Kristin Sandal Berg

Inflammation and Risk Prediction of Major Complications Following Cardiac Surgery

Thesis for the degree of Philosophiae Doctor

Trondheim, August 2015

Norwegian University of Science and Technology
Faculty of Medicine
Department of Laboratory Medicine, Children's and Women's Health

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Science and Technology

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ISBN 978-82-326-1104-1 (print)
ISBN 978-82-326-1105-8 (digital)
ISSN 1503-8181

Doctoral theses at NTNU, 2015:2015:224

Printed by NTNU Grafisk senter

Inflammasjon og prediksjon av alvorlige komplikasjoner etter hjertekirurgi

Hjertekirurgi utløser en betennelsesreaksjon i kroppen, som står sentralt i utviklingen av flere alvorlige komplikasjoner etter operasjonen. Nyresvikt og hjertesvikt er to av de vanligste alvorlige organkomplikasjonene, mens den mest alvorlige komplikasjonen er død. Formålet med avhandlingen var å utvikle matematiske modeller som kunne forutsi risikoen for død og for nyresvikt etter hjertekirurgi, og å undersøke om økt betennelse i kroppen før hjerteoperasjon var assosiert med akutt hjertesvikt etter operasjon.

Med utgangspunkt i variable som er enkle å registrere i klinisk arbeid og tilgjengelige i forkant av operasjonen utviklet vi risikomodeller som kunne forutsi risikoen for død og for nyresvikt etter hjerteoperasjon. Modellene var nøyaktige og enkle å bruke. Testing viste at modellene med stor sannsynlighet vil fungere godt også for fremtidige pasienter. Både for pasienter, pårørende og helsepersonell er det interessant å kunne forutsi risikoen for å utvikle bestemte komplikasjoner etter en hjerteoperasjon. Nøyaktig informasjon om risikoen før et inngrep kan også brukes til å avgjøre om noen pasienter skal få en annen behandling og til fordeling av ressurser, i tillegg til å være viktig i kvalitetssikringsarbeid. For å undersøke sammenhengen mellom betennelse i kroppen før hjerteoperasjon og hjertesvikt etter operasjonen, målte vi fire betennelsesmarkører i blodprøver som ble tatt før hjertekirurgi. De fire markørene var C-reaktivt protein (generell betennelsesmarkør), laktoferrin (markør for aktivering av nøytrofile granulocytter, forsvarsceller i det medfødte immunforsvaret), neopterin (markør for aktivering av monocytter, forsvarsceller i det medfødte immunforsvaret) og det terminale komplement kompleks (markør for aktivering av komplement). Vi fant en sammenheng mellom forhøyet konsentrasjon av neopterin før operasjon og hjertesvikt etter operasjon. Dette gir ny kunnskap om hvilke deler av betennelsesresponsen som er medvirker utviklingen av hjertesvikt etter hjerteoperasjon.

Metoder: Risikomodellene er basert på data fra omtrent 5000 pasienter som gjennomgikk åpen hjertekirurgi ved St. Olavs Hospital, Trondheim, i årene 2000-2007. Det ble brukt logistisk regresjon til å utvikle risikomodellene. Sammenhengene mellom betennelse i kroppen før operasjon og hjertesvikt etter operasjon ble undersøkt ved hjelp av data og blodprøver fra omtrent 1000 pasienter som gjennomgikk hjertekirurgi i årene 2008-2010 ved St. Olavs Hospital, Trondheim. Logistisk regresjon ble brukt til å analysere sammenhengen mellom fire betennelsesmarkører og hjertesvikt etter hjertekirurgi.

Kandidat: Kristin Sandal Berg
Institutt: Institutt for laboratoriemedisin, barne- og kvinnesykdommer
Hovedveileder: Vibeke Videm
Biveileder: Roar Stenseth
Finansieringskilde: Samarbeidsorganet mellom Helse Midt-Norge RHF og NTNU

Ovennevnte avhandling er funnet verdig til å forsvares offentlig for graden PhD i klinisk medisin.
Disputas finner sted i Auditoriet KA11, Kunnskapssenteret, NTNU torsdag 27.08.2015, kl. 12:15.

If you have knowledge,
let others light their candles in it.

Margaret Fuller

Contents

Acknowledgements

The work of this thesis was carried out at the Department of Laboratory Medicine, Children's and Women's Health, Norwegian University of Science and Technology, Trondheim, Norway, in close collaboration with the Department of Cardiothoracic Anaesthesia and Intensive Care and the Department of Cardiothoracic Surgery, St. Olavs University Hospital, Trondheim, Norway.

I am indebted to the Medical Student Research Programme for giving me the opportunity to start doing research during my medical studies, and the Norwegian Research Council who funded the Medical Student Research Programme. Moreover, I am obliged to the Liaison Committee between the Central Norway Regional Health Authority and the Norwegian University of Science and Technology, from whom I received funding as a PhD student. I would also like to thank the other funding sources, namely the Norwegian Council on Cardiovascular Diseases, St. Olavs University Hospital and the Simon Fougner Hartmann Family Foundation.

I would like to thank the staff at the Department of Cardiothoracic Anaesthesia and Intensive Care and the Department of Cardiothoracic Surgery, St. Olavs University Hospital, who has contributed to this thesis through their work. The patients deserve special thanks for their vital contribution to this thesis.

I would like to express my sincere gratitude towards my principal supervisor, Vibeke Videm. Her great knowledge, positive spirit, understanding and working capacity have been a great inspiration to me. She has listened to me and challenged me, and I have learned so much from her. Because of her help and support, my encounter with medical research has been an inspiring and pleasant course.

I am very grateful to my co-supervisor Roar Stenseth. His clinical and research experience has been very important for my work. His meticulous work with the quality database of cardiac surgical patients operated at St. Olavs University Hospital has been invaluable. Furthermore, I appreciate his good humour, kindness and support and for finding time to answer my questions during days filled with critically ill patients and even during his holidays.

Special thanks also to my co-authors Hilde Pleym and Alexander Wahba for their support, their important views and comments, and help to improve the quality of my work. Hilde also collected most of the blood samples used in the study of inflammatory biomarkers. Many thanks to Guri Greiff, Roar Stenseth and Lise Bjella, who also helped collecting blood samples when Hilde was not available.

I am very thankful for the excellent technical assistance provided by the bioengineers Nina Sandberg, Oddrun Kari Storrø and Nina Nasirzadeh. Their efficiency and accuracy impress me, and I value their friendship and kindness. Nina Sandberg and Oddrun Kari Storrø also introduced me to laboratory work and shared from their great knowledge.

I would like to express my gratitude towards other past and present members of the Cardiac Surgery Outcome Study group, Yunita Widyastuti, Tone Bull Enger and Guri Greiff, and my past office mate, Inga Thorsen Vengen. Their company, friendship and help have meant a lot to me. Particularly the sharing of experiences with Guri has been important during my time as a PhD student.

Furthermore, I greatly appreciated the good company of everyone belonging to the yellow lunchroom on the second floor in the east wing of the Laboratory centre.

All my friends deserve special thanks for their love and support, and for making my life a pleasant and eventful journey. In particular I would like to thank Ane-Kristine, who has been an important inspiration and my travel companion and friend along the road of research from the very beginning when we first applied for the Medical Student Research Programme together. I am also very grateful to the student choir Bilyd for providing a wonderful break from work and studies the last decade.

I would like to express my gratitude towards my parents, Gunnhild and Arne, for their love and support, for always encouraging my curiosity and doing their best to answer my questions ever since I was a little girl. I am grateful for the love and support from my brothers Brede, Runar and Vebjørn, and the rest of my family.

I am very grateful to my husband Asbjørn for always supporting me and sharing my joys without envy. His love, enthusiasm and understanding have meant a lot to me. Finally, I want to thank my wonderful children, Sigrid and Eskil, for their unconditional love and for enriching my life.

List of papers

The work of this thesis is based on the three following papers:

Paper I

Berg KS, Stenseth R, Pleym H, Wahba A, Videm V. **Mortality risk prediction in cardiac surgery: comparing a novel model with the EuroSCORE.** Acta Anaesthesiol Scand. 2011;55(3):313-21.

Paper II

Berg KS, Stenseth R, Wahba A, Pleym H, Videm V. **How can we best predict acute kidney injury following cardiac surgery?: a prospective observational study.** Eur J Anaesthesiol. 2013;30(11):704-12.

Paper III

Berg KS, Stenseth R, Pleym H, Wahba A, Videm V. **Neopterin predicts cardiac dysfunction following cardiac surgery.** Interact Cardiovasc Thorac Surg. 2015 (in press).

Abbreviations and Definitions

AKI : Acute kidney injury

AVR : Aortic valve replacement

CABG : Coronary artery bypass grafting

eGFR : Estimated glomerular filtration rate

ROC curve : Receiver operating characteristic curve

STS : Society of Thoracic Surgeons

Summary

Background

In cardiac surgery improvements in quality of care have been a major focus for several decades. Statistical models for predicting the risk of operative mortality and other adverse outcomes have been an important part of this work. Risk prediction models may be used to inform patients of the risk of the planned operation, to adjust complication rates to enable a valid comparison between institutions or surgeons, and to identify potential fields of improvement. Risk prediction models were often less accurate when applied to other patient populations than the one they were derived from. No risk prediction models for mortality or acute kidney injury following cardiac surgery had been developed using data from Norwegian patients.

The surgical trauma and the artificial surfaces in the cardiopulmonary bypass circuit evoke a systemic inflammatory response. The inflammatory response includes activation of leukocytes, endothelium, and plasma cascade systems including the complement system, coagulation and fibrinolysis. Inflammation is thought to play a pivotal role in the development of major complications following cardiac surgery.

Cardiac dysfunction following open-heart surgery is a clinical syndrome where reduced cardiac output results in insufficient oxygen delivery to the tissues. It is thought to be induced by ischaemia and reperfusion, as well as inflammation, increasing the myocardial oxidative stress. Several inflammatory biomarkers, including C-reactive protein (general marker of inflammation), lactoferrin (neutrophil activation marker), neopterin (monocyte/macrophage activation marker) and the terminal complement complex (complement activation marker), had previously been associated with adverse cardiac outcomes in ischaemic heart disease.

Aims

One aim was to develop local risk prediction models for operative mortality and acute kidney injury following cardiac surgery. Another aim was to investigate whether increased preoperative inflammation was associated with the development of cardiac dysfunction following open-heart surgery.

Methods

For developing the risk prediction models for mortality and acute kidney injury we included all 5029 adult patients who underwent open-heart surgery at St. Olavs University Hospital, Trondheim from 2000 through 2007. We applied multivariable logistic regression for model development, and the models were internally validated using bootstrapping methods. For investigating whether increased preoperative inflammation was associated with cardiac dysfunction following open-heart surgery we included 1018 consecutive patients who underwent open-heart surgery at St. Olavs University Hospital, Trondheim, Norway from 1 April 2008 to 19 April 2010. We applied enzyme immunoassay to measure the preoperative concentration of C-reactive protein, lactoferrin, neopterin and the terminal complement complex in plasma. Logistic

regression was used for the statistical analysis, and we adjusted for clinical variables previously associated with postoperative cardiac dysfunction.

Results and discussion

The mortality risk prediction model consisted of eight preoperative variables easily obtainable in clinical practice: Age, degree of urgency for surgery, female gender, serum creatinine concentration, chronic pulmonary disease, chronic cardiac insufficiency, previous cardiac surgery, and type of operation. The acute kidney injury risk model included eleven easily available preoperative variables: age, body mass index, lipid lowering treatment (protective effect), hypertension, peripheral vascular disease, chronic pulmonary disease, haemoglobin concentration, serum creatinine concentration, previous cardiac surgery, emergency operation, and operation type. Both the mortality and the acute kidney injury risk models displayed good discrimination and calibration in our population.

We found that neopterin was associated with cardiac dysfunction after cardiac surgery, and this association remained significant also after adjustment for clinical variables associated with postoperative cardiac dysfunction.

Conclusions

Our local preoperative risk models predicted mortality and acute kidney injury accurately, and were generally robust. Our findings regarding neopterin and cardiac dysfunction support the hypothesis of the role of inflammation and oxidative stress in the development of postoperative cardiac dysfunction.

Trondheim April 2015,

Kristin Sandal Berg

Introduction

Historical aspects

Cardiac surgery

Cardiac surgery had long been prevented by the lack of proper anaesthetics, and its history began late in the 19th century. The first reports on cardiac surgery were on repair of heart wounds resulting from stabbing. The German surgeon Ludwig Rehn successfully performed the first heart operation, the suture of a heart wound, 9 September 1896 in Frankfurt, Germany [1]. The patient was a 22-year old man who had been stabbed in the heart two days earlier, and his condition had worsened when Dr. Rehn decided to operate on him. He discovered a 1.5 cm hole in the right ventricle, and decided to suture the wound. The patient recovered, and Dr. Rehn reported this case the year after. Ten years later he published the results of 124 operations on heart wounds, with a mortality of 60% [1].

Early in the 20th century surgery on heart valves was introduced, and later also surgery on congenital defects. However, up to the 1940s cardiac surgery remained experimental; the number of patients was low and the mortality rates were high [1, 2]. In the 1940's the volume of cardiac surgery slowly increased in the USA, but without the cardiopulmonary bypass it was confined to a small number of procedures like closure of a patent ductus arteriosus, coarctation repair, mitral commisurotomy and the Blalock-Taussig shunt [1, 2]. The Blalock-Taussig shunt was used to alleviate cyanotic heart defects: a branch of either the carotid or subclavian artery was connected to the pulmonary artery so that the lungs would receive more of the deoxygenated blood.

Cardiopulmonary bypass

Cardiopulmonary bypass devices can perform gas exchange and pump the blood after cannulation of the right atrium or the venae cavae and the aorta. Thus, the blood bypasses the heart and lungs, and is delivered to the rest of the body. This permits the surgeon to operate inside the heart and to keep the heart and lungs still. The invention of cardiopulmonary bypass therefore revolutionised cardiac surgery [1, 2]. The first successful attempt of using complete cardiopulmonary bypass took place 6 May 1953

by John Gibbon, an American surgeon who has been given much of the credit for the development of cardiopulmonary bypass [1-3]. The patient was a female student with an atrial septal defect, and she was still alive and well fifty years after the operation. In the 1950's open-heart surgery was still offered to very few patients. Around 1970 the coronary artery bypass grafting procedure (CABG) gained acceptance, and since then improvements were made to the cardiopulmonary bypass devices and protection of the myocardium during the procedure [3]. By the 1980's cardiac surgery was considered standard care [3].

Risk of major complications following cardiac surgery

Since the 1980's the mortality rates have decreased. The reported 30-day mortality for CABG procedures in the Society of Thoracic Surgeons (STS) database (USA) was 3.2% between 1980 and 1990 [4], 3.1% for CABG procedures performed between 1997 and 1999 [5], and 2.3% between 2002 and 2006 [6]. For comparison, a study of survival after CABG in a Norwegian population operated between 2003 and 2006 found a 30-day mortality rate of 0.8% [7].

As the mortality rates decreased, the patients' preoperative status worsened in several aspects. Between 1980 and 1990 22.7% of the CABG patients in the STS database were older than 70 years [4], whereas 22.0% were aged 75 years or older between 2002 and 2006 [6]. A comparison of proportions having certain preoperative morbidities in the STS database in 1980 to 1990 and 2002 to 2006 is given in Table 1.

Table 1. Comparison of preoperative morbidity in coronary artery bypass grafting patients in the Society of Thoracic Surgeons' database in the years 1980-1990 and 2002-2006, USA.

Preoperative morbidity	**1980-1990** [4]	**2002-2006** [6]
Diabetes mellitus	17.9%	36.3%
Chronic pulmonary disease	3.2%	20.0%
New York Heart Association class IV	23.6%	21.3%
Cerebrovascular disease	1.38%	13.6%

The decrease in operative mortality occurring concomitantly with the increase in preoperative morbidity is often referred to as the risk paradox in cardiac surgery. This phenomenon has often been attributed to the great efforts made to improve quality of care.

Cardiac surgery is still associated with a risk of postoperative complications and mortality. Systemic inflammation following open-heart surgery is thought to play a pivotal role in the development of complications [8]. Inflammation is the body's reaction to tissue injury, and the cardiac operation represents a major trauma to the body. Moreover, blood comes into contact with foreign surfaces in the cardiopulmonary bypass circuit. A comparison of on-pump versus off-pump cardiac surgery indicated that cardiopulmonary bypass is responsible for the activation of the complement cascade [9]. Furthermore, off-pump cardiac surgery was associated with lower plasma levels of some pro-inflammatory markers, such as interleukin-8 and tumour necrosis factor-α [9]. However, C-reactive protein and interleukin-6 appeared to be similarly elevated in both patient groups [9]. Ischaemia and reperfusion is another powerful inflammatory stimulus, occurring with the aortic cross-clamping and declamping [10]. During reperfusion the free radical activity also increases [11]. This thesis focuses on three major postoperative complications after cardiac surgery in adults, namely mortality, acute kidney injury (AKI), and cardiac dysfunction.

Mortality

Death is the most serious complication following cardiac surgery. Mortality is often measured as 30-day-mortality or in-hospital mortality or a combination of both, referring to death occurring within 30 days postoperatively or during the primary hospital stay, respectively. Postoperative mortality following open-heart surgery ranges from less than 1% to almost 5% [12, 13], depending on case mix, surgical procedures included in the analyses, and quality of care.

Among the most common causes of death following CABG are cardiac failure, respiratory failure, haemorrhage, neurologic injury and dysrhythmia [14]. In patients requiring prolonged ventilation following cardiac surgery, the most important determinant for mortality is multi-organ dysfunction [15]. It is generally acknowledged

that the mortality risk increases with increasing age and preoperative morbidity in major organ systems such as the kidneys, lungs, heart, peripheral arteries and nervous system [16]. Naturally, critically ill patients who are likely to die without surgery have a high postoperative mortality risk [16]. Moreover, complex surgical procedures carry a greater risk than standard CABG [16].

Acute kidney injury

Most patients develop a slight reduction in renal function after heart surgery, but this is rarely detected by serum creatinine measurements [17]. However, AKI, a more severe degree of renal function deterioration, is one of the most common serious organ complications after open-heart surgery, and occurs in 3-24%, depending on the definition [18-21]. AKI severe enough to require dialysis occurs in 1.1-3.9% [21-24]. Postoperative AKI is associated with increased mortality and morbidity [20, 21, 23, 25], and the mortality rates among patients who experience AKI ranges from 26 to 43%, and for patients who require renal replacement therapy the mortality is 38-64%. AKI following open-heart surgery increases the risk of later developing chronic kidney disease in patients with normal kidney function preoperatively [26]. Moreover, it increases the long-term mortality, and may lead to progression of previously acquired chronic kidney disease [26].

The Acute Kidney Injury Network have proposed that AKI should be defined as an absolute increase in serum creatinine of 26.4 μmol/L (0.3 mg/dL) or more, a relative increase in serum creatinine of 50% or more, or urine output less than 0.5 mL/kg per hour for more than six hours [27]. Even so, it takes some time to develop a detectable increase in serum creatinine, and the diagnosis of AKI is often delayed [20, 28].

The reasons for AKI following cardiac surgery remain to be further elucidated [8]. Proposed mechanisms include ischaemia and reperfusion, inflammation, neuro-hormonal activation and endogenous and exogenous toxins [29]. Both cardiopulmonary bypass time and haemodynamic instability during the cardiac operation have been associated with postoperative renal dysfunction, suggesting that systemic inflammation as well as ischaemia and reperfusion may be involved [21]. Inflammation is crucial in mediating the deleterious effects of ischaemia and reperfusion causing kidney injury

[30]. Neuro-hormonal activation includes the release of adrenal medullary hormones and activation of the renin-angiotensin-aldosterone system, affecting renal perfusion [29]. Contrast media used in angiography are potent exogenous renal toxins [29]. Free haemoglobin from haemolysis due to mechanical stress in the cardiopulmonary bypass circuit is one of the hypothesized endogenous renal toxins [29].

Cardiac dysfunction

In the setting of cardiac surgery, a postoperative reduction in ventricular function is commonly seen, and ventricular function is often deteriorating for several hours after surgery before recovery [31]. The clinical syndrome of cardiac dysfunction is characterised by reduced cardiac output leading to insufficient blood delivery to the tissues, and is sometimes also referred to as the low cardiac output syndrome [32]. In 5-12% of patients it is severe enough to require intravenous administration of several inotropic agents or an intra-aortic balloon pump to maintain sufficient blood pressure [33-35], and strongly influences mortality, ranging from 7 to 38% depending on case mix, type of surgery and end-point definition [32, 36]. Postoperative heart failure was by far the most common mode of death in a study from 1998, concerning coronary artery bypass grafting patients in northern New England, USA, applying to 64.8% of in-hospital deaths [14]. In a Swedish study of 5-year survival after cardiac surgery from 2007, cardiac death was the most common cause of death, applying to 50.4% of overall mortality, and 69.5% of deaths in patients who had suffered postoperative heart failure [36].

Cardiac dysfunction following open-heart surgery is often considered as myocardial stunning, evoked by ischaemia and reperfusion injury [37, 38], and also local and systemic inflammation [39]. Myocardial stunning is the mechanical cardiac dysfunction occurring after ischaemia and reperfusion, notwithstanding re-established normal or near-normal blood flow and without any sign of irreversible damage [40]. As cardiac surgery involves controlled ischaemia and reperfusion of the heart, followed by restored myocardial perfusion, postoperative cardiac dysfunction has often been regarded as myocardial stunning when there is no evidence of irreversible damage [37]. The proposed mechanisms of myocardial stunning involve oxidative stress and calcium overload, leading to modification or damage of the contractile apparatus and decreased

calcium responsiveness [38]. The recovery from myocardial stunning occurs slowly as new contractile proteins are synthesized or modified proteins are repaired [38].

The greatest reduction in left ventricular function in stunning most often occurs immediately after reperfusion [37]. However, after open-heart surgery, the left ventricular function often decreases for several hours postoperatively, suggesting that the underlying mechanisms may be partially different from those of myocardial stunning [39]. Elevated levels of the pro-inflammatory cytokine interleukin-6 measured immediately after cardiopulmonary bypass has been correlated with poor ventricular function, measured as changes in wall motion score postoperatively compared to the preoperative wall motion score [41]. Similarly, lower levels of interleukin-6 were associated with improvement in ventricular function measured as improvement in wall motion score in the same study [41]. As cardiac surgery evokes an inflammatory reaction, it is likely that inflammation also may be part of the underlying mechanisms responsible for cardiac dysfunction following open-heart surgery [39].

Risk prediction in cardiac surgery

Risk prediction models in cardiac surgery aim at identifying patients at increased risk for a certain outcome, and provide an objective measure of individual risk. This information may be used to adjust complication rates for factors related to the patient in assessment of quality of care, instead of comparing doctors or institutions by crude complication rates [4, 42, 43].

Preoperative risk prediction may be used to provide objective information to health care providers, as well as patients and their next of kin. In some cases, it can also be used to identify subjects at increased risk of complications before surgery, in order to enable alternative treatment or enhance perioperative monitoring to reduce the risk of serious complications or death [8, 28, 44].

Mortality risk prediction

1980's

One of the first works on preoperative mortality risk stratification was published in 1983 by Paiement and co-workers [45]. It was a simple system of risk classification

based on the presence of eight risk factors, assigning patients to three different risk groups.

In 1986, the Health Care Financing Administration, USA, released a report on mortality data from institutions performing open-heart surgery in the USA to enable comparison of quality of care between institutions [43]. The report failed to adjust for important factors that could explain variation in mortality rates, and its release was heavily criticized [4, 42, 43]. More sophisticated risk prediction in cardiac surgery emerged as a response to this, as most thoracic surgeons and other health care providers considered comparison based on unadjusted mortality rates as unfair and misleading.

Data collection and analysis for development of risk prediction models takes time, and in 1989 Parsonnet and colleagues were the first to publish a risk stratification system as a response to the Health Care Financing Administration's release of mortality data [42]. Using data from 3500 cardiac operations at one single centre they applied multivariable logistic regression to develop a risk prediction model. The model included 15 preoperative predictors with assigned weights in whole numbers for simple calculation of expected 30-day operative mortality. Models in which the predicted mortality is calculated by adding the assigned weights of the risk factors are often referred to as additive models. Parsonnet's model was validated in a British population in 1992, and was evaluated to perform well by the authors despite over-prediction of risk particularly in high-risk patients [46]. In 1997 the Parsonnet model was also validated in a French population, where its discrimination was evaluated as poor [47]. Moreover, two of the variables, "catastrophic states" and "other rare circumstances", were criticized for not being objective enough [47].

1990's

In the first half of the 1990's, risk prediction in heart surgery was dominated by North American studies (Figure 1), and the main aim was to enable evaluation of the quality of care given, applying risk-adjusted mortality data to compare different institutions [4, 48-52]. As a direct response to the Health Care Financing Administration's release of mortality rates from cardiac surgery units, the STS in the USA established a large national database, commonly referred to as the STS database [4]. The main objective

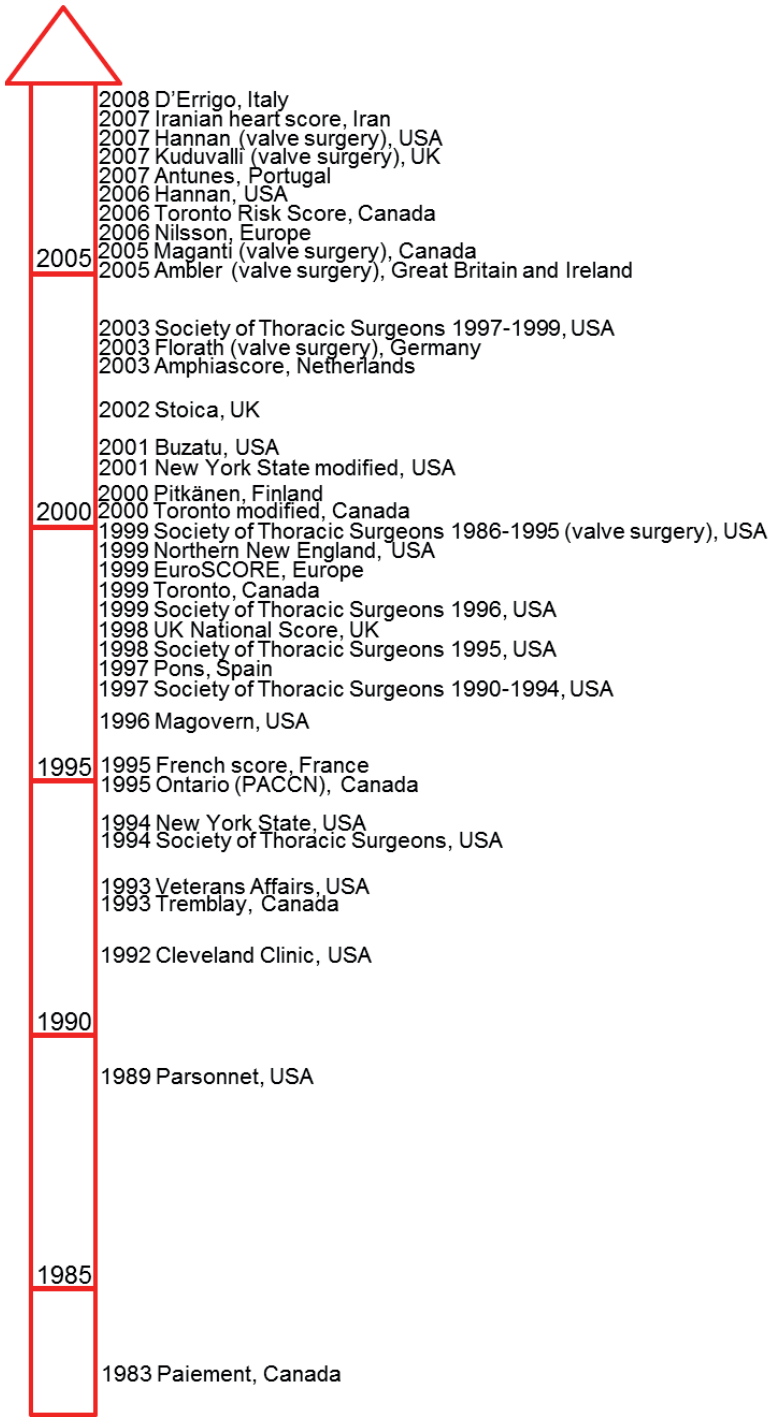

Figure 1. Publication of mortality risk prediction models in cardiac surgery. Abbreviations: PACCN - Provincial Adult Cardiac Care Network, Ontario.

was to enable a fair and valid comparison of institutions performing heart surgery based on patient risk factors in order to improve cardiac surgery outcomes. In 1994, the first STS risk model was published. Whereas the most common method for model development was multivariable logistic regression [42, 48, 50, 53], the STS model was developed using Bayesian theory [4]. Data on 78,927 patients operated between 1984 and 1990 was used for model development and validation, and the model included 21 risk variables.

During the 1990's development of risk prediction models in cardiac surgery reached Europe, and in the second half of the decennium several European studies were published [13, 54-56] along with North American studies [57-59].

The STS in the USA continued its work with the database, and the number of participating centres increased substantially [60]. In 1997, the second publication of STS mortality risk models for coronary artery bypass surgery were published, using logistic regression for model development [61], and new models were published also in 1998 [62] and 1999 [63]. In 1999 they also published a mortality risk prediction model for valve surgery [64].

The European System for Cardiac Operative Risk Evaluation (EuroSCORE) [13] became the most widely used among the European models [12, 65-68]. The EuroSCORE database was randomly split into a developmental dataset (n = 13 302) and a validation dataset (n = 1497). Potential risk factors for mortality following cardiac surgery were identified and evaluated by an expert panel, aiming to find risk factors useful in the risk model. Univariable testing was applied to the resulting variables, and significant variables were entered in the multivariable logistic regression model. The number of risk factors was then reduced by deleting non-significant factors one at a time, beginning with the one with the highest *p*-value. The final model consisted of 17 preoperative risk factors considered to be objective and easily obtainable in clinical practice.

2000-2008

After the turn of the millennium risk prediction in open-heart surgery continued to raise interest (Figure 1). Previously most models were developed for all types of cardiac

surgery, or for coronary artery bypass surgery only, but now several separate models for valve surgery were developed [32, 69-72]. Validation of previous models was also commonly seen: In a study from 2006, Nilsson and colleagues found that the Parsonnet model still performed well in a Swedish population [73]. Nevertheless, several other studies published between 2000 and 2008 found that it over-predicted the risk of mortality [12, 65, 74, 75]. The over-prediction of risk was often attributed to changes in medical and surgical treatment and patient mix during the time since it was developed [12, 65, 74, 75]. During the 2000's, the EuroSCORE performed well in some places [73, 75, 76] while others found that its performance had worsened [12, 65, 74]. The EuroSCORE had been used in cardiac surgery in Norway for several years, and it was indicated that it overestimated the risk of mortality [7].

Also after 2000, logistic regression was by far the most common method for model development [5, 12, 32, 65, 68-72, 76-81]. Simplified, one could say that logistic regression assumes a linear relationship between the explanatory variables and the logarithmically transformed risk of the outcome. A few studies using artificial neural networks were also published, seeking to improve the accuracy of risk prediction as artificial neural networks are based on a concept mimicking the networks of neurones in the brain, and not relying on an assumption of linearity [82, 83]. Nilsson and colleagues used the large EuroSCORE database to develop a system for mortality risk prediction applying artificial neural networks, and its discrimination was better than the logistic EuroSCORE both in the developmental dataset and in an independent population [83]. Except for a small Iranian study, none had included intraoperative variables [81], as most studies had focused on developing tools to adjust mortality rates for the purpose of quality assurance work.

As the EuroSCORE was widely used and its performance was varying, it seemed appropriate to us to validate it in a Norwegian population, as well as developing a locally adjusted mortality risk prediction model. It would also be interesting to perform an assessment of the possible gain by including intraoperative risk factors.

Acute kidney injury risk prediction

In 1992 Tuman et al. and Higgins et al. published models for prediction of morbidity, and these were some of the first models predicting morbidity rather than mortality, recognizing that morbidity may be just as important when assessing quality of care in cardiac surgery [50, 84].

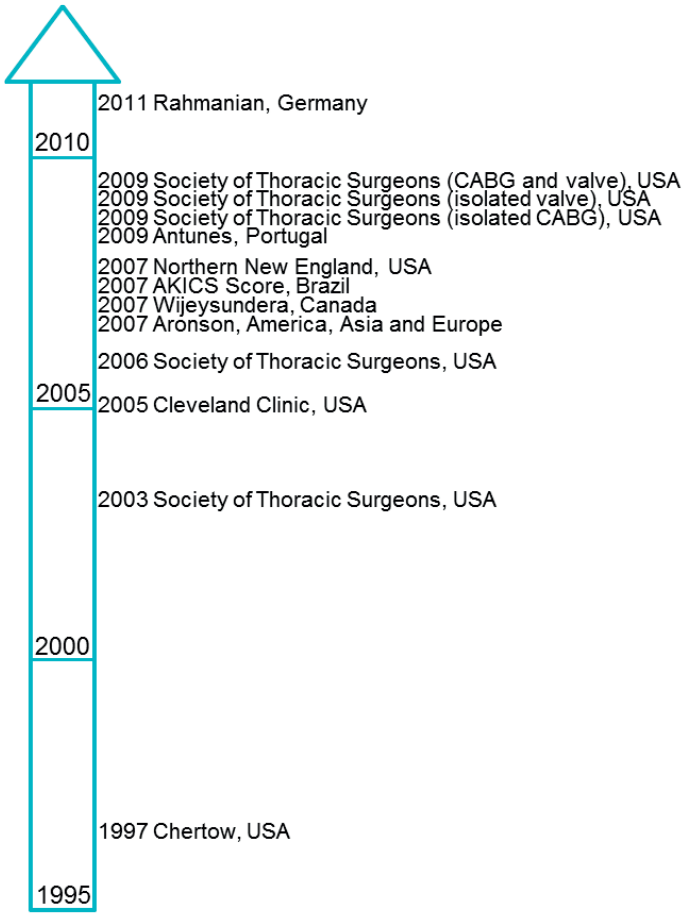

Figure 2. Publication of models for prediction of acute kidney injury. Abbreviations: CABG – coronary artery bypass grafting, AKICS – acute kidney injury following cardiac surgery.

One of the first models to predict renal failure, defined as the need for postoperative dialysis, was published in 1997 by Chertow et al. (Figure 2) [22]. Risk prediction of postoperative renal injury was dominated by North American studies (Figure 2) [5, 6, 18, 22, 85-89], and even the multi-national study by Aronson et al. from 2007 was dominated by contributors in the USA, although it included centres in 17 countries in America, Asia and Europe [90]. However, in 2007 a small Brazilian study was used to develop an AKI risk score [25]. Furthermore, in 2009 a Portuguese

group published several models for prediction of major morbidity end-points following heart surgery, including AKI, and in 2011 a group in Germany published a model for prediction of postoperative dialysis [24].

The most commonly used end-point definition was renal failure requiring postoperative dialysis [22, 24, 85, 88, 89]. Otherwise various end-point definitions were applied, and some of them represented quite serious forms of AKI, such as estimated glomerular filtration rate below 30 [18], or a two-fold increase in serum creatinine [6, 86, 87]. Definitions including milder forms of AKI were also used by a few, applying either relative or absolute increases in postoperative serum creatinine compared with the preoperative value, or using a threshold value of postoperative serum creatinine [5, 12, 25, 90]. None of the mentioned publications had used the definition of AKI proposed by the Acute Kidney Injury Network [27, 28], or any other standardized definition [6, 18, 22, 24, 85-89].

Two studies had included intraoperative risk factors, such as time on cardiopulmonary bypass, in their risk models [25, 90]. Most studies included a measure of preoperative kidney function. Serum creatinine concentration was the most widely used [6, 12, 25, 85-88], whereas two research groups had used creatinine clearance estimated by the Cockcroft-Gault formula [22, 89, 91]. We found that a comparison of models using different end-point definitions and different measures of preoperative kidney function would be of interest, as well as assessment of the added value of intraoperative variables.

Inflammatory biomarkers and cardiac dysfunction

Cardiac surgery evokes an inflammatory response, which is considered important in the development of postoperative complications [10]. The inflammatory reaction has often been attributed to the surgical trauma itself, contact with foreign surfaces in the cardiopulmonary-bypass circuit and ischaemia and reperfusion injury due to aortic cross-clamping (Figure 3) [8, 92, 93]. The inflammatory response includes activation of plasma cascade systems including the complement system [10, 94, 95] coagulation and fibrinolysis [96], endothelium [97] and leukocytes [10, 95, 98], with release of multiple cytokines [10, 97, 98] and altered expression of adhesion molecules [97].

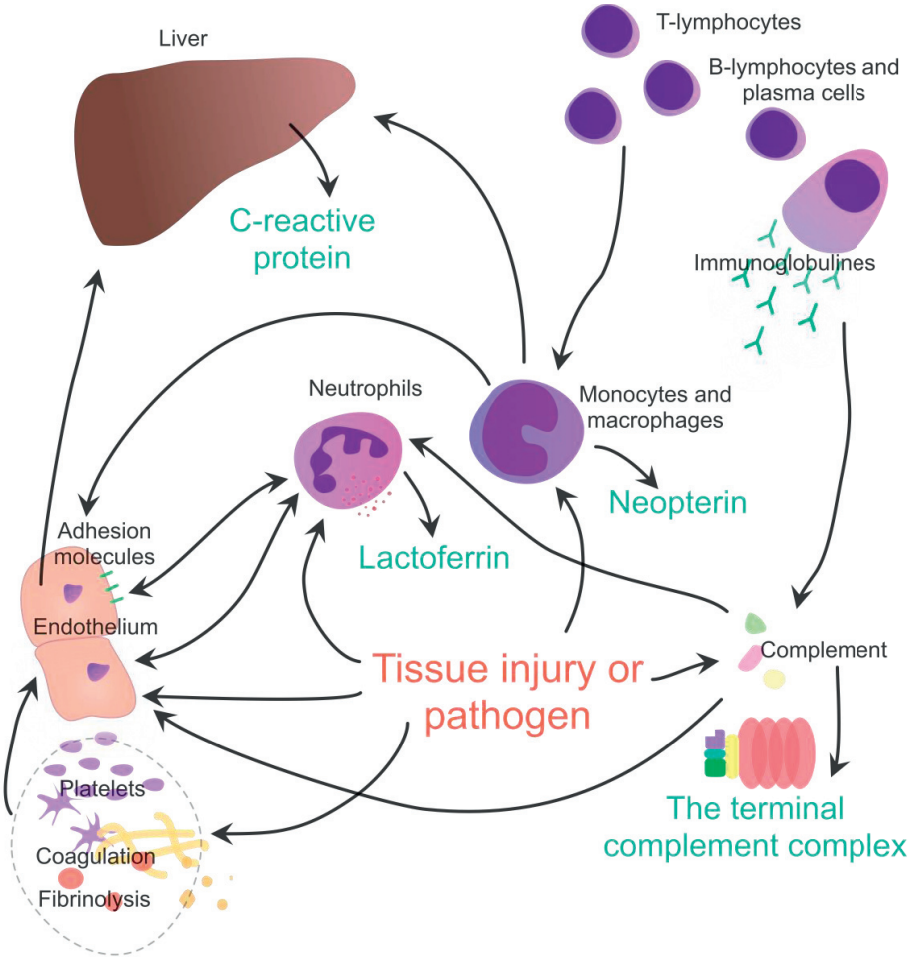

Figure 3. Simplified overview of inflammation with focus on the pathways relevant to this thesis. The surgical trauma and the surfaces in the cardiopulmonary bypass circuit activate several inflammatory pathways, including leukocytes, endothelium, platelets and plasma cascade systems including complement, coagulation and fibrinolysis. The adaptive immune system, including lymphocytes and immunoglobulins, interacts with the innate immune system through cytokines and more directly via complement activation. The inflammatory biomarkers investigated in this thesis are highlighted in green.

C-reactive protein is secreted by the liver in response to pro-inflammatory cytokines. Lactoferrin is released by activated neutrophils. Neopterin is released by activated monocytes and macrophages after stimulation with interferon-γ from T-lymphocytes. The terminal complement complex is the end-product of the complement cascade.

In some patients, a greatly enhanced inflammatory response is seen, which may contribute to the development of several complications. It may present as coagulopathy, as coagulation is tightly interlinked with inflammation [8]. Cardiopulmonary bypass may cause platelet activation and dysfunction, and disturbs the delicate balance between coagulation and fibrinolysis [99]. Coagulation is activated by several mechanisms, leading to consumption of coagulation factors and triggering of fibrinolysis, and may present as severe bleeding, commonly without a surgical focus [99].

Increased inflammation may also be associated with organ dysfunction [8]. Need for prolonged ventilation, often due to pulmonary failure, is fairly common after cardiac surgery [100]. There is evidence of neutrophil sequestering in the pulmonary capillaries and endothelial damage after cardiac surgery [101]. The kidneys may also be affected by inflammation, and postoperative AKI is not uncommon [18]. The pro-inflammatory cytokine tumour necrosis factor has been indicated to induce renal infiltration of inflammatory cells, renal cell apoptosis and renal vasoconstriction, resulting in reduced glomerular filtration rate [102]. Endothelial dysfunction may lead to reduced cerebral vasodilation and neurologic dysfunction [103]. In some cases a permanent stroke may result [5], although emboli are probably the most common cause of stroke after cardiac surgery. If production of anti-inflammatory cytokines dominate the inflammatory response following cardiac surgery, immunosuppression and postoperative infections may result [104].

Several important molecules in inflammatory pathways have been shown to have cardio-depressant effects [105]. These include interleukins-1 and -8, complement component 3a and tumour necrosis factor-α [105]. Furthermore, the myocardium itself has been shown to produce large amounts of pro-inflammatory cytokines following cardiac surgery with cardiopulmonary bypass [41].

The degree of inflammatory response after cardiac surgery is difficult to evaluate directly. In order to investigate the inflammatory response inside the body we need markers of the ongoing processes. The term biomarker refers to biological markers, or medical signs, that can be observed from outside the patient, indicating something about the state inside the patient [106]. In this thesis, the term biomarker refers to a molecular

biomarker. In order to explore some potential underlying pathways of postoperative inflammation, we have focused on four molecular biomarkers measured in plasma, reflecting different aspects of inflammation. A blood sample requires puncturing of a blood vessel, but is easily obtainable and less invasive than inserting catheters or removing tissue.

C-reactive protein

C-reactive protein is widely used as a general marker of inflammation, and is an acute phase protein secreted by the liver and adipose tissue in response to inflammatory stimuli (Figure 3) [107]. Its plasma concentration may increase more than 1000-fold following a moderate inflammatory stimulus, and its production is stimulated by the pro-inflammatory cytokine interleukin-6 [108]. Interleukin-6 increases during cardiac surgery [41]. In clinical practice, C-reactive protein is usually quantified above 5 mg/L. For analysis of low-grade inflammation one must apply a more sensitive assay, quantifying C-reactive protein also below 5 mg/L. This is often referred to as high-sensitivity C-reactive protein or hsCRP.

C-reactive protein and its association with cardiac disease have been thoroughly studied both in population-based studies and high-risk individuals, showing associations with both reduced ventricular function and ischaemic heart disease [109-117].

C-reactive protein has been associated with heart failure in several population-based studies, excluding participants with established coronary heart disease at baseline to minimize the possibility that elevated C-reactive protein was due to a recent myocardial infarction [114-116, 118]. Cesari et al. assessed 2225 persons aged 70 to 79 years, and found an association between C-reactive protein and congestive heart failure [114]. However, the association was not significant after adjusting for established risk factors [114]. C-reactive protein was not associated with coronary heart disease or stroke in the same population, whereas interleukin-6 was a significant predictor of congestive heart failure, coronary heart disease and stroke [114]. On the other hand, Gottdiener et al. found that C-reactive protein was a strong predictor of congestive heart failure in a population-based study of 5888 subjects older than 65 years, also after adjustment for atherosclerotic disease and other conventional risk factors [115].

In a population-based study of 5691 participants, Kardys et al. found that C-reactive protein was associated with heart failure in men after exclusion of participants with coronary heart disease at baseline, and after adjustment for established risk factors [116]. The association was not significant in women after adjustment for established risk factors for heart failure, and it was suggested that the reason could be that heart failure in men tend to result from ischaemic heart disease, whereas women more often have heart failure due to hypertension [116].

Arroyo-Espliguero and co-workers found that higher levels C-reactive protein was associated with poor functional status and that it correlated with left ventricular ejection fraction in patients with chronic stable angina pectoris [109]. The same research group also found an association between C-reactive protein levels and the risk of adverse cardiac events in patients with chronic stable angina pectoris [110]. However, they found no association between C-reactive protein and adverse cardiac events in patients with chronic stable angina pectoris when testing C-reactive protein and neopterin in the same analysis [113]. Furthermore, Videm and colleagues found no association between C-reactive protein and coronary artery stenosis in a study of 234 patients, where a wide selection of inflammatory markers representing numerous aspects of inflammation was evaluated [112]. Thus, association studies of C-reactive protein and cardiovascular disease have demonstrated somewhat conflicting results, particularly studies assessing C-reactive protein and other inflammatory markers concomitantly [111-114, 116]. It is uncertain whether C-reactive protein is solely a marker of inflammation, or whether it exerts an effect on the cardiovascular system leading to disease progression and complications. Nevertheless, we found that it was of interest to investigate its relation to cardiac dysfunction following open-heart surgery.

Lactoferrin

Lactoferrin is an iron-binding protein in the transferrin family found in external secretions and in the secondary granules of neutrophil granulocytes (Figure 3) [119]. The amount of lactoferrin in blood is usually low [120]. It correlates with neutrophil counts and probably arises from neutrophil degranulation [120]. The plasma lactoferrin concentration increases during inflammation. Lactoferrin possesses anti-microbial properties, and is proposed to have anti-inflammatory and immunomodulatory effects

[119]. The mechanisms are suggested to be through limiting the iron concentration at inflammatory sites [121], binding bacterial endotoxin [122] and inhibiting production of oxygen-free radicals by neutrophils [123].

Neutrophils are activated during cardiac surgery with cardiopulmonary bypass, and the concentration of lactoferrin in plasma increases [95, 124, 125]. A French research group compared polymorphonuclear leukocyte counts and lactoferrin concentrations in myocardial venous blood with that of peripheral venous and arterial blood during and after cardiopulmonary bypass [126, 127]. Their findings suggested that activated neutrophils may be sequestered in the myocardium or the myocardial vascular bed during cardiopulmonary bypass and to a greater extent following reperfusion upon the release of the aortic cross clamp [126, 127]. It was also suggested that reperfusion induced additional neutrophil degranulation [126, 127].

Few studies have assessed the association of lactoferrin and cardiovascular disease. However, higher levels of lactoferrin have previously been associated with coronary artery stenosis [112], and fatal ischaemic heart disease in patients with newly diagnosed diabetes mellitus type 2 [128]. These findings suggest that neutrophil granulocytes and lactoferrin may be involved in the development of cardiac dysfunction following open-heart surgery. This biomarker is less well studied than for instance C-reactive protein [129]. It remains to be investigated whether there is an association between preoperative lactoferrin concentration as a measure of preoperative activation of neutrophils and the development of postoperative cardiac dysfunction.

Neopterin

Neopterin is released from activated macrophages and monocytes after stimulation with interferon-γ from activated T-lymphocytes (Figure 3) [130, 131], and is often considered a marker of monocyte activation and activation of the cellular immune system. The release of neopterin by macrophages is correlated with the release of hydrogen peroxide, and is thus related to the amount of oxidative stress generated [132].

One of the first associations between neopterin and cardiac function was published by Barani et al. in 2006, who found an association between neopterin and left ventricular ejection fraction and diastolic left ventricular diameter in patients with critical limb

ischaemia [133]. Later, Estevez-Louriero and co-workers found that neopterin was associated with left ventricular ejection fraction and cardiac dysfunction in patients with chronic stable angina pectoris [129].

Neopterin has also been associated with coronary artery disease [111-113]. Vengen et al. found that neopterin predicted the risk of death from ischaemic heart disease in patients with type 2 diabetes mellitus [111], and Videm et al. found that neopterin was associated with significant coronary artery stenosis [112]. Avanzas and colleagues found that neopterin was associated with adverse cardiac events in patients with chronic stable angina pectoris during a one-year follow-up [113].

Neopterin has been shown to induce contractile dysfunction in isolated perfused rat hearts [134]. Although the effective concentration in that study was higher than neopterin levels occurring in vivo, it was suggested that long-term influence of lower levels of neopterin could lead to cardiac dysfunction in humans [134]. This effect could possibly be mediated through oxidative stress. An in vitro study has shown that neopterin enhanced the oxidative effect of hydrogen peroxide, measured by chemoluminescence and the bactericidal effect of hydrogen peroxide, suggesting that the neopterin molecule possesses oxidative properties [135].

Thus, neopterin has been shown to be a promising marker in cardiovascular research, and we found that it would be interesting to evaluate the association between neopterin and cardiac dysfunction after cardiac surgery.

The terminal complement complex

The complement system belongs to the innate immune system and consists of more than 30 proteins in plasma and on cell surfaces [136]. It can be activated mainly via three pathways, the classical, alternative and the lectin pathways (Figure 4) [136]. These activation cascades involve cleavage of several proteins, resembling the cascades of coagulation and fibrinolysis [136]. Terminal complement activation leads to the formation of the terminal complement complex, or membrane attack complex (Figure 4) [136]. The complement proteins included in the terminal complement complex is designated C5b, C6, C7, C8 and C9 (C5b-9) [136]. The main functions of the complement system include defence against microorganisms, clearance of apoptotic

cells and immune complexes, and bridging the innate and adaptive immune system [136]. If complement is activated in tissue injury it can cause further damage by the generation of the membrane attack complex, and chemotaxis and activation of leukocytes [137].

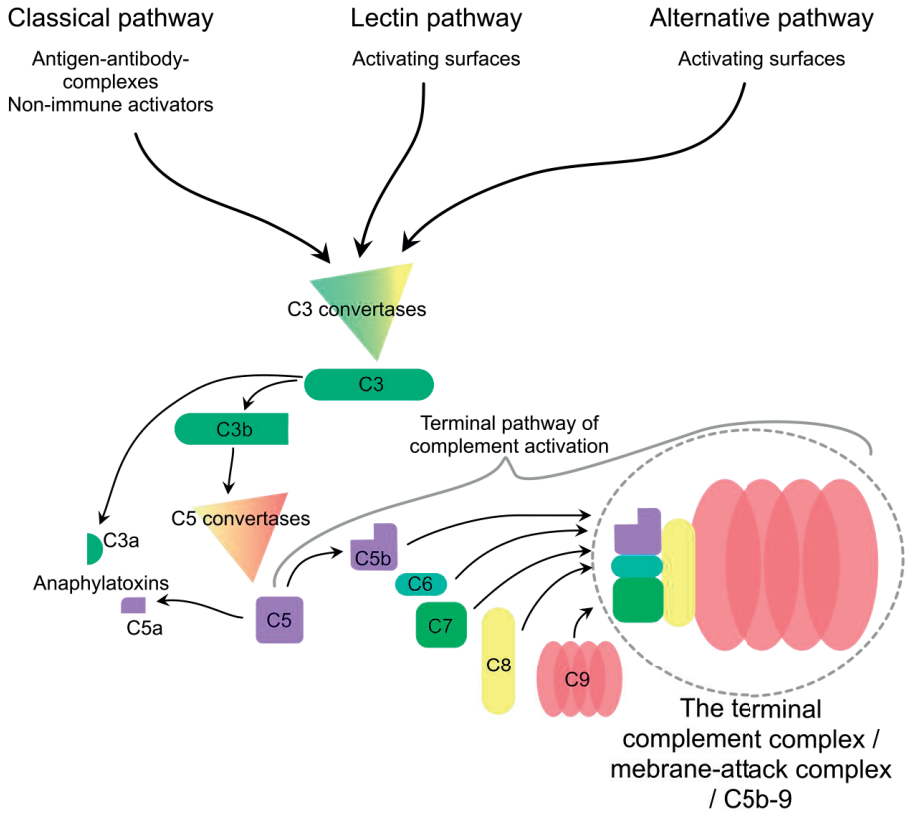

Figure 4. Simplified overview of the complement system. The complement system can be activated via three pathways, the classical, the alternative and the lectin pathways. Activation results in cleavage of a series of proenzymes into active enzymes, a cascade reaction of enzyme activation. Some of the smaller cleavage products, such as C3a and C5a, are called anaphylatoxins. Anaphylatoxins may attract and activate neutrophils and other inflammatory cells. Terminal complement activation results in formation of the terminal complement complex (C5b-9), which is capable of inducing cell lysis.

Numerous studies have shown that the complement system is activated during cardiac surgery with cardiopulmonary bypass [124, 125, 138]. Terminal complement activation also occurred after cardiac surgery with cardiopulmonary bypass, with levels decreasing

during the first eight hours after admission to the intensive care unit [138]. The initial pathways of complement were activated again eight hours after admission to the intensive care unit, but without formation of the terminal complement complex [138].

Terminal complement activation has been hypothesized to play an important role in myocardial damage following ischaemia and reperfusion. Ito et al. used rabbits with and without the ability to generate C6, a part of the terminal complement complex, to study infarction size after 30 minutes and two hours of coronary artery occlusion and area of reflow, and its relation to complement activation. They found that congenitally C6-deficient animals had smaller infarction size after 30 minutes of coronary occlusion, and larger area of reflow after two hours of coronary occlusion compared to animals who were C6-competent. Histological examination with immunocytochemical staining showed that the terminal complement complex accumulated at the border of the infarction after 30 minutes of ischaemia, while after two hours of ischaemia the terminal complement complex was deposited throughout the infarcted myocardium. Their results suggested that terminal complement activation may lead to reperfusion injury in rabbits [139].

It was, however, less certain if this was the case in humans. No increased myocardial terminal complement activation was found during cardiac surgery and in the reperfusion period in a study of ten patients without heart failure, ten patients with ischaemic heart failure and ten patients with idiopathic non-ischaemic heart failure [140]. The results indicated that complement was deposited in the myocardium preoperatively [140]. On the contrary, a randomised controlled trial of the soluble complement receptor type I, an inhibitor of the classical complement pathway, showed that the soluble complement receptor inhibited complement activity effectively and reduced mortality and the frequency of myocardial infarction in male patients, but not in female patients [141]. Moreover, a sub-analysis of patients undergoing coronary artery bypass grafting combined with valve surgery demonstrated an elimination of a need for an intra-aortic balloon pump postoperatively [141].

Furthermore, circulating levels of the terminal complement complex have also been associated with coronary heart disease and cardiac dysfunction following myocardial

infarction. Videm et al. found that lower plasma levels of the terminal complement complex were associated with coronary artery stenosis [112]. Furthermore, in a study of 74 patients with acute occlusion of the left anterior descending coronary artery treated with primary percutaneous coronary intervention, Haahr-Pedersen and colleagues demonstrated that lower plasma concentration of the terminal complement complex also was associated with cardiac dysfunction following acute myocardial infarction [142]. Clark et al. found higher levels of circulating terminal complement complex in 36 patients with congestive heart failure compared with 12 age-matched controls [143]. Patients with poor functional status and patients who had an adverse outcome within six months had higher levels of complement activity in plasma compared with patients who remained event-free during follow up or patients with better functional class [143].

The role of the terminal complement complex in human cardiac disease is still debated. It remains to be investigated whether preoperative complement activity is associated with cardiac dysfunction after open-heart surgery.

Hypotheses

The overall hypothesis of this thesis was that mortality and AKI could be accurately predicted using preoperative variables collected routinely in clinical practice, and that increased preoperative inflammation could affect the development of postoperative cardiac dysfunction.

The specific hypotheses to be tested in this thesis were:

1. Operative mortality and AKI following adult cardiac surgery may be accurately predicted from a set of variables collected in clinical routine work.
2. Inclusion of intraoperative variables will improve the accuracy of outcome prediction.
3. The definition of the AKI end-point is important for which risk factors are found to be significant and for their effect size.
4. More accurate estimates of kidney function will yield more precise predictions of AKI risk after adult cardiac surgery.
5. Local risk prediction models for mortality and AKI after open-heart surgery will be more accurate in our population than some previously published risk prediction models.
6. One or several of the inflammatory biomarkers C-reactive protein, lactoferrin, neopterin and the terminal complement complex are associated with cardiac dysfunction following cardiac surgery in adults.

Aims

The first main aim of this thesis was to investigate whether it was possible to predict mortality and AKI after open-heart surgery accurately to provide precise information on operative risk to patients and health care providers.

Despite fairly accurate statistical models it remains difficult to foresee which patients will develop a certain complication or not, and an explanation could be that unmeasured factors play an important part in the development of complications after cardiac surgery. Inflammation is hypothesized to play a pivotal role in the development of several complications following open-heart surgery the second main aim was to investigate whether increased preoperative inflammation could affect the development of postoperative cardiac dysfunction.

Death is the most serious complication following cardiac surgery, and the aims of Paper I were therefore:

1) To develop a local preoperative mortality risk prediction model for cardiac surgery and assess its performance in our population.
2) To investigate if the inclusion of intraoperative variables could enhance predictive ability of the model.
3) To assess the performance of the additive and the logistic EuroSCORE in our population, and compare them to a local preoperative risk model.

AKI is one of the most common and most serious morbidities that may arise after cardiac surgery. The aims of Paper II were therefore:

4) To develop a local preoperative risk prediction model for postoperative AKI following cardiac surgery and assess its performance in our population.
5) To investigate how the model would change with changes in the end-point definition.
6) To assess whether intraoperative variables and more accurate estimates of kidney function could improve the model.
7) To validate the previously published risk models for AKI by Antunes et al. [144] and Brown et al. [18] in our population.

Cardiac dysfunction is also a common and serious complication following heart surgery. The inflammatory response following cardiac surgery may cause severe dysfunction in several organs, such as the heart, and inflammation has also been demonstrated to be of importance in the development of heart disease outside the setting of cardiac surgery. The aim of Paper III was therefore:

8) To investigate whether the four inflammatory biomarkers C-reactive protein, lactoferrin, neopterin and the terminal complement complex were associated with cardiac dysfunction after heart surgery.

Patients and methods

Data

This thesis is founded on two different cohorts of cardiac surgical patients at St. Olavs University Hospital in Trondheim, Norway. In the first cohort we included all adult patients who underwent open-heart surgery from 1 January 2000 to 31 December 2007 (n = 5029). The second cohort included all adult patients undergoing cardiac surgery from 1 April 2008 to 19 April 2010 and who gave their informed consent (n = 1055). The data were collected prospectively as part of the department's quality assurance routines, and a selection of the variables are listed in Table 2. The data were quality assured and stored in a local database.

Table 2. Selection of the variables that were registered in the database

Variable type	Example of variables
Patient characteristics and history	Age, gender, degree of urgency for surgery, recent myocardial infarction or angina, endocarditis or aortic disease
Other diseases and risk factors	Chronic pulmonary disease, hypertension, peripheral vascular disease, diabetes mellitus, previous cardiac surgery and smoking status
Preoperative blood tests and examinations	Creatinine, haemoglobin, potassium, liver transaminases and myocardial infarction markers, electrocardiographic findings, angiographic findings and measurements and echocardiographic findings and measurements
Medication	Diuretics, angiotensin converting enzyme inhibitors and receptor blockers, calcium antagonists, beta-blockers, statins, antiarrhythmic agents and antiplatelet and anticoagulant treatment
Surgical data	Type of operation and description of procedures
Intraoperative measurements	Temperature, blood pressure, time on cardiopulmonary bypass, pump flow, type of cardioplegia, need for inotropic support, defibrillation, red blood cell transfusion and pacemaker, and electrocardiographic changes
Postoperative complications	Re-operation, bleeding, myocardial infarction, acute cardiac dysfunction, arrhythmias, acute kidney injury, infections, intubation time, re-intubation, cerebrovascular incident, death
Postoperative blood tests	Similar to those performed before surgery
Postoperative treatment	Blood transfusions and inotropic medication

We collected preoperative peripheral blood samples from all patients included in the second cohort, using the arterial line. The blood samples were centrifuged within six hours, and were kept on ice before centrifugation. Plasma, serum and buffycoat were stored at -80°C until analysis. For the second cohort, informed consent was obtained for all participants. The need for informed consent for the first cohort was waived by the Norwegian Data Inspectorate.

Of 1149 patients eligible for the second cohort, 21 did not consent, 32 were unable to consent due to emergency surgery, seven were unable to consent due to language problems, and 57 had missing blood samples. We also excluded 14 patient samples due to the following reasons: One had infectious blood, three had active endocarditis, two underwent off-pump surgery, one did not have data on the end-point, and seven samples due to an identification error preventing coupling with clinical data. Thus, 1018 patients were included in the further analyses.

Data from the first cohort was used in Paper I and Paper II, and data from the second cohort was used in Paper III. Variable definitions for all three papers are listed in Table 3. Both projects were approved by the Norwegian Data Inspectorate and the Regional Research Ethics Committee in Medicine, Trondheim, Norway. The surgical team was experienced and very stable over the study period, and there were no relevant changes in the use of cardiopulmonary bypass.

Risk model development

The model development started with formulation of hypotheses for selection of variables that could predict the outcome. The hypotheses were based on clinical knowledge and previous research publications, and the hypothesized predictors also had to be relevant for a sufficient proportion of the patients. We did not perform any univariable screening of the predictors, as this increases the risk of over-fitting the model to the developmental dataset, and potentially reduces the predictive ability in future datasets [145]. The risk prediction models were developed using logistic regression, and the entire dataset was used for model development, as splitting the dataset into smaller subsets reduces the power and increases the risk of over-fitting [145].

An underlying assumption of binary logistic regression is that a continuous explanatory variable is linearly related to the log odds of the outcome [145]. Odds are defined as the probability for the outcome divided by one minus the probability for the outcome. The assumption of linearity was tested by including spline functions of the continuous variables in the model. Spline functions include several terms, and can fit almost any shape of the relationship because the approach allows several breaking points in the curve displaying the relationship between the variable and the log odds of the outcome. The deviation from linearity of the spline function was assessed, and if it was significant, the continuous variable was transformed. For practical purposes in clinical work we transformed non-linearly related continuous variables to categorical variables for development of risk prediction models, whereas logarithmic transformation was used for non-linear inflammatory biomarkers in Paper III.

Another underlying assumption of logistic regression is that the effects of the explanatory variables can be added together, i.e. that there is no statistical interaction [145]. This means that the effect of age is not different for different categories of another variable. For example, if a patient is 80 years old and his serum creatinine is 150 μmol/L, his risk of the outcome is not greater or smaller than what would be expected if one added the effect of these two variables. Interactions were tested by adding cross-products of the potential interactions to the model.

Overly-influential observations are extreme observations that strongly influence the model, and potentially will reduce the predictive ability of the model in future datasets [145, 146]. To identify influential observations one can use plots of various residuals and look for extreme outliers. To determine whether an observation is overly-influential, one can re-fit the model, but leave out this observation, and notice how much the coefficients in the model changes [146, p. 245].

When these requirements were taken care of, the number of predictors in the model was reduced using backward step-down, retaining variables according to Akaike's Information Criterion. To achieve robust estimates of the coefficients, and to reduce the risk of over-fitting, the reduced models were bootstrapped (n = 400) [145]. Bootstrapping refers to a method for creating new datasets based on the original one by

Table 3. Variable definitions for Paper I, II and III

Variable	Definition	Paper
Demographics		
Age	Years (continuous)	II
	Above 68 years (no/yes)	I
Female gender	(no/yes)	I, II
Body mass index	kg/m^2 (continuous)	I
	Above or equal to 30 kg/m^2 (no/yes)	II
Smoking	Current smoker or quit less than 6 months ago (no/yes)	I
Medication		
ACE-inhibitor use	Treated with angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker (no/yes)	II
Lipid-lowering treatment	Treated with statins (no/yes)	II
Intercurrent or previous disease		
Diabetes mellitus	Receiving medication (no/yes)	I, II
Hypertension	Receiving medication or diastolic blood pressure above 90 mmHg (no/yes)	I, II
Left ventricular hypertrophy	Diagnosed by electrocardiography or echocardiography (no/yes)	I
Endocarditis	Receiving antibiotic treatment for endocarditis (no/yes)	II
Previous myocardial infarction	Previously undergone myocardial infarction (no/yes)	III
Previous cardiac surgery	Previously undergone cardiac surgery (no/yes)	I, II, III
Chronic cardiac insufficiency/heart failure	Medical treatment for chronic cardiac insufficiency /chronic heart failure (no/yes)	I, II, III
Pulmonary hypertension	Systolic pulmonary arterial pressure (PAP) > 40 mmHg or mean PAP > 25 mmHg, echocardiography or catheterisation (no/yes)	I, III
Chronic pulmonary disease	Use of bronchodilating agents or forced expiratory volume in 1 s (FEV_1) < 75% (no/yes)	I, II
Peripheral vascular disease	Intermittent claudication, carotid stenosis or abdominal aortic aneurysm (no/yes)	I, II
Preoperative renal dysfunction	Creatinine concentration > 140 µmol/L or dialysis (no/yes)	III
Blood samples		
Creatinine	Above 140 µmol/L (no/yes)	I
	Three categories (< 100 µmol/L; 100 – 140 µmol/L; > 140 µmol/L)	II
Creatinine clearance	Estimated creatinine clearance by the Cockcroft-Gault formula [91], three categories (> 90 mL/min; 60 – 90 mL/min; < 60 mL/min)	II
eGFR	Estimated glomerular filtration rate by the four-variable Modification of Diet in Renal Disease formula for standardised creatinine values [147], three	II

	categories (> 90 mL/min/1.73m^2; 60 – 90 mL/min/1.73m^2; < 60 mL/min/1.73m^2)	
Haemoglobin	Haemoglobin concentration (g/dL), (continuous)	I, II, III
Operation related		
Degree of urgency for surgery	Three categories: standard waiting list, operation within two weeks, operation within 24 h	I
	Emergency operation, within 24 h (no/yes)	II
	Urgent operation, within two weeks (no/yes)	III
Operation type	Four categories: 1: Coronary artery bypass grafting (CABG) or atrial septum defect. 2: Aortic valve replacement (AVR) only, AVR and CABG combined, non-ischaemic mitral valve surgery or aneurysm in the ascending aorta 3: Dissection of the ascending aorta and ventricular septum rupture. 4 Miscellaneous surgery[1].	I, II
	Three categories: 1: Coronary artery bypass grafting (CABG) or atrial septum defect. 2: Aortic valve replacement (AVR) only, AVR and CABG combined, non-ischaemic mitral valve surgery or aneurysm in the ascending aorta 3: Dissection of the ascending aorta, ventricular septum rupture and miscellaneous surgery[1]	III
Intraoperative variables		
Cardiopulmonary bypass	Cardiopulmonary bypass during surgery (no/yes)	I
Cardiopulmonary time	Time on cardiopulmonary bypass per 10 minutes (continuous)	I, II
Fluid balance	Fluid balance during surgery (tertiles)	I
	Above 3500 mL (no/yes)	II
Inotropic support	On clinical indication during surgery (no/yes)	I, II
Plasma transfusion	On clinical indication during surgery (no/yes)	I, II
Red blood cell transfusion	On clinical indication during surgery (no/yes)	I, II
Vasoconstrictor use	On clinical indication during surgery (no/yes)	I, II
Main end-points		
Death	Death within 30 days after surgery or during the same hospital stay (no/yes)	I
Acute kidney injury	A 50% or greater increase in creatinine concentration, an absolute increase in creatinine of 26.4 µmol/L or more, or a new requirement for dialysis (no/yes)	II
Cardiac dysfunction	Need for more than one inotropic agent or an intra-aortic balloon pump (no/yes)	III

[1]Miscellaneous surgery consisted of various operations like mitral valve surgery in combination with CABG or AVR, AVR in combination with procedures other than CABG or aneurysm of the ascending aorta, and other cardiac surgery like pericardiectomy and removal of cardiac tumours.

sampling with replacement [146, pp. 87-88]. Each sample contains the same number of patients, but the patient composition will differ between each sample in order to better represent the entire population of possible cardiac surgery patients.

Model validation and comparison

The models were internally validated, in part by using bootstrapping methods (n = 400). Calibration of a model describes how the predicted risk for the outcome compares with the actual risk for the outcome throughout the range of predicted risks [145, 148]. If the model generally predicts a risk that is too high or too low the calibration is poor. The calibration of the model can be studied by examining the calibration curves (including the intercept and slope), which display the model calibration.

Shrinkage refers to how the calibration curves deviates from the ideal 45° line because of over-fitting to the developmental dataset [145, 146]. An estimated shrinkage factor should be above 0.85, corresponding to an error in future predictions less than 15%.

Discrimination is the model's ability to differentiate between patients who have the outcome or not. The receiver operating characteristic (ROC) curve is a plot of the sensitivity versus one minus specificity throughout the range of possible cut-off points [146, 149]. The c statistic is a widely used measure of predictive model discrimination, and for binary outcomes it is identical to the area under the ROC curve [145]. A c statistic above 0.80 is considered good [146]. We also compared the c statistic of the different models with the same end-point, applying the method proposed by DeLong et al., for correlated non-parametric areas under ROC curves [150].

Comparison of model c statistics is, however, considered an insensitive measure of improvement in discrimination [145, 151, 152], and we therefore also used the integrated discrimination improvement [152] for assessing differences in discrimination between models in Paper II and Paper III. The integrated discrimination improvement is a measure of the estimated difference in average sensitivity minus the estimated difference in average specificity across all possible cut-offs, and was developed primarily to detect the added value of a new predictive marker [152].

The Hosmer Lemeshow test assesses the goodness-of-fit of the model, which is a comparison of the predicted risk and the number of actual outcomes in equally sized groups, where subjects are grouped according to predicted risk [153]. The number of groups is usually ten, and should not be less than six. The lowest number of groups in our tests was nine. The test is similar to a Chi-square test, and the null hypothesis is that the model fits the data. A *p*-value below 0.05 means that the model predicts a risk for the outcome that is different from what is observed, and usually *p*-values greater than 0.10 are considered adequate.

Previously published risk prediction models

We calculated the risk according to previously published risk prediction models in our patients, assessed the performance of these models, and compared them to our local risk prediction models. We validated the models with the Hosmer-Lemeshow test, ROC curves and calculation of the c statistic, and compared the c statistics of the previously published models to that of our own model for the relevant end-point.

Paper I

Preoperative model

In Paper I, we first developed a preoperative model for prediction of early mortality after open-heart surgery. The end-point was in-hospital mortality, defined as death occurring during the same hospital admission or within 30 days after surgery. The in-hospital mortality was 2.7%, corresponding to 135 of 5029 patients.

Sixteen preoperative variables were hypothesized to be important predictors of in-hospital mortality: Age, found best to be modelled as above the median age of 68 years, female gender, body mass index, diabetes mellitus, smoking, hypertension, chronic cardiac insufficiency, peripheral vascular disease, chronic pulmonary disease, left ventricular hypertrophy, pulmonary hypertension, preoperative haemoglobin concentration, serum creatinine above 140 μmol/L, previous cardiac surgery, degree of urgency, and type of operation. The operation types were grouped according to average risk, so that surgical procedures in the same group would have approximately the same risk of mortality.

Left ventricular ejection fraction was also considered an important predictor of in-hospital mortality, but it was measured either by catheterisation or echocardiography, and the exact value was only registered in 4043 of 5029 patients (80.4%). Thus, the quality of the left ventricular ejection fraction variable was considered too poor to be used for logistic regression model development as a continuous variable, and was therefore excluded. Instead we used the presence or absence of chronic cardiac insufficiency as a potential predictor. Chronic cardiac insufficiency was defined as receiving medical treatment for this condition. In Paper III, chronic cardiac insufficiency is referred to as chronic heart failure, which was a more commonly used term. The preoperative risk model was developed and validated according to the description above in the "Risk model development" section.

Intraoperative models

To investigate whether the inclusion of intraoperative variables could yield a more accurate model we added five intraoperative variables to the final preoperative model: whether the patient was on cardiopulmonary bypass, need for inotropic support, vasoconstrictor use, intraoperative fluid balance, red blood cell transfusion, and plasma transfusion.

As 2.7 % of our patients were operated without cardiopulmonary bypass, we developed a second intraoperative model excluding patients operated without cardiopulmonary bypass. Since the time on cardiopulmonary bypass was considered important for outcome prediction, we exchanged information on whether the patient was on cardiopulmonary bypass with cardiopulmonary bypass time. The two intraoperative models were developed and validated, and the performance of the preoperative and the two intraoperative models was compared by comparing the c statistics of the models, and with ROC curves and other plots.

Previously published models

The additive EuroSCORE was calculated prospectively for every patient during data collection and stored in the database, whereas the logistic EuroSCORE was calculated retrospectively from the registered variables. We found matching definitions for all variables except neurological dysfunction. This variable was excluded from the

calculation of the predicted probability, which means it was set to be absent for all patients. If data on other variables were missing for individual patients, it was set to the alternative giving the lowest risk. The exact left ventricular ejection fraction was, as previously stated, not registered in 986 patients, but was always registered if the patient had reduced ventricular function according to the referring cardiologist. Hence, when left ventricular ejection fraction data were missing, it was also set to the category giving the lowest risk. The EuroSCOREs were validated and compared to our local preoperative model.

Paper II

In Paper II we developed a local model for risk prediction of AKI following cardiac surgery, and evaluated how alternative end-point definitions, alternative measures of preoperative kidney function and the inclusion of intraoperative variables would change the model. We also assessed the performance of two previously published models in our dataset.

The main end-point of Paper II was AKI after open-heart surgery, defined as either a relative increase in creatinine of at least 50% after surgery compared with the concentration before surgery, an absolute increase in serum creatinine of 26.4 μmol/L (0.3 mg/dL) or more, or a new requirement for dialysis. This definition may be regarded as a slight modification of the criteria proposed by the Acute Kidney Injury Network [27, 28], as the urine output criterion was not included, and we allowed a longer time span than 48 hours postoperatively for an increase in creatinine to occur. Preoperative creatinine was measured 1-2 days before surgery except in emergency patients, where it was measured on admission. Postoperative creatinine was measured several times postoperatively, and we registered creatinine values from the first postoperative day, the maximum creatinine value during the hospital stay, and creatinine at discharge. To calculate the end-point the maximum postoperative creatinine value was used.

According to this definition 633 patients (12.7%) suffered AKI. Exclusion criteria for the analyses included in Paper II were preoperative dialysis (n = 9) and missing information on preoperative or maximum postoperative creatinine concentration (n = 42). No specific alterations in preoperative or operative procedures were done based on

preoperative kidney function. Aprotinin was used in most patients with rupture of the ventricular septum or dissection of the ascending aorta, and some patients with endocarditis (less than 182 patients or 3.6%).

Primary model

Sixteen variables were hypothesized as important predictors of AKI after open-heart surgery: Age, female sex, body mass index, lipid lowering treatment, ACE inhibitor use, diabetes mellitus, hypertension, chronic cardiac insufficiency, endocarditis, peripheral vascular disease, chronic pulmonary disease, haemoglobin concentration, serum creatinine, previous cardiac surgery, emergency operation, and type of operation. The operation types were grouped according to average risk, so that surgical procedures in the same group would have approximately the same risk of AKI. Body mass index did not fulfil the assumption of linearity in the logit, and was therefore modelled as two categories: below/equal to 30 kg/m^2 or above 30 kg/m^2. The preoperative risk model for prediction of AKI was developed and validated according to the description above.

Alternative models

We developed several alternative models to investigate how the model and its performance would change with alternative end-point definitions, more accurate kidney function estimates or inclusion of intraoperative variables. The models were developed and validated as described above.

Alternative end-point definitions

Three models were developed using alternative end-point definitions: 1) A 50% or greater increase in serum creatinine or a new requirement for dialysis after surgery; 2) an absolute increase in creatinine of 26.4µmol/L (0.3 mg/dL) or more, and 3) a 25% or greater increase in creatinine. The two first definitions are variations of the AKI Network definition; the third is regarded as mild AKI. Only 65 patients had a new requirement of dialysis postoperatively, and therefore we did not develop a separate model for this end-point, as it would greatly increase the risk of over-fitting the model to the dataset if more than a few potential explanatory variables were included.

More accurate kidney function estimates

We calculated the estimated glomerular filtration rate (eGFR) applying the four-variable Modification of Diet in Renal Disease formula for standardised creatinine [147], and the estimated creatinine clearance applying the Cockcroft-Gault formula [91]. We developed two alternative models to Model I, exchanging serum creatinine with either eGFR or creatinine clearance. Because of strong correlation, we excluded the variables used to calculate eGFR (age and sex) and creatinine clearance (age, sex and body mass index) from the development of these models. Because of non-linearity in the logit eGFR and creatinine clearance were divided into three categories (above 90; 90 to 60; below 60 mL/min/1.73 m^2) and (above 90; 90 to 60; below 60 mL/min), respectively.

There were quite a few females with normal creatinine and reduced eGFR: Among the females who had creatinine < 100 μmol/L (n = 1164), 9.5% had eGFR > 90, 49.1% had eGFR 60 – 90, and 41.3% had eGFR < 60. Thus, eGFR or creatinine clearance would perhaps be a more accurate measure of preoperative kidney function particularly in women. We therefore compared the discrimination of Model I with the discrimination of the eGFR and creatinine clearance models when used for females only.

Intraoperative variables

Six intraoperative variables were added to Model I: Time on cardiopulmonary bypass, inotropic support, vasoconstrictor use, fluid balance above 3500 mL, red blood cell transfusion and plasma transfusion. We excluded patients operated without cardiopulmonary bypass (n = 136, 2.7%) from the development of the intraoperative model. Because only 1% of the patients received platelets without concomitant plasma transfusion we did not include platelet transfusion as a separate risk factor in the intraoperative model.

Previously published models

We evaluated the performance of two previously published models, Antunes and colleagues' model for renal failure [144] and Brown and colleagues' model for renal insufficiency [18], in our dataset. The models' predicted probabilities were calculated retrospectively. The two models were chosen because they had been developed using end-point definitions comparable to the main end-point definition we had used, and the

relevant variables matched with variables in our database, except that data were missing on preoperative white blood cell count to be used for Brown's model [18].

Paper III

In Paper III we evaluated the association between the four inflammatory biomarkers C-reactive protein, lactoferrin, neopterin and the terminal complement complex, and cardiac dysfunction after open-heart surgery in order to explore some of the underlying mechanisms of cardiac dysfunction following cardiac surgery. Cardiac dysfunction was defined as the need for more than one inotropic agent or an intra-aortic balloon pump occurring after the operation and until the patient was discharged from the department, and occurred in 95 (9.3%) patients according to this definition. Patients with signs of intercurrent infection other than endocarditis and high levels of C-reactive protein were normally not considered for elective heart surgery.

Biomarkers

The four inflammatory biomarkers were analysed in plasma using enzyme immunoassay (EIA). C-reactive protein is considered a general marker of inflammation, and is measured in all patients submitted for heart surgery. We used a high-sensitivity method for C-reactive protein measurement (sometimes denoted hs-CRP) using a commercial kit (Quantikine Human C-Reactive Protein Immunoassay, R&D Systems, Inc, Minneapolis, USA). Lactoferrin was analysed as previously described [154], whereas neopterin and the terminal complement complex were analysed using commercial kits (Neopterin ELISA, GenWay Biotech Inc, San Diego, USA) and (MicroVue SC5b-9 Plus EIA, Quidel Corporation, San Diego, USA), respectively.

Statistical analysis

We used logistic regression to evaluate the association of the four biomarkers with cardiac dysfunction after cardiac surgery. We first performed an unadjusted analysis, analysing each fluid-phase marker separately. Then we performed an adjusted analysis including the eight preoperative clinical variables found to be significant predictors of cardiac dysfunction in a previous study from our group [33]: Previous myocardial infarction, previous cardiac surgery, chronic heart failure, pulmonary hypertension, preoperative renal dysfunction, preoperative haemoglobin concentration, urgent

operation and operation type. All four biomarkers were fitted in the same model with the clinical variables. The model was then tested for linearity in the logit, overly-influential observations, interactions and collinearity.

Preoperative renal dysfunction was strongly correlated with neopterin, and was therefore removed from the model. We evaluated alternative models with and without renal dysfunction and serum creatinine as a sensitivity analysis, to make sure we did not fail to account for a possible confounding effect of preoperative renal dysfunction on neopterin levels and cardiac dysfunction. Several other researchers have identified age and sex as important predictors of postoperative cardiac dysfunction [155, 156], and we therefore performed an alternative analysis including age and sex, even though these variables previously were not significant in our population [33]. In order to fulfil the assumption of linearity in the logit neopterin and the terminal complement complex were transformed by natural logarithm. Only inflammatory markers that were significant after adjustment for clinical variables were included in the further analyses.

To ensure that the results were consistent also after adjustment with a more general risk prediction model we performed a sensitivity analysis. We used the EuroSCORE II to calculate the predicted risk, and performed a logistic regression analysis including neopterin and the predicted risk according to the EuroSCORE II as adjustment variable [157].

We performed a likelihood ratio test to evaluate if the significant biomarker could improve the prediction of cardiac dysfunction after heart surgery compared with clinical variables alone. Since the risk model in this study was based on a previously published model from our research group, we did not use backward elimination to reduce the number of risk factors. We also compared model discrimination between clinical variables alone and clinical variables and neopterin by calculating the c statistic [150] and the integrated discrimination improvement [152].

General statistics

Continuous variables are given as mean with 95% confidence intervals for normally distributed data and median with 95% confidence intervals for non-normally distributed data, and categorical variables are given as frequency with percentage, unless otherwise

stated. For inter-group comparisons we have used the Mann-Whitney U-test and Pearson's Chi Square test. Linear correlation was assessed with Pearson's correlation coefficient. For statistical analyses and modelling we have applied the statistic software R (versions 2.10.1 and 2.12.0, R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria), SPSS (versions 16.0 and 18.2, SPSS Inc., Chicago, IL and IBM Corporation, Armonk, NY, USA), SigmaPlot (versions 11.0 and 13.0, Systat Software Inc., San Jose, CA, USA), and MiniTab (versions 15.1.30.0 and 17, MiniTab Inc., State College, PA, USA). R was used for the logistic regression model development and validation, SPSS was used for data and variable handling and simple statistical analyses, SigmaPlot was used for comparing the areas under the receiver operating characteristic curves and for creating horizontal scatterplots with error bars, and MiniTab was used for calculating 95% confidence intervals for medians.

Summary of results

Paper I

The preoperative mortality risk prediction model is summarised in Table 3, and consisted of eight risk factors for early mortality after cardiac surgery: age above 68 years, degree of urgency, female gender, serum creatinine above 140 µmol/L, chronic pulmonary disease, chronic cardiac insufficiency, previous cardiac surgery, and type of operation.

Table 4. Mortality risk prediction model

Variable	Coefficient	Odds ratio	95% conficence interval	*p*-value
Age above 68 years	1.141	3.128	(2.026-4.831)	< 0.0001
Degree of urgency				
Standard waiting list		1.000	Reference	
Operation within 2 weeks	0.397	1.487	(0.969-2.306)	0.11
Operation within 24 hours	1.927	6.870	(3.525-13.389)	< 0.0001
Female gender	0.541	1.718	(1.130-2.612)	0.03
Serum creatinine > 140 µmol/L	1.234	3.434	(2.007-5.874)	< 0.0001
Chronic pulmonary disease	0.783	2.189	(1.440-3.327)	0.0001
Chronic cardiac insufficiency	0.690	1.994	(1.293-3.076)	0.002
Previous cardiac surgery	0.782	2.185	(1.147-4.165)	0.006
Operation type				
CABG or atrial septum defect		1.000	Reference	
Pure AVR, AVR and CABG, non-ischaemic mitral valve surgery, or aneurysm of ascending aorta	0.621	1.861	(1.176-2.946)	0.01
Dissection of ascending aorta, or ventricular septum rupture	1.906	6.723	(2.918-15.489)	< 0.0001
Miscellaneous	1.483	4.407	(2.614-7.430)	< 0.0001
Intercept	-6.045			

The strongest associations were between the outcome and type of operation, degree of urgency, age above 68 years and serum creatinine above 140 µmol/L. The c statistic was 0.857 (0.823-0.891), denoting good discriminatory ability, and the Hosmer-Lemeshow test indicated satisfactory goodness-of-fit (p = 0.62). The calibration curves showed that it predicted mortality accurately throughout the dataset, except for the 1% of patients at extremely high risk, where it was somewhat less accurate. The estimated

shrinkage factor was 0.93, indicating that the model also would predict mortality accurately in future datasets.

The intraoperative mortality risk prediction models showed that time on cardiopulmonary bypass, the need for inotropic support and red blood cell transfusion during surgery were the most important intraoperative predictors of early mortality. The c statistic was 0.877 (0.823-0.891) for the model without cardiopulmonary bypass time, and 0.866 (0.833-0.898) for the model including cardiopulmonary bypass time. These two models also showed good performance, but did not substantially improve the accuracy compared to the preoperative model ($p > 0.10$ for all comparisons). The Hosmer-Lemeshow test showed adequate goodness-of-fit ($p = 0.34$ and 0.75, respectively).

The logistic and the additive EuroSCORE displayed good discrimination, with c statistic of 0.821 (0.785-0.857) and 0.846 (0.810-0.881), respectively. The difference in c statistics was significant between the logistic EuroSCORE and our local preoperative model ($p = 0.02$), but not between the additive EuroSCORE and our local preoperative model ($p = 0.40$). The Hosmer-Lemeshow test was significant for both the logistic and the additive EuroSCORE, ($p = 0.0008$ and $p < 0.0001$, respectively), denoting that the EuroSCOREs predicted a mortality that was significantly different from the observed mortality (Figure 5), indicating poor calibration.

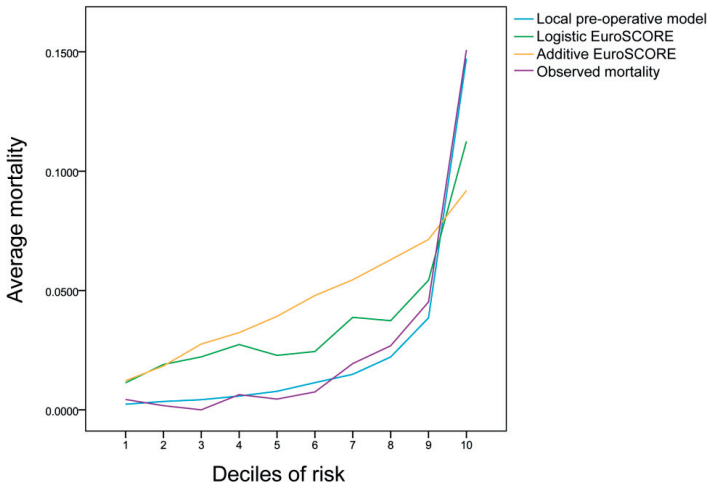

Figure 5. Observed and predicted mortality across the deciles of estimated risk for the local preoperative mortality risk model and the additive and the logistic EuroSCORE.

Paper II

Primary model

The primary model for prediction of AKI is summarised in Table 5 and consisted of eleven preoperative risk factors for AKI following cardiac surgery.

Table 5. Acute kidney injury risk prediction model

Variable	Coefficient	Odds ratio	95% confidence interval	*p*-value
Age (per 10 years increase)	0.479	1.614	(1.430-1.821)	< 0.0001
Body mass index above 30 kg/m^2	0.276	1.318	(1.023-1.697)	0.03
Lipid lowering treatment	-0.322	0.725	(0.589-0.891)	0.005
Hypertension	0.440	1.553	(1.273-1.894)	< 0.0001
Peripheral vascular disease	0.291	1.338	(1.003-1.786)	0.04
Chronic pulmonary disease	0.569	1.767	(1.391-2.244)	< 0.0001
Haemoglobin concentration (g/dL)	-0.176	0.839	(0.787-0.894)	< 0.0001
Serum creatinine				
Below 100 µmol/L		1.000	Reference	
100 – 140 µmol/L	1.031	2.805	(2.229-3.529)	< 0.0001
Above 140 µmol/L	2.308	10.058	(7.047-14.356)	< 0.0001
Previous cardiac surgery	0.775	2.171	(1.512-3.118)	< 0.0001
Emergency operation	0.731	2.077	(1.383-3.119)	0.0007
Operation type				
CABG and atrial septum defect		1.000	Reference	
AVR, AVR and CABG, non-ischemic mitral valve surgery, aneurysm of the ascending aorta	0.688	1.989	(1.555-2.543)	< 0.0001
Dissection of ascending aorta or rupture of the ventricular septum	1.632	5.116	(2.377-11.012)	< 0.0001
Miscellaneous	0.971	2.640	(1.885-3.696)	< 0.0001
Intercept	-3.867			

Important predictors of postoperative AKI were age, body mass index above 30 kg/m^2, lipid lowering treatment (protective effect), hypertension, peripheral vascular disease, chronic pulmonary disease, haemoglobin concentration, serum creatinine (below 100 µmol/L, 100-140 µmol/L and above 140 µmol/L), previous cardiac surgery, emergency

operation and operation type. The strongest predictors of AKI were elevated serum creatinine, operation type, emergency operation and age.

The c statistic for the model was 0.819 (0.801-0.837), denoting good discrimination. The Hosmer-Lemeshow test *p*-value was 0.17, indicating adequate goodness-of-fit, and the estimated shrinkage factor was 0.976, indicating an estimated error in future predictions of 2.4%. The calibration curves showed that the model was well calibrated for patients with a predicted risk of AKI of less than 0.50, while the 4.4% of patients with predicted probability above 0.50 the model somewhat overestimated the risk of AKI.

Alternative models

The alternative models are summarised in Figure 6, showing variables and corresponding odds ratios with 95% confidence intervals.

Alternative end-point definitions

The models developed using alternative end-point definitions of AKI included the same variables, and the coefficient estimates changed very little (Figure 6). Creatinine and body mass index were best modelled as continuous variables in the model where AKI was defined as ≥ 50% increase in creatinine or a new requirement for dialysis, and peripheral vascular disease was no longer significant. The model where AKI was defined as an increase ≥ 26.4 μmol/L (0.3 mg/dL) or more in creatinine was very similar to the primary model.

The model for prediction of an increase in creatinine ≥ 25% included the same variables. However, certain coefficients changed somewhat, particularly for body mass index (0.435 versus 0.276 in the primary model) and creatinine (0.584 and 0.881 versus 1.031 and 2.308 in the primary model). Validation showed satisfactory discrimination and goodness-of-fit.

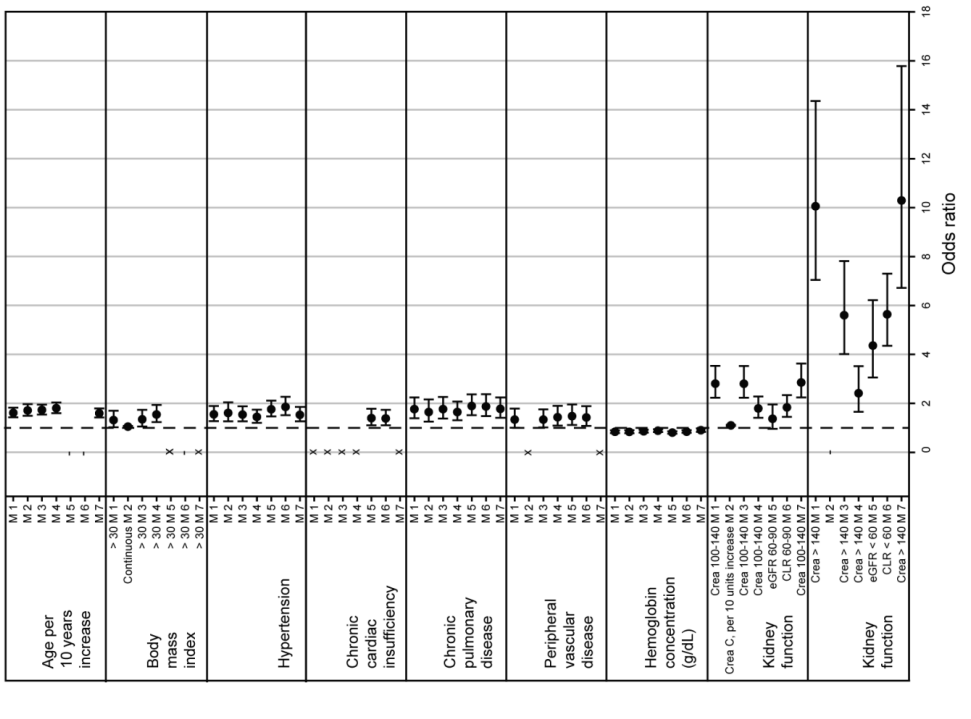
Variables and model number
Odds ratio
Age per 10 years increase
Body mass index
Hypertension
Chronic cardiac insufficiency
Chronic pulmonary disease
Peripheral vascular disease
Hemoglobin concentration (g/dL)
Crea C, per 10 units increase
Kidney function
Kidney function
Lipid lowering treatment
Previous cardiac surgery
Emergency operation
Operation type 2
Operation type 3
Operation type 4
Intraoperative variables
Inotropic support 7
Red blood cell transfusion 7
CPB time per 10 minutes 7
Odds ratio
Variable not significant in model
Variable not included in model

Figure 6. Model comparison. Plot showing odds ratios with 95% confidence intervals for all variables in all models. Odds ratios are grouped according to variable to enable comparison among models, and the models are indicated with numbers from 1 to 7. The models differ regarding which end-point was used, how kidney function was estimated, and whether intraoperative variables were included.

End-points: *1) ≥ 50% increase in creatinine, an absolute increase in creatinine ≥ 26.4 µmol/L or a new requirement for dialysis, 2) ≥ 50% increase in creatinine or a new requirement for dialysis after surgery; 3) an absolute increase in creatinine ≥ 26.4 µmol/L, and 4) ≥ 25% increase in creatinine.*

Models: M1*: Model I, endpoint 1),* ***M2****: End-point 2),* ***M3****: end-point 3),* ***M4****: end-point 4),* ***M5****: end-point 1) and creatinine exchanged with estimated glomerular filtration rate[147],* ***M6****: end-point 1) and creatinine exchanged with creatinine clearance [91],* ***M7****: end-point 1) and intraoperative variables included.*

Abbreviations: CLR*: creatinine clearance (mL/min),* ***Crea****: Creatinine (µmol/L),* ***Crea C****: Creatinine, continuous (µmol/L),* ***eGFR****: estimated glomerular filtration rate (mL/min/1.73m^2),* ***Operation type 2****: Aortic valve replacement (AVR), AVR and coronary artery bypass grafting, non-ischemic mitral valve surgery, aneurysm of the ascending aorta,* ***Operation type 3****: Dissection of ascending aorta or rupture of the ventricular septum,* ***Operation type 4****: Miscellaneous surgery.*

More accurate kidney function estimates

Figure 6 illustrates that the models where creatinine was exchanged with eGFR or creatinine clearance were quite similar to the primary model. There were only minor differences with regard to significant predictors and coefficients. In the eGFR model body mass index was not a significant predictor, whereas it was excluded from the creatinine clearance model. Lipid lowering treatment was not significant in the eGFR model, and chronic cardiac insufficiency was a significant predictor in both the eGFR and the creatinine clearance model. Age was excluded from both models, and gender was not a significant predictor in the primary model.

The c statistic was 0.793 (0.774-0.812) for the eGFR model, and 0.801 (0.782-0.819) for the creatinine clearance model. These models with a more accurate estimate of kidney function than creatinine showed slightly poorer discrimination than the primary model (p = 0.05 and 0.02 respectively). When comparing discrimination by the integrated discrimination improvement this difference was even greater (-0.0379 for the eGFR model, -0.0317 for the creatinine clearance model, $p < 0.0001$ for both comparisons). In females, discrimination by the primary model (c statistic = 0.798 (0.764-0.832)), the eGFR model (c statistic = 0.779 (0.742-0.832)) and the creatinine

clearance model (c statistic = 0.773 (0.735-0.812) were not significantly different (p = 0.11 and 0.06 respectively).

Intraoperative variables

Cardiopulmonary bypass time, inotropic support and red blood cell transfusion were important intraoperative predictors of AKI. The intraoperative model was not more accurate than the primary model (p = 0.22) when comparing c statistics, whereas the integrated discrimination improvement showed that the intraoperative model had better discrimination than the primary model (0.0290, $p < 0.0001$).

Model comparison

Figure 6 shows that the primary model was robust, and that generally the models consisted of the same risk factors and the odds ratios for each variable were similar, but some of the odds ratios for the various estimates of kidney function changed somewhat.

Previously published models for acute kidney injury

The Hosmer-Lemeshow test p-values for Brown's and Antunes' models were < 0.0001, denoting poor goodness-of-fit in our population. The c statistic of Brown's model was 0.653 (0.630-0.676), suggesting that it had poor discrimination in our population. Antunes' model displayed acceptable discrimination with a c statistic of 0.740 (0.718-0.762). The previously published models had significantly poorer discrimination than our primary model for prediction of AKI ($p < 0.0001$ for both comparisons). The integrated discrimination improvement was -0.1960 when comparing Antunes' model with our primary model, and -0.192 for the comparison with Brown's model ($p <$ 0.0001 for both comparisons).

Comparison of preoperative models for prediction of mortality and acute kidney injury

Figure 7 illustrates that the preoperative model for mortality risk prediction and the primary (preoperative) model for prediction of AKI showed both similarities and differences, both with regard to significant predictors and coefficient estimates. The two models included 13 predictors altogether, of which six were significant in both models (age, creatinine, chronic pulmonary disease, previous cardiac surgery, degree of urgency for surgery and operation type). Two were only significant in the mortality model

(female gender and chronic cardiac insufficiency), and five were only significant in the AKI model (body mass index, lipid lowering treatment, haemoglobin concentration, hypertension and peripheral vascular disease).

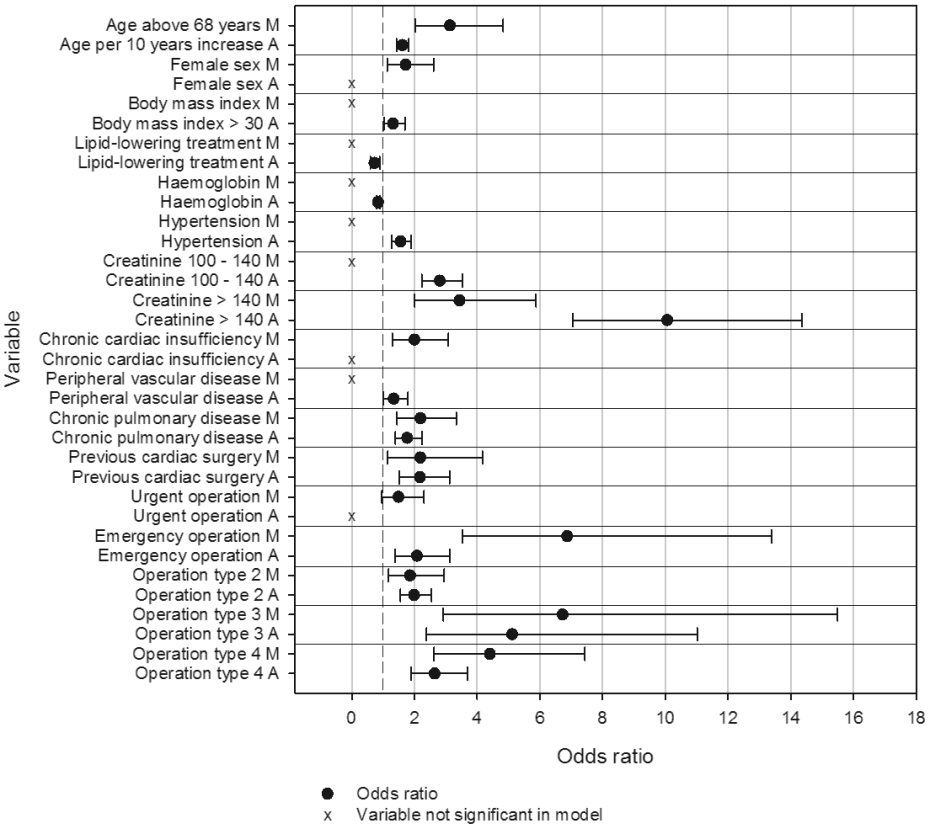

*Figure 7. Comparison of risk models for mortality and AKI. Plot showing odds ratios with 95% confidence interval listed according to variable to enable comparison between the models. The letter **M** or **A** following each variable name indicate the mortality model and the AKI model, respectively. Operation types are defined in Table 4 and Table 5.*

It is difficult to compare age directly between the two models because age was transformed to a categorical variable in the mortality model, but age was highly significant in both models ($p < 0.0001$) (Table 4 and Table 5). Creatinine was more

strongly associated with AKI than with mortality, whereas the odds ratios for chronic pulmonary disease and previous cardiac surgery were quite similar. Emergency operation and the operation types had an even greater impact on the mortality risk than on the risk of AKI.

Paper III

In the unadjusted analysis, both C-reactive protein and neopterin were significantly associated with cardiac dysfunction after open-heart surgery (Table 6). However, after adjustment for urgent operation, operation type, previous cardiac surgery, chronic heart failure, pulmonary hypertension, previous myocardial infarction and preoperative haemoglobin concentration, neopterin was the only biomarker that remained significant ($p = 0.0005$) (Table 6). Neopterin was correlated with C-reactive protein ($R = 0.27$, $p < 0.0005$).

Preoperative renal dysfunction was strongly correlated with neopterin ($R = 0.37$, $p < 0.0005$), and was therefore removed from the model. The sensitivity analysis demonstrated that there was no difference in odds ratios after renal dysfunction or creatinine was removed from the model. Neither renal dysfunction nor serum creatinine were significant risk factors for cardiac dysfunction in our dataset. We also developed an alternative model including age and sex, but the odds ratios for the biomarkers were essentially unchanged (data not shown).

There was a significant association of neopterin with cardiac dysfunction also when the EuroSCORE II was used for adjustment ($p = 0.03$). The neopterin model adjusted using the variables from the local risk model for postoperative cardiac dysfunction had a higher c statistic than the neopterin model adjusted using the EuroSCORE II (0.883 (0.779-0.874) vs. 0.776 (0.726-0.825), $p = 0.02$) in our patients.

According to the likelihood ratio test, neopterin improved the model fit ($p < 0.0001$). Neopterin increased the model c statistic from 0.817 (0.770 – 0.863) to 0.833 (0.779 – 0.874) ($p = 0.07$) (Figure 8), and the integrated discrimination improvement was 0.014 ($p = 0.02$), when comparing the model containing only clinical variables with the model including neopterin as well, suggesting that neopterin increased discrimination somewhat.

Variable	Unadjusted analysis			Adjusted analysis[1]			Final model[2]		
	OR	95% confidence interval	*p*-value	OR	95% confidence interval	*p*-value	OR	95% confidence interval	*p*-value
C-reactive protein	1.02	(1.01-1.04)	< 0.0001	1.01	(1.00-1.03)	0.07	-	-	-
Lactoferrin	1.00	(1.00-1.00)	0.57	1.00	(1.00-1.00)	0.85	-	-	-
Neopterin[3]	3.23	(2.10-4.97)	< 0.0001	2.38	(1.33-4.24)	0.003	2.73	(1.65-4.51)	0.0005
Terminal complement complex[3]	1.34	(0.95-1.90)	0.099	1.31	(0.88-1.95)	0.19	-	-	-

[1]All four biomarkers in the same model, adjusted for urgent operation, operation type, previous cardiac surgery, chronic heart failure, pulmonary hypertension, previous myocardial infarction and preoperative haemoglobin concentration.
[2]Final model adjusted for urgent operation, operation type, previous cardiac surgery, chronic heart failure, pulmonary hypertension, previous myocardial infarction and preoperative haemoglobin concentration.
[3]Transformed using natural logarithm.

Figure 8 illustrates that inclusion of neopterin particularly increased discrimination for a group of patients. These were 380 (37.3%) patients with predicted risks between 2.5% and 6.4%. This corresponded approximately to the 25th percentile, and the 60th percentile, i.e. the patients with intermediate predicted risks, having a few risk factors for cardiac dysfunction.

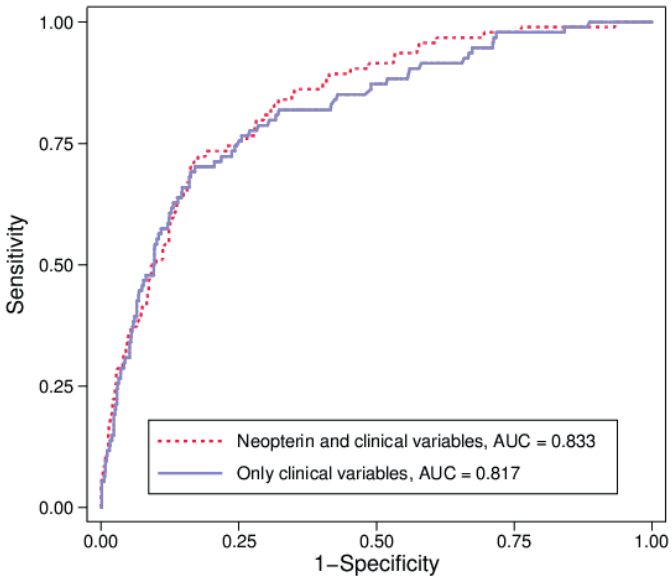

Figure 8. Receiver operating characteristic curve for neopterin and clinical variables compared with clinical variables alone.

Discussion

Main findings

In Paper I, we developed a preoperative risk prediction model for operative mortality following cardiac surgery. The model included eight risk factors routinely registered in clinical practice and displayed good discrimination and calibration in our population. The mortality risk prediction model was more accurate than the logistic and the additive EuroSCORE.

In Paper II, we found that AKI after cardiac surgery could be accurately predicted using eleven preoperative risk factors easily obtainable in clinical routine work. Serum creatinine concentration yielded more accurate predicted risks than eGFR or creatinine clearance, and inclusion of intraoperative variables improved the model slightly.

In Paper III we found that elevated neopterin levels before cardiac surgery was an independent predictor of postoperative cardiac dysfunction.

Methodological considerations

Logistic regression

We used multivariable binary logistic regression analysis for development of the risk prediction models, and for investigating the association between the inflammatory biomarkers and cardiac dysfunction. It is a widely used method, but it has certain limitations. Firstly, the linearity assumption in logistic regression may be a limitation, since it assumes a linear relationship between the explanatory variables and the logarithmically transformed risk of the outcome. If the assumption of linearity generally does not fit the data, the predictions from the logistic regression model will not be accurate. It is therefore important to test the model's calibration and discrimination. As mentioned, artificial neural networks is an alternative method not relying on an assumption of linearity [82, 83]. However, logistic regression is available in most statistics software packages, while artificial neural networks require programming of the network in order to make predictions.

Study period

For development of the risk prediction models for mortality and AKI we used data collected from 2000 through 2007. Time has passed since then, and we cannot exclude that recent changes in treatment strategies and patient characteristics may influence the performance of the models. Thus, the risk models ought to be calibrated in a more recently collected dataset before application in contemporary cardiac surgery.

The data used for the analyses of association between the inflammatory biomarkers and cardiac dysfunction after open-heart surgery were collected more recently, from 2008 to 2010.

Sample size and single centre

We have used data from only one surgical centre, serving the population of Middle Norway, and that influences the generalizability of our findings. Thus, the risk models for operative mortality and AKI following cardiac surgery must be calibrated if they are to be applied elsewhere [158]. The findings regarding the association between neopterin and cardiac dysfunction ought to be evaluated in other cardiac surgical populations as well.

The number of observations in the dataset, as well as the number of patients with the outcome, is of consequence to the number of risk factors that can be included in the model development process [145]. This includes interaction terms that ought to be tested and the number of categories for categorical variables [145]. Most mortality risk prediction models from multi-centre data published since 2008 were developed from a larger patient sample than ours [6, 86, 87, 157, 159-169]. Compared with other single-centre models published since 2008, the size of our sample was 5029 patients, some studies being larger [170], and some being smaller [171-175]. Therefore, we had to be more careful when selecting potential predictors than if we had a larger sample size. The sample size limited the number of categories in continuous variables that was transformed due to not fulfilling the linearity assumption. Another option could have been to transform variables in the risk prediction models using natural logarithm or another method of transformation, but that would make the clinical application and interpretation more difficult, especially to clinicians with little statistical experience.

The sample size also affected the number of categories in categorical variables. It is possible that our models would have been even more accurate if we could have used more categories. Even so, both the mortality and AKI risk prediction models displayed good discrimination and calibration.

Data collection

Collection of clinical data used in this thesis was done prospectively by the treating doctors. The data were controlled several times during registration, and then quality assured by a senior anaesthesiologist before storage in a local database. Despite the thorough quality assurance, we cannot exclude that errors have occurred at some point during registration.

Due to the great efforts made in quality assurance of data collection, the problem with missing information on some variables was generally small. Therefore, we did not use any strategy to deal with missing values, such as multiple imputation.

The blood samples were marked with identification tags at the arterial sampling time before transportation to the laboratory, but we cannot exclude that identification errors occurred.

End-points

Mortality

We measured operative mortality as a combination of in-hospital mortality and 30-day mortality, using linkage to the Norwegian Cause of Death Registry to obtain information on 30-day mortality. In-hospital mortality is often the easiest to register as it does not require investigation of patient status after discharge. However, as there is a trend towards shorter hospital stays more patients are likely to die after the index hospitalization. A German study of mortality among CABG patients in the 1990's found that mortality rates according to 30-day mortality and in-hospital were similar [176]. However, a recent study from Great Britain and Ireland found that 28% of patients that died within 30 days of surgery died after hospital discharge [168]. This number will probably vary between institutions, but one may miss a considerable proportion of patients by including only in-hospital mortality.

It has also been demonstrated that the instantaneous mortality hazard is still increased beyond 30 days after cardiac surgery, and it was suggested that longer follow-up times should be used [176]. A postoperative risk score for 90-day mortality after cardiac surgery was published in 2013 [174]. Measuring mortality during a certain time frame following cardiac surgery requires appropriate gathering of mortality data, such as linkage to national cause of death registries, to be valid [176]. Furthermore, the longer the time-frame after surgery, the more likely it becomes that deaths that are not related to the cardiac operation are also included. It would have been interesting to evaluate if our model for operative mortality would predict 90-day mortality accurately.

Acute kidney injury

We applied a definition of AKI after cardiac surgery that could be regarded as a slight modification of the AKI Network definition because we allowed a longer time span than 48 hours for the maximum creatinine value to occur.

Another common definition of AKI is the Risk/Injury/Failure/Loss/End-stage definition, often referred to as the RIFLE criteria [177]. The AKI Network definition represents a modification of the Risk/Injury/Failure/Loss/End-stage definition [27, 177]. The greatest differences are that the AKI Network definition does not include any eGFR decrease, it does include an absolute increase in serum creatinine as well as the relative increase of 50% or more, and the time frame is limited to 48 hours [27, 177].

We applied a standardized and validated definition of AKI, except that we allowed a longer time span than 48 hours for the maximum creatinine to occur, since the postoperative increase in creatinine is often delayed [20]. A more recently proposed definition of AKI is the Kidney Disease: Improving Global Outcomes definition [178]. This definition is similar to the AKI Network definition, but it allows a time frame of seven days for the relative increase in serum creatinine, instead of 48 hours [27, 178]. The Kidney Disease: Improving Global Outcomes definition is more in line with the definition of AKI that we used in Paper II, and it would probably have been more appropriate to refer to that definition. However, it was published after we had started the work with the AKI risk model, and we were not aware of the new proposal for an AKI definition at that time.

Another study compared the Risk/Injury/Failure/Loss/End-stage definition and the AKI Network definition in 1881 cardiac surgical patients, and showed that more than 40% of AKI events occurred after 48 hours [179]. Moreover, when the AKI Network definition was applied within seven days of surgery it demonstrated superior discrimination with regard to in-hospital mortality compared to the Risk/Injury/Failure/Loss/End-stage definition [179]. The same study also compared the proportion of patients developing AKI according to the Risk/Injury/Failure/Loss/End-stage definition and the AKI Network and Kidney Disease: Improving Global Outcomes definitions [179]. The study showed that the AKI Network definition and the Kidney Disease: Improving Global Outcomes definition resulted in the same frequency and staging of AKI [179]. Moreover, the Risk/Injury/Failure/Loss/End-stage definition and the AKI Network included many of the same patients, but the two definitions did not overlap completely [179].

Sampaio et al. compared the three AKI classifications in 321 patients undergoing cardiac surgery [180]. They found that the incidence and risk factors for AKI after cardiac surgery varied according to the definition used [180]. AKI was associated with increased mortality regardless of which classification was used [180]. They also found that the Kidney Disease: Improving Global Outcomes definition demonstrated improved prognostic power with regard to a composite end-point of mortality, need for dialysis and prolonged hospital stay [180].

It would have been interesting to evaluate how the different AKI classifications would change the AKI risk model in our data. However, our results regarding minor changes in end-point definition indicated that it had little impact on the model.

Cardiac dysfunction

We defined cardiac dysfunction as the need for more than one inotropic agent or an intra-aortic balloon pump occurring after the operation and until the patient was discharged from the department. This definition was dependent upon the clinical judgement of the necessity to start therapy with inotropic agents or an intra-aortic balloon pump in order to maintain sufficient perfusion. Thus, it was not based on objective criteria that could be measured reliably in every patient or in other institutions.

Similar definitions of postoperative cardiac dysfunction have been used by others [32, 34]. A more objective definition could possibly be based on measures of inadequate tissue perfusion, such as mixed venous oxygen saturation [35]. However, that would require the insertion of a pulmonary artery catheter in all patients, which was done in less than 30% of our patients. Cardiac dysfunction is a clinical syndrome, and the definition we applied was considered to include the relevant patients. If we had measured the mixed oxygen saturation in all patients, it would have been interesting to evaluate the relation between the mixed oxygen saturation and the need for more than one inotropic agent or an intra-aortic balloon pump.

Risk prediction models may teach us useful things in clinical research. For instance it is important that the definition of the end-point and the predictors are clinically relevant and easy to use to enable clinical application of the results [181]. Moreover, the variable definitions are important for comparison of results between institutions and for interpretation of results [181].

Variable definitions

Some of the continuous variables had to be transformed because the assumption of linearity during logistic regression modelling was not fulfilled. For the risk prediction models we chose to transform them to categorical variables to preserve the intuitive clinical interpretation of the odds ratios. When categorizing a variable, some information is lost, and we cannot exclude that the models would have been even more accurate if we had been able to keep the continuous variables as such.

In the analysis of the biomarkers, neopterin and the terminal complement complex had to be logarithmically transformed in order to fulfil the linearity assumption. This affected the interpretation of the odds ratios, but was not expected to affect model accuracy.

The variable operation type included four categories covering all cardiac surgery performed in our institution in the study period. Thus, several operation categories were merged to reduce the number of categories because we had to take into account the sample size and the number of patients with the outcome. Operation types with approximately the same risk of the outcome were grouped together, and were consistent

in all three studies, except that in Paper III we used only three categories instead of four due to smaller sample size. The merged operation type variable was highly significant, and our models performed well, but our way of dealing with the operation types may be unusual. We could have used a similar approach as in the EuroSCORE II, using isolated CABG as the reference category, and adding weight for other or additional procedures [157]. Such an approach could possibly have been more intuitive to many surgeons.

Several models have been developed for separate operation types, most frequently for CABG and valve surgery [6, 86, 87, 162-165, 167, 182]. This strategy could perhaps be better for modelling the pathophysiology, because the pathophysiological mechanisms underlying coronary heart disease, heart valve disease and other cardiac pathologies are somewhat different. Thus, the handling of operation types is likely to affect the modelling of all other risk factors included in the model as well. Even so, using four categories of operation types rendered a more general model than if we had developed separate models for each type of operation. It enabled us to predict mortality and AKI in all cardiac surgery patients, which was our aim. However, it is likely that the models were less accurate in subgroups of operation types than if we had included only this type of surgery. It is also possible that this will affect the accuracy of predictions if the model is applied in data from other surgical centres with different procedure-specific mortality.

Moreover, given our sample size, it was not feasible to split the data into even smaller datasets to make separate models for several operation types. Another approach could have been to extend the study period to include more patients, but the longer time that passes from beginning to end of the study, the more likely it is that the study population and treatment strategies changes during the study period.

We have used several categorical variables that in part rely on clinical judgement, such as the ones included in previous or intercurrent diseases, the degree of urgency for surgery and some of the intraoperative variables concerning treatment. For instance, not all patients underwent spirometry, and the diagnosis of chronic pulmonary disease was partly based on whether the patient was receiving bronchodilating medication. Another example is the intraoperative use of blood product transfusions and inotropic agents.

We endeavoured to use as reliable and objective variable definitions as possible, but treatment strategies will likely differ somewhat between institutions and may influence the risk prediction models and their performance elsewhere. Thus, validation and calibration of the risk prediction models before use is warranted. Furthermore, we cannot exclude that local factors not accounted for could impact the results regarding the inflammatory biomarkers, and re-evaluation of the hypothesis in another patient population is desirable.

In our data, the exact value of left ventricular ejection fraction and pulmonary arterial pressure was not registered in all patients, and two different methods of measurement were used (catheterization and echocardiography). As stated in the Patients and methods section, the quality of the left ventricular ejection fraction variable was considered too poor to be used as a continuous variable. The diagnosis of pulmonary hypertension was based on measurement of pulmonary arterial pressure. Although the exact value was not registered in all patients, we believe that the large majority of patients with pulmonary hypertension according to the criteria were identified.

Comparing model discrimination

In Paper I we compared the discrimination of our preoperative risk prediction model with that of the intraoperative models and the logistic and additive EuroSCORE by comparing the models' c statistics. As mentioned, this method is considered to be a conservative test [152]. In Paper II and III we used the integrated discrimination improvement as well as the c statistic, and in Paper III we also used the likelihood ratio test.

If we had used the integrated discrimination improvement in Paper I as well, it is not unlikely that the intraoperative model would have displayed significantly better discrimination than the preoperative model. Recently it has been argued that comparison of c statistics is an invalid measure of improvement in discrimination when applied to two regression models fitted in exactly the same patients [183]. It has also been recommended that the likelihood ratio test is used instead of, and not along with comparison of c statistics, to avoid double testing of the same hypothesis [184]. When several tests are used to test the same hypothesis, problems may arise if the different

tests yield contradicting conclusions [184]. If we had been aware of the recommendation of using the likelihood ratio test instead of comparison of c statistics from the beginning, we might have considered not using the c statistic at all. Even so, ROC curves are useful for descriptive evaluation of model performance, and are suitable for visualizing where the differences in discrimination are greatest [184]. Moreover, comparing model discrimination by comparing c statistics has become a widely used method that many researchers are familiar with. Therefore journal reviewers will often ask for it if it is not already included in a submitted manuscript.

External and temporal validation and recalibration

We validated our models internally using bootstrapping methods. As a considerable sample size is required to include a sufficiently large number of patients with the end-point [185], we have not yet been able to evaluate our models applying temporal validation. Temporal validation refers to validating the risk prediction model in a patient sample derived from the same population, but from a different time period. However, temporal validation and model updating should be feasible within the foreseeable future.

External validation is the only method that assesses the generalizability of the risk prediction model as it evaluates the model performance in a different patient population [186]. It is therefore considered the gold standard of validation[186]. Unfortunately we did not have access to an independently collected dataset from another institution, but it would certainly be of great interest to assess our risk prediction models in a different dataset.

Significant predictors in risk models for mortality and acute kidney injury

Significant predictors in the primary risk prediction models

Six variables were significant in both the mortality and AKI models: Age, creatinine, chronic pulmonary disease, previous cardiac surgery, degree of urgency for surgery and operation type. Two variables, female gender and chronic cardiac insufficiency, were only significant in the mortality model. Five risk factors were only significant in the AKI model: Body mass index, lipid lowering treatment, haemoglobin concentration, hypertension and peripheral vascular disease.

Various studies have identified different risk factors and different weighting, and this may seem somewhat confusing. Moreover, it may be more difficult to convince clinicians of the reliability of the risk prediction model when known risk factors are left out. However, it is important to underline that risk prediction is not the same as assessing causal relationships. The model-building strategy in risk prediction modelling focuses on developing a model that make the most accurate predictions, without taking potential confounders, colliders and mediators into account. Therefore, odds ratios of risk prediction models should not be used for making causal inferences. On the other hand, it is possible to predict an outcome accurately using very few risk factors, even if some known causal risk factors are left out. The modelling process will identify the variables that contain the most statistical information in order to make a precise prediction of the outcome risk in the dataset used. That being said, variables that carry a large amount of statistical information are also likely to be important for development of a certain complication.

Thus, in both the mortality and AKI risk prediction models only a few variables were not significant predictors of the other end-point. This indicates that the mechanisms underlying AKI and death probably are partly overlapping. Serum creatinine was an important predictor of both end-points, and patients who suffered from postoperative AKI had increased mortality. Other researchers have also found an association between postoperative AKI and mortality [20, 21, 26], and this may account for some of the similarity in predictors between the two end-points. However, postoperative AKI or chronic kidney disease has not been shown to be among the most common causes of death in cardiac surgical patients [14, 36]. As mentioned in the Introduction, some of the most common causes of death following CABG are cardiac failure, respiratory failure, haemorrhage, neurologic injury and dysrhythmia [14]. The kidneys are crucial in maintaining homeostasis, and therefore impaired renal function will affect the entire organism.

Age and creatinine were strongly associated with both mortality and AKI. A direct comparison of respective odds ratios is difficult because they were not modelled in the same way in both models. Age was dichotomized in the mortality model and continuous in the AKI model, and creatinine was modelled with two categories in the mortality

model and three categories in the AKI model. However, the *p*-values for age and creatinine were all below 0.0001 in both models.

The need for emergency surgery (operation within 24 hours) was more strongly associated with mortality than with AKI. Emergency surgery was probably associated with higher mortality partly because a considerable proportion of these patients would have died without immediate surgery, and some of them instead died during surgery. The association between emergency surgery and AKI also indicates that the emergency patients who survive the operation have an increased risk of AKI. Potential mechanisms increasing the AKI risk particularly in emergency patients include haemodynamic instability, which may enhance inflammation and oxidative stress to the kidneys.

The presence of chronic pulmonary disease was associated with both AKI and death. Chronic obstructive pulmonary disease has been associated with systemic inflammation and dysfunction of other organ systems [187], and may in turn lead to death. However, the association between chronic pulmonary disease and kidney disease has not been thoroughly examined. Van Gestel et al. found that chronic pulmonary disease was associated with chronic kidney disease [188]. Although the underlying mechanisms of chronic kidney disease may differ from those of AKI, our findings indicate that there could be an association between chronic pulmonary disease and AKI as well.

The surgery-related risk factors operation type, previous cardiac surgery and degree of urgency, and their association with both end-points are partly associated with cardiopulmonary bypass time. Cardiopulmonary bypass time is in turn connected with systemic inflammation [8, 9, 92, 93]. The systemic inflammation includes activation of leukocytes [10, 95, 98], endothelium [97] and plasma cascade systems including complement [10, 94, 95] and coagulation [96].

The mortality risk prediction model showed better discrimination than the AKI risk prediction model, with c statistics 0.857 (0.823-0.891) and 0.819 (0.801-0.837), respectively. A possible reason could be that mortality is a more clear-cut end-point than is AKI. Although objective criteria were used, kidney function spans over a continuum while mortality is dichotomous by nature. This could influence the possibility to predict the outcome accurately, because for AKI the model must predict

how large the increase in creatinine will be. In binary logistic regression the end-point must be dichotomous, and perhaps the model fits better with a naturally dichotomous end-point.

Intraoperative variables

Significant intraoperative predictors of both mortality and AKI were time on cardiopulmonary bypass, the need for inotropic support and red blood cell transfusion. We neither found a significant improvement in discrimination according to comparison of model c statistics for the mortality nor the AKI risk prediction models. However, the c statistics indicated that discrimination was somewhat improved by adding information about intraoperative variables. In Paper II we used the integrated discrimination improvement in addition to the comparison of model c statistics, which showed a significant improvement in discrimination. It is possible that the mortality risk prediction model including intraoperative variables would have shown significantly better discrimination according to the integrated discrimination improvement. However, how large a clinically relevant integrated discrimination improvement should be remains to be agreed on.

Our findings suggest that both mortality and AKI can be accurately predicted before surgery, although intraoperative factors, and particularly time on cardiopulmonary bypass, play an important part [189, 190]. It is possible that many of the preoperative risk factors are related to the intraoperative risk factors. For instance operation type and the need for emergency surgery could be good surrogate markers for long duration of cardiopulmonary bypass and need for inotropic support during surgery.

Alternative kidney function estimates and end-point definitions

In the EuroSCORE II, preoperative kidney function was included as creatinine clearance estimated by the Cockcroft-Gault formula [91, 157]. In Paper II, we investigated whether more accurate measures of kidney function could improve the AKI risk prediction model. Although there was a discrepancy in kidney function estimated from creatinine and eGFR, the models including creatinine clearance or eGFR were not as accurate as the model including creatinine. This difference disappeared when we evaluated the model in women only.

A possible explanation could be that each logistic regression model is adapted to the explanatory variables it includes. Thus, the model uses the available information and the coefficients of the other predictors are adapted to the information included in the creatinine variable. It is possible, despite correlation, that the variables used for calculating eGFR and creatinine clearance could not be entirely replaced by eGFR and creatinine clearance alone. Therefore, the primary model would appear as the most accurate since it included those predictors separately instead. Thus, contrary to our hypothesis, these models with more accurate estimates of kidney function than creatinine showed slightly poorer discrimination than the primary model.

Minor changes in end-point definition did not alter the AKI risk prediction model much. The model for prediction of a 25% or greater increase in creatinine included the same risk factors as the primary model, and some of the coefficients changed somewhat. Our results suggested that preoperative kidney function was more important for development of moderate AKI than for mild AKI. Furthermore, body mass index appeared to be more important for mild AKI than for moderate AKI. As changes in the end-point definition had little impact on the model, one may speculate that the mechanisms for milder and more severe forms of AKI are similar.

The usefulness and pitfalls of risk prediction models

Preoperative risk evaluation can be used for individual patient counselling and risk stratification. When predicting risk for individual patients, it is important to emphasize that the predicted risk corresponds to the risk in a group of similar patients, and that each individual will or will not have the complication in question. It is recommended that the risk prediction model c statistic ought to be greater than approximately 0.8 in order to be applicable to individual patients [146, page 247].

It is also possible to use predicted risk for assignment of patients at unacceptable risk to alternative treatment. However, risk prediction models tend to be less accurate in high-risk individuals, including our own models, and therefore it may not be appropriate to base the choice of treatment solely on predicted risk [85, 191]. This may be so because high-risk patients are a diverse group, and many of them have rare risk factors that

cannot be included in a risk model, but may have great impact on the individual patient's risk [192]. Thus, sound clinical judgement is of great importance.

Preoperative risk prediction can be used to identify patients at high risk for a certain complication, and enable initiation of early treatment or even prevent the complication [28]. The list of possible interventions to reduce the risk of AKI includes avoidance of nephrotoxic substances such as intravenous contrast media, non-steroid anti-inflammatory drugs and aminoglycoside antibiotics, or delaying surgery until recovery following a renal insult [28]. Moreover, meticulous monitoring and optimum control of haemodynamics and correction of anaemia preoperatively may decrease the risk of AKI [28]. Avoidance of prolonged aortic cross-clamping and cardiopulmonary bypass times, and minimizing intravascular haemolysis are also thought to be beneficial [28]. However, none of the suggested interventions have shown any considerable effect [28], and no pharmacological treatment has yet been proved effective [193, 194].

In research, risk prediction can be used to identify patients at a greater risk of the outcome one is interested in, in order to increase power if the sample size is limited by external factors like available resources. Often the outcome is rare, and if one applies a risk prediction model to include only high-risk patients, the incidence of the outcome is likely to increase. Thus, the total number of patients investigated can be lower than if one is to include all patients.

Since health care resources are limited, risk prediction models may facilitate a more sensible use of resources by allocating them to those patients who are more likely to benefit from the use of additional resources for monitoring and supportive treatment [8]. Risk models can also be used for adjusting complication rates when comparing surgeons or institutions in quality assurance work [148]. Thus, the impact of a model making systematically erroneous predictions can be considerable, and may affect choice of treatment for individual patients, resource allocation and ranking of surgeons or hospitals.

Local versus multi-centre models

The results of Paper I showed that the EuroSCORE displayed poor fit despite good discrimination in our population, whereas our own preoperative mortality risk model

displayed both good calibration and discrimination, and was better than the EuroSCORE. A similar observation was made by Antunes and colleagues [12].

The data from which the EuroSCORE was developed was collected in 1995 [16], hence the poor fit in our population might arise from changes in patient characteristics and treatment. If discrimination of a model is poor, the model is not suitable for risk prediction and cannot easily be improved. On the other hand, if calibration is poor while discrimination is good, one can recalibrate the model to improve prediction, without forsaking discrimination [145, 158]. Our results indicated that the EuroSCORE might not be suitable for individual preoperative risk evaluation in our population without recalibration, but it can still be used to classify patients in broad risk groups. However, after we validated the old EuroSCORE, the updated EuroSCORE II has been published [157]. It has not been validated in our patient population yet, but we expect that it will be better calibrated than the old EuroSCORE, and it would certainly be of interest to validate the EuroSCORE II in our population.

Developing risk models from data collected from many hospitals and several countries will enable risk prediction and adjustment of complication or mortality rates when benchmarking hospitals across a wide geographical range. However, the coefficients in such models will represent an average of the influence and composition of risk factors for all contributing centres, and risk prediction may be less accurate when applied to single centre data than locally developed models. Previous studies have also shown that locally developed risk models were often more accurate than models developed elsewhere [12, 65, 68, 171], and may therefore be preferred for preoperative risk evaluation and for assigning patients to alternative treatment based on risk evaluation.

A locally developed model may not be suitable for use outside the institution where it was developed. Preoperative risk evaluation as well as internal assessment and inter-institutional comparison are important to improve the quality of treatment, and to provide patients with optimum care and counselling. All risk prediction models, regardless of origin, should be validated before implementation in clinical or administrative work. Risk prediction models also need to be updated regularly to ensure

the eligibility of the predictions as changes may occur in patient characteristics and treatment strategies.

Do we need a model for each end-point?

Our results indicate that the pathophysiological mechanisms for different complications are likely to be partly different, partly overlapping. This suggests that any risk model developed to predict a diverse range of outcomes would be prone to inaccuracy, because the coefficients would represent the average impact of the risk factors, and all the predictors might not be significant predictors of all the end-points if they were analysed separately. This is supported by Antunes et al. and the STS cardiac surgery risk models. Antunes et al. found that models for different postoperative morbidity end-points differed with regard to the composition of predictors and their respective coefficients [144]. In the risk models of the STS the coefficients of the predictors varied between the models for different end-points [6, 86, 87]. Thus, a universal model that can accurately predict several end-points is probably unattainable.

In clinical work, time is limited and most clinicians do not have the time to calculate the predicted risks for many end-points preoperatively. A good solution could be that the risk predictions could be integrated in a computerised data collection form, using the registered information to automatically calculate the predicted probability for each of the complications. Another alternative could be to use the model predicting the most interesting end-point only. In administrative work or research one is not dependent upon predicting the risk preoperatively. Given that the data are stored in a computerised database, the predicted risks for a large number of patients can easily be calculated simultaneously.

However, it is still uncertain whether clinicians trust their own clinical judgement more than they would trust a risk prediction model. Experienced clinicians have accumulated a large amount of knowledge that is used for making decisions and judgements every day. A risk prediction model cannot take all possible risk factors into account. Its predictors are normally confined to some of the most common and powerful risk factors and rare risk factors that may have a great impact on individual patient risk will never achieve significance in a risk prediction model developed from several thousand

patients. A comparison of a risk prediction model and surgeons' risk predictions showed that both surgeons and a risk prediction model displayed similar discriminatory ability for mortality following AVR [192]. However, surgeons tended to over-estimate the mortality risk in high-risk patients [192].

Parsimonious models versus large number of risk factors

In 2009, Ranucci et al. published a mortality risk score for cardiac surgery using only three variables: the Age, creatinine and ejection fraction score [172]. Thus, this model represents the parsimonious extreme in mortality risk prediction, as opposed to the STS risk models, which include a large number of risk factors [6, 86, 87]. Interestingly, the parsimonious risk model by Ranucci et al. was just as accurate when validated in a separate cohort from the same area as were the complex STS models when validated in the separate validation cohort [6, 86, 87, 172]. Our research group has also evaluated the discrimination of the Age, creatinine and ejection fraction score for operative mortality in our population, and the c statistic was 0.762 (0.707-0.816) [33]. For comparison, the c statistic of the logistic and the additive EuroSCORE was 0.821 (0.785-0.857) and 0.846 (0.810-0.881) in our data, respectively. Thus, the parsimonious risk model showed somewhat poorer discrimination than the EuroSCORE in our population. The Age, creatinine and ejection fraction score was also compared to the additive and logistic EuroSCORE in a large Italian study [191]. There it demonstrated somewhat better discrimination than the EuroSCOREs, although not significantly better, and none showed particularly impressive discrimination [191]. The slightly better discrimination of the Age, creatinine and ejection fraction score compared with the EuroSCORE in an Italian population also illustrates the importance of taking geographical differences into account when using risk prediction models.

Inflammation and cardiac dysfunction

Preoperative neopterin and postoperative cardiac dysfunction

As inflammation caused by cardiac surgery is thought to play a pivotal role in the development of several postoperative complications [8], we hypothesized that increased preoperative inflammation would affect the development of postoperative cardiac dysfunction. We found an association between increased preoperative neopterin and

cardiac dysfunction following open-heart surgery. The finding ought to be tested in other populations of cardiac surgical patients in order to test its validity. Even so, it is possible that increased preoperative inflammation and elevated plasma neopterin in particular, render the heart more vulnerable to further inflammation and oxidative stress induced by cardiac surgery. It remains to be investigated whether the plasma concentration of neopterin is correlated with the amount of myocardial neopterin.

The sensitivity analysis with adjustment using the EuroSCORE II confirmed that our findings regarding neopterin were robust and did not depend on the way of adjustment. It also showed that the local model was more accurate for predicting postoperative cardiac dysfunction than the EuroSCORE II, which was developed to predict postoperative mortality.

Reasons for elevated neopterin

Acute infection, autoimmune diseases and malignancy can also cause an elevation in plasma neopterin levels [130]. As mentioned in the Introduction, atherosclerosis and left ventricular dysfunction have been associated with elevated neopterin [111-113, 129, 133]. In patients with intercurrent infections, elective cardiac surgery is normally postponed until recovery, and patients with active endocarditis were excluded from the study in Paper III. Information on autoimmune diseases and cancer was not included in our data. Therefore, we were not able to identify patients with autoimmune diseases or malignancy. However, the total number of affected patients is expected to be low. We statistically adjusted for atherosclerosis and left ventricular dysfunction, which are frequently observed in patients undergoing heart surgery.

Neopterin is subject to renal elimination, and the molecule is biologically stable in the circulation [130]. Thus, an increase in neopterin concentration can also be caused by impaired renal function. Therefore we performed sensitivity analyses of neopterin where we adjusted for serum creatinine as well as the other clinical variables, but the results were not altered in this alternative analysis. The robustness of our results suggests that neopterin is not merely a marker of poor kidney function, and that the elevations in neopterin concentration were more than what would be expected from increases caused by reduced renal clearance. Neopterin may have an effect on the

development cardiac dysfunction itself, or be a marker of a process increasing its production, such as the activation of the cellular immune system.

C-reactive protein

After adjustment for the clinical variables C-reactive protein was no longer significantly associated with postoperative cardiac dysfunction. C-reactive protein was somewhat correlated with neopterin in our study. We analysed C-reactive protein and neopterin in the same model, and it could be that C-reactive protein contributed with little additional information that was not already provided by neopterin. Furthermore, neopterin is considered a more specific marker of activation of monocytes and the cellular immune system in inflammation, while C-reactive protein is a more general marker of inflammation. C-reactive protein has been associated with ischaemic heart disease and chronic cardiac dysfunction in previous studies. Therefore, another possible explanation is that C-reactive protein represents some of the information included in some of the clinical covariates, such as urgent operation, chronic heart failure and previous myocardial infarction.

C-reactive protein circulates as a pentamer, but the monomeric form is more active [195]. A recent study showed that the monomeric form of C-reactive protein, as opposed to the circulating pentameric form, was found in inflamed atherosclerotic plaques and in myocardial infarction lesions in humans, [195]. We are measuring the circulating pentamer, and it does not necessarily correspond with the concentration of monomeric, pro-inflammatory C-reactive protein at sites of inflammation. This could perhaps partly explain the varying results in the literature regarding associations of C-reactive protein and cardiac end-points.

The preoperative level of C-reactive protein should reflect low-grade inflammation, as patients with signs of intercurrent infection and elevated C-reactive protein were not eligible for elective cardiac surgery. For most patients the C-reactive protein concentration was therefore not expected to be measurable by routine methods used in clinical practice.

Inflammation and risk prediction

Cardio-renal syndromes

The risk factors of the outcomes in this thesis, namely mortality, AKI and cardiac dysfunction, were partly overlapping. AKI following cardiac surgery has been strongly associated with increased mortality [20, 21, 26], however cardiac problems have been found to be the most common cause of death [14, 36]. It is possible that the increased mortality in part is mediated through a failing heart. It is also possible that AKI reduces the clearance of substances such as neopterin, which may increase the cardiac stress.

The heart and the kidneys are closely interrelated, and disturbances in the functioning of one organ affect the other. The main routes of influence are changes in cardiac output and renal perfusion, haemodynamic changes, disturbances in fluid and electrolyte balance, neuroendocrine activation and inflammation [196, 197].

In recent years, there has been a focus on concomitant dysfunction in several organs, and among these are the cardio-renal syndromes [198]. There are five categories of cardio-renal syndromes [198]: (1) Acute decrease in heart function leading to acute kidney dysfunction. (2) Chronic reduction in heart function leading to kidney dysfunction. (3) AKI leading to cardiac dysfunction. (4) Chronic kidney disease leading to worsening of heart function. (5) Systemic disease, such as sepsis, leading to dysfunction in both the heart and the kidneys.

Cardiac surgical patients already have a disease of the heart, and therefore AKI following open-heart surgery may be considered as cardio-renal syndrome type 1 [197]. The systemic inflammatory response following cardiac surgery show similarities with that in sepsis, and it is possible that some of the mechanisms underlying type 5 cardio-renal syndrome are also present in cardiac dysfunction and AKI following cardiac surgery [199]. The two entities may also be parts of multiple organ failure.

Improvement of risk prediction

In Paper I and Paper II we found that three intraoperative variables, namely time on cardiopulmonary bypass, need for inotropic support and red blood cell transfusion, slightly improved discrimination of the risk prediction models. In Paper III we found

that inclusion of neopterin yielded somewhat more accurate predictions of cardiac dysfunction following open-heart surgery.

Inclusion of intraoperative variables could make the model more complex, and it precludes risk prediction before surgery. Thus, the slight improvements in model performances do not justify the use of the intraoperative models instead of the preoperative ones in our population.

Potentially useful new biomarkers ought to be thoroughly evaluated before implementation in the clinic, and one must compare the benefit with the increased expenses of the analysis of this biomarker. Therefore, we do not suggest the measurement of neopterin in all patients before cardiac surgery at present.

However, our findings regarding the association between preoperative neopterin levels and postoperative cardiac dysfunction support the hypothesis that inflammation and oxidative stress are involved in the development of cardiac dysfunction after open-heart surgery, and suggest that further investigation of this potential relationship is warranted.

It is possible that risk prediction can be further improved by including markers of several hypothesized pathways, or by including information on genetic information on molecules involved in underlying pathways. Such studies can be used to further investigate findings from animal models as part of translational research.

Future studies

Our risk prediction models ought to be validated and updated in a more recent cohort from our institution. To assess the generalizability of the risk models, external validation is also necessary. It would also be interesting to assess how preoperative risk prediction could impact on treatment choice.

In order to further investigate the potential underlying mechanisms of dysfunction in the heart and kidneys, it would be interesting to compare levels of inflammatory markers in venous blood from the heart and kidneys with that of peripheral circulation during and after cardiac surgery. This could be done by placing catheters in the renal vein and coronary sinus, and then compare the concentration of inflammatory markers in the renal vein or coronary sinus with the concentration in systemic circulation (i.e. radial

artery). This investigation could increase the knowledge about organ-specific activation of inflammatory cells or humoral immunologic factors like complement during and after cardiac surgery.

If one exposed mice to small amounts of neopterin over a longer time, one could measure if there was any effect on the cardiac function. Despite that murine macrophages do not produce neopterin; neopterin has been shown to affect contractility in rat hearts [134]. Probably also more suitably investigated in an animal model, the relationship between circulating neopterin and its presence in the myocardium or other organs could be tested.

Conclusions

Paper I:

1) We developed a local preoperative mortality risk prediction model for cardiac surgery that displayed good discrimination (c statistic 0.857 (0.823-0.891)) and calibration in our population. It consisted of eight risk factors for operative mortality after cardiac surgery: age above 68 years, degree of urgency, female gender, serum creatinine above 140 µmol/L, chronic pulmonary disease, chronic cardiac insufficiency, previous cardiac surgery, and type of operation.
2) The inclusion of intraoperative variables did not significantly improve the predictive ability of the model.
3) The additive and the logistic EuroSCORE were poorly calibrated in our population, yet the discrimination was good with c statistic of 0.846 (0.810-0.881) and 0.821 (0.785-0.857), respectively. Our local mortality risk prediction model displayed significantly better discrimination than the logistic EuroSCORE ($p = 0.02$).

Paper II:

4) We developed a well-calibrated and accurate local preoperative risk prediction model for AKI following cardiac surgery (c statistic 0.819 (0.801-0.837)). The model included eleven important risk factors for AKI after open-heart surgery: age, body mass index above 30 kg/m^2, lipid lowering treatment (protective effect), hypertension, peripheral vascular disease, chronic pulmonary disease, haemoglobin concentration, serum creatinine (below 100 µmol/L, 100-140 µmol/L and above 140 µmol/L) , previous cardiac surgery, emergency operation, and operation type.
5) The model changed little with changes in the end-point definition.
6) Intraoperative variables slightly improved model discrimination and more accurate estimates of kidney function slightly decreased the model accuracy.
7) The previously published risk model for AKI by Antunes et al. [144] displayed poor fit but adequate discrimination in our population (c statistic 0.740 (0.718-0.762)), and the AKI model by Brown et al. [18] displayed poor fit and discrimination in our population (c statistic 0.653 (0.630-0.676)).

Paper III:

8) Preoperative neopterin was associated with cardiac dysfunction after open-heart surgery.

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Paper I

Acta Anaesthesiol Scand 2011; **55**: 313–321
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ACTA ANAESTHESIOLOGICA SCANDINAVICA
doi: 10.1111/j.1399-6576.2010.02393.x

Mortality risk prediction in cardiac surgery: comparing a novel model with the EuroSCORE

K. S. Berg[1], R. Stenseth[2,3], H. Pleym[2,3], A. Wahba[2,4] and V. Videm[1,5]
Departments of [1]Laboratory Medicine, Children's and Women's Health, [2]Circulation and Medical Imaging, Norwegian University of Science and Technology, Trondheim, Norway, Departments of [3]Cardiothoracic Anaesthesia and Intensive Care, [4]Cardiothoracic Surgery and [5]Immunology and Transfusion Medicine, St. Olav University Hospital, Trondheim, Norway

Background: Several models for prediction of early mortality after open-heart surgery have been developed. Our objectives were to develop a local mortality risk prediction model, compare it with the European System for Cardiac Operative Risk Evaluation (EuroSCORE), and investigate whether the addition of intra-operative variables could enhance the accuracy of risk prediction.
Methods: All 5029 patients undergoing open-heart surgery in 2000–2007 were included in the study. Logistic regression with bootstrap methods was used to develop a pre-operative risk prediction model for in-hospital mortality. Next, several intra-operative variables were added to the pre-operative model. Calibration and discrimination were assessed, and the model was internally validated for prediction in future datasets. We thereafter compared the pre-operative model with the additive and logistic EuroSCOREs.
Results: Our pre-operative model included eight risk factors that are routinely registered in our department: age, gender, degree of urgency, operation type, previous cardiac surgery, and renal, cardiac, and pulmonary dysfunction. The model estimated mortality accurately throughout the dataset except in the 1% of patients at extremely high risk, in which mortality was somewhat overestimated. The estimated shrinkage factor was 0.930. The areas under the receiver operating characteristic curve for our pre-operative model and the logistic EuroSCORE were 0.857(0.823–0.891) and 0.821(0.785–0.857) ($P = 0.02$). There was no significant difference in performance between the pre-operative and the intra-operative model ($P > 0.10$).
Conclusion: Our pre-operative model was simple and easy to use, and showed good predictive ability in our population. Internal validation indicated that it would accurately predict mortality in a future dataset.

Accepted for publication 20 December 2010

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During the last decades, the interest for predicting early mortality after cardiac surgery has been increasing, and a number of models have been developed.[1–10] Risk prediction models are used to adjust the mortality rate according to the patients' risk profile when comparing surgeons or institutions, and to assign patients to risk groups.

Risk prediction models can be developed from data from one surgical centre or data from several surgical centres and even different countries. When comparing different risk prediction models, it is important to assess the models' discrimination and calibration. Discrimination is the model's sensitivity and specificity and refers to the model's ability to discriminate between subjects with high and low risk for the outcome.[11] Calibration is a measure of how well the predicted outcomes compare with the observed outcomes.[11]

One of the most widely used pre-operative risk prediction models in European cardiac surgery is the European System for Cardiac Operative Risk Evaluation (EuroSCORE), which exists both as an additive and a logistic model.[6,12] An additive model is ideal for bedside application, as each factor is weighted and one can easily sum up the predicted risk in percentage, whereas the logistic model normally requires the use of a calculator or a computer to solve the logistic regression equation to calculate the predicted probability for the outcome. The EuroSCORE's performance has been evaluated and compared with other risk prediction models.[1,3,4,8,13–19] Several groups have found that it was the most accurate risk prediction model, compared with many other models.[14,16] In recent years, however, several studies have indicated that the EuroSCORE over-estimates the risk of mortality for

the majority of patients,[1,3,15,17] particularly when compared with new, locally developed models. This was also suspected to be true in our institution. To our knowledge, the performance of the EuroSCORE has never been formally assessed in Norway.

As local models were often more accurate than the EuroSCORE,[1,8,17] our aims were to develop a local risk prediction model and assess its performance, compare it with the EuroSCORE, and assess the performance of the EuroSCORE in our population.

Pre-operative risk assessment gives valuable information about the likely outcome, but intra-operative events may also influence outcome. Therefore, our objective also was to develop a risk prediction model including some intra-operative variables. This approach would allow us to investigate which intra-operative factors contributed significantly to the risk of mortality, and compare the pre-operative model with the intra-operative models to quantify the difference in predictive ability before and immediately after surgery.

Methods

Data

Between 2000 and 2007, all 5029 adult patients undergoing open-heart surgery at the St. Olav University Hospital in Trondheim, Norway, were included in the study. Data on patient characteristics, like other diseases and risk factors, pre-operative blood tests and examinations, medication, surgical data and intra-operative measurements, and post-operative complications, blood tests and treatment were collected prospectively, quality assured, and stored in a local database, as part of the department's quality assurance routine. The project was approved by the Regional Ethics Committee and The Norwegian Data Inspectorate.

The end point of the present study was in-hospital mortality, defined as mortality during the same hospital stay or within 30 days after surgery. One hundred and thirty-five of the 5029 (2.7%) patients died according to this definition.

Analysis

Model development. We first developed a pre-operative model for mortality risk prediction. We then investigated whether addition of selected intra-operative variables could improve outcome prediction after surgery.

The entire dataset was used for model development, as internal validation with bootstrapping methods are preferred over data-splitting methods.[20] The selection of explanatory variables was performed on the basis of clinical knowledge and an hypothesis of their potential influence on the outcome, as univariable screening of variables enhances the risk of over-fitting the model and reduces the predictive ability in future datasets.[20] We also took into account whether the variables were relevant for a sufficient proportion of the patients. The full models (including all the hypothesised variables) were tested for interactions, linearity in the logit for continuous variables, and overly influential observations. The variable definitions are given in Table 1.

Model I. Sixteen variables considered to be clinically relevant were entered into a logistic regression model. The variables were age, found to be best modelled as above the median age of 68 years (yes/no), female gender (yes/no), body mass index (continuous), diabetes mellitus (yes/no), smoking (yes/no), hypertension (yes/no), chronic cardiac insufficiency (yes/no), peripheral vascular disease (yes/no), chronic pulmonary disease (yes/no), left ventricular hypertrophy (yes/no), pulmonary hypertension (yes/no), pre-operative haemoglobin concentration (continuous), serum creatinine above 140 μmol/l (yes/no), previous cardiac surgery (yes/no), degree of urgency (within 24 h/within 2 weeks/standard waiting list), and type of operation [1. coronary artery bypass grafting (CABG) or atrial septum defect (ASD); 2. aortic valve replacement (AVR) only, AVR and CABG combined, non-ischaemic mitral valve surgery, or aneurysm in the ascending aorta; 3. dissection of the ascending aorta or ventricular septum rupture; and 4. miscellaneous surgery].

The operation types were grouped according to average risk for the different surgical procedures, so that the surgical procedures in each group would have approximately the same risk. The risk estimation was based on the frequency of patients who died after each surgical procedure.

The left ventricular ejection fraction (LVEF) was measured either by catheterisation or by echocardiography, and the exact value was registered in 4043 of 5029 patients (80.4%). Because exact LVEF was missing for 986 patients and different methods were used, it was excluded. Instead, the presence

Table 1

Variable definitions.

Variable	Definition
Age above 68 years*	No/yes
Female gender	No/yes
Body mass index (kg/m^2)	Continuous variable
Diabetes mellitus	Receiving medication (no/yes)
Smoking	Current smoker or quit less than 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	Electrocardiography or echocardiography (no/yes)
Pulmonary hypertension	Systolic pulmonary arterial pressure (PAP) >40 mmHg or mean PAP >25 mmHg, echocardiography or catheterisation (no/yes)
Haemoglobin	Haemoglobin concentration (mmol/l), continuous variable
Creatinine above 140 μmol/l	Serum creatinine above 140 μmol/l (no/yes)
Previous cardiac surgery	Previously undergone cardiac surgery (no/yes)
Degree of urgency	3 categories: standard waiting list, operation within 2 weeks, operation within 24 h
Type of operation	4 categories: 1. Coronary artery bypass grafting (CABG) or Atrial septum defect (ASD). 2. Aortic valve replacement (AVR) only, AVR and CABG combined, non-ischaemic mitral valve surgery or aneurysm in the ascending aorta 3. Dissection of the ascending aorta or Ventricular septum rupture. 4. Miscellaneous†
CPB	Cardio-pulmonary bypass during surgery (no/yes)
CPB time (per 10 min)	Time on cardio-pulmonary bypass
Inotropic support	On clinical indication during surgery (no/yes)
Vasoconstrictor	On clinical indication during surgery (no/yes)
Fluid balance	Tertiles of fluid balance during surgery
Red cell transfusion	On clinical indication during surgery (no/yes)
Plasma transfusion	On clinical indication during surgery (no/yes)
Death	Death within 30 days after surgery or during the same hospital stay

*68 years was the median age.
†Miscellaneous surgery consisted of various operations like 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 like pericardiectomy and removal of cardiac tumours.

or absence of chronic cardiac insufficiency, defined as medically treated, was entered as a potential explanatory variable.

A limited backwards step-down procedure, retaining variables according to Akaike's Information Criterion, reduced our model to eight significant variables. The model was bootstrapped ($n = 400$) to achieve more robust estimates of the coefficients and reduce the risk of over-fitting.[20]

Next, internal validation with bootstrapping ($n = 400$) methods was performed. The discriminatory ability was assessed by calculating the area under the receiver operating characteristic (ROC) curve. Calibration was assessed by comparing actual and predicted probabilities by bootstrapping ($n = 400$). We also estimated the optimism of the model if it was applied on a future dataset, also known as the shrinkage factor. A shrinkage factor over 0.85 is considered satisfactory.[20] The Hosmer–Lemeshow test was used to assess the goodness-of-fit. A *P*-value above 0.05 is generally considered as indicating a satisfactory goodness-of-fit.

Model II. To investigate whether outcome prediction could be improved, we added the following six intra-operative variables to the final pre-operative model: whether the patient was on cardiopulmonary bypass (CPB) (yes/no), need for inotropic support (yes/no), vasoconstrictor use (yes/no), intra-operative fluid balance (tertiles), red cell transfusion (yes/no), and plasma transfusion (yes/no).

This second model was then reduced by limited step-down, and the coefficients were bootstrapped. The final intra-operative model contained seven variables, and was validated and calibrated as described above.

Model III. As 2.7% of our patients were operated on without CPB, the time on CPB could not be included as a variable in model II. We therefore fit an alternative intra-operative model, including only the patients operated on with CPB, and replacing CPB (yes/no) with the time on CPB. The previously described statistical procedures were used to develop and validate this model.

The three models were compared statistically by the likelihood test. The models were also compared both with ROC curves and other plots displaying predicted and actual probability across the deciles of risk. Furthermore, we compared the area under the ROC curves (AUC) for the three models apply-

ing the method for non-parametric correlated AUCs, proposed by DeLong et al.[21]

Comparison with external risk prediction models. We compared our model with the additive and logistic EuroSCOREs.[6,12] The additive EuroSCORE was scored for each patient during data collection and stored in the database, and the logistic EuroSCORE was calculated retrospectively from the variables registered in the database. We found matching definitions for all variables except neurological dysfunction, and this variable was not included in the calculations. If data were missing for individual patients, it was set to the alternative giving the lowest risk, including the 986 patients where LVEF was not precisely registered. LVEF was always precisely measured when the patient had reduced ventricular function according to the referring cardiologist. Thus, when LVEF was not precisely measured, the patient usually had a normal or near-normal left ventricular function.

The EuroSCOREs were validated with the Hosmer–Lemeshow test, ROC curves, and AUCs. To compare the different scores, we calculated the predicted probability (expected mortality) for all, and compared them with the observed mortality in each decile of risk. We also compared the AUCs for the different models by the DeLong's method.[21]

Data are given as mean with 95% confidence interval for continuous variables, and as frequency (percentage) for categorical variables, unless otherwise stated. The χ^2-test and the Mann–Whitney *U*-test were used for inter-group comparisons. The statistic software R (version 2.10.1, R Foundation*), SPSS (version 16.0, SPSS Inc., Chicago, IL), SigmaPlot (version 11.0, Systat Software Inc., San Jose, CA), and MiniTab (version 15.1.30.0, Minitab Inc., State College, PA) were applied for statistical analyses and modelling.

Results

Patient characteristics

Three thousand seven hundred and forty (74.4%) of the 5029 patients were men, 1289 (25.6%) were women, and the median age was 67.6 (67.1–68.1) years. Six hundred and fifty-one patients (12.7%) had diabetes, 1260 patients (25.1%) were smokers, and the mean BMI was 26.6 (26.5–26.7) kg/m^2. Four thousand eight hundred and ninety-three patients (97.3%) were operated on with CPB, the median CPB time was 69 (67–70) minutes, and the median per-operative fluid balance was 2873 ml, ranging from −875 to 19690 ml. Three thousand five hundred and thirty-eight patients (70.4%) underwent coronary surgery only or closure of an ASD (0.7%), 1068 patients (21.2%) underwent an AVR, AVR and CABG combined, non-ischaemic mitral valve surgery, or operation for an aneurysm of the ascending aorta, 94 patients (1.9%) were operated for dissection of the ascending aorta or rupture of the ventricular septum, and 329 patients (6.5%) underwent miscellaneous surgery. Miscellaneous surgery consisted of various operations like 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 like pericardiectomy and removal of cardiac tumours. Characteristics of the patients who survived or died are given in Table 2. The mortality did not change significantly during the 8 years of data collection ($P = 0.33$).

Among the group of patients with chronic cardiac insufficiency, the mean LVEF was 48.1 (46.5–49.7)% in patients evaluated by catheterisation, and 41.3 (40.2–42.4)% in patients evaluated by echocardiography. In the group without chronic cardiac insufficiency, the mean LVEF was 65.3 (64.8–65.8)% in patients evaluated by catheterisation, and 51.3 (50.7–52.0)% in patients evaluated by echocardiography. The inter-group difference was 17.2 (15.6–18.9)% for catheterisation and 10.1 (8.8–11.3)% for echocardiography ($P < 0.0005$).

Novel risk prediction models

Table 3 summarises the risk prediction models. The Hosmer–Lemeshow test showed an adequate goodness-of-fit for all of the models (Model I: $P = 0.62$, Model II: $P = 0.34$, Model III: $P = 0.75$). The models showed good discrimination with AUC of 0.857 (0.823–0.891) for Model I, 0.877 (0.843–0.910) for Model II, and 0.866 (0.833–0.898) for Model III. The differences in AUCs were not statistically significant ($P > 0.10$ for all comparisons).

The shrinkage factor was 0.930 for Model I, 0.936 for Model II, and 0.942 for Model III. This indicates that all three models would predict mortality accurately in a future dataset, with an estimated error of 7.0% for Model I, 6.4% for Model II, and 5.8% for Model III. As shown in the calibration curves (Fig. 1), all three models were well calibrated for the group of patients with a predicted

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

Table 2

Patient characteristics.

Characteristic*	Survived	Died
Age (years, median)	66.0 (65.7–66.2)	70.5 (68.4–72.5)
Female gender	1236 (25.3%)	53 (39.3%)
Body mass index (kg/m^2)	26.6 (26.5–26.7)	26.6 (25.7–27.5)
Diabetes mellitus	635 (13.0%)	16 (11.9%)
Smoking status	1230 (25.1%)	30 (22.2%)
Hypertension	2379 (48.6%)	76 (56.3%)
Chronic cardiac insufficiency	734 (15.0%)	54 (40.0%)
Peripheral vascular disease	511 (10.4%)	25 (18.5%)
Chronic pulmonary disease	656 (13.4%)	44 (32.6%)
Left ventricular hypertrophy	969 (19.9%)	46 (34.6%)
Pulmonary hypertension	417 (8.5%)	38 (29.0%)
Haemoglobin concentration (mmol/l)	8.50 (8.47–8.52)	7.98 (7.81–8.15)
Serum creatinine (μmol/l)	96.2 (94.8–97.5)	126.3 (112.2–140.4)
Previous cardiac surgery	273 (5.6%)	18 (13.3%)
Degree of urgency		
Standard waiting list	2686 (54.9%)	44 (32.6%)
Operation within 2 weeks	1965 (40.2%)	49 (36.3%)
Operation within 24 h	242 (4.9%)	42 (31.1%)
Operation type		
Coronary surgery only or closure of an ASD	3493 (71.4%)	45 (33.3%)
Aortic valve replacement (AVR), non-ischaemic mitral valve surgery, AVR and CABG combined or operation for an aneurysm of the ascending aorta	1032 (21.1%)	36 (26.7%)
Operation for dissection of the ascending aorta or rupture of the ventricular septum	71 (1.5%)	23 (17.0%)
Miscellaneous	298 (6.1%)	31 (23.0%)
CPB	4772 (95.5%)	132 (97.8%)
CPB time (min, median)	68 (67–69)	139 (119–160)
Inotropic support	1100 (22.5%)	85 (63.4%)
Vasocontrictor use	3714 (75.9%)	116 (85.9%)
Fluid balance (ml, median)	2865 (2844–2890)	3285 (3018–3530)
Red cell transfusion	681 (13.9%)	82 (60.7%)
Plasma transfusion	392 (8.0%)	56 (41.5%)

*All variables have the same definition as in Table 1, unless otherwise stated.

probability <0.25, but above this level the calibration decreased. However, only 1.0% of the patients had a predicted probability of in-hospital mortality above 0.25. The corrected calibration curves showed that if the models were calibrated with their respective shrinkage factors, all three models would be well calibrated for the small group of high-risk patients, but less well calibrated for the majority of patients, compared with the current models.

Performance of the EuroSCORE in our population

The Hosmer–Lemeshow test was highly significant for both the logistic and the additive EuroSCORE (P-values $= 0.0008$ and <0.0001, respectively), indicating that the predicted mortality was significantly different from the observed mortality in our population (Fig. 2). The EuroSCOREs showed good discrimination; the logistic EuroSCORE had an AUC of 0.821 (0.785–0.857), and the additive EuroSCORE had an AUC of 0.846 (0.810–0.881). The difference in AUC was significant for Model I and the logistic EuroSCORE ($P = 0.02$), but not for Model I and the additive EuroSCORE ($P = 0.40$).

Discussion

We have shown that mortality after open-heart surgery may be accurately predicted from eight risk factors that are easily collected as part of ordinary clinical routines. In the present population, our model was more accurate than the EuroSCORE.

Local models

The performance of our models was good throughout the dataset, except for calibration for the 1.0% of patients at extremely high risk. The low number of patients may explain the poor calibration in this group. If we calibrated our models with their respective shrinkage factors, the calibration became better for this small group. However, patients with a mortality risk above 0.25 are always subject to a thorough individual consideration pre-operatively, and the necessity of a score with excellent calibration in this range is limited.

Our findings suggest that the intra-operative variables added to Model I contained valuable information for mortality risk prediction. The time on CPB seemed to be an especially important predictor, as well as the use of inotropic support

Table 3

Risk prediction models for in-hospital mortality following open-heart surgery.

Variable*	Coefficient	OR	95% CI	*P*-value	Died (*n*)
Model 1 ($n = 4969$)					
Age above 68 years	1.141	3.128	(2.026–4.831)	<0.0001	101
Degree of urgency					
Standard waiting list		1.000	Reference		
Operation within 2 weeks	0.397	1.487	(0.969–2.306)	0.11	49
Operation within 24 h	1.927	6.870	(3.525–13.389)	<0.0001	42
Female gender	0.541	1.718	(1.130–2.612)	0.03	53
Serum creatinine (>140 μmol/l)	1.234	3.434	(2.007–5.874)	<0.0001	39
Chronic pulmonary disease	0.783	2.189	(1.440–3.327)	0.0001	44
Chronic cardiac insufficiency	0.690	1.994	(1.293–3.076)	0.002	54
Previous cardiac surgery	0.782	2.185	(1.147–4.165)	0.006	18
Operation type					
CABG or ASD		1.000	Reference		
Pure AVR, AVR and CABG, non-ischaemic mitral valve surgery, or aneurysm of ascending aorta	0.621	1.861	(1.176–2.946)	0.01	36
Dissection of ascending aorta, or ventricular septum rupture	1.906	6.723	(2.918–15.489)	<0.0001	23
Miscellaneous	1.483	4.407	(2.614–7.430)	<0.0001	31
Intercept	−6.045				
Model 2 ($n = 4954$)					
Age above 68 years	1.013	2.754	(1.722–4.404)	<0.0001	101
Degree of urgency					
Standard waiting list		1.000	Reference		
Operation within 2 weeks	0.299	1.348	(0.879–2.068)	0.20	49
Operation within 24 h	1.461	4.310	(2.113–8.792)	<0.0001	42
Serum creatinine (>140 μmol/l)	1.153	3.169	(1.837–5.468)	<0.0001	39
Chronic pulmonary disease	0.865	2.375	(1.533–3.680)	0.0007	44
Operation type					
CABG or ASD		1.000	Reference		
Pure AVR, AVR and CABG, non-ischaemic mitral valve surgery, or aneurysm of ascending aorta	0.520	1.682	(1.014–2.788)	0.05	36
Dissection of ascending aorta, or ventricular septum rupture	1.235	3.437	(1.339–8.825)	0.02	23
Miscellaneous	1.396	4.039	(2.373–6.875)	<0.0001	31
Inotropic support	0.996	2.706	(1.759–4.164)	<0.0001	85
Red cell transfusion	1.125	3.080	(1.962–4.837)	<0.0001	82
Intercept	−6.186				
Model 3 ($n = 4830$)					
Age above 68 years	1.187	3.279	(2.026–5.306)	<0.0001	98
Degree of urgency					
Standard waiting list		1.000	Reference		
Operation within 2 weeks	0.252	1.287	(0.789–2.099)	0.30	47
Operation within 24 h	1.662	5.270	(2.738–10.146)	<0.0001	41
Serum creatinine (>140 μmol/l)	1.060	2.887	(1.620–5.146)	0.0003	30
Chronic pulmonary disease	0.782	2.187	(1.412–3.386)	0.002	53
Chronic cardiac insufficiency	0.709	2.032	(1.326–3.114)	0.002	84
Red cell transfusion	0.810	2.247	(1.369–3.690)	0.004	80
CPB time†	0.140	1.150	(1.116–1.185)	<0.0001	
Intercept	−7.026				

*Variable definitions are listed in Table 1.
†CPB time per 10 min.

and the need for red cell transfusion. Even so, the pre-operative model without these variables displayed good predictive ability. One reason may be that the operation type provides a good estimate of the expected time on CPB and to some degree also the need for inotropic support and red cell transfusions. There was no significant difference in the AUC of the three models, suggesting that prediction immediately after surgery is not significantly better than that before surgery. The operation types were grouped according to average risk for the different surgical procedures, and this may also have contributed to the good accuracy displayed by our pre-operative model.

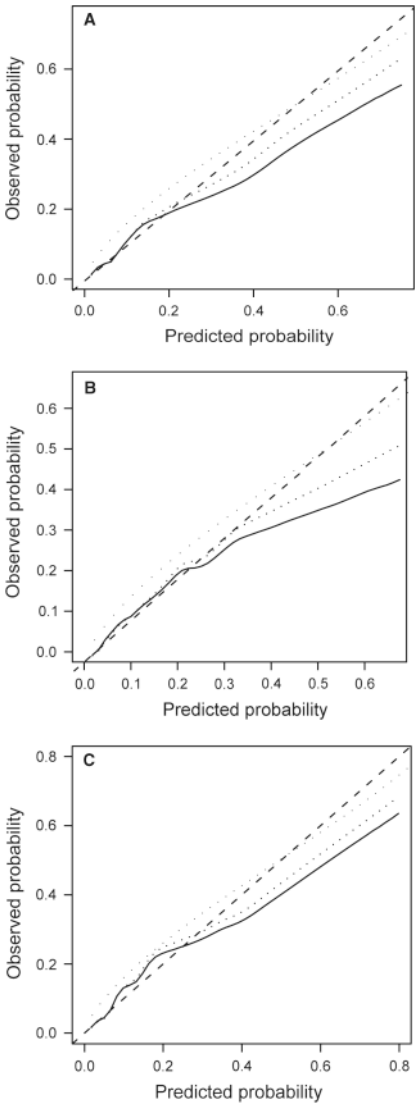

Fig. 1. Calibration curves for Model I (panel A), Model II (panel B), and Model III (panel C). The curves display the apparent fit (continuous line), the bias corrected fit (dotted line), the ideal fit (dashed line), and the fit if the models were corrected with their respective shrinkage factors (spaced dots).

Comparison with the EuroSCORE

The EuroSCOREs showed satisfactory discriminatory ability, but predicted a higher mortality than that observed for the low-risk patients and a lower mortality than observed for the high-risk patients.

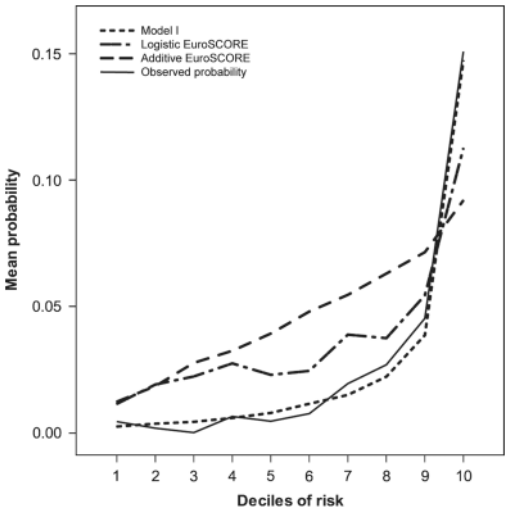

Fig. 2. Observed and predicted probabilities across the deciles of estimated risk for Model I and the additive and logistic EuroSCOREs.

This is in accordance with the findings of others,[1,15,17] and indicates that the EuroSCORE may not be useful for risk prediction in our patient population.

The logistic EuroSCORE had significantly lower discriminatory ability than our Model I. The current EuroSCORE was developed from data collected in 1995,[22] and changes in treatment and patient characteristics may explain the poor fit in our population. However, the EuroSCORE may still be used to assign patients to treatment categories or classify them in broad risk groups. Currently, the EuroSCORE2010 project aims at collecting new sets of data from a large number of European hospitals to improve the existing EuroSCORE.[†]

Collecting data from many hospitals and several countries is ideal for deriving a model suitable for risk prediction and benchmarking across a wide geographical range. However, a model based on a diverse population also estimates the average influence from various risk factors, and might thereby be more prone to inaccuracy in specific risk groups. On the other hand, a locally developed model may be more accurate for risk prediction and classification of local patients, but may not be useful for benchmarking outside the institution

[†]*https://euroscore2010.org/FurtherEuroscoreInfo.aspx*

where it was developed. Both internal assessment and inter-institutional comparison are important to provide patients with optimal counselling and treatment, and we would recommend the use of both local and more general models concomitantly. All risk prediction models, whether local or more general, should be validated before implementation in clinical or administrative work.

All the significant risk factors in our pre-operative model were among the 17 risk factors included in the EuroSCORE,[6] except for chronic cardiac insufficiency, which was entered instead of LVEF. Our model was more accurate than the EuroSCORE, with fewer risk factors, which suggests that one can predict mortality accurately with simpler measures. Results from other institutions support this conclusion.[1]

Study limitations

It might be argued that LVEF should have been included in the modelling, but because it was not precisely registered in 986 cases and different measurement methods were used, LVEF was excluded. The patients with chronic cardiac insufficiency had significantly lower ejection fractions than the patients without, and to some extent this variable carries similar information as LVEF. Moreover, registration is simple and non-invasive, but perhaps less objective than the measurement of LVEF.

Our models were derived from a patient population in Middle Norway, operated at only one surgical centre. Risk prediction models often show poorer performance in other populations than the one they were derived from. Our models were not externally validated, and therefore the use of our models may not be appropriate for other institutions. However, the performance of the models suggests that they will be suitable for use in our institution. The data collection started in 2000, and we cannot exclude that calibration may be poorer when applied to future patients, even if the estimated shrinkage factors were low. Validation in a more recently collected dataset is therefore warranted.

Conclusion

Our models were simple and easy to use, and showed good predictive ability in our population. Based on the estimated shrinkage factors, our models should also accurately predict mortality in a future population. The EuroSCOREs displayed satisfactory discriminatory ability, but overestimated the risk for the low-risk patients, and underestimated the risk for the high-risk patients. The intra-operative models included useful information for mortality risk prediction, but did not perform significantly better than the pre-operative model. After validating the local pre-operative model in a more recently collected dataset, we will probably be using both our local model and the EuroSCORE in our institution.

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Address:
Vibeke Videm
Department of Immunology and Transfusion Medicine
St. Olav University Hospital
NO-7006 Trondheim
Norway
e-mail: vibeke.videm@ntnu.no

Paper II

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Paper III

Neopterin predicts cardiac dysfunction following cardiac surgery[1]

Authors: Kristin S Berg[1,3], Roar Stenseth[2,3], Hilde Pleym[2,4], Alexander Wahba[2,5], Vibeke Videm[1,6].

[1]Department of Laboratory Medicine, Children's and Women's Health, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway

[2]Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway

[3]Department of Cardiothoracic Anaesthesia and Intensive Care, St. Olavs University Hospital, Trondheim, Norway

[4]Clinic of Anaesthesia and Intensive Care, St. Olavs University Hospital, Trondheim, Norway

[5]Department of Cardiothoracic Surgery, St. Olavs University Hospital, Trondheim, Norway

[6]Department of Immunology and Transfusion Medicine, St. Olavs University Hospital, Trondheim, Norway

Word count: Abstract: 350, manuscript text: 2682 (excluding tables, figure legends and references)

Corresponding author:

Vibeke Videm

Department of Immunology and Transfusion Medicine

St. Olavs University Hospital

NO-7006 Trondheim

Norway

Tel: +47 725 73 321

Fax: +47 725 76 426

vibeke.videm@ntnu.no

[1] This work was supported by the Norwegian Council on Cardiovascular Diseases, the Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU), St. Olavs University Hospital and the Simon Fougner Hartmann Family Foundation.

Abstract

Objectives: Oxidative stress following ischaemia and reperfusion, as well as inflammation, are thought to be important for the development of cardiac dysfunction after cardiac surgery. Our main objective was to investigate whether the inflammatory biomarkers C-reactive protein (CRP), lactoferrin, neopterin and the terminal complement complex (TCC) were associated with cardiac dysfunction after cardiac surgery. Another objective was to assess whether the biomarkers could improve prediction of postoperative cardiac dysfunction compared with clinical variables only.

Methods: Blood samples and clinical data from 1018 consecutive patients undergoing cardiac surgery from 1 April 2008 to 19 April 2010 at St. Olavs University Hospital, Trondheim, Norway, were collected prospectively. The end-point was postoperative cardiac dysfunction, defined as the need for more than one inotropic agent or an intra-aortic balloon pump (IABP) occurring after the operation and until the patient was discharged from the department. CRP, lactoferrin, neopterin and TCC were analysed in plasma, and we used logistic regression to evaluate the association of the biomarkers with postoperative cardiac dysfunction. We adjusted for the following clinical variables previously associated with postoperative cardiac dysfunction: urgent operation, operation type, previous cardiac surgery, chronic heart failure, pulmonary hypertension, previous myocardial infarction and haemoglobin. The likelihood ratio test, the integrated discrimination improvement and receiver operating characteristic (ROC) curves were used to assess whether the biomarkers could improve prediction of postoperative cardiac dysfunction compared with clinical variables alone.

Results: Neopterin was the only biomarker significantly associated with postoperative cardiac dysfunction (odds ratio 2.73, 95% confidence interval 1.65 - 4.51) after adjustment for clinical variables. Neopterin improved risk prediction of cardiac dysfunction following heart surgery compared with clinical variables alone according to the likelihood ratio test ($p < 0.0001$) and the integrated discrimination improvement ($p = 0.02$), particularly for patients with intermediate risks.

Conclusions: Neopterin was associated with cardiac dysfunction following cardiac surgery, and improved the accuracy of risk prediction of postoperative cardiac dysfunction. At present we do not

suggest that neopterin should be measured routinely before heart surgery, but our findings support the hypothesis of the role of oxidative stress and inflammation in development of cardiac dysfunction following heart surgery.

Keywords: Cardiac surgery, cardiac dysfunction, biomarker, inflammation.

Introduction

Cardiac dysfunction after heart surgery is a clinical syndrome characterized by insufficient delivery of blood to the tissues because of reduced cardiac output. A reduction in ventricular function is commonly seen following cardiac surgery, often worsening for several hours postoperatively before recovery [1]. In 5-11% of patients the cardiac dysfunction is severe enough to require treatment with several inotropic drugs or an intra-aortic balloon pump [2-4], and cardiac dysfunction was the most common cause of death after coronary artery bypass grafting in a study from New England, USA [5].

Many factors contribute to the development of cardiac dysfunction following heart surgery, and oxidative stress following ischaemia and reperfusion, as well as inflammation, seem to be crucial [6, 7]. The inflammatory markers C-reactive protein (CRP), lactoferrin, neopterin and the terminal complement complex (TCC) have all been associated with coronary artery disease or the development of reduced ventricular function following ischaemia [8-10]. An overview of further relevant publications regarding these markers is given in the Supplementary Table 1. Several models for prediction of cardiac dysfunction following cardiac surgery have been published, but most of them were based on clinical variables alone [2, 4, 5].

In order to explore some of the inflammatory pathways that might underlie the development of cardiac dysfunction, our main aim was to investigate whether the inflammatory biomarkers CRP, lactoferrin, neopterin and TCC were associated with cardiac dysfunction after cardiac surgery. The secondary aim was to assess whether one or several of the biomarkers could improve the accuracy of risk prediction of cardiac dysfunction after heart surgery compared with a model based on previously published clinical variables [2].

Our hypothesis was that CRP, lactoferrin, neopterin or TCC would be associated with cardiac dysfunction after cardiac surgery, and that one or several of the biomarkers would improve the accuracy of risk prediction compared with clinical variables alone.

Materials and methods

The project was approved by The Regional Research Ethics Committee in Medicine (Project number 4.2007.1528), Trondheim, Norway, on 27 June 2007, (Chairperson Arne Sandvik), and by the Norwegian Data Inspectorate. The present work is part of the Cardiac Surgery Outcome Study (CaSOS).

Data

In this prospective study, all adult patients undergoing cardiac surgery from 1 April 2008 to 19 April 2010 at St. Olavs University Hospital, Trondheim, Norway, were considered eligible for inclusion in the study. Patient characteristics, other diseases and risk factors, blood tests, perioperative data and data on postoperative factors and complications were collected prospectively, quality assured by a senior anaesthesiologist, and stored in a local database as part of the department's quality assurance work.

We collected preoperative peripheral arterial blood samples from consecutive patients. The samples were kept on ice for maximum six hours before they were centrifuged, and stored at -80°C until analysis. Of 1149 eligible patients, 21 did not consent, 32 and seven were unable to consent due to emergency surgery and language problems, respectively, and 57 had missing blood samples. We also excluded 14 patient samples: One had infectious blood, three had active endocarditis, two underwent off-pump surgery, one did not have data on the end-point, and seven samples due to an identification error preventing coupling with clinical data. Thus, 1018 patients were included in the further analyses. Patients with signs of intercurrent infection (other than endocarditis) and elevated levels of CRP were normally not considered for elective heart surgery. Data from the same cohort have been used in another publication on clinical and genetic risk factors for fluid overload following heart surgery [11] as part of CaSOS.

The end-point of the study was cardiac dysfunction after cardiac surgery, defined as the need for more than one inotropic agent or an intra-aortic balloon pump (IABP) occurring after the operation and until the patient was discharged from the department. The same end-point definition was used in a

previously published paper from our group [2]. Ninety-five patients (9.3%) acquired cardiac dysfunction after heart surgery according to this definition.

Biomarkers

In order to explore some of the underlying mechanisms of cardiac dysfunction following cardiac surgery, four biomarkers related to inflammatory pathways were analysed in plasma using enzyme immunoassay (EIA).

CRP is considered a general marker of inflammation, and is measured in all patients admitted for heart surgery. The routine method of CRP measurement used in clinical practice quantifies CRP above 5 mg/L, but quantification of CRP concentrations under 5 mg/L is also important when assessing low-grade preoperative inflammation. We therefore used a high-sensitivity method for CRP measurement (sometimes denoted hsCRP) using a commercial kit (Quantikine Human C-Reactive Protein Immunoassay, R&D Systems, Inc, Minneapolis, USA).

Lactoferrin is considered a marker of activation of neutrophil granulocytes, and was analysed as previously described [12]. Neopterin may be seen as a marker of activated monocytes and the cellular immune system, and was analysed using a commercial kit (Neopterin ELISA, GenWay Biotech Inc, San Diego, USA). TCC, also referred to as C5b-9, is a marker of complement activation, and was analysed using a commercial kit (MicroVue SC5b-9 Plus EIA, Quidel Corporation, San Diego, USA).

Statistical analysis

We used logistic regression to evaluate the association of the four biomarkers with cardiac dysfunction after cardiac surgery. We first analysed each fluid-phase marker separately (unadjusted analysis), and thereafter included the eight preoperative clinical variables found to be significant predictors of cardiac dysfunction in a previous study from our group (Table 1) (adjusted analysis) [2]. All four biomarkers were fitted in the same model with the clinical variables. The model was then tested for linearity in the logit, overly-influential observations, interactions and collinearity.

Preoperative renal dysfunction was removed from the model because of strong correlation with neopterin. As sensitivity analyses we evaluated alternative models with and without renal dysfunction and serum creatinine. Although previously not significant in our population [2], several others have identified age and sex as important predictors of postoperative cardiac dysfunction [4, 13], and we therefore performed a sensitivity analysis including age and sex. Neopterin and TCC were transformed by natural logarithm to fulfil the assumption of linearity in the logit. Only fluid-phase markers that were significant after adjustment for clinical variables were included in the further analyses.

We then tested if the significant fluid-phase marker could improve the prediction of cardiac dysfunction after heart surgery compared with clinical variables alone by performing a likelihood ratio test. Since these clinical variables were previously found to be significant in a risk prediction model for postoperative cardiac dysfunction from the same institution, we did not use backward step-down to reduce the number of predictors in the present study. Further testing of model stability and generalizability is described in the Supplementary data. We compared model discrimination by calculating the area under the receiver operating characteristic (ROC) curve (AUC) [14], and the integrated discrimination improvement (IDI) [15]. The IDI was developed to evaluate average differences in sensitivity and specificity between models with and without new markers using average predicted probabilities, as differences in AUC are insensitive measures of improvement in discrimination [15].

General statistics

Descriptive statistics are given as median with 95% confidence intervals for continuous data, and frequencies with percentages for categorical data. For between-group comparisons we used the Mann Whitney U-test for continuous data, and Pearson's chi square test for categorical data. Linear correlation was assessed with Pearson's correlation coefficient. Statistical analyses were performed using the statistical software R (version 12.2.0; R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria), IBM SPSS (version 18.0; IBM Corporation, Armonk, New York, USA), Minitab 17 (Minitab Ltd., Coventry, United Kingdom) and Sigma Plot 13.0 (Systat Software Inc., San Jose, California, USA).

Results

Patient characteristics are given in Table 2. In the unadjusted analysis, both CRP and neopterin showed significant associations with cardiac dysfunction following cardiac surgery (Table 3). However, neopterin was the only significant biomarker after adjustment for urgent operation, operation type, previous cardiac surgery, chronic heart failure, pulmonary hypertension, previous myocardial infarction and preoperative haemoglobin concentration ($p = 0.0005$) (Table 3, Supplementary Table 2). Neopterin was correlated with CRP ($R = 0.27$, $p < 0.0005$).

Preoperative renal dysfunction was removed from the model because of strong correlation with neopterin ($R = 0.37$, $p < 0.0005$). Neither renal dysfunction nor serum creatinine were significant predictors of cardiac dysfunction in these patients, and the sensitivity analysis showed no difference in odds ratios after removal of renal dysfunction or creatinine from the model. As another sensitivity analysis we also developed an alternative model including age and sex, but the odds ratios for the biomarkers were essentially unchanged (data not shown).

The likelihood ratio test showed that neopterin improved the model fit ($p < 0.0001$). When comparing the model containing only clinical variables with the model including neopterin as well, neopterin increased the model AUC from 0.817 (0.770 – 0.863) to 0.833 (0.779 – 0.874) ($p = 0.07$) (Figure 1), and the IDI was 0.014 ($p = 0.02$), indicating that neopterin increased discrimination.

Figure 1 illustrates that neopterin increased discrimination for a group of patients in particular. These were 380 (37.3%) patients with predicted risks between 2.5% and 6.4%. This corresponded approximately to the 25^{th} percentile, and the 60^{th} percentile, i.e. the patients with intermediate predicted risks, having a few risk factors for cardiac dysfunction.

Discussion

Preoperative neopterin levels were associated with cardiac dysfunction after cardiac surgery, also after adjustment for clinical variables. Moreover, neopterin improved the accuracy of prediction of cardiac dysfunction compared with clinical variables alone.

Cardiac dysfunction after cardiac surgery

Cardiac dysfunction following cardiopulmonary bypass is thought to result from myocardial stunning due to ischaemia and reperfusion [16], and in part also from local and systemic inflammation [7]. It has been proposed that the mechanism involves generation of reactive oxygen species and impaired calcium homeostasis, with damage of the sarcolemma, modification of contractile proteins and reduced calcium sensitivity [6].

Neopterin

Neopterin is released from activated macrophages and monocytes after stimulation with interferon-γ from activated T-lymphocytes [17, 18], and may be seen as a marker of activation of monocytes and the cellular immune system. Neopterin has been associated with left ventricular ejection fraction and cardiac dysfunction in patients with chronic stable angina pectoris [9], and with left ventricular ejection fraction and diastolic left ventricular diameter in patients with critical limb ischaemia [19].

Neopterin has been shown to induce contractile dysfunction in isolated perfused rat hearts [20]. Although the effective concentration in that study was higher than neopterin levels occurring in vivo, it was suggested that long-term influence of lower levels of neopterin could lead to cardiac dysfunction in humans [20]. This effect could possibly be mediated through oxidative stress. A previous study has shown that neopterin enhanced the oxidative effect of hydrogen peroxide *in vitro* [21].

Our findings suggest that neopterin could play a role in the development of cardiac dysfunction. We measured neopterin before the cardiac operation, and registered if the patient had cardiac dysfunction postoperatively. It is uncertain whether it was the specific effects of neopterin that enhanced the risk of

postoperative cardiac dysfunction, if neopterin acted as a marker of inflammation in general or activation of macrophages and the cellular immune system, or whether the association was caused by something else. However, it is possible that elevated levels of neopterin before surgery enhanced the effects of oxidative stress resulting from ischaemia and inflammation after aortic cross-clamping and cardiopulmonary bypass during heart surgery.

Other causes of elevated neopterin

Elevated levels of neopterin have been associated with acute infection, autoimmune diseases and malignancy [18], as well as atherosclerosis [22] and left ventricular dysfunction [9]. We excluded patients with active endocarditis, and normally patients with intercurrent infections were not eligible for elective cardiac surgery until recovery. We did not have data to identify autoimmune diseases or malignancy, but the total number of affected patients is expected to be low. Atherosclerosis and left ventricular dysfunction are frequently observed in patients undergoing heart surgery, and were adjusted for.

Neopterin is biologically stable in the circulation and is eliminated by the kidneys [18]. Thus, impaired kidney function could also cause an increase in neopterin concentration. Therefore we also analysed neopterin with adjustment for serum creatinine as well as the other clinical variables, but this did not change the results.

Improvement of risk prediction

Neopterin improved the accuracy of prediction of cardiac dysfunction after cardiac surgery. This was statistically significant according to the likelihood ratio test and the IDI, and almost significant according to the comparison of AUC for the model with and without neopterin ($p = 0.07$). Differences in AUC are conservative measures of improvement in discrimination when comparing risk prediction models, yet ROC curves may be useful for describing the discrimination [15, 23]. Figure 1 indicated that neopterin improved risk prediction especially for patients with intermediate risk of postoperative cardiac dysfunction. The added value for high-risk and low-risk patients was less important. For the high-risk patients it could be that the added effect of several risk factors overshadowed the effect of

neopterin. Implementation of new biomarkers in clinical practice should rely on thorough research and evidence of its usefulness, and an evaluation of the benefit compared with the increased expenses. Presently we therefore do not suggest that neopterin should be measured routinely before heart surgery. However, our findings support the hypothesis of the role of oxidative stress and inflammation in development of cardiac dysfunction following heart surgery.

C-reactive protein

CRP was not significant after adjustment for the clinical variables. Previous studies of the association between CRP and several cardiac end-points have shown conflicting results [8-10, 22, 24]. In our study CRP was somewhat correlated with neopterin, and this could weaken the association between CRP and cardiac dysfunction when CRP and neopterin were analysed in the same model. Moreover, CRP is considered a more general marker of inflammation than is neopterin. It is also possible that CRP represents some of the information included in the clinical variables, such as urgent operation, chronic heart failure and previous myocardial infarction, as CRP has been associated with ischaemic heart disease and chronic heart failure in previous studies [10, 24].

CRP circulates as a pentamer, but a recent study showed that the more active monomeric form of CRP, and not the pentameric form, was found in inflamed atherosclerotic plaques and in myocardial infarction lesions in humans [25]. The different results regarding associations of CRP and cardiac end-points could be partly explained by the fact that we are measuring the circulating pentamer, which not necessarily corresponds with the concentration of monomeric, pro-inflammatory CRP at sites of inflammation.

As patients with signs of intercurrent infection and elevated CRP were not eligible for elective cardiac surgery, the preoperative level of CRP should reflect low-grade inflammation, and for most patients it was not expected to be measurable by routine methods used in clinical practice.

Strengths and limitations

Strengths of the present study include the large number of patients and the completeness of data, with few missing observations. However, we cannot exclude that there exist some unknown confounders

that we have not adjusted for. Unfortunately we did not have complete data on left ventricular ejection fraction, and we could therefore not control for the severity of chronic heart failure. Another limitation is that the end-point definition was partly based on clinical judgment, and was therefore less specific than end-points such as mortality or myocardial infarction. The use of data from one institution only may also have introduced a bias.

Conclusion

Neopterin was associated with cardiac dysfunction following cardiac surgery, and improved the accuracy of risk prediction of cardiac dysfunction after heart surgery, especially in patients with intermediate risk. At present we do not suggest that neopterin should be measured routinely before heart surgery, but our findings support the hypothesis of the role of oxidative stress and inflammation in development of cardiac dysfunction following heart surgery.

Funding

This work was supported by the Norwegian Council on Cardiovascular Diseases, the Liaison Committee between the Central Norway Regional Health Authority (RHA) and the Norwegian University of Science and Technology (NTNU), St. Olavs University Hospital and the Simon Fougner Hartmann Family Foundation.

Acknowledgements

We thank Guri Greiff and Lise Bjella for their help with including patients to the study, and Nina Sandberg for excellent technical assistance.

Table 1. Variable definitions[1]

Variable	Definition
Urgent operation[2]	1: Standard waiting list 2: Need for operation within one week
Operation type[3]	1: CABG[4] only or repair of atrial septum defect 2: AVR[5] only, AVR and CABG combined, repair of aneurysm in the ascending aorta or non-ischaemic mitral valve surgery 3: Miscellaneous procedures such as mitral valve surgery combined with CABG or AVR, AVR combined with other procedures than CABG, operation for dissection of the ascending aorta or rupture of the ventricular septum, and other cardiac surgery such as removal of cardiac tumours and pericardectomy
Previous cardiac surgery	No/yes
Chronic heart failure	Receiving medication (no/yes)
Pulmonary hypertension	Systolic pulmonary arterial pressure (PAP) > 40 mmHg or mean PAP > 25 mmHg, echocardiography or catheterisation (no/yes)
Previous myocardial infarction	No/yes
Preoperative renal dysfunction	Creatinine concentration > 140 μmol/L or dialysis (no/yes)
Preoperative haemoglobin concentration	g/dL (continuous)

[1]Previously published risk factors for cardiac dysfunction after open-heart surgery in our population [2].

[2]Compared with the previously published definition we used only two categories instead of three because no patients underwent emergency surgery in our cohort.

[3]Compared with the previously published definition we used only three categories instead of four due to only one patient needing elective surgery for dissection of the ascending aorta and none undergoing surgery for rupture of the ventricular septum.

[4]Coronary artery bypass grafting

[5]Aortic valve replacement

Table 2. Patient characteristics

Characteristics	Adequate cardiac function (n = 923)	Cardiac dysfunction (n = 95)	*p*-value
Age	67 (67 – 68)	72 (69 – 75)	0.002
Female sex	261 (28.3%)	21 (22.1%)	0.20
Urgent operation	413 (44.7%)	44 (46.3%)	0.77
Operation type			< 0.0001
CABG[1] and ASD[2]	624 (67.6%)	31 (32.6%)	
AVR[3], AVR and CABG, non-ischaemic mitral valve surgery and aneurysm of the ascending aorta	235 (25.5%)	38 (40%)	
Miscellaneous procedures	64 (6.9%)	26 (27.4%)	
Previous cardiac surgery	33 (3.6%)	13 (13.7%)	< 0.0001
Chronic heart failure	79 (8.6%)	40 (42.1%)	< 0.0001
Pulmonary hypertension	59 (6.4%)	30 (31.6%)	< 0.0001
Previous myocardial infarction	417 (45.2%)	54 (56.8%)	0.03
Preoperative renal dysfunction	34 (3.7%)	7 (7.4%)	0.08
Preoperative haemoglobin concentration (g/dL)	14.2 (14.0 – 14.3)	13.9 (13.3 – 14.3)	0.12

C-reactive protein (mg/L)	1.8 (1.6 – 2.0)	3.4 (2.2 – 6.0)	< 0.0001
Lactoferrin (µg/L)	130.1 (123.4 – 138.3)	124.2 (117.7 – 143.3)	0.88
Neopterin (nmol/L)	7.0 (6.8 – 7.3)	9.4 (8.5 – 10.1)	< 0.0001
Terminal complement complex (ng/mL)	165.2 (157.3 – 173.5)	179.5 (147.0 – 209.5)	0.13

Data are given as median (95% confidence interval) or frequencies (percentage). *P*-values were obtained using the Mann-Whitney U test or the Pearson's chi square test, as appropriate.

[1]Coronary artery bypass grafting

[2]Atrial septum defect

[3]Aortic valve replacement

Table 3. Analysis of biomarkers

	Unadjusted analysis			Adjusted analysis[1]			Final model[2]		
Variable	OR	95% confidence interval	*p*-value	OR	95% confidence interval	*p*-value	OR	95% confidence interval	*p*-value
C-reactive protein	1.02	(1.01 - 1.04)	< 0.0001	1.01	(1.00 - 1.03)	0.07	-	-	-
Lactoferrin	1.00	(1.00 – 1.00)	0.57	1.00	(1.00 – 1.00)	0.85	-	-	-
Neopterin[3]	3.23	(2.10 - 4.97)	< 0.0001	2.38	(1.33 - 4.24)	0.003	2.73	(1.65 - 4.51)	0.0005
Terminal complement complex[3]	1.34	(0.95 - 1.90)	0.099	1.31	(0.88 - 1.95)	0.19	-	-	-

[1]All four biomarkers in the same model, adjusted for urgent operation, operation type, previous cardiac surgery, chronic heart failure, pulmonary hypertension, previous myocardial infarction and preoperative haemoglobin concentration.

[2]Final model adjusted for urgent operation, operation type, previous cardiac surgery, chronic heart failure, pulmonary hypertension, previous myocardial infarction and preoperative haemoglobin concentration.

[3]Transformed using natural logarithm

Figure legends

Figure 1. Receiver operating characteristic curves for the model with only clinical variables (urgent operation, operation type, previous cardiac surgery, chronic heart failure, pulmonary hypertension, previous myocardial infarction and preoperative haemoglobin concentration) and the model with neopterin and clinical variables. AUC: Area under the receiver operating characteristic curve.

Conflict of interest

Conflict of interest: none declared.

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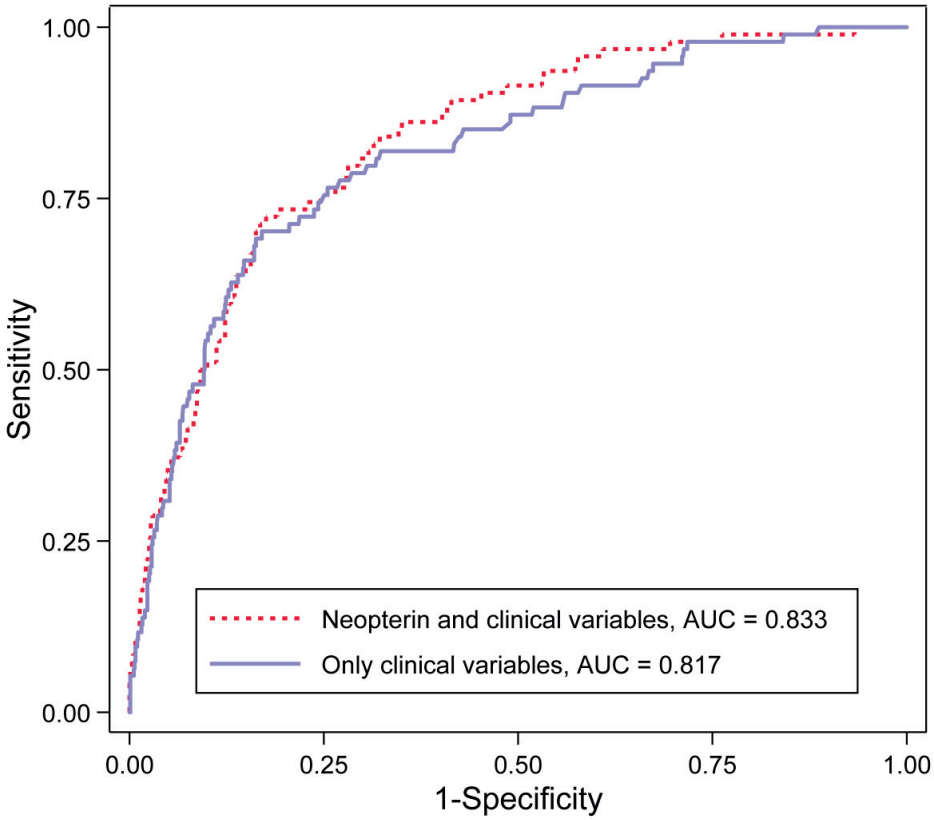
1.00
0.75
0.50
0.25
0.00
Sensitivity
0.00
0.25
0.50
0.75
1.00
1-Specificity
Neopterin and clinical variables, AUC = 0.833
Only clinical variables, AUC = 0.817

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