4%)	321 (11.2%)	297 (11.6%)	0.35
Sepsis	26 (0.8%)	19 (0.7%)	11 (0.4%)	0.17
Multi-organ failure	58 (1.9%)	61 (2.1%)	59 (2.3%)	0.50
30-day mortality	73 (2.3%))	52 (1.8%)	59 (2.3%)	0.30

Categorical variables are given in n (%), continuous variables in median (95% confidence interval). Differences across the study period were tested with χ^2 and Kruskal-Wallis tests for categorical and continuous data, respectively. *Creatinine clearance calculations based on formula from Cockcroft and Gault (223). Abbreviations: CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass.

Intraoperatively, there was a marked increase in the proportion of patients receiving intraoperative red cell transfusion, inotropic support and vasoconstrictor therapy. Median duration of CPB increased steadily over the study period. Nevertheless, the incidence of postoperative complications and in-hospital mortality remained unchanged. There was a reduction in the duration of postoperative hospital stay.

5.3.2 Long-term survival and mortality following cardiac surgery

The median time to censoring was 6.4 years with a maximum of 14.99 years. A total of 2,044 patients (23.9%) died. Of the patients who survived the first 30 days postoperatively but died within the follow-up time, 47.0% (men: 45.9%, females: 49.5%), were officially classified as suffering a cardiovascular death, as opposed to 92.4% of the patients who died within 30 days postoperatively (n=184).

5.3.2.1 Observed survival and mortality

The overall observed 30-day, 1-, 3- and 5-year mortality rates were 2.2%, 4.4%, 8.2% and 13.8%, respectively and did not change significantly during the follow-up period. Conversely, the observed survival rates calculated by the Kaplan-Meier method were 95.7%, 86.9% and 69.3% after 1, 5 and 10 years, respectively, and differed significantly amongst different surgical interventions as classified by EuroSCORE II ($p<0.001$). Similarly, patients undergoing AVR and combined AVR and CABG showed significant differences compared to isolated CABG (Figure 8). Despite a linear increase in observed long-term mortality across patients undergoing isolated CABG, isolated AVR and combined AVR and CABG (HR 1.00, HR 1.39 (1.17-1.64) and 1.59 (1.39-1.82), respectively), observed mortality in AVR-patients undergoing concomitant CABG did not differ significantly from that of isolated AVR ($p=0.48$).

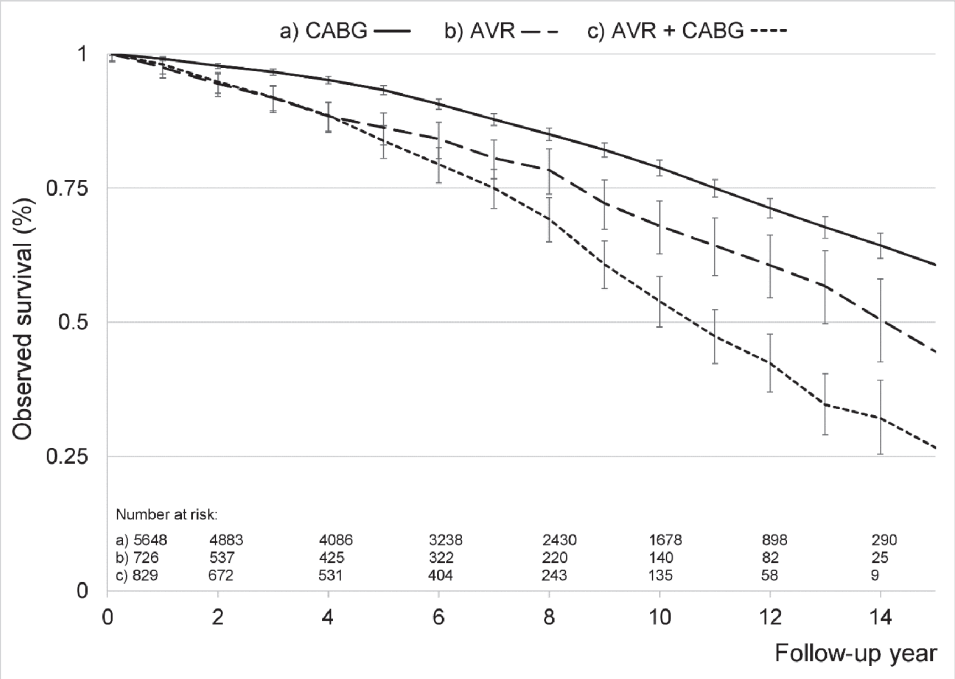

Figure 8: Long-term observed survival. Unadjusted Kaplan-Meier survival curves for patients undergoing coronary artery bypass grafting (CABG) and/or aortic valve replacement (AVR). The number at risk (n) at the start of even follow-up years are provided.

5.3.2.2 Relative survival and mortality

Relative survival

When adjusting for the expected survival in a similar subset of the general Norwegian population, the 1-, 5- and 10-year relative survival rates were estimated to 97.8%, 98.8% and 94.9%, respectively. However, when excluding patients who died within 30 days postoperatively (n=184), there was a survival benefit in cardiac surgery patients compared to the reference population: Observed survival during the first four years of follow-up was higher than expected survival (relative survival >1 (>100%), Figure 9A). Survival during the three subsequent years was similar to that of the background population (relative survival =1). Overall relative survival decreased from the eighth year and onwards; however, the reduction in survival started earlier and was greater amongst females and patients undergoing combined CABG and AVR.

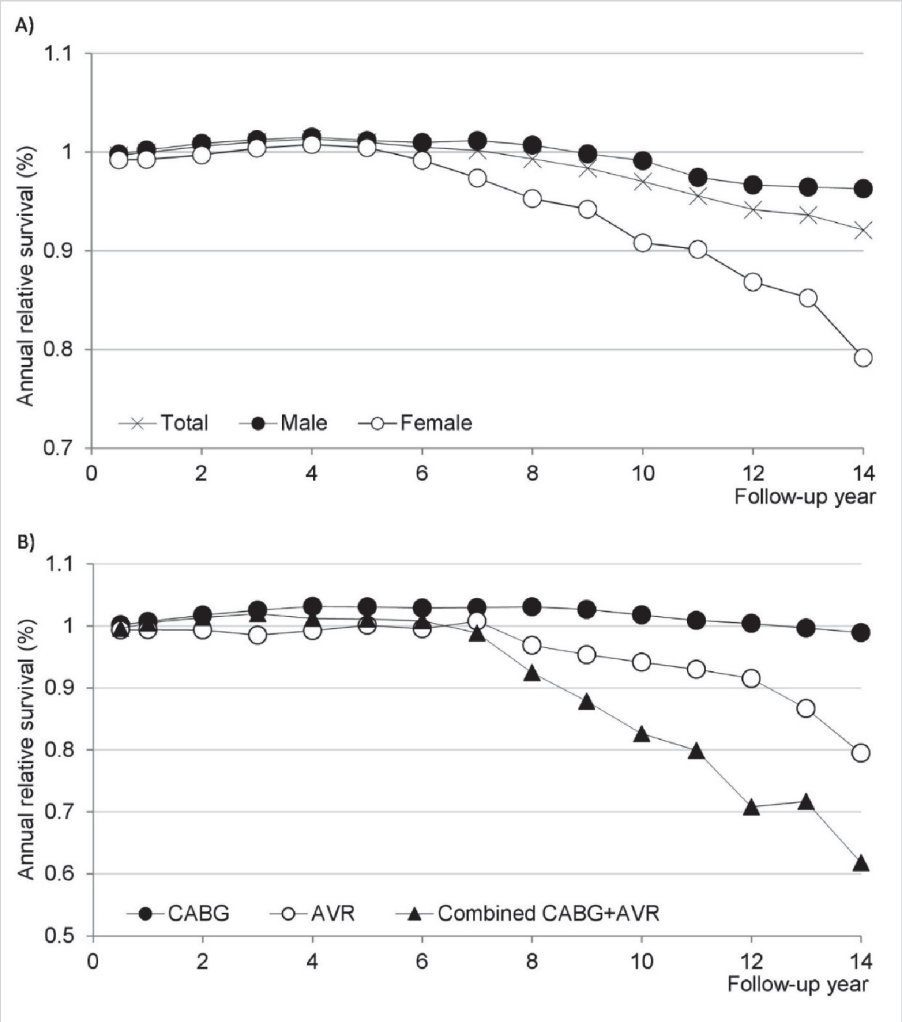

Figure 9: Annual relative survival amongst cardiac surgery patients surviving the first 30 postoperative days; A) shown in total (n=8,380) and separately for males (n=6,244) and females (n=2,136); B) shown for patients undergoing isolated coronary artery bypass grafting (CABG, n=5,593), isolated aortic valve replacement (AVR, n=699) and combined CABG and AVR (n=809). A relative survival >1 indicates a survival advantage in the study cohort.

Isolated CABG, AVR and combined AVR and CABG showed similar relative survival from the first throughout the seventh year of follow-up (Figure 9B). However, from the eighth year of follow up, survival was most reduced for combined AVR and CABG, moderately reduced for isolated AVR, whereas relative survival remained >1 for isolated CABG throughout the

follow-up period. After the tenth year of follow up, the numbers at risk were too small for statistical analysis.

When compared to older reports on patients undergoing CABG or valve replacement from other centres (106, 207), patients undergoing cardiac surgery in Trondheim showed somewhat improved observed and relative survival (Figure 10).

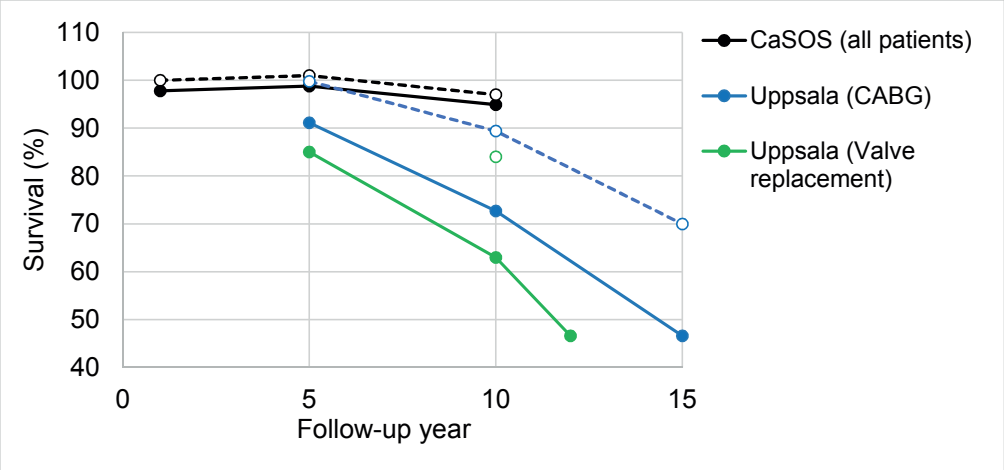

Figure 10: Comparison of long-term follow-up in cardiac surgery patients from Trondheim (CaSOS), Norway, and Uppsala, Sweden (106, 207). Observed survival is shown with continuous lines, relative survival with dashed lines and hollow circles. Results from all cardiac surgery patients in CaSOS (years 2000-2014, in black) are provided together with survival in Swedish heart surgery patients undergoing CABG (years 1970-1989, in blue) (106) and heart valve replacement (years 1980-1992, in green) (207). Relative survival after heart valve replacement was provided for AVR patients only, and was given as a point estimate for ten years following surgery. Abbreviations: AVR; Aortic valve replacement, CABG; coronary artery bypass grafting, CaSOS; Cardiac Surgery Outcome Study.

Relative mortality

When comparing the overall observed and expected number of deaths in patients who were still alive after 30 postoperative days, patients undergoing cardiac surgery from 2000 through 2014 did not have significantly different mortality compared to the general population (overall SMR 1.02, 95% CI 0.97-1.06). However, subgroup analyses showed that females (SMR 1.17, 95% CI 1.07-1.27) and patients aged <70 years (SMR 1.77, 95% CI 1.52-2.04 for <60 years and SMR 1.17, 95% CI 1.06-1.29 for 60-69 years, Figure 11) had a significantly higher relative

mortality when adjusting for background mortality. For men, patients aged >70 years showed a survival benefit. Females aged ≥ 80 years may have a similar survival advantage, however, the number of cases in this age group was small (n=288).

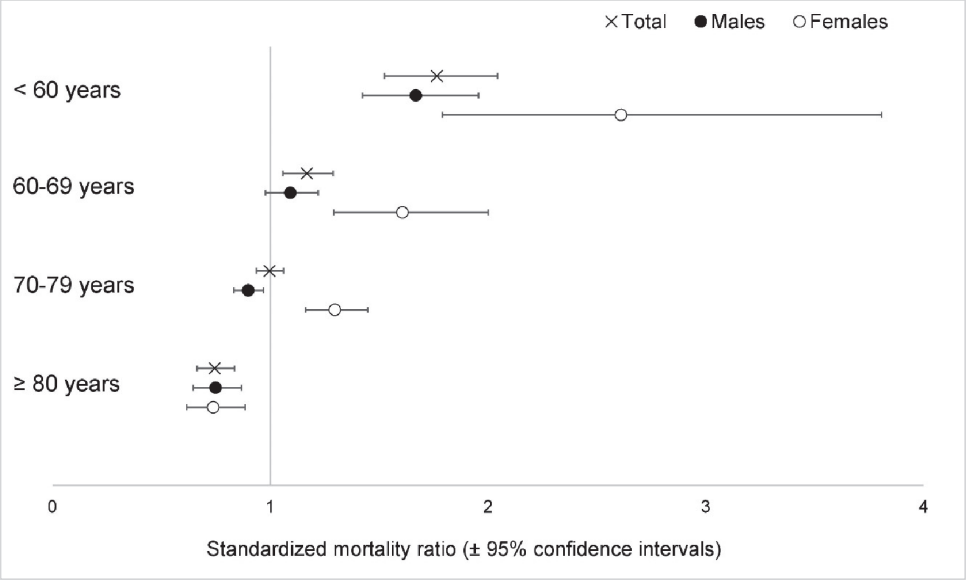

Figure 11: Standardized mortality ratios (SMR) stratified by gender and age group (n=8,380). A SMR > 1 indicates that there was a higher number of observed deaths amongst cardiac surgery patients than expected, based on data from the general population.

Furthermore, stratification by surgical procedure showed that patients undergoing AVR, both isolated and with concomitant CABG, had a higher relative mortality compared to isolated CABG ($p<0.001$ for both). The findings remained similar when adjusting for intergroup differences in age and sex distributions. Concomitant CABG in AVR-patients was not associated with a higher relative mortality ($p=0.24$).

5.3.3 Risk factor analysis

Long-term observed mortality

Observed mortality increased with age (HR per 5 years 1.46 (1.41-1.50), $p<0.001$). Gender was not an independent predictor of observed long-term mortality following cardiac surgery ($p=0.09$). Pre-existing chronic heart failure, chronic pulmonary disease, preoperative serum

creatinine concentrations >140 μmol/L, peripheral vascular disease, diabetes and current tobacco smoking were associated with increased risk of long-term mortality (Table 12). Mortality increased linearly with increased complexity and number of procedures performed. There were non-proportional hazards over time, as shown by fitting piecewise hazard ratios (Table 12). The type of surgical procedure, co-existing chronic pulmonary disease and reduced kidney function seemed to play more dominant roles during the first year. There were only small changes when using cardiovascular death as the outcome.

A comparison of male and female risk profiles revealed significant differences: Females were significantly older compared to men (Table 13). Furthermore, females generally had worse cardiovascular risk profiles, with a higher prevalence of diabetes, hypertension, left ventricular hypertrophy and heart failure. They also more often presented with chronic pulmonary disease. On the contrary, they showed a lower prevalence of preoperative kidney dysfunction, peripheral vascular disease and previous myocardial infarction. Females more seldom had a history of previous cardiac surgery, but on the other hand, they were more often scheduled for an emergency operation. They less often underwent isolated CABG, but more frequently underwent more complicated surgical procedures and generally spent longer time on CPB.

Table 12. Risk factors associated with observed mortality.

Predictor	Time period							
	Complete follow-up		≤ 1 year		1-5 years		> 5 years	
	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)
Age per 5 years	1.46	(1.41-1.50)***	1.25	(1.14-1.36)***	1.37	(1.30-1.44)***	1.53	(1.46-1.60)***
Female gender	0.91	(0.82-1.02)	1.07	(0.77-1.48)	0.68	(0.56-0.83)***	1.03	(0.88-1.20)
Surgical category:								
1) Isolated CABG (reference)	1.00	---	1.00	---	1.00	---	1.00	---
2) 1 non-CABG procedure	1.49	(1.29-1.73)***	2.41	(1.59-3.64)***	1.56	(1.20-2.03)***	1.33	(1.04-1.68)*
3) 2 surgical procedures	1.53	(1.37-1.71)***	2.06	(1.44-2.93)***	1.60	(1.32-1.94)***	1.44	(1.21-1.71)***
4) ≥ 3 surgical procedures	1.94	(1.51-2.48)***	2.43	(1.20-4.90)*	2.17	(1.47-3.20)***	1.53	(1.00-2.33)*
Chronic cardiac insufficiency	1.61	(1.44-1.79)***	1.89	(1.38-2.60)***	1.78	(1.48-2.15)***	1.49	(1.26-1.77)***
Chronic pulmonary disease	1.70	(1.52-1.89)***	2.38	(1.75-3.25)***	1.69	(1.40-2.04)***	1.52	(1.27-1.81)***
Serum creatinine >140 µmol/L	2.07	(1.77-2.42)***	3.02	(2.02-4.53)***	2.00	(1.54-2.60)***	1.85	(1.43-2.41)***
Diabetes mellitus	1.58	(1.40-1.78)***	1.63	(1.15-2.32)**	1.50	(1.23-1.84)***	1.66	(1.38-1.99)***
Peripheral vascular disease	1.69	(1.50-1.91)***	1.16	(0.77-1.73)	1.95	(1.60-2.37)***	1.65	(1.37-2.00)***
Current smoking	1.42	(1.29-1.57)***	1.54	(1.12-2.11)**	1.25	(1.05-1.48)*	1.41	(1.21-1.64)***

Hazard ratios are given for the complete follow-up period, as well as piecewise for the 1st year (n=8,380), 1st-5th year (n=7,704) and >5th year (n=5,207) of follow-up.

*p<0.05; **p<0.01; ***p<0.001. Abbreviations: HR, hazard ratio; CI, confidence interval.

Table 13. Comparison of patient characteristics between genders.

Characteristic	Female (n=2,211)	Male (n=6,353)	P-value
Age (years)	71 (70-71)	66 (66-66)	<0.001
Body mass index (kg/m^2)	26.4 (26.2-26.6)	26.7 (26.6-26.8)	0.001
Ever smoker	961 (48.2%)	3,656 (63.6%)	<0.001
Diabetes mellitus	343 (15.5%)	847 (13.3%)	0.01
Hypertension	1,290 (58.3%)	3,307 (52.1%)	<0.001
History of atrial fibrillation	285 (12.9%)	807 (12.7%)	0.83
Peripheral vascular disease	197 (8.9%)	715 (11.3%)	0.002
Previous myocardial infarction	810 (36.6%)	3,058 (48.1%)	<0.001
Left ventricular hypertrophy	781 (39.4%)	1,343 (24.2%)	<0.001
NYHA class III/IV	1,573 (71.2%)	4,189 (66.0%)	<0.001
Chronic heart failure	472 (22.5%)	899 (16.6%)	<0.001
Chronic pulmonary disease	387 (17.5%)	933 (14.7%)	0.002
Kidney dysfunction	69 (3.1%)	317 (5.0%)	<0.001
Acute preoperative heart failure	22 (1.2%)	63 (1.2%)	0.99
Acute surgery (<24 hours)	139 (6.3%)	318 (5.0%)	0.02
Urgent surgery (<2 weeks)	853 (38.6%)	2,682 (42.2%)	0.003
Redo operation	55 (2.5%)	282 (4.4%)	<0.001
Surgical category			<0.001
1) Isolated CABG	1,136 (51.4%)	4,512 (71.0%)	
2) 1 non-CABG procedure	478 (21.6%)	593 (9.3%)	
3) 2 surgical procedures	529 (23.9%)	1,088 (17.1%)	
4) ≥ 3 surgical procedures	68 (3.1%)	160 (2.5%)	
Serum creatinine (µmol/L)	76 (76-77)	90 (90-91)	<0.001
Beta-blockers**	1.525 (69.0%)	4,854 (76.4%)	<0.001
Diuretics**	778 (35.2%)	1,567 (24.7%)	<0.001
Statins**	1,489 (67.4%)	4,942 (77.8%)	<0.001
Cardiopulmonary bypass time (min)	82 (80-83)	78 (77-79)	0.003

Categorical variables are given in n (%), continuous variables in median (95% confidence interval). Differences between genders were tested with χ^2 test and Mann-Whitney U-test for categorical and continuous data, respectively. **Medication before referral for surgery. Abbreviations: CABG, coronary artery bypass grafting; NYHA, New York Heart Association Functional Classification (class I-IV).

In the sensitivity analysis of the effect of gender on observed survival, propensity score matching resulted in 1,493 pairs of females and males with standardized differences ≤ 9% for all covariates (Figure 12). In this matched subgroup, gender emerged as a predictor of observed mortality (HR=0.81, 95% CI (0.70-0.93), p=0.004). This association was not reproduced when evaluating cardiovascular mortality (p=0.43).

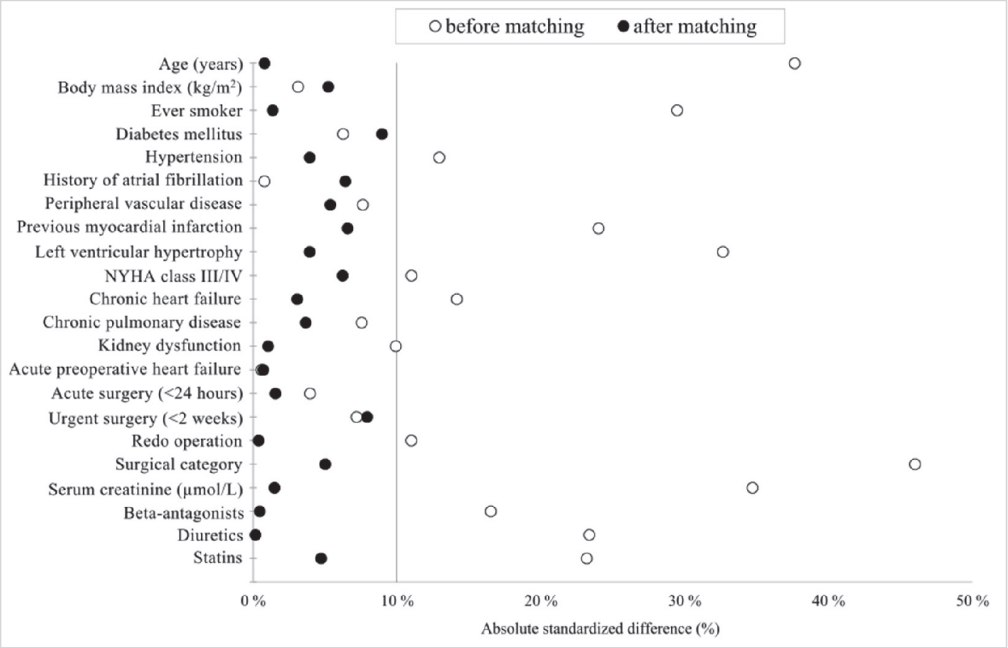

Figure 12. Standardized difference plot. Absolute standardized differences in covariate means between female and male cardiac surgery patients before and after propensity score matching on preoperative covariates.

Long-term relative mortality

When adjusting for background mortality, relative mortality was higher in females (RMR 1.35 (1.19-1.54), p<0.001) and younger patients (RMR decreased with increasing age; per 5 years 0.81 (0.79-0.84), p<0.001). The multivariate analysis showed that the remaining predictors of observed mortality as summarized in Table 12 were also associated with relative mortality (Figure 13). Results were consistent also for the subgroup analysis designated to CABG- and AVR-patients.

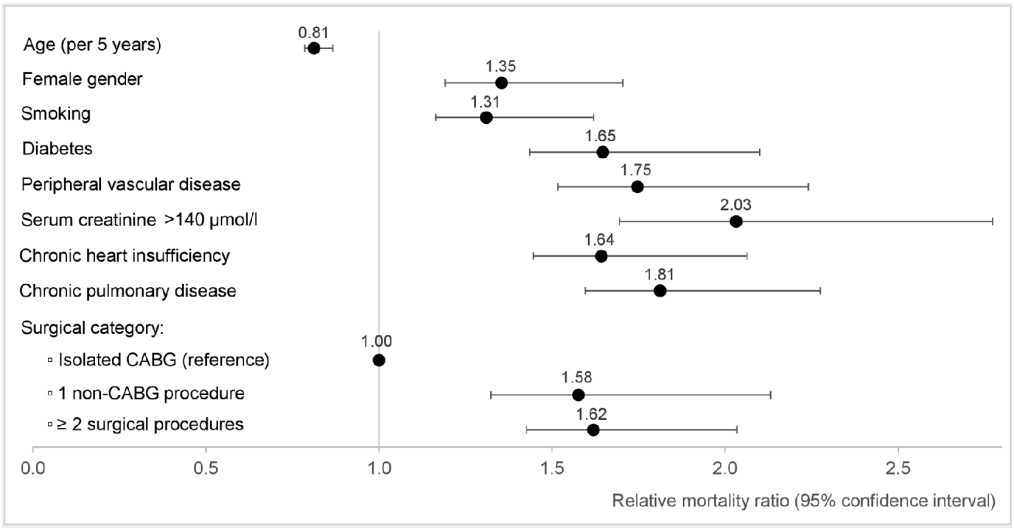

Figure 13. Predictors of relative mortality. Estimated relative mortality ratios (RMR) with corresponding 95% confidence intervals (CI) for predictor variables of long-term relative mortality in patients undergoing cardiac surgery. A ratio > 1 indicates more observed deaths than expected. Abbreviations: CABG, coronary artery bypass grafting.

5.4 Joint findings of all papers

From all primary cases undergoing cardiac surgery in Trondheim between 2000 through 2014 (n=8,564), 8,455 patients had complete information with regards to postoperative acute kidney injury, cardiac dysfunction and fluid overload. Whereas the incidence of CSA-AKI remained unchanged (p=0.80), the incidences of postoperative fluid overload and cardiac dysfunction have increased (p<0.001) across the study period (Figure 14).

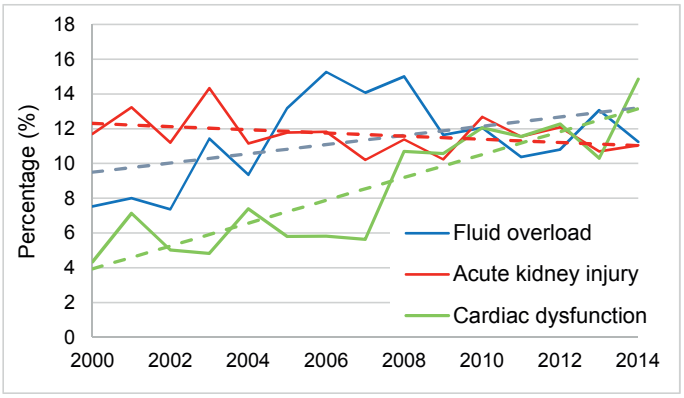

Figure 14. Yearly incidences of postoperative acute kidney injury (red), cardiac dysfunction (green) and fluid overload (blue) in patients undergoing cardiac surgery in Trondheim between 2000 through 2014. Linear trends are indicated with dashed lines.

From all patients suffering postoperative fluid overload, 12.2% developed CSA-AKI, 12.6% patients developed postoperative cardiac dysfunction, whereas 16.4% developed both CSA-AKI and cardiac dysfunction (Figure 15). This left 58.9% of patients with isolated postoperative fluid overload without renal and/or heart dysfunction. As a sensitivity analysis, repeated analysis in patients undergoing cardiac surgery between 2008 through 2010 (as applied in Papers 1 and 2; n=988) showed similar results, where 69.7% of patients with postoperative fluid overload had neither postoperative cardiac dysfunction nor AKI.

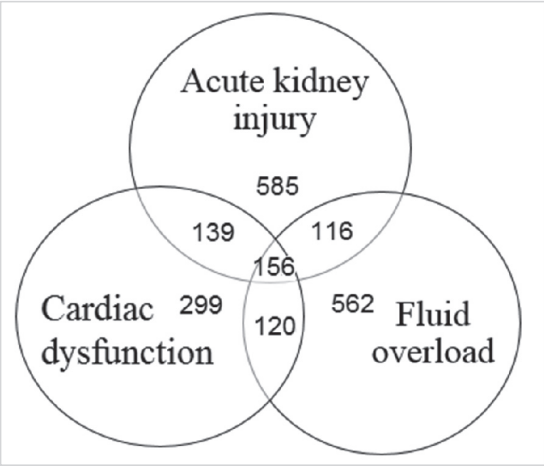

Figure 15. The distribution of patients suffering acute kidney injury (n=988), fluid overload (n=954) and cardiac dysfunction (n=706) following cardiac surgery. These three adverse outcomes can be seen as both distinct and partially overlapping syndromes.

51.5% of patients dying within 30 days postoperatively suffered postoperative fluid overload, compared to only 10.6% of the patients surviving. Both 30-day mortality (7.2% vs. 0.9%, $p<0.001$) and long-term mortality ($p<0.001$) was significantly higher in patients with postoperative fluid overload. These remained significant despite adjusting for age, gender, CSA-AKI and postoperative cardiac dysfunction.

6. Discussion

6.1 Short-term outcomes following cardiac surgery

Despite previously reported associations between postoperative organ dysfunction and the progression into multi-organ failure or death, our findings suggest that postoperative complications may not always be multiple sides to the same story.

6.1.1 Fluid overload – a surrogate marker?

In Paper 1, we identified a SNP in the *UMOD* gene, related to renal function, which was associated with postoperative fluid overload. Furthermore, a genetic risk score consisting of 14 SNPs related to different inflammatory and vascular pathways was linearly related to an increased risk of postoperative fluid overload. Our findings highlight two important approaches to the understanding of perioperative fluid accumulation: First, our findings support the idea of a subclinical susceptibility to abnormal fluid handling. Whereas observable clinical risk factors such as heart and renal dysfunction may only partially explain the risk of postoperative fluid overload, there may also exist genetic variations related to minor alterations in inflammatory and molecular signalling pathways that contribute in explaining the individual risk. Second, the present findings underscore the multifactorial pathogenesis of perioperative fluid accumulation, where analysing the joint influence of several factors improved outcome prediction. The data support that clinical and genetic risk factors act additively on the overall risk, where each factor alone may only exert a minor influence on the outcome.

Fluid accumulation has been associated both with AKI and heart failure (224-226). Whether fluid overload is a secondary consequence or a contributor to the development of postoperative cardiac and renal injury is difficult to discriminate, and there probably exist complex interactive effects (225). However, although correlated with the development and prognosis of cardiac and renal dysfunction, fluid overload may also be a separate syndrome with different underlying causes. The linear association between the genetic risk score and the risk of postoperative fluid overload indicates that there exists multiple pathways that might be involved. In the joint analysis investigating the coexistence of postoperative fluid overload, cardiac dysfunction and AKI, 59% and 70% of patients with fluid overload in the full study cohort and biomarker dataset

(years 2008-2010), respectively, had neither postoperative cardiac nor renal dysfunction. Thus, postoperative fluid overload does not merely represent a surrogate marker for postoperative renal and/or cardiac dysfunction, and using it as a primary outcome measure identifies a larger group of patients needing heightened vigilance.

In the present study cohort, patients with postoperative fluid overload generally had a higher risk of short- and long-term mortality. Fluid overload has also been recognized as a determinant of postoperative outcome and longer stay in the intensive care unit (227). Nevertheless, the current approach to perioperative fluid therapy is subject to substantial variation and remains a highly debated subject. Despite several investigations of liberal, restricted and goal-directed fluid management strategies, the "right amount" remains uncertain (228, 229).

The polygenic susceptibility to abnormal perioperative fluid handling and potential interactive effects of clinical and genetic risk factors underscore why adjusting fluid therapy to each individual patient is a challenging task. Improved knowledge about factors contributing to the increased risk of abnormal perioperative fluid handling may raise awareness and caution amongst clinicians, because apparent low-risk individuals based on clinical evaluation may also be exposed through other factors, including genetic risk factors.

However, even with awareness about the hazards of fluid overload, patients must be treated with pressors and fluid therapy in order to maintain adequate tissue oxygenation. This requires careful and dynamic monitoring of circulating volume, cardiac output and vascular resistance. Routine cardiovascular monitoring of factors such as blood pressure, heart rate and urine output are not reliable predictors of intravascular fluid status and should not represent the rational guide to fluid therapy (229). Moreover, it has been shown that central venous pressure and pulmonary capillary wedge pressure, both being invasive measurements frequently used in cardiac surgery patients, were inaccurate in predicting fluid responsiveness and unsuitable to guide fluid management (230, 231).

Fluid therapy must be titrated against a patient's response. The demonstrated involvement of genetic risk factors may support the modern approach of goal-directed fluid therapy (GDT) in cardiac surgery patients in order to balance the risk of fluid overload with that of hypovolemia. GDT is usually combined with flow algorithms directed at pre-defined hemodynamic targets

including flow-based goals (such as cardiac index or stroke volume index), or markers of tissue perfusion or oxygen consumption (such as central venous oxygen saturation or lactate concentration). Although concensus on optimal targets and monitoring methods have yet not been reached, GDT facilitates individualized fluid therapy and has shown convincing potential with reduced postoperative morbidity and hospital length of stay in cardiac surgery patients (232).

6.1.2 Acute kidney injury

In Paper 2, we found that a multimarker assessment of preoperative plasma NT-proBNP, neopterin and lactoferrin was significantly associated with CSA-AKI. The biomarkers remained independently associated with CSA-AKI despite adjustment for age, gender, body mass index above 30 kg/m^2, preoperative renal function and surgical procedure. All together, the combination of clinical and biomarker data underscore the complex, multifactorial nature of CSA-AKI including cardio-renal as well as inflammatory pathways.

NT-proBNP is an inactive cleavage product from the prohormone proBNP, and is released into the circulation in response to ventricular wall stress. The strong association with CSA-AKI underlines the importance of hemodynamic stress and increased venous pressure in the pathogenesis of renal dysfunction. This is in line with previous findings (158) and supports the changing clinical trend from mainly concentrating on inadequate renal perfusion as the most important determinant of CSA-AKI, towards acknowledgement of the importance of high venous pressure in causing reduced renal function. Interestingly, addition of clinical variables related to preoperative cardiac function, including reduced left ventricular ejection fraction or a diagnosis of chronic heart failure, did not alter the results.

Neopterin and lactoferrin can both be related to inflammatory pathways. Neopterin is released from activated macrophages. It may act as a marker for general inflammation; for progressive atherosclerosis; or it may be directly related to CSA-AKI through its stimulation of reactive oxygen species production (233). The exact underlying mechanisms for the association of neopterin with CSA-AKI warrant further investigation.

Lactoferrin is released from secondary granules in neutrophils and correlates with the amount and activity of neutrophils. In the univariate analysis, AKI cases showed lower baseline lactoferrin concentrations. This may be related to its iron-binding properties, limiting the deleterious effects of red blood cell destruction and subsequent release of free iron into the circulation (184). After adjustment for clinical variables, lower lactoferrin concentrations were associated with an increased risk of CSA-AKI with a p-value of 0.04. However, application of bootstrapping techniques to assess the uncertainty of the sample estimates indicated that the influence of lactoferrin must be evaluated in larger datasets.

6.1.3 Novel biomarkers of postoperative complications

The findings of this thesis have pointed out potentially important pathways in the development of postoperative fluid overload and CSA-AKI. This may enhance our understanding of underlying mechanisms and susceptibilities to postoperative complications. The identified biomarkers may contribute to resolve the unexplained observed variability, raise new hypotheses of underlying causal pathways and offer clues to new potential targets in the prevention and treatment of postoperative complications.

The complexity of the underlying pathways for postoperative fluid overload and CSA-AKI emphasizes the need for a comprehensive preoperative evaluation of cardiac surgery patients. Application of standard treatment regimens is not always beneficial, and substantial inter-individual variations call for need of prevention and therapeutic strategies tailored according to the patient's pathogenic profile. Our findings support the changing approach to the study of pathogenic mechanisms and treatment strategies from “one size fits all” to a new era of individualized medicine. Different pathogenic pathways may be differently weighted in different individuals, which highlights the importance of a simulteaneous assessment in order to accurately identify all patients at increased risk. It may also indicate that any isolated intervention may not be sufficient in order to prevent adverse outcomes in the general population of cardiac surgery patients.

The aim of this thesis was not to propose a new preoperative screening programme or to design new prediction tools for clinical use. However, we do not exclude that some of these biomarkers may have a clinical potential in the future. Before eventual translation into clinical practice,

replication of data is necessary. Furthermore, for both genetic and plasma biomarkers, other biomarkers may exist which were not analysed. We cannot exclude that the included biomarkers may act as markers of other clinical risk factors which we did not take into account. Finally, clear evidence of a clinical impact of improved risk stratification, such as altered provider behaviour and/or improved patient outcomes, must be demonstrated.

6.2 Long-term outcomes following cardiac surgery

In Trondheim, observed short-, medium- and long-term mortality in patients undergoing cardiac surgery has remained more or less constant during the study period from 2000 through 2014. Furthermore, patients undergoing cardiac surgery showed a survival comparable to or better than the general Norwegian population for seven years following operation. Subsequently, there was a modest reduction in overall annual survival, which was more pronounced in females as well as patients undergoing other procedures than isolated CABG. Increasing observed mortality with ageing was due to population risk, and the female survival advantage in the general population was lost in patients undergoing cardiac surgery. Confirming these findings, the ratio of observed and expected deaths was higher for females, in younger age groups and in patients undergoing AVR. Multivariate survival analyses indicated that the same predictor variables associated with observed mortality remained significant predictors of relative mortality.

6.2.1 Observed and relative survival and mortality

The good long-term survival in cardiac surgery patients underscores a continued patient benefit from this intervention in appropriately selected patients. Despite an increasing trend of more patients with increased comorbidity and complex disease being referred to cardiac surgery, long-term survival remained unchanged during the study period. Furthermore, patients undergoing cardiac surgery in Trondheim showed somewhat improved observed and relative survival compared to data from older reports in Sweden (106, 207).

The comparison of long-term survival considered patients surviving the first 30 postoperative days, thus different in-hospital mortality rates should not have influenced our results. However, it is important to take into consideration that the older reports on relative long-term survival

were performed on patients undergoing CABG and valvular surgery between the 1970s and 1990s. Furthermore, the comparison of survival rates has not taken into account the patients' risk profiles. Reasons for improved long-term survival compared with previous findings therefore remain uncertain, but might be related to different risk profiles, geographically or temporally, improvements in medical care or advances in secondary prevention.

6.2.2 Risk factor analysis

6.2.2.1 Age

In accordance with previous studies, higher age was an independent predictor of observed long-term mortality, whereas relative survival analyses revealed that the effect of age was attributable to the population risk (105-107). Relative mortality increased with decreasing age, even after adjustment for smoking and comorbidities. Thus, the need for cardiac surgery at a young age may indicate severe underlying cardiovascular disease, which is progressive and conveys an increased risk for future cardiovascular events.

In general, older patients have reduced functional reserves as well as more systemic and cardiovascular comorbidities; thus having a higher operative risk (120, 234). However, if they survive the early postoperative phase, our observations support that they show excellent long-term results (234). This might be reasoned by careful evaluation of older patients before surgery with respect to comorbidities and preoperative function, and indicates that chronological age alone should not preclude patients from cardiac surgery.

6.2.2.2 Gender

The role of gender as a risk factor for long-term mortality is debated (235). In the present multivariate analysis, gender did not significantly affect observed long-term mortality, despite significant differences in the preoperative risk profiles between genders. Our initial findings comply with previous observations where adjustment for potential predictors in a multivariate analysis eliminated the gender differences seen in observed mortality rates (236). However, results from the propensity score matched analysis suggest that a gender difference may have been masked by residual confounding. In general, female patients showed a worse risk factor profile. Nevertheless, in the matched pairs of females and males, females had a reduced observed mortality. Importantly, our data suggest that this association may be confounded by

the better life expectancy of women, because female patients had a significantly higher mortality risk relative to their expected level of survival. Thus, the female survival advantage observed in the general population was obliterated. This phenomenon was also found in an old study from Norway (237). Underlying reasons may be that females suffer more aggressive heart disease (238), or delayed diagnosis and referral in women which allows time for the development of more severe disease and comorbidities prior to intervention.

6.2.2.3 Surgical procedure

Patients undergoing procedures other than isolated CABG showed higher long-term observed and relative mortality. However, a temporal assessment of relative survival across follow-up time showed that up to the seventh year, cumulative survival was comparable in all patient groups. CABG-patients showed a sustained survival benefit throughout follow-up compared to the general population, whereas there was a trend of reduced relative survival in patients undergoing AVR from the eighth year. Overall relative mortality was significantly higher in patients undergoing isolated AVR or combined AVR and CABG, also after adjustment for different distributions of age, gender and comorbidities. Reduced relative survival in AVR patients was also described in an old report from Sweden (107). Here, the mortality risk increased markedly from the fourth year of follow-up.

There was no significant difference in mortality between patients undergoing AVR and combined AVR and CABG. Due to the low number of patients in these two groups, further investigation is warranted. However, our findings comply with previous reports, where adjustment for age, gender and other risk factors eliminated concomitant CABG in AVR patients as an independent predictor of mortality (107, 239, 240). A trend of reduced relative survival in combined AVR-CABG patients compared to isolated AVR was seen after 8-10 years (107), but as in our study, this difference did not reach statistical significance.

Compared to isolated CABG, the reduced survival in patients undergoing AVR may be causal (i.e. imply a more aggressive disease), act as a marker for a high-risk patient profile, or be related to follow-up care and suboptimal secondary prevention. Further investigation of this patient group might be valuable for improving outcomes following cardiac surgery.

6.2.3 Improving long-term cardiovascular mortality

The high prevalence of cardiovascular risk factors, disease and death in the general population supports our findings, showing that except for gender and age, the predictors of observed and relative mortality were the same. The temporal survival trends and multivariate predictor analyses highlight the prognostic importance of systemic and cardiovascular risk factors above surgical factors.

The favourable long-term outcomes might coincide with the gradual reduction in cardiovascular mortality in the Norwegian population; from 41% in 2000 to 29% in 2014 (102). Several attempts have been made in order to evaluate the major contributors to reduced cardiovascular mortality. International data show that between half and two-thirds of the decline in coronary heart disease mortality can be attributable to reduced levels of the main risk factors of cardiovascular disease, i.e. smoking prevalence, serum cholesterol levels and systolic blood pressure (241, 242). Studies on contributions to reduced cardiovascular mortality have not been performed specifically in the Norwegian population. However, consistent with international findings, data from the Norwegian population show that there has been a decrease in cholesterol levels, blood pressure and smoking (243). The positive trend is opposed by an increase in overweight and type 2 diabetes (244). However, in similar data from USA, this increase was not strong enough to counteract the significant effect of the reduction in cholesterol levels and smoking (241).

The remaining one-third to half of the decline in coronary heart disease mortality may be explained by other factors, including secondary prevention among high-risk patients. Improved medical treatment, including acetylsalicylic acid, beta blockers, angiotensin-converting-enzyme inhibitors and statins, has been identified as the most important (241). The use of revascularization for chronic angina was estimated to contribute with 5% of the reduction in cardiovascular deaths from 1980 to 2000 (241).

The good long-term results in cardiac surgery patients may therefore be largely explained by the optimization of patient risk profiles and advances in pharmaceutical treatment. Patients undergoing cardiac surgery suffer severe and progressive heart disease with a continuous risk of symptomatic events and mortality. The beneficial effects of operation will decline over time,

thus risk factor control and secondary prevention remains the cornerstone for improving the long-term prognosis of these patients.

To date, there are guidelines ensuring that all patients undergoing CABG are routinely started on lipid-lowering treatment and antiplatelet therapy postoperatively, with additional recommendations for optimizing treatment of diabetes, hypertension and heart failure. Nevertheless, Hlatky and colleagues demonstrated that patients undergoing CABG are suboptimally medically treated following revascularisation compared with patients undergoing PCI (245). This might suggest an opportunity to further improve the quality of care and hence long-term outcomes after surgical revascularization. However, whether this observation applies to cardiac surgery patients in Norway as well, remains to be demonstrated.

The hazards of hyperlipidaemia (246) and benefits of lipid-lowering treatment (247) have also been demonstrated in AVR-patients, but not yet routinely implemented in clinical practice. Trials of antiplatelet therapy and antihypertensive treatment have also been called for (248). Improving secondary prevention strategies might therefore represent an opportunity to further improve the quality of care and hence long-term outcomes after AVR.

6.3 Methodological considerations

6.3.1 Data quality and variable sample

As Shahian *et al.* put it: "No risk adjustment model is better than the data upon which it is based" (142). The Trondheim Heart Surgery Database was established in 1992 for the purpose of internal quality control. Since then, investments in form of time and personal efforts have led to expansion into a high-quality database with both clear clinical and research purposes. The broad range of available variables represents a major strength of CaSOS. On the other hand, it also challenges collaboration and external validation in other research centres, which may not have the same routine registration of the needed variables.

Routine registration of relevant variables alters correspondingly with developments in cardiac surgery. Some variables have been left out because these have not shown to be of either clinical or research value, whereas some new variables have been included due to renewed interest in

how these might affect patient outcomes, as well as the need to match variables with comparable international databases. We therefore do not have information on all variables for all study participants.

Previous work from CaSOS has described that data on left ventricular ejection fraction (LVEF) were unavailable. In Paper 2, LVEF was included, which may cause some confusion: In order to overcome the potentially important limitation of not taking LVEF into consideration, an estimation approach was made based on the available data: Throughout the study period (2000-2014), LVEF has been determined by means of either single-planed left ventricular angiography or noninvasively through two-dimensional echocardiography. However, over time echocardiography has become the main modality. As it has previously been shown that LVEF obtained by echocardiography tends to be lower compared to angiographic measurements (249), a regression equation was derived based on patients undergoing cardiac surgery in Trondheim from 2000 through 2011 who had their LVEF measured by both methods. This enabled us to estimate echocardiographic LVEF values in patients who underwent angiography only. Patients with no clinical indication for LVEF measurement either way were assumed a LVEF of > 50%. Due to the potential inaccuracy of the estimation, LVEF was categorized into > 50%, 31-50%, 21-30% and ≤ 20%.

6.3.2 Study period

Papers 1 and 2 comprised patients undergoing cardiac surgery between 2008 and 2010. Paper 3, on the other hand, included patients from 2000 through 2014. During this long study period, several factors may have changed: Surgical techniques might have advanced. The distribution of risk factors and their influence might have been altered. Perioperative care might have improved. To get an overview, we compared baseline patient characteristics across time. However, such changes may also indicate that predictors of long-term mortality alter with time. This can have influenced our results, and separate analyses for separate time periods might yield different inclusion and weighting of risk factors. For example, gender bias in surgery referral and its impact on postoperative adverse outcome have been reported in previous studies (236). However, subsequent increased attention and focus on female patients may have diminished the role of gender on postoperative outcomes in more recent years. Furthermore, we were not able to assess the effect of novel strategies including the implementation of routine antiplatelet

therapy, lipid-lowering treatment or new anticoagulant agents on surgical outcomes. We cannot exclude possible influence from these factors on patient outcome.

6.3.3 End-point definitions

Postoperative fluid overload

There is no international consensus which defines a cut-off for postoperative fluid overload. Fluid overload has been a debated topic within critically-ill and septic patients. However, pre-existing data specifically in adult cardiac surgery patients are sparse. In general, investigations on postoperative fluid overload have used arbitrary cut-off values (83-85, 250), which limits the generalisation of results. Some reports have quantified cumulative fluid balance in relation to the baseline body weight, with a cut-off for fluid overload defined as a net positive fluid balance per kilogram of baseline bodyweight ≥ 10% (227, 251). In our study, we used a cut-off corresponding to the 90th percentile of the study population. This corresponded to 80.40 ml/kg, equivalent to 8% when calculated as net fluid balance (litres) / bodyweight (kilograms). In a preliminary analysis, a cut-off of 10% in our study population produced similar results to those presented in this thesis (data not shown). However, this stricter end-point definition resulted in only 41 patients (4.0%) with fluid overload, limiting in-depth statistical analyses.

Postoperative AKI

The strategy of defining postoperative kidney dysfunction has been controversial. Whereas early studies tended to use dialysis-dependent renal failure as the primary outcome measure, there has been a general acknowledgement that even smaller changes in renal function may have considerable effect on the postoperative outcome. This led to the definition of AKI, where consideration to data on serum creatinine values and urine output also take into account patients with milder degrees of renal dysfunction. Many attempts have been made to reach an international consensus, and the Acute Dialysis Quality Initiative (ADQI) Risk/Injury/Failure/Loss/End-stage (RIFLE) (252), AKI Network (AKIN) (253) and KDIGO (169) classifications represent different strategies. Sampaio *et al*. showed how the incidence of AKI and different risk factors varied significantly according to the diagnostic criteria (254). The lack of uniform definitions may explain the difficulty in reproducing previous results and

consequently hampering the progression in improving identification, prevention and treatment of CSA-AKI.

The KDIGO criteria represent a compromise between its two forerunners, in which the absolute increase in serum creatinine within 48 hours is retained from the AKIN definition, whereas the timeframe for a ≥ 50% increase in serum creatinine was set to seven days as included in the RIFLE criteria. Comparison studies of the three different outcome definitions have proved the KDIGO approach the highest prognostic power for cardiac surgery patients (254) as well as in other hospitalized patients (255-257).

In Paper 2, AKI was defined based on the criteria proposed by the KDIGO-guidelines. However, we made a slight modification as we allowed for a longer postoperative time span than 48 hours for an absolute increase ≥ 26 μmol/l or seven days for a ≥ 50% increase in serum creatinine concentration to occur. Furthermore, data on urine output were not available. Whereas the diagnostic utility of urine output alone have yielded variable results, combining data on serum creatinine and urine output has shown a superior role in predicting dialysis and mortality during hospitalization and over the following year (258).

Long-term mortality

In Paper 3, our primary endpoint was observed mortality, which includes all deaths during follow-up. As a sensitivity analysis, we repeated the analyses for cardiovascular mortality as recorded in the Cause of Death records. However, due to the risk of classification errors in cause-of-death records (259, 260), relative survival analyses, permitting comparison with data from the general population, were applied in order to adjust for mortality of other causes in the cardiac surgery patients. Our secondary endpoint was therefore set to relative mortality.

Cardiac surgery is seldom curative, but aims to reduce symptoms, and improve quality of life and the patients' life expectancy. We investigated the risk of mortality from the day of surgery up to the censoring date; 31 December 2014. However, we did not have information on other long-term outcomes following hospital discharge, such as new cardiovascular events, improvement in symptomatology or quality of life. These may also be important outcome measures when evaluating the success and benefits of cardiac surgery.

6.3.4 Selection of biomarkers

In this thesis, investigated biomarkers were selected based on hypotheses of underlying causal mechanisms. Although the published genetic risk score emerged as an independent risk factor of postoperative fluid overload, and the multi-marker assessment of NT-proBNP, neopterin and lactoferrin was significantly associated with CSA-AKI, the lists of biomarkers were not definite: There may be many other genetic variations or plasma biomarkers involved. Furthermore, we cannot prove causality from the present association studies, and the associated biomarkers may act as markers for other causal pathways.

In contrast to hypothesis-driven candidate gene studies, GWAS allow the testing of thousands of genes simultaneously and represents a more unbiased approach. During the last decade, there has been an increasing interest in GWAS, which is currently considered the gold-standard for identifying novel genetic associations. However, GWAS require very large study populations and are therefore restricted to large research centres or multi-centre settings. Furthermore, GWAS may not be able to select causal SNPs or rare genetic variations. Thus, larger candidate gene studies can be considered a useful supplementation. Nevertheless, validation of our findings remains crucial in the process of identifying true genetic risk factors.

6.3.5 Statistical strategy

Selection of the appropriate statistical strategy with respect to the data and specified scientific problem sets the foundation for good research and publications. For Paper 1 and 2, we defined binary outcome variables which were explored using logistic regression. In Paper 3, we applied survival analyses.

Logistic regression analysis

Awareness of underlying premises and limitations is essential for the interpretation of results. In logistic regression, covariates are assumed to be linearly associated with the outcome variable. Thus, continuous variables were assessed with splines and plots. Deviations from the linearity assumption can be overcome with transformation of the relevant covariate or categorisation. Whereas transformation can complicate the ease of interpretation amongst clinicians, categorisation can potentially lead to the loss of valuable information. For the

purpose of this thesis, we chose the strategy that would most accurately describe the association with the outcome. If there was considerable deviation from linearity, we applied logarithmic transformation of the variable. Persisting linearity deviation justified the need for categorisation.

In the investigation of preoperative plasma biomarkers associated with CSA-AKI, neopterin and lactoferrin were log transformed. NT-proBNP was best modelled categorized into two levels. However, the interpretation of log transformed variables may be less straightforward. If clinical application becomes of current interest in the future, categorisation may be a more convenient alternative. Otherwise, computer-based calculations may overcome this problem.

Survival analyses

Cox PH modelling is regarded as the traditional approach for analysing survival data where time to event is available. In addition to the linearity assumption of continuous covariates, Cox PH modelling builds on the proportionality assumption, which implicates that the hazards (risk estimates) of the covariates are constant over time. In Paper 3, deviations from the proportionality assumption were explored with separate modelling of long-term mortality in cardiac surgery patients for categorized time periods (< 1, 1-5 and > 5 years).

Modelling of excess and relative mortality represents two different approaches of adjusting for background mortality, being defined as the difference (additive model) and ratio (multiplicative model) of observed and expected mortalities, respectively. It is not intuitive which approach fits the data better, and the information provided by each strategy may be complementary (261). However, when modelling excess mortality, excess mortality is presumed to be positive (observed mortality > expected mortality) at all time points. Models that force excess mortality to be positive may suffer convergence problems when fitted to data where the observed excess mortality rate is zero or less than zero (262). This was the case in the present study cohort, and thus using the multiplicative model with estimation of relative survival was considered more appropriate.

6.3.6 Applications of clinical prediction modelling

The usefulness of clinical prediction models can be described from a clinical, economic as well as from a research perspective:

Although risk prediction tools cannot replace clinical judgement and decision-making, they serve as important adjuncts for clinicians. They also facilitate early identification of high-risk patients, enabling heightened vigilance and early optimization of perioperative care, prevention of injury and early initiation of treatment strategies.

Risk stratification tools may also aid planning of the operational programme and contribute to an ethical and fair allocation of hospital resources. Whereas low-risk patients may be considered for earlier discharge, high-risk patients might be triaged to more specialized hospital services, such as the intensive care unit, earlier patient visit postoperatively or closer outpatient follow-up following discharge.

Accurate identification of high-risk patients may also enhance research on novel preventive and treatment strategies, through recruitment of relevant patients (inclusion criteria) (263), as appropriate covariate adjustment in randomized clinical trials (264, 265) as well as in investigations of novel biomarkers (266). Prediction models can also be useful in comparisons of quality of care and outcomes across different surgeons and centres. Variations in risk factors and poor model performance may reflect different practices.

The optimal balance between parsimony and complexity in model building must therefore be adjusted to its purpose. There is a wide span of more generalized multi-centre models to local models, as well as models designed for prediction of overall mortality or composite outcomes, to models constructed for prediction of specific outcomes.

In Paper 2, we compared the performance of three pre-existing prediction models for CSA-AKI with our novel model. Model performance may differ substantially between derivation and validation cohorts for several reasons, including overfitting of the model to the derivation cohort, missing important predictor variables, variations in variable and endpoint definitions, and differences in the patient cohort case mix. Therefore, model performance in the derivation cohort may be overly optimistic and may not perform equally well in other patient cohorts. There exists several methods on how to update prior predictive models using data from the validation cohort. Nevertheless, when a validation study shows disappointing results,

researchers are often tempted to reject the initial model and to develop a new predictive model using the new data.

The external, pre-existing models showed good performance in predicting CSA-AKI in our study cohort, with good discriminative ability and goodness-of-fit. The local AKI-model previously developed by CaSOS had high discriminative ability, but poor goodness-of-fit. Whereas the pre-existing models were originally designed for clinical prediction, the intention of Paper 2 was not to produce clinical prediction models to be adopted in the everyday clinic. The aim was to find the most accurate way of adjusting for clinical variables in the present study cohort, with less concern to the risk of overfitting. Thus, a novel model was developed for comparison, which showed superior performance set side by side with previous AKI models. As a sensitivity analysis, we also repeated the analyses of plasma biomarkers in the pre-existing models. The robustness of our results, independent of the clinical modelling strategy, underscores the independent value of the biomarkers.

6.3.7 Improvement of clinical prediction modelling

Addition of intraoperative variables

In Paper 1, we found a parsimonious model consisting of age, intraoperative red cell transfusion and time spent on CPB showing good prediction of postoperative fluid overload. The strategy for the clinical modelling was to derive the most accurate set of clinical variables for confounder adjustment. The clinical adjustment therefore included variables that were only attainable postoperatively.

In Paper 2 and 3, we chose to restrict the investigation to preoperative clinical variables. Even though addition of intraoperative variables might improve predictive ability, such as demonstrated for CSA-AKI (210, 267), we considered that a possible improvement would not outweigh the gains of preoperative risk prediction. Even though the purpose of the clinical model in Paper 2 was focused on accurate confounder adjustment, selecting preoperative variables only may also indicate the potential of the preoperative biomarkers in future prediction modelling. Furthermore, important intraoperative risk factors are likely to be at least partially correlated to preoperatively available variables: Surgical category, preoperative

haemoglobin and urgency level may be good surrogate markers for duration of CPB, need for intraoperative red cell transfusion and need for inotropic support, respectively.

Addition of novel biomarkers

One of the main aims of this thesis was to assess the incremental value of novel biomarkers, on top of predictors that are already readily available. Clinical predictors were selected based on a literature review and hypotheses of potential causal pathways. Identification of novel biomarkers could help to expand our understanding of the underlying pathogenesis and may unveil additional molecular pathways that can contribute to perioperative fluid accumulation and/or renal injury.

Use of several tests may lead to inconsistent conclusions. It has therefore been emphasized that one statistical test is sufficient for assessing one hypothesis (268). In Paper 1, the hypothesis that the added novel biomarkers possess incremental predictive information was tested with the likelihood ratio test. Whereas comparison of the AUC in two regression models fitted in the same dataset has been demonstrated to be inappropriate when evaluating the incremental contribution of a new marker, the likelihood ratio test has shown to be superior(268, 269).

Even if a marker provides a significant incremental value, it is important to assess the magnitude of the incremental information to determine if the marker is of practical clinical value. Thus, there is an ongoing shift in biomarker research; from being based solely on statistical significance, to assessment of the magnitude of observed effects and hence their clinical significance. Whereas ROC curves are useful for characterizing the predictive accuracy of competing predictive models, the IDI and NRI were launched as newer measures thought to be more sensitive than improvements in the AUC (270). It has therefore been proposed to analyse the potential of a new predictor using a staged approach: First assessing the significance of a predictor within a predictive model, then evaluate the increment in AUC in a descriptive setting, and finally to evaluate its impact on clinical decision making (219, 268). With the continuous acquisition and development of statistical skills during the work of this thesis, this strategy was adopted for Paper 2.

However, the methods for assessing the incremental value of novel biomarkers still remain debated (271). Both the IDI and NRI have been criticized for being over-optimistic (272). Kerr

et al. have discussed shortcomings of these novel statistical measures which should be taken into consideration: The categorical NRI necessitates the use of risk categories and thresholds, which should have clear clinical implications and be motivated on clinical grounds. The continuous NRI, or NRI(>0), has been recommended in situations where the primary focus is on the strength of the marker rather than model performance (273). However, the continuous NRI has been criticized for having an unfamiliar scale and lacking a threshold for meaningful or sufficient degree of improvement (274). A continuous NRI > 0.6, around 0.4 and < 0.2 have been suggested as thresholds for strong, intermediate and weak effects, respectively (219). However, these thresholds have not been interpreted in relation to the clinical benefit of the novel marker.

These limitations became evident in Paper 2. There is no consensus on clinical thresholds for AKI-risk. Pencina and colleagues have proposed that in situations with no established cut-offs, using the event rate as the default risk threshold may not be unreasonable (275). Thus, we defined three categories of low, intermediate and high risk, corresponding to < 10%, 10-20% and >20% predicted risk calculated from the clinical model, respectively. Additionally, we compared these results with cut-offs at < 25%, 25-50% and > 50% predicted risk, as applied by a previous study investigating the impact of preoperative cystatin C on AKI risk prediction (198). Due to lack of clear risk thresholds for CSA-AKI, we also calculated the continuous NRI. All methods indicated a potential usefulness of the biomarkers in prediction of CSA-AKI.

Although the wide array of statistical methods applied in the papers of this thesis may contradict the abovementioned argument that several tests can lead to inconsistent conclusions, this is reflective of the continuous development in statistical methodology and lacking consensus. Until a consensus has been reached and clear guidelines are proposed on how to test different hypotheses, one may also argue that sensitivity analyses are important to offer a relative comparison of different methods and test the robustness of the results derived using different methodologies. Nevertheless, before clinical implementation, both validation in an independent cohort and an impact study on the effect of biomarker inclusion on provider behaviour and patient outcomes are necessary.

6.3.8 Predictive versus explanatory modelling: Association versus causation

It has been asserted that the terms "predictive" and "explanatory" in the context of statistical modelling may be conflated (276). Explanatory and predictive modelling are two dimensions with both distinct and overlapping qualities. Whereas studies with explanatory purposes focus on individual risk factors and statistical inference, predictive studies put more emphasis on the overall predictive power, comparison across alternative models and finding the combination of variables that best predicts the outcome. In the latter, the role of individual risk factors is less critical, and variables may be included that are not causally related to the outcome. As summarized by Schmueli (276); a priori determination of the main study aim as either explanatory or predictive is essential for conducting adequate modelling, as it affects every aspect of model building and evaluation.

The focus of this thesis was to explore pathogenic aspects of postoperative complications. A better understanding of risk factors and causal mechanisms may lay foundation for the future development of novel preventive and treatment strategies. Thus, we employed an explanatory approach to statistical modelling.

In explanatory modelling, the role of the underlying theory is very strong, and the reliance on data and statistical modelling is strictly "through the lens of the theoretical models" (276). Present causal hypotheses were justified after examination of existing literature and clinical experience, as described for the selection of biomarkers and clinical variables in the present papers. Specialized causal inference methods for observational data, including causal diagrams (277), propensity scores (278, 279), probability trees (280) and structured equation modelling (281) have gained increased attention. Nevertheless, association-based statistical models remain the most commonly used methods for testing causal hypotheses (276), and were also applied in this thesis.

However, the restricted availability of all potentially relevant variables, as well as challenges with complex interactions, collinearity and measurement errors, may limit the strength of the explanatory model. Causal inference would only be possible if all factors that influence the outcome were known. Important limitations with observational studies include the risk of

spurious findings, as well as unmeasured and residual confounding. In the present studies, there may exist additional risk factors that affect the outcome which we have not controlled for, and which can have influenced our results. Furthermore, associated predictors may carry information from one or several other predictors and act as surrogate markers for other causal relationships.

Even though association may not necessarily translate into causation, this does not imply that explanatory and predictive models are inconsistent or incompatible (282). Predictive modelling may also contribute to scientific theory development: Being developed in large and rich datasets, they may enable the uncovering of complex relationships and patterns that are difficult to hypothesize from scratch. The genetic and plasma biomarkers identified in Papers 1 and 2, respectively, have raised curiosity into different pathways that might be involved and may lay the foundation for the design of follow-up studies on underlying causality. Thus, prediction models can uncover potential new causal mechanisms, lead to the generation of new hypotheses and suggest improvements to existing explanatory models (276).

Although explicit specification of the study aim is essential, it has been recommended that researchers should report both the explanatory and predictive qualities of their model (276). Even if prediction is not the main task, the predictive qualities of a model should be reported alongside its explanatory power so that it can be fairly evaluated in terms of its capabilities and compared to other models. As shown in Paper 2, the multimarker biomarker analysis may not only provide insight into underlying pathophysiology, but might also be of potential clinical usefulness, as indicated through the AUC, IDI and NRI analyses. The clinical implications and cost-effectiveness resulting from including biomarker data remain to be demonstrated. Conversely, a predictive model might not require causal explanation in order to be scientifically useful, however, reporting its relation to causal theory is important for purposes of theory building.

6.3.9 Validity and generalisability of results

Discrimination and calibration are important measures to evaluate model performance in the studied dataset. However, optimism is a well-known problem of predictive models. Thus, internal validation is important to obtain an honest estimate of performance for patients that are

similar to those in the development sample. In the present thesis, internal validation was performed using bootstrapping methods. Bootstrapping is a resampling technique where samples are drawn from the original dataset with replacement, to create new sample sets of the same size as the original data set. Testing of the pre-specified model in the bootstrapped data sets enables assessment of the shrinkage factor and calculation of bias-corrected (overfitting-corrected) estimates. An alternative strategy would be to split data into a development and validation sample. However, due to greater variability and risk of bias with this method, bootstrapping has been pointed out as the recommended strategy (283).

By external validation, analyses are repeated in an independent validation cohort (differing temporally and/or geographically) and compared with the original performance estimates. Similar performance in independent study cohorts increases the generalisability of the findings.

The present thesis was based on single-centre studies. The findings remain to be externally validated. The generalisability of our findings may be challenged by differences in the selection of patients, definition of variables, surgical techniques and perioperative care. Furthermore, our study population comprises all patients undergoing cardiac surgery in Trondheim, which constitutes a heterogeneous study cohort: Patients undergoing different surgical interventions may have different cardiovascular diseases with different underlying pathogeneses. They may also have different risk profiles with different weighting of underlying risk factors. First, this might affect the robustness of our findings. Second, we cannot exclude that our results were influenced by the specific patient case-mix and different compositions of patients may challenge the reproducibility in other study cohorts.

Registration and enrolment of consecutive patients undergoing cardiac surgery in Trondheim, Norway, is still ongoing, enabling future validation of findings.

7. Conclusions

1. A SNP in the *UMOD* gene, possibly related to abnormal fluid handling in the kidneys, was associated with an increased risk of postoperative fluid overload following cardiac surgery.
2. A genetic risk score comprising 14 genetic risk variants, related to alterations in inflammatory and vascular pathways, was linearly associated with an increased risk of postoperative fluid overload, strengthening the evidence of a polygenic and cumulative susceptibility to postoperative fluid overload.
3. Advanced age, longer duration of CPB and use of intraoperative red cell transfusion emerged as clinical risk factors for postoperative fluid overload following cardiac surgery.
4. Both the genetic risk variant and the genetic risk score remained associated with postoperative fluid overload despite adjustment for conventional clinical variables.

5. Preoperative levels of neopterin, CRP, cystatin C and NT-proBNP were significantly higher in patients developing CSA-AKI. Neopterin, lactoferrin, cystatin C and NT-proBNP remained significant in a simultaneous test of all biomarkers.
6. A parsimonious clinical prediction model with age, gender, obesity, surgical category and preoperative renal function was identified as appropriate adjustment for conventional clinical variables.
7. Neopterin and NT-proBNP emerged as independent preoperative predictors of CSA-AKI. A protective effect of lactoferrin on CSA-AKI warrants further investigation. Inclusion of multimarker data into the clinical model provided a significant increment in predictive utility for CSA-AKI.

8. Observed mortality rates have remained constant in patients operated and followed up between 2000 through 2014.
9. Relative survival analyses show that patients undergoing cardiac surgery show excellent results, with comparable survival to the general population the first seven years of follow-up. Thereafter, there was a gradual reduction in relative survival, being more prominent in females and patients undergoing other procedures than isolated CABG.
10. When adjusting for the expected mortality in the general population, we identified three patient groups which may be relevant targets for improving long-term outcomes: Younger patients, females and patients undergoing other procedures than isolated CABG.

8. Future studies

The findings summarized in the present thesis remain to be validated in an independent study population. Continued data collection and analyses of patients who underwent cardiac surgery between 2010 and 2014 enables validation of the genetic biomarkers explored in Paper 1. However, since the study was designed back in 2008, methods for investigating genetic associations have advanced. GWAS have emerged as the gold-standard for exploring genetic associations to diseases, and have been applied in the invesigation of genetic risk factors for postoperative AKI (284), atrial fibrillation (285), venticular dysfunction (286) and perioperative myocardial infarction (287) in cardiac surgery patients. However, to this date, there is no published GWAS on the risk of perioperative fluid accumulation following cardiac surgery. Since our study supports a genetic basis for postoperative fluid overload, it would be interesting to conduct an unbiased GWAS to further disclose the potential for an underlying genetic susceptibility. This might also help to identify additional genetic variants that may be involved.

Furthermore, our study did not offer a direct functional analysis to validate the genetic associations. A transcriptional network analysis of susceptibility loci may help to explain how genetic variants contribute to perioperative fluid accumulation. However, a functional analysis in the setting of postoperative complications following cardiac surgery has its limitations. Due to the difficulties in performing an animal model of postcardiac surgery complications, experimental models of acute renal ischemia/reperfusion injury may be more applicable. On the other hand, this might limit the clinical relevance and translatability to the cardiac surgery population.

The findings from Paper 2 also remain to be replicated in an independent study cohort. With regards to the plasma biomarkers and risk for AKI, it would be interesting to explore the biologic mechansisms underlying the associations between neopterin and NT-proBNP with CSA-AKI. An animal model investigating the effect of injected neopterin to the renal arteries might disclose a potential direct effect of neopterin on renal function. Furthermore, a larger study sample to assess the association between baseline plasma lactoferrin and CSA-AKI might help to clarify the observations made in the present study.

In Paper 3 on long-term survival in cardiac surgery patients, our findings indicated that patients undergoing AVR had a higher relative mortality over follow-up time compared to CABG patients. We could not demonstrate any significant changes in patients undergoing AVR and CABG compared to isolated AVR, however, the groups were too small for further exploration. A larger study, preferably also multi-centered, would be needed in order to further investigate intergroup differences.

Furthermore, it would be interesting to compare long-term outcomes following cardiac surgery with results from PCI and TAVI. The Norwegian Registry for Invasive Cardiology was established in 2012, and during the period from 01.01.2013-01.01.2015, all the eight hospitals in Norway performing coronary angiography and PCI have committed themselves to registrating all consecutive patients undergoing these procedures. Comparable to the Heart Surgery Database, this register includes information on indications, risk factors, medical health condition, important findings, as well as more specific information about the procedure(s) performed and complications. Similar registrations for catheter-based treatment of valvular disease are currently being established.

Even though the present study comprised a follow-up period of more than 14 years, the median time was 6.4 years. Longer observational time is needed to further explore long-term mortality in cardiac surgery patients exceeding 8-10 years and longer. Continued follow-up is also important in order to continue the monitoring and quality-control of long-term results following cardiac surgery in future patients.

9. References

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Papers 1-3

Paper 1

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Genetic and clinical risk factors for fluid overload following open-heart surgery

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Background: Post-operative fluid overload following cardiac surgery is associated with increased morbidity and mortality. We hypothesised that genetic variations and pre-operative clinical factors predispose some patients to post-operative fluid overload.

Methods: Perioperative variables were collected prospectively for 1026 consecutive adults undergoing open-heart surgery at St. Olavs University Hospital, Norway from 2008–2010. Post-operative fluid overload was defined as a post-operative fluid balance/kg ≥ the 90th percentile of the study population. Genotyping was performed for 31 single-nucleotide polymorphisms related to inflammatory/vascular responses or previously associated with complications following open-heart surgery. Data were analysed using logistic regression modelling, and the findings were internally validated by bootstrapping ($n = 100$).

Results: Homozygous carriers of the common G allele of rs12917707 in the *UMOD* gene had a 2.2 times greater risk of post-operative fluid overload ($P = 0.005$) after adjustment for significant clinical variables (age, duration of cardiopulmonary bypass, and intraoperative red cell transfusion). A genetic risk score including 14 single-nucleotide polymorphisms was independently associated with post-operative fluid overload ($P = 0.001$). The number of risk alleles was linearly associated with the frequency of fluid overload (odds ratio per risk allele 1.153, 95 % confidence interval 1.056–1.258). Nagelkerke's R^2 increased with 7.5% to a total of 25% for the combined clinical and genetic model. Hemofiltration did not reduce the risk.

Conclusion: A common variation in the *UMOD* gene previously shown to be related to renal function was associated with increased risk of post-operative fluid overload following cardiac surgery. Our findings support a genetic susceptibility to disturbed fluid handling following cardiac surgery.

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Perioperative fluid accumulation following cardiac surgery is related to increased durations of stay in the intensive care unit and hospital,[1,2] incidence of acute kidney injury,[1] myocardial oedema formation and extracellular lung water accumulation with subsequent cardiorespiratory dysfunction,[2,3] and 90-day mortality.[4] Protocol-driven administration of fluid during cardiac surgery may be excessive and devastating for some patients. Despite consideration to relevant clinical risk factors such as myocardial and renal dysfunction as well as procedure-related factors such as complicated, prolonged surgery and sedation, identification of patients who are more likely to accumulate fluid and should receive an adjusted fluid therapy regimen is difficult.

The pathogenesis behind post-operative fluid overload is complex. Renal excretion function and fluid accumulation in third-space tissues are important factors. Cardiopulmonary bypass (CPB) triggers a systemic inflammatory response resulting in endothelial injury and microvascular leakage.[5] In certain patients, the so-called capillary leak syndrome develops, characterised by a pathological shift of proteins and fluid towards previously 'dry tissues'.[6] A perioperative dilemma arises, where more fluid is required to keep adequate circulatory volume and tissue perfusion, while third-space tissues are being increasingly swamped.

Genetic risk variants associated with alterations in hemodynamic and inflammatory pathways during and after cardiac surgery may increase an individual's susceptibility to post-operative fluid overload. The genetic alterations may act together with clinical risk factors causing an altered immune profile preoperatively that primes the immune system and

facilitates an enhanced inflammatory and vascular reaction triggered by CPB.[7–9]

We hypothesised that there exist clinical as well as genetic markers associated with fluid accumulation during and after open-heart surgery. These may be related to alterations in inflammatory and haemodynamic signalling pathways contributing to increased susceptibility to microvascular instability and injury, causing some patients to develop post-operative fluid overload. Based on this hypothesis, our aim was to investigate the relationship between clinical variables, the genetic profile with respect to 31 single-nucleotide polymorphisms (SNPs) and post-operative fluid overload.

Patients and methods

Data collection

Study cohort. Consecutive patients undergoing open-heart surgery at St. Olavs University Hospital, Trondheim, Norway, from April 2008 through April 2010 were included ($n = 1026$ after exclusion of 21 patients who did not consent, 32 and 7 patients who were unable to consent because of emergency operation or language problems, respectively, 57 patients with missing blood samples, 8 patients with pre-operative endocarditis, and 3 patients who underwent off-pump surgery). Patient characteristics and perioperative data were prospectively recorded in a local database. Buffycoats from pre-operative blood samples were kept at –80°C before analysis.

Study end-point and selection criteria. The anaesthetic and fluid therapy regimen during surgery is described in Supporting Information Appendix S1. The operations for all patients were completed before 17:00 h. Post-operative fluid balance was recorded as cumulative net fluid balance from anaesthesia induction until the first post-operative morning, resulting in an observational period of 16 (range 13–19) hours for all patients. The cut-off for an increased post-operative fluid balance, denoted post-operative fluid overload, was the 90th percentile of post-operative fluid balance per kilogram body weight in the study cohort, corresponding to 80.40 ml/kg. With five participants missing records of fluid balance, there were 102 cases with fluid overload and 919 controls ($n = 1021$). Intraoperative hemofiltration may affect post-operative fluid balance directly through removal of excess fluid as well as indirectly by modifying the inflammatory response. In a supplementary analysis of clinical predictor variables, patients who were subjected to hemofiltration intraoperatively ($n = 57$) were therefore excluded. In the remaining patients ($n = 964$), the 90th percentile for post-operative fluid balance was 81.88 ml/kg.

Validation cohort. Patients undergoing open-heart surgery from January 2006 through December 2007 ($n = 1110$) were used as a validation cohort for the clinical predictors associated with post-operative fluid overload. In this cohort, 147 (13.2%) patients developed post-operative fluid overload. No pre-operative blood samples were available from these patients.

The project was approved by The Norwegian Data Inspectorate and The Regional Research Ethics Committee in Medicine, Trondheim, Norway (Chairperson Arne Sandvik) on 27 June 2007 (Project number 4.2007.1528). All patients in the study cohort gave written informed consent. Given the anonymised data, the need for informed consent from the validation cohort was waived by the local ethical committee.

SNP selection and genotyping

The included 31 SNPs in 21 genes had either been (1) described as functional in mediators central to inflammatory and vascular responses, (2) previously associated with adverse outcomes following cardiac surgery, or (3) identified in genome-wide association studies of adverse outcomes following cardiac surgery (Supporting Information Appendix S2). A wide selection of genes was included to evaluate the combined effect of several SNPs that may predispose to post-operative fluid overload. Genotyping for the lactoferrin (*LTF*) gene was carried out by Sanger sequencing.[10] Twenty-seven other SNPs were analysed by Centre for Integrative Genetics (CIGENE, Norwegian University of Life Sciences, Ås, Norway) using Sequenom MassArray technology (Sequenom, San Diego, CA, USA).

Statistical analysis

Statistical analyses were performed using SPSS (version 20.0, SPSS, Inc., Chicago, IL, USA), Stata (version 12.1, StataCorp, College Station, TX, USA), SigmaPlot (version 12.0, Systat Software, San Jose, CA, USA), Minitab (version 16.2.3, Minitab, State College, PA, USA), and the 'rms', 'Hmisc', and 'xtable' packages of *R* statistical software (version 3.0.0, R Foundation*). For the simultaneous analysis

*http://www.r-project.org

of many SNPs, we applied HyperLasso (The European Bioinformatics Institute†).[11]

Data are given as medians (because of non-normal distribution of several variables) or odds ratios (ORs) with 95% confidence intervals (CIs), or as frequencies. Baseline patient characteristics for participants with post-operative fluid overload and their controls were compared using the Mann–Whitney *U*-test or χ^2 test. For assessment of linear correlations among variables, Pearson's correlation coefficient (R) was calculated after evaluation of scatterplots to assure that linear correlation analysis was appropriate. Logistic regression analyses were used to identify clinical predictors of post-operative fluid overload, to assess the association between genetic polymorphisms and post-operative fluid overload, and to adjust the genetic model for clinical predictors. Different models were compared using likelihood ratio tests. All statistical tests were two-sided, and P-values ≤ 0.05 were considered statistically significant.

There are many potentially important clinical factors that may influence perioperative fluid accumulation, and it is important to adjust for these when investigating genetic effects. We followed a three-step procedure: First, we identified significant clinical predictors from a wide selection of variables (Supporting Information Appendix S3) and found the optimal way to represent these. Second, we validated the clinical predictors in the validation cohort to evaluate the presence of overfitting. Third, we used the clinical model to adjust and test for the independency of genetic risk factors related to post-operative fluid overload.

Clinical predictors of post-operative fluid overload (Step 1). Multivariate logistic regression was applied to evaluate pre-operative and perioperative clinical covariates potentially associated with post-operative fluid overload. Two separate models were developed: one including all patients ($n = 1021$) and a supplementary model where patients subjected to intraoperative hemofiltration were excluded ($n = 964$). The selection of candidate predictor variables (Supporting Information Appendix S3) was guided by clinical knowledge and literature, a method recommended to avoid overfitting and confounders as found with selection based on univariate analyses.[12] The models were checked for deviations from the linearity assumption, pre-defined interactions, and overly influential observations. Predefined interactions between hemofiltration and other clinical variables were investigated in a model with all patients where hemofiltration was included as a potential predictor variable.

Backward limited stepdown was performed, and the final predictors were internally validated by bootstrap resampling (400 runs). Tolerance and variance inflation factors were calculated to evaluate collinearity. Performance of the final model was evaluated with calculation of the estimated shrinkage factor, a calibration plot, Hosmer–Lemeshow goodness-of-fit test, and calculation of the area under the receiver operating curve (AUC) (Supporting Information Appendix S1).

The identified clinical predictors were externally validated in the validation cohort (Step 2). Because the models were based on different patient cohorts, exact levels of significance between the models could not be calculated. The clinical predictors and discriminative ability of the models in the complete study cohort, the study cohort with hemofiltrated patients excluded, and the validation cohort were compared by the extent of overlapping of the 95% CIs for the bootstrapped ORs and AUC, respectively.

Genetic risk variants associated with post-operative fluid overload. After evaluation of genotyping quality, two different approaches to the genetic analyses were taken. First, we investigated SNPs strongly associated with post-operative fluid overload. Second, we created a predictive model with a subset of SNPs possibly related with the risk of post-operative fluid overload. The association between genotypes and increased post-operative fluid balance was assessed using penalised maximum likelihood-based logistic regression in a simultaneous analysis of all SNPs with *HyperLasso*. Simultaneous analysis of several SNPs enables detection even of those with small impact on the end-point. In contrast with single-SNP analyses, testing the joint influence of all reduces the risk of false-positives (Supporting Information Appendix S1).[11]

When searching for strongly associated SNPs, the total type I error in *HyperLasso* was set to 0.05 to limit the family-wise error rate. The initial analysis was based on an additive genetic model, followed by investigation of the dominant and recessive models. Further analysis of associated SNPs was carried out with ordinary logistic regression. When investigating a subset of SNPs possibly related to the risk of post-operative fluid overload, we aimed at includ-

†*http://www.ebi.ac.uk/projects/BARGEN*

ing all relevant SNPs using more lax criteria in *HyperLasso* (total type 1 error of 0.1).

Both genetic analyses were first performed on the original data set. The genetic associations and the definition of risk alleles and optimal genetic model for each SNP were validated by retesting in bootstrapped data sets ($n = 100$). SNPs that were significant in more than 20% of the data sets were included in the genetic risk score. The genetic risk score was calculated by adding the number of risk alleles carried by each study participant and had a theoretical range from 0 (no risk alleles) to twice the number of SNPs included in the score.

Finally, we investigated whether the associated SNPs or the genetic risk score were independent predictors of post-operative fluid balance by adding the identified significant clinical predictors to the logistic regression analysis (Step 3). The proportion of explained variance for each model was assessed using Nagelkerke's R^2, which is a pseudo-R^2 statistic for logistic regression used to evaluate improvement when comparing models. Higher Nagelkerke's R^2 indicates a better model.

Results

Study participants with post-operative fluid overload were older, had a lower body mass index, and were more often female and less frequently used lipid lowering treatment (Table 1). They more often had chronic cardiac insufficiency, left ventricular hypertrophy, and peripheral vascular disease. They also had lower pre-operative haemoglobin values and creatinine clearances, and more often went through a challenging surgical procedure with longer CPB times. They received more intraoperative red cell and plasma transfusions. Intraoperative fluid balance was significantly higher among patients developing post-operative fluid overload, and there was a high correlation between intraoperative and post-operative fluid balance per kg body weight ($R = 0.73$). Both the predicted mortality based on EuroSCORE II and the observed mortality was higher among the cases.

Hemofiltration

The patients treated with hemofiltration had significantly worse kidney function than the remaining patients [serum creatinine 161.0 (136.8–174.5) µmol/l vs. 79.0 (78.0–80.0) µmol/l, $P < 0.001$, creatinine clearance 39.7 (33.7–50.8) ml/min vs. 89.2 (85.7–91.2) ml/min, $P < 0.001$]. However, use of hemofiltration did not differ between cases with post-operative fluid balance below (8.8%) and above (5.2%) the 90th percentile ($P = 0.13$). Exclusion of hemofiltrated patients ($n = 57$) did not alter the clinical model (Table 2). None of the interactions with hemofiltration were significant. The clinical models including and excluding hemofiltrated patients had AUCs of 0.797 (0.746–0.847) and 0.793 (0.739–0.846). Intraoperative hemofiltration was not considered a relevant exclusion criterion, and further analyses included the full study cohort ($n = 1021$).

Clinical predictors

Longer CPB time, increasing age, and need for intraoperative red cell transfusion were associated with an increased risk of post-operative fluid overload (Table 2). The model showed excellent goodness-of-fit (Hosmer–Lemeshow test; $P = 0.85$) was well calibrated, and the shrinkage factor was 0.88. The AUC of 0.797 indicated good discrimination. For the validation cohort, age was not a significant predictor variable ($P = 0.41$). The calibration plot, however, showed good prediction, and the AUC did not differ significantly from that of the study cohort [0.785 (0.742–0.829)]. An alternative clinical risk model substituting serum creatinine concentration with estimated creatinine clearance was tested; however, this model was not better than the original model. The linear correlation between serum creatinine and creatinine clearance was moderate ($R = -0.41$).

SNPs associated with post-operative fluid overload

All SNPs were successfully genotyped (missing < 3% except for rs2010963 with 7.7% missing). The genotypes were in Hardy–Weinberg equilibrium ($P > 0.05$), except for SNPs in the *LTF* gene. Simultaneous testing identified two SNPs, rs12917707, G > T in the locus of the uromodulin (*UMOD*) gene and rs353625, A > G in the *CD44* gene, as significant predictors of post-operative fluid overload ($P < 0.05$) with the rare alleles being protective. They were significant in 63 and 62 of the 100 bootstrapped datasets, respectively. When adjusting for the clinical covariates, only rs12917707 remained significant. Because of few homozygous cases for the rare allele, both SNPs were recoded into recessive traits for the common allele associated with an increased risk. Further analysis with ordinary logistic regression confirmed rs12917707 as an independent predictor, where homozygous carriers of the G allele had a 2.24 times greater risk of post-operative fluid over-

Table 1

Patient and operative characteristics.

	Cases, $n = 102$	Controls, $n = 919$	*P*-value
Age (years)	74 (72.0–75.5)	67 (66.0–67.5)	< 0.001
Female gender	47 (46.1%)	231 (25.1%)	< 0.001
Body mass index (kg/m^2)	23.8 (23.0–24.6)	27.4 (27.1–27.6)	< 0.001
Previous or current smoker	47 (47.0%)	514 (56.3%)	0.08
Diabetes mellitus	9 (8.8%)	133 (14.5%)	0.12
Hypertension	57 (55.9%)	573 (62.4%)	0.20
Use of lipid-lowering treatment	66 (64.7%)	775 (84.3%)	< 0.001
Use of angiotensin-converting-enzyme inhibitors	43 (42.2%)	360 (39.2%)	0.56
Chronic cardiac insufficiency	18 (17.7%)	100 (10.9%)	0.04
Left ventricular hypertrophy	36 (35.3%)	206 (22.4%)	0.004
NYHA class 3 or 4	70 (68.6%)	576 (62.7%)	0.24
Peripheral vascular disease	23 (22.6%)	130 (14.2%)	0.02
Chronic pulmonary disease	18 (17.7%)	123 (13.4%)	0.24
Preoperative haemoglobin (mmol/l)	8.3 (8.0–8.4)	8.8 (8.7–8.8)	< 0.001
Serum creatinine (μmol/l)	79.0 (74.5–84.0)	81.0 (80.0–82.0)	0.16
Estimated glomerular filtration rate* (ml/min/1.73 m^2)	77.1 (72.8–81.2)	79.4 (78.1–80.7)	0.26
Creatinine clearance† (ml/min)	68.1 (63.5–72.8)	91.2 (89.1–93.3)	< 0.001
Previous cardiac surgery	11 (10.8%)	32 (3.5%)	< 0.001
Urgent operation‡	50 (49.0%)	409 (44.5%)	0.39
Operation type			< 0.001
CABG or atrial septum defect correction	30 (29.4%)	629 (68.4%)	
AVR only, AVR and CABG combined, non-ischaemic mitral valve surgery or operation for aneurysm of the ascending aorta	57 (55.9%)	218 (23.7%)	
Miscellaneous§	15 (14.7%)	72 (7.8%)	
Time on cardiopulmonary bypass (min)	109 (98.5–120.0)	76 (74.0–78.0)	< 0.001
Intraoperative hemofiltration	9 (8.8%)	48 (5.2%)	0.13
Intraoperative inotropic support	39 (38.2%)	207 (22.5%)	< 0.001
Intraoperative vasoconstrictor use	100 (98.0%)	877 (95.6%)	0.25
Fluid balance intraoperatively (ml)	3583 (3385–3818)	2690 (2648–2733)	< 0.001
Fluid balance post-operatively (ml)	6692 (6285–7130)	3931 (3862–4000)	< 0.001
Intraoperative red cell transfusion	57 (55.9%)	139 (15.1%)	< 0.001
Intraoperative plasma transfusion	35 (34.3%)	113 (12.3%)	< 0.001
Predicted mortality (EuroSCORE II)	5.1% (4.2–6.1%)	2.3% (2.2–2.4%)	< 0.001
Died	11 (10.8%)	20 (2.2%)	< 0.001

Continuous variables: median (95% confidence interval); categorical variables: *n* (%). The logistic EuroSCORE II is given in median percentage risk (min. – max. %).
*Estimated glomerular filtration rates calculated using Modification of Diet in Renal Disease formula.[21]
†Creatinine clearance calculations based on formula from Cockcroft and Gault.[22]
‡Urgent operations comprised prioritised patients in which the elective operation was performed within 2 weeks.
§Mitral valve surgery in combination with CABG or AVR, AVR in combination with procedures other than CABG or operation for aneurysm of the ascending aorta and other cardiac surgery such as pericardectomy or removal of cardiac tumours.
AVR, aortic valve replacement; CABG, coronary artery bypass grafting; NYHA class, The New York Heart Association Functional Classification (I–IV).

load (Table 3). rs12917707 was not significantly associated with creatinine clearance ($P = 0.14$).

Genetic risk score

The selected subset consisted of 14 SNPs (bold in Supporting Information Appendix S1). The observed score was 7–24 (theoretical range 0–28) and was linearly associated with the frequency of post-operative fluid overload (Fig. 1). In the unadjusted logistic regression analysis, every additional risk genotype was associated with a 12% increased risk of developing post-operative fluid overload (Table 2). The genetic risk score remained a significant predictor of post-operative fluid overload when adjusting for clinical variables, and there was no evidence of collinearity. Neither the ORs for the two significant SNPs nor for the genetic risk score changed significantly when the alternative model with creatinine clearance was used for adjustment.

Inclusion of the genetic risk score into the clinical model significantly improved the performance of the model ($P < 0.001$, Table 2). Nagelkerke's R^2 increased with 7.5% to a total of 25% for the combined clinical and genetic model. The cumulative relationship between clinical risk factors and the number of genetic risk variants on the risk of post-operative

fluid overload is illustrated in Fig. 2. In alternative combined clinical and genetic models, either preoperative renal and chronic heart failure, or left ventricular hypertrophy and kidney disease were included as adjustment factors in addition to the three-variable clinical model because of their close relation to fluid accumulation. There was no improvement by these more complex models ($P = 0.71$ and 0.91). Neither the distribution of the genetic risk score ($P = 0.69$) nor of the SNPs rs129117707 ($P = 0.55$) or rs353625 ($P = 0.29$) were different among the patients receiving hemofiltration compared with those who did not.

Table 2

Logistic regression models for prediction of post-operative fluid overload following open-heart surgery.

	Odds ratio (95% CI)	*P*-value
Complete study cohort ($n = 1021$*)		
CPB time (per 10 min)	1.151 (1.091–1.213)	< 0.001
Age (per 5 years)	1.327 (1.141–1.544)	< 0.001
Intraoperative red cell transfusion	3.852 (2.426–6.114)	< 0.001
Hemofiltrated patients excluded ($n = 964$*)		
CPB time (per 10 min)	1.143 (1.077–1.213)	< 0.001
Age (per 5 years)	1.329 (1.154–1.531)	< 0.001
Intraoperative red cell transfusion	3.990 (2.451–6.494)	< 0.001
Validation cohort ($n = 1110$*)		
CPB time (per 10 min)	1.249 (1.193–1.307)	< 0.001
Age (per 5 years)	1.047 (0.937–1.169)	0.41
Intraoperative red cell transfusion	4.742 (2.927–7.682)	< 0.001
Genetic models in complete study cohort†		
Univariate analysis		
Genetic risk score	1.119 (1.037–1.207)	0.004
Adjusted analysis		
Genetic risk score	1.153 (1.056–1.258)	0.001
CPB time (per 10 min)	1.152 (1.097–1.209)	< 0.001
Age (per 5 years)	1.310 (1.148–1.494)	< 0.001
Intraoperative red cell transfusion	3.755 (2.322–6.071)	< 0.001

*After exclusion of incomplete cases, clinical models were based on 1020, 963 and 1109 participants, respectively.
†After exclusion of incomplete cases, the nested model was based on 959 participants. Likelihood ratio test between univariate and adjusted model: $P < 0.001$.
CI, confidence interval; CPB, cardiopulmonary bypass.

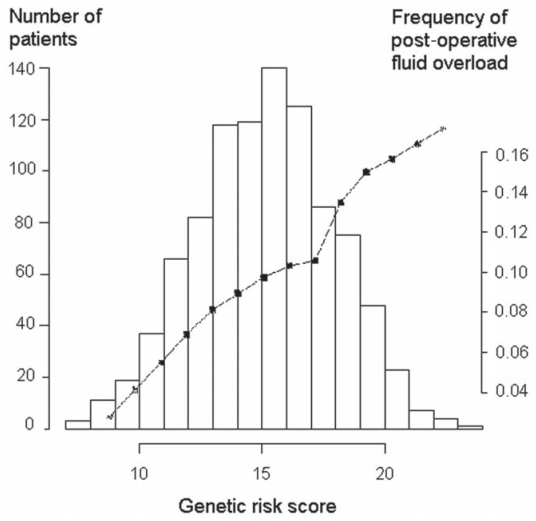

Fig. 1. Genetic risk scores in patients with post-operative fluid overload. Left y*-axis represents distribution of the genetic risk scores (histogram). Right* y*-axis shows proportion of patients with a specific risk score suffering from post-operative fluid overload (line graph).*

Table 3

Logistic regression analyses for association of rs12917707 (*UMOD*) and rs353625 (*CD44*) with fluid overload following open-heart surgery.

SNP and genotype	Genotype frequencies		Logistic regression*		Adjusted logistic regression*†	
	Controls	Cases	Odd ratio (95% CI)	*P*-value	Odd ratio (95% CI)	*P*-value
rs12917707 (G > T)						
TT/TG	285 (31.2%)	20 (19.6%)	1.00 [reference]	–	1.00 [reference]	–
GG	628 (68.8%)	82 (80.4%)	1.861 (1.119–3.094)	0.017	2.237 (1.271–3.937)	0.005
rs353625 (A > G)						
GG/GA	429 (47.5%)	39 (38.6%)	1.00 [reference]	–	1.00 [reference]	–
AA	474 (52.5%)	62 (61.4%)	1.439 (0.944–2.193)	0.091	1.434 (0.905–2.271)	0.12

*After exclusion of incomplete cases, the models for rs12917707 and rs353625 were based on 1015 and 1004 participants, respectively.
†Adjusted for cardiopulmonary bypass time, age and use of intraoperative red cell transfusion.
CI, confidence interval.

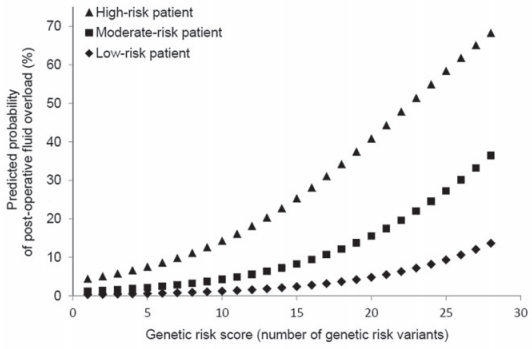

Fig. 2. Probability of post-operative fluid overload according to patient risk profile. Examples of influence of genetic risk score on predicted probability of post-operative fluid overload according to clinical risk profile. An increasing number of risk variants gives a non-linear increase in probability of fluid overload. Low-risk patient: 65 years old, cardiopulmonary bypass (CPB) time 45 min, no intraoperative red cell transfusion; moderate-risk patient: 75 years old, CPB time 98 min, no intraoperative red cell transfusion; high-risk patient: 75 years old, CPB time 98 min, received intraoperative red cell transfusion.

Discussion

CPB duration, older age, and use of red cell transfusion during surgery were significant clinical predictors of post-operative fluid overload following open-heart surgery. A SNP in the *UMOD* gene locus (rs12917707) was independently associated with post-operative fluid overload. A genetic risk score incorporating 14 SNPs related to inflammatory/haemodynamic pathways also emerged as a significant predictor. These findings support the hypothesis that both clinical and genetic factors play central roles in the pathogenesis of post-operative fluid overload.

Genetic determinants of post-operative fluid overload

Renal function is central in the pathogenesis behind post-operative fluid overload. During cardiac surgery, the kidneys are subjected to unstable circulation because of CPB with non-pulsatile flow as well as systemic inflammation but must respond with increased metabolism and fluid excretion because of increased fluid intake perioperatively. In this study, a SNP located upstream of the *UMOD* gene (rs12917707) was consistently associated with the risk of post-operative fluid overload. The *UMOD* gene encodes uromodulin, the most abundant urine protein of healthy individuals. Uromodulin is produced in Henle's loop.[13] When bound to the apical segment, it influences cell surface events and renal reabsorption of sodium and potassium, but its functions in more detail are still unclear[13] and functional studies are warranted.

Several studies have shown that a set of closely linked common SNPs located near the *UMOD* transcription start site, including rs12917707, are strongly associated with renal function.[14–18] The location of these SNPs suggests that they may influence uromodulin transcriptional activity. The rare alleles of these SNPs have repeatedly been related to lower uromodulin concentrations and protection against the development of hypertension, chronic kidney disease, and progression to end-stage renal disease.[14–18] The lacking correlation between rs12917707 and creatinine clearance may be explained by the complex interplay between several factors affecting renal function. Nevertheless, our study showing a protective effect against post-operative fluid overload is consistent with the previously described associations.

In the genetic analyses, rs353625 in the *CD44* gene was associated with post-operative fluid overload. CD44 is an adhesion molecule involved in leukocyte activation, leukocyte migration, and engagement of the acquired immune system. The expression of CD44 has previously been associated with various inflammatory and autoimmune diseases,[19] and it is conceivable that CD44 may play a role in a polygenic susceptibility to disturbed fluid handling. However, we could not confirm rs353625 as an independent predictor of post-operative fluid balance.

Inter-individual variations in post-operative fluid balance may be explained by a combination of clinical variables and a polygenic inheritance. The genetic risk score was constructed using looser criteria (total alpha = 0.1) in order to avoid missing out relevant SNPs, which increases the risk of false-positives. When testing with stricter criteria (total alpha = 0.05 or including only the 5 or 10 SNPs most frequently significant in the bootstrapped data sets), the alternative risk scores were consistently significant but with slightly changing OR (data not shown). The final list of SNPs is not definite, and other genetic risk variants may be involved. Although each individual SNP may only have a modest association with post-operative fluid balance and may not be directly causal, our findings give indications of potentially important pathways for post-operative fluid accumulation. The score was not intended for genetic screening of patients but to

investigate underlying mechanisms behind post-operative fluid overload and the relevance of a genetic susceptibility. The trend showing a cumulative effect of genetic risk variants on post-operative fluid overload should be further investigated.

Clinical predictors of post-operative fluid overload

Patients suffering post-operative fluid overload more often presented with reduced heart and kidney function pre-operatively. However, with the parameters available in the present study, a parsimonious model consisting of three other easily available variables showed good ability to identify patients with increased risk of post-operative fluid overload. Increasing age is related to higher comorbidity, more fragile blood vessels, and smaller functional reserves, predisposing to more unstable fluid handling. Blood transfusion may directly lead to increased fluid balance because of increased volume and osmotic effects but can also act indirectly by triggering inflammatory pathways and hence influence vascular permeability. Several clinical factors may be associated with the need for red cell transfusion, e.g., pre-operative anaemia, small body size/female gender, or complicated surgery, but it is not possible to deduce the importance of each one from our study. Increased CPB time results in greater exposure of blood to the extracorporeal circuit with subsequent enhanced inflammation, but also a longer period of potential peripheral hypoperfusion and ischaemia, which increases the risk of post-operative kidney dysfunction and prolonged myocardial stunning. Increased CPB time is also directly related to more complicated surgical procedures and increased fluid administration.

The fact that other variables with clear relevance to the end-point, such as creatinine clearance, did not become significant in the multivariate risk model does not imply that they are without clinical importance. However, advanced age, use of red cell transfusion, and CPB time may act as informative markers for many aspects related to post-operative fluid overload and thus be a better combination to predict post-operative fluid overload, rendering other clinically relevant variables superfluous in the model.

The clinical model was developed to identify adjusting variables for the genetic risk variants associated with post-operative fluid accumulation. Age was not significantly related to post-operative fluid overload in the validation cohort comprising patients operated a few years before the main study cohort. The reason for this discrepancy is unknown, but we may speculate that increased administration of norepinephrine during more recent years has resulted in a more restrictive fluid therapy. This suggestion is supported by the observation that post-operative fluid overload was somewhat more frequent in the validation cohort. Hence, the improved handling of other related conditions through vasopressors may render age a more important risk factor for post-operative fluid overload in the more recent cohort. Nevertheless, thorough internal and external validation of the clinical model confirmed that the identified predictor variables were appropriate for adjustment of the genetic analyses.

Hemofiltration

Somewhat surprisingly, use of hemofiltration during surgery did not affect post-operative fluid balance. Because the use of hemofiltration was comparable in patients with and without post-operative fluid overload, the effect of the reduced kidney function in the patients undergoing hemofiltration was also distributed between the patients with and without the outcome. The genetic risk score and the SNPs associated with fluid overload were not related to use of hemofiltration. These results support our decision not to exclude patients receiving hemofiltration from the study.

Whereas hemofiltration is considered standard care in paediatric surgery, the efficacy and use of hemofiltration during adult cardiac surgery is controversial.[20] The proposed benefits during adult cardiac surgery include reduced hemodilution, lower concentrations of circulating inflammatory mediators, and improved cardiac function.[20] Hemofiltration is applied to conserve fluid balance and avoid unnecessary transfusions, blood loss, and need of drug support immediately following cardiac surgery. By reducing the inflammatory reaction and directly restoring a normal fluid and electrolyte balance, hemofiltration was thought to reduce the risk of post-operative fluid overload. Hemofiltration was used on clinical discretion in high-risk patients with clinical kidney dysfunction and/or high-risk surgery with prolonged CPB. However, our study indicates that the efficacy may be lower than proposed. Because of the limited study size and subsequent risk of false-negative findings, larger studies are warranted to confirm our results. The importance of hemofiltration with respect to blood loss and reduction of transfusions was not investigated in this study.

Limitations

Patients operated at different times during the day would have been administered unequal total volumes of fluid during the time frame for measuring the cumulative net fluid balance. However, intraoperative and post-operative fluid balances were highly correlated, indicating that fluid accumulation started early in the course for most patients. Furthermore, the importance of inter-individual variations was reduced because only patients with the most extreme fluid accumulation (> 90th percentile) were defined as having the endpoint and by using a no/yes outcome variable. Fluid balance per kilogram body weight was chosen in order to take body size and thus different fluid distribution volumes into consideration.

The present study was not designed to describe causal relationships between single predictors and outcome, which would only be possible if all factors that influence post-operative fluid overload were known. The aim of the clinical model was to identify suitable adjustment for the investigation of a possible genetic susceptibility for post-operative fluid overload. There may exist other important variables related to post-operative fluid overload that were not registered in our database, such as the total volume of infused cardioplegia or use of post-operative sedation. However, these variables would – at least partially – be covered by the type of surgical procedure and pre-operative organ dysfunctions. CPB time was chosen as marker for operation type, as these variables were highly correlated and CPB time was more informative with relation to post-operative fluid overload. Residual confounding among the clinical factors or between clinical and genetic factors cannot be excluded. The patients included in the study were heterogeneous and underwent a wide range of surgeries. However, the genetic factors underlying the tendency to fluid overload are probably similar and not a function of the surgical procedures. The actual fluid balance, on the other hand, will be influenced by the patients' clinical conditions, intraoperative and post-operative management, which is why substantial work was performed in order to select and validate appropriate clinical adjustment variables for the genetic analyses. Within the group of patients with post-operative fluid overload, one may speculate that the genetic profile of patients undergoing more complex procedures could be different from that of patients undergoing the lowest risk operations (coronary artery bypass grafting or atrial septum defect correction). A subgroup analysis indicated that this was not the case ($P = 0.66$); however, the study was not powered for such subgroup analysis, and a larger study would be necessary to investigate this hypothesis.

The main limitation of the genetic analyses was the size of the study population, resulting in few homozygous individuals for the rare allele of some SNPs. Furthermore, blood samples from another cohort were not available for validation of our genetic findings. Nevertheless, to our knowledge, this is the first investigation of genetic determinants of post-operative fluid overload, thus laying the foundation for further studies. More research is warranted to validate and investigate the causal relationship between the genetic risk variants and increased fluid accumulation before new tests or management strategies can be advocated. However, our results indicate that it will probably be difficult to accurately predict fluid overload following cardiac surgery without considering genetic predispositions.

Conclusion

A common SNP in the *UMOD* gene (rs12917707) protected against the risk of fluid overload following cardiac surgery, independent of age, CPB time and use of intraoperative red cell transfusion. A genetic risk score comprising 14 SNPs related to inflammatory and haemodynamic pathways showed that the contribution from several genetic risk variants was linearly associated with an increased risk of post-operative fluid overload. The findings support a genetic susceptibility to disturbed fluid handling following cardiac surgery.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Further description of methods.

Appendix S2. Selection of SNPs and selection rationale.

Appendix S3. Variable definitions and predefined interactions.

Appendix 1: Further description of methods

Anesthesia and fluid therapy during open-heart surgery

A standard anesthetic technique with morphine and scopolamine for premedication and with fentanyl, thiopental, diazepam and cisatracurium for induction was used. Anesthesia was maintained with isoflurane and small additional doses of fentanyl as required (total fentanyl dose approximately 10µg/kg). During bypass anesthesia was maintained with propofol.

The average patient received 500ml of acetated Ringer's solution before bypass. The perfusion circuit was primed with 1500-1800ml of acetated Ringer's solution. Up to 1000ml of cold crystalloid hyperkalemic cardioplegia was given, alternatively a smaller volume of cold blood cardioplegia was given in patients with more complex surgical procedures or anticipated longer CPB time. Additional perioperative crystalloids and blood products were given at the discretion of the attending anesthesiologist.

Prolonged postoperative sedation and ventilation was considered in patients with clinical signs of postoperative organ failure. In cases with cerebral complications, vasoconstrictors were given at discretion of the attending anesthesiologist. Similarly, in the presence of postoperative myocardial stunning or heart failure, a need for ionotropics was considered.

Statistical analysis

Identification and internal validation of clinical predictors of postoperative fluid overload

In order to identify a more parsimonious model, backward limited stepdown was performed based on Akaike's information criterion. The expected shrinkage factors were calculated both for the full and reduced model, and were used to assess the stability of the models both with respect to the number of covariates and potential application on future observations. A shrinkage factor above 0.85 indicates that a model is well calibrated for future data. A calibration plot was constructed and was used together with the Hosmer-Lemeshow goodness-of-fit test to evaluate model calibration. The discriminative ability of the model was assessed by the area under the receiver operating curve (AUC) An AUC greater than 0.7 is considered acceptable and an AUC greater than 0.8 is considered good.

Genetic analyses

In preliminary analyses, genotyping quality was assessed by missingness for each SNP, and deviation from Hardy-Weinberg equilibrium using a standard χ^2 test. Genetic associations were then assessed applying *HyperLasso*. The total type 1 error was specified, thus accounting for testing of multiple SNPs in the same patients. In contrast to univariate analyses, where there is a risk of spurious associations, simultaneous analyses strengthen signals from true, causal associations and weaken false signals[1].

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Appendix 2

Selection of SNPs and selection rationale

SNP ID	Gene	Gene selection rationale	SNP selection rationale			References
			Identified by previous association studies	Identified by genome wide association studies (GWAS)	Identified through pathway analysis	
rs2115763	***BCO2***	Proinflammatory		X	X	1
rs353625	***CD44***	Proinflammatory	X		X	2
rs13038305	***CST2***	Immunomodulatory		X	X	3-5
rs7933007	*CXCR5*	Proinflammatory	X		X	2,6
rs1799983	*eNOS*	Released under oxidative stress; involved in vasomotor regulation	X		X	7
rs17609240	*GSDMA/ORMDL3*	Proinflammatory		X	X	8
rs17379472	*HSP-A1L*	Chaperone activity; protects against inflammation and oxidative stress			X	9,10
rs5498	***ICAM 1***	Proinflammatory	X		X	11,12
rs1861494	*IFN-γ*	Proinflammatory			X	13
rs1800872	*IL-10*	Antiinflammatory	X		X	7,11
rs1800896	***IL-10***	Antiinflammatory	X		X	7,11
rs1800871	***IL-10***	Antiinflammatory	X		X	7,11
rs1834481	***IL-18***	Proinflammatory		X	X	1
rs11209026	*IL23-R*	Proinflammatory and immunomodulatory			X	14
rs1800795	***IL-6***	Proinflammatory	X		X	7,11
rs4073	*IL-8/CXCL8*	Proinflammatory			X	11
rs2227306	*IL-8/CXCL8*	Proinflammatory			X	11
rs10662431	***LTF***	Antimicrobial, antiinflammatory and immunomodulatory			X	15-17
rs1126478	***LTF***	Antimicrobial, antiinflammatory and immunomodulatory			X	15-17
rs1126477	*LTF*	Antimicrobial, antiinflammatory and immunomodulatory			X	15-17
rs17078878	*LTF*	Antimicrobial, antiinflammatory and immunomodulatory			X	15-17
rs243865	*MMP-2*	Immunomodulatory	X		X	18
rs12119788	*MRP-14 =S100A9*	Proinflammatory		X	X	19,20
rs4673	***p22phox***	Superoxide production	X		X	7

		under oxidative stress and respiratory burst; proinflammatory					
rs2107538	***RANTES/CCL5***	Proinflammatory				X	11
rs1805193	***SELE***	Proinflammatory	X			X	12
rs12917707	***UMOD***	Potential facilitator of inflammation		X		X	3,21
rs699947	*VEGF-A*	Increases vascular permeability and leakage	X			X	3,7,22
rs2010963	*VEGF-A*	Increases vascular permeability and leakage	X			X	3,7,22
rs3025039	*VEGF-A*	Increases vascular permeability and leakage	X			X	3,7,22
rs833061	*VEGF-A*	Increases vascular permeability and leakage	X			X	3,7,22

SNPs given in bold were included in the genetic risk score for postoperative fluid overload

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Appendix 3

Variable definitions and predefined interactions	
Preoperative variables	
Age	Years, continuous variable
Female gender	No/yes
Diabetes mellitus	Receiving medication (no/yes)
Smoking	Current smoker or quit < 6 months ago (no/yes)
Hypertension	Receiving medication or diastolic blood pressure above 90 mmHg (no/yes)
Chronic cardiac insufficiency	Medical treatment (no/yes)
Peripheral vascular disease	Intermittent claudication, carotid stenosis or abdominal aortic aneurysm (no/yes)
Chronic pulmonary disease	Use of bronchodilating agents or $FEV_{1.0}$ <75% (no/yes)
Left ventricular hypertrophy	Based on electrocardiography or echocardiography (no/yes)
Pulmonary hypertension	sPAP>40mmHg or mPAP>25 mmHg (no/yes)
Preoperative hemoglobin	Hemoglobin concentration (mmol/l), continuous variable
Preoperative serum creatinine*	Creatinine concentration (µmol/L), continuous variable
Previous cardiac surgery	No/yes
Degree of urgency	2 categories: (1) standard waiting list, (2) operation within 2 weeks
NYHA class	2 categories: (1) NYHA class I or II, (2) NYHA class III or IV
Intraoperative variables	
CPB time	Per 10 minutes, continuous variable
Red cell transfusion	On clinical indication during surgery (no/yes)
Plasma transfusion	On clinical indication during surgery (no/yes)
Intraoperative hemofiltration	No/yes *(tested in alternative model only)*
Predefined interactions:	
General interactions:	
Age and chronic pulmonary disease	
Peripheral vascular disease and chronic cardiac insufficiency	
Peripheral vascular disease and preoperative hemoglobin concentration	
Chronic cardiac insufficiency and preoperative hemoglobin concentration	
Age and degree of urgency	
Interactions regarding intraoperative hemofiltration (tested in alternative model only):	
Hemofiltration and preoperative serum creatinine	
Hemofiltration and intraoperative plasma transfusion	
Hemofiltration and age	

CPB: cardiopulmonary bypass; $FEV_{1.0}$: forced expiratory volume in one second; mPAP: mean pulmonary artery pressure; NYHA class. New York Heart Association Functional Classification (I-IV); sPAP: systolic pulmonary artery pressure

*In an alternative model, estimated creatinine clearance (mL/min) based on Cockroft-Gault's equation replaced serum creatinine concentration.

Paper 2

A preoperative multimarker approach to evaluate acute kidney injury following cardiac surgery

Short title: Acute kidney injury in cardiac surgery

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Conflicts of interest:

None.

ABSTRACT

Objective(s): To investigate whether a multimarker strategy combining preoperative biomarkers representing distinct pathophysiological pathways enhances preoperative risk assessment of acute kidney injury following cardiac surgery (CSA-AKI) and elaborates knowledge of underlying pathogenesis.

Design: Prospective, cohort study.

Setting: Single-center tertiary referral hospital.

Participants: 1,015 adults undergoing cardiac surgery with cardiopulmonary bypass.

Interventions: CSA-AKI was defined as ≥ 50% increase in serum creatinine concentration, absolute increase ≥ 26 µmol/l or new requirement for dialysis. Pre- and perioperative information until hospital discharge was registered. Preoperative plasma levels of C-reactive protein, terminal complement complex, neopterin, lactoferrin, N-terminal pro-brain natriuretic peptide (NT-proBNP) and cystatin C were determined using enzyme immunoassays. Biomarkers were selected based on causal hypotheses of underlying mechanisms and were related to inflammatory, hemodynamic or renal signaling pathways.

Measurements and Main Results: 100 patients (9.9%) developed CSA-AKI. Higher baseline plasma concentrations of neopterin and NT-proBNP were independently associated with CSA-AKI ($p=0.04$ and $p<0.001$, respectively). Lower baseline plasma lactoferrin concentrations were observed in patients with CSA-AKI ($p=0.05$). Compared to clinical risk assessment, addition of these biomarkers provided a slight, but significant increment in predictive utility (area under the curve 0.81-0.83, likelihood ratio test $p<0.001$). A net of 12% of patients were correctly reclassified, and improved prediction was especially seen in patients with intermediate risk (56% correct reclassification).

Conclusions: Preoperative hemodynamic, renal and immunologic function play central roles in the pathogenesis of CSA-AKI. Our findings add evidence to the potential of a multimarker approach in order to improve preoperative prediction of CSA-AKI.

Key words: Acute kidney injury, cardiac surgery, preoperative biomarkers, risk prediction

INTRODUCTION

The multifactorial pathogenesis of cardiac-surgery associated-acute kidney injury (CSA-AKI) is related to a complex interaction between baseline predisposition, hemodynamic disturbances, nephrotoxic insults and inflammatory responses. Several prediction models have been developed, combining preoperative clinical characteristics to identify patients at risk of CSA-AKI.[1] In the clinical setting, preoperative prediction of CSA-AKI may facilitate patient consulting, clinical decision-making and commencement of primary preventive strategies, before significant damage has taken place (Figure 1). Furthermore, it may facilitate a fair allocation of hospital resources. For research purposes, there has been a call for novel interventions in the early prevention of AKI in high-risk patients, and preoperative risk modeling may both contribute to enhanced identification of at-risk patients eligible for trial-specific intervention as well as recognition of central pathways and thus potential treatment targets.

However, it has been shown that existing models are of insufficient precision with a tendency of underestimating the AKI risk.[2, 3] Furthermore, most investigators have used dialysis-dependent AKI or severe AKI as their endpoint, whereas even smaller changes in renal function are associated with cardiovascular adverse events and reduced survival.[4-6]

The limited ability to identify patients who develop CSA-AKI may indicate that some patients have a subclinical vulnerability to AKI that is not predictable through clinical risk factors alone. We hypothesized that there exist preoperative biomarkers more closely related to the baseline risk that may improve our ability to predict CSA-AKI. Some preoperative biomarkers (natriuretic peptides, cystatin C) have already been suggested to provide additional or superior information about the risk for AKI following adult cardiac surgery.[7, 8] However, a single biomarker may not provide sufficient precision because of the multifactorial pathogenesis and heterogeneous patient populations. Therefore, we hypothesized that a multimarker approach reflecting different potentially pathogenic mechanisms, including inflammatory, hemodynamic or renal signaling pathways, could provide complementary information, improve risk stratification and increase our understanding of the complex pathology behind CSA-AKI. Thus, the aim of this prospective study was to investigate the associations of preoperative plasma C-reactive protein (CRP), terminal complement complex (TCC), neopterin, lactoferrin, N-terminal pro-brain natriuretic peptide (NT-proBNP) and cystatin C with the risk of AKI following cardiac surgery.

METHODS

The study was part of the Cardiac Surgery Outcome Study (CaSOS). Approval was given by The Norwegian Data Inspectorate and The Regional Research Ethics Committee in Medicine (Project number 4.2007.1528), Trondheim, Norway on 27.06.2007. Written informed consent was obtained from all patients.

Consecutive adult patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) at St. Olavs University Hospital in Trondheim, Norway, from 04.01.2008 through 04.19.2010 were enrolled. Patients on preoperative dialysis or with missing preoperative serum creatinine data were excluded. All exclusions are outlined in Figure S1, Supplementary Methods 1 (SM1), leaving 1,015 patients for analysis. Information on patient and procedural characteristics, laboratory tests, and postoperative factors until hospital discharge was prospectively registered as part of the department's quality assurance work. Plasma from preoperative blood samples was stored at -80˚C for later analysis. The same study cohort was included in three previous investigations from CaSOS.[9-11]

The end-point was postoperative AKI defined as ≥ 50% increase after surgery from the baseline serum creatinine concentration, an absolute increase ≥ 26 µmol/l or a new requirement for dialysis, including all stages of AKI as defined by the *Kidney Disease: Improving Global Outcomes* (KDIGO)-guidelines.[12] A slight modification from the original definition was used, as data on urine output was unavailable and we allowed a longer time span than 48 hours for an absolute increase or 7 days postoperatively for a doubling in serum creatinine concentration to occur. Changes in serum creatinine from baseline were based on the maximum postoperative concentration, as previously employed in CaSOS.[13]

Plasma biomarkers

Biomarker selection was based on causal hypotheses. We hypothesized that inflammatory, hemodynamic and renal signaling pathways are central mechanisms in the preoperative increased risk for CSA-AKI, and biomarkers for these pathways were chosen (Table 1).

Preoperative plasma concentrations of CRP, neopterin, TCC, lactoferrin, cystatin C and NT-proBNP were analysed using enzyme immunoassays. High-sensitivity CRP, TCC, neopterin and lactoferrin were estimated as earlier described.[11] NT-proBNP and Cystatin C were measured using commercial kits (Human NT-proBNP ELISA Kit, MyBioSource, Inc., San Diego, CA, USA; RayBio Human Cystatin C ELISA, RayBiotech Inc., Norcross, GA, USA; and BioVendor Human Cystatin C ELISA, Biovendor Research and Diagnostic Products, Brno, Czech Republic).

Statistical analysis

Data are described using median with 95% confidence intervals (CI) or frequencies with percentages, as appropriate. Outcome comparisons were performed with the Mann-Whitney U-test or Chi-square test. Pearson's correlation coefficients were used to test linear correlation between biomarkers, where coefficients between 0.1-0.3, 0.3-0.6 and >0.6 were considered to indicate weak, moderate and strong correlation, respectively. Statistical analyses were performed using Stata (version 13.1, StataCorp LP, Lakeway Drive, USA), R (version 3.2.2, Foundation for Statistical Computing, Vienna, Austria) and Minitab 17 (Minitab Ltd, Coventry, UK).

Associations between biomarkers and the incidence of CSA-AKI were investigated using an explanatory approach,[21] following a 3-step procedure applying logistic regression: (1) Unadjusted analyses of each biomarker separately; (2) Simultaneous testing of all biomarkers; (3) Multivariate analyses adjusting for relevant preoperative clinical predictors.

Biomarkers

The linearity assumption was tested by plots and splines. Biomarkers were natural log-transformed when appropriate. NT-proBNP was dichotomized with a cut-off value >125 pg/ml as recommended for preoperative screening in moderate-to-high risk patients.[22, 23] Biomarkers with p-value <0.10 were included into the multivariate analyses.

Clinical variables

The purpose of the clinical model was to represent clinical risk factors appropriately and adjust for them in order to explore underlying independent associations of biomarkers predicting CSA-AKI. To avoid spurious results we compared four different approaches applying (1) the clinical predictors derived previously in our centre (CaSOS' CSA-AKI model);[13] (2) the additive Cleveland clinical risk score;[24] (3) the UK any-stage AKI risk calculator;[25] and (4) by constructing a parsimonious model based on novel hypotheses of mechanisms. The Cleveland clinical risk score was originally developed to predict dialysis-dependent AKI, but has proven applicable at different severity levels of CSA-AKI.[26, 27] The different models are outlined in Table S1 in Supplementary Methods (SM1).

For novel model development, the selection of potential clinical predictor variables was guided by an extensive literature review and clinical knowledge, as recommended to avoid overfitting and confounding with selection based on univariate analyses.[28, 29] Backward limited stepdown was performed and the final predictors were internally validated by bootstrap resampling (200 runs). As a sensitivity analysis, alternative models with different indicators of pre-existing renal dysfunction (serum creatinine, creatinine clearance, or estimated GFR based on creatinine or creatinine and cystatin C combined[20]) and heart function (ejection fraction, diagnosis of chronic heart failure or New York Heart Association (NYHA) Functional Classification) were tested. Models were checked for linearity, predefined interactions and overly-influential observations. Model discrimination was evaluated using the area under the receiver-operating characteristic curve (AUC). Goodness-of-fit was assessed with a plot of observed vs. predicted outcomes and Hosmer-Lemeshow (HL) test. Predicted performance of the final model in future data sets was evaluated using the estimated shrinkage factor and a calibration plot.

Final model selection was based on comparison of the AUC, HL test, log likelihood, Akaike and Bayesian information criteria (AIC and BIC) (section 1.3 in SM1).

Multivariate analysis

Finally, we analyzed whether the biomarkers remained significant predictors of CSA-AKI when adjusting for clinical variables. Predefined interactions between covariates were tested. As a sensitivity analysis, biomarkers were adjusted with each of the clinical models in order to assess the robustness of the associations and reduce the risk of overfitting. The model including novel biomarkers was compared to the clinical model without biomarkers by a likelihood ratio test. Final estimation was performed on 1,005 patients after excluding eight patients (0.8%) with incomplete data for all biomarkers and two overly-influential cases. Model estimates are presented with bootstrapped confidence intervals (200 runs). Estimated risk levels were plotted as predicted probabilities across decile groups of predicted risk with the observed proportions of AKI cases superimposed. Model discrimination and goodness-of-fit was evaluated as described above.

The incremental contribution of the biomarkers to the final clinical model was assessed by improvements in AUC, the integrated discrimination improvement (IDI) and the continuous and categorical net reclassification improvement (NRI) (for a more detailed explanation, see section 1.4 in SM1). In order to evaluate the effect of the biomarkers on AKI risk classification, patients were grouped into low (< 10%), intermediate (10-20%) and high (>20%) risk groups according to their calculated AKI risk by the clinical model before addition of the biomarkers. Pencina and colleagues have proposed that in situations with no established cut-offs, using the event rate as the default risk threshold may not be unreasonable,[30] which was therefore done in the present study. The NRI was calculated as the sum of improvement for cases and controls. Due to lack of clear risk thresholds for CSA-AKI, we also calculated the “continuous NRI” or NRI(>0). The continuous NRI has been recommended in situations where the primary focus is on the strength of the marker rather than model performance.[31] A continuous NRI >0.6, around 0.4 and <0.2 are considered strong, intermediate and weak, respectively.[32]

RESULTS

100 patients (9.9%) developed postoperative AKI following cardiac surgery. Patients with CSA-AKI were older and more frequently presented with diabetes, hypertension, chronic pulmonary disease and peripheral vascular disease (Table 2). AKI cases more often had pre-existing left ventricular hypertrophy or chronic cardiac failure; however, they did not present with lower ventricular ejection fractions. They had significantly higher baseline serum creatinine concentrations. Patients suffering CSA-AKI more often had a history of previous cardiac surgery. They generally underwent more complex surgical procedures, spent longer time on CPB and more frequently received intraoperative inotropic support. Detailed comparisons of AKI cases and controls are provided in Table 2.

Biomarkers

Baseline concentrations of fluid-phase markers are given in Table 3. Neopterin, CRP, cystatin C and NT-proBNP concentrations were higher ($p<0.001$ for all) in patients developing CSA-AKI. Lactoferrin concentrations were lower in AKI cases, but this difference did not reach statistical significance ($p=0.05$). Neopterin, lactoferrin, NT-proBNP and cystatin C remained significant in a simultaneous test of all biomarkers. Correlations amongst the different markers were weak (Table S2a in Supplementary Material 2 (SM2)).

Clinical variables

The final parsimonious clinical model used to adjust for biomarkers in the multivariate analysis comprised age, body mass index above 30 kg/m^2, female gender, multiple surgical procedures and preoperative serum creatinine level. By overall judgement, this model provided better fit than any of the previously published prediction models for CSA-AKI (Table S3a in SM2): Besides being simpler, it showed lower AIC, intermediate BIC, high discrimination (AUC 0.800 (95% CI 0.758-0.842)) and excellent goodness-of-fit (HL test, $p=0.47$). Alternative models from the sensitivity testing including data on chronic heart failure, NYHA class, diabetes, pulmonary disease or urgency level of operation did not improve model performance (data not shown).

Multivariate analysis

Neopterin, NT-proBNP and lactoferrin emerged as independent predictors of CSA-AKI (Table 3). Cystatin C was moderately correlated with serum creatinine levels (R=0.48), but did not provide significant information above that of preoperative creatinine concentrations (p=0.15). Substitution of creatinine with cystatin C did not improve model performance. However, when estimating GFR using the combined creatinine-cystatin C equation, the model showed somewhat better fit (Table S4 in SM2). Thus, in the final model, estimated GFR based on serum cystatin C and creatinine concentrations substituted serum creatinine concentration alone (Table 4).

Neopterin was correlated with serum creatinine (R=0.56), however neopterin remained significant also after adjusting for kidney function (neopterin/creatinine ratio). This adjustment did not alter any results, thus the parsimonious model without adjustment was kept. In well-calibrated clinical models, NT-proBNP and neopterin consistently emerged as independent predictors of CSA-AKI (Table S3b in SM2). Initially, increased serum lactoferrin concentrations were associated with a protective effect on CSA-AKI (Table 3); however, this association was no longer significant after bootstrapping the estimates in the final model with adjustment for clinical variables (Table 4, p=0.08).

Addition of baseline NT-proBNP, lactoferrin and neopterin levels to the clinical variables improved model fit (Table 4, LR test p<0.001). The final model showed a median predicted CSA-AKI risk of 6.3% (95% CI 5.8-7.0%, range 0.3%-81.3%) (Figure 2). AKI-cases showed a median predicted risk of 21.5% (95% CI 17.1-26.1) in the baseline model, and 27.2% (95% CI 22.0-32.3) in the combined model. In comparison, patients without CSA-AKI had median predicted risks of 6.1% (95% CI 5.7-6.6) and 5.3% (95% CI 4.8-5.8) based on the baseline and combined models, respectively.

The continuous NRI was 0.55 (0.34-0.75). When comparing the effect of including biomarkers on AKI risk categories, a net 12% of all patients were reclassified correctly when combining

biomarkers and clinical variables (categorical NRI 0.12, p=0.05, section 2.4 in SM2). 10% were due to AKI-patients being correctly reclassified to higher risk groups (Table S5 in SM2). A subgroup analysis was performed in the intermediate risk group (predicted risk 10-20%, n=175 (17%)). Among AKI cases (n=22), 11 patients were correctly upgraded in risk category, whereas 5 incorrectly downgraded, yielding a net correct reclassification in 6 out of 22 AKI-patients (27%). Correspondingly, among non-AKI cases (n=153), 71 patients were correctly downgraded, whereas 26 incorrectly put into a higher risk category, yielding a correct net reclassification of 45 (29%) non-AKI cases. The overall NRI in the intermediate group was therefore 56%.

The combined risk model demonstrated excellent agreement between predicted and observed risks (Figure 2, HL test p=0.87). There was an incremental increase in the AUC from 0.806 (95% CI 0.764-0.847) with clinical variables only, to 0.832 (95% CI 0.791-0.873, p=0.05) when including biomarkers. The improvement in prediction seen in the intermediate risk group, as demonstrated with reclassification, was also confirmed by the AUC graph (Figure 3).

DISCUSSION

In the present study, neopterin and NT-proBNP emerged as independent predictors of CSA-AKI. The statistical significance of the protective effect of higher baseline lactoferrin concentrations was inconsistent, which may be due to the sample size. Cystatin C was not superior to serum creatinine as an indicator of preoperative renal function. However, estimated GFR combining both creatinine and cystatin C provided subtle improvement in model performance. Inclusion of biomarkers provided significant incremental information regarding the risk of CSA-AKI beyond traditional, clinical risk factors. Improved prediction was especially seen in patients with intermediate AKI risk, where a net of 27% of AKI cases and 29% of control patients were correctly reclassified (overall NRI 56%).

We explored biomarkers that may help understand and predict CSA-AKI preoperatively. Whereas later prediction including perioperative variables and biomarkers may enhance risk stratification and identification of injury and impaired renal function,[13, 35] we find that preoperative prediction is of greater clinical utility.

Clinical utility

A comprehensive analysis of the added incremental value of novel predictors confirmed that preoperative information about NT-proBNP, neopterin and lactoferrin significantly enhanced the predictive ability for CSA-AKI. Findings remained robust independent of the clinical risk model used for adjustment, underscoring the independency of the biomarkers from clinical risk factors. The additive role of each biomarker confirmed our hypothesis regarding the importance of including biomarkers from different pathophysiological pathways.

Previous studies on preoperative biomarkers of AKI have only considered high-risk patients.[7, 8] Shlipak and colleagues investigated the impact of preoperative cystatin C on AKI risk prediction, where addition of cystatin C to a baseline clinical model increased AUC from 0.70 to 0.72 ($p<0.01$) and led to a NRI of 21%.[8] Similarly, Patel and coworkers assessed preoperative BNP in prediction of CSA-AKI, where addition of BNP increased AUC from 0.67 to 0.68 (LR test

p=0.01) compared to the baseline, clinical model.[7] The continuous NRI in all patients with AKI was calculated to 0.18. Of note, improved risk prediction was most prominent in controls. The modest effects seen in these single biomarker analyses may indicate that a single biomarker may not provide sufficient precision. A combination of preoperative variables and biomarkers was identified in the present study, which provided an overall better fit compared to previous studies.

Addition of multiple biomarkers to the clinical model increased the AUC from 0.81 to 0.83. It has been shown that increases in AUC are smaller if the baseline model has a high AUC.[30] The likelihood ratio test was significant, which is a better criterion when evaluating the incremental contribution of a new marker.[36, 37] Furthermore, the present data demonstrate a significant impact on reclassification of AKI patients, and a continuous NRI of 0.55 indicating a moderate-to-strong effect size. Assessment of the AUC and NRI both indicated that prediction was particularly improved in patients with intermediate AKI risk, where a purely clinical judgement may be more difficult.

Nevertheless, the focus of this study was not to design a definite prediction model for clinical use, but to assess the importance of a multimarker strategy and the combined strength of the added biomarkers. It has been recommended that studies of an explanatory character report both the explanatory and predictive qualities of the final model, so that it can be fairly evaluated in terms of its capabilities and compared to other models.[21] With the modest increase in AUC and uncertain clinical implications gained by correct reclassification, the cost-effectiveness remains to be demonstrated. The previous failure to identify effective intervention for AKI has placed continued emphasis on optimizing supportive treatment and avoiding secondary renal injury, such as watchful hemodynamic monitoring and restraining from nephrotoxins (Figure 1). However, improved understanding of underlying biology may also facilitate identification and development of novel treatment strategies.

Potential mechanisms

Preoperative cardiac and renal function are well-known predictors of CSA-AKI, where altered function may disturb their intricate regulation of cardiac output, volume status and vascular tone, affecting hemodynamic stability and end-organ perfusion.[38] Furthermore, preoperative prediction becomes complicated by individual variations in the inflammatory response.

Renal function

Preoperative renal function has been pointed out as the most important determinant of CSA-AKI.[39] Nevertheless, estimation methods for renal function are debated. In the present study, plasma cystatin C showed moderate correlation with serum creatinine. However, as opposed to earlier findings,[8] preoperative cystatin C did not enhance prediction of CSA-AKI. Reasons for these conflicting results could be that the previous study was restricted to high-risk patients, and consequently, the incidence of AKI cases was considerably larger (36%).[8] The present inclusion of consecutive cardiac surgery patients and consequent smaller frequency of AKI cases may render smaller differences in alternative measures of renal function more difficult to detect. However, estimating GFR using combined data on creatinine and cystatin C concentrations improved model fit slightly compared to creatinine-based estimates alone, indicating that these markers are indeed complementary. This complies with previous findings from Inker showing that the combined creatinine–cystatin C equation performed better than equations based on either markers alone for detection of chronic kidney disease.[20]

Cardiac function

Patients with a baseline NT-proBNP concentration >125 pg/ml showed a higher risk of CSA-AKI, despite adjusting for relevant clinical disorders. This is in accordance with previous findings,[7, 40, 41] underlining the importance of hemodynamic stress in the pathogenesis of CSA-AKI. NT-proBNP is released into the circulation in situations with volume expansion or pressure overload. Inadequate renal perfusion has traditionally been considered the most important event in the course of CSA-AKI.[39] However, there has been an increasing focus towards the importance of increased venous pressure in causing reduced renal function and a higher risk of

AKI in patients with acute or chronic heart failure.[7] The lack of association with ejection fraction in our study strengthens this view. Furthermore, the lacking association between diagnoses of chronic heart failure or NYHA class with the risk of CSA-AKI underscores the importance of a more objective marker to identify patients at risk.

Inflammation

CPB activates a systemic inflammatory response which is modulated by many factors and processes including tissue injury, blood exposure to the artificial surface of the CPB, ischemia/reperfusion injury, perioperatively administered drugs and hemodilution, as well as individual variations. In the present study, we sought to investigate the role of baseline inflammation by including key mediators of different inflammatory pathways: CRP, TCC, lactoferrin and neopterin represent activation of general inflammation, the complement system, neutrophils and macrophages, respectively. Their different roles were supported by the low correlations among them.

Neopterin emerged as an independent predictor of CSA-AKI. Neopterin has previously been associated with the risk for cardiovascular events,[42] including cardiac dysfunction following cardiac surgery.[11] The underlying mechanisms, however, are not clear. Neopterin is released from activated macrophages and monocytes. Its only weak-to-moderate correlation with CRP may suggest that neopterin more specifically acts as a marker of active atherosclerosis and aggressive cardiovascular disease in cardiac surgery patients.[42] However, neopterin may also be directly related to CSA-AKI. Neopterin increases the generation of radical oxygen species and enhances oxidative stress,[42] and may thus exacerbate the renal insult following cardiac surgery due to ischemia/reperfusion and inflammation caused by intraoperative aortic cross-clamping and CPB.

In the univariate analysis, AKI cases showed lower baseline lactoferrin concentrations. Being released from secondary granules in neutrophils, lactoferrin correlates with the amount and

activity of neutrophils. Lactoferrin possesses a range of functions, including immunomodulation and iron-binding.[43] After adjustment for clinical variables, lactoferrin emerged as a significant AKI predictor with a p-value of 0.04. However, application of bootstrapping techniques to assess the uncertainty of the sample estimates indicated that the influence of lactoferrin must be evaluated in larger datasets.

There may exist other biomarkers that were not investigated in this study. Although the design of the study does not allow us to draw conclusions about underlying mechanisms, the combination of biomarkers underscore preoperative cardio-renal function and inflammatory properties as key pathways for CSA-AKI. The pathogenic pathways may be differently weighted in each individual, highlighting the importance of evaluating multiple biomarkers with distinct pathophysiological backgrounds simultaneously, in order to improve the understanding and prediction of CSA-AKI.

In conclusion, neopterin and NT-proBNP emerged as independent preoperative predictors of CSA-AKI. Higher baseline lactoferrin concentrations may exert a protective effect on CSA-AKI, but further investigation is warranted. Inclusion of the biomarkers into a parsimonious clinical prediction model with age, gender, obesity, surgical category and preoperative renal function provided a significant increment in predictive utility for CSA-AKI. Improved prediction was especially seen in patients with intermediate AKI risk. Further studies are needed to explore whether there exist other useful biomarkers. Findings from the present study underline the importance of a multimarker approach in order to improve preoperative prediction of CSA-AKI.

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FIGURE LEGENDS

Figure 1: Clinical course of acute kidney injury (AKI). Initiation of AKI can involve hemodynamic changes in GFR, sub-clinical tubular injury, or both processes occurring simultaneously. A short time window may exist where specific therapy might reverse AKI; however, this treatment may need to be tailored to the nature of the injury and risk profile of the patient. Established AKI requires days to weeks for recovery, and the emphasis during this period should be on supportive therapy and the avoidance of secondary renal injury that may result in non-recovery of renal function or chronic kidney disease (CKD). These remain the main strategies in this patient group to date. (Figure and legend reproduced with modifications from Prowle JR. Acute kidney injury: an intensivist's perspective. Pediatr Nephrol (2014; 29:13-21.). With permission from Springer.)

Figure 2: Predictiveness curve of the combined model with clinical variables and biomarkers. The excellent goodness-of-fit is demonstrated by the agreement between the black line, showing mean predicted risk of acute kidney injury (left y-axis) along decile groups of predicted risk, and the black dots, indicating the observed risk in the respective groups. The horizontal line shows the overall incidence of acute kidney injury (9.9%). The distribution of predicted risk in the study population (right y-axis) is sketched with a grey, dashed line.

Figure 3: Comparison of the area under the receiver-operating characteristic curve (AUC) of the clinical model without biomarkers, with the model including N-terminal pro-brain natriuretic peptide (NT-proBNP), lactoferrin and neopterin.

Table 1: Overview over the origin, function and application of analyzed biomarkers

Biomarker	Origin	Indicator of	Current clinical application
C-reactive protein	Produced by hepatocytes in response to circulating interleukin-6.	Inflammation; acute-phase-reactant.	Diagnosis and monitoring of inflammatory and infectious diseases, prediction of cardiovascular disease.[14]
Neopterin	Produced by activated macrophages and monocytes.	Inflammation; cellular immune response.	Novel biomarker; suggested in monitoring of inflammatory and infectious diseases.[15]
Terminal complement complex	End product of the complement cascade; assembled by its five late components; C5b-9.	Inflammation; complement activation.	Novel biomarker; suggested in evaluation of biocompatibility of treatments such as CPB.[16]
Lactoferrin	Produced and released by neutrophils.	Inflammation; neutrophil activation.	Novel biomarker; suggested in monitoring of inflammatory and infectious diseases.[17]
NT-proBNP	Released from ventricular myocytes in response to myocardial stretch.	Hemodynamic function; diastolic heart dysfunction and volume overload.	Diagnostics of acute dyspnea[18] and monitoring of heart failure.[19]
Cystatin C	Produced by all nucleated cells at a constant rate.	Renal function; estimated glomerular filtration rate.	Estimation of kidney function and diagnosis of chronic kidney disease.[8, 20]

NT-proBNP; N-terminal pro-brain natriuretic peptide.

Table 2: Patient and perioperative characteristics

	AKI-cases (n=100)	Non-AKI cases (n=915)	*P*-value
Age (years)	74 (71-76)	67 (66-68)	<0.001
Female gender	28 (28.0%)	254 (27.8%)	0.96
Body mass index (kg/m^2)	28.3 (27.3-29.4)	26.9 (26.6-27.2)	0.004
Smoker	45 (45.0%)	513 (56.1%)	0.04
Diabetes mellitus	21 (21.0%)	121 (13.2%)	0.03
Lipid-lowering treatment	69 (69.0%)	763 (83.4%)	<0.001
Hypertension	74 (74.0%)	548 (59.9%)	0.006
ACE inhibitor use	49 (49.0%)	347 (37.9%)	0.03
Left ventricular hypertrophy	38 (38.0%)	203 (22.2%)	<0.001
Chronic cardiac failure[a]	20 (20.0%)	101 (11.0%)	0.009
Ventricular ejection fraction[b]			0.13
1) > 50%	51 (51.0%)	563 (61.5%)	
2) 31-50%	41 (41.0%)	312 (34.1%)	
3) 21-30%	7 (7.0%)	37 (4.0%)	
4) ≤ 20%	1 (1.0%)	3 (0.3%)	
NYHA class III or IV	70 (70.0%)	571 (62.4%)	0.14
Peripheral vascular disease	22 (22.0%)	131 (14.3%)	0.04
Chronic pulmonary disease	27 (27.0%)	115 (12.6%)	<0.001
Endocarditis	1 (1.0%)	2 (0.2%)	0.17
Urgent operation (within 2 weeks)	51 (51.0%)	400 (43.7%)	0.16
Previous cardiac surgery	11 (11.0%)	34 (3.7%)	0.001
Surgical risk groups			<0.001
1) Isolated CABG	35 (35.0%)	609 (66.6%)	
2) 1 procedure non-CABG	13 (13.0%)	114 (12.5%)	
3) 2 surgical procedures	44 (44.0%)	168 (18.4%)	
4) ≥ 3 surgical procedures	8 (8.0%)	24 (2.6%)	
Haemoglobin (g/dl)	13.4 (13.0-13.8)	14.1 (14.0-14.2)	<0.001
Serum creatinine (µmol/l)	98 (92-105)	80 (79-81)	<0.001
Creatinine clearance[c] (ml/min)	71 (65-77)	91 (89-93)	<0.001
Estimated glomerular filtration rate[d] (ml/min/1.73 m^2)	68 (63-73)	88 (86-89)	<0.001

CPB time (min)	117 (106-129)	76 (74-79)	<0.001
Intraoperative inotropic support	42 (42.0%)	203 (22.2%)	<0.001
Intraoperative vasoconstrictor use	97 (97.0%)	876 (96.0%)	0.61
Fluid balance intraoperatively	3048 (2825-3275)	2740 (2695-2783)	0.002
Intraoperative red blood cell transfusion	42 (42.0%)	155 (16.9%)	<0.001
Intraoperative plasma transfusion	39 (39.0%)	110 (12.0%)	<0.001
EuroSCORE II	5.5 (4.7-6.4%)	2.2 (2.1-2.4%)	<0.001
30-day mortality	5 (5.0%)	6 (0.7%)	<0.001

Continuous variables are presented as median (95% CI), categorical variables as n (%). ACE, angiotensin-converting enzyme; CABG, coronary artery bypass grafting; CPB, cardiopulmonary bypass; EuroSCORE, European Risk Stratification Score System; NYHA class, The New York Heart Association Functional Classification (I-IV). Conversion factor for serum creatinine in mg/dl to µmol/l: x 88.4.

[a]The diagnosis of chronic heart failure was based on history and clinical evaluation by an attending cardiologist.

[b]Estimated (see supplementary methods, Supplementary Material 1)

[c]Creatinine clearance calculations based on formula from Cockcroft and Gault.[33]

[d]Estimated glomerular filtration rates calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Creatinine Equation (2009).[34]

Table 3: Preoperative plasma concentrations of biomarkers and logistic regression results[a]

Biomarker	Missing (n)	Baseline serum concentrations		Step 1: Univariate biomarker analysis	Step 2: Multimarker analysis	Step 3: Adjusted multimarker analysis[d]
		AKI-cases (n=100)	Non-AKI cases (n=905)			
Lactoferrin (µg/l)[b]	2	125 (111-149)	149 (142-158)	0.05	0.04	0.05
Neopterin (nmol/l)[b]	6	10.5 (9.4-11.9)	7.4 (7.2-7.6)	<0.001	0.001	0.03
C-reactive protein (mg/l)[b]	2	5.8 (4.4-7.7)	2.5 (2.3-3.0)	<0.001	0.70	0.40
Terminal complement complex (ng/l)	3	188 (168-208)	174 (167-181)	0.47	---	---
Cystatin C (mg/l)[b]	4	1.07 (0.98-1.18)	0.85 (0.83-0.87)	<0.001	0.001	0.15
NT-proBNP (pg/ml)[c]	3	313 (230-488)	108 (99-119)	<0.001	<0.001	0.001

Two overly-influential cases were excluded. Multivariate analyses were performed on cases with complete data on all biomarkers (n=1,005).

[a]P-values at each of the three steps of the statistical analysis (see methods for further details).

[b]Natural log-transformed.

[c]Dichotomized at a cut-off value of >125 pg/ml.

[d]With adjustment for gender, age, body mass index ≥ 30 kg/m^2, surgical category and preoperative serum creatinine.

Table 4: Nested logistic regression analysis comparing the clinical model without and with biomarkers

	Clinical model			Clinical model with biomarkers		
Predictor parameters	OR	95% CI	P-value	OR	95% CI	P-value
Age (per 5 years)	1.19	1.02-1.39	0.03	1.09	0.93-1.27	0.30
Female gender	0.56	0.33-0.96	0.04	0.54	0.31-0.96	0.03
Body mass index >30 kg/m^2	2.12	1.25-3.61	0.006	2.51	1.47-4.28	0.001
Multiple surgical procedures	3.63	2.30-5.73	<0.001	3.22	1.87-5.54	<0.001
Estimated GFR[a] (per 5 ml/min per 1.73m^2)	0.84	0.79-0.89	<0.001	0.91	0.85-0.97	0.006
NT-proBNP >125 pg/ml	---	---	---	2.86	1.63-5.01	<0.001
Neopterin[b]	---	---	---	2.70	1.45-5.02	0.002
Lactoferrin[b]	---	---	---	0.70	0.47-1.04	0.08
Model evaluation parameters						
AUC (95% CI)	0.806 (0.764-0.847)			0.832 (0.791-0.873)		
Akaike information criterion	538.7			510.4		
Bayesian information criterion	568.1			554.6		

Final model parameters with bootstrapped confidence intervals (200 runs). The nested analysis was performed on cases with complete data for all biomarkers (n=1,005). AUC, area under the receiver-operating characteristic curve; CI, confidence interval; GFR, glomerular filtration rate; NT-proBNP, N-terminal pro-brain natriuretic peptide; OR, odds ratio.

[a]Calculations were based on the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine-cystatin C single equation (2012): $135\times\min(Scr/\kappa, 1)^{\alpha}\times\max(Scr/\kappa, 1)^{-0.601}\times\min(Scys/0.8, 1)^{-0.375}\times\max(Scys/0.8, 1)^{-0.711}\times0.995^{Age}$ [×0.969 if female][×1.08 if black], where Scr is serum creatinine, Scys is serum cystatin C, κ is 0.7 for females and 0.9 for males, α is −0.248 for females and −0.207 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.[20]

[b]Natural log-transformed.

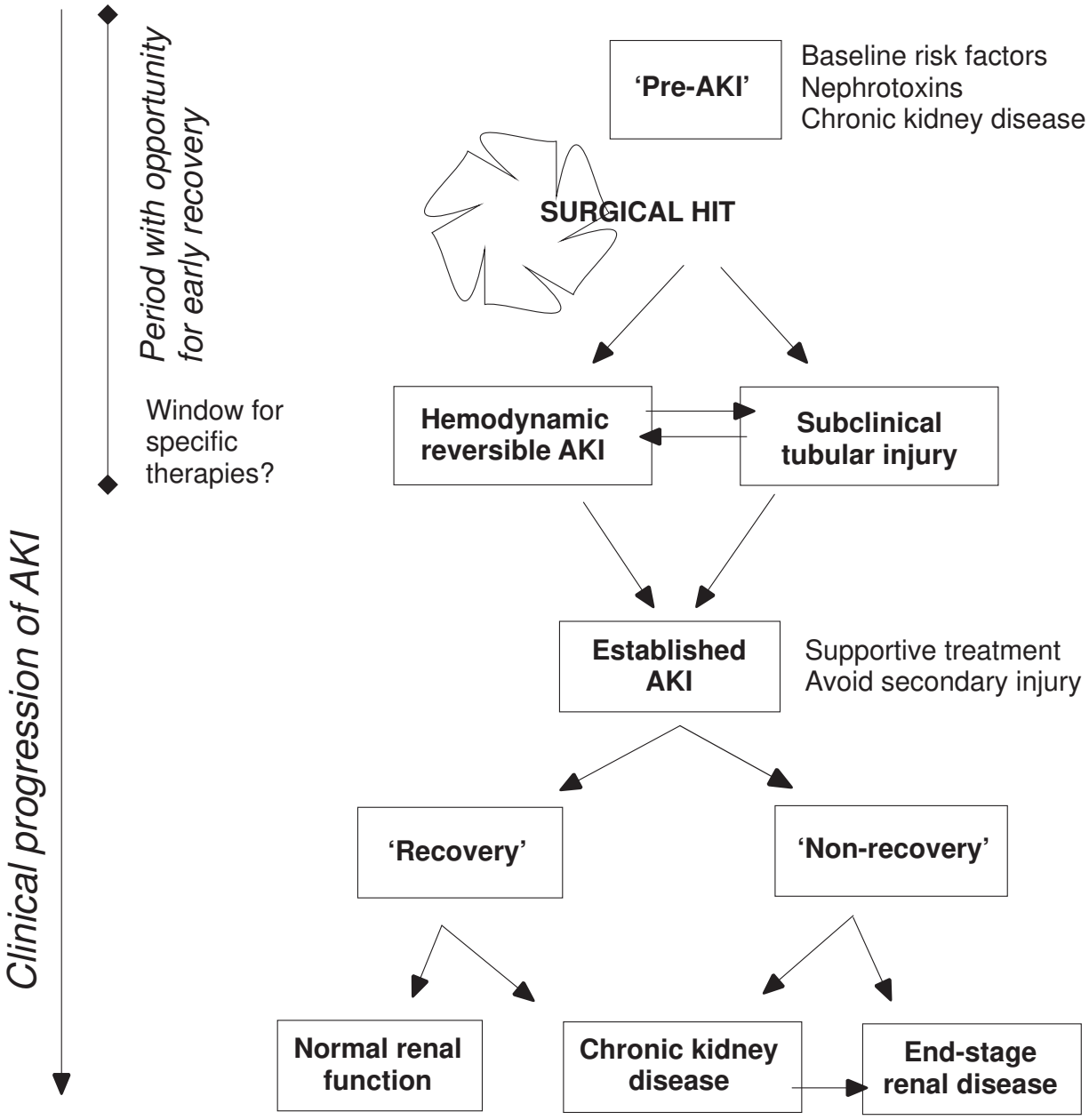
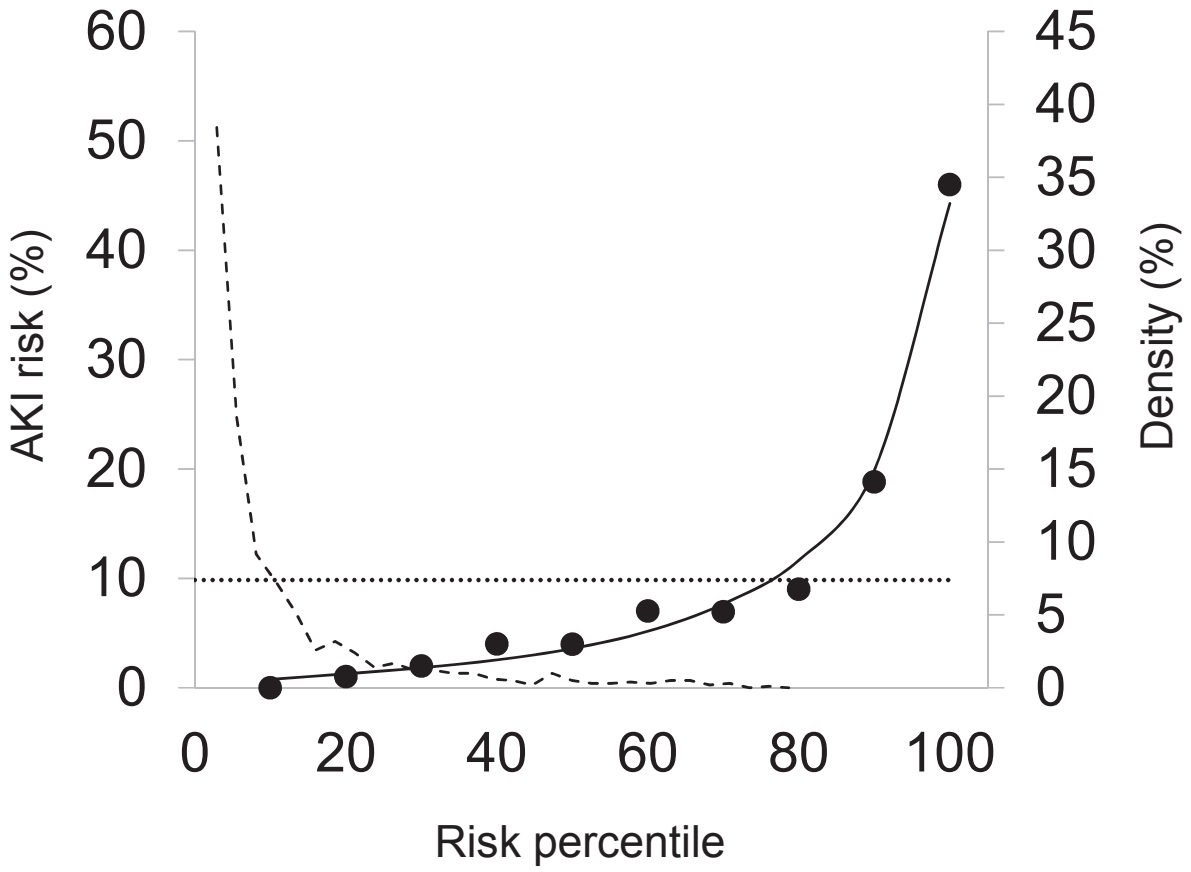

Clinical progression of AKI
Period with opportunity for early recovery
Window for specific therapies?
'Pre-AKI'
Baseline risk factors
Nephrotoxins
Chronic kidney disease
SURGICAL HIT
Hemodynamic reversible AKI
Subclinical tubular injury
Established AKI
Supportive treatment
Avoid secondary injury
'Recovery'
'Non-recovery'
Normal renal function
Chronic kidney disease
End-stage renal disease

Figure 1


60
50
40
30
20
10
0
AKI risk (%)
45
40
35
30
25
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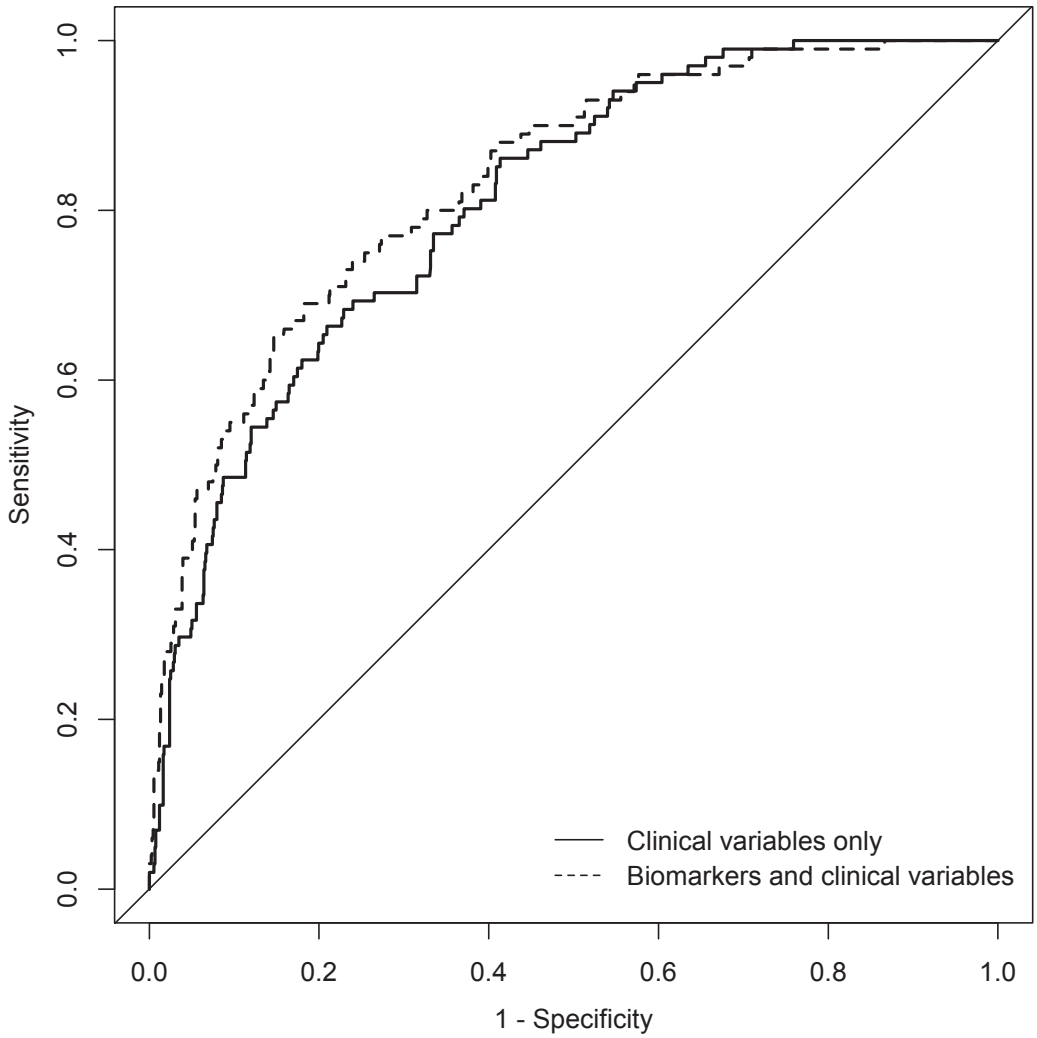
15
10
5
0
Density (%)
0
20
40
60
80
100
Risk percentile

Figure 2


Sensitivity
1 - Specificity
0.0
0.2
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0.6
0.8
1.0
Clinical variables only
Biomarkers and clinical variables

Figure 3

Supplementary Material 1: Supplementary methods

1.1 Patient selection

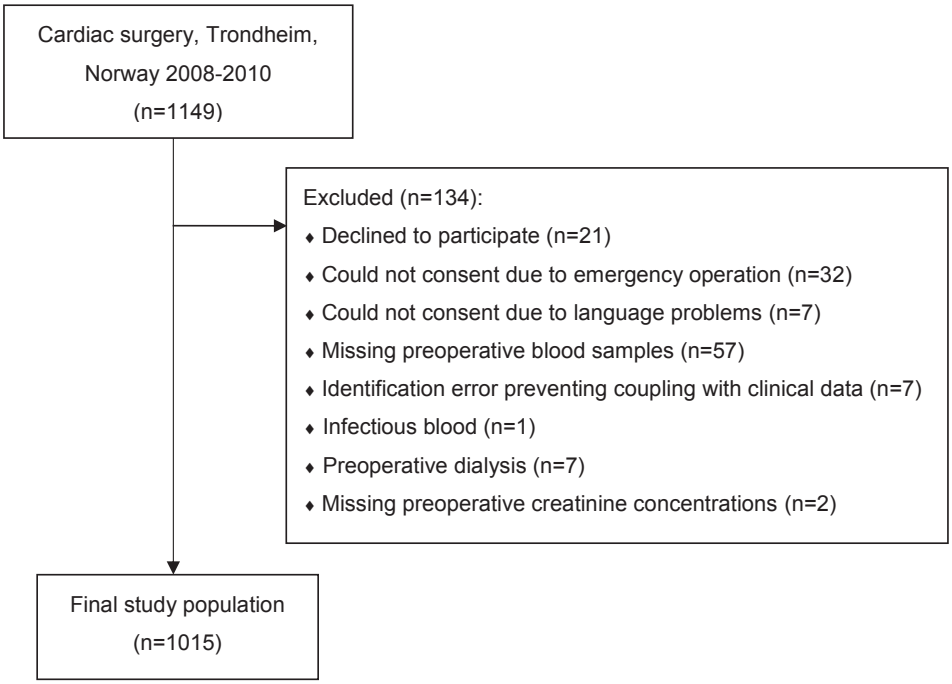

Figure S1: Patient inclusion and exclusion to the study

1.2 Measurements of ventricular ejection fraction

In patients registered into the Trondheim Heart Surgery Database, left ventricular ejection fraction (LVEF) has been determined by means of single-planed left ventricular (LV) angiography or noninvasively through two-dimensional echocardiography. Over time, echocardiography has become the main modality.

It has previously been shown that LVEF obtained by echocardiography tends to be lower compared to angiographic measurements.[1] A regression equation was derived based on patients undergoing cardiac surgery in Trondheim from 2000 through 2011 who had their LVEF measured by both methods. Estimated echocardiographic LVEFs were then calculated for those patients in the present study who underwent angiography only (n=194 (19%)). 566 patients (56%) underwent preoperative echocardiography. The remaining 255 patients (25%) had no clinical indication for LVEF measurement, and thus the LVEF was assumed to be >50%.

1.3 Selection of clinical adjustment variables

Table S1: Variables included in clinical risk models for acute kidney injury

CaSOS' CSA-AKI risk model[a]	Cleveland clinical risk score[b]	UK any-stage AKI risk calculator[c]	Novel model[d]
Age	Female gender	Age - < 60 years† - 60-74 years - ≥ 75 years	Age
BMI >30 kg/m^2	Congestive heart failure	Female gender	Female gender
Lipid-lowering treatment	Left ventricular ejection fraction <35%	BMI (kg m^{-2}) - <20.0 - 20-0-24.9 - 25.0-29.9† - 30.0-34.9 - ≥ 35.0	BMI >30 kg/m^2
Hypertension	Preoperative use of IABP	Smoking - Never smoked† - Ex-smoker - Current smoker	≥ 2 surgical procedures
Peripheral vascular disease	Chronic obstructive pulmonary disease (medically treated)	Dyspnoea - NYHA class I† - NYHA class II - NYHA class III - NYHA class IV	Preoperative creatinine
Chronic pulmonary disease	Insulin-dependent diabetes	Diabetes	
Haemoglobin concentration	Previous cardiac surgery	Peripheral vascular disease	
Preoperative creatinine - Below 100 µmol/l† - 100 to 140 µmol/l - Above 140 µmol/l	Emergency surgery	Hypertension	
Previous cardiac surgery	Surgery type - CABG only† - Valve only - CABG + Valve - Other cardiac surgeries	Haemoglobin (g/dl) - <10.0 - 12.0-11.9 - ≥ 12.0†	
Emergency surgery	Preoperative creatinine* - < 1.2 mg/dl† - 1.2-2.1 mg/dl - ≥ 2.1 mg/dl	GFR (mL/min per 1.73 m^2) - <30.0 - 30.0-59.9 - 60.0-89.9† - ≥ 90.0	
Surgery type - CABG and ASD† - AVR, AVR and CABG, non-ischaemic mitral valve surgery, aneurysm of ascending aorta - Dissection of ascending aorta, rupture of the ventricular septum - Miscellaneous		PCI prior to surgery	
		Triple vessel disease	
		Ejection fraction - Good (≥ 50%)† - Fair (30-49%) - Poor (<30%)	
		Operative priority - Elective† - Urgent - Emergency surgery	
		Surgery type - CABG only† - Valve only - CABG + Valve - Other/multiple cardiac surgeries	

Three previously described clinical models were validated in the present study cohort. This table provides an overview over the AKI predictors included in the published models, as well as the variables constituting the final clinical model used for adjustment of the biomarker analysis. ASD, atrium septum defect; AVR, aortic valve replacement; BMI, body mass index; CABG, coronary artery bypass grafting; CSA-AKI, cardiac surgery-associated acute kidney injury; IABP, intra-aortic balloon pump; GFR, glomerular filtration rate; NYHA, The New York Heart Association Functional Classification (I-IV); PCI, percutaneous coronary intervention.

[†]Defined as reference categories

*Categories correspond to <106.1 µmol/l, 106.1 µmol/l-185.64 µmol/l and >185.64 µmol/l. Conversion factor from mg/dl to µmol/l; x 88.4.

[a]**CaSOS' CSA-AKI risk model:** Berg KS, Stenseth R, Wahba A, et al.: How can we best predict acute kidney injury following cardiac surgery?: A prospective observational study. Eur J Anaesthesiol. 30:704-712, 2013.

[b]**Cleveland clinical risk score:** Thakar CV, Arrigain S, Worley S, et al.: A clinical score to predict acute renal failure after cardiac surgery. J Am Soc Nephrol. 16:162-168, 2005.

Due to incomplete registration of medical treatment for chronic obstructive lung disease, we included all patients registered with chronic obstructive lung disease. The risk score was originally developed for dialysis-dependent renal failure, but was presently applied on all patients with CSA-AKI.

[c]**UK any-stage AKI risk calculator**: Birnie K, Verheyden V, Pagano D, et al.: Predictive models for kidney disease: improving global outcomes (KDIGO) defined acute kidney injury in UK cardiac surgery. Crit Care. 18, 2014.

Information about PCI prior to surgery and triple vessel disease was not available, and were therefore excluded from the calculations.

[d]For a detailed description of novel model development, see the main article.

Model performance and model selection strategies

Model discrimination, i.e. the extent to which the model successfully separates between cases and non-cases, was evaluated by the area under the receiver-operating characteristic curve (AUC). The AUC may indicate that a model can have an excellent discriminative ability, however, it gives no information about calibration. Calibration refers to the agreement between observed and predicted outcomes and may be assessed by the Hosmer-Lemeshow test. A high p-value (>0.05) indicates appropriate calibration.

Log likelihood is an estimate of the probability of observing the data given the parameter estimates and the specified model. However, adding more terms to a model will usually improve the fit and thus lead to the acceptance of more complex models. The Akaike and Bayesian information criteria (AIC and BIC, respectively) are useful goodness-of-fit statistics to select the model in a set of candidate models giving the best balance between model fit and complexity. Both penalize for the number of estimated parameters, where BIC penalizes more for additional parameters and thus minimizes the risk of overfitting. The best model is generally the one that minimizes both AIC and BIC.

1.4 Assessing the incremental value of novel predictors

The increase in the area under the receiver operating characteristics curve (AUC), integrated discrimination improvement (IDI) and net reclassification improvement (NRI) provide complementary information and have been recommended as three parallel measures that form the "first line of assessment" in pre-screening of novel biomarkers.[2]

IDI is a measure of the separation in predicted probabilities between events and non-events. However, IDI depend on the incidence of the outcome of interest. Thus, the relative IDI (rIDI), defined as the increase in discrimination slopes divided by the slope of the old model, was calculated.[3] A rIDI ≥ the inverse number of variables in the baseline model indicates that the added predictors performed better than the average contribution of each clinical variable.

The NRI is derived by calculating the net proportion of events and non-events reclassified correctly with the new risk algorithm. Net correct reclassification for patients suffering CSA-AKI is calculated as the proportion of AKI cases in the test dataset who are correctly reclassified to a higher risk category minus the proportion of cases that are incorrectly reclassified to a lower risk category. Correspondingly, for the controls, the proportion of patient incorrectly being reclassified to higher risk categories are subtracted from the proportion being correctly reclassified to lower risk categories. The overall categorical NRI is then the sum of the net correct reclassification in cases and controls.

Use of categorical NRI should be based on risk thresholds which have clear clinical implications and are motivated on clinical grounds.[4] Pencina and colleagues have proposed that in situations with no established cut-offs, using the event rate as the default risk threshold may not be unreasonable.[5] Thus, we defined three categories of low, intermediate and high risk, corresponding to < 10%, 10-20% and > 20% predicted risk calculated from the clinical model, respectively. Additionally, we compared these results with cut-offs at < 25%, 25-50% and > 50% predicted risk, as applied by a previous study investigating the impact of preoperative cystatin C on AKI risk prediction.[6] Due to lack of clear risk thresholds for CSA-AKI, we also calculated the "continuous NRI" or NRI(>0). The continuous NRI has been recommended in situations where the primary focus is on the strength of the marker rather than model performance.[4] A continuous NRI >0.6, around 0.4 and <0.2 are considered strong, intermediate and weak, respectively.[2]

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Supplementary Material 2: Supplementary results

2.1 Correlations among variables

Table S2: Pearson's correlation coefficients amongst a) biomarkers and b) different indicators of renal function

a)

	CRP	TCC	Lactoferrin	Neopterin	Cystatin C	NT-proBNP
C-reactive protein (CRP)	1.00					
Terminal complement complex (TCC)	0.03	1.00				
Lactoferrin	-0.05	0.03	1.00			
Neopterin	0.32***	0.05	0.01	1.00		
Cystatin C	0.14***	0.09**	0.00	0.40***	1.00	
NT-proBNP	0.14***	0.01	-0.03	0.27***	0.30***	1.00

b)

	Creatinine	Creatinine clearance	Cystatin C	eGFR-cr	eGFR-cr+cys
Creatinine	1.00				
Creatinine clearance[a]	-0.51***	1.00			
Cystatin C	0.48***	-0.32***	1.00		
eGFR-cr[b]	-0.79***	0.80***	-0.47***	1.00	
eGFR-cr+cys[c]	-0.63***	0.64***	-0.85***	0.81***	1.00

*p=0.05, **p<0.01, ***p<0.001.

[a]Creatinine clearance calculated from the Cockcroft-Gault equation.[1]

[b]eGFR-cr: Estimated glomerular filtration rate calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (2009).[2]

[c]eGFR-cr+cys: Estimated glomerular filtration rate calculated with CKD-EPI creatinine-cystatin C single equation (2012): $135\times\min(Scr/\kappa, 1)^{\alpha}\times\max(Scr/\kappa, 1)^{-0.601}\times\min(Scys/0.8, 1)^{-0.375}\times\max(Scys/0.8, 1)^{-0.711}\times0.995^{Age}$ [×0.969 if female][×1.08 if black], where Scr is serum creatinine, Scys is serum cystatin C, κ is 0.7 for females and 0.9 for males, α is −0.248 for females and −0.207 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.[3]

2.2 Alternative multivariate models

Table S3: Comparison of AKI risk model performance in patients undergoing cardiac surgery

	CaSOS' CSA-AKI risk model		**Cleveland clinical risk score**		**UK any stage AKI calculator**	**Novel model**
	Calculated predicted probability (%)	Predictor variables	Calculated risk score (range 0-17)	Predictor variables	Calculated predicted probability (%)	Predictor variables
a) Model statistics						
Degrees of freedom	2	13	2	12	2	6
Log likelihood	-274.7	-259.7	-284.2	-276.8	-275.8	-264.3
AIC	553.5	545.3	572.5	577.6	555.6	540.7
BIC	563.3	609.2	582.3	636.5	565.4	570.2
AUC	0.802	0.816	0.745	0.772	0.778	0.805
(95% CI)	(0.759-0.845)	(0.775-0.858)	(0.694-0.796)	(0.721-0.823)	(0.729-0.827)	(0.763-0.847)
Hosmer-Lemeshow test	0.004	0.02	0.40	0.56	0.11	0.46
b) Biomarker evaluation	Level of significance					
Lactoferrin[a]	0.15	0.10	0.12	0.08	0.06	**0.05**
NT-proBNP[b]	**<0.001**	**0.002**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Neopterin[a]	0.12	**0.03**	**0.02**	**0.004**	0.07	**0.04**
C-reactive protein[a]	0.43	0.80	0.41	0.57	0.75	0.38
Cystatin C[a]	0.11	0.12	**0.03**	**0.04**	0.09	0.13

a) Summary of the performance of the clinical risk models and model comparison statistics. b) Summary of the multimarker analysis for the biomarkers when adjusted with each of the clinical models. Significant biomarkers are shown in bold.

Clinical models were compared in 1,004 patients with complete data on all variables for all models. Comparison of models including biomarkers was performed on n=996, after exclusion of 8 patients with incomplete biomarker data. The CaSOS' CSA-AKI model, Cleveland clinical risk score and UK any stage AKI calculator were tested by adding the calculated risk score as a continuous variable in a multivariate model with the biomarkers. Additionally, we tested the alternative strategy using the variables described by the CaSOS' CSA-AKI model and Cleveland clinical risk score in new model versions with recalculated model coefficients.

AIC, Akaike information criterion; AUC, area under the receiver-operating characteristic curve; BIC, Bayesian information criterion; HL test, Hosmer-Lemeshow test; NT-proBNP, N-terminal pro-brain natriuretic peptide.

[a]Natural log-transformed. [b]Dichotomized with a cut-off >125 pg/ml

2.3 Alternative indicators of renal function

Table S4: Comparison of novel AKI risk prediction models with different indicators of renal function

Predictor variable	**Odd ratios with level of significance**			
	Creatinine	Creatinine clearance	eGFR-cr	eGFR-cr+cys
Female gender	0.64	.54*	.55*	.53*
Age (per 5 years)	1.12	1.04	1.05	1.06
Body mass index >30 kg/m^2	2.68***	3.21***	2.58***	2.48***
Multiple surgical procedures	3.46***	3.42***	3.33***	3.36***
NT-proBNP >125 pg/ml	2.90***	2.90***	2.87***	2.82***
Neopterin[a]	2.87***	3.33***	2.62**	2.59**
Lactoferrin[a]	.67*	.68*	.69*	.68*
Serum creatinine (per 10 µmol/l)	1.08	-	-	-
Creatinine clearance[b] (ml/min)	-	0.99	-	-
eGFR-cr[c] (ml/min per 1.73 m^2)	-	-	.98*	-
eGFR-cr+cys[d] (ml/min per 1.73 m^2)	-	-	-	.91**
Model comparison estimates				
Log likelihood	-251.8	-252.9	-250.8	-250.0
Akaikes information criterion	521.6	523.9	519.7	518.0
Bayes information criterion	565.9	568.1	563.9	562.2

*0.05, **<0.01, ***<0.001. NT-proBNP, N-terminal pro-brain natriuretic peptide.

[a]Natural log-transformed.

[b]Calculated from the Cockcroft-Gault equation.[1]

[c]eGFR-cr: Estimated glomerular filtration rate calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine equation (2009).[2]

[d]eGFR-cr+cys: Estimated glomerular filtration rate calculated with the CKD-EPI creatinine-cystatin C equation (2012).[3]

As shown by the model comparison statistics, there were only small differences. Parameter estimates for novel biomarkers were consistent independently of how renal function was modelled. However, the model using estimated GFR based on both creatinine and cystatin C showed somewhat better performance and was thus used as the final model for clinical adjustment of the multivariate biomarker analysis.

2.4 The incremental value of added biomarkers

The AUC difference indicated a marginally significant improved discrimination (p=0.05). However, AUC comparison is considered a conservative measure, and it is considered especially difficult to improve baseline models with large AUCs.[4] Nevertheless, there was a significant improvement in the ability to separate between AKI events and non-events (IDI=0.06 (0.03-0.08), p<0.001). The calculated relative IDI of 0.37 indicates that the incremental contribution by the biomarkers was higher than the average contribution from each clinical variable (1/5=0.20).

The continuous NRI was 0.55 (0.34-0.75). The categorical NRI showed a net reclassification of 12% (p=0.05), where a net of 10% of AKI cases were correctly reclassified to higher risk categories (Table 4). Inclusion of biomarker data had the largest impact in the intermediate risk group (predicted risk 10-20%, n=175), where a net of 27% and 29% of AKI and non-AKI cases were reclassified into higher and lower risk categories, respectively. The overall NRI in the intermediate group was therefore 56%.

A similar analysis for risk categories at cut-offs 25% and 50% resulted in a NRI of 23% (p<0.001). The estimates were of greater magnitudes, but fewer patients reached the higher risk categories. A net of 24% of AKI cases were correctly reclassified to higher risk categories (Table 4). Inclusion of biomarker data had the largest impact in the intermediate risk group (predicted risk 25-50%, n=68)). From 23 AKI cases, a net of 9 patients were correctly reclassified to a higher risk group (12-3, 39%), whereas a net of 9 from 45 non-AKI cases were correctly reclassified to a lower risk category (15-4, 20%). Thus, there was an overall NRI of 59% in the intermediate risk group using these alternative cut-offs.

Table S5: Comparison of risk classification for AKI based on clinical variables only and combined biomarkers and clinical variables.

A) Main analysis

		Risk classification with biomarkers and clinical variables			
		< 10% risk	10-20% risk	> 20% risk	Total
	Risk classification with clinical variables only				
AKI	< 10% risk	20	**8**	**2**	*30*
cases	10-20% risk	*5*	6	**11**	*22*
	> 20% risk	*2*	*4*	42	*48*
	Total	27	18	55	100
Non-AKI	< 10% risk	614	*52*	*4*	670
cases	10-20% risk	**71**	56	*26*	153
	> 20% risk	**5**	**20**	59	84

	Total	690	128	89	907
B) Sensitivity analysis					
		Risk classification with biomarkers and clinical variables			
		< 25% risk	25-50% risk	> 50% risk	Total
	Risk classification with clinical variables only				
AKI cases	< 25% risk	49	**18**	**0**	67
	25-50% risk	*3*	8	**12**	23
	> 50% risk	*0*	*3*	7	10
	Total	52	29	19	100
Non-AKI cases	< 25% risk	827	*24*	*0*	851
	25-50% risk	**15**	26	*4*	45
	> 50% risk	**0**	**3**	8	11
	Total	842	53	12	907

Reclassification tables when using cut-offs at A) 10% and 20% predicted AKI risk; and B) 25% and 50% predicted AKI risk. Values represent number of patients (n). Correct reclassification is indicated with bold; incorrect reclassification in italics. AKI, acute kidney injury.

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Paper 3

PLOS | ONE

RESEARCH ARTICLE

Reduced Long-Term Relative Survival in Females and Younger Adults Undergoing Cardiac Surgery: A Prospective Cohort Study

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Abstract

Objectives

To assess long-term survival and mortality in adult cardiac surgery patients.

Methods

8,564 consecutive patients undergoing cardiac surgery in Trondheim, Norway from 2000 until censoring 31.12.2014 were prospectively followed. Observed long-term mortality following surgery was compared to the expected mortality in the Norwegian population, matched on gender, age and calendar year. This enabled assessment of relative survival (observed/expected survival rates) and relative mortality (observed/expected deaths). Long-term mortality was compared across gender, age and surgical procedure. Predictors of reduced survival were assessed with multivariate analyses of observed and relative mortality.

Results

During follow-up (median 6.4 years), 2,044 patients (23.9%) died. The observed 30-day, 1-, 3- and 5-year mortality rates were 2.2%, 4.4%, 8.2% and 13.8%, respectively, and remained constant throughout the study period. Comparing observed mortality to that expected in a matched sample from the general population, patients undergoing cardiac surgery showed excellent survival throughout the first seven years of follow-up (relative survival ≥ 1). Subsequently, survival decreased, which was more pronounced in females and patients undergoing other procedures than isolated coronary artery bypass grafting (CABG). Relative mortality was higher in younger age groups, females and patients undergoing aortic valve replacement (AVR). The female survival advantage in the general

Competing Interests: The authors have declared that no competing interests exist.

population was obliterated (relative mortality ratio (RMR) 1.35 (1.19–1.54), p<0.001). Increasing observed long-term mortality seen with ageing was due to population risk, and younger age was independently associated with increased relative mortality (RMR per 5 years 0.81 (0.79–0.84), p<0.001)).

Conclusions

Cardiac surgery patients showed comparable survival to that expected in the general Norwegian population, underlining the benefits of cardiac surgery in appropriately selected patients. The beneficial effect lasted shorter in younger patients, females and patients undergoing AVR or other procedures than isolated CABG. Thus, the study identified three groups that need increased attention for further improvement of outcomes.

Introduction

Factors associated with mortality following adult cardiac surgery can be patient- or procedure-related [1]. During the last decades, there has been a consistent focus on improving surgical techniques, pre- and postoperative care, resulting in reduced operative mortality [2, 3]. However, parallel to therapeutic advances, life expectancy in industrialized countries increases; people tend to get older and have increased comorbidity and more health issues when being referred to cardiac surgery [4]. Thus, it is desirable to obtain information on late mortality in order to capture whether there is a sustained mortality reduction following cardiac surgery.

Recent studies have provided reports of all-cause mortality and conveyed information on potential predictors of long-term mortality following cardiac surgery [2, 5–12]. Age has consistently emerged as the most important risk factor. However, long-term mortality in cardiac surgery patients must be seen in context with the background mortality in the general population. Reports from the 1980–90s adopted relative survival analysis in order to assess the excess and relative mortality associated with cardiac disease in operated patients [13–18]. Since then, techniques and equipment have evolved, and the introduction of endovascular- and catheter-based methods together with changing patient demographics might have influenced the target population for cardiac surgery.

Even though cardiac surgery has shown improved short-term outcomes over the last decades, it is seldom curative. Patients undergoing cardiac surgery suffer from severe cardiac disease and usually have several cardiovascular risk factors and co-existing comorbidities. We therefore hypothesized that long-term survival following cardiac surgery has remained unchanged. The aim of this study was to analyse observed and relative long-term survival in patients who underwent cardiac surgery in Trondheim, Norway, from 2000 through 2014. We have explored potential prognostic factors for long-term mortality for a follow-up period of up to 14 years, with special focus on the effects of age, gender and surgical procedure.

Methods

Trondheim Heart Surgery Database

Since 1992, adult patients undergoing cardiac surgery in Trondheim have been registered consecutively into the Trondheim Heart Surgery Database as part of the local quality-assurance work. Patient- and procedure-related preoperative characteristics, intraoperative and postoperative events and factors, as well as laboratory values have been registered prospectively. The

present study was part of the Cardiac Surgery Outcome Study (CaSOS), which has used the database as a foundation for investigating different complications following adult cardiac surgery. Previously published investigations include risk assessment for prolonged postoperative ventilation [19], increased length of stay in the intensive care unit [20], postoperative heart dysfunction [21, 22], short-term mortality [23], postoperative fluid overload [24], postoperative acute kidney injury [25] and postoperative bleeding complications [26, 27]. CaSOS was approved by the Norwegian Data Inspectorate and the Regional Research Ethics Committee in Medicine (project number 4.2007.1528), Trondheim, Norway on 27 June 2007. The need for informed consent was waived up to April 2008, after which all patients have given their informed consent.

The present part of CaSOS was based on consecutive patients who underwent cardiac surgery in Trondheim, Norway between 1.1.2000 and 31.12.2014. Only the first entry into the data registry during the study period was used for the survival analyses. Patients undergoing off-pump coronary artery bypass (n = 130), transcatheter aortic valve insertion (TAVI, n = 109) and surgery for a thoraco-abdominal aortic aneurysm (n = 22) were excluded from the CaSOS database. As remaining cardiac surgery patients still comprise a heterogeneous group, a subgroup analysis was performed where patients undergoing isolated coronary artery bypass grafting (CABG, n = 5,648), isolated aortic valve replacement (AVR, n = 726) or combined AVR and CABG (n = 829) were compared.

Endpoint

Data on cause and date of death through December 2014 were obtained through linkage to the Norwegian Cause of Death Registry. Causes of death were provided according to the International Statistical Classification of Diseases and Related Health Problems, 10th Revision (ICD-10) [28]. The unique national registration numbers assigned to each Norwegian citizen enabled accurate linkage. 42 (<0.05%) temporary residents could not be coupled to the Cause of Death Registry, leaving 8,564 patients for the analysis. The primary endpoint of this study was all-cause mortality, referred to as observed long-term mortality. The secondary endpoint was to explore mortality specifically seen in cardiac surgery patients, estimated as relative long-term mortality (see below).

Statistical analysis

Temporal trends were analysed across year of surgery continuously as well as categorized into 3 time periods (2000–2004, 2005–2009 and 2010–2014). Unless otherwise specified, categorical variables are described as n (%); continuous variables as median (95% confidence intervals (CI)). Differences across time periods were tested using the χ^2 and Kruskal-Wallis tests for categorical and continuous data, respectively. Changes in mortality rates during the study period were assessed with a chi-square test for departure from the trend line [29]. P-values <0.05 were considered significant. All statistical analyses were performed with Stata (version 13.1, StataCorp LP, Lakeway Drive, USA), Minitab 17 (Minitab Ltd, Coventry, UK) and R (version 3.2.2 64x, R Foundation, http://www.r-project.org).

Observed survival and mortality. Observed cumulative survival and hazard rates were calculated using the Kaplan-Meier and Nelson-Aalen estimators, respectively. Univariate survival analyses, with time since operation as the time variable and death (no/yes) as the event, were performed with the Kaplan-Meier method and log-rank test. Different interventions were compared pairwise while correcting for multiple comparisons using the Bonferroni method. As a sensitivity analysis, calculations were repeated for inclusion up to 2006 or 2010, as well as when using cardiovascular death (ICD-10 chapter IX, block I00-I99) as the outcome variable.

Relative survival and mortality. Long-term survival and mortality in cardiac surgery patients must be seen in context with that expected in the general population. Relative survival was calculated as the ratio between the observed and expected survival rates [30] and presented graphically over follow-up time. For the complete follow-up time, relative mortality was calculated as the ratio between the observed and expected number of deaths (multiplicative hazard model), providing so-called standardized mortality ratios (SMR). Expected survival and mortality rates were calculated from lifetables compiled from the Norwegian population stratified on age, sex and calendar year, obtained from the Human Mortality Database [31]. Subgroup analyses were performed across gender and pre-defined age groups ($<$60 years, 60–69 years, 70–79 years and $\geq$ 80 years).

Predictors of observed and excess mortality. Previous studies have pointed out differences in predictors of short- and long-term mortality [6]. Thus, after estimating observed and relative mortality rates, patients who died within 30 days postoperatively, classified as short-term mortality, were excluded from the analysis of prognostic factors.

Potential predictors of observed mortality were investigated using multivariate Cox proportional hazards (PH) modelling. The selection of candidate predictor variables was guided by clinical knowledge and literature, a method recommended to avoid overfitting and confounders as found with selection based on univariate analyses [32]. General demographics (age, gender, body mass index), procedure-related factors (surgical procedure, redo-operation, emergency level), comorbidity and smoking (never/former vs. current smoker) were included into the models block-wise. Surgical procedures were categorized in accordance with EuroScore II's definition into isolated CABG, 1 non-CABG procedure, 2 surgical procedures or $\geq$ 3 surgical procedures, where isolated CABG was defined as the reference category [3]. As cardiac surgery patients still constitute a heterogeneous group, a sensitivity analysis was performed by including patients only undergoing CABG and/or AVR.

A secondary analysis was performed to further investigate female gender as a risk factor for long-term mortality. Gender differences in preoperative risk factors were compared with the Mann-Whitney U-test or χ^2 test for continuous and categorical variables, respectively. Thereafter, a balancing propensity score was developed using logistic regression with gender as the outcome, including the following explanatory variables: Age, body mass index, smoking status, diabetes, hypertension, preoperative history of atrial fibrillation, peripheral vascular disease, chronic pulmonary disease, previous myocardial infarction hypertension, left ventricular hypertrophy, NYHA functional class, diagnosis of chronic heart failure, kidney disease, preoperative serum creatinine, use of beta-antagonists, statins or diuretics before scheduled for surgery, previous cardiac surgery, emergency level of operation, acute preoperative heart failure, and type of surgical procedure. We used 1:1 greedy matching with a calliper width 0.25*the standard deviation of the propensity score to form female-male pairs. Covariate balance was evaluated using standardized differences, where an absolute standardized difference in the covariate mean for a variable $\leq$ 10% indicated acceptable balance. Analyses were performed using boost [33] and psmatch2 [34] programs in Stata. Following adequate balance of preoperative risk factors, Cox PH modelling for all-cause and cardiovascular mortality was repeated in the matched dataset.

Deviations from the proportionality assumption were assessed graphically and by inclusion of interaction terms between the predictors and time. Separate parameter estimates for pre-specified time periods ($<$1, 1–5 and $>$5 years) were compared in order to assess time-dependent effects. Model fit and complexity were compared using log likelihood, the Bayesian and Akaike information criterions (BIC and AIC, respectively). The likelihood ratio test was used to guide final model selection. Goodness-of-fit was evaluated with Harrell's concordance (C)

statistic and Somer's D correlation coefficient, both measures of the concordance of ranked predicted and observed outcomes [35].

In order to evaluate factors associated with long-term relative mortality, we applied multiplicative modelling of relative mortality as described by Pohar *et al*. [36, 37] using the relsurv package in R [38]. Goodness-of-fit was tested by means of the Brownian Bridge process. Differences in relative mortality between patients with different covariate levels are expressed as relative mortality ratios (RMR).

Results

Temporal trend analysis

Of the 8,564 patients undergoing cardiac surgery in Trondheim from 2000 through 2014, 2,211 (25.8%) were female. The mean yearly number of patients who underwent cardiac surgery was 571 (minimum 486-maximum 671). The annual reduction of total cases was reflected by a steady decline in the number of isolated CABG performed, from 470 (74.5% of yearly procedures) in 2000 to 294 (58.1%) in 2014 (Fig 1A). The reduction in CABG was not compensated by an increase in other procedures.

Median age at the time of surgery was 67.8 years and was constant during follow-up ($p = 0.25$). The distribution of age across the study period is visualized in Fig 1B. Females were significantly older compared to men (71.7 years versus 66.3 years, $p<0.001$). Further gender differences are summarized in S1 Table.

A detailed comparison of preoperative, surgical and postoperative factors across the study period is provided in S2 Table. Median age, the proportion of females, smokers and acute surgeries remained constant. Patients admitted to cardiac surgery during more recent years presented with better renal function. However, patients tended to present with more comorbid diseases, such as diabetes (11.9–14.8–15.3% for 2000–2004, 2005–2009 and 2010–2014, respectively, $p<0.001$) and chronic obstructive lung disease (14.2–13.4–19.2%, $p<0.001$). More patients presented with acute cardiac insufficiency requiring either ionotropic therapy or intra-aortic balloon pump before surgery (0.6–0.9–1.6%, $p = 0.001$). There was an increasing proportion of patients scheduled for urgent surgery (within 2 weeks) during more recent years (39.7–41.2–43.3%, $p = 0.02$).

There was a marked increase in the proportion of patients receiving intraoperative red cell transfusion (13.9–18.8–23.9%, $p<0.001$), inotropic support (24.3–23.5–30.2%, $p<0.001$) and vasoconstrictor therapy (67.8–92.8–97.4%, $p<0.001$) intraoperatively. Median duration of cardiopulmonary bypass increased steadily over the study period (72-79-85 minutes, $p<0.001$). Nevertheless, the incidence of postoperative complications remained unchanged, and there was a reduction in the duration of postoperative hospital stay (7-6-5 days, $p<0.001$).

Mortality following cardiac surgery

The median time to censoring was 6.4 years with a maximum of 14.99 years. A total of 2,044 patients (23.9%) died, corresponding to an observed mortality rate of 22.5% and 28.0% for males and females, respectively ($p<0.001$). Of the patients who survived the first 30 days postoperatively but died within the follow-up time, 47.0% (men: 45.9%, females: 49.5%), were officially classified as suffering a cardiovascular death, as opposed to 92.4% of the patients who died within 30 days postoperatively ($n = 184$). The overall mean survival time was 11.6 years (95% CI 11.5–11.7).

Observed mortality. The overall observed 30-day, 1-, 3- and 5-year mortality rates were 2.2%, 4.4%, 8.2% and 13.8%, respectively (Fig 1C). There were no significant changes across follow-up year ($p = 0.45$, $p = 0.78$, $p = 0.33$ and $p = 0.88$, respectively). Conversely, the observed

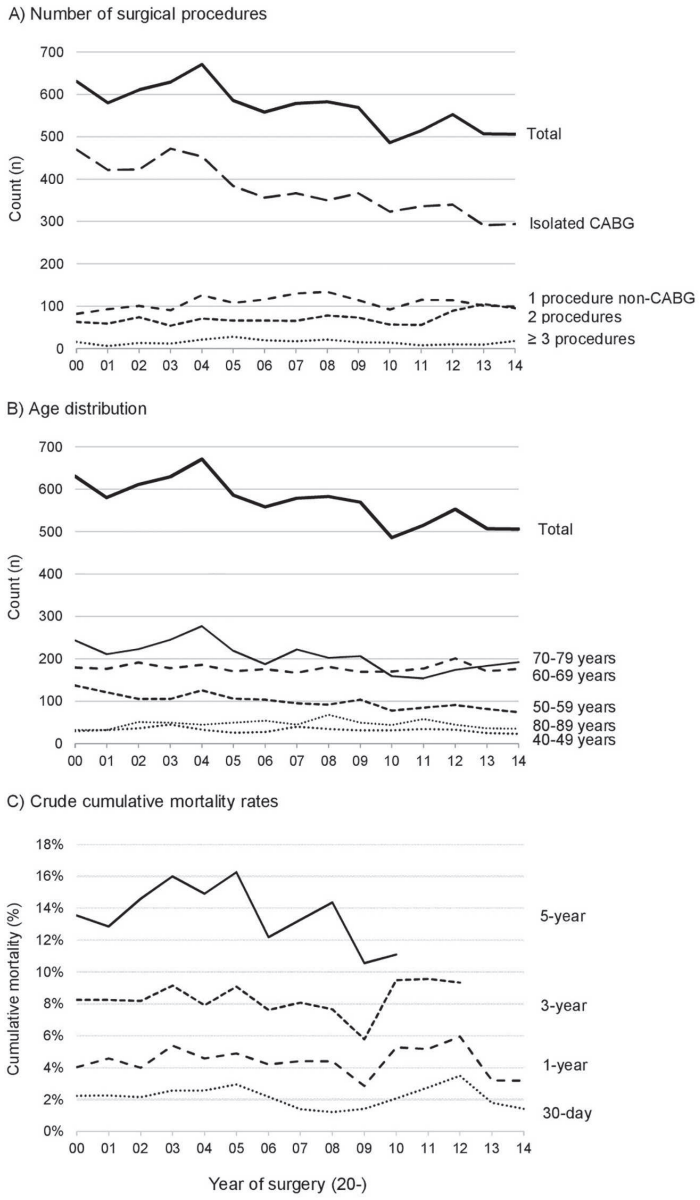

Fig 1. Overview over cardiac operations performed at the Department of Cardiothoracic Surgery, Trondheim, Norway, from 2000 through 2014. A) Stratified on the surgical procedure(s) performed. B) Stratified on age at operation day. For A and B, the total number of procedures is given as reference. C) Cumulative observed mortality rates 30 days, 1, 3 and 5 years following surgery.

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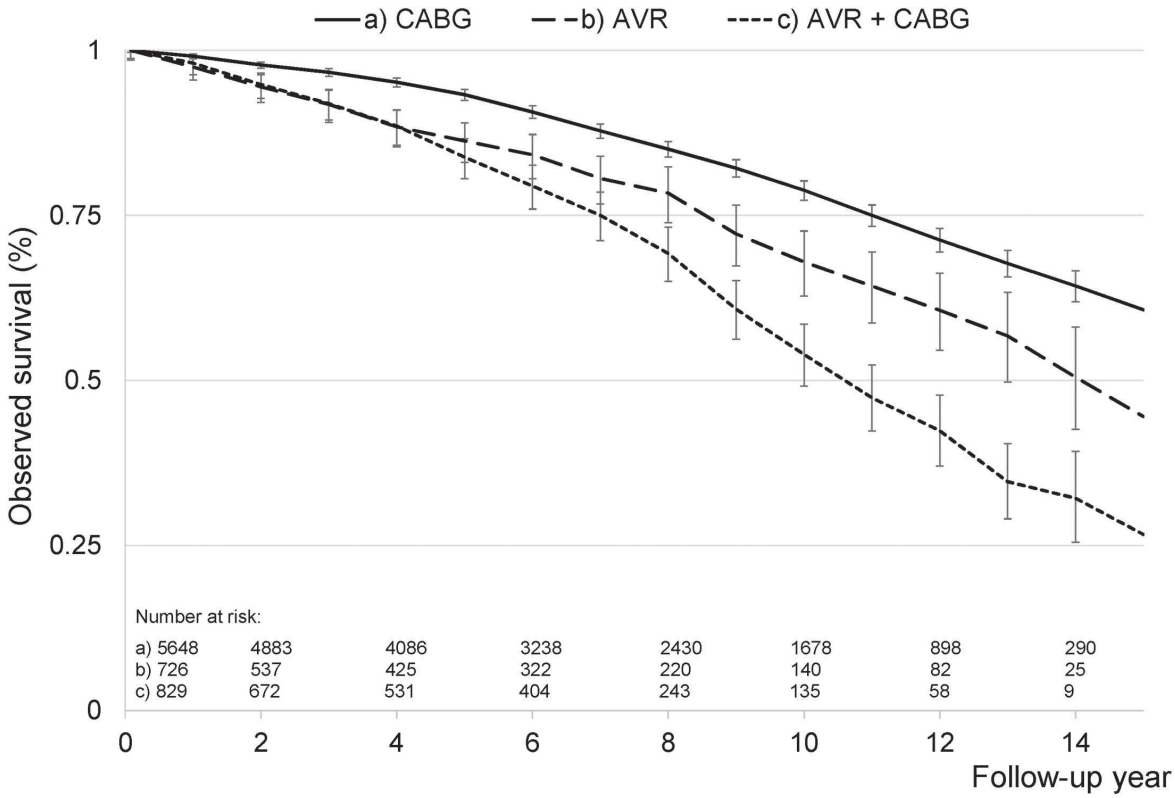

Fig 2. Long-term observed survival. Unadjusted Kaplan-Meier survival curves for patients undergoing coronary artery bypass grafting (CABG) and/or aortic valve replacement (AVR). The number at risk (n) at the start of even follow-up years are provided.

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survival rates calculated by the Kaplan-Meier method were 95.7%, 86.9% and 69.3% after 1, 5 and 10 years, respectively, and differed significantly amongst different surgical interventions as classified by EuroSCORE II ($p<0.001$, S1 Fig). Similarly, patients undergoing AVR and combined AVR and CABG showed significant differences compared to isolated CABG (Fig 2). Despite a linear increase in observed long-term mortality across patients undergoing isolated CABG, isolated AVR and combined AVR and CABG (HR 1.00, HR 1.39 (1.17–1.64) and 1.59 (1.39–1.82), respectively), observed mortality in AVR-patients undergoing concomitant CABG did not differ significantly from that of isolated AVR ($p = 0.48$). Comparable trends were seen when using inclusion of patients up to 2006 and 2010, as well as when using cardiovascular death as the outcome variable.

Relative survival. When adjusting for the expected survival in a similar subset of the general Norwegian population, the 1-, 5- and 10-year relative survival rates were estimated to 97.8%, 98.8% and 94.9%, respectively. However, when excluding patients who died within 30 days postoperatively (n = 184), there was a survival benefit in cardiac surgery patients compared to the reference population: Observed survival during the first four years of follow-up was higher than expected survival (relative survival >1, Fig 3A). Survival during the three subsequent years was comparable to that of the background population (relative survival = 1).

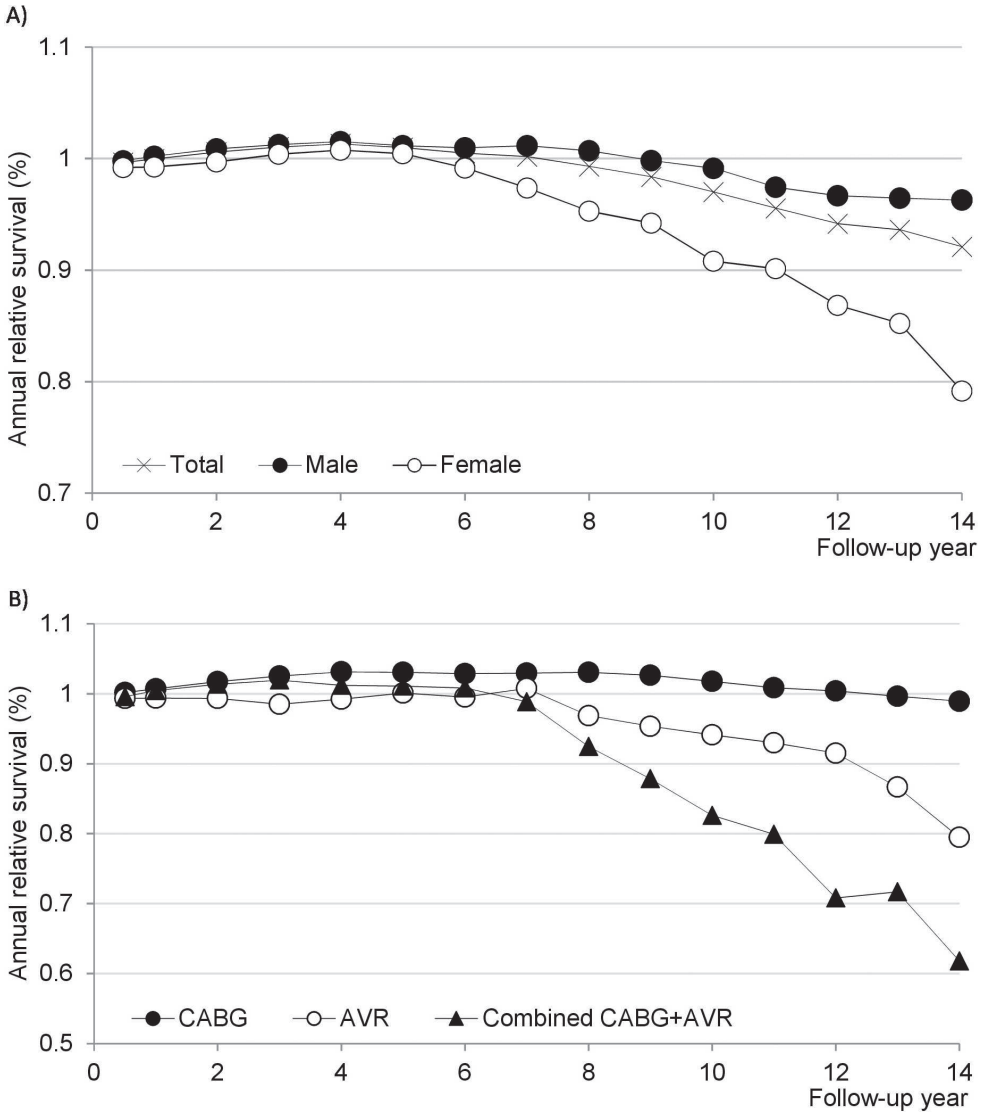

Fig 3. Annual relative survival amongst cardiac surgery patients surviving the first 30 postoperative days. A) Shown in total (n = 8,380) and separately for males (n = 6,244) and females (n = 2,136). B) Shown for patients undergoing isolated coronary artery bypass grafting (CABG, n = 5,593), isolated aortic valve replacement (AVR, n = 699) and combined CABG and AVR (n = 809). A relative survival >1 indicates a survival advantage in the study cohort.

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Overall relative survival decreased from the eighth year and onwards; however, the reduction in survival started earlier and was greater amongst females.

Isolated CABG, AVR and combined AVR and CABG showed similar relative survival from the first throughout the seventh year of follow-up (Fig 3B). However, from the eighth year of follow up, survival was most reduced for combined AVR and CABG, moderately reduced for

isolated AVR, whereas relative survival remained >1 for isolated CABG. After the tenth year of follow up, the numbers at risk were too small for statistical analysis.

Relative mortality. When comparing the overall observed and expected number of deaths in patients who were still alive after 30 postoperative days, we found that patients undergoing cardiac surgery from 2000 through 2014 did not have significantly different mortality compared to the general population (overall SMR 1.02, 95% CI 0.97–1.06, Table 1). However, subgroup analyses showed that females (SMR 1.17, 95% CI 1.07–1.27) and patients aged <70 years (SMR 1.77, 95% CI 1.52–2.04 for <60 years and SMR 1.17, 95% CI 1.06–1.29 for 60–69 years) had a significantly higher relative mortality when adjusting for background mortality. For men, patients aged >70 years showed a survival benefit. There was a trend that females aged ≥ 80 years may have a similar survival advantage, however, the number of cases in this age group was small.

Furthermore, stratification by surgical procedure showed that patients undergoing AVR, both isolated and with concomitant CABG, had a higher relative mortality compared to isolated CABG ($p<0.001$ for both). The findings remained comparable when adjusting for intergroup differences in age and sex distributions. Concomitant CABG in AVR-patients was not associated with a higher relative mortality ($p = 0.24$).

Risk factor analysis. The multivariate Cox PH model showed that observed mortality increased with age (HR per 5 years 1.46 (1.41–1.50), $p<0.001$). Gender was not an independent predictor of observed long-term mortality following cardiac surgery ($p = 0.09$). However, following propensity score matching, resulting in standardized differences ≤ 9% for all covariates (S2 Fig), gender emerged as a predictor of observed mortality (HR = 0.81, 95% CI (0.70–0.93), $p = 0.004$) in the matched 1,493 pairs of females and males (n = 2,986). This association was not reproduced when evaluating cardiovascular mortality ($p = 0.43$).

Pre-existing chronic heart failure, chronic pulmonary disease, preoperative serum creatinine concentrations >140 μmol/L, peripheral vascular disease, diabetes and current tobacco smoking were associated with increased risk of long-term mortality (Table 2). Mortality increased linearly with increased complexity and number of procedures performed. There were non-proportional hazards over time, as shown by fitting piecewise hazard ratios (Table 2). The type of surgical procedure, co-existing chronic pulmonary disease and reduced kidney function seemed to play more dominant roles during the first year. The predictor estimates remained

Table 1. Standardized mortality ratios stratified on gender and age group (n = 8,380).

Age group	Total					Males				Females			
	Number at risk (n)	Observed deaths (n)	Expected deaths (n)	SMR	95% CI	Observed deaths (n)	Expected deaths (n)	SMR	95% CI	Observed deaths (n)	Expected deaths (n)	SMR	95% CI
< 60 years	2,081 (24.8%)	179	101	1.77	(1.52–2.04)	152	91	1.67	(1.42–1.96)	27	10	2.61	(1.79–3.81)
60–69 years	2,636 (31.5%)	394	337	1.17	(1.06–1.29)	314	287	1.09	(0.98–1.22)	80	50	1.61	(1.29–2.00)
70–79 years	2,999 (35.8%)	995	998	1.00	(0.94–1.06)	676	752	0.90	(0.83–0.97)	319	246	1.30	(1.16–1.45)
≥ 80 years	664 (7.9%)	292	392	0.74	(0.66–0.84)	175	234	0.75	(0.65–0.87)	117	158	0.74	(0.62–0.89)
Total	8,380	1,860	1,828	1.02	(0.97–1.06)	1,317	1,364	0.97	(0.91–1.02)	543	464	1.17	(1.07–1.27)

CI; confidence interval, SMR; standardized mortality ratio.

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Table 2. Risk factors associated with observed mortality.

Predictor	Time period							
	Complete follow-up		≤ 1 year		1–5 years		> 5 years	
	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)
Age per 5 years	1.46	(1.41–1.50)***	1.25	(1.14–1.36)***	1.37	(1.30–1.44)***	1.53	(1.46–1.60)***
Female gender	0.91	(0.82–1.02)	1.07	(0.77–1.48)	0.68	(0.56–0.83)***	1.03	(0.88–1.20)
Surgical category:								
1) Isolated CABG (reference)	1.00	—	1.00	—	1.00	—	1.00	—
2) 1 non-CABG procedure	1.49	(1.29–1.73)***	2.41	(1.59–3.64)***	1.56	(1.20–2.03)***	1.33	(1.04–1.68)*
3) 2 surgical procedures	1.53	(1.37–1.71)***	2.06	(1.44–2.93)***	1.60	(1.32–1.94)***	1.44	(1.21–1.71)***
4) ≥ 3 surgical procedures	1.94	(1.51–2.48)***	2.43	(1.20–4.90)*	2.17	(1.47–3.20)***	1.53	(1.00–2.33)*
Chronic cardiac insufficiency	1.61	(1.44–1.79)***	1.89	(1.38–2.60)***	1.78	(1.48–2.15)***	1.49	(1.26–1.77)***
Chronic pulmonary disease	1.70	(1.52–1.89)***	2.38	(1.75–3.25)***	1.69	(1.40–2.04)***	1.52	(1.27–1.81)***
Serum creatinine >140 μmol/L	2.07	(1.77–2.42)***	3.02	(2.02–4.53)***	2.00	(1.54–2.60)***	1.85	(1.43–2.41)***
Diabetes mellitus	1.58	(1.40–1.78)***	1.63	(1.15–2.32)**	1.50	(1.23–1.84)***	1.66	(1.38–1.99)***
Peripheral vascular disease	1.69	(1.50–1.91)***	1.16	(0.77–1.73)	1.95	(1.60–2.37)***	1.65	(1.37–2.00)***
Current smoking	1.42	(1.29–1.57)***	1.54	(1.12–2.11)**	1.25	(1.05–1.48)*	1.41	(1.21–1.64)***

Hazard ratios are given for the complete follow-up period, as well as piecewise for the 1st year (n = 8,380), 1st-5th year (n = 7,704) and >5th year (n = 5,207) of follow-up. HR; hazard ratio, CI; confidence interval.

*p<0.05

**p<0.01

***p<0.001. CABG; coronary artery bypass grafting.

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comparable when selecting patients included until 2006 or 2010. Furthermore, there were only small changes when using cardiovascular death as the outcome variable (S3 Table).

When adjusting for background mortality, relative mortality was higher in younger patients (RMR per 5 years 0.81 (0.79–0.84), p<0.001) and increased with female gender (RMR 1.35 (1.19–1.54), p<0.001). The multivariate analysis showed that the predictors of observed mortality mentioned above were also associated with relative mortality (Fig 4). Results were consistent also for the subgroup analysis including only CABG and AVR patients (S3 Fig).

Discussion

In the present study cohort, short- and long-term observed mortality rates remained constant throughout the study period. Comparing observed mortality to that expected in a matched sample from the general population, patients undergoing cardiac surgery showed excellent survival throughout the first seven years of follow-up. Subsequently, there was a modest reduction in overall annual survival, which was more pronounced in female patients as well as patients undergoing other procedures than isolated CABG. Confirming these findings, the ratio of observed and expected deaths was higher for females, in younger age groups and in patients undergoing AVR, independently of concomitant CABG. Multivariate survival analyses indicated that the same predictor variables associated with observed mortality remained significant predictors of relative mortality.

Long-term survival

The good long-term survival in cardiac surgery patients underscores a continued patient benefit from this intervention in appropriately selected patients. Despite an increasing trend of more comorbid and complex patients being referred to cardiac surgery, long-term survival

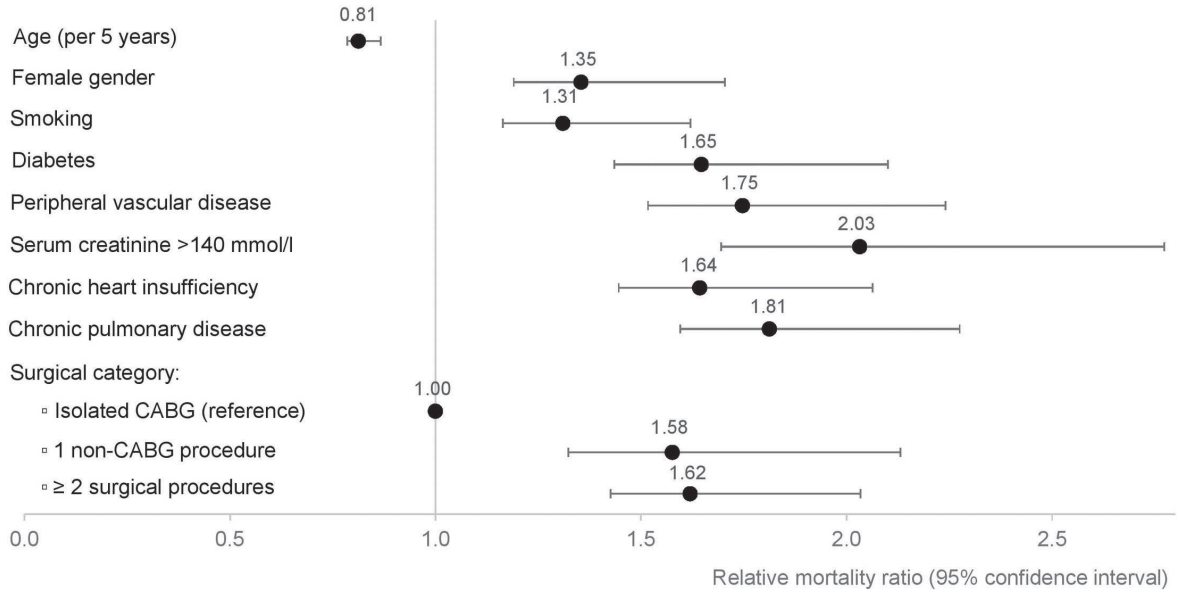

Fig 4. Predictors of relative mortality. Estimated relative mortality ratios (RMR) with corresponding 95% confidence intervals (CI) for predictor variables of long-term relative mortality in patients undergoing cardiac surgery. A ratio of 1 indicates no difference. CABG, coronary artery bypass grafting.

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remained unchanged throughout the study period. Furthermore, patients undergoing cardiac surgery in Trondheim also showed similar and even somewhat improved observed and relative survival rates compared to older reports from other centres [15, 16].

Older reports on relative long-term survival were performed on patients undergoing CABG and valvular surgery between the 1970s and 1990s. However, the comparison of present survival rates with older reports have not taken into account the patients' changing risk profiles. Reasons for the sustained or even improved long-term survival therefore remain uncertain, but might be related to different risk profiles, geographically or temporally, advances in secondary prevention or improvements in medical care.

Age

In accordance with previous studies, higher age was as an independent predictor of observed long-term mortality, and relative survival analyses revealed that the effect of age was attributable to the population risk [13, 14, 16]. On the contrary, relative mortality increased with decreasing age, also after adjustment for smoking and comorbidities. The need for cardiac surgery at a young age may indicate severe cardiovascular disease, which is progressive and conveys an increased risk for future cardiovascular events.

In general, older patients have reduced functional reserves as well as more systemic and cardiovascular comorbidities; thus having a higher operative risk [39, 40]. However, if they survive the early postoperative phase, our observations support that they show excellent long-term results [40]. Our study indicates that co-existing comorbidities remain the major determinants of surgical eligibility, not chronological age as such; underlining the importance of detailed operative risk stratification in older patients.

Gender

The role of gender as a risk factor for long-term mortality is debated [41]. In the present multivariate analysis, gender did not significantly affect observed long-term mortality, despite significant differences in the preoperative risk profiles between genders. Our initial findings comply with previous observations where adjustment for potential predictors in a multivariate analysis eliminated the gender differences seen in observed mortality rates [42]. However, results from the propensity score matched analysis suggest that a gender difference may have been masked by residual confounding. In general, female patients showed a worse risk factor profile. Nevertheless, in the matched pairs of females and males, females had a reduced observed mortality. Importantly, our data suggest that this association may be confounded by the better life expectancy of women, because female patients had a significantly higher mortality risk relative to their expected level of survival. Thus, the female survival advantage observed in the general population was obliterated. This phenomenon was also found in an old study from Norway [18], and might be explained by more aggressive heart disease in females [43].

Surgical procedure

Patients undergoing procedures other than isolated CABG showed higher long-term observed and relative mortality. However, a temporal assessment of relative survival across follow-up time showed that up to the seventh year, cumulative survival was comparable in all patient groups. CABG-patients showed a sustained survival benefit throughout follow-up compared to the general population, whereas there was a trend of reduced relative survival in patients undergoing AVR from the eighth year. Overall relative mortality was significantly higher in patients undergoing isolated AVR or combined AVR and CABG, also after adjustment for different distributions of age, gender and comorbidities. Reduced relative survival in AVR-patients was comparable to an old report from Sweden [13]. Here, the mortality risk increased markedly from the fourth year of follow-up.

There was no significant difference in mortality between patients undergoing AVR and combined AVR and CABG. Due to the low number of patients in these two groups, further investigation is warranted. However, our findings comply with other reports, where adjustment for age, gender and other risk factors eliminated concomitant CABG in AVR-patients as an independent predictor of mortality [13, 44, 45]. It has previously been described a trend of reduced relative survival in combined AVR-CABG patients after 8–10 years compared to isolated AVR [13], but as in our study, this difference did not reach statistical significance.

Compared to isolated CABG, the reduced survival in patients undergoing AVR may be causal (i.e. implying a more aggressive disease), act as a marker for a high-risk patient profile, or be related to follow-up care and suboptimal secondary prevention. Further investigation of this patient group might be important for improving outcomes following cardiac surgery.

With the present covariates, the prognostic difference amongst CABG- and AVR-patients persisted despite adjusting for different preoperative risk profiles. Postoperatively, all patients undergoing CABG are routinely started on lipid-lowering treatment and antiplatelet therapy, with additional guidelines for optimizing treatment of diabetes, hypertension and heart failure. The hazards of hyperlipidaemia [46] and benefits of lipid-lowering treatment [47] have also been demonstrated in AVR-patients, but not yet routinely implemented in clinical practice. Trials of antiplatelet therapy and antihypertensive treatment has also been called for [48]. Improving secondary prevention strategies might therefore represent an opportunity to further improve the quality of care and hence long-term outcomes after AVR.

Altogether, the temporal survival trends and multivariate predictor analysis highlight the prognostic importance of systemic and cardiovascular risk factors above surgical factors.

Despite more complex preoperative risk profiles, long-term mortality remained unchanged. This might comply with the gradual reduction in cardiovascular mortality in the Norwegian population; from 41% in 2000 to 29% in 2014 [49]. Nevertheless, it remains the number one cause of death in the population. This supports our finding that the predictors of observed mortality and relative mortality were the same. Patients undergoing cardiac surgery suffer severe and progressive heart disease with a continuous risk of symptomatic events and mortality. The beneficial effects of operation will decline over time, thus risk factor control remains the cornerstone for improving the long-term prognosis of these patients.

Study limitations

This is a single-centre study based on data from cardiac surgery patients in Trondheim, Norway, as well as expected survival rates from the Norwegian population. Thus, the results may not apply to other institutions and countries. Furthermore, patients undergoing cardiac surgery may have been included in the expected survival rates. As the prevalence of cardiac surgery in the general population is low, this will have had little impact on our estimates.

Despite a total follow-up period of up to 14 years, the median follow-up was 6.4 years. Sensitivity analyses for data between 2000–2006 and 2000–2010 did not alter the main results, indicating that our original findings are robust with respect to inclusion period. Furthermore, neither survival trends nor predictors of long-term mortality changed when using cardiovascular death as an alternate endpoint to all-cause mortality. However, due to the risk of classification errors in cause-of-death records [50, 51], relative survival analyses, permitting comparison with data from the general population, were applied in order to adjust for mortality of other causes in the cardiac surgery patients.

The causal mechanisms underlying increased mortality in younger patients, females and patients undergoing AVR cannot be deduced from the present study. Despite adjustment for varying risk profiles, we cannot exclude that associated predictors may act as markers for other causal relationships and risk factors which were not accounted for in our analysis. The gender differences in preoperative risk profiles and results from repeated calculations in the propensity score matched subset of patients (n = 2,896) may indicate that there was residual confounding. On the other hand, propensity score matching results in smaller groups for comparison due to difficulties in matching of patients with the most extreme scores. Furthermore, the paradoxical combination of more preoperative risk factors in females but reduced observed mortality may indicate that the association was confounded by the longer life expectancy in females, as indicated by our relative survival analyses. The improved relative survival in older patient groups might be due to a chance effect in small groups when the numbers at risk were reduced towards the end of follow-up.

Cardiac surgery patients constitute a heterogeneous group. In order to reduce patient heterogeneity, patients undergoing off-pump surgery have not been enrolled into CaSOS. Consequently, mortality data from the Norwegian Cause of Death Registry were not available for these patients. A sensitivity analysis only including patients undergoing CABG and/or AVR showed consistent results with the complete study cohort. Nevertheless, patients undergoing AVR still constitute a heterogeneous population ranging from young patients with bicuspid valve disease, to elderly patients with degenerative disease complicated by coexisting comorbidities. The low number of patients in these subgroups limited the pairwise comparison and depth of the subgroup analysis. Our main aim was therefore to identify common effects in a large patient group, where the majority suffers cardiovascular disease with many common risk factors.

The present study did not provide any direct information on the survival of surgical patients in comparison with similar patients who did not undergo cardiac surgery. We did not have

data on other long-term outcomes, such as complications like bleeding and thromboembolism or quality of life.

Conclusions

Despite more complex preoperative risk profiles, the observed 30-day, 1-, 3- and 5-year mortality rates in patients undergoing cardiac surgery in Trondheim, Norway, have remained constant both throughout the study period and compared to older reports. Overall, cardiac surgery patients showed comparable survival to that expected in the general Norwegian population, underlining the benefits of cardiac surgery in appropriately selected patients. However, the beneficial effect was more short-lived and relative mortality increased with lower age, in female patients, and patients undergoing AVR or other procedures than isolated CABG. The present findings therefore identify three patient groups that should receive increased attention in order to further improve patient outcomes. A key to improving patient outcomes might lie in closer attention to the underlying, chronic disease and implementation of appropriate preventive strategies.

Supporting Information

S1 Fig. Long-term observed survival. Unadjusted Kaplan-Meier survival curves stratified on the surgical procedure as classified by EuroSCORE II. Due to the low number of patients, the two latter surgical groups were combined (2 and $\geq$ 3 surgical procedures). The number at risk (n) at the start of even follow-up years are provided.
(TIF)

S2 Fig. Standardized difference plot. Absolute standardized differences in covariate means between female and male cardiac surgery patients before and after propensity score matching on preoperative covariates.
(TIF)

S3 Fig. Predictors of long-term relative mortality. Comparison of predictor estimates when modelling long-term relative mortality in patients undergoing isolated CABG, isolated AVR or combined AVR and CABG (n = 7,203, hollow circles), with the complete patient samples stratified on EuroSCORE II's weighted procedures (n = 8,564, black circles). Patients who died within 30 days following surgery have been excluded. *For EuroSCORE's categories, the two latter surgical groups were combined (2 and $\geq$3 surgical procedures) due to small patient groups.
(TIF)

S1 Table. Comparison of patient characteristics between genders.
(DOCX)

S2 Table. Comparison of patient characteristics across time.
(DOCX)

S3 Table. Risk factors associated with observed cardiovascular mortality.
(DOCX)

Author Contributions

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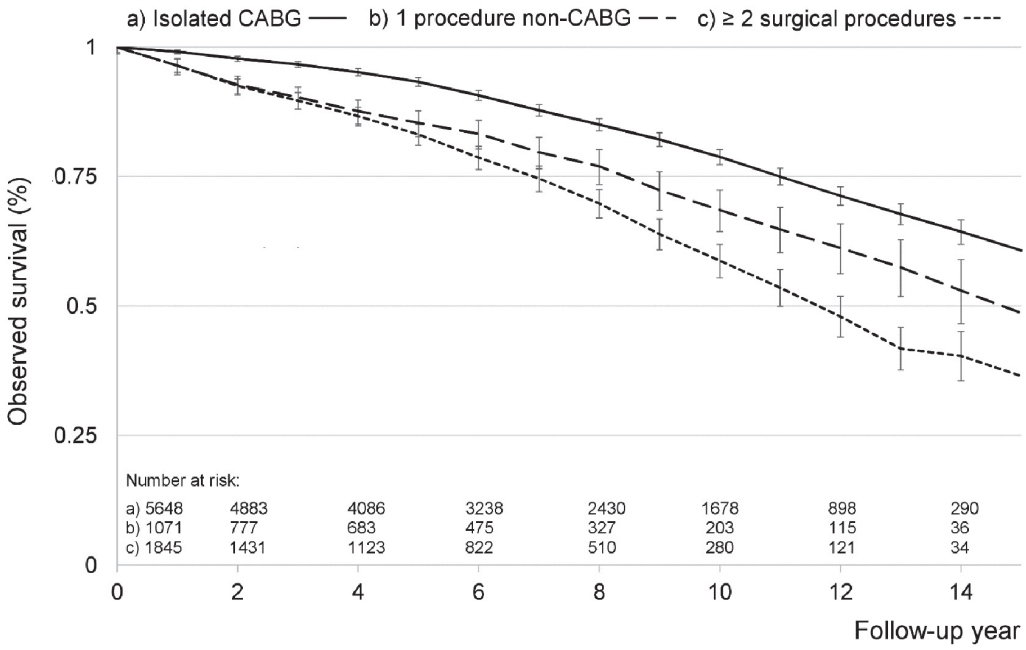
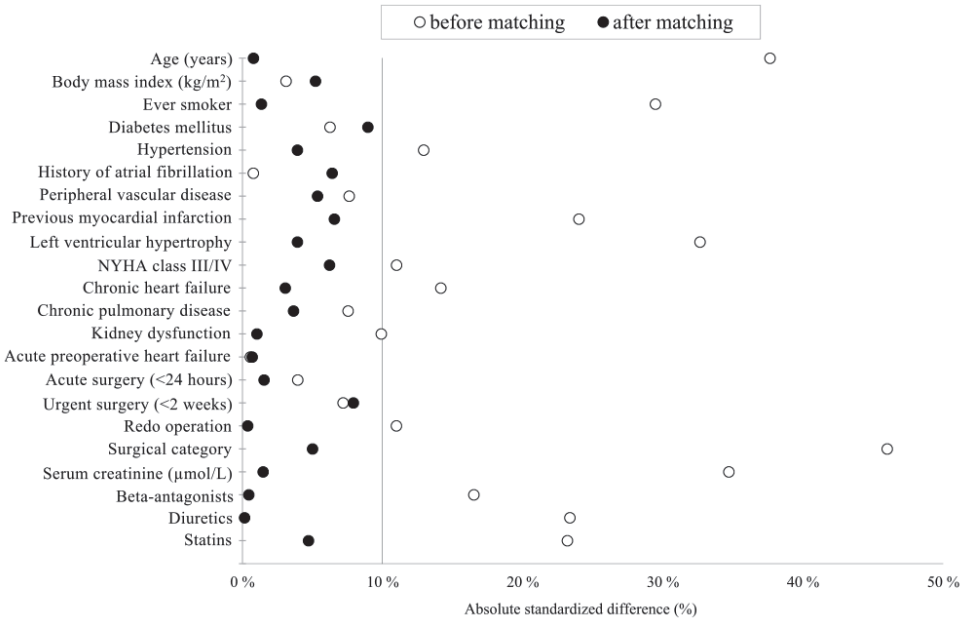
S1 Fig. Long-term observed survival.



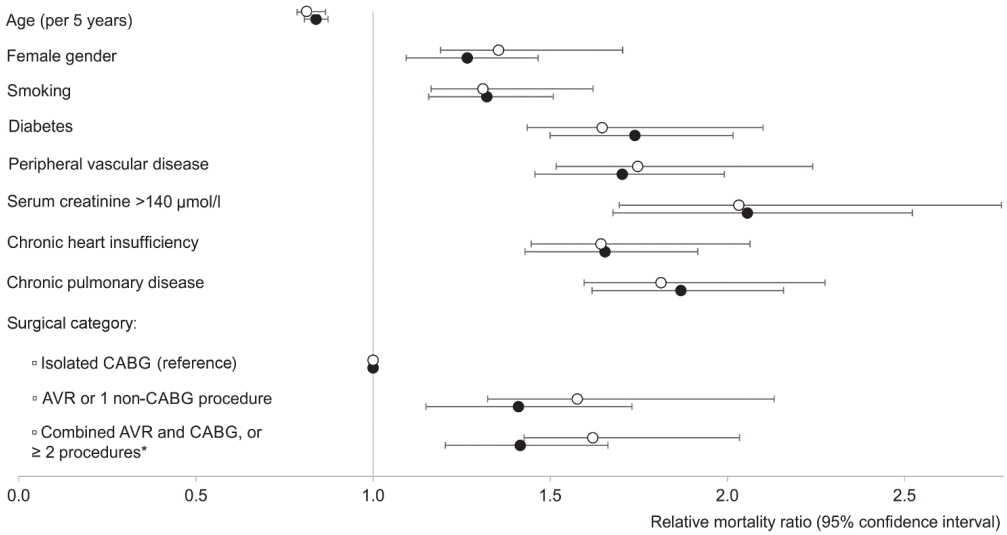
Unadjusted Kaplan-Meier survival curves stratified on the surgical procedure as classified by EuroSCORE II. Due to the low number of patients, the two latter surgical groups were combined (2 and ≥ 3 surgical procedures). The number at risk (n) at the start of even follow-up years are provided.

S2 Fig. Standardized difference plot.



Absolute standardized differences in covariate means between female and male cardiac surgery patients before and after propensity score matching on preoperative covariates.

S3 Fig. Predictors of long-term relative mortality.



Comparison of predictor estimates when modelling long-term relative mortality in patients undergoing isolated CABG, isolated AVR or combined AVR and CABG (n = 7,203, hollow circles), with the complete patient samples stratified on EuroSCORE II's weighted procedures (n = 8,564, black circles). Patients who died within 30 days following surgery have been excluded. *For EuroSCORE's categories, the two latter surgical groups were combined (2 and ≥3 surgical procedures) due to small patient groups.

S1 Table. Comparison of patient characteristics between genders.

Characteristic	Female (n=2,211)	Male (n=6,353)	P-value
Age (years)	71 (70-71)	66 (66-66)	<0.001
Body mass index (kg/m^2)	26.4 (26.2-26.6)	26.7 (26.6-26.8)	0.001
Ever smoker	961 (48.2%)	3,656 (63.6%)	<0.001
Diabetes mellitus	343 (15.5%)	847 (13.3%)	0.01
Hypertension	1,290 (58.3%)	3,307 (52.1%)	<0.001
History of atrial fibrillation	285 (12.9%)	807 (12.7%)	0.83
Peripheral vascular disease	197 (8.9%)	715 (11.3%)	0.002
Previous myocardial infarction	810 (36.6%)	3,058 (48.1%)	<0.001
Left ventricular hypertrophy	781 (39.4%)	1,343 (24.2%)	<0.001
NYHA class III/IV	1,573 (71.2%)	4,189 (66.0%)	<0.001
Chronic heart failure	472 (22.5%)	899 (16.6%)	<0.001
Chronic pulmonary disease	387 (17.5%)	933 (14.7%)	0.002
Kidney dysfunction	69 (3.1%)	317 (5.0%)	<0.001
Acute preoperative heart failure	22 (1.2%)	63 (1.2%)	0.99
Acute surgery (<24 hours)	139 (6.3%)	318 (5.0%)	0.02
Urgent surgery (<2 weeks)	853 (38.6%)	2,682 (42.2%)	0.003
Redo operation	55 (2.5%)	282 (4.4%)	<0.001
Surgical category			<0.001
1) Isolated CABG	1,136 (51.4%)	4,512 (71.0%)	
2) 1 procedure non-CABG	478 (21.6%)	593 (9.3%)	
3) 2 surgical procedures	529 (23.9%)	1,088 (17.1%)	
4) ≥ 3 surgical procedures	68 (3.1%)	160 (2.5%)	
Serum creatinine (μmol/L)	76 (76-77)	90 (90-91)	<0.001
Beta-blockers**	1.525 (69.0%)	4,854 (76.4%)	<0.001
Diuretics**	778 (35.2%)	1,567 (24.7%)	<0.001
Statins**	1,489 (67.4%)	4,942 (77.8%)	<0.001
Cardiopulmonary bypass time (min)	82 (80-83)	78 (77-79)	0.003

Categorical variables are given in n (%), continuous variables in median (95% confidence interval). Differences between genders were tested with χ^2 test and Mann-Whitney U-test for categorical and continuous data, respectively. **Medication before referral for surgery. CABG; coronary artery bypass grafting, NYHA; New York Heart Association Functional Classification (class I-IV).

S2 Table. Comparison of patient characteristics across time.

	Time period			P-value
	2000-2004 (n=3,122)	2005-2009 (n=2,875)	2010-2014 (n=2,567)	
Preoperative characteristics				
Age (years)	67 (66.5-67.0)	67 (67.0-67.5)	67 (66.5-67.0)	0.22
Female gender	796 (25.5%)	774 (26.9%)	641 (24.9%)	0.23
Body mass index (kg/m^2)	26.3 (26.2-26.4)	26.8 (26.7-26.9)	26.8 (26.6-27.0)	<0.001
Ever smoker	1,686 (54.0%)	1,525 (53.0%)	1,406 (54.8%)	0.44
Diabetes mellitus	371 (11.9%)	426 (14.8%)	393 (15.3%)	<0.001
History of atrial fibrillation	1,517 (48.6%)	1,314 (45.7%)	1,084 (42.2%)	<0.001
Peripheral vascular disease	353 (11.3%)	289 (10.1%)	270 (10.5%)	0.28
Previous myocardial infarction	1,437 (46.0%)	1,298 (45.2%)	1,132 (44.1%)	0.35
Chronic pulmonary disease	443 (14.2%)	384 (13.4%)	493 (19.2%)	<0.001
Chronic heart failure	505 (16.2%)	402 (14.0%)	419 (16.3%)	0.024
Kidney dysfunction	172 (5.5%)	111 (3.9%)	103 (4.0%)	0.003
Acute preoperative heart failure	19 (0.6%)	26 (0.9%)	40 (1.6%)	0.001
Acute surgery (<24 hours)	161 (5.2%)	155 (5.4%)	141 (5.5%)	0.84
Urgent surgery (<2 weeks)	1,240 (39.7%)	1,183 (41.2%)	1,112 (43.3%)	0.02
Redo operation	155 (5.0%)	119 (4.1%)	63 (2.5%)	<0.001
Surgical category:				<0.001
1) Isolated CABG	2,240 (71.2%)	1,824 (63.4%)	1,584 (61.7%)	
2) 1 non-CABG procedure	321 (10.3%)	348 (12.1%)	402 (15.7%)	
3) 2 surgical procedures	493 (15.8%)	602 (20.9%)	522 (20.3%)	
4) ≥ 3 surgical procedures	68 (2.2%)	101 (3.5%)	59 (2.3%)	
Serum creatinine (μmol/l)	95 (95-96)	82 (81-82)	81 (80-82)	<0.001
Creatinine clearance* (ml/min)	73.2 (72.3-74.1)	87.3 (86.1-88.5)	89.8 (88.5-91.0)	<0.001
Hemoglobin (g/dl)	13.8 (13.7-13.8)	13.8 (13.8-13.9)	14.0 (13.9-14.0)	<0.001
Preoperative medications				
Antiarrythmics	42 (1.4%)	70 (2.4%)	62 (2.4%)	0.003
Beta-blockers	2,512 (80.5%)	2,168 (75.5%)	1,699 (66.2%)	<0.001
Diuretics	766 (24.6%)	769 (26.8%)	810 (31.6%)	<0.001
Statins	2,189 (70.1%)	2,264 (78.9%)	1,978 (77.1%)	<0.001
Intraoperative characteristics				
Cardiopulmonary bypass time (min)	72 (71-73)	79 (78-81)	85 (84-87)	<0.001
Intraoperative red cell transfusion (no/yes)	433 (13.9%)	539 (18.8%)	613 (23.9%)	<0.001
Use of inotropic support (no/yes)	759 (24.3%)	676 (23.5%)	776 (30.2%)	<0.001
Use of vasoconstrictors (no/yes)	2,115 (67.8%)	2,667 (92.8%)	2,501 (97.4%)	<0.001
Postoperative factors				
Postoperative hospital stay (days)	6.5 (6.5-6.5)	6 (6-6)	5.5 (5-5.5)	<0.001
Pneumothorax	92 (3.0%)	90 (3.1%)	104 (4.1%)	0.05

Myocardial infarction	203 (6.5%)	160 (5.6%)	146 (5.9%)	0.25
Acute kidney injury	386 (12.4%)	321 (11.2%)	297 (11.6%)	0.35
Sepsis	26 (0.8%)	19 (0.7%)	11 (0.4%)	0.17
Multi-organ failure	58 (1.9%)	61 (2.1%)	59 (2.3%)	0.50
30-day mortality	73 (2.3%))	52 (1.8%)	59 (2.3%))	0.30

Categorical variables are given in n (%), continuous variables in median (95% confidence interval). Differences across the study period were tested with χ^2 and Kruskal-Wallis tests for categorical and continuous data, respectively. *Creatinine clearance calculations based on formula from Cockcroft and Gault [1]. CABG; coronary artery bypass grafting.

References:

[1] Cockcroft DW, Gault MH. *Prediction of creatinine clearance from serum creatinine*. Nephron 1976;**16**:31-41.

S3 Table: Risk factors associated with observed cardiovascular mortality†

Predictor	Time period							
	Complete follow-up		≤ 1 year		1-5 years		> 5 years	
	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)	HR	(95% CI)
Age per 5 years	1.43	(1.37-1.50)***	1.27	(1.14-1.42)***	1.29	(1.20-1.39)***	1.58	(1.48-1.68)***
Female gender	0.98	(0.84-1.14)	1.13	0.76-1.69)	0.68	(0.51-0.92)*	1.12	(0.92-1.36)
Surgical category:								
Isolated CABG (reference)	1.00	---	1.00	---	1.00	---	1.00	---
1 non-CABG procedure	1.59	(1.28-1.98)***	2.49	(1.48-4.19)***	2.10	(1.43-3.08)***	1.20	(0.88-1.64)
2 surgical procedures	1.78	(1.52-2.09)***	2.34	(1.51-3.63)***	2.11	(1.58-2.82)***	1.55	(1-24.1.93)***
≥ 3 surgical procedures	2.20	(1.55-3.11)***	3.53	(1.64-7.58)***	2.60	(1.46-4.63)***	1.63	(0.95-2.79)
Chronic cardiac insufficiency	1.84	(1.58-2.15)***	1.94	(1.31-2.87)***	2.04	(1.55-2.68)***	1.72	(1.39-2.13)***
Chronic pulmonary disease	1.46	(1.24-1.72)***	2.08	(1.41-3.06)***	1.39	(1.04-1.87)*	1.31	(1.04-1.66)*
Serum creatinine >140 µmol/L	2.17	(1.73-2.71)***	3.02	(1.83-4.98)***	1.81	(1.21-2.72)**	2.18	(1.59-3.00)***
Diabetes mellitus	1.56	(1.31-1.85)***	1.61	(1.04-2.50)*	1.56	(1.16-2.12)**	1.52	(1.20-1.94)***
Peripheral vascular disease	1.90	(1.60-2.25)***	0.97	(0.57-1.65)	2.48	(1.86-3.31)***	1.89	(1.49-2.40)***
Current smoking	1.41	(1.22-1.63)***	1.72	(1.16-2.55)**	1.30	(1.00-1.69)*	1.42	(1.17-1.72)***
Complete data (n)	8356		8356		7682		5188	
All-cause deaths during interval (n)	1856		182		601		789	
Circulatory deaths during interval† (n)	872		119		261		492	

Hazard ratios are given for the complete follow-up period, as well as piecewise for the 1st year (n=8,380), 1st-5th year (n=7,704) and >5th year (n=5,207) of follow-up. CABG; coronary artery bypass grafting, CI; confidence interval. HR; hazard ratio. *p<0.05; **p<0.01; ***p<0.001. †As classified according to the International Classification of Diseases (ICD)-10 (chapter IX, block I00-I99) and registered in the Norwegian Cause of Death Registry.