Riikka Rimpiläinen

MINIMIZED CARDIOPULMONARY BYPASS IN EXTRACORPOREAL CIRCULATION

A CLINICAL AND EXPERIMENTAL COMPARISON WITH CONVENTIONAL TECHNIQUES
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MINIMIZED CARDIOPULMONARY BYPASS IN EXTRACORPOREAL CIRCULATION
A clinical and experimental comparison with conventional techniques

Academic dissertation to be presented with the assent of the Faculty of Medicine of the University of Oulu for public defence in Auditorium 7 of Oulu University Hospital, on 27 May 2011, at 12 noon

UNIVERSITY OF OULU, OULU 2011
Rimpiläinen, Riikka, Minimized cardiopulmonary bypass in extracorporeal circulation. A clinical and experimental comparison with conventional techniques
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Abstract
Cardiac surgery with cardiopulmonary bypass (CPB) results in hemodilution, systemic inflammatory response, activation of coagulation and fibrinolysis, and microembolization, which may all contribute to postoperative organ dysfunction. As an attempt to attenuate these side effects, the use of minimized cardiopulmonary bypass (MCPB) systems has increased. Compared to conventional CPB (CCPB), they are characterized with reduced artificial surface area and blood-air interface. The goal of these alterations has been to reduce systemic inflammation, preserve coagulation function and minimize the need for blood transfusions.

This study was aimed at determining whether or not MCPB attenuates the adverse effects of CPB. In study I, the safety, feasibility and effect on transfusion requirements of MCPB was investigated in unselected coronary artery bypass surgery (CABG) patients. In studies II and III, the incidence of retinal microembolism after CABG and aortic valve replacement (AVR) surgery with MCPB was compared to that of CCPB by means of fluorescein angiography. Furthermore, in studies II and III, the effect of MCPB on systemic inflammation, coagulation, endothelial activation and injury, as well as on platelet activity, was compared to those of CCPB. In study IV, the effect of MCPB on intestinal mucosal damage following CPB was compared to CCPB in a porcine model of prolonged CPB.

MCPB appeared as safe and feasible as CCPB in unselected CABG patients (Study I). MCPB was associated with decreased retinal microembolism compared to CCPB in CABG patients (Study II). Conversely, the difference in retinal microembolism in AVR patients was not statistically significant (Study III). MCPB was associated with a decrease in neutrophil activation in CABG and AVR patients as compared to CCPB. However, there were no differences in coagulation, endothelial activation and injury, or in platelet activity (Studies II, III). There were no differences in markers of intestinal mucosal damage between MCPB and CCPB following prolonged CPB in the experimental model (Study IV).

The results of this study suggest that MCPB may be used safely with CABG patients, with beneficial effects on hematocrit, and attenuated neutrophil activation. In CABG patients, MCPB is associated with reduced retinal microembolism, suggesting a decreased embolic load to the brain. The clinical feasibility of MCPB requires further technical evolution in the management of valve surgery. The results of the animal model support previous concerns regarding intestinal mucosal damage during CPB.

Keywords: cardiac surgery, cardiopulmonary bypass, embolism, minimized cardiopulmonary bypass, retinal fluorescein angiography, systemic inflammatory response
Rimpiläinen, Riikka, Miniperfuusioteknikka – Kliininen ja kokeellinen vertailu perinteiseen sydänkeuhkokoneeseen.
Oulun yliopisto, Lääketieteellinen tiedekunta, Kliinisen lääketieteen laitos, Anesthesiologia,
Kirurgia, PL 5000, 90014 Oulun yliopisto
Oulu

Tiivistelmä

Tutkimuksen tavoitteena oli selvittää voidaanko miniperfuusiolla lievittää sydän keuhkokoneen hai toja. Ensimmäisessä osatyössä selvitettiin miniperfuusion käyttökelpoisuutta ja vaikutusta verensiirtotarpeeseen ohitusleikkauspotilailla valikoimattomassa aineistossa. Toisessa ja kolmannessa osatyössä selvitettiin silmänpohjan mikroembolioiden ilmaantuvuutta miniperfu sion ja perinteisen sydänkeuh kokoneen käytön jälkeen ohitusleikkauspotilailla ja aortalämpä lieikkauspotilailla. Toisessa ja kolmannessa osatyössä selvitettiin lisäksi miniperfuusion vaikutuksia yleistynyt tulehdusvasteen voimakkuuteen, hyytymisjärjestelmään sekä endoteelin aktivaatioon perinteiseen sydänkeuh kokoneeseen verrattuna. Neljännessä osatyössä verrattiin kokeellisessa mallissa miniperfuusion ja perinteisen sydänkeuh kokoneen vaikutuksia suoliston limakalvon eheyteen.


Asiasanat: embolia, fluoresseiniangiografia, miniperfuusioteknikka, sydänkeuh kokone, sydänkirurgia, yleistynyt tulehdusvaste
Acknowledgements

This work was carried out at the Department of Anaesthesiology in co-operation with the Department of Surgery, Division of Cardiotoracic and Vascular Surgery, and at the Cardiotoracic Research Laboratory of the Department of Surgery, Oulu University Hospital, during 2007–2011.

I am deeply grateful to Professor Tero Ala-Kokko, MD, for his persistent encouragement and sound advice during this work.

Professor Tatu Juvonen, MD, deserves my sincerest gratitude for his unfailing trust and exceptional support.

I wish to acknowledge Professor Seppo Alahuhta, MD, for his positive attitude as the head of the Department of Anaesthesiology during this study.

Docent Mika Valtonen, MD, and Docent Kari Kuttila, MD, who kindly agreed to be the official reviewers of this work, deserve my sincere gratitude. Their valuable advice and constructive comments were appreciatively received and have improved the outcome.

My deepest thanks belong to my co-worker and husband Docent Jussi Rimpiläinen, MD, who has firmly helped me to navigate through this project despite my transient suspicions and disbeliefs. I am forever indebted to him for his return to the heavy engines of the Cardiotoracic Research Laboratory to enable the experimental work in this study.

Docent Päivi Laurila, MD, and Docent Martti Lepojärvi, MD, are warmly thanked for the opportunity to conduct this work in their departments.

I am sincerely grateful to Juha Koskenkari, MD, PhD, for all the essential contribution and help. Jan-Ola Wistbacka, MD, PhD, deserves my special appreciation for sharing his broad insight in minimized cardiopulmonary bypass.

For unlimited technical, intellectual and mental support during these years, I am deeply grateful to my friends and co-workers Merja Vakkala, MD, PhD, Tiina Erkinaro MD, PhD, and Sanna Meriläinen, MD. I am very grateful to Nina Hautala, MD, PhD, for rewarding co-operation; Professor Tuomo Karttunen, MD, for patient guidance and co-operation in addition to his expertise; Pasi Ohtonen, MSc, for biostatistical advice.

I also express my warm thanks to my all other excellent co-authors Docent Fausto Biancari, MD, Docent Kai Kiviluoma, MD, Docent Eeva-Riitta Savolainen MD, Docent Heljä-Marja Surcel, MD, Pirjo Mustonen, MD, PhD, Martti Mosorin, MD, Juha Nissinen, MD, PhD, Hanna Jensen, MD, PhD, Eija Rimpiläinen, MD, Matti Pokela, MD, PhD, Pertti Loponen, MD, PhD, Simo-
Pekka Koivisto, MD, Kari Teittinen, MD, for their assistance, expertise and valuable co-operation.

Docent Vesa Anttila, MD, deserves my special thanks for his sincere help and encouraging advice during this work.

I am sincerely grateful to Michael Spalding, MD, PhD, for his thorough and excellent language revision, and for the most beneficial communication.

Sylvi Savolainen, SN, Tarja Lamberg, SN, and Sinikka Sälkiö, SN are deeply valued for their irreplaceable assistance. I warmly thank the staff of cardiac operation theatre and the staff of postoperative intensive care unit for their help in the practical implementation during this study. I also owe my warm thanks to the rest of the excellent research team in the Cardiothoracic Research Laboratory: Fredrik Yannopoulos MS, Tuomas Mäkelä MS, Kirsi Alestalo MS, as well as Seija Seljänperä, RN, are truly thanked.

My colleague cardiac anaesthesiologists, as well as cardio-thoracic surgeons deserve my deepest gratitude for all their help and contribution during this work.

I owe particular thanks to Tuomas, Anni and Salla Rimpiläinen for their patience and understanding during these busy years. Salla’s earnest technical assistance, as well as her unlimited supply of positive ambiance have been crucial during the course of this work. I am most grateful to grandparents Rimpiläinen for their valuable help in the household maintenance and logistics during this work. I thank my parents Marja and Juhani Hirvonen, as well as my grandparents Kerttu and Jaakko Hirvonen, for a lifelong encouragement and support. I owe my heartfelt thanks to my dear sister Liisa Hirvonen, for all those treasured days together.

I am most indebted to the deep snow in the summits of Valais and Lyngen for setting the perspective, and to The National for providing the perfect pace.

This work was financially supported by grants provided by Oulu University Hospital, The Finnish Foundation for Cardiovascular Research and the Sigrid Juselius Foundation, which are all gratefully acknowledged.

Oulu April 2011

Riikka Rimpiläinen
Abbreviations

AF  Atrial fibrillation
AKI  Acute kidney injury
AKIN  Acute Kidney Injury Network
ATP  Adenosine triphosphate
C3  Complement 3
CABG  Coronary artery bypass grafting
CCPB  Conventional cardiopulmonary bypass
CK-MBm  Myocardial creatinine kinase
COPD  Chronic obstructive pulmonary disease
CPB  Cardiopulmonary bypass
CRP  C-reactive protein
DO2i  Systemic oxygen delivery index
EC  Endothelial cell
eNOS  Endothelial nitric oxide synthase
F1+2  Prothrombin fragment 1+2
GP  Glycoprotein
Hcr  Hematocrit
ICU  Intensive care unit
IL-6I  Interleukin 6
IL-8  Interleukin 8
iNOS  Inducible nitric oxide synthase
IR  Ischemia-reperfusion
LBP  Lipopolysaccharide binding protein
LPS  Lipopolysaccharide
MAP  Mean arterial pressure
MCPB  Minimized cardiopulmonary bypass
MI  Myocardial infarction
MIF  Macrophage migration inhibitory factor
MOF  Multiorgan failure
MPO  Myeloperoxidase
MRI  Magnetic resonance imaging
NAD+  Nicotinamide adenine dinucleotide
NF-κB  Nuclear factor κB
nNOS  Neural nitric oxide synthase
NO  Nitric oxide
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>ONCABG</td>
<td>Coronary artery bypass on cardiopulmonary bypass</td>
</tr>
<tr>
<td>OPCAB</td>
<td>Off pump coronary artery bypass</td>
</tr>
<tr>
<td>PAI</td>
<td>Plasminogen activator inhibitor</td>
</tr>
<tr>
<td>PAR</td>
<td>Protease activated receptor</td>
</tr>
<tr>
<td>PC</td>
<td>Phosphorylcholine</td>
</tr>
<tr>
<td>PCI</td>
<td>Percutaneous coronary intervention</td>
</tr>
<tr>
<td>PMEA</td>
<td>Poly[2-methoxyethyl acrylate]</td>
</tr>
<tr>
<td>PMN</td>
<td>Polymorphonuclear leukocyte</td>
</tr>
<tr>
<td>PMX</td>
<td>Polymyxin B-immobilized fiber</td>
</tr>
<tr>
<td>POCD</td>
<td>Post-operative cognitive decline</td>
</tr>
<tr>
<td>Qspl</td>
<td>Splanchnic blood flow</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cell</td>
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<tr>
<td>RIFLE</td>
<td>Risk Injury Failure Loss End-stage</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>SCAD</td>
<td>Small capillary and arteriolar dilatation</td>
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<tr>
<td>sGPV</td>
<td>Soluble plasma glycoprotein V</td>
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<tr>
<td>SIRS</td>
<td>Systemic inflammatory response syndrome</td>
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<tr>
<td>TAT</td>
<td>Thrombin-antithrombin complex</td>
</tr>
<tr>
<td>TCD</td>
<td>Transcranial Doppler</td>
</tr>
<tr>
<td>TF</td>
<td>Tissue factor</td>
</tr>
<tr>
<td>TFPI</td>
<td>Tissue pathway inhibitor</td>
</tr>
<tr>
<td>TJ</td>
<td>Tight junction</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like receptor</td>
</tr>
<tr>
<td>TM</td>
<td>Thrombomodulin</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor α</td>
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<tr>
<td>TRALI</td>
<td>Transfusion-related acute lung injury</td>
</tr>
<tr>
<td>vWF:Ag</td>
<td>von Willebrand factor antigen</td>
</tr>
<tr>
<td>VO₂i</td>
<td>Systemic oxygen consumption index</td>
</tr>
<tr>
<td>ZO-1</td>
<td>ZO-1 protein</td>
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List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:


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Original publications
1 Introduction

Modern cardiac surgery with cardiopulmonary bypass (CPB) is characterized by decreasing complication rates in combination with increased patient risk profile and surgical complexity (Ferguson et al. 2002, Baillot et al. 2009). Due to the increase in referral of older patients with a higher rate of comorbidity for more complex and prolonged cardiac surgery, there is a need to understand the mechanisms leading to end-organ injury after cardiac surgery and CPB, and to identify strategies to attenuate them. Considering the progression in costs and load on the healthcare system, even low-risk cardiac surgery patients may benefit from strategies to attenuate the adverse effects of CPB, perhaps allowing for more success with a "fast-track" protocol to reduce the need for time in the intensive care unit (ICU).

Despite advances in perfusion components, anesthesiologic procedures and surgical techniques, cardiac surgery with CBP is still not without side effects and is associated with various pathophysiologic changes. CPB has been clearly shown to be associated with systemic inflammatory response leading to postoperative organ dysfunction (Levy et al. 2003). Surgical trauma, blood interaction with extracorporeal circuit artificial surfaces, and reinfusion of pericardial suction blood, activate humoral and cellular inflammatory cascades, which may further contribute to end-organ injuries (Paparella et al. 2002). These may manifest themselves as bleeding disorders, myocardial dysfunction, respiratory failure, renal and neurologic dysfunction, altered liver function and ultimately multiple organ failure (Butler et al. 1993, Levy et al. 2003).

Cerebral microembolism is a recognized consequence of CPB and it has been suggested that it is associated with postoperative neurologic injuries and cognitive dysfunction (Pugsley et al. 1994). Splanchnic vulnerability during CPB has become a subject of interest, as the current understanding of gastrointestinal physiology implicates the gastrointestinal tract in the pathogenesis of the systemic inflammatory response syndrome (SIRS) and multiple organ failure (MOF) (Ackland et al. 2000).

A number of different strategies, including surgical techniques, such as off-pump coronary artery bypass surgery (CABG) and endovascular interventions, and new pharmacologic agents have been employed in an attempt to minimize the deleterious effects of CPB and inflammatory activation. Not all patients are suitable candidates, however, for interventional techniques or off-pump surgery. To counteract these problems, CPB technology has evolved with advances in
oxygenator design, biocompatible surface coatings, centrifugal pumps, and, more recently with reductions in circuit size.

In an attempt to attenuate the adverse effects of CPB, the use of minimized cardiopulmonary bypass (MCPB) systems has recently gradually increased. Compared to conventional CPB, the artificial surface area has been reduced and a hard shell venous reservoir has been excluded or replaced with a collapsible venous reservoir to avoid the blood-air interface. The goal of these alterations has been to reduce systemic inflammation while preserving platelet function and minimizing the need for blood transfusions due to diminished priming volume. As these circuits lack a venous reservoir, intravascular filling may be maintained more physiologically compared to conventional CPB. This, along with a well-maintained hematocrit level (Benedetto et al. 2009), provides a theoretical basis for a better preservation of tissue perfusion.

The aim of this research was to evaluate the effects of minimized CPB on the adverse effects of CPB compared to CCPB. The safety and feasibility of MCPB as well as the techniques’ potential in reducing hemodilution and requirement for blood product transfusions were investigated in unselected coronary artery surgery patients (I). The capacity of MCPB for reducing microembolism compared to CCPB was evaluated in elective patients undergoing coronary artery surgery (II), and aortic valve replacement with or without coronary revascularization (III). Furthermore, the effects of MCPB in systemic inflammation, in the activation of the coagulation cascade, as well as in endothelial and platelet activation during CPB was assessed in elective patients undergoing coronary artery surgery (I), and aortic valve replacement with or without coronary revascularization (III) in comparison to CCPB. Finally, the effects of prolonged MCPB and CCPB on intestinal mucosal integrity were compared in an experimental porcine model (IV).
2 Review of the literature

2.1 Adverse effects of cardiopulmonary bypass

2.1.1 Systemic inflammatory response syndrome

The systemic immune response induced by non-infectious agents is called systemic inflammatory response syndrome (SIRS). At present, the immune system is defined in the context of “danger model” as innate and adaptive immunity. Anything that causes tissue stress or damage is sensed as danger, which instantly triggers the innate immune system. In addition to exogenous pathogen-associated molecular patterns, endogenous alarmins can activate immune cells. Alarmins are molecules produced in stressed or damaged tissues exposed to trauma, ischaemia, haemorrhage or other conditions of altered homeostasis (Castellheim et al. 2009). Innate immunity is the instinctive first line of defense against injury, and represents the body’s attempt to protect itself from danger. Adaptive immunity develops as a result of encountering a pathogen, is specific and long-term (Castellheim et al. 2009).

SIRS is organized and executed by innate immunity influenced by the neuroendocrine system (Castellheim et al. 2009). Irrespective of the cause, SIRS involves a qualitatively similar network of highly sophisticated events consisting of humoral, cellular and hemostatic factors. While aiming to be protective, this vital defense mechanism may at times become exaggerated, ultimately damaging the host it is meant to protect.

SIRS is a frequent condition after CPB, occurring in 50% of patients undergoing elective cardiac surgery (Delannoy et al. 2009). Undoubtedly, some degree of inflammatory response present a relevant physiologic host response to surgical trauma and CPB, and often manifests itself merely as simple pyrexia, leukocytosis, tachycardia, hypotension and an excessive accumulation of interstitial fluid, which is seen in even in low-risk patients with uncomplicated cardiac surgery and CPB (Taylor et al. 1996). Less commonly, but increasingly, due to an evolving patient profile, SIRS accompanied by other adverse effects of cardiac surgery and CPB, exceeds the physiologic reserve of the patient, resulting in multiple organ dysfunction and - in severe cases - death (Baillot et al. 2009).

The key components and mechanisms involved in the immune response in SIRS following cardiac surgery requiring CPB are described herein.
Danger signals in cardiac surgery and CPB

Cardiac surgery with CPB represents a multifactorial model of systemic stress and is associated with several conditions, which release alarmins to trigger the body’s host defense: blood components are exposed to nonendothelial surfaces and abnormal shear stress (Butler et al. 1993) (Fig. 1). Blood flow is altered from pulsatile to laminar (Vainionpää et al. 1989). Rapid hemodilution leads to intercompartmental fluid shifts and a dilution of vital plasma proteins. These insults along with the tissue damage in the operative field (Prondzinsky et al. 2005), a possible retransfusion of cardiotomy suction blood (Aldea et al. 2002), hypothermia (Grünenfelder et al. 2000) and aortic cross clamping act as alarmins that are recognized by pattern-recognition receptors such as Toll-like receptors (Castellheim et al. 2009). Pattern-recognition receptors trigger the production of inflammatory mediators, and following complex events are commenced with overlapping plasma protease pathways each generating active proinflammatory mediators, which further activate leukocytes, vascular endothelial cells and platelets (Castellheim et al. 2009). The immune response following cardiac surgery and CPB is to some extent undoubtedly vitally important, but may result in organ dysfunction when it becomes exaggerated. The resulting organ dysfunction is generally temporary and constrained, but may become severe and clinically significant in those patients with limited functional reserve, resulting in postoperative morbidity and mortality (Levy et al. 2003).
Fig. 1. A schematic presentation of the danger signals and consequent pathophysiologic pathways following cardiac surgery and CPB. Modified from Castellheim A, Brekke O-L, Espevik T et al (2009), Innate Immune response to danger signals in systemic inflammatory response syndrome and sepsis. Immunology 69: 479–491.
Toll-like receptors and nuclear factor κB

Toll-like receptors (TLRs) are a family of transmembrane receptors which recognize different pathogens and some endogenous danger signals and activate transcriptional elements such as nuclear factor κB (NF-κB) (Valen et al. 2009, Kawai et al. 2010). NF-κB is a family of transcription factors implicated in the regulation of a large number of disease states and physiologic phenomena. They are activated by numerous stimuli and contribute to the transcription of genes involved in inflammation, apoptosis, cell proliferation and differentiation, organ development and tumorigenesis (Valen et al. 2009). TLRs and NF-κB are distinguished in the complex transcriptional networks of the immune response as they both represent relatively few intracellular signaling pathways which regulate the expression of an extensive amount of inflammatory mediators.

The complement system

The complement system has fundamental clinical implications in the context of tissue injury and inflammation. Activation of the complement system as a result of cardiac surgery with or without CPB has been demonstrated by a decrease in plasma C3, C4, C5 and C1 inhibitor levels and increase of anaphylotoxins C3d and C5a with anaphylactic and chemotactic activity (Ascione et al. 2000, Diegeler et al. 2000). The alternative complement pathway via C3d is particularly accelerated by CPB, and this is a result of contact activation (Tarnok et al. 1999).

The coagulation and fibrinolytic systems

Traditionally, the complement and coagulation systems are described as separate cascades. Both proteolytic cascades are composed of serine proteases, however, which belong to a complex inflammatory network, and exhibit similar characteristics regarding the specialized functions of their activators and inhibitors (Amara et al. 2010). Increasing evidence points to extensive cross-talk between the two systems, particularly as thrombin has been shown to be capable of generating C5a (Huber-Lang et al. 2006). The present immunological understanding suggests that the complement and coagulation systems are an indivisible complex serine protease system, rather than just two separate overlapping pathways (Levi et al. 2004, Amara et al. 2010, Levi et al. 2010).
Parallel to the complement system, the coagulation/fibrinolytic pathway becomes activated. The tissue factor (TF) has been proposed to be the key initiator of inflammation-induced thrombin generation. This is supported by the observations that blocking the TF activity abolishes inflammation-induced coagulation in experimental sepsis models, whereas inhibition of the contact system has no effect on thrombin production (Levi et al. 2004). On exposure to blood, TF binds to factor VIIa, resulting in excessive thrombin formation as well as hyperfibrinolysis as discussed later in chapter 2.1.3. Coagulation activation generates proteases, which not only further mediate the coagulation process, but also induce signaling pathways which mediate inflammatory responses. Most importantly, coagulation proteases bind to protease activated receptors (PARs), a group of transmembrane, G-protein-couplet receptors localized on endothelial cells, mononuclear cells, platelets, fibroblasts and smooth muscle cells (Coughlin et al. 2000). PARs further upregulate several crucial inflammatory responses, such as the production of reactive oxygen species and cell adhesion molecules, and the activation of platelets (Coughlin et al. 2000). Activated platelets play an important role in inflammation, such as in the release of various proinflammatory cytokines and in the support of leukocyte rolling, adhesion and transmigration (Levi et al. 2004). Similar to the role played by the components of the coagulation process, activators and inhibitors in the fibrinolytic cascade play a major role in inflammation-induced coagulation, in particular in cell adhesion and migration and in cytokine production (Levi et al. 2010).

**Cytokine response**

Cytokines are small proteins produced by many different cell types involved in the immune system. They are used as intercellular messengers by the innate immunity to attract inflammatory cells to the site of injury (Castellheim et al. 2009). Cytokines may mediate both proinflammatory and anti-inflammatory pathways during the cause of inflammation and their target cells include leukocytes, endothelial cells, epithelial cells in the intestine and lungs, and organ-specific cells, such as hepatocytes, which then produce acute phase proteins (Castellheim et al. 2009). Tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-8, IL-12, IL-17 and macrophage migration inhibitory factor (MIF) are among the main proinflammatory cytokines, while IL-10 belongs to the principal anti-inflammatory mediators. As an example of the complexity of the system, some proinflammatory cytokines e.g. IL-6, may exert anti-inflammatory
properties depending on their concentration (Castellheim et al. 2009). Thus, the properties of cytokine release as a part of the immune response cannot be understood just by determining the components of the response, or the relationship between the components. This fundamental feature may explain the variability between individual studies investigating the cytokine response during and after cardiac surgery and CPB, despite the large number of papers published (Franke et al. 2005, Diegeler et al. 2000, Matata et al. 2000, Ascione et al. 2000, Narayan et al. 2010). Furthermore, although increased levels of proinflammatory cytokines have generally been associated with a negative postoperative course, this correlation has not been validated. In general, plasma levels of proinflammatory cytokines TNF-α, IL-6 and IL-8 are shown to elevate after cardiac surgery and CPB in several studies, with variable degrees and correlations to cardiac surgery with or without CPB.

**Acute phase proteins**

Proinflammatory cytokines stimulate liver cells to synthesize a broad spectrum of plasma proteins with specific functions mediating the immune response (e.g. C-reactive protein (CRP), fibrinogen, fVIII, C3, C4). During the acute phase reaction of innate immunity, the production of these acute phase proteins is upregulated, while the expression of other proteins such as albumin is temporarily decreased. The iron-binding plasma proteins are increased, which - together with an enhanced iron uptake in macrophages - decrease the plasma free iron concentration to inhibit bacterial growth (Castellheim et al. 2009).

**Cellular components**

Circulating polymorphonuclear leukocytes (PMNs), commonly called neutrophils, are recruited to the site of injury by soluble inflammatory mediators, such as cytokines, chemokines and matrix metalloproteinases. Recently the pathways leading from injury to SIRS were further elucidated, as it was shown that circulating mitochondrial peptides are released as a result of severe trauma (Zhang et al. 2010), and they promote PMNs to migrate and degranulate in the absence of bacterial infection or other stimuli (Zhang et al. 2010, Raoof et al. 2010). Considering that mitochondria are evolutionary endosymbionts derived from bacteria and may bear bacterial molecular patterns, it may not be that
confusing that SIRS carries clinical characteristics that are virtually indistinguishable from sepsis.

The migration of neutrophils from blood to inflamed tissues is an essential component of innate immunity and a key contributing factor to the pathogenesis of inflammatory disorder. The neutrophil adhesion cascade is a complex process including sequential and overlapping stages. Neutrophil adhesion involves leukocyte rolling on the vascular endothelium, followed by leukocyte firm attachment, and eventually migration through the vascular wall (Woodfin et al. 2010). The neutrophil rolling cascade has been demonstrated to also exist in CPB-related inflammation through real-time imaging in experimental models (Duebener et al. 2001, Alaoja et al. 2006). Endothelial cells (ECs) provide the critical substrate for attachment and motility of leukocytes. Adherent neutrophils crawl on the venular endothelium to the preferred site (Woodfin et al. 2010). This is followed by firm attachments between ECs and neutrophils, provided by adhesion molecules expressed on ECs, leukocytes and platelets (Woodfin et al. 2010). The expression of neutrophil surface and soluble ECs adhesion molecules has been suggested to reflect the degree of neutrophil and endothelial activation, and has been shown to become elevated as a result of cardiac surgery with CPB (Grünenfelder et al. 2000, Eikemo et al. 2004), but there exists somewhat conflicting results as to how this correlates with cardiac surgery without CPB (Wei et al. 2003, Wehlin et al. 2004).

Ischemia – reperfusion

The return of perfusion into ischemic tissue may inconsistently contribute to tissue damage. This phenomenon described 50 years ago (Jennings et al. 1960) is called ischemia-reperfusion (IR) injury. The consequences of myocardial IR injury cannot be detached from the CPB-related immune response, as aortic cross-clamping induces myocardial ischaemia and these two events overlap and interconnect.

Releasing the aortic cross-clamp is followed by reperfusion with blood which is fully anticoagulated, immunologically primed and characterized with a very high partial oxygen pressure. As a result the myocardium is exposed to dramatic extremes of ischemia and reperfusion during cardiac surgery. Ischemia induces the accumulation of intracellular sodium (Na+), hydrogen and calcium (Ca2+) ions, culminating in tissue acidosis (Turer et al. 2010). In reperfusion, pH is mainly balanced with Na+-dependent regulatory mechanisms, which lead to
intracellular sodium accumulation. This is followed by enhanced Na\(^+\)-Ca\(^+\)-exchange leading to intracellular Ca\(^+\)-overload in cardiac myocytes. The result is hypercontractility, adenosine triphosphate (ATP) depletion, ultrastructural damage to mitochondria and myocardial stunning (Nayler et al. 1981).

Effective myocardial function depends primarily on oxidative energy production and to secure the large demand of energy, cardiac myocytes host a high density of mitochondria. As a result of Ca\(^+\)-overload, the rapid normalization of pH and oxidative stress, the electromechanical gradient of the inner mitochondrial membrane is disturbed. This results in the release of reactive oxygen species (ROS) and apoptosomes to switch on apoptosis (Turer et al. 2010). Reactive oxygen species (oxygen free radicals) are highly reactive and can quickly overwhelm a cell’s endogenous free radical scavenging system. During the innate immunity response, an additional load of ROS is generated by activated neutrophils (Castellheim et al. 2009). Improper ROS production leads to peroxidation of lipid cell membranes and impairment of the membrane-bound enzymes, causing cell and tissue injury (Castellheim et al. 2009), which predispose to postpump myocardial dysfunction.

It may be difficult to distinguish the causal proportions of IR-injury and amplified immune response in the development of clinical organ dysfunction after cardiac surgery. In low-risk on-pump CABG patients with or without aortic cross-clamp, however, the inflammatory activation and the myocardial injury appear in a similar fashion (Narayan et al. 2010). This suggests that the observed changes in inflammation and myocardial injury were due to a large degree to CPB and surgical trauma rather than to IR-injury.

**Endothelial dysfunction and NO**

Vascular endothelium provides a critical and multifunctional base not only for the innate immune response but also for the ischemia-reperfusion injury. The endothelium participates in the synthesis of inflammatory mediators and is a target for some of the mediators released. The endothelium releases vasoactive mediators which regulate vascular tone and permeability. Thus, it maintains the local balance between pro- and anti-inflammatory factors as well as pro- and anticoagulant factors.

Endothelium is the major source of nitric oxide (NO), which has a significant impact on acute inflammation and critical illness. NO is produced by nitric oxide synthase (NOS) expressed in three isoforms. Neuronal NOS (nNOS) and
endothelial NOS (eNOS) are constitutively expressed while inducible NOS (iNOS) is mainly expressed after activation of the immune system. nNOS and eNOS are responsible for a low physiologic NO production, which elicits vasodilatation with beneficial and protective effects (Balligand et al. 1997). NO is relatively unreactive in physiologic concentrations and acts as a signaling molecule and neurotransmitter regulating mitochondrial respiration and host defense. NO maintains normal hemostasis by reducing neutrophil adhesion, platelet aggregation, relaxation of vascular smooth muscle and preservation of mucosal integrity. At high concentrations NO becomes harmful, generating vasodilatation and increasing permeability. Peroxynitrite, a derivate of NO, can initiate lipid peroxidation and interfere with the functions of ion channels, signaling proteins, receptors, enzymes and transcription factors through protein reactions (Balligand et al. 1997). Furthermore, NO can decrease intracellular nicotinamide adenine dinucleotide (NAD\(^+\)) stores by activation of poly(ADP-ribose) polymerase, and thus affect cell signaling pathways at various levels such as receptor function, enzymatic activity, transcription factor activity and gene expression (Castellheim et al. 2009). Ultimately, NO inhibits mitochondrial respiration, which leads to decreased ATP production and consequently to increased ROS production. The persistent formation of peroxynitrite as a result of excessive ROS production may inhibit the respiratory cycle irreversibly (Castellheim et al. 2009). Taken together, NO may be assumed to be one of the key participants in initiating multiple organ dysfunction in amplified inflammatory response.

2.1.2 Endotoxemia

Endotoxin is a lipopolysaccharide toxin from the outer membrane of gram-negative bacteria and is recognized as a danger signal by the immune system. It is produced by intestinal flora and is normally confined to the lumen of the intestine by a barrier of endothelial cells. After entering the circulation, endotoxins bind to the lipopolysaccharide-binding protein (LBP), of monocytes, macrophages and ECs. LBP interacts with various signaling pathways, such as Toll-like receptor 4 and leads to the production of cytokines and accelerates innate immunity (Kats et al. 2010). Endotoxin is believed to be a major contributor in the development of the inflammatory response (Danner et al. 1991).

Transient systemic endotoxemia as a result of cardiac surgery and CPB has been clearly demonstrated in various studies (Andersen et al. 1987, Riddington et
al. 1996, Bouter et al. 2002, Aydin et al. 2003), but there is still some controversy as to the origin of endotoxin. Endotoxemia seems to mainly be associated with CPB, as endotoxin levels observed in off-pump surgery are comparatively low (Aydin et al. 2003). Comparison of the different endotoxin studies is difficult and confused by a lack of standardized measurement tests (Kats et al. 2010).

A simultaneous increase in gut permeability (Oudemans-van Straaten et al. 1996a) supports the hypothesis that endotoxin originates in the intestinal wall as a result of CPB-related tissue ischemia. Furthermore, higher levels of systemic endotoxin have been shown to associate with the degree of inflammatory response and with increased oxygen consumption after cardiac surgery (Oudemans-van Straaten et al. 1996b). Systemic endotoxins are normally cleared from the circulation by Kupffer cells and sinusoidal endothelial cells in the liver (Uhrig et al. 2005). This clearance may be affected during CPB, while reticuloendothelial cells are greatly overloaded by the clearance of cellular debris and aggregated proteins as a further-by-product of the CPB circuit (Kats et al. 2010). Endogenous endotoxin immunity encompasses antibodies against endotoxins which each individual carries from early fetal life via maternal transfer and which is strengthened by subsequent exposure to endotoxins throughout their life (Kats et al. 2010).

Antibodies against the inner core region of endotoxins may be determined, and this has been used to quantify the endogenous endotoxin immunity. Interestingly, there exists a marked variability in endogenous endotoxin immunity, and preoperative low levels of endotoxin antibodies have been shown to be independently related to adverse outcome in cardiac surgery with CPB (BennettGuerrero et al. 1997, HamiltonDavies et al. 1997, Rothenburger et al. 2001, Mathew et al. 2003). This suggests that endotoxin and its regulation are essential elements in the pathophysiology of SIRS during cardiac surgery and encourages researchers to seek possible therapeutic strategies aimed at reducing either endotoxin release, or its effect.

2.1.3 Coagulopathy

The hemostatic system maintains blood in a fluid state under normal conditions and responds to vascular endothelial injury with a rapid formation of a clot. Blood coagulation is a highly refined defense mechanism to detect injury and prevent exsanguination to maintain survival (Davie et al. 2003). It is a sophisticated balance of procoagulant, anticoagulant, fibrinolytic and antifibrinolytic activities.
Coagulation cascade

Bearing in mind the increasing evidence for extensive cross-talk between the inflammatory and coagulation systems as discussed above, a cell-based model of coagulation (Hoffman et al. 2001) is applied here to define hemostasis. Hemostasis can be divided in three overlapping stages: 1) Initiation, 2) amplification and 3) propagation. In the initiation phase the silent endothelium is stimulated to become an activate surface by injury or exposure to a signaling molecule. Endogenous heparin molecules are discarded from the endothelial cell surface and anticoagulant factors thrombomodulin and antithrombin are locally downregulated. Tissue factor (TF) is exposed and binds and activates circulating Factor VII (fVII). Activated Factor VII (fVIIa) activates Factor V to fVa and Factor X (fX) to fXa, which further activates prothrombin (fII) to thrombin to initiate clot formation. During the amplification and propagation of the coagulation, activated platelets adhere to the endothelium, facilitated by von Willebrand factor (vWF), via glycoprotein (GP) Ib receptors. Once thrombin is formed, this will activate additional platelets. Subsequently, factors I, XI and VIII are activated. A positive feedback cycle is switched on and aggravates thrombin formation. Platelets are activated by thrombin and play a critical role in the amplification of the coagulation cascade by providing a thrombogenic surface. Thrombin converts fibrinogen to fibrin monomer, which is then polymerized into a clot stabilizer by thrombin-activated factor XIII. The coagulation process is limited to the site of vascular injury by serine protease inhibitors including proteins C and S, tissue factor pathway inhibitor (TFPI) and antithrombin. The fibrinolytic system is activated concurrently, resolving the clot as a part of the wound healing and tissue remodeling (Hoffman et al. 2001, Levy et al. 2010).

CPB affects coagulation

Cardiac surgery requiring CPB significantly alters the above highly balanced system of coagulation, predisposing patients to coagulopathy and perioperative bleeding. The incidence of severe bleeding in cardiac surgery exceeds 10%, and approximately 4% of patients are subjected to re-exploration for excess bleeding (Despotis et al. 2001). Despite improved biocompatibility, the CPB surface is
detected as foreign by circulating blood components, which activates coagulation and fibrinolytic cascades. Hence, establishment of thorough anticoagulation before blood contact with the CPB surface constitutes an essential component of CPB management. This is, as a rule, achieved using heparin. Heparin binds to antithrombin and potentiates antithrombin to inactivate thrombin, fXa and fIX. Despite the use of heparin, a significant generation of thrombin occurs during CPB (Boisclair et al. 1993, Knudsen et al. 1996). Thrombin mediates several hemostatic actions in addition to the principal function of cleaving fibrinogen to fibrin. Amongst them, it activates platelets via thrombin-specific receptors and stimulates endothelial cells to produce von Willebrand factor to enhance platelet aggregation (Edmunds et al. 2006). 

The origins of progressive thrombin generation during CPB have been uncertain and under speculation. It has earlier been reasoned that the contact of blood with the CPB circuit via the intrinsic coagulation pathway was the main contributor to this (Wachtfoleg et al. 1987). More recent data suggests, however, an equally important trigger via the material-independent fVIIa/TF pathway (Boisclair et al. 1993, Philippou et al. 1995) in response to vessel wall injury during cannulation, coronary arteriotomy, monocyte activation and the retransfusion of pericardial shed blood (Hattori et al. 2005, Boisclair et al. 1993, Philippou et al. 2000, Aldea et al. 2002). In addition, the so-called “blood-borne” TF (in the form of tissue factor-containing monocytes, platelets and multicellular-derived cell-derived microparticles flowing freely within the systemic circulation) could be delivered to sites of vascular injury and contribute to the amplification and propagation of thrombosis (Mackman et al. 2007).

Hyperfibrinolysis

A rapid and robust generation of thrombin is accompanied with a profound state of hyperfibrinolysis (Holloway et al. 1988, Despotis et al. 2001). Tissue plasminogen activator levels increase significantly instantly after the initiation of CPB, while plasminogen activator inhibitor (PAI)-1 levels remain unchanged (Chandler et al. 2003). The heightened state of fibrinolysis is considered to be a major participant in CPB-related coagulopathy, which has resulted in the development of several anti-fibrinolytic pharmacotherapies as an attempt to decrease perioperative bleeding.
Platelets and plasma coagulation factors

Several mechanisms contribute to platelet dysfunction as a result of CPB (Woodman et al. 1990). Heparin alters platelet function even before CPB (Khuri et al. 1995). After the initiation of CPB, the number of platelets rapidly depletes, exceeding the decrease explainable by hemodilution alone (Holloway et al. 1988). Thrombin-mediated platelet activation leads to their rapid clearance from the circulation (Despotis et al. 1999). Furthermore, several authors have reported a significant alteration of platelet surface antigens during CPB (Wahba et al. 1996, Wenger et al. 1989), including the enhanced expression of adhesion molecule P-selectin to promote platelet-leukocyte and mutual aggregation (Wahba et al. 2000).

Additional hemostatic abnormalities related to CPB include the consumption and dilution of plasma coagulation factors and platelets after the initiation of CPB (Despotis et al. 2008). Any possible hypothermia will also alter the function of both plasma coagulation factors and platelets (Parr et al. 2003).

Inhibition of coagulation

Activation of the coagulation system is regulated by 3 major anticoagulant pathways: antithrombin, the protein C system and tissue factor pathway inhibitor (TFPI) (Levi et al. 2004). During inflammation-induced activation of coagulation, the function of all 3 pathways can be impaired. As another indication of the intimate association between inflammation and coagulation, antithrombin, the protein C system and TFPI are also known for their anti-inflammatory and for their cell protective effects (Loubele et al. 2010). Antithrombin activity is reduced after cardiac surgery with CPB and a low postoperative, but not preoperative, level of antithrombin is associated with adverse events after cardiac surgery (Ranucci et al. 2005, Garvin et al. 2009). TFPI is the main inhibitory mechanism of TF-fVIIa complex (Levi et al. 2004). The predominant data reported demonstrates TFPI’s equally important role in inflammation and tissue repair, as it’s administration has been shown to have some benefits in the management of severe clinical community acquired pneumonia (Laterre et al. 2009) and carry a role in vascular remodeling following shear stress injury (Ekstrand et al. 2010).

The causes of coagulopathy during cardiac surgery requiring CPB are multifactorial, and despite many years of investigations, still await to be full clarification. This lack of understanding may partly explain why this common
adverse effect persists today. A more thorough understanding of the causal mechanisms, deeper insight into the modulation of inflammation and coagulation along with a specific, bed-side point-of-care monitoring of coagulation may lead to more targeted management strategies and better outcomes.

2.1.4 Hemodilution

Hemodilutional anemia is an unavoidable consequence of CPB using conventional priming volumes. The degree of hemodilutional anemia is related to the patients’ preoperative hemoglobin level and the priming volume of CPB. Potential advantages of hemodilution during CPB include a reduction in blood viscosity and thus improved microcirculatory flow, as well as a decreased requirement for transfusions during CPB. Excessive hemodilution may, however, compromise oxygen delivery at the tissue level.

Several large databased studies have identified an association between lowest hematocrit during CPB and postoperative morbidity and mortality (Fang et al. 1997, Zindrou et al. 2002, Karkouti et al. 2005). Morbidity and mortality have been demonstrated to increase when hematocrit levels are below 22% and 23% (Habib et al. 2003, Defoe et al. 2001). The lowest hematocrit on CPB has been reported to be an independent risk factor for renal (Karkouti et al. 2009, Habib et al. 2003) and neurologic (Karkouti et al. 2005, Mathew et al. 2007) injury. It is, of course, difficult to draw absolute conclusions based on these studies, as low hematocrit triggers red blood cell (RBC) transfusion and makes it difficult to decide which of these is the actual cause of the adverse outcomes.

Preoperative anemia and CPB priming volume are the primary determinants of the degree of hemodilutional anemia. Preoperative anemia is highly prevalent in cardiac surgery populations (Karkouti et al. 2008) and it is the single most important determinant of perioperative RBC transfusion (Khanna et al. 2003), which have many side effects (Murphy et al. 2007). Preoperative anemia may also have independent harmful effects in outcome (Karkouti et al. 2008). The knowledge of the association between preoperative anemia and adverse outcomes in cardiac surgery has recently encouraged to diagnosing and correcting preoperative hemoglobin status, which is further discussed in chapter 2.3.2.
2.1.5 Blood transfusion

Cardiac surgical procedures account for a significant amount of allogeneic blood transfusion use despite advances in surgical techniques and pharmacological methods for the reduction of blood loss. Allogeneic blood transfusion in cardiac surgery accounted for 9.0% of all blood supplied by the national blood service in Finland in 2008 (Annual report of Finnish Red Cross Blood Service).

An enhancement of oxygen-carrying capacity (Weiskopf et al. 1998), improved hemostasis with blood component therapy (Boneu et al. 1987) and volume support for cardiac output (Ferraris et al. 2007) are accepted benefits of blood transfusions. The transfusion of allogeneic red blood cells is, however, increasingly recognized to be associated with several adverse outcomes after cardiac surgery, including an increased risk of postoperative infection (Zacharias et al. 1996, Murphy et al. 2007, Hortal et al. 2009), long-term morbidity and mortality risk (Engoren et al. 2002, Kuduvalli et al. 2005, Murphy et al. 2007) in addition to the risk of transfusion-related transmittable diseases.

Transfusion related immunomodulation

Due to advances in transfusion medicine, blood transfusions carry a minimal risk for the transmission of diseases and concerns regarding the risks associated with blood transfusion and blood products have therefore shifted to the transmission of noninfectious hazards. The noninfectious serious hazards of transfusion are a very broad category, which include everything from transfusion reactions to the less understood complications such as transfusion related immunomodulation (Hendrickson et al. 2009). The transfusion of RBCs has been demonstrated to modulate the immune response in organ transplant patients, and there is a general concern exists that blood transfusions may also suppress the immune response also in other conditions such as cancer and sepsis (Blumberg et al. 1994). The extent and type of immune deficit remains controversial, but leukocytes and soluble factors mediated by leukocytes are considered as possible contributors (Ferraris et al. 2007). This has been supported by the findings of randomized controlled trials in cardiac surgery patients which have reported a significant reduction of in-hospital death and infection rate in transfused patients receiving leukocyte-depleted RBC transfusion, compared to in-hospital in recipients of non-leukocyte-depleted RBC transfusions (van de Watering et al. 1998, Bilgin et al. 2004).
A distinctive manifestation of transfusion-related immunomodulation is transfusion-related acute lung injury (TRALI) and this may be the most common cause of transfusion-related morbidity and mortality (Benson et al. 2009). It has been described with the infusion of most blood products including RBCs (including leukocyte-depleted), fresh frozen plasma and platelets, but is more common with plasma-containing blood products (Benson et al. 2009). The incidence of TRALI is extremely variable, and presumably underreported due to difficulties in differential diagnosis in critically ill patients with their many other risk factors for acute lung injury. In clinical terms, TRALI results in hypoxia and non-cardiogenic pulmonary edema typically within a few hours after transfusion. The development of TRALI most likely requires prior activation of the pulmonary vascular endothelium and adherence of neutrophils by endogenous stimuli (i.e. sepsis, surgery). As a second event, the transfusion of antibodies within the transfusion products results in neutrophil-mediated cytotoxicity of the vascular endothelium, causing capillary leak and acute lung injury (Benson et al. 2009).

In conclusion, as blood products should be viewed as a limited resource carrying both risks and benefits, they should be carefully allocated and efforts should be made in order to develop approaches for blood conservation and to improve patient outcomes (Shann et al. 2006).

2.1.6 Microembolism

Emboli, which may be gaseous or particulate, can be divided into macroembolism and microembolism: macroemboli occlude flow in arteries with a diameter of 200 µm or greater, whereas microemboli occlude flow in smaller arteries, arterioles and capillaries (Blauth et al. 1995). Microembolism is a recognized consequence of CPB. Microemboli act as a foreign surface and activate the inflammatory, complement and coagulation cascades. As the microemboli protrude against the endothelial capillary wall, they induce endothelial damage, which causes further tissue factor expression and subsequent platelet activation and thrombus generation (Barak et al. 2005) (Gorbet et al. 2004). Tissue ischemia takes place distal to the obstructing microemboli depending on the sensitivity of the tissue in question to hypoxic conditions. Gaseous microemboli are unlikely to cause significant prolonged ischemia, but solid microemboli may obstruct cerebral capillaries for prolonged periods leading to focal ischemic damage. In addition to ischemia, the simultaneous inflammatory response may further aggravate the
injury, as cerebral tissue injury resulting from emboli has been demonstrated to be accelerated by the inflammatory response (Reasoner et al. 1997, Helps et al. 1991).

**Manifestations**

Autopsy studies reporting CPB-related cerebral microembolism during the era of membrane oxygenators revealed thousands of focal dilatations in terminal brain arterioles and capillaries (SCADs) sized 10–70 µm which were considered to have manifested as a consequence of fat or air emboli (Moody et al. 1990). Additional experimental studies have identified platelet-fibrin microaggregates in retinal vessels after CPB, which were associated with microfocal ischemic neuronal injury (Blauth et al. 1988). Cerebral microembolism during and after cardiac surgery with CPB has been documented with transcranial Doppler ultrasound (TCD) technology (Braekken et al. 1997), with retinal angiography (Ascione et al. 2005, Blauth et al. 1988) and with autopsy studies (Moody et al. 1990, Brown et al. 2000). The number of microembolic signals is significantly higher in patients undergoing valve replacement than those undergoing CABG (Braekken et al. 1997). Cerebral microvascular embolic load has been demonstrated to correlate with the duration of CPB (Brown et al. 2000), and to be diminished but not avoided by processing the mediastinal scavenged blood (Kincaid et al. 2000), and using an arterial line filter (Pugsley et al. 1994). The arterial filter removes particles larger than 40 µm, but as the capillaries are only about 8–10 µm in diameter they can easily be obstructed with smaller emboli.

As can be reasoned, CPB-related microembolism is not a phenomenon restricted to the brain, although it is less investigated in other organs. Earlier histological studies have revealed embolized material has in the several other organs in humans (Orenstein et al. 1982) as has experimental work in animals (Brondén et al. 2006).

**The source of microemboli**

The source of microemboli as a result of CPB is thought to be multifactorial (Blauth et al. 1995), and presumably both gaseous and biological as well as nonbiological particulate emboli are involved (Markus et al. 1993). Biological particles arise from components of the circulation (aggregates of RBCs, leucocytes, platelets, denatured protein, fibrin) and the operative field (thrombus,
fat, cellular aggregates, atheroma, valve debris, muscle fragments) (Arrowsmith et al. 2000). Nonbiological particles arise from CPB circuit and cardiotomy reservoir and from foreign material introduced into the operative field (Arrowsmith et al. 2000). Venous air during CPB is common and the perceived ability of venous hard shell reservoirs to remove such air can easily lead to a misunderstanding of the venous air being consequently benign. This air however, has a tendency to dissolve into microbubbles detectable in the arterial line distal to the arterial filter (Willcox et al. 1999). More air is introduced in the systemic circulation during valve surgery, and - not surprisingly - systemic embolization is therefore more common during valve surgery than CABG (Braekken et al. 1997).

2.2 Clinical organ dysfunction

2.2.1 Myocardial injury

Cardiac surgery is always associated with some degree of direct myocardial trauma following the handling of the heart. This is usually well tolerated, but ischemia-reperfusion injury, inflammatory response, left ventricular over-distension, and increased cardiac workload may contribute to the development of perioperative myocardial injury (MI). Cardiac specific markers such as troponin-T, troponin-I and creatinine kinase MBm (CK-MBm) have been used to quantify the myocardial injury, but some degree of myocardial enzyme release almost always occurs as a result of direct surgical trauma and manipulation, particularly in valve surgery (Thygesen et al. 2007). Due to the difficulties and differences in diagnostic criteria, the reported incidence of perioperative myocardial infarction following CABG has varied from 3% to 21%, which has encouraged the production of a universal classification for myocardial infarction following CABG (Thygesen et al. 2007). By convention, increases of biomarkers (preferably troponin) greater than 3 x 99th percentile of the upper reference limit, plus either new pathological Q waves or a new LBBB, or an angiographically documented new graft or native coronary artery occlusion, or imaging evidence of a new loss of viable myocardium have been designated as defining CABG-related myocardial infarction (Thygesen et al. 2007). Recently, the incidence of perioperative MI after CABG in a large prospective trial was reported to be 3.3% (Serruys et al. 2009).
Ischemia-reperfusion injury

Cardiac surgery with CBP is typically associated with cardioplegic arrest to provide a still and blood-free operating field. This is achieved by inducing an ischemic arrest to the heart by cross-clamping the aorta and thereby preventing coronary artery perfusion. Global ischemia of the heart is detrimental and cardioprotection during this ischemia involves the application of a hyperkalemic cardioplegic solution to induce rapid myocardial depolarized arrest and induce an inanimate diastolic state. This technique has been the cornerstone of cardiac protection (albeit with a number of modifications – continuous or intermittent, crystalloid or blood, hypothermic or warm, with or without various additives) for over 30 years (Chambers et al. 2010). The hyperkalemic cardioplegic arrest is reversible with reperfusion. Short periods (seconds to a few minutes) of myocardial ischemia accompanied with this technique are generally well tolerated, but may occasionally lead to evident ischemia-reperfusion injury with prolonged recovery (Braunwald et al. 1985).

Sequelae of myocardial injury

Ischemia is a progressive process and as the ischemic duration increases, the cellular and molecular events become more severe, and without timely reperfusion they may eventually lead to cell death (Chambers et al. 2010). After a longer ischemic duration (prolonged aortic cross-clamping, maldistribution of cardioplegic solution) or in case of readily ischemic heart, the reperfusion following aortic declamping may provoke a profound ischemia-reperfusion injury. This is associated with transient or irreversible postischemic contractile dysfunction and tendency to postoperative arrhythmias. Atrial fibrillation (AF) is the most common cardiac arrhythmia, which occurs after cardiac surgery. Postoperative AF is usually well-tolerated, but may result in hemodynamic instability and subjective discomfort, and is associated with a higher risk of operative morbidity, prolonged hospitalization, and increased cost compared with patients remaining in sinus rhythm (Hogue et al. 2005). In addition, postoperative AF is associated with a 2- to 3-fold increase in the postoperative risk for stroke (Epstein et al. 2005).
2.2.2 Renal injury

Acute kidney injury (AKI) is a serious complication after cardiac surgery and is associated with an increase in short-term mortality (Chertow 1998). To classify the severity of acute kidney injury, present epidemiological studies use the consensus definitions of the Risk Injury Failure Loss End-stage (RIFLE) or the Acute Kidney Injury Network (AKIN) (Bellomo et al. 2004). AKI develops in 2% to 30% of patients undergoing cardiac surgery, depending on the definition (Rosner et al. 2006). The overall risk of AKI requiring dialysis is 1.0% among CABG patients and 2.0% among valvular surgery patients (Chertow et al. 1997). The long-term survival of cardiac surgical patients is proportional to the severity of the AKI, when assessed as peak changes in serum creatinine (Hobson et al. 2009). In addition, the duration of AKI after cardiac surgery is directly proportional to long-term mortality (Brown et al. 2010).

Renal injury following cardiac surgery appears to be related to pre-existing renal dysfunction, diabetes, older age, female sex, hypertension, chronic obstructive pulmonary disease, peripheral vascular disease, left ventricular dysfunction, emergency operation, prolonged CPB duration and hemodilution <25% (Rosner et al. 2006).

No systematic studies have been undertaken to investigate the pathophysiologic changes in patients with AKI after cardiac surgery. The pathogenesis most likely involves multiple pathways, and the release of labile iron contributing to oxidation from reactive oxygen species (Rosner et al. 2006). Mechanical stress in the CPB circuit causes hemolysis, which results in the release of hemoglobin from lysed RBCs into the plasma and may thus be a crucial factor in contributing to renal injury (VermeulenWindsant et al. 2010).

2.2.3 Neurologic injury

Neurologic abnormalities after cardiac surgery are a dreaded complication and when severe, may completely overcast the benefits of an otherwise successful operation. Neurologic complications are of particular concern because of their impact on hospital stay, mortality, health care costs, and quality of life (Roach et al. 1996, Arrowsmith et al. 2000, Newman et al. 2001a, Newman et al. 2001b). Despite the many considerable advances in surgical, CPB and anesthetic techniques over the last several decades, postoperative stroke following cardiac surgery remains a serious problem.
Classification

Postoperative neurologic injury can be classified in several ways. In the American College of Cardiology / American Heart Association guidelines for CABG surgery, postoperative neurologic deficits have been divided into two types (Eagle et al. 2004): Type I deficits are those associated with major, focal neurologic deficits, stupor, and coma; type II deficits are characterized by deterioration in intellectual function or memory, confusion, agitation, memory deficits and seizures without evidence of focal injury (Table 1). Based on localization, the injury may also be classified as focal, global or diffuse-, multifocal (Taylor et al. 1998).

Incidence

The most frequently cited data on stroke incidence following cardiac surgery is that of Roach and colleagues (Roach et al. 1996), who reported a multi-institutional prospective cohort study of 2108 patients aimed at determining the true neurologic outcome after CABG in the United States. The incidence of type I deficits was reported as 3.1% and that of type II deficits as 3.0% (Roach et al. 1996). However, it must be taken into account that the incidence of cerebral complications after cardiac surgery is related to increased age, and where as the rate of stroke is under 2% in patients under 60, it is over 5% in patients aged 70–79 and over 7% in patients over 80 after CABG surgery (Roach et al. 1996). This is noteworthy considering the increasing age of patients undergoing cardiac surgery. More recent data from the SYNTAX trial, which compared percutaneous coronary intervention (PCI) and CABG for treating patients with previously untreated three-vessel or left main coronary artery disease in 1800 randomly assigned patients, reported the incidence of stroke at 12 months 2.2% in the CABG group versus 0.6% in the PCI group (Serruys et al. 2009).

Risk factors

Other clinical variables which identify the risk for neurologic complications include systolic hypertension, prior stroke, diabetes, female sex, and atherosclerosis of the ascending aorta (Roach et al. 1996, Hogue et al. 2001, Newman et al. 2001, Arrowsmith et al. 2000, van Dijk et al. 2000). Patients undergoing open-chamber cardiac surgery, such as aortic or mitral valve surgery,
are believed to be at higher risk for adverse cerebral outcomes than CABG (Wolman et al. 1999). These procedures are generally associated with an increased risk of embolism from atherosclerotic plaques, thrombi and gaseous bubbles that are created by the intraoperative entrapment of air within the cardiac chambers. The incidence of neurologic events has been shown to increase as the complexity of the surgical procedure increases (Wolman et al. 1999, Hogue et al. 2001), with the highest incidence of 16% for type I and 7.3% for type II deficits in combined intracardiac and CABG procedures (Wolman et al. 1999).

**Type I outcomes**

Postoperative major focal neurologic complications lead to significant morbidity and mortality, and they contribute to increased therapeutic costs and impairment of patient quality of life. These complications are associated with a 10-fold increase in hospital mortality and prolonged intensive care and hospital stay (Roach et al. 1996, Wolman et al. 1999). Oropharyngeal aspiration and reintubation are more frequent (Wolman et al. 1999). Only 31% of patients affected by type I cerebral injury return home (Wolman et al. 1999).

**Type II outcomes**

Type II outcomes are comparable with postoperative cognitive decline (POCD), which has been evaluated in a large number of studies in cardiac patients (Newman et al. 2006). POCD is generally defined as a substantial decrease in individual cognitive performance postoperatively, affecting the activities of daily living (e.g. shopping, domestic work, preparation of meals, bodily care, and dressing) (Moller et al. 1998). Although Roach et al based type II deficits on clinical data and hospital discharge summaries, POCD is more often diagnosed with repeated administration of a group of psychometric tests, as they are believed to be much more sensitive for detecting subtle cognitive changes (Murkin et al. 1995). Therefore, it is not surprising that studies evaluating POCD with neuropsychological tests have consistently reported a higher incidence of POCD than the incidence of type II deficits found by Roach et al. There still exists, however, substantial diversity between the reported of incidences of POCD after cardiac surgery, varying between 20% and 70% (Newman et al. 2006). This may be partially explained by several difficulties associated with perioperative
psychometric testing which may limit the precision of the tests (Rasmussen et al. 2001).

**Mechanisms**

Magnetic resonance imaging (MRI) has revealed brain swelling in patients after cardiac surgery with CPB (Harris et al. 1993), but not after off-pump CABG (Anderson et al. 1999). The mechanisms leading to neurologic damage after CPB are most likely multifactorial, but the primary factors believed to be involved are cerebral embolism and hypoperfusion exacerbated by the systemic inflammatory response and ischemia-reperfusion injury (Roach et al. 1996, Hogue Jr et al. 1999, Arrowsmith et al. 2000, Pugsley et al. 1994, Ahonen et al. 2004, Newman et al. 2006, Hogue et al. 2006). An atherosclerotic ascending aorta is the most significant predictive factor of adverse cerebral outcome after cardiac surgery (Roach et al. 1996), which may be assumed because it provides a potential source of solid emboli. Embolization of atheroma or other debris from the ascending aorta may result from cannulation or decannulation, clamping or declamping, and additionally from a falsely directed cannula jet (Ura et al. 2000). Epiaortic ultrasound is superior to simple palpation, and is a safe and useful method for detecting atherosclerosis in the ascending aorta (Bolotin 2005). When combined with appropriate modificationsto the operative technique based on epiaortic ultrasound findings, it may be effective in reducing the postoperative incidence of stroke when severe atherosclerosis is present (Zingone et al. 2006). Macroembolism may also result from infrequent accidental massive air embolism.

Since the discovery of abundant cerebral microembolic signs (SCADs) after cardiac surgery in human autopsy studies, it has been suspected that they are associated with neurological deficits after CPB (Moody et al. 1990). Asymptomatic cerebral embolic signals detected using a transcranial Doppler (TCD) seem to predict a risk of stroke in several clinical settings (King et al. 2009). To date, studies using neuropsychological testing have failed to show any differences in the incidence of POCD between patients undergoing coronary bypass surgery with or without CPB, despite of the significantly reduced cerebral embolic load assessed with TCD (Liu et al. 2009, Lund et al. 2003). Considering the possibility that the true incidence of POCD is much higher than that previously believed (Rasmussen et al. 2001), instead of a few hundred patients, thousands of patients would be needed to prove or exclude whether two different
management strategies had an impact on the incidence of POCD (van Dijk & Kalkman 2009).

Other potential mechanisms of cerebral injury during CPB include major reductions in CPB flow, mean arterial pressure (MAP) and/or hematocrit, thus affecting cerebral oxygen delivery. In addition to pressure and flow, the actual cerebral blood flow during CPB is determined by other factors, such as temperature, acid-base management strategy (changes in arterial carbon dioxide tension), anesthetic agents and depth of anesthesia, packed cell volume and oxygen saturation (Arrowsmith et al. 2000). Perioperative hyperglycemia may amplify the cerebral damage associated with cardiac surgery (Li et al. 1996). CPB-related systemic inflammation has been shown to be associated with an accentuated cerebral inflammatory response (Hindman et al. 2001, Jungwirth et al. 2010), which has generally been speculated to contribute to cerebral injury after cardiac surgery, but there is not yet enough evidence to confirm this (Jungwirth et al. 2010). Despite extensive efforts and investigations, a detailed knowledge of the pathophysiology of mechanisms involved in neurologic damage after CPB is still warranted in order to design potentially effective neuroprotective strategies, as neurologic damage remains the most important complication after cardiac surgery

Table 1. Type and possible mechanisms of brain injury following cardiac surgery and CPB.

<table>
<thead>
<tr>
<th>Type of cerebral damage</th>
<th>Mechanisms of cerebral damage during CPB</th>
</tr>
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<tbody>
<tr>
<td>Type I injury</td>
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<tr>
<td>Focal neurological damage</td>
<td>Macroembolism</td>
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<tr>
<td>Stupor / coma</td>
<td>Dislodging of atheromatous debris due to aortic manipulation</td>
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<tr>
<td></td>
<td>Diseased carotid arteries</td>
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<tr>
<td></td>
<td>Air embolism</td>
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<tr>
<td></td>
<td>Atrial fibrillation</td>
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<tr>
<td>Type II injury</td>
<td></td>
</tr>
<tr>
<td>Deterioration of intellectual and cognitive function</td>
<td>Microembolism</td>
</tr>
<tr>
<td>Confusion, agitation</td>
<td>Fat particles</td>
</tr>
<tr>
<td>Memory deficits</td>
<td>Platelet aggregates</td>
</tr>
<tr>
<td></td>
<td>Air bubbles</td>
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<tr>
<td></td>
<td>Fragments of CPB surface material</td>
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<tr>
<td></td>
<td>Hypoperfusion</td>
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<tr>
<td></td>
<td>Inflammatory response</td>
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</table>
2.2.4 Gastrointestinal injury

Gastrointestinal perfusion is often compromised earlier than the other vascular beds during conditions with increased demands on the circulation to maintain tissue oxygen delivery. Cardiopulmonary bypass with its related hemodilution and nonpulsatile flow challenges the splanchnic circulation which lacks precise autoregulatory control. Although gastrointestinal complications following cardiac surgery appear with low incidence (0.53%–1.5%), they carry a significant mortality rate (22.5%–61%) (Dancona et al. 2003, Mangi et al. 2005, Khan et al. 2006) even when recognized early and treated appropriately. The interest in the splanchnic circulation during CPB, however, goes beyond preventing abdominal complications, as the current understanding of gastrointestinal physiology implicates the gastrointestinal tract in the pathogenesis of SIRS and multiple organ failure (MOF) (Ackland et al. 2000).

Splanchnic perfusion

CPB has been implicated in causing splanchnic ischemia by inducing regional differences in intestinal blood flow. Previous studies have shown that while global splanchnic perfusion is reasonably well maintained during CPB (Tao et al. 1995, Gårdebäck et al. 2002), splanchnic oxygen consumption is enhanced (Braun et al. 2004), and intestinal permeability increases (Oudemans-van Straaten et al. 1996a, Sun et al. 2008). There have been numerous attempts in order to clarify peripheral mucosal tissue perfusion and oxygenation during CPB with conflicting results (Kuttila et al. 1991, Ohri et al. 1994, Tao et al. 1995, Uusaro et al. 1995, Sicsic et al. 1998, Haisjackl et al. 1999, Nygren et al. 2006, Dong et al. 2009). This is presumably due to the great methodological challenges in defining splanchnic microcirculation: results of a single site of measurement may not be applicable to overall tissue oxygenation. The possible changes in splanchnic blood flow may, however, be amplified by CPB-induced dysfunction in mesenteric endothelial cells with consequent hyperreactivity to α-agonist agents (Doguet et al. 2004).

Intestinal barrier function

The disruption of intestinal barrier function and translocation of micro-organisms and endotoxin have been suggested to lead to a release of proinflammatory cytokines potentially promoting the systemic inflammatory response resulting
from surgical stress and artificial surfaces, and ultimately predisposing to multiple organ dysfunction (Ackland et al. 2000, Riddington et al. 1996). This hypothesis was more recently supported by results of an experimental study demonstrating that as a result of CPB, the intestinal tight-junction proteins occludin and ZO-1 proteins are down-regulated and the formation and function of tight junctions is altered (Sun et al. 2008).

Intestinal barrier function is primarily governed by the integrity of the intestinal epithelium. Intestinal epithelium integrity depends on the result of a dynamic equilibrium between tissue injury and tissue repair mechanisms such as epithelial restitution and proliferation. Factors that may affect gut barrier integrity include enterocyte apoptosis and necrosis, as well as disruption of tight junctions that play a key role in maintaining epithelial monolayer integrity (Keita et al. 2010). Intercellular tight junctions regulate the permeability of ions, macromolecules and cells via the paracellular route and serve as a barrier against the paracellular penetration of pathogenic bacteria and toxic luminal antigens including endotoxins (VanItallie et al. 2004, Furuse et al. 2006).

**Tight junction proteins**

Intramembranous tight junction strands consist of transmembrane proteins, such as occludin (Furuse et al. 1993), tricellulin (Ikenouchi et al. 2005) and claudins (Morita et al. 1999). The region of cytoplasm underlying the tight junction contains several multimolecular protein complexes (including ZO-1 protein), which are involved in scaffolding the transmembrane proteins and in the regulation of cytoskeletal organization and several other vital functions (Guillemot et al. 2008, Itoh et al. 1999). Accumulated evidence indicates that the claudins are the key components in determining the barrier properties of the tight junctions (VanItallie et al. 2004).

Claudins are a multigene family, consisting of at least 24 members in mammals (Krause et al. 2008). They are 20-27 kDa proteins containing two extracellular loops with variably charged amino acid residues and two short intracellular trails (Krause et al. 2008). In most cell types, several claudin types are co-expressed in single cells. Claudins comprise a number of essential barrier components and their properties appear to be essential for the normal functioning of various organs (Krause et al. 2008). There is substantial evidence that tight junction permeability is affected by several pathological stimuli, such as hypoxia (Witt et al. 2003), cytokines (Nusrat et al. 2000), and sepsis (Li et al. 2009).
The redistribution of claudins appears to be related to altered tight junction structure in pathological conditions such as sepsis (Li et al. 2009), active Crohn’s disease (Zeissig et al. 2007), and in acalculous and calculous cholecystitis (Laurila et al. 2007). CPB has been shown to decrease the expression of the tight junction proteins occludin and ZO-1 with a simultaneous increase in intestinal permeability in an experimental model (Sun et al. 2008). The intestinal expression of claudins after CPB has not been reported.

### 2.2.5 Multiorgan dysfunction

In the evaluation of survival after cardiac surgery, up to 36% of the patients have longer intensive care unit (ICU) stays related to multiorgan dysfunction (Kollef et al. 1995, Ryan et al. 1997, Engoren et al. 2000, Kern et al. 2001, Hein et al. 2006). This group of cardiac surgery patients is associated with disproportionate consumption of hospital resources and with a high mortality (Kollef et al. 1995, Ryan et al. 1997, Engoren et al. 2000, Kern et al. 2001, Hein et al. 2006). Mortality is dependent on renal, respiratory, and cardiac failure, as well as age, elevated APACHE II scores, and reexploration (Hein et al. 2006). Long-term survival analyses have demonstrated a significantly lower survival in patients with longer ICU stay (Hein et al. 2006), although the 6-month to 3-year long-term survival is again comparable with survival in cardiac surgery patients without prolonged ICU stay (Hein et al. 2006). In those patients requiring a prolonged ICU stay, long-term survival has proven to be better for cardiac versus general surgery patients due to the mostly curative nature of cardiac surgery (Combes et al. 2003). The functional status and quality of life among the survivors after prolonged ICU stay is however encouraging (Pappalardo et al. 2004, Lagercrantz et al. 2010). Considering the changing profile of cardiac surgery patients, as older and more co-morbid patients with more limited physiologic reserves are being referred for more complex and longer procedures, it is of paramount interest to implement all the methods available to maximize organ function, improve survival and resource utilization.

### 2.3 Strategies to attenuate the adverse effects of cardiopulmonary bypass

A number of different strategies, including pharmacological attempts, blood conservations interventions, myocardial protection strategies and new CPB
technologies have been employed to minimize the injurious effects of CPB and inflammatory response (Table 2.).

Table 2. Strategies to attenuate the unfavorable effects of CPB and their possible beneficial effects.

<table>
<thead>
<tr>
<th>Strategies</th>
<th>Possible beneficial effects</th>
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<tbody>
<tr>
<td>Pharmacological approaches</td>
<td></td>
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<tr>
<td>Corticosteroids</td>
<td>Attenuation of inflammatory response</td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>Inhibition of fibrinolysis</td>
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<tr>
<td>Blood conservation</td>
<td></td>
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<tr>
<td>Preoperative approaches</td>
<td>Optimization of preoperative Hb-level</td>
</tr>
<tr>
<td>Cell Salvage</td>
<td>Reduction of loss of RBCs</td>
</tr>
<tr>
<td>Antifibrinolytic drugs</td>
<td>Attenuating of inflammatory response</td>
</tr>
<tr>
<td>Point-of-care monitoring</td>
<td>Inhibition of fibrinolysis</td>
</tr>
<tr>
<td>Topical hemostatic agents</td>
<td>Optimizing the use of blood products</td>
</tr>
<tr>
<td>Myocardial conditioning</td>
<td>Reduction of bleeding</td>
</tr>
<tr>
<td>Mechanical approaches</td>
<td></td>
</tr>
<tr>
<td>Biocompatible surfaces</td>
<td>Attenuation of blood-surface interface</td>
</tr>
<tr>
<td>Membrane oxygenators</td>
<td>Reduction of embolic load</td>
</tr>
<tr>
<td>Non-occlusive pumps</td>
<td>Reduction of blood trauma</td>
</tr>
<tr>
<td>Arterial filters</td>
<td>Reduction of embolic load</td>
</tr>
<tr>
<td>Off-pump cardiac surgery</td>
<td>Avoidance of CPB</td>
</tr>
<tr>
<td>Minimized circuits</td>
<td>Reduction of blood contact with non-endothelial surfaces</td>
</tr>
</tbody>
</table>

2.3.1 Pharmacological approaches

Corticosteroids

The use of corticosteroids in the cardiac surgery setting utilizing CPB has been a topic for debate for several decades (Chaney et al. 2002). Corticosteroids attenuate complement activation in vitro (Moat et al. 1993), but in vivo affects on complement activation during CPB have been reported as being inconsistent (Tennenberg et al. 1986, Jansen et al. 1991, Engelman et al. 1995). Most existing studies investigating the effect of corticosteroids during CPB have been underpowered to effectively assess clinically important outcomes (Jansen et al. 1991, Engelman et al. 1995, von Spiegel et al. 2002). In the lack of substantial
evidence, the use of steroids has generally been avoided in the fear of their potential side-effects such as an increased risk for postoperative infection. Two recent meta-analyses of randomized controlled clinical trials showed that the use of corticosteroid prophylaxis reduced the incidence of postoperative atrial fibrillation, postoperative bleeding and the length of ICU and hospital stay, without any significant effects on mortality or postoperative infections (Whitlock et al. 2008, Cappabianca et al. 2010). The results are, however, confounded by the different dose regimens of corticosteroids and different complexities of surgery. The effect of corticosteroids on postoperative bleeding and transfusion requirements has also been demonstrated in a randomized controlled trial of 189 patients (Oliver Jr et al. 2004). Furthermore, intravenous hydrocortisone was shown to reduce the incidence of postoperative AF in a randomized controlled study of 241 patients underoing CABG with CPB (Halonen et al. 2007). Neither of these studies demonstrated any complications such as postoperative infections, associated with the use of corticosteroids. The available evidence remains insufficient to make conclusive statements as to the major safety questions regarding corticosteroid use during CPB, and any possible optimal steroid type, dose and frequency are not well established.

**Aprotinin**

In addition to its influence on hemostasis, aprotinin has been demonstrated to exert multiple effects on inflammation and ischemia-reperfusion injury (McEvoy et al. 2007). It has been shown to decrease neutrophil and macrophage activation and chemostasis, attenuate the release and activation of proinflammatory cytokines (Mojcik et al. 2001, Bull et al. 2003), and to reduce oxidative stress. Higher doses of aprotinin have been suspected to be associated with lower risk of adverse cerebrovascular outcome and a reduced need for use of vasoactive drugs (Royston et al. 2006). It has subsequently been shown, however, to be associated with increased organ dysfunction and a trend towards increase mortality (Fergusson et al. 2008). The potent inhibition of the endogenous fibrinolytic system by aprotinin was speculated to cause thrombotic complications in susceptible organs. This again demonstrates the strong inter-relations between the coagulation, fibrinolytic, inflammation and complement cascades and the difficulties in attempting to selectively influence in such a complex system.
2.3.2 Blood conservation interventions

Blood products should be used cautiously, acknowledging their risks and benefits. Preoperative and perioperative interventions, which are likely to reduce bleeding and transfusion requirements should be applied in the treatment of cardiac surgical patients, especially in cases with known risk factors for bleeding (Ferraris et al. 2007). These indicators for risk include 1) advanced age, 2) low preoperative RBC volume, 3) preoperative antiplatelet or antithrombotic drug, 4) reoperative or complex procedures, 5) emergency operations, and 6) noncardiac patient comorbidities (Ferraris et al. 2007).

Preoperative approaches

The preoperative oral administration of iron is safe and low cost and has been shown to reduce the number of patients requiring RBC transfusions in colorectal surgery (Lidder et al. 2007) and bilateral knee replacement arthroplasty (Na et al. 2011) but has not been comprehensively studied in cardiac surgery. Preoperative intravenous iron administration has been suggested as a faster and more efficient therapy, but again this has not been properly evaluated (Beris et al. 2008). Preoperative human recombinant erythropoietin therapy combined with oral iron is widely used in patients contraindicated for RBC transfusions. This therapy is usually initiated 4 to 6 weeks before surgery and requires several hospital visits. It cannot therefore be directly applied into cardiac surgery patients with preoperative anemia. Lately, a short-term high-dose preoperative erythropoietin administration has been suggested as a safe and easy method of correcting preoperative hemoglobin status and reducing the need for RBC transfusions in off-pump cardiac surgery (Weltert et al. 2010). Although no large-scale safety studies for erythropoietin have been performed in cardiac surgical patients, available evidence suggests an acceptable safety profile and it is considered as a reasonable alternative for anemic elective patients in conjunction with iron therapy several days before operation (Ferraris et al. 2007).

Cell salvage

The use of intraoperative cell salvage and autologous blood transfusion have become valuable methods for blood conservation. Cardiotomy suction has been used in cardiac surgery for many years to reduce blood loss. It involves the
intraoperative suction of pericardial blood, which is then redirected to the CPB circuit. The re-transfusion of cardiotomy blood has, however, been associated with impaired hemostasis (DeHaan et al. 1995) and increased inflammatory activation (Aldea et al. 2002). Pericardial suction blood has furthermore been identified as the primary source of fat emboli during CPB (Appelblad et al. 2002, Jewell et al. 2003) and has been associated with postoperative cognitive dysfunction (Djaiani et al. 2007). Hence, the direct reinfusion of unprocessed pericardial blood exposed to pericardial and mediastinal surfaces should be avoided (Shann et al. 2006) and cell salvage has become a common procedure during cardiac operations.

The primary goal of cell salvage is to reduce the need for allogeneic blood transfusion and its associated risks for complications. The principle of cell salvage is comprised of three phases – the collection of blood cells from the surgical field, the subsequent washing and then re-infusion. This can be achieved with several commercially available devices. The collected blood is centrifuged and filtered across a membrane, resulting in the removal of free hemoglobin, plasma, platelets, white blood cells and hemoglobin (Carless et al. 2010). Salvaged red blood cells are re-suspended in saline with a hematocrit of 50–80%, which is then autotransfused back to the patient.

The risk of several potential harms associated with cell salvage has decreased with technical advances (Domen et al. 1998, Carless et al. 2010) and cell salvage has proven to be safe and effective in reducing the need for allogeneic blood transfusions in cardiac surgery (Wang et al. 2009, Moskowitz et al. 2010) and its use is recommended during the entire operative period (Wang et al. 2009).

**Antifibrinolytic drugs**

Antifibrinolytic pharmacotherapies have been widely adopted for cardiac surgery (Henry 2007). The lysine analogues tranexamic acid and the less potent epsilon-aminocaproic acid inhibit fibrinolysis by inhibiting plasminogen by binding to the lysine binding sites on the plasminogen molecule. Aprotinin is a bovine protein which inhibits proteases with active serine residues, especially plasmin, resulting in an attenuation of the inflammatory responses, fibrinolysis, and thrombin generation. They have all been shown to be effective in significantly reducing total blood loss and transfusion requirements (Ferraris et al. 2007). Recently, aprotinin was withdrawn from the market after observational trials and a randomized clinical trial reported increased organ dysfunction and a trend
towards increased mortality in patients receiving aprotinin (Fergusson et al. 2008). Although slightly less potent, there is a strong evidence to support the use of lysine analogues for reducing total blood loss and the number of patients who require RBC transfusion after cardiac surgery (Ferraris et al. 2007).

**Point-of-care coagulation monitoring**

Routine laboratory-based coagulation tests (e.g. prothrombin time / International Normalized Ratio, activated partial thromboplastin time, fibrinogen) and platelet numbers have traditionally been used to assess the patients’ current coagulation status. They have, however, several limitations in the acute perioperative setting: there is always a delay from the time of blood sampling to obtaining results, coagulation tests are determined in plasma rather than the whole blood and the assays are performed at a standard temperature of 37°C regardless of the patient’s temperature. In addition, no information on platelet function is available. Point-of-care coagulation monitoring devices (i.e. thromboelastography, thromboelastometry) assessing the viscoelastic properties of whole blood may overcome several limitations of routine coagulation tests (Luddington et al. 2005), and may be beneficial in providing optimal blood conservation when used as a part of the perioperative multimodality approach in cardiac surgery (Ferraris et al. 2007).

**Topical hemostatic agents**

Numerous commercial topical hemostatic agents are available for use during cardiac surgery. The efficacy of topical hemostatics has not been extensively studied in large, randomized, placebo-controlled prospective studies and they also carry risks with their use. Topical sealants, such as fibrin glue, used to assist in the repair of high risk complex cardiac and aortic procedures may be effective as adjuncts to bleeding (Ferraris et al. 2007).

**2.3.3 Myocardial conditioning**

The expression “conditioning” of the heart refers to therapeutical attempts to harness myocardial natural endogenous protective mechanisms characterized by increased tissue resistance towards ischemia and reperfusion. An abundance of experimental evidence exists for different strategies of myocardial conditioning
that have been shown to protect against ischemia reperfusion injury locally, and against its consequences, which may affect organs remote from the site of injury (Valen et al. 2009). Different conditioning approaches include pharmacological preconditioning, ischemic preconditioning, ischemic postconditioning and remote ischemic preconditioning. Several different approaches to implement myocardial conditioning have been introduced into a clinical setting, but the translation of promising results from experimental studies to clinical practice have so far failed (Kaukoranta et al. 1997, Venugopal et al. 2009, Rahman et al. 2010). Although myocardial adaptation to ischemia - especially in the form of ischemic preconditioning - is clinically attractive, it is not yet applicable in practical cardiac surgery until the molecular mechanisms involved in the process have been more clearly determined (Valen et al. 2009).

2.3.4 Mechanical approaches

It was recognized relatively early during the routine use of CPB that an improvement in the hemocompatibility of circuit materials could significantly contribute to the reduction of extreme reactions. Over the past decades, numerous advancements in equipment and techniques in CPB management have been introduced with notable improvements in morbidity and mortality (Murphy et al. 2009).

Biocompatible surface materials

Surfaces coatings have been produced in order to alleviate the blood-surface interface in CPB circuits. At first, the artificial surfaces were coated with heparin, which has been proven to have many other biocompatible properties, in addition to its well-known anti-thrombotic effect (Li et al. 2009). Heparin bonded circuits are still widely used and have been shown to decrease blood loss and transfusion requirements and to attenuate the inflammatory response (Mangoush et al. 2007). More recently, surface coating materials have been developed which contain surface modifying additives such as phosphorylcholine, resembling cell surface proteins, in order to reduce surface adsorption of blood plasma proteins. There are several commercial materials with different surface coating available, and bioengineering continues to produce technological advancements. It has been difficult to estimate the effectiveness of modern surface materials due to the small numbers involved in the studies published with a wide variability in heparin
management (Murphy et al. 2009). Heparin-coated bypass circuits are considered to be potentially beneficial according to the recent guidelines published on the practice of CPB in adults (Shann et al. 2006).

Oxygenators

A gradual evolution from film to bubble oxygenators and ultimately to hollow-fiber membrane oxygenators has occurred over the last 50 years. Modern hollow-fiber membrane oxygenators require the diffusion of gas through a permeable membrane which separates blood and oxygen (Murphy et al. 2009). Membrane oxygenators have mostly replaced bubble oxygenators in modern clinical practice due to earlier reports with reduced cerebral emboli, better biocompatibility and reduced blood utilization (Murphy et al. 2009). Even current oxygenators, however, are not yet fully capable of sufficiently removing gaseous microemboli when challenged with air in the inflow (Guan et al. 2009).

Occlusive and nonocclusive pumps

A critical component of the CPB system is the arterial blood pump. At present, clinical use is divided quite evenly between nonocclusive centrifugal and occlusive roller pumps (Murphy et al. 2009). With occlusive roller pumps the propulsion of blood is achieved by the action of two rollers sequentially compressing a segment of tubing causing a forward movement of blood. Thus, the degree of hemolysis is related to both the time and exposure of the blood to the shear stress generated by the roller pump. It has been speculated and demonstrated in vitro, that particulate emboli may be generated by microfragmentation of the inner surface of the tubing when the roller contacts the tubing (spallation) (Peek et al. 2000). Regardless of the type of the arterial pump, roller pumps are used for suction systems and for delivering cardioplegic solution. Centrifugal pumps are nonocclusive pumps which function by producing a constrained cortex within a polycarbonate structure resulting in the forward movement of blood. In addition to the speed of rotation, the flow rate depends on preload from the blood resource (reservoir of patient) and afterload produced by afterload (downstream resistance).

Several studies have reported less hemolysis with the centrifugal pump compared to the roller pump in vitro (Murphy et al. 2009). A number of relatively small clinical trials have compared the two pump types in relation to emboli
generation, blood trauma and clinical trauma with no clear consensus to promote one or the other (Murphy et al. 2009).

**Arterial filters**

Arterial line filters are incorporated in the CPB circuit in order to reduce the embolic load delivered to the patient. They have been demonstrated to be effective (Padayachee et al. 1988) and should be used in CPB circuits (Shann et al. 2006, Murphy et al. 2009). Although the recommendations encouraging to the use of arterial filter are based on studies performed in the era of bubble oxygenators (Padayachee et al. 1988), arterial filters have been demonstrated to reduce embolic load also when incorporated in CPB circuits including the most modern hollow-fibre membrane oxygenators (Guan et al. 2009).

As many of the harmful effects of CPB have been traced to leukocyte activation, it has been reasoned that temporary removal of leukocytes around the time of CPB might limit some of this damage. This hypothesis has led to the development of leukocyte filters incorporated within the CPB circuit. Although attenuation of neutrophil adhesion (Chen et al. 2002), better endothelial function (Chen et al. 2004), better lung function (Karaiskos et al. 2004) and improved cerebral protection following hypothermic circulatory arrest (Rimpiläinen et al. 2000) have all been achieved, the effects of leukocyte filters have not translated into significant clinical benefit (Gott et al. 1998, Warren et al. 2007). There is also evidence that leukocyte depletion during CPB may, in fact, activate leukocytes (Koskenkari et al. 2006, Ilmakunnas et al. 2005). Although the current evidence is still rather scarce, leukocyte depleting filters have not been incorporated in routine cardiac surgical practice.

**Hemofiltration**

Hemofiltration, or ultrafiltration, uses a combination of convection and osmosis under a pressure gradient to remove fluid and low molecular weight molecules from plasma, including proinflammatory mediators. The clinical benefit of filtration attempts on the immune response associated with CPB have been disappointing in adult cardiac surgery, although with some evidence of reduced time to tracheal extubation (Oliver Jr et al. 2004).

The interest in the removal of endotoxin in order to attenuate the progression of the biological cascade of sepsis has led to the development of medical devices
aimed at eliminating endotoxin. Two commercial extracorporeal hemoperfusion devices are currently available. A polymyxin B-immobilized fiber column cartridge (PMX cartridge) is an extracorporeal hemoperfusion device which uses polymyxin-B, an antibiotic with a high affinity for endotoxin, fixed to fibers packed in the cartridge. This leads to effective binding of endotoxin to fibers when blood is perfused through the cartridge. The use of BMX-cartridge has been researched for severe intra-abdominal sepsis (Vincent et al. 2005, Cruz et al. 2009) and sepsis-related acute lung injury (Kushi et al. 2005) with promising preliminary results. Polymyxin B-immobilized endotoxin-removal in CPB has been shown to attenuate the inflammatory response and pulmonary and myocardial injury in an experimental model (Ohki 2008), but has not been studied in humans. An LPS adsorber consists of porous polyethylene discs to which is bound a specific polypeptide that binds to the lipopolysaccharide of gram negative bacteria. The discs are enclosed in a polycarbonate hub with connections for blood supply. There are very few reports on the use of the LPS adsorber, and its efficacy has not been shown. Two studies have investigated the effect of LPS adsorber during CPB without demonstrating any significant effects on blood endotoxin or inflammatory response, (Blomquist et al. 2009) or on renal function (DeSilva et al. 2010).

2.3.5 Off-pump coronary surgery

In the mid 1990s, interest emerged in performing CABG without the use of CPB (OPCAB), in order to reduce the postoperative complications associated with CPB (Ferguson et al. 2002). Despite favorable evidence the adoption of OPCAB has been variable, and appears to have reached a plateau in recent years. In 2008, the proportion of CABG procedures performed OPCAB was 22% in the U.S. (Data Analyses of the Society of Thoracic Surgeons National Adult Cardiac Database, 2008, http://www.sts.org) and 10,3% in Germany (Gummert et al. 2010). The proportion of OPCAB procedures in Finland has not recently been systematically analyzed. In our institution, OPCAB accounts for slightly over 50% of CABG procedures.

Technique

Initially viewed as experimental, OPCAB techniques are now established as an acceptable alternative to on-pump CABG (ONCAB). Accurate coronary bypass
grafting on the beating heart is technically very challenging. This may be
facilitated by commercial suction stabilizers and advanced understanding of
maneuvering the heart, which have enabled positioning and taming of
systodiatolic movement without compromising cardiac output. Optimal
positioning and visibility is especially critical in revascularization of less
accessible lateral and posterior walls of the heart.

**Clinical benefits**

al. 2007, Angelini et al. 2009 Møller et al. 2010) and several meta-analyses
2009) and large observational studies (Hannan et al. 2007, Li et al. 2008, Chu et
al. 2009) have been undertaken to compare outcome after OPCAB ONCAB. The
evidence considering in-hospital mortality is somewhat conflicting, with most
studies demonstrating a similar outcome after OPCAB and ONCAB, whereas a
few observational studies demonstrate a mortality benefit for OPCAB (Hannan et
al. 2007, Li et al. 2008, Chu et al. 2009). There is some evidence through
observational studies that the short-term mortality benefit for OPCAB may be
emphasized in high-risk patients (Demaria et al. 2002, Panesar et al. 2006,
DiMauro et al. 2007, Mishra et al. 2008, Puskas et al. 2009), although
contradictory results exist through randomized studies (Møller et al. 2010). On
the basis of several studies, mid-term and long-term survival appears comparable
between OPCAB and ONCAB (Williams et al. 2005, Motallebzadeh et al. 2007,
In a recent large randomized study, the 1-year mortality rate from cardiac causes
was slightly higher in the OPCAB group compared with the ONCAB group
(Shroyer et al. 2009).

Large retrospective analyses have shown that OPCAB may be associated
with a reduced incidence of stroke compared to ONCAB (Sedrakyan et al. 2006,
Brizzio et al. 2010). No adequately powered prospective randomized trials have
shown reduction in stroke with OPCAB compared with ONCAB. A large cohort
study analyzed temporal patterns of perioperative strokes following OPCAB and
ONCAB, and found that OPCAB significantly reduced the risk of early
postoperative stroke (Nishiyama et al. 2009). Early strokes may be presumed to

55
result from manipulations of the aorta (Roach et al. 1996, Ura et al. 2000, Zingone et al. 2006) and with the release of particulate matter from the CPB circuit (Bowles et al. 2001, Liu et al. 2009). Atheroembolism may also occur with the use of OPCAB due to tangential clamping of the ascending aorta during proximal graft attachment. In high-risk patients with a severely calcified ascending aorta this may, however, be avoided by a "non-touch" technique with complete avoidance of aortic clamping and connecting the grafts to the internal mammarian artery (Kim et al. 2002).

In general, studies comparing OPCAB and ONCAB show a reduction in the use of allogeneic blood transfusions with OPCAB (Puskas et al. 2003, Cheng et al. 2005, Wijeysundera et al. 2005). OPCAB and ONCAB appear to be associated with a similar degree of perioperative myocardial injury (Wijeysundera et al. 2005, Feng et al. 2009, Möller et al. 2010, Shroyer et al. 2009). No prospective randomized trials have shown a reduction in stroke with OPCAB compared to ONCAB. Large retrospective analyses have shown that OPCAB may have a reduced incidence of neurologic complication compared to ONCAB (Puskas et al. 2008, Brizzio et al. 2010). Results concerning postoperative atrial fibrillation (Wijeysundera et al. 2005, Möller et al. 2010), and acute kidney injury (Massoudy et al. 2008, Nigwekar et al. 2009, Shroyer et al. 2009) are also inconsistent. Some studies report reduced mortality and morbidity in high-risk patients with OPCAB compared to ONCAB (Puskas et al. 2008, Puskas et al. 2009) but contradictory results have also been reported (Möller 2010).

The initial enthusiasm for OPCAB was later slightly reduced due to concerns as to the completeness of revascularization, the rate of myocardial infarction, and long-term graft patency (Khan et al. 2004, Caputo et al. 2005, Williams et al. 2005, Hannan et al. 2007). In the largest randomized controlled study with 2203 patients investigating off-pump versus on-pump CABG 1-year composite outcomes, completeness of revascularization and graft patency were significantly worse with off-pump than with on-pump surgery (Shroyer et al. 2009). This study has been criticized for the reported high rate of conversion of OPCAB to ONCAB which was suspected to mirror inexperience in performing OPCAB. Similar findings of less complete revascularization and a relative increase in the long-term incidence of acute myocardial infarction following OPCAB compared to ONCAB (Hueb et al. 2010) have, however, been reported in other trials. It has been speculated as to whether the absence of the anticoagulatory effect of CPB may have contributed to the reduced graft patency following OPCAB. It may also be that in some cases the applicability of OPCAB is limited by its technically
demanding profile to provide complete revascularization, at least with coronary lesions with complex anatomy (Halkos et al. 2010).

Taken together, in the light of current evidence, OPCAB is a safe alternative to ONCAB, with similar outcomes in low-risk patients. The benefits of OPCAB are presumably more apparent in patients at a higher risk of complications from CPB, such as those with a severely calcified ascending aorta. It has been alluded to in large observational studies, it may be that the greatest benefit of OPCAB will be seen in high-risk patients, but this remains to be clarified by large randomized trials with high-risk patients.

### 2.3.6 Minimized circuits

**Differences as compared to conventional cardiopulmonary bypass**

The primary perfusion system for CPB today is an open system, consisting of an oxygenator and a hard-shell reservoir with standard shape and volume for the collection of venous blood (Fig 2). Open systems are characterized by direct blood–air contact in the reservoir and passive siphon drainage of venous return. The current era with its deteriorating risk-profile in the population referred for cardiac surgery has led to a search for a less aggressive and more biocompatible CPB. This has resulted in the development and use of more condensed circuits, so called minimized cardiopulmonary bypass systems (MCPB). The primary idea was a closed low prime volume circuit consisting of a centrifugal pump and a membrane oxygenator as the only components (Curtis et al. 2010). Several commercial modifications have become available with various differences between them, especially with respect to the existence of a collapsible reservoir and an air removal device. In minimized cardiopulmonary bypass systems the artificial surface area has been reduced and a hard shell venous reservoir has been excluded or replaced with a collapsible venous reservoir to avoid the blood-air interface (Curtis et al. 2010). The goal of these alterations has been to reduce SIRS while preserving platelet function and minimizing the need for allogeneic blood products due to a diminished priming volume.

In MCPB circuits the venous blood returns by active vacuum assisted drainage instead of gravity as in the CCPB. The concept of a vacuum assisted venous return and the exclusion of a venous hard shell reservoir requires meticulous air handling strategies including the precise sealing of the venous
cannula, with careful management of drug and fluid administration and the collection of blood samples. In addition, the integration of an air removal device to the circuit system is preferable. With the reservoir removed in minimized systems, the insufficient venous return may not be compensated for with additional volume. Thus, MCPB systems require a particular differentiated volume management and present new challenges for the clinical application of CPB (Curtis et al. 2010).

**Clinical benefits**

Several investigators have demonstrated that the surface reduction and the avoidance of a direct blood–air interface by removal of the hard shell reservoir is associated with less systemic inflammatory activation as a consequence of CPB (Lindholm et al. 2004, Bical et al. 2006, Fromes et al. 2002). Furthermore,
MPCB systems are associated with a better preservation of hematocrit (Remadi et al. 2006, Valtonen et al. 2007), as well as significant reductions in blood loss and transfusion rate (Remadi et al. 2006, Biancari et al. 2009, Benedetto et al. 2009, Zangrillo et al. 2010). MCPB is presented with somewhat less of a degree of myocardial injury as compared to CCPB (Remadi et al. 2004, Skrabal et al. 2007, Castiglioni et al. 2009, Zangrillo et al. 2010). To date, no prospective randomized trials with enough power to show the benefit of MCPB on postoperative neurologic dysfunction or on overall outcome compared to CCPB have been reported. A meta-analysis including 1619 patients comparing the outcome after MCPB and CCPB reported a diminished stroke rate following CABG after MCPB (0.7%) as compared to CCPB (3.4%) (Zangrillo et al. 2010). A recent single-center retrospective report of 2243 patients who had undergone elective or urgent CABG with MCPB showed a stroke incidence of 2.2% and a 30-day mortality of 2.3% (Puehler et al. 2011). The mechanisms behind the suggested cerebral protection are unclear, but they have been speculated to relate to better tissue oxygenation and a reduced embolic load.
3 Aims of the present research

The aim of this study was to determine the role of minimized cardiopulmonary bypass in attenuating the adverse effects of extracorporeal circulation in comparison to CCPB.

The specific goals were to ascertain answers to the following questions:

1. Is minimized cardiopulmonary bypass safe and feasible, and does it reduce the transfusion requirements compared to conventional cardiopulmonary bypass in unselected coronary artery bypass surgery patients? (Study I).
2. Does minimized cardiopulmonary bypass reduce retinal microembolization compared to conventional cardiopulmonary bypass in elective coronary artery surgery patients (Study II) and in patients undergoing elective aortic valve replacement with or without combined coronary revascularization? (Study III).
3. Does minimized cardiopulmonary bypass attenuate systemic inflammation, coagulation, endothelial activation and injury, and platelet activity compared to conventional cardiopulmonary bypass in elective coronary artery bypass surgery (Study II) and in elective aortic valve replacement with or without combined coronary revascularization? (Study III).
4. Is prolonged cardiopulmonary bypass associated with intestinal mucosal damage, and can this be attenuated by using minimized cardiopulmonary bypass compared to conventional cardiopulmonary bypass in an experimental porcine model? (Study IV).
4 Material and methods

This section summarizes the material and methods of the studies with a detailed description in the original publications at the end of this volume. The first study (I) was a retrospective matched cohort study, studies II and III were prospective, randomized clinical trials, while the final (IV) study was a randomized experimental animal model.

4.1 Material and study designs

Study I involved 466 consecutive patients admitted for elective or urgent isolated on-pump CABG in the Vaasa Central Hospital during the years 2005–2009. Study II involved 40 patients admitted for elective on-pump CABG in the Oulu University Hospital during the years 2007–2008. Study III included 18 patients undergoing elective combined aortic valve replacement (AVR) with CABG and 22 patients undergoing elective AVR in Oulu University Hospital during the years 2007–2009. The experimental study (IV) involved 22 female domesticated pigs (6–7 weeks, mean weight 32.5 kg (SD 3.5)). The first study (I) was a propensity score matched cohort study based on an institutional database excluding patient identification data. Based on the principles of the Local Ethics Committee exemption from consent was obtained because all data were extracted retrospectively for study purposes from clinical records. The two other clinical studies (II-III) were prospective, randomized trials that were approved by the Oulu University Ethics Committee (2006) and written informed consent was obtained from all patients. The fourth study (IV) was a prospective randomized experimental study using a porcine model, and all including experiments were performed in compliance with the guidelines of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986) and in compliance with the European Union Directive 86/609/EEC (1997). The research protocol of the fourth study (IV) was approved by the Animal Care and Use Committee of the University of Oulu.

The first study included a consecutive series of 466 patients who underwent isolated CABG, employing either minimized or conventional CPB. In the randomized prospective clinical studies (II-III), patients with antithrombotic (excluding acetylsalicylic acid) and immunosuppressive medication, unstable coronary artery disease, significant left ventricle dysfunction (EF <35%), severe renal or liver failure and diabetes were excluded (II). Randomization was carried
out by using numbered sealed envelopes. The study designs are summarized in Table 3.

### Table 3. Materials and study designs.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study design</th>
<th>Patients and material (n)</th>
<th>CPB design</th>
<th>Focus</th>
<th>Primary endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Retrospective matched cohort study</td>
<td>Unselected isolated CABG (466)</td>
<td>Sorin ECC.O™ vs. Sorin Dideco Avant™ + centrifugal/roller pump</td>
<td>MCPB’s suitability in higher risk patients</td>
<td>Hemodilution, transfusion requirements</td>
</tr>
<tr>
<td>II</td>
<td>Prospective randomized clinical study</td>
<td>Elective CABG (40)</td>
<td>Terumo ROCSafe™ vs. Sorin Dideco Avant™ roller pump</td>
<td>Microembolism, Inflammatory activation, coagulation, platelet activation, endothelial activation</td>
<td>Retinal microembolism, inflammatory, coagulation, platelet activation and endothelial markers</td>
</tr>
<tr>
<td>III</td>
<td>Prospective randomized clinical study</td>
<td>Elective AVR with or without CABG (40)</td>
<td>Terumo ROCSafe™ vs. Sorin Dideco Avant™ roller pump</td>
<td>Microembolism, Inflammatory activation, platelet activation, endothelial activation</td>
<td>Retinal microembolism, inflammatory and platelet activation, endothelial markers</td>
</tr>
<tr>
<td>IV</td>
<td>Experimental randomized study</td>
<td>Experimental porcine model of prolonged CPB (22)</td>
<td>Sorin ECC.O™ vs. Sorin Dideco Avant™ roller pump</td>
<td>Intestinal mucosal integrity</td>
<td>Histopathological score, tight junction proteins</td>
</tr>
</tbody>
</table>

### 4.2 Anesthesia and cardiopulmonary bypass

#### 4.2.1 Anesthesia

Anesthesia protocols were based primarily on the normal standards and practices of the Vaasa Central Hospital and Oulu University Hospital during the years 2005–2009. In study I, in addition to combined general anesthesia, thoracic epidural anesthesia was used at the discretion of the anesthesiologist for patients with anticipated pulmonary or other significant risk. Anesthesia was induced with iv boluses of fentanyl (3–3.5 µg/kg) and propofol (0.8–2 mg/kg), followed by incremental doses of...
fentanyl (up to a total dose of 7–10 µg/kg) or an infusion of alfentanil (30–40 µg/kg/h), and a continuous infusion of propofol 1–2 mg/kg/h. Patients were ventilated with 40–60% O₂ supplemented with isoflurane or sevoflurane. Muscle relaxation was achieved with pancuronium 0.1mg/kg iv clonidine 1.5–2 µg/kg was given as a slow iv bolus at induction of anesthesia to patients without thoracic epidural anesthesia.

In studies II and III anesthesia was induced with a fentanyl bolus (3–7.5 µg/kg) and propofol (5–8 mg/kg/h) and alfentanil (0.05 mg/kg/h) infusions until intubation. Muscle relaxation was achieved with pancuronium 0.1mg/kg mg i.v. After intubation, anesthesia was maintained with inhaled sevoflurane (1.5–2%) combined with propofol (1–2 mg/kg/h) and alfentanil (0.025–0.05 mg/kg/h) infusions.

In study IV, animals were deprived of food but not water for 24 h before the experiment. After sedation with intramuscular ketamine hydrochloride (350 mg) and midazolam (45 mg), an auricular vein was cannulated. Anesthesia was induced with intravenous thiopental (5–10 mg/kg). Following intubation with a 6-mm cuffed endotracheal tube, the animals were mechanically ventilated using a volume-controlled ventilator (Servo 900C, Siemens, Solna, Sweden). Tidal volume was maintained at 10 ml/kg while the respiratory rate was adjusted to maintain PaCO₂ levels between 4.5 and 5.5 kPa. Fractional inspired oxygen concentration was adjusted to maintain PaO₂ levels between 13 and 20 kPa, and an end-expiratory pressure of 5 cmH₂O was used. Anesthesia was maintained with a continuous infusion of fentanyl at 25 µg/kg/hour (with an initial bolus of 25 µg/kg) and midazolam (0.25 mg/kg/hour) combined with isoflurane (0.5%). Muscle relaxation was achieved with a continuous infusion of pancuronium (0.2 mg/kg/hour).

4.2.2 Hemodynamic monitoring and support

In studies I-III, all patients received hemodynamic monitoring, fluid therapy and glycemic control according to the routine practices of the two hospitals. Cardiac index was maintained above 2.2 l/min/m² by optimizing preload and, if necessary, with an infusion of dobutamine. Vasopressors (phenylephrine, followed by norepinephrine) were administered to maintain a mean arterial pressure above 60 mmHg.

In study IV, an arterial catheter was inserted via the left femoral artery and a pulmonary artery thermodilution catheter was introduced via the left femoral vein. In addition, an orogastric tube and a urinary catheter were inserted.
Throughout the protocol, the animals received 0.9% saline at a constant rate of 10 ml/kg/hour. Additional fluid (Ringer’s lactate and hydroxyethyl starch with a ratio of 2:1) was administered to keep the pulmonary artery wedge pressure between 5 and 8 mmHg. The blood glucose level was maintained between 5 and 7 mmol/l by an intravenous infusion of 30% glucose if necessary. Electrocardiography was continuously monitored. No vasoactive therapy was used in study IV.

4.2.3 Blood conservation and blood transfusions

In study I, patients received aprotinin, which was withdrawn from the market during the study period, thereafter it was replaced with tranexamic acid. In study I, acetylsalicylic acid was discontinued 3–5 days before elective surgery, and in studies II-III 10 days before surgery. In studies II-III, tranexamic acid (50 mg/kg) was administered in both groups to minimize blood loss. Red blood cells (RBCs) were administered to maintain haemoglobin level above 80 g/l throughout the perioperative period (I-III). No blood transfusions were given in study IV.

4.2.4 Cardiopulmonary bypass

CPB components

The technical data on different CPB techniques is presented in Table 4. A heat exchanger was used in all settings for core cooling and warming. The composition of conventional CPB was quite similar in all studies. It consisted of a phosphorylcholine (PC) coated 1/2” tubing circuit, including a membrane oxygenator with an open hard shell dual (I-III) or single (IV) chamber cardiomy reservoir, and a roller pump. However, 65% of patients in study I also had a centrifugal pump with conventional CPB. A 40 µm arterial filter was included in the conventional CPB in studies I-III.

In studies I and IV, minimized CPB included a 3/8” customized phosphorylcholine-coated circuit (ECC.O™, Sorin Group, Italy) with a venous air removal device and an integrated centrifugal pump. A 40 µm coated arterial line filter was included in the minimized CPB circuit in study I.

In studies II and III, minimized CPB comprised a customized circuit with 3/8” tubing (ROCsaf™, Terumo Europe NV, Leuven, Belgium) consisted of poly[2-methoxyethyl] acrylate (PMEA) coated lines, a membrane oxygenator, a
manual venous line bubble trap, a 40 μm arterial line filter, a collapsible reservoir bag, and a centrifugal pump.

In all studies, the circuits were primed with a crystalloid solution containing 50–75 mg of heparin. With MCPB circuits, retrograde autologous priming was performed at the initiation of the CPB to displace from 150ml (IV) to 300ml (I-III) of the crystalloid prime. Heparin 3 mg/kg was administered (I-III) or 5 mg/kg (IV), with additional boluses if necessary to maintain an activated clotting time of more than 480 seconds. After the termination of CPB, heparinization was reversed with protamine sulfate (3 mg/kg) (I-III).

Table 4. System components of the different CPB techniques used in studies I-IV.

<table>
<thead>
<tr>
<th>Component</th>
<th>MCPB Study I</th>
<th>MCPB Studies II-III</th>
<th>MCPB Study IV</th>
<th>CCPB Studies I-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Device</td>
<td>Stöckert, SIII ECC.O™</td>
<td>Terumo, System 1 ROCSafe™</td>
<td>Stöckert, SIII</td>
<td>Stöckert SC/SIII/SS</td>
</tr>
<tr>
<td>Pump</td>
<td>Centrifugal, Stöckert SCP™</td>
<td>Centrifugal, Sams™ SCP™</td>
<td>Centrifugal, Stöckert SCP™</td>
<td>Centrifugal/Roller I SCP™</td>
</tr>
<tr>
<td>Tubing</td>
<td>3/8”, P.h.i.s.i.o™ coating</td>
<td>3/8”, Terumo X-coating™</td>
<td>3/8”, P.h.i.s.i.o™ coating</td>
<td>1/2”, P.h.i.s.i.o™ coating</td>
</tr>
<tr>
<td>Oxgenator</td>
<td>Eos™, Sorin</td>
<td>Capiox™SX-18, Terumo</td>
<td>Eos™, Sorin</td>
<td>DidecoD903Avani™</td>
</tr>
<tr>
<td>Blood reservoir</td>
<td>Soft-shell reservoir</td>
<td>Soft-shell reservoir, Terumo X-coating™</td>
<td>Soft-shell reservoir</td>
<td>Dideco Dual Chamber Reservoir, P.h.i.s.i.o™ coating</td>
</tr>
<tr>
<td>Arterial filter</td>
<td>Dideco D733, 40μm</td>
<td>Pall, AV 6, 40μm</td>
<td>-</td>
<td>Dideco 734, 40μm I-III</td>
</tr>
<tr>
<td>Venous air removal</td>
<td>Automated</td>
<td>Manual Terumo BT15™</td>
<td>Manual</td>
<td>-</td>
</tr>
<tr>
<td>Static prime volume</td>
<td>480ml</td>
<td>800ml</td>
<td>480ml</td>
<td>1500ml I-III</td>
</tr>
<tr>
<td>Foreign surface area</td>
<td>12 900cm²</td>
<td>25 978cm²</td>
<td>12 500cm²</td>
<td>≈ 27 775cm² I-III</td>
</tr>
<tr>
<td>Cardioplegia</td>
<td>30 °C continuous Ringer-KCl-MgCl₂</td>
<td>12°C (III) - 18°C (II) continuous Ringer-KCl-MgCl₂</td>
<td>-</td>
<td>12°C (III) - 18°C (II) continuous Ringer-KCl-MgCl₂</td>
</tr>
</tbody>
</table>

Cannulations and venting

The further experimental protocol regarding study IV is described in chapter 4.3.2. In studies I-III, standard aortic and venous cannulations were performed.
after systemic heparinization. In the MCPB groups, a size 32/37 Fr. venous two-stage cannula (Maquet Cardiovascular), with an additional snare ligature was used to avoid air leak from the cannulation site. In studies I and II, aortic root venting was used, and in study I, the vented blood was collected to a cell saver. In study III, a vent was placed into the left ventricle via the pulmonary vein in both groups. In the MCPB groups in studies II-III, a small roller pump was integrated into the system to direct the vented blood into a collapsible reservoir bag, from which it could be further directed to the venous line prior to a bubble-detector. Air and related blood from the collapsible reservoir bag were transferred to the reservoir of a cell saver machine.

**Air handling**

In the ECC.O™ MCPB circuit used in study I, a side-inlet 120 µm filter-containing venous bubble trap was combined with an Air Purge Control (APC) module. In this system, an ultrasonic bubble detector triggers a roller pump draining the air-contaminated blood off the top of the bubble trap through a purge line into a soft-shell reservoir. The side-inlet structure creates centrifugal flow, which collects air at the top center of the filter housing.

If air is detected in the venous line in the Terumo ROCsafe™ (II-III) circuit by the ultrasound-controlled bubble detector, the speed of the centrifugal pump is reduced to a “coast speed” of 1500 rpm, creating neither forward nor reverse flow. This avoids negative pressure in the venous line during closure of electronic venous occluder, which automatically closes the venous line if the speed of the centrifugal pump is reduced to 1500 rpm. This allows for manual de-airing of the venous bubble trap. In study IV, any air detected in the venous line was dealt with manually.

**Cardioplegia**

In studies I-II, cardiac protection was maintained with retrograde continuous 18°C (II) - 30°C (I) blood-Ringer-KCl-MgCl cardioplegia through a cannula in the coronary sinus in both groups. In study III, retrograde and antegrade continuous 12°C blood-Ringer-KCl-MgCl cardioplegia was used through a cannula in coronary sinus and coronary ostiae in both groups.
**Suction blood management**

In studies I-II, all pericardial suction blood was directed into a cell saver (Dideco Electa™) and was processed before reinfusion. In the CPB group in study III (open-chamber surgery), the pericardial suction and vent blood were directed into the upper part of a dual chamber reservoir, from where it was further directed into a cell saver to the point it was assessed clinically reasonable without causing hypovolemia. The volume collected into the cell saver included cardiotomy suction blood from the surgical field, volume from the continuous cold cardioplegia and a considerable part was volume remaining in the bypass circuit after CPB. After termination of CPB, the MCPB circuits were kept on “stand-by” using saline substitution and, at the end, this remaining saline-diluted volume was processed in the cell saver.

**4.3 Experimental protocols**

**4.3.1 Retinal fluorescein angiography (II, III)**

At the time of the premedication patients received tropicamide and phenylephrine eye drops for optimal pupil dilatation. A Heidelberg Retina Angiograph II™ (HRA 2) camera was used in the vertical alignment (Fig 3). Retinal fluorescein angiographs were performed on both eyes (1) after the induction of anesthesia and (2) instantly after determination of CPB. In both groups standardized angiographs were performed in each eye sequentially. (1) After assisting personnel retracted the eyelids and the optical settings were modified, a black and white image without a contrast medium was obtained. (2) Next, a bolus dose of 5 ml of 20% sodium fluorescein was administrated into the central venous line. (3) Thereafter a digital fluorescein angiography image was obtained. (4) In 4 minutes, the angiograph was repeated. The cornea were kept moist with a flush of saline every 10 seconds. The images taken before and after CPB were used as within-patient controls. The images were analyzed by an ophthalmologist, blinded to the CPB circuit used.
4.3.2 Animal model of prolonged CPB (IV)

Surgical preparations

In study IV, the protocol was carried out in 20 animals, and 2 animals that served as sham controls underwent the experimental protocol excluding the CPB. The abdominal cavity of animals was exposed through a midline laparotomy. The superior mesenteric artery and celiac trunk were exposed and transit time ultrasonic flow probes (Transonic Systems, Ithaca, N.Y., USA) were carefully secured around the vessels. A catheter was inserted into the portal vein. Baseline intestinal biopsies (PRECPB) representing all layers of the intestinal wall were obtained from the terminal ileum and ascending colon and intestinal walls and the biopsy sites were carefully sutured. Thereafter, the abdominal wall was reapproximated but the fascia was left open. A right anterolateral thoracotomy was performed in the fourth intercostal space to expose the right atrium for
venous cannulation. The right thoracic vessels were ligated and cut, and the pericardium was opened. After systemic heparinization (5 mg/kg), a 12F arterial cannula and a 24F venous cannula were inserted into the ascending aorta and the right atrial appendage, respectively. The normal porcine body temperature of 39°± 0.5°C was maintained until the induction of CPB using an operating table heater, warmed fluids, and a blanket.

**Experimental protocol**

After the surgical preparations were completed, the animals were allowed to stabilize for 60 minutes before the baseline measurements were obtained. Mild hypothermic CPB was then initiated and maintained for 4 hours (240 minutes). Thereafter, post-procedural biopsies (CPB240min) from the terminal ileum and ascending colon were obtained and the animals were terminated using intravenous pentobarbital (60 mg/kg).

**Hemodynamic measurements**

Arterial, pulmonary and central venous pressures were continuously monitored with quartz pressure transducers and a monitor. All pressure transducers were simultaneously zeroed at the level of the heart. At baseline, cardiac output was measured using the thermodilution catheter and indexed to weight (CI). During CPB, CI was calculated from pump flow. Regional blood flows of the superior mesenteric artery and celiac trunk (Qspl) were measured continuously with the transit time flow probes attached to a flow meter.

**Oxygen delivery and consumption (IV)**

Systemic oxygen delivery index (DO₂i) was calculated as \[CI \times Hb \times SaO_2 \times 0.0139\] and systemic oxygen consumption index (VO₂i) as \[CI \times Hb \times (SaO_2 – SvO_2) \times 0.0139\]. Qspl was indexed to body weight and presented as ml/kg/min (Qspli). Gut oxygen delivery index (GutDO₂i) was calculated as \[Qspli \times Hb \times SaO_2 \times 0.0139\], and splanchnic oxygen consumption index (VO₂i) as \[Qspli \times Hb \times (SaO_2 – Portal vein O_2 saturation) \times 0.0139\].
4.4 Laboratory analyses

4.4.1 Blood sample collection (II, III, IV)

In studies II and III, blood samples were collected at 8 time points: 1) before the induction of anesthesia, 2) 45 minutes after the start of CPB, 3) after protaminization, 4) 2 hours and 5) 4 hours after the termination of CPB and in the morning of the 6) first, 7) second and 8) third postoperative day. Separated plasma were stored at -70°C.

In study IV, arterial blood samples were drawn every 30 minutes to analyze blood gas values, pH, electrolytes, glucose- and hemoglobin concentrations with an i-STAT Analyzer; i-STAT Corporation, East Windsor, NJ. Central venous and portal vein samples were taken every 60 minutes to measure SvO₂ and portal vein saturation (SpO₂). In addition, systemic arterial and portal venous lactate concentrations were analyzed every 120 minutes with an i-STAT Analyzer.

The time points for the analysis of markers for coagulation, endothelial activation and injury, and thrombin dependent platelet activity in studies II and III are presented in Tables 5–6. Commercial ELISA kits were used for the analysis of following hemostatic markers: TAT (Enzygnost® Tat) and F1+2 (Enzygnost® F1+2) from Dade Behring, Marburg Germany; sGPV (Asserachrom® sGPV) and TM (Asserachrom Thrombomodulin) from Diagnostica Stago, Asnières, France. vWF:Ag was analyzed using the immuno-turbidometric method (STA-LIATEST, Diagnostica Stago, Asnières, France). All the analyses were performed in duplicates. Intra-assay coefficients of variation were as follows: TAT 7.1%, F1+2 7.6%, sGPV 6.1%, TM 17.4% and vWF:Ag 3.0%. Cytokine concentrations were measured using commercial ELISA kits for TNF-a, IL-6 and IL-8 (Duoset, Genzyme Diagnostics, Cambridge, USA) according to the manufactures instructions. The lower detection limits for the tests were 10 pg/ml for TNF-a and IL-6 and 15 pg/ml for IL-8. PMN elastase (BioVendor, Modrice, Czech Republic) and MPO (HyCult biotechnology, Eindhoven Area, Netherlands) were measured by the ELISA method with a lower detection limit of 3 ng/ml for PMN and 0.5 ng/ml for MPO. All other tests were performed using routine laboratory methods in our institution.
4.4.2 Markers of systemic Inflammation, coagulation and endothelial activity and injury (II, III)

The time points for the analysis of markers for coagulation, endothelial activation and injury, and thrombin dependent platelet activity in studies II and III are presented in Tables 5–6. Commercial ELISA kits were used for the analysis of following hemostatic markers: TAT (Enzygnost® TAT) and F1+2 (Enzygnost® F1+2) from Dade Behring, Marburg Germany; sGPV (Asserachrom® sGPV) and TM (Asserachrom Thrombomodulin) from Diagnostica Stago, Asnières, France. vWF:Ag was analyzed using the immuno-turbidometric method (STA-LIATEST, Diagnostica Stago, Asnières, France). All the analyses were performed in duplicates. Intra-assay coefficients of variation were as follows: TAT 7.1%, F1+2 7.6%, sGPV 6.1%, TM 17.4% and vWF:Ag 3.0%. Cytokine concentrations were measured using commercial ELISA kits for TNF-a, IL-6 and IL-8 (Duoset, Genzyme Diagnostics, Cambridge, USA) according to the manufactures instructions. The lower detection limits for the tests were 10 pg/ml for TNF-a and IL-6 and 15 pg/ml for IL-8. PMN elastase (BioVendor, Modrice, Czech Republic) and MPO (HyCult biotechnology, Eindhoven Area, Netherlands) were measured by the ELISA method with a lower detection limit of 3 ng/ml for PMN and 0.5 ng/ml for MPO. All other tests were performed using routine laboratory methods in our institution.
Table 5. Markers of systemic inflammation (CRP, C3, IL-6, IL-8, TNFα, PMN elastase, MPO), coagulation (TAT, F1,2,) and endothelial activity and injury (vWF:Ag, TM), and platelet activity (sGPV) in study I. CRP: C-reactive protein; C3: Complement C3; IL-6: Interleukin 6; IL-8: Interleukin 8; TNFα: Tumor necrosis factor α; PMNelast: Polymorphonuclear elastase; MPO: Myeloperoxidase; TAT: Thrombin-antithrombin complex; F1,2: Prothrombin fragment 1,2; vWF:Ag: von Willebrand factor antigen; TM: Thrombomodulin; sGPV: Soluble plasma glycoprotein.

<table>
<thead>
<tr>
<th>Preop</th>
<th>CPB+ 45min</th>
<th>CPB+ 15min</th>
<th>CPB+ 2h</th>
<th>CPB+ 4h</th>
<th>1. POD</th>
<th>2. POD</th>
<th>3. POD</th>
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<td>CRP</td>
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<tr>
<td>PMN elast</td>
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<tr>
<td>vWF:Ag</td>
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<td>vWF:Ag</td>
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<tr>
<td>TM</td>
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<tr>
<td>sGPV</td>
<td>sGPV</td>
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<td>sGPV</td>
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</tbody>
</table>
Table 6. Markers of systemic inflammation (CRP, C3, IL-6, IL-8, TNFα, PMN elastase, MPO), coagulation (TAT, F1,2,) and endothelial activity and injury (vWF:Ag, TM), and platelet activity (sGPV) in study II. CRP: C-reactive protein; IL-6: Interleukin 6; IL-8: Interleukin 8; TNFα: Tumor necrosis factor α; PMN elast: Polymorphonuclear elastase; MPO: Myeloperoxidase; vWF:Ag: von Willebrand factor antigen; TM; Thrombomodulin, sGPV: Soluble plasma glycoprotein.

<table>
<thead>
<tr>
<th>Preop</th>
<th>CRP</th>
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<th>CRP</th>
<th>CRP</th>
<th>CRP</th>
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<td>IL-6</td>
<td>IL-6</td>
<td>IL-6</td>
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<tr>
<td>CPB+ 15min</td>
<td>IL-6</td>
<td>IL-6</td>
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<tr>
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<tr>
<td>TNFα</td>
<td>TNFα</td>
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<tr>
<td>PMN elast</td>
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<tr>
<td>vWF:Ag</td>
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<td>vWF:Ag</td>
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<td>vWF:Ag</td>
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<tr>
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<tr>
<td>sGPV</td>
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<td>sGPV</td>
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</tr>
</tbody>
</table>

Hematocrit corrections (II, III)

In studies II-III, hematocrit values varied significantly between the measurement points during and after CPB (17% to 46%). Plasma concentrations of the measured inflammatory, coagulation and endothelial variables were therefore corrected for hematocrit by relating every measurement to a standard hematocrit value of 40% according to the following formula: Corrected concentration = measured concentration × (standard hematocrit / measured hematocrit). sGPV was related to patients platelet count at the measuring point according to the following formula: sGPV ng/ml / thromb109.

4.5 Histopathologic analysis and immunohistochemistry (IV)

4.5.1 Mucosal damage score

In study IV, biopsies from the terminal ileum and ascending colon were fixed in neutral buffered 10% formalin for 3 days, embedded in paraffin and cut into 5-µm thick sections that were stained in hematoxylin and eosin. Histopathologic evaluation under light microscope at 40X and 100X included measurements of the height of villi and crypts using a calibrated ocular micrometer, quantification
of the amount of inflammatory cells and estimation of the microvascular filling, all of these features being assessed separately in mucosal, submucosal, muscular and serosal layers. Mucosal injury was assessed by an unbiased experienced pathologist. The histologic criteria used to assess epithelial damage included increased eosinophilic staining intensity of epithelial cells, karyopyknosis, karyolysis and karyorrhexis, cell disruption, subepithelial edema and detachment of cells from the basal lamina. Morphologic damage was expressed as a mucosal damage grade (Table 7).

Table 7. Mucosal epithelial damage score to quantify the degree of changes in epithelial integrity.

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degeneration</td>
<td>0-5% 6-10% 11-50% 51-75% 76-100%</td>
</tr>
<tr>
<td>Defect</td>
<td>0% 0-5% 6-25% 26-50% 50-75%</td>
</tr>
</tbody>
</table>

4.5.2 Immunohistochemistry

In study IV, immunohistochemistry was used to investigate epithelial expression of Ki-67 as a measure of proliferation as well as claudin-1, -2, -3, -4, -5, and -7 as elements of tight junctions. The sections were dewaxed in xylene and rehydrated in graded alcohol series to PBS, pre-treated with TRIS/EDTA and heated in a microwave oven for 17 minutes. The endogenous peroxidase activity was quenched by incubating the specimen for 15 minutes with DAKO Peroxidase Block. Primary antibodies were purchased from Zymed Laboratories Inc. (San Francisco, CA, USA) for detection of claudins and from Immunotech (Marseille, France) for detection of cell proliferation (Ki-67). Polyclonal rabbit anti-claudin 1 (clone JAY.8), monoclonal mouse anti-claudin 2 (clone 12H12), polyclonal rabbit anti-claudin 3 (clone Z23.JM), monoclonal mouse anti-claudin 4 (clone 3E2C1), monoclonal mouse anti-claudin 5 (clone 4C3C2) and polyclonal rabbit anti-claudin 7 (clone ZMD.241) and monoclonal mouse anti-MIB-1 (Ki-67, clone MIB-1) were used as primary antibodies. After a 30 minute incubation with the primary antibody (dilution 1:50 for anti-claudin 1, 2, 3, 4, 5, and 7 and 1:25 for anti-MIB-1), staining was completed with the EnVision Detection Systems Peroxidase/DAB (rabbit/mouse) for automated systems (Dako, Glostrup, Denmark) with Dako Autostainer (Dako, Glostrup, Denmark). The sections were
lightly counterstained with haematoxylin and mounted with Eukitt (Kindler, Freiburg, Germany). Negative control stains were carried out by substituting phosphate buffered saline for the primary antibodies. The immunoreactivity was assessed quantitatively. In each section, multiple readings of immunoreactive staining were obtained at 500 µm intervals and the mean of these 15-20 readings for each section provided a staining intensity for that section. Staining was categorized into five classes (0 = absent, 1 = weak, 2 = moderate, 3 = strong, 4 = very strong) in terms of intensity and extent (Table 8). For epithelial cell proliferation rate, approximately 300 epithelial cells in each sample were evaluated and the Ki-67 index was calculated as the percentage of cells showing nuclear Ki-67-expression. All assessments were performed blinded to the group and sampling time point by two investigators.

**Table 8. Combined score for the assessment of immunohistochemical staining in claudin proteins.**

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Quantity</th>
<th>Combined score (=Intensity+Quantity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 = weak</td>
<td>0 = no positivity</td>
<td>0 = absent</td>
</tr>
<tr>
<td>2 = moderate</td>
<td>1 = &lt;25%</td>
<td>1-2 = weak</td>
</tr>
<tr>
<td>3 = strong</td>
<td>2 = 25-50%</td>
<td>3-4 = moderate</td>
</tr>
<tr>
<td>4 = very strong</td>
<td>3 = 50-75%</td>
<td>5-6 = strong</td>
</tr>
<tr>
<td></td>
<td>4 = &gt;75%</td>
<td>7-8 = very strong</td>
</tr>
</tbody>
</table>

**4.6 Statistical analysis**

A professional medical biostatistician was consulted in the performance of statistical analyses. Analyses were performed using SPSS (versions 16.0.1-18.0.1, SPSS Inc., Chicago, Ill., USA) and SAS (version 9.1.3, SAS Institute Inc., Cary, NC., USA) statistical software packages. Summary measurements are expressed as mean and standard deviation (SD) or as median and 25th – 75th percentiles. Categorical variables are displayed as frequency distributions (n) and simple percentages (%). Either the Student t test or Mann-Whitney U test was used to assess the distribution of variables between the study groups. Differences between two related measurements were analyzed using Paired samples t-test or Wilcoxon signed rank test as appropriate. The SAS procedure Mixed was used for repeated measurements. Because the measurement intervals in study IV were uneven due
to variability in the duration of preparations of the experimental model, spatial exponential covariance structure was defined in repeated statement. Univariate comparison between groups for categorical variables was made using the $\chi^2$-test and Fisher’s exact test when appropriate. Complete independence was assumed across animals (by random statement). Reported $P$-values are as follows: $P_t$ indicates change over time, $P_g$ indicates a level of difference between the groups and $P_{tg}$ indicates interaction between the groups and time. In study IV, $P_{\text{group}}$ indicates the distribution of variables between the study groups and $P_{\text{time}}$ indicates the level of difference between two measurements. Two-sided $P$-values are reported.

4.6.1 Propensity score analysis (I)

Propensity score analysis is a statistical technique used to eliminate investigational bias especially in relation to preoperative patient characteristics, which is one of the key criticisms of retrospective studies. The goal of propensity score analysis is to balance two nonequivalent groups on observed covariates to get more accurate estimates of the effects of a treatment on which the two groups differ (Luellen 2005). In study I, propensity score analysis was performed in order to correct the evident imbalances in terms of clinical characteristics and operative risk of the study groups.

Logistic regression with backward selection was performed to calculate the risk of the patients who were to be assigned either to conventional or minimized CPB study group. Receiver operating characteristic (ROC) curve analysis was used to estimate the area under the curve of the model, predicting the probability of assignment to any of the study groups. The calculated propensity score was employed for one-to-one matching. Matching between study group pairs was done according to a difference in the propensity score <0.005. Because of marked difference in the preoperative risk, a one-to-one matching based on logistic EuroSCORE was used additionally (difference between subjects <1.0%). A $P < 0.05$ was considered statistically significant. The developed propensity score showed a satisfactory area under the ROC curve (Hosmer- Lemeshow test: $P = 0.22$, area under the curve: 0.744; 95% CI 0.686–0.802; S.E. 0.030; $P < 0.0001$).
5 Results

5.1 Comparability of the study groups

Despite using the propensity score method to eliminate major imbalances between the study groups in terms of clinical characteristics in study I, patients in the MCPB group still had a somewhat increased operative risk. Logistic EuroSCORE showed itself to be a strong predictor of in-hospital mortality (area under the ROC curve: 0.83, 95% CI 0.68-0.97; S.E. 0.74; \( P = 0.002 \)). Because of this, a one-to-one matching, according to logistic EuroSCORE, was performed. The demographic data of patients in study I is presented in Table 9. There was a trend towards more female sex and smaller body weight in the MCPB group, despite matching.

In study II, 3 patients were excluded from the study due to protocol violations. 18 patients in the MCPB group and 19 patients in the CCPB group completed the study out of 40 enrolled patients. All patients in study III completed the study. The preoperative characteristics did not differ significantly between groups in studies II and III, although the Euroscore risk scores were somewhat higher in MCBP patients in study III (additive Euroscore 5.7 (SD 2.3) vs. 4.6 (SD 2.3), logistic Euroscore 5.1 (SD 3.7) vs. 3.8 (SD 2.4)).

In study IV, 3 animals in both experimental groups were excluded from the analyses due to surgical difficulties prolonging the laparotomy time or causing excessive bleeding. There were no statistically significant differences between the two groups at baseline.

5.2 Perioperative data

In study I, significantly more patients in the CCPB group received aproitin compared to the MCPB patients, and this difference remained after propensity score and logistic Euroscore matching. Patients in the MCPB group had a significantly higher lowest hematocrit during CPB compared to CCPB group. Duration on CPB was similar in both groups. In the CCPB group, the perfusion flow and perfusion pressure were significantly higher than in MCPB group (Table 10).

By means of epiaortic ultrasound, the ascending aorta was assessed as normal (aortic wall <2 mm) in all patients in studies II and III. In study II, the perioperative data was similar in both groups, apart from the hematocrit, which was significantly higher in the MCPB group at all time points and group MCPB
received significantly less red blood cell transfusions compared to the CCPB group (Fig. 4, Table 11).

In study III, Hematocrit was similarly significantly higher during CPB and 2 hours post CPB in the MCPB group, but this difference disappeared at 4 hours post CPB (Fig 4, Table 12). The amount of shed blood collected to the cell saver was significantly higher in the MCPB group (3558 ml) compared to the CCPB group (917 ml). The perioperative red blood cell transfusion rates were similar in both groups (Table 12). The amount of intraoperative platelet transfusion was higher in the MCPB group (Table 12), with a similar trend in the frozen plasma transfusion rate (difference not significant). The cumulative use of vasopressor therapy (norepinephrine) during the first 24 hours was somewhat higher in the MCPB group compared to the CCPB group (difference not significant). The cumulative use of inotropic therapy (dobutamine) during the first 24 hours was similar in both groups.

Fig. 4. Hematocrit concentrations in minimized (MCPB) and conventional (CCPB) CPB groups in studies II and III. Median and interquartile range. Reported P-values are as follows: Pt indicates change over time, P_g indicates a level of difference between the groups and P_{tg} indicates interaction between the groups and time.
Table 9. Demographic data on study I patients. MI: Myocardial infarction; LVEF: Left ventricular ejection fraction; Values are reported as mean±standard deviation; Values in parentheses are percentages.

<table>
<thead>
<tr>
<th></th>
<th>Overall series</th>
<th>Propensity score matched</th>
<th>Logistic Euroscore matched</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCPB n=365</td>
<td>MCPB n=101</td>
<td>CCPB n=77</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67.4±9.1</td>
<td>74.2±8.3</td>
<td>72.8±8.0</td>
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<tr>
<td>Females</td>
<td>56 (15.3)</td>
<td>28 (27.7)</td>
<td>12 (15.6)</td>
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<tr>
<td>Weight</td>
<td>85±13</td>
<td>80±13</td>
<td>83±10</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>30 (8.2)</td>
<td>20 (19.8)</td>
<td>10 (13.0)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>70 (19.2)</td>
<td>30 (29.7)</td>
<td>11 (14.3)</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>86.1±21.3</td>
<td>91.9±23.7</td>
<td>90.6±22.5</td>
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<tr>
<td>Cerebrovascular disease</td>
<td>23 (6.3)</td>
<td>17 (16.8)</td>
<td>6 (7.8)</td>
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<tr>
<td>Neurological dysfunction</td>
<td>4 (1.1)</td>
<td>2 (2.0)</td>
<td>0 (0)</td>
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<td>MI &lt;3 months</td>
<td>84 (23.0)</td>
<td>32 (31.7)</td>
<td>15 (19.5)</td>
</tr>
<tr>
<td>Extracardiac arteriopathy</td>
<td>42 (11.5)</td>
<td>16 (15.8)</td>
<td>5 (6.5)</td>
</tr>
<tr>
<td>Previous cardiac surgery</td>
<td>12 (3.3)</td>
<td>2 (2.0)</td>
<td>2 (2.6)</td>
</tr>
<tr>
<td>LVEF&gt;50%</td>
<td>304 (83.3)</td>
<td>76 (75.2)</td>
<td>65 (84.4)</td>
</tr>
<tr>
<td>30-49%</td>
<td>55 (15.1)</td>
<td>21 (20.8)</td>
<td>10 (13.0)</td>
</tr>
<tr>
<td>&lt;30%</td>
<td>6 (1.6)</td>
<td>4 (4.0)</td>
<td>2 (2.6)</td>
</tr>
<tr>
<td>Unstable angina pectoris</td>
<td>50 (13.7)</td>
<td>15 (14.9)</td>
<td>9 (11.7)</td>
</tr>
<tr>
<td>Emergency operation</td>
<td>27 (7.4)</td>
<td>6 (5.9)</td>
<td>3 (3.9)</td>
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<tr>
<td>Additive EuroSCORE</td>
<td>4.0±3.0</td>
<td>5.9±3.2</td>
<td>4.7±2.8</td>
</tr>
<tr>
<td>Logistic EuroSCORE (%)</td>
<td>4.6±7.1</td>
<td>8.5±10.0</td>
<td>5.2±5.7</td>
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</table>
Table 10. Perioperative data and clinical outcome on study I patients. Hct: Hematocrit; CS: Cell saver; ICU: Intensive care unit; RBC: red blood cells. Values are reported as mean ± standard deviation; Values in parentheses are percentages.

<table>
<thead>
<tr>
<th>Overall series</th>
<th>Propensity score matched</th>
<th>Logistic Euroscore matched</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCPB</td>
<td>MCPB</td>
</tr>
<tr>
<td>No aortic x-clamping</td>
<td>27 (7.4)</td>
<td>24 (23.8)</td>
</tr>
<tr>
<td>Aortic x-clamp time (min)</td>
<td>71.3±31.1</td>
<td>53.6±36.0</td>
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<tr>
<td>CPB time (min)</td>
<td>96.7±28.1</td>
<td>101±34.6</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>338 (92.6)</td>
<td>76 (75.2)</td>
</tr>
<tr>
<td>Tranexamic acid</td>
<td>27 (7.4)</td>
<td>23 (22.8)</td>
</tr>
<tr>
<td>Lowest Hct during CPB</td>
<td>0.23±0.04</td>
<td>0.26±0.04</td>
</tr>
<tr>
<td>Lowest CPB flow (L/min)</td>
<td>4.0±0.4</td>
<td>3.0±0.6</td>
</tr>
<tr>
<td>Highest CPB flow (L/min)</td>
<td>4.7±0.4</td>
<td>3.9±0.6</td>
</tr>
<tr>
<td>CS reinfused (ml)</td>
<td>948±368</td>
<td>738±407</td>
</tr>
<tr>
<td>Extubation time (hours)</td>
<td>6.6±15.0</td>
<td>9.8±30.5</td>
</tr>
<tr>
<td>Reintubation</td>
<td>8 (2.2)</td>
<td>10 (9.9)</td>
</tr>
<tr>
<td>ICU length of stay (days)</td>
<td>1.5±2.2</td>
<td>2.3±4.4</td>
</tr>
<tr>
<td>Postop. blood loss (ml)</td>
<td>614±326</td>
<td>707±514</td>
</tr>
<tr>
<td>Packed RBC (units)</td>
<td>0.6±1.7</td>
<td>1.6±3.1</td>
</tr>
<tr>
<td>30-day mortality</td>
<td>5 (1.4)</td>
<td>3 (3.0)</td>
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<tr>
<td>Atrial fibrillation</td>
<td>131 (37.0)</td>
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<tr>
<td>Stroke</td>
<td>7 (1.9)</td>
<td>0 (0)</td>
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</tbody>
</table>
In study IV pump flow during CPB was lower in the MCPB group than in the CCPB group (Pg <0.05). Larger portion of systemic blood flow was directed to the splanchnic region in the MCPB group (Pt*g <0.05), and Qspl decreased comparably in both groups (Fig 5) Systemic and gut DO2i values declined similarly in both groups. Gut VO2i was stable in both groups, and portal venous O2 (SpO2) saturation declined in line with the systemic mixed venous O2 saturation. Due to the surgical preparations, baseline systemic and portal venous lactate concentrations were somewhat elevated in both groups as well as in the sham animals. After baseline, lactate values normalized in sham animals, with no significant changes from baseline in the study groups. Systemic lactate concentrations, however, were consistently higher in the CCPB than in the MCPB group (P_g<0.05).

![Fig. 5. Cardiac output and pump flow (left) and splanchnic flow (right) indexed to weight and time between baseline and at 240 minutes of CPB in the study groups and in the two sham animals (median and 25th, 75th percentiles).](image)

### 5.3 Clinical outcome

In study I, clinical outcome between propensity score matched and logistic Euroscore matched pairs appeared similar following MCPB and CCPB. The amount of transfused blood products and postoperative blood loss were without a significant intergroup difference, as well as extubation time and length of stay in the intensive care unit. In logistic Euroscore matched pairs, reintubation occurred more often in MCPB group. No strokes appeared in MCPB patients. Postoperative atrial fibrillation occurred similarly in both groups after propensity score matching and logistic Euroscore matching. The 30-day mortality was equally low in both groups (Table 10).
Studies II and III were not powered to measure clinical outcome. In study II, the postoperative outcome was uneventful in 35/37 patients. Patients in the MCPB group had lower ICU and hospital stay lengths ($P = 0.05$). Two patients in the CCPB group suffered a postoperative stroke verified with a CT scan. The first patient suffered from a mild right hemiparesis after extubation and the CT scan performed on the second postoperative day revealed a left frontoparietal cortical infarct. The second patient suffered from prolonged delirium and confusion and the CT scan performed on the fifth postoperative day revealed a right temporal infarct. Both these patients recovered rapidly.

In study III, 1 patient in each group required reoperation for hemostasis, and the postoperative mediastinal blood loss was similar in both groups (Table 12). 4 patients (20%) in the MCPB group and 3 patients (15%) in the CCPB group had a prolonged ICU stay of more than 72 hours. No in-hospital mortality occurred. There was no intergroup difference in renal function measured with creatinine and Cystatin C release until the 3rd postoperative day. Acute kidney injury (defined as 1.5-fold increase in creatinine) occurred in 3 patients in the MCPB group and 1 patient in the CCPB group. Myocardial damage defined as Troponin I release occurred similarly in both groups. No postoperative strokes occurred in either group, and 1 patient in the MCPB group suffered from postoperative delirium.

Table 11. Perioperative data in study II. RBC: Red blood cells; ICU: Intensive care unit. Values are reported as mean ± standard deviation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>MCPB group</th>
<th>CCPB group</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grafts per patient</td>
<td>4.4 ± 1.19</td>
<td>4.3 ± 1.16</td>
<td>.99</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>117 ± 20</td>
<td>121 ± 24</td>
<td>.52</td>
</tr>
<tr>
<td>Aortic crossclamp time (min)</td>
<td>89 ± 19</td>
<td>95 ± 22</td>
<td>.36</td>
</tr>
<tr>
<td>Cell salvage reinfusion (ml)</td>
<td>799 ± 271</td>
<td>640 ± 562</td>
<td>.28</td>
</tr>
<tr>
<td>RBC total (units)</td>
<td>0.05 ± 0.23</td>
<td>1.25 ± 1.05</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Postoperative blood loss (ml)</td>
<td>589 ± 147</td>
<td>532 ± 152</td>
<td>.31</td>
</tr>
<tr>
<td>ICU length of stay (days)</td>
<td>1.06 ± 0.24</td>
<td>1.47 ± 0.84</td>
<td>0.05</td>
</tr>
<tr>
<td>Hospital length of stay (days)</td>
<td>5.47 ± 1.19</td>
<td>6.04 ± 3.41</td>
<td>0.05</td>
</tr>
</tbody>
</table>
Table 12. Perioperative data in study III. Values are reported as mean ± standard deviation. CS: Cell salvage; RBC: Red blood cells; OR: Operating room.

<table>
<thead>
<tr>
<th>Variable</th>
<th>MCPB group n = 20</th>
<th>CCPB group n = 20</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPB time (min)</td>
<td>153 ± 41</td>
<td>142 ± 30</td>
<td>1.0</td>
</tr>
<tr>
<td>Aortic crossclamp time (min)</td>
<td>115 ± 36</td>
<td>104 ± 30</td>
<td>.35</td>
</tr>
<tr>
<td>CS collected (ml)</td>
<td>3558 ± 2159</td>
<td>917 ± 1165</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>CS reinfusion (ml)</td>
<td>1520 ± 969</td>
<td>344 ± 460</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Packed RBC in OR (%)</td>
<td>55</td>
<td>50</td>
<td>1.00</td>
</tr>
<tr>
<td>Packed RBC in OR (unit)</td>
<td>0.9 ± 1</td>
<td>0.8 ± 1</td>
<td>.82</td>
</tr>
<tr>
<td>Packed RBC total (%)</td>
<td>60</td>
<td>75</td>
<td>.51</td>
</tr>
<tr>
<td>Fresh frozen plasma in OR (%)</td>
<td>25</td>
<td>5</td>
<td>.18</td>
</tr>
<tr>
<td>Fresh frozen plasma in OR (unit)</td>
<td>1.1 ± 1.0</td>
<td>0.25 ± 1.1</td>
<td>.09</td>
</tr>
<tr>
<td>Fresh frozen plasma total (%)</td>
<td>42</td>
<td>15</td>
<td>.08</td>
</tr>
<tr>
<td>Platelets in OR (%)</td>
<td>25</td>
<td>0</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Platelets in OR (unit)</td>
<td>0.45</td>
<td>0</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Platelets total (%)</td>
<td>47</td>
<td>20</td>
<td>.10</td>
</tr>
<tr>
<td>Postoperative blood loss (ml)</td>
<td>689 ± 336</td>
<td>620 ± 322</td>
<td>.45</td>
</tr>
<tr>
<td>Atrial fibrillation (%)</td>
<td>40</td>
<td>50</td>
<td>.75</td>
</tr>
<tr>
<td>Norepinephrine (µg/kg/24h)</td>
<td>113 ± 98</td>
<td>73 ± 107</td>
<td>.07</td>
</tr>
<tr>
<td>Dobutamine (mg/kg/24h)</td>
<td>1.47 ± 1.5</td>
<td>1.47 ± 1.8</td>
<td>.42</td>
</tr>
</tbody>
</table>

5.4 Retinal fluorescein angiography (II, III)

Baseline angiographs were normal in all patients. In study II, postperfusion angiograph could not be obtained in one CCPB patient. Retinal fluorescein angiographs were reported normal or abnormal, with abnormal postperfusion microvascular damage that was not present in preperfusion angiograph (Figure 6). In postperfusion angiographs in study II, retinal microembolic perfusion defects were detected in 2 out of 18 in the MCPB group and 9 out of 18 in the CCPB group (11% vs. 50%, difference 39%, 95% confidence interval 8.7%–61% 9, \( P = 0.029 \)). Both patients with a postoperative stroke in the CCPB group had perfusion defects in postperfusion angiographs.

In study III, postperfusion angiograph could not be obtained in 1 patient in both groups. Retinal fluorescein angiographs were reported normal or abnormal, with abnormal postperfusion microvascular damage that was not present in preperfusion angiograph. In postperfusion angiographs, retinal microembolic perfusion defects were detected in 7 out of 19 in the MCPB group and 12 out of
19 in the CCPB group (37% vs. 63%, difference 26%, 95% CI−5%−51%, \( P = 0.194 \)).

All abnormal postperfusion fluorescein angiographs revealed hyperfluorescence (an abnormal presence of fluorescein or an increase in normal fluorescence in the fluorescein angiogram) in the parafoveal region (Figure 6). Vascular abnormalities (such as aneurysms and dilation) at the edge of the foveal avascular zone were detected in most of the abnormal postperfusion fluorescein angiographs simultaneously with the leakage of fluorescein (hyperfluorescence of fluorescein in the extravascular space) (Figure 6). These changes probably result from embolization-induced vessel occlusion, which damages the inner blood-retina barrier and causes the leakage of fluorescein.

![Retinal fluorescein angiography.](image)

**Fig. 6. Retinal fluorescein angiography. Circles pointing out the sites of the microembolic signals after conventional CPB in study II.**

### 5.5 Inflammatory, coagulation and endothelial markers (II, III)

**Markers of systemic inflammation**

Markers of neutrophil activity, PMN elastase and MPO increased markedly during surgery and CPB. In study II, PMN-elastase concentration was lower 15 minutes and 2 hours after CPB in the MCPB group (\( P_t = <0.0001, P_g = 0.12, P_{tg} = 0.03 \)) (Figure 7). In study III, PMN elastase concentration was significantly lower in the MCPB group 15 minutes and 2 hours after CPB (Figure 7). Other
measured markers of inflammation (C3, IL-6, IL-8, TNF-α), however, were without a significant intergroup difference in studies II and III.

![Graph showing PMN elastase concentrations in MCPB and CCPB groups in studies II and III.](image)

**Fig. 7.** PMN elastase concentrations in MCPB and CCPB groups in studies II and III. Values are in median and interquartile range. Reported $P$-values are as follows: $P_t$ indicates change over time, $P_g$ indicates a level of difference between the groups and $P_{tg}$ indicates interaction between the groups and time.

**Markers of coagulation, endothelial injury and activation, and platelet activation**

In study II, markers for coagulation (TAT, F1+2) were increased significantly in response to the surgical procedure regardless of the CPB circuit used.

In study II, markers for endothelial activation and injury (vWF:Ag, TM) increased during CPB without a significant difference between groups. In study III, vWF:Ag was significantly lower in the MCPB group 15 minutes after CPB ($P_t = <0.0001$, $P_g = 0.27$, $P_{tg} = 0.0005$), but TM increased in a similar fashion after CPB in both groups.

In studies II and III, thrombin dependent platelet activity (sGPV) increased significantly in response to the surgical procedure regardless of the CPB circuit used.

### 5.6 Histopathologic evaluation (IV)

Light microscopy examination of baseline biopsies revealed intact ileal mucosal structure and minor focal epithelial degeneration in colon samples in all groups. There were no changes relative to the baseline biopsies in sham animals in either ileal or colon biopsies at the end of the experiment at 240 minutes. No differences
in the height of villi or crypts, amount of inflammatory cells or microvascular filling in the CPB240min biopsies in relation to baseline were recorded in either study group. In both groups, CPB240min ileal biopsies revealed mild focal degeneration of mucosal epithelium ($P_{\text{time}} = 0.07$). The epithelial alterations were more evident in colonic control biopsies in both CPB groups ($P_{\text{time}} < 0.05$), manifesting as moderate focal damage of colonic surface epithelial cells. In sites with damage present, epithelial cells were shrunken and nuclei showed pyknosis and karyolysis and there were foci showing detachment of the epithelial cells from the basal lamina (Fig. 8). This damage was only present on the luminal epithelium and the crypts remained intact. Morphologic damage, expressed as mucosal damage grade, was more severe in colonic than ileal biopsies and there were no significant differences between the MCPB and CCPB groups (Fig. 9). The ileal epithelial cell proliferation rate (Ki-67 index) remained constant despite CPB in both groups (Fig. 10). In colonic specimens, epithelial cell proliferation rate showed a slight increase after CPB without an intergroup difference (Fig. 10). In sham animals, the proliferation rate stayed constant in both ileal and colonic biopsies.
Fig. 8. Micrographs of hematoxylin/eosin (HE)-stained sections of colon biopsies of animals in the MCPB (first row), CCPB (second row) groups and in a SHAM animal (third row) at baseline (left) and at 240 minutes on CPB (right). Bar = 250 µm.
Fig. 9. Mucosal damage scores (median, 25th, 75th percentiles) of ileal (left) and colon (right) biopsies at baseline (PRECPB) and at 240 minutes of CPB (CPB 240min) in the study groups and in the two sham animals.

Fig. 10. Median (25th, 75th percentiles) percentages of epithelial cells staining positively for Ki-67 as a marker of cell proliferation at baseline (PRECPB) and at 240 minutes of CPB (CPB 240min) in the study groups and in the two sham animals.
5.7 Immunohistochemistry (IV)

The mucosal epithelial cell immunostaining of claudins (CL) was presented as follows: CL1 was clearly cytosolic-bound, expressing abundantly in both ileal and colonic epithelium. Immunostaining of CL2 was more membrane-bound, expressing in ileal villi excluding the villus tips that were more faintly stained. In the colon, CL2 was expressed only in the deeper proliferative layer of the mucosa. CL3 and CL7 were constantly expressed in both the cytoplasms and the membranes of epithelial cells in ileal and colonic samples. CL4 staining was slightly more membrane-bound and its expression was stronger on the apical parts in both ileal and colon samples. CL5 was evenly stained in cytoplasms and membranes and, similarly to CL4, the staining was fainter towards the basal parts of the epithelium. Immunostaining of CL1, 2, 3, 5 and 7 revealed no differences with relation to group or time. Ileal membrane-bound CL4 decreased slightly during CPB in both experimental groups ($P_{\text{time}} = 0.05$) and in colon samples this decrease was found in both membrane- and cytoplasmic-bound CL4 without any significant differences between groups (cytoplasmic-bound $P_{\text{time}} < 0.05$, membrane-bound $P_{\text{time}} < 0.05$) (Fig 11).
Fig. 11. Median (25th, 75th percentiles) combined scores of cells staining positively for claudins in ileum and colon at baseline (PRECPB) and at 240 minutes of CPB (CPB 240min) in the study groups and in the two sham animals. CL1, claudin-1; CL2, claudin-2; CL3, claudin-3; CL4, claudin-4; CL5, claudin-5; CL7, claudin-7.
Fig. 12. Micrographs of sections of colon biopsies showing immunohistochemical staining for claudin-4 (CL4) in the MCPB (first row), CCPB (second row) groups and in a sham animal (third row) at baseline (left) and at 240 minutes on CPB (right). Bar = 250µm.
6 Discussion

6.1 Main findings and strengths of the study

The first study included a consecutive series of unselected CABG patients, managed with minimized or conventional CPB according to the hospital's practice for patient selection. This study demonstrated that minimized CPB is as safe and feasible as conventional CPB when treating unselected coronary artery bypass surgery patients. We were, however, not able to show any beneficial effect on blood product transfusion requirements with MCPB in this patient category, despite a better preservation of hematocrit during CPB.

Study II confirmed in a prospective randomized study the earlier findings of less hemodilution and a reduced need for blood product transfusions with MCB in CABG patients. In addition, study II demonstrated for the first time that retinal microembolic load related to CPB is significantly attenuated with MCPB compared to CCPB in patients undergoing CABG. Our method of measuring microembolization by means of retinal fluorescein angiography provides a more precise way to evaluate cerebrovascular microembolism compared to the more widely used TCD, which is more sensitive to artifacts. Retinal fluorescein angiography also includes a visual examination of the tissue damage caused by microemboli. Study III showed less patients with retinal microembolism with MCPB compared to CCPB in AVR with or without coronary revascularization, but the difference was not statistically significant. Furthermore, there was a significantly higher rate of blood collected by the cell saver and reinfused in the MCPB group, as well as a higher rate of intraoperative platelet transfusions.

Studies II and III demonstrated prospectively that MCPB is associated with decreased activation of polymophonuclear leukocytes, measured with PMN elastase, compared to CCPB in patients undergoing elective CABG and AVR with or without combined coronary revascularization. Other measured markers of systemic inflammation, coagulation, endothelial activation and injury, and platelet activity were similar between MCPB and CCPB.

Study IV demonstrated for the first time that prolonged CPB is associated with intestinal mucosal damage, which was observed similarly in minimized CPB and conventional CPB as indicated by increased epithelial proliferation and decreased expression of tight junction protein claudin 4 in colon. Based on this work, the effects of MCPB on intestinal mucosal stability are similar to those of
CCPB at least in an animal model. With a unique animal model, it is possible to observe splanchnic events during and after surgery, and thus provide information that cannot be obtained for human trials.

6.2 Limitations of the study

First study was a retrospective study, which always presents a risk for nonequivalent study groups. This was also evident in our study, and it was attempted to eliminate the investigational bias by using a matched pair analysis. Even after this, the groups remained somewhat nonequivalent, which may affect the reliability of the study. A prospective, randomized study would unquestionably have been a more exact method for a reliable evaluation of the intended variables, but such data was deemed to be unfeasible to collect within a reasonable time period in Finland.

All other studies were prospective randomized studies, carried out in a non-blinded manner, which may also introduce an investigational bias. The measurements for the primary outcomes in all three studies, however, were performed blinded to the treatment group after the end of the study period.

An experimental animal model was chosen for study IV, as this is the only reliable method to evaluate the effects of prolonged CPB on intestinal mucosal histopathology. Histopathology is widely accepted as a reliable method of assessing tissue injury when performed in a blinded manner by an experienced pathologist. The results obtained from animal models must always be interpreted carefully, however, and can not be directly applied in humans. Despite many anatomic and physiologic similarities between the porcine and human digestive and cardiovascular systems, caution must be taken when applying our findings to patients. The expression of tight junction proteins was assessed quantitatively based on immunohistochemical staining, and an additional, more accurate method of quantification might have been more creditable. Furthermore, a follow-up time would have allowed for the detection of clinical manifestations of impaired intestinal barrier function.

The sample size in all studies was relatively small. In study I, however, this was assessed as adequate to evaluate the safety and feasibility of the MCPB technique and produce reliable results in terms of hemodilution and blood product requirements. In studies II and III this was based on power analysis with retinal microvascular damage as outcome measure. It may be, however, that the sample sizes were inadequate to measure secondary outcomes (markers of systemic
inflammation, coagulation, endothelial activation and injury, and platelet activation). The fact that in study IV the amount of animals was decided on without conducting a power analysis is a limitation. This may have affected our results, thus the observed differences between groups were small. We probably could have produced more reliable results by increasing the sample size, but this is often limited by resources in a large animal model.

6.3 Hemodilution and transfusion requirements (I, II, III)

6.3.1 CABG

The Vaasa Central Hospital is a relatively small institution with limited reserves of blood products. This necessitates meticulous blood conservation with regards to every patient undergoing cardiac surgery. The Vaasa Central Hospital was the first center in Finland to conduct a routine use of minimized CPB in cardiac surgery, and at the time of planning of study I, it was the only hospital in Finland with a reasonable perspective of the technique. It was of great value to be able to produce a systematic evaluation on the safety and efficacy of using MCPB in the Vaasa Central Hospital before starting this technique in our own institution (The Oulu University Hospital).

Based on the literature and clinical experience, better preservation of hematocrit and a reduced requirement for blood products would be the most obvious benefits using minimized CPB. Therefore, at the Vaasa Central Hospital, patients with female sex, small body weight and a low preoperative hematocrit were primarily recruited to be perfused with MCPB. As a result of this clinical patient selection, the bias especially in relation to body weight and the use of aprotinin could not totally be eliminated in the study, even after using propensity score and logistic Euroscore matching. This makes it difficult to draw any definite conclusions on the effect of MCPB in reducing the need for blood products, which was one of the primary endpoints of this study. As other clinical outcome measurements after matching were observed to be similar after CPB and MCPB, it was concluded that MCPB is as safe and feasible as CCPB with unselected coronary artery bypass surgery patients. With this finding, we were encouraged to begin to use minimized CPB in our institution.

Study II confirmed earlier findings of less hemodilution and a reduced need for blood product transfusions associated with MCB in CABG patients.
(Benedetto et al. 2009). This benefit was not observed, however, in study III with similar requirements for perioperative RBC transfusions and a higher rate of perioperative platelet and FFP transfusions.

Clotting factors and platelets have been shown to be severely depleted in salvaged blood (Burman et al. 2002). This explains the higher intraoperative transfusion rate for platelets and fresh frozen plasma as well as the lack of a reduction in the red blood cell transfusion rate in the MCPB group. The intense use of cell saver suction may have caused relative hypovolemia and thus explain the trend towards an increased need for the intraoperative use of vasopressor drugs in the MCPB group. Due to the demand of extensive additional use of cell saver suction, we feel the potential biological benefits of MCPB were lost in this series of valve cardiac surgery. We speculate that the reason for the large volume of blood collected to the cell saver was largely due to our strategy of continuous ante- and retrograde cold blood cardioplegia together with left ventricle venting via the pulmonary vein, which proved insufficient to ensure operative visibility, necessitating the extensive use of cell saver suction.

### 6.3.2 Valve surgery

The establishment of the MCPB circuit in the management of valve surgery appears somewhat more problematic compared to CABG, which is reflected by several different venting approaches, even within groups using similar commercial setups. Most of the studies published comparing MCPB with CCPB in AVR have used the MECC™ (Maquet Cardiopulmonary, Germany) MCPB system (Remadi et al. 2004, Castiglioni et al. 2007, Castiglioni et al. 2009, Yilmaz et al. 2010) of the several commercially available MCPB circuits. Additionally ECC.O™ (Sorin Group, Italy) has been utilized successfully in AVR (Issitt et al. 2008), and there is one previous report on ROCSafe™ (Terumo Europe) in aortic surgery (Kutschka et al. 2009). Remadi and colleagues (Remadi et al. 2004) first reported the successful use of the MECC™ circuit in AVR using a pulmonary artery vent, which transferred vented blood to a cell saver. During the study, they later added a suction system to the MECC™ system, to direct the vented blood to a vacuum bag, from where it could then be reinfused to the venous line. They reported a reduced intraoperative RBC transfusion rate and better platelet preservation, as well as attenuated myocardial and renal injury with MCPB compared to CCPB. Castiglioni and colleagues have published two reports (Castiglioni et al. 2007, Castiglioni et al. 2009) on the MECC™ system in AVR,
using a double vent in the pulmonary artery and via the pulmonary vein to the left ventricle, the vent blood being collected into a vacuum bag from which it is immediately possible to reinfuse into the venous line. Both studies report reduced RBC transfusions, better platelet preservation and reduced chest tube drainage with MCPB compared to CCPB. Yilmaz and colleagues quite recently reported a non-randomized study evaluating the MECC™ system in combined CABG and AVR (Yilmaz et al. 2010). They used a pulmonary artery vent with additional sump suction directly through the aortotomy if necessary. Both vents were connected to a bubble trap in the venous line. In agreement with previous reports on the MECC™ system, they reported a reduced requirement for blood products with MCPB compared to CCPB. Issit and colleagues published an institutional report of 50 consecutive unselected aortic surgery patients using the ECC.O system with beneficial experiences (Issitt et al. 2008). After careful consideration, they had decided on a left ventricle vent inserted via aortotomy with an additional aortic root vent. Both vents were attached to a soft-shell reservoir, from which the vented blood could be reinfused into the venous line with an incorporated automated air-removal device. Although the results could not be compared to CCPB, the series represented a blood product transfusion rate of 12%, which is lower than in aortic surgery on average. Kutschka et al. reported the use of the ROCSafe™ system in aortic surgery (Kutschka et al. 2009) in a very similar technique to that used in our series. They used a left ventricle vent via the pulmonary vein, the vent blood being directed to a reservoir bag through a roller pump. They reported no problems in managing the vented blood, and reported a slightly reduced requirement for blood products with MCPB without a significant reduction in chest tube drainage. It is noteworthy that none of the studies above report the amount of blood collected to the cell saver. We can only assume that they must have been lower than that in our study, as the capacity of MCPB to reduce the transfusion requirements was conserved. In contrast to other previous reports using MCPB on valve surgery, we used continuous cold blood cardioplegia, which increases the amount of vented blood, and may thus partly explain the problems encountered with venting in this study.

6.4 Retinal microembolism in CABG (II)

The retinal fluorescein angiography findings in study II demonstrated for the first time that the retinal microembolic load related to CPB is less with MCPB than it is with CCPB in CABG patients. As the retina develops as an outgrowth of the
embryonic brain and the retinal artery is a branch of the cerebral artery, these findings suggest that microembolic load to the brain following CPB may be attenuated by MCPB. The findings of study II support a previous observation obtained with the less direct, but more easily accessible TCD (Liebold et al. 2006). Compared with TCD, however, retinal imaging provides a more definitive method to evaluate cerebrovascular microembolism and additionally enables visualization of the tissue damage caused by microemboli. Although histopathologic and imaging studies suggest that retinal vascular signs are closely related to pathological microvascular changes in other organs (Schneider et al. 1993, Liew et al. 2008), it is not clear to what extent the retinal microvasculature reflects the cerebral and other microvascular beds when investigating acute consequences of systemic microembolization. Given the morphological and functional similarities due to their common origin from the internal carotid artery, however, retinal microvasculature may be assumed to quite reliably mirror the cerebral microvasculature. Therefore, retinal fluorescein angiography may provide a minimally invasive tool to assess the cerebral microvasculature.

The reduction of retinal microvascular embolism in the MCPB group may have resulted from technical improvements in minimized circuits. The conventional CPB systems can also be modified towards more advanced circuits (e.g. applying a centrifugal pump and a softshell reservoir) than those used in our study. Our aim was, however, to compare the open hardshell CPB system widely used and accepted in European and Scandinavian practice with a more modern MCPB system. The concept of MCPB with its vacuum assisted venous return and exclusion of a venous hard shell reservoir requires meticulous air handling strategies including the precise sealing of the venous cannula, with careful management of drug and fluid administration and collecting of blood samples. In addition, as done in our series, integration of an air removal device to the circuit system is recommended. With these precautions the minimized circuit concept provides a safe air management. Venous air during conventional CPB is common and the perceived ability of venous hard shell reservoirs to remove such air can easily lead to a misunderstanding of venous air being consequently benign. This, however, has a tendency to dissolve into micobubbles detectable in the arterial line distal to the arterial filter (Willcox et al. 1999). Air appearing in minimized CPB circuits cannot be ignored and left untouched, which in addition to other MCPB specific air-eliminating precautions, may be one reason for the reduction of embolic load.
Hemodilution increases cerebral blood flow (Sungurtekin et al. 1999), which may potentiate the microembolic load to the brain at the commencement of CPB. Due to a reduction in the non-physiologic surface area and retrograde priming, minimized CPB is associated with significantly less hemodilution in comparison to a conventional circuit, which was also observed in our study. The difference in hemodilution is emphasized at the commencement of CPB when the prime is introduced to the patient. When initiating conventional CPB the patient receives a large volume of crystalloid fluid in a very short time and the upper body is blanched. With minimized CPB the mixing of blood and prime occurs outside the body with retrograde and antegrade priming. This leads to a marked difference in hematocrit variation amplitude during the first minutes of CPB between the two systems before the extracorporeal circulation has stabilized. Interestingly, the highest embolic count is noted with TCD at the very beginning of CPB (Motallebzadeh et al. 2007).

6.5 Retinal microembolism in valve surgery (III)

Study III showed no statistically significant difference in retinal microembolism between MCPB and CCPB in AVR with or without coronary revascularization, although fewer patients had retinal microembolism in the MCPB group. Our study showed a considerably higher amount of retinal microvascular defects following AVR compared to previous reports in CABG patients (Ascione et al. 2005), and considering the tendency to lowered retinal microvascular defects in the MCPB group, we cannot eliminate that this study was after all inadequately powered to detect statistically significant differences in retinal microembolism between the two circuit types.

Valve surgery is associated with more potential sources of both gaseous and particulate emboli compared to CABG. An additional blood-air interface in the operating field, increased demand for left ventricle venting, dislodging of atheromatous debris during valve resection, and possible reinfusion of pericardial suction blood, are all potential sources for microemboli. The source of the retinal microvascular emboli observed in this study cannot reliably be detected with retinal fluorescein angiography. It has been shown by means of TCD that open-heart surgery is associated with a larger number of both gaseous and solid emboli compared to CABG (Abu-Omar et al. 2004), and the increase in retinal microvascular defects may be assumed to result from both types of emboli. The capacity of MCPB circuits to reduce cerebral microembolism in CABG has been
speculated to result from the minimized air interface and meticulous air handling in order to avoid air entrainment with the exclusion of a venous hard shell reservoir. In the concept of open-chamber cardiac surgery, the “closed” nature of MCPB circuits is unavoidably lost. The safety issues regarding air removal in open-chamber operations have been addressed in several commercial MCBP models with an integration of an air removal-system, providing safe air-handling strategies also in open-heart surgery (Yilmaz et al. 2010, Issitt et al. 2008, Kutschka et al. 2009). AVR and other open chamber procedures demand, however, increased left ventricle venting to ensure adequate visibility of the operative field. Venting of open-heart chambers is often associated with more air in the vent line, and the removal of this air challenges the MCPB circuit more than in CABG procedures, and must therefore be addressed appropriately in order to preserve the biological benefits of the MCPB circuit. Due to our institution’s routine practice of continuous cold blood cardioplegia, we chose a type of vent to allow appropriate venting to ensure visibility. In our series, left ventricle venting via pulmonary vein during aortic cross-clamp turned out to be somewhat laborious, necessitating continuous careful controlling of the returning vented blood from the reservoir bag to the venous line in an air-free manner. This easily led to the blood becoming a challenge to operative visibility, which necessitated the additional use of cell saver suction in clearing the operative field. We can not rule out the possibility of the large amounts of air-containing vent blood transferred from the reservoir bag to the circuit creating gaseous microemboli, and speculate whether this may have been one reason for the higher frequency of retinal microvascular defects in MCPB and CCPB, compared to CABG patients.

6.6 Neurologic sequelae of retinal microembolism

The clinical significance of microvascular alterations observed and their dissimilar frequency in patients undergoing two different CPB circuit systems needs to be further elucidated. Asymptomatic embolic signals detected using TCD seem to predict stroke risk in several settings (King et al. 2009), but this correlation has not been comprehensively evaluated in cardiac surgery due to the large study population needed. Furthermore, the mechanism of postoperative stroke is only in part from microembolism, while the more common mechanisms are related more to embolism of larger particles (e.g. atherosclerosis of the ascending aorta) or to pre-existing carotid or intracranial arterial disease. Although the retinal microcirculation may mirror the cerebral microcirculation,
the susceptibility of retinal and cerebral nerve tissue to ischemic injury from microembolization is in part determined by the presence of arterial collaterals which are generally absent in the retina but plentiful in the brain. This is also indirectly demonstrated in our study in which 31% of patients had retinal microembolization and of those only two patients in the CCPB group developed stroke. So far studies using neuropsychological testing have failed to show any differences in the incidence of POCD between patients undergoing CABG with or without CPB, despite the significantly reduced cerebral embolic load in OPCAB patients demonstrated with TCD (Lund et al. 2003, Liu et al. 2009). Considering the difficulties in defining the real incidence of POCD, these studies may lack the statistical power to reliably assess the connection with CPB-related microembolism and cognitive outcome (van Dijk & Kalkman 2009). Although the postoperative stroke rate is low (1–2%) for elective CABG, there are some indications that MCPB may be related to an even lower postoperative stroke rate compared to CCPB. A prospective study of 1674 patients undergoing CABG found that in the MCPB group the stroke risk was decreased in comparison with the CCPB group (2.3% vs. 4.1%, p <0.05) (Puehler et al. 2009). Similarly, a recent meta-analysis showed that neurologic damage after cardiac surgery was significantly lower in the MCPB group (0.7% vs. 3.4%, P = 0.008) (Zangrillo et al. 2010). Although study I was not powered to measure postoperative neurologic damage, it is worth noting that even in the overall series with markedly high operative risk, there were no strokes in patients treated with MCPB.

6.7 Inflammatory markers (II, III)

CPB induces a complex acute phase reaction culminating in adhesions of PMNs, platelets and endothelial cells, causing transendothelial migration of PMNs and fluid extravasation which can lead to ischemia and microvascular tissue damage. PMNs activated by complement and cytokines play a major role in CPB-induced inflammation and tissue destruction by degranulating free oxygen radicals and proteases (Paparella et al. 2002). This study confirmed earlier findings of a reduced release of PMN elastase with MCPB compared to conventional CPB (Fromes et al. 2002, Lindholm et al. 2004, Ohata et al. 2007, Svitek et al. 2009). MPO, a similar marker of the degranulation of activated PMNs, had peaked prior to elastase release (CPB+45min vs. CPB+15min) without any intergroup difference. This may be explained by earlier findings with MPO being released already by circulating PMNs (Biasucci et al. 1996), while the degranulation of
elastase by PMNs appears to be dependent on adherent endothelial cells stimulated with cytokines (Topham et al. 1998). Thus elastase may more precisely reflect the decisive phase of leukocyte adhesion. Contradictory to previous studies (Fromes et al. 2002), our study demonstrated a CPB and surgery induced activation of complement and proinflammatory cytokines without a statistically significant intergroup difference.

It could be speculated, that the attenuated release of PMN elastase in the MCPB group was related to the larger amount of blood collected to the cell saver in study III. This has been deemed to be somewhat doubtful, as the profile of measured inflammatory mediators associated with these two different CPB systems was very similar to study II, in which there was an equal amount of blood collected to the cell saver.

6.8 Markers for coagulation, endothelial activation and injury, and platelet activity (II, III)

Stimulation of the coagulation cascade and thrombin-induced activation of platelets was observed to a similar extent regardless of the CPB method used. Likewise, markers of endothelial activation and injury increased comparably in both groups in response to CPB in study II. In study III, vWF:Ag as a marker of endothelial activation and injury was significantly lower in the MCPB group 15 minutes after CPB. TM was released quite similarly in both groups, however, and it is difficult to dismiss the effect of the larger amount of blood collected to the cell saver which was observed in study III, on the vWF:Ag concentration in the MCPB group.

Considering the tendency towards lower values of coagulation and endothelial markers in the MCPB group in study II, we cannot unequivocally state that it was not adequately powered to detect changes within these variables since - prior to our study - there have been no published reports on the effects of ROCSafe™ modification on coagulation and endothelial dysfunction.

We did not find any difference in thrombin-induced activation of platelets measured with sGPV between the two CPB systems.

These findings on markers of coagulation, endothelial activation, and platelet activity, indicate that these pathophysiologic events may not be attenuated by using the ROCSafe™ modification of MCPB.
6.9 Intestinal mucosal integrity (IV)

The major finding of the present study is that during prolonged CPB, gut mucosal integrity was affected similarly by MCPB and CCPB. McIlwain and colleagues (McIlwain et al. 2010) reported similar, although more severe, alterations of porcine jejunal biopsies following 8 hours of extracorporeal membrane oxygenation (ECMO). The pathophysiologic mechanisms causing CPB-related gastrointestinal dysfunction are largely unresolved and multifaceted, and their attenuation may require more than minimized circuits are able to provide.

Our results support previous concerns of CPB-related gastrointestinal dysfunction. There is some evidence (Khan et al. 2006), that patients with prolonged CPB (over 120 minutes) are at a greater risk of developing postoperative gastrointestinal complications, suggesting that splanchnic integrity may be more threatened by a longer duration of CPB. After 240 minutes of CPB we found intestinal mucosal damage in all animals except one in the MCPB group. The abnormalities were only moderate, however, and given the intestinal epithelial restitution capacity even after severe damage following hemorrhagic shock (Chang et al. 2005), it may be assumed that injuries of this degree would not have caused evident intestinal ischemia. Still, it is possible that epithelial barrier function, which is primarily governed by the integrity of intestinal epithelium, was affected.

CPB has previously been shown to decrease the expression of tight junction proteins occludin and ZO-1 with a simultaneous increase in intestinal permeability (Sun et al. 2008). To our knowledge, there are no studies published concerning the intestinal expression of claudins after CPB. We investigated the expression and localization of claudins 1, -2, -3, -4, -5 and 7 and found that the immunostaining of intestinal apical CL 4 protein was decreased after prolonged CPB. A decreased and redistributed expression of CL 4 protein has previously been reported in human active ulcerative colitis (Oshima et al. 2008), norovirus infection (Troeger et al. 2009) and in murine polymicrobial sepsis (Li 2009, Clark 2009). The role of CL 4 is strongly related to barrier function, as an overexpression of CL 4 has been shown to increase the paracellular barrier (VanItallie & Anderson 2001). We did not detect any changes in immunostaining of CL 1, -2, -3, -5 and 7 in response to CPB. Except for CL 2, a similar finding has been reported in jejunal biopsies of septic mice (Clark et al. 2009). Since our study had no follow-up time, the distribution of claudins after CPB remains to be elucidated. The loss of expression of the related tight junction proteins occludin
and ZO-1 has been shown to further decrease in the 2 hours after CPB (Sun et al. 2008). The colonic Ki-67 expression rate, as a measure of cell proliferation, was slightly increased after prolonged CPB. This is in line with other observed alterations of the mucosal structure and the changes in tight junction proteins in colon samples and further indicates that CPB has induced a significant epithelial destruction necessitating prompt regeneration.

The specific pathophysiologic mechanisms endangering the gastrointestinal integrity during CPB were not addressed in this study. In our protocol, splanchnic oxygen delivery further decreased after 120 minutes of CPB in both study groups. We speculate that this was not due only to hemodilution but also to increased vascular resistance in the splanchnic region in response to prolonged CPB, even in the absence of vasoconstrictive agents. Vasopressor therapy is generally required during prolonged CPB and taking into account the CPB-related mesenteric endothelial dysfunction (Doguet et al. 2004) and increased splanchnic contractile response to α1-adrenergic agonists (Doguet et al. 2004, Odwyer et al. 1997), this may contribute to an even more substantial hypoperfusion of the splanchnic region in extended duration CPB.
7 Clinical implications and future perspectives

According to the findings of this study, MCPB may be used safely with elective CABG patients, with a better preserved hematocrit, and with evidence of attenuated neutrophil activation.

In CABG patients, MCPB is associated with reduced retinal microembolism compared to conventional CCPB, suggesting a decreased embolic load to the brain after MCPB. Until more substantial evidence of the neuroprotective capacity of the decreased embolic load related to MCPB is available, its potential in decreasing cerebral and systemic microembolism ought not to be overlooked and should be further examined. Should MCPB contribute to decrease in postoperative neurologic damage, the mechanism for preventing microembolism and the significance of retinal changes should be further studied.

The present work provides important additional information on the hypothesis of impaired splanchnic integrity during CPB. These findings support the previous concerns about the intestinal mucosal injury during CPB. Based on this study, prolonged CPB is associated with intestinal mucosal damage in colon, independent of the CPB technique used.

The findings of this study encourage the use of MCPB over conventional CPB techniques with CABG patients. Adaptation of the MCPB technique in the management of valve surgery patients is more challenging compared to CABG. Further studies and technical improvements are required to ensure the uncomplicated employment of MCPB in the management of patients exposed to more complex cardiac surgery, these being the populations most expected to benefit from CPB optimization.
8 Conclusions

Based on this work, the following conclusions can be made:

1. Minimized cardiopulmonary bypass is as safe and feasible as conventional cardiopulmonary bypass with unselected coronary artery bypass surgery patients, but according to this study there was no beneficial effect on blood product transfusion requirements in this patient category (Study I).

2. Minimized cardiopulmonary bypass reduces retinal microembolization compared to conventional cardiopulmonary bypass in elective coronary artery bypass surgery patients (Study II). Conversely the difference in retinal microembolism in elective AVR surgery patients with or without combined coronary revascularization was not statistically significant (Study III).

3. Minimized cardiopulmonary bypass decreases the activation of polymophonuclear leukocytes in patients undergoing elective coronary artery bypass surgery and aortic valve surgery with or without combined coronary revascularization, compared to conventional cardiopulmonary bypass. There were no differences, however, in the other markers of systemic inflammation, coagulation, endothelial activation and injury, or in platelet activity (Studies II and III).

4. In the experimental model, markers of intestinal mucosal integrity were affected similarly with prolonged minimized and conventional cardiopulmonary bypass (Study IV).
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