Oxygen demand and neuronal biomarker release during extracorporeal circulation

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Cover illustration: AI-generated picture of a mechanical heart with inspiration from the style of Leonardo da Vinci

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“Persistence overshadows even talent as the most valuable resource in shaping the quality of life”

-Tony Robbins

Till Clara och Filip

ABSTRACT

Extracorporeal circulation (ECC) has revolutionized open-heart surgery, but its application has also extended beyond cardiac procedures into other medical domains, including locoregional cancer treatment, where ECC is combined with chemotherapy. The overall aim of this thesis was to explore patient physiological response and alternative approaches in extracorporeal circulation to increase understanding of procedure related side effects.

In *Paper I,* a randomized controlled non-inferiority trial, we investigated the possibility of replacing the current erythrocyte-based prime solution with a crystalloid-based one, while ensuring sufficient oxygen delivery for patients undergoing isolated limb perfusion (ILP). We found no significant benefits of continuing adding erythrocytes to the prime solution for ILP, a finding leading to practice and guideline changes. In *Paper II* the oxygen demand in an isolated extremity was measured and described for the first time. Based on the findings, recommendations for nadir oxygen delivery during ILP was provided. Patients treated with ILP can develop transient peripheral nerve damage, and in *Paper III,* we explored if this could be detected using novel neuronal biomarkers. We found the biomarkers measurable and significantly increased during ILP. However, in this small feasibility study, we found no correlation to clinical outcomes. In *Paper IV,* a randomized controlled trial, we investigated the release pattern for biomarkers associated with brain injury in patients undergoing open-heart surgery randomized to either a standard or a high ECC flow rate. We found several correlations between treatment characteristics and the levels of biomarkers, however, there was no reduction in biomarkers with the higher flow rate as we had hypothesized.

In conclusion, the use of ECC has emerged as a crucial component in modern healthcare. While originally applied in heart surgery, ECC has also been integrated into novel cancer treatments. This thesis underscores the importance of continued research in ECC methodology and physiological reactions to enhance the technique and minimize associated side effects.

Keywords: Extracorporeal circulation, isolated limb perfusion, oxygen delivery, neuronal biomarkers, open-heart surgery, cardiopulmonary bypass flow rate.

SAMMANFATTNING PÅ SVENSKA

Varje år utförs ca 2 miljoner hjärtoperationer i världen. För att möjliggöra detta krävs att hjärtat tillfälligt stoppas, och under denna tid övertas andning och cirkulationen av en hjärt-lungmaskin. Sedan introduktionen 1953 av hjärt-lungmaskinen har utvecklingen fört med sig flera typer av cirkulationspumpar (extrakorporeal cirkulation) för olika ändamål. Främst används dessa som stöd till allvarligt sviktande cirkulation och andning, men hjärt-lungmaskinen har även öppnat upp för nya behandlingsalternativ, däribland cancerbehandling. Isolerad hyperterm perfusion är en regional cancerbehandling som möjliggör tillförsel av en mycket hög koncentration av cellgift till bara en del av kroppen, exempelvis en arm eller ett ben, med en mycket liten risk för biverkningar i resten av kroppen.

Syftet med denna avhandling var att utforska basal fysiologi och alternativa tillvägagångssätt för att öka förståelsen och minska risken för biverkningar vid användandet av hjärt-lungmaskin under isolerad hyperterm perfusion och öppen hjärtkirurgi.

I *delarbete I,* undersöktes möjligheten att ersätta den nuvarande blodbaserade vätskelösning som används i hjärt-lungmaskinen mot en vanlig koksaltsbaserad vätskelösning vid isolerad hyperterm perfusion. Studien visade inga fördelar med att tillsätta blod till lösningen, och detta har nu ändrat internationella riktlinjer för denna typ av behandling. *Delarbete II* var en explorativ studie där vi för första gången kunde mäta syrgasbehovet i en isolerad extremitet under isolerad hyperterm perfusion. Utifrån detta kunde vi presentera en rekommenderad lägsta nivå för syrgasleverans under denna typ av behandling. I samband med att nervceller skadas eller påverkas så frisätter dessa celler särskilda ämnen som sedan kan mätas i vanliga blodprov, vilket kan ge en uppfattning av skadans omfattning. I *delarbete III* undersöktes om det var möjligt att mäta dessa markörer i en isolerade extremitet under isolerad hyperterm perfusion. Resultatet visade en signifikant stegring av dessa markörer i den behandlade extremiteten, vilket visar att även skador på perifera nerver utsöndrar dessa markörer till blodet. I denna mindre studie kunde vi dock inte påvisa att nivån av markörer i blodet korrelerade till skadans omfattning.

Hjärt- lungmaskinens pumpflöde vid hjärtoperationer anpassas till patientens kroppsyta. Studier har generellt visat fördelar med ett högre pumpflöde, företrädesvis för njurarna, men i *delarbete IV* var syftet att undersökta hur hjärnan påverkades av ett högre flöde genom att mäta och jämföra nivåerna av hjärnskademarkörer hos patienter som opererades med antingen ett standardflöde eller ett högre flöde. Resultatet visade ingen skillnad mellan grupperna i nivån av utsöndrade hjärnskademarkörer, men flera samband mellan hjärnskademarkörsnivå och olika patient- och behandlings egenskaper kunde identifieras.

Sammanfattningsvis har användandet av extrakorporeal cirkulation expanderat väsentligt bortom sitt ursprungliga syfte inom hjärtkirurgin och etablerats som en nödvändig del även inom annan avancerad sjukvård, så som exempelvis cancerbehandling. Denna avhandling understryker därav vikten av fortsatt forskning för en förfinad metodik och ökad förståelsen av de fysiologiska reaktionerna vid extrakorporeal cirkulation med syfte att minimera biverkningar.

LIST OF PAPERS

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

I. Corderfeldt A, Nielsen S, Katsarelias D, Hjärpe A, Mattsson J, Olofsson Bagge R.
Is blood a necessary component of the perfusate during isolated limb perfusion – a randomized controlled trial.
International Journal of Hyperthermia 2019; 36(1): 793-799

II. Corderfeldt Keiller A, Holmén A, Hansson C, Ricksten S-E, Bragadóttir G, Olofsson Bagge R.
Non-invasive and invasive measurement of skeletal muscular oxygenation during isolated limb perfusion.
Perfusion 2023; 38(5): 1019-1028

III. Corderfeldt Keiller A, Axelsson M, Bragadóttir G, Blennow K, Zetterberg H, Olofsson Bagge R.
A prospective feasibility trial exploring novel biomarkers for neurotoxicity after isolated limb perfusion.
Perfusion 2023 Nov 7: 2676591231213506 Online ahead of print.

IV. Corderfeldt Keiller A, Axelsson M, Bragadóttir G, Lannemyr L, Wijk J, Blennow K, Zetterberg H, Olofsson Bagge R.
The impact of cardiopulmonary bypass flow rate on brain injury biomarkers during cardiac surgery – a randomized controlled trial.
Manuscript submitted

CONTENTS

ABBREVIATIONS

ACT	Activated clotting time
ANOVA	Analysis of variance
BBB	Blood-brain barrier
BSA	Body surface area
CABG	Coronary artery bypass grafting
CaO_2	Arterial oxygen content
CNS	Central nervous system
CPB	Cardiopulmonary bypass
CR	Complete response
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
DO_2i	Indexed oxygen delivery
DO_2	Oxygen delivery
ECC	Extracorporeal circulation
EDTA	Ethylenediaminetetraacetic acid
GFAP	Glial fibrillary acidic protein
GDP	Goal-directed perfusion
GFR	Glomerular filtration rate
HLM	Heart-lung machine
ILP	Isolated limb perfusion

LVEF	Left ventricular ejection fraction
MAP	Mean arterial pressure
NfL	Neurofilament Light
NIRS	Near-infrared spectroscopy
O_2ER	Oxygen extraction ratio
ORR	Overall response rate
P-value	Probability value
PD	Progressive disease
PR	Partial response
PVC	Polyvinyl chloride
Q	Flow (L/min)
RAP	Retrograde autologous priming
r_{rm}	Repeated-measures correlation coefficient
rSO_2	Regional tissue oxygen saturation
Simoa	Single Molecule Array
SD	Stable disease
TNF-α	Tumor necrosis factor-alpha
UV	Ultraviolet
VO_2i	Indexed oxygen consumption
VO_2	Oxygen consumption

PROLOGUE

As a perfusionist, I function as a consultant in diverse aspects of blood perfusion, possessing a unique expertise in circulatory devices tailored for diverse purposes, often for the critical ill patient. We operate as a hybrid between engineering and medicine and are educated and trained in encompassing anatomy and physiology, circulation, fluid dynamics, mechanical engineering, materials, and research. Being part of and gaining insight into numerous exciting areas alongside enthusiastic and skilled professionals in my daily work is a source of great pleasure. It makes me feel both honored and humble, continually sparking my insatiable curiosity. As a perfusionist I am a part of so many different surgical procedures, life-altering interventions, and crucial decisions every day so it might not be surprising that my thesis also is diverse, and encompasses cancer treatment, extremity perfusion, peripheral nerve injury, cardiac surgery, brain injury and cognitive decline. All these diverse areas, however, share a common factor—extracorporeal circulation. In this, I have a role to perform the absolute best possible perfusion for the individual connected to the tubings at the other end, with the least possible negative impact. That is my strive with this thesis — to optimize perfusion and its outcomes with the least possible side effects for our patients. Still an area for improvement, more than 70 years since the inception of extracorporeal circulation.

1 INTRODUCTION

Safe and efficient use of extracorporeal circulation (ECC) requires a comprehensive understanding of the human body's physiological principles, pathophysiological processes, as well as a profound understanding of the pharmacological agents used and the technological intricacies of the extracorporeal circulatory circuit. The goal is to achieve optimal perfusion and gas exchange while balancing among a range of multi-faceted factors, including surgical and mechanical aspects, fluid dynamics, air-handling, body temperature and acid base levels. The outcome is further complicated by the systemic inflammatory response induced by ECC. Although techniques and knowledge continues to advance, ECC remains an emerging field that requires further understanding.

1.1 Extracorporeal circulation

Extracorporeal circulation, as the term implies, refers to the circulation of blood outside of the body. In the late 19th century, pioneering experiments were initiated in pursuit of a method to oxygenate blood outside of the human body[1]. Due to these early innovative efforts, we can now offer secure devices for extracorporeal circulation that supports failing organs for both short- and long-term use. The heart-lung machine (HLM), for example, facilitates "cardio" and "pulmonary" bypass, providing a blood- and motionless environment that is crucial for complex open-heart surgery. It temporarily shoulder the physiological functions of the heart and lungs, ensuring adequate oxygenation of the blood enabling intricate surgery by temporarily bypassing the operated organ. The term extracorporeal circulation is a broad term including all pumps that facilitate blood circulation outside the body. The pumps are titled differently based on their specific purpose.

1.2 Heart-lung machine historical overview

It is obvious that any operative procedure upon the heart could be performed better if that organ were temporarily relieved of its function of pumping blood. For example, if the flow of blood through the heart and lungs could be safely stopped for 30 minutes, it is conceivable that a new field of cardiac surgery might be developed. In order to maintain life during such a temporary cessation of blood flow through the heart and lungs, it is necessary to assume the function of these organs by some other means.

- John H. Gibbon Jr, 1939[2]

In the mid-20th century, open-heart surgery had not yet become a reality. Safe surgical methods for operating on a motionless empty heart for an extended period, while the rest of the patient's body was perfused with oxygenated blood, was still not developed. In 1953, after pioneering work dedicated in the development of a machine capable of fully supporting the circulation, Dr. John Gibbon Jr. made history[3]. An 18-year-old female student from Philadelphia, USA, suffering from a large atrial septal defect was on cardiopulmonary bypass for 45-minutes, facilitated by his invention of the HLM, while a primary closure of the defect was successfully performed by Dr. Gibbon[4]. In July 1954, the world's second successful open-heart surgery with the HLM was performed in Sweden, at Karolinska University hospital in Stockholm. Dr. Gibbons' close friend and surgeon colleague Dr. Clarence Crafoord removed a myxoma from the left atrium of a 42-year-old female who was on cardiopulmonary bypass for 33 minutes. She recovered fully and lived to reach her 91st birthday[5]. Since then, the field of extracorporeal circulation has made steady and rapid progress, particularly with extensive multidisciplinary efforts in patient safety and the development of bypass devices for both short- and long-term support[6]. Although cardiopulmonary bypass (CPB) technology continues to advance, and efforts are being made to minimize its complications, it remains an emerging field that requires further refinement.

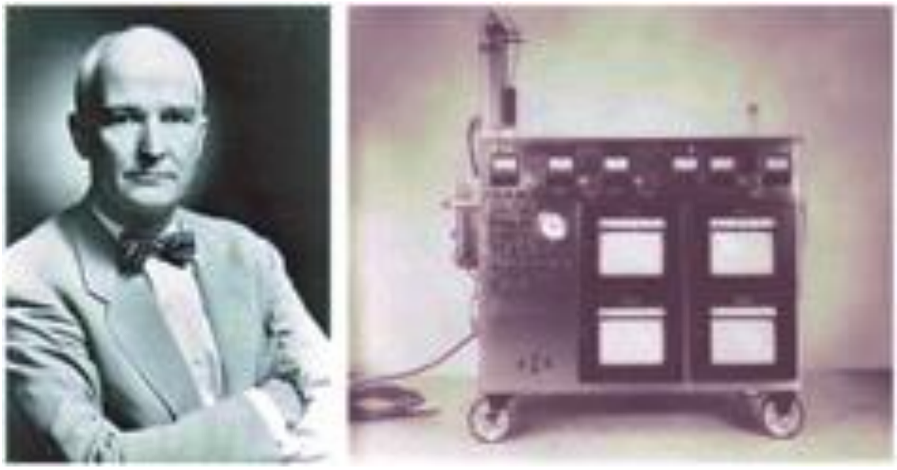

Figure 1. John H. Gibbon, Jr 1955 (left), and the heart-lung machine model II built by IBM, which was used by Gibbon during his first successful heart surgery on May 6, 1953 (right). Reproduced with courtesy of Thomas Jefferson University, Archives & Special Collections, Philadelphia.

Figure 2. Modern setup of heart-lung machine and monitoring during heart surgery 2022. Photo: Anna Corderfeldt Keiller.

1.3 Cardiopulmonary bypass

During typical cardiopulmonary bypass (CPB) procedures at Sahlgrenska University hospital, the surgeons open the chest and expose the ascending aorta, where the arterial cannula is inserted and secured. The primed and de-aired arterial tubing from the HLM is connected to the arterial cannula. A straight dual-stage venous cannula is placed in the right atrium, draining venous blood from both vena cava superior and inferior (although various cannulations techniques are used depending on the surgical procedure). The primed venous tubing from the HLM is connected to the venous cannula. The cardiopulmonary bypass circuit is thereby established. When going on CPB the venous clamp is gradually opened by the perfusionist, slowly allowing the venous blood to passively drain from the patient down to a reservoir. While simultaneously starting the arterial roller pump and gradually extracting the same amount out of the reservoir, pushing it through the oxygenator and back into the patient via the arterial tubing. The pump flow is gradually increased until reached "full flow", which is individually calculated for every patient. The process of initiating CPB requires rigorous attention, along with careful consideration of the patient's hemodynamic condition[7]. The blood flow from the HLM is generally delivered in a continuous flow, although the HLM has the capability to also provide a pulsatile flow. Ongoing research has explored the potential benefits of continuous versus pulsatile flow for the patient. Presently, the global consensus leans towards favoring continuous flow, and recent research indicates no inferiority for pulsatile flow during ECC[8, 9]. In most cardiac surgeries, the heart must be temporarily stopped and kept still during the procedure to provide a bloodless and motionless surgical field. To accomplish this without causing ischemia to the heart, the ascending aorta is temporarily clamped transversely, so called cross-clamp (below the arterial cannula), and cold cardioplegia is administered from the HLM through a thin cannula anchored in the ascending aorta (below the cross-clamp) into the coronary arteries. Because of the hyperkalemic content of the cardioplegia solution, the heart intentionally and temporarily undergoes diastolic arrest for myocardial protection during the cross-clamp period[7, 10].

After the completion of the surgery, the aortic clamp is removed, enabling the return of blood circulation into the coronary vessels, flushing away the remnants of the cardioplegia. The myocardium is recirculated, and the rhythmic beating of the heart resumes spontaneously. As the heart strengthens, the circulation is gradually transitioned back to the patient, resulting in the gradual weaning of the patient from the HLM. The surgeons conclude the surgery by removing the cannulas and closing the chest.

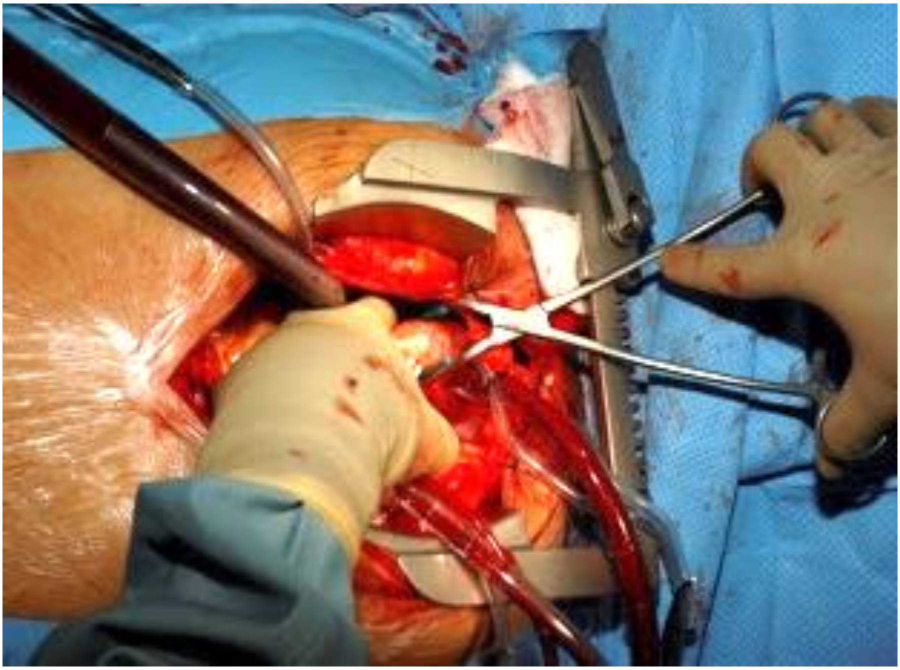

Figure 3. Cannulas for cardiopulmonary bypass established, CPB in full flow rate and the ascending aorta is about to be cross clamped. Photo courtesy of Dr. Martin Silverborn, Sahlgrenska University hospital, Gothenburg.

1.4 Hardware in extracorporeal circulation

Advancements in heart-lung machine hardware and associated components have significantly lowered the occurrence of complications during CPB. Clinical perfusionists can now actively monitor the body's metabolic demands, allowing for proactive interventions to ensure sufficient organ perfusion, oxygen supply, and anticoagulation. There are substantial evidence supporting improved outcomes due to these advancements[9].

1.4.1 Cannulas

Cannulas establish the connection between the patient and the HLM. Cannula size is measured in French (Fr), with the size gauged by its outer diameter. Cannulas are mostly constructed from polyvinyl chloride (PVC) and are sometimes, depending on purpose, wire-reinforced to prevent kinking. The size of the arterial cannula for adult use usually range between 17-24 Fr, and when placed in the aorta ascendence, it could either be a straight malleable cannula or a stiff cannula with a curved tip. The selected size for the arterial cannula depends on the required pump flow. The higher the flow, the larger the cannula. The pressure drop across the cannula should be minimized to reduce the risk of harm to the aortic wall caused by a forceful and concentrated jet stream of blood flowing through a restricted opening[11]. The venous cannulas are larger in diameter, typically 24-40 Fr and placed depending on the surgical procedure. The two main options for venous cannulation are bicaval cannulation and dual-stage cannulation. Bicaval cannulation is often used in mitral valve repairs, and involves the use of two malleable cannulas, one cannula inserted into the superior vena cava and one cannula inserted in the inferior vena cava, both cannulas joined together with a Y-connector to the venous tubing. The second option, often used in coronary artery bypass grafting (CABG) or aortic valve replacement, employs a single dual-stage cannula, inserted into the right atrium, facilitating the drainage of blood from both the superior and the inferior vena cava. Venous blood is drained through gravity to the venous reservoir. The option to apply vacuum allows for either enhanced drainage or the flexibility to downsize the venous cannula[7, 9].

1.4.2 Tubing

Most of the tubing in the CPB circuit is made of phthalate-free PVC, selected for its durability and resistance to tearing. For certain components of the circuit, as in the roller pump house, silicone is used due to its compressibility, enabling the roller pump to effectively move the blood forward. The diameter of the tubing is measured in inches. For adults undergoing CPB, the venous tubing has a diameter of 3/8 or 1/2 inch, and the arterial line is typically 3/8 inch[12, 13]. The interaction of blood with the CPB circuit triggers a systemic inflammatory and coagulation response, and this reaction may potentially be reduced by applying biocompatible coating to the tubing, such as ionic or covalent heparin. For this reason, the tubing can be either coated or non-coated[9].

1.4.3 Reservoir

The reservoir serves as a large mixing chamber for drained venous blood, mediastinal blood from the cardiotomy suckers and blood from the venting lines. Before it enters the reservoir, the blood undergoes defoaming filtration, to eliminate foam and bubble formation. Filters also segregate blood from debris, including fat, bone particles and clotting components. Drugs and fluids necessary for the perfusion are added to the reservoir[9].

1.4.4 Pump

During CPB, there is the option of either a centrifugal or roller pump. At Sahlgrenska University hospital the roller pump is used during CPB. The roller pump has a rotating arm with two rollers that compress a section of the silicone tubing to generate a forward flow of the blood. The roller pump is not afterload sensitive and do not decrease in pump flow if there is an increase in resistance. A centrifugal pump consists of an impeller, a rotating disc with blades. As the impeller spins rapidly within the pump housing, it generates a centrifugal force moving the blood outward creating positive pressure, propelling the blood through the pump and forward. The centrifugal pump is afterload sensitive and decreases in forward flow if the resistance increase[7, 9]

1.4.5 Oxygenator

In the CPB system, a microporous polypropylene hollow fiber membrane oxygenator, facilitates gas exchange. The blood flows external of the fiber, while gas traverse within the fiber. Because of effective diffusion resulting from the microporous characteristics of the membrane, oxygen is introduced to the blood and carbon dioxide is removed without the mixture of blood and gas[11, 14]. Integrated in the oxygenator is an arterial filter, which is recommended as a standard practice, and leads to a decreased embolic burden to the patient[15, 16].

1.4.6 Heater-cooler unit

The heater-cooler unit (HCU) is an essential part of the CPB. Tubing from the HCU is connected to the oxygenator. In normothermic CPB the HCU maintains the patient’s body temperature and facilitates effective cooling and warming during deep hypothermic circulatory arrest. Further, the HCU is also used to cool the cardioplegia. This is accomplished by circulating water at a specific temperature around the fibers of the oxygenator and the coils of the cardioplegia system[9].

1.5 Conduct of extracorporeal circulation

1.5.1 Prime

The CPB circuit is primed before being connected to the patient. This must be done meticulously to eliminate all air before proceeding with CPB. Administration of priming solutions results in hemodilution, potentially influencing the coagulation and causing fluid extravasation into the tissues[17]. Consensus on the ideal priming solution is lacking. However, recent survey findings indicate that crystalloids are the favored choice for priming solutions in European centers[18], including Sahlgrenska University hospital, where the priming volume for heart surgery contains 1300 mL of Ringer-Acetate, and 700 mL (500 mL Ringer-Acetate, 100 mL Tribonate and 100 mL Albumin) for isolated limb perfusion (ILP). In a small sized patient, the hemodilution of the priming solution will substantially lower the hematocrit. In those cases, retrograde autologous priming (RAP) of the CPB circuit has been shown to decrease the need for allogeneic blood transfusions[19]. RAP diminishes hemodilution by allowing passive exsanguination of arterial and venous lines before initiating CPB, allowing the patient's blood to displace the priming solution from the circuit. This may reduce the prime volume with approximately 500 mL, but its effectiveness is dependent on the patient's hemodynamic management.

1.5.2 Anticoagulation

Heparin is utilized as the anticoagulant to prevent clotting of the blood within the ECC circuit. The administration of heparin is individualized based on the procedure, the patient's medical story and weight, typically 300-400 units per kilogram. Activated clotting time (ACT) is closely monitored throughout the procedure, with a target range usually of > 480 seconds before initiating CPB and maintaining this during CPB, with the goal to achieve sufficient anticoagulation to prevent clot formation while balancing the risk of bleeding. At the conclusion of CPB, the anticoagulant effect is reversed using protamine sulfate to restore normal clotting function and minimize the risk for bleeding postoperative[9].

1.5.3 Pump flow management

The desired pump flow during CPB has traditionally been calculated based on body surface area (BSA) and modified based on the temperature selected for the procedure. In a normothermic context, the pump flow is typically set within the range of 2.2 – 2.8L/min/m^2. However, depending solely on the BSA method have limitations as it may not adequately ensure the delivery of sufficient oxygen, for instance in individuals with obesity or low hematocrit[20]. Recent research advocate increasingly for the goal to maintain an indexed oxygen delivery (DO_2i) > 330 ml/min/m^2 at 36 °C during CPB to more effectively meet the metabolic requirements[21-23], and a meta-analysis showed a reduction in the length of hospital stay when using goal-directed perfusion (GDP)[24].

1.5.4 Goal-directed perfusion

The strategi of GDP has gained popularity worldwide and is recommended in both US[23] and European[9] guidelines. In modern HLMs, values for DO_2i, indexed oxygen consumption (VO_2i) and oxygen extraction ratio (O_2ER) are continuously displayed. This enhances the perfusionist's capability to optimize the pump flow in accordance with the patient's metabolic demands.

1.5.5 Body surface area

The most frequently used formula for calculating body surface area is the Du Bois formula, which was introduced in 1916[25], and is the formula used in this thesis. The formula is as follows:

$$BSA\ (m^2) = height^{0.725}(cm) \times weight^{0.425}(kg) \times 71.84/10\,000$$

This formula is integrated into the data program for the ECC and is automatically calculated when height and weight are entered. For a quick approximation of BSA in acute situations, the simple formula; height + weight – 60 / 100 is often used.

For calculation of the extremity surface area, the patient's limb circumference was measured at every 5 cm, and the mean circumference was then multiplied with the length of the leg/arm. For a quick approximation of extremity surface area, a leg estimates 18% and an arm 9% of total BSA according to Wallace Rule of Nine[26]

1.5.6 Mean arterial pressure

The ideal mean arterial pressure (MAP) for ensuring sufficient tissue perfusion during CPB remains undetermined[27]. Specifically, uncertainty exist regarding the lower limit of a safe perfusion pressure, with some researchers recommending lower levels (40-50 mmHg) and others advocating for higher levels (70-80 mmHg)[28, 29]. Clinicians commonly aim to sustain a MAP between 50-70 mmHg for the majority of adult patients, based on data supporting it as the lower limit of cerebral autoregulation[30].

1.5.7 Oxygen demand

The oxygen demand is determined by the oxygen consumption. The consumption of oxygen (VO_2) is regulated by tissue metabolism, maintaining a standard DO_2:VO_2 ratio of 5:1. The resulting saturation of mixed venous oxygen (SvO_2) is dependent on this ratio. When there is a moderate decrease in systemic DO_2 without a corresponding change in VO_2, each deciliter of arterial blood draws more oxygen, resulting in a decrease in SvO_2. A significant reduction in DO_2 leads to insufficient oxygen supply for metabolic needs, initiating anaerobic metabolism and causing lactic acidosis. This situation typically occurs when the DO_2:VO_2 ratio drops below 2:1. Consequently, effective management aims to sustain DO_2 at least double the

VO_2, preferably five times. Mixed venous oxygen saturation (SvO_2) plays a crucial role in monitoring and controlling CPB pump flow, as it accurately reflects the ratio of oxygen consumption (VO_2) to oxygen delivery (DO_2), also known as oxygen extraction ratio (O_2ER)[31].

1.5.7.1 Oxygen delivery

DO_2 represents the quantity of oxygen supplied to the tissues per minute, calculated as the product of arterial oxygen content (CaO_2) multiplied with cardiac output. Oxygen is found in the blood in two forms: dissolved in the plasma (about 2%) and bound to hemoglobin (Hb) within red blood cells (about 98%). The mathematical formulas used to determine DO_2 in a patient undergoing CPB are as follow[32]:

$$\mathbf{DO_2 = (CaO2) \times pump\ flow\ (Q)\ L/min}$$

$$\mathbf{CaO_2 = 1.36 \times Hb\ (g/L) \times SaO2\ (\%) \times 0.01 + PaO2 \times 0.23}$$

Where: 1.36 mL is the volume of oxygen carried by 1 g of 100% saturated hemoglobin and PaO_2 x 0.23 is the oxygen dissolved in 1L of plasma[32]. The amount of DO_2 calculated from the formula is then divided with body surface area for DO_2i.

1.5.7.2 Oxygen consumption

The total oxygen consumption (VO_2) by different organs is the combined amount of oxygen they utilize. This can be directly measured or calculated based on pump flow (Q) L/min and arterial and venous content in the blood. The mathematical formulas used to determine VO_2 in a patient undergoing CPB are as follow[32]:

$$\mathbf{VO_2 = Q \times (CaO_2 - CvO_2)}$$

$$\mathbf{CvO_2 = 1.36 \times Hb\ (g/L) \times SvO2\ (\%) \times 0.01 + PvO2 \times 0.23}$$

The amount of VO2 calculated from the formula is then divided with body surface area for VO2i.

1.5.7.3 Oxygen extraction ratio

The O_2ER is defined by the amount of oxygen consumed (VO_2) by tissues, as blood circulate through the body, expressed as a fraction of (DO_2). It indicates the difference in oxygen content between arterial blood entering the tissues and venous blood leaving the tissues. The mathematical formula used to determine O_2ER in a patient undergoing CPB are as follow[32]:

$$\mathbf{O_2ER = VO_2/DO_2}$$

1.6 Monitoring

The cornerstone of a perfusionist's patient care lies in monitoring physiological patient parameters and CPB performance parameters. While it is straightforward and common practice to monitor fundamental factors such as arterial blood pressure, pump flow, and blood gas analysis, there are technologies that offer a more comprehensive understanding of our patient care practices.

1.6.1 Acid-base management

Metabolic acidosis, resulting from tissue hypoxia or an electrolyte imbalance, may increase the risk of tissue injury and organ dysfunction during and after CPB. Blood gas analyses are conducted on regular intervals with alpha-stat strategy (samples analyzed at 37 °C)[33], during routine and normothermic CPB, but are continuously monitored on complicated cases, and with pH-stat strategy (analyzes the samples on actual temperature)[33] on hypothermic cases.

1.6.2 Lactate

In aerobic conditions, energy is produced by breaking down glucose into pyruvate though glycolysis. Pyruvate enters the cell mitochondria where it is converted into more energy by cellular respiration. In anaerobic conditions, pyruvate gets converted to lactate instead of going into cellular respiration. Lactate production increases when the demand for energy and oxygen exceeds the supply. Lactate is commonly perceived as a waste product, yet it serves as a valuable energy source. Lactate is transported through the bloodstream to organs with higher oxygen levels and undergoes recycling via the Cori cycle. Through this process, lactate is converted back into pyruvate, and through gluconeogenesis, glucose is synthesized. This glucose acts as a vital fuel source for tissues during periods of heightened energy demand[34]. When hypoxia persists over a prolonged period, lactate levels in the body continue to rise. With ongoing hypoxia, the demand for energy is still high, leading to increased glycolysis and lactate production[34]. Lactate levels are closely monitored to evaluate the adequacy of oxygen delivery during procedures involving ECC.

1.6.3 Near-infrared spectroscopy

In 1977, Jöbsis initially introduced the potential use of infrared spectroscopy as a real-time, non-invasive, continuous measurement tool for tissue oxygen saturation (rSO_2)[35]. Near-infrared spectroscopy (NIRS) assesses mean tissue oxygen saturation, representing hemoglobin saturation in a combination of venous, capillary, and arterial blood. The average tissue hemoglobin is biologically distributed with an arterial/venous ratio of approximately 25:75, a pattern consistent across normoxia, hypoxia, and hypocapnia[36, 37]. Consequently, the NIRS signal predominantly reflects tissue venous oxygen saturation. Research has shown that NIRS, in addition to cerebral oxygen monitoring, can serve as a non-invasive method for measuring muscular oxygenation, allowing the detection of skeletal muscle ischemia in extremities and other peripheral tissues[38-41]. At Sahlgrenska University hospital, NIRS is routinely used in complex procedures during CPB and serves as a non-invasive complement for measuring extremity oxygenation during isolated limb perfusion.

1.7 Isolated limb perfusion

Isolated limb perfusion (ILP) is a regional cancer treatment option primarily for patients with melanoma and sarcoma localized to an extremity (leg or arm). The ILP technique was first introduced by Creech and Krementz in 1958[42]. It is performed by surgical isolation of the extremity where the blood circulation of the extremity is connected to an ECC. A high dose of chemotherapy is then administered to the isolated extremity while minimizing the systemic exposure and associated toxicity. ILP has proven to be effective and safe with a an overall response rate (ORR) of 80 %[43, 44] for patients with melanoma, with low rates of regional and systemic toxicity[45].

ILP is a recommended treatment for patients with melanoma in-transit metastasis, which has spread from its primary tumor throughout the intradermal and subcutaneous lymphatic channels, linking the primary site to the nearby lymph nodes. When dealing with a limited number of removable lesions, it is recommended to perform a straightforward surgical excision[46]. But for patients with unresectable in-transit metastases, like multiple, bulky, or rapidly recurring metastases, ILP is a recommended treatment. The primary goal of the treatment is to establish regional control and minimize the risk for cancer recurrence[46].

Sahlgrenska University hospital is the only center offering this treatment in Sweden and is also an international referral center, and approximately 20-25 ILP's are performed annually.

Figure 4. Melanoma in-transit metastases on upper thigh of the left leg. Photo courtesy of Roger Olofsson Bagge. Patient consent given for publication.

1.7.1 Malignant melanoma

Melanoma is a malignancy originating from melanocytes, the pigment-producing cells situated within the basal layer of the epidermis. Melanin produced by these cells absorbs harmful ultraviolet (UV) radiation. Exposure to UV radiation can trigger deoxyribonucleic acid (DNA) damage within skin cells, usually addressed by cellular repair mechanisms. However, deficiencies in these repair processes, whether originating from genetic factors or environmental influences, can ultimately result in the development of invasive melanoma[47, 48]. Worldwide cancer statistics indicated approximately 325 000 new patients with melanoma and 57 000 deaths due to melanoma in the year 2020[49]. In Sweden, approximately 4 500 individuals are diagnosed with melanoma each year, leading to approximately 500 deaths annually[50].

Figure 5. ILP about to start on bulky tumor of malignant melanoma with in-transit metastasis in right arm. Photo: Anna Corderfeldt Keiller, Sahlgrenska University hospital, Gothenburg. Patient consent given for publication.

1.7.2 Technique of ILP

In the context of performing ILP on a leg, the procedure is most often done by a femoral approach, involving the dissection and cannulation of the corresponding artery and vein. For arms, the brachial artery and vein is most commonly used. To achieve complete limb isolation, the remaining collateral vessels are compressed using an Esmarch rubber band. The cannulas are then connected to tubings from the ECC, and the perfusion is started. The total perfusion time is approximately 90 minutes, consisting of a 30-minute warming phase (raising ingoing blood to 40 ˚C), a 20-minute chemotherapy administration, and an additional 40 minutes for the circulation of the chemotherapy, resulting in a total of 60 minutes for the circulation of the chemotherapy. Throughout this process, the tissue temperature is maintained at 40˚C and measured through thermistors at two different locations in the limb. At the end of the perfusion, the limb is irrigated with 3000 mL of Ringer's acetate solution to remove any chemotherapy before the circulation is restored[51].

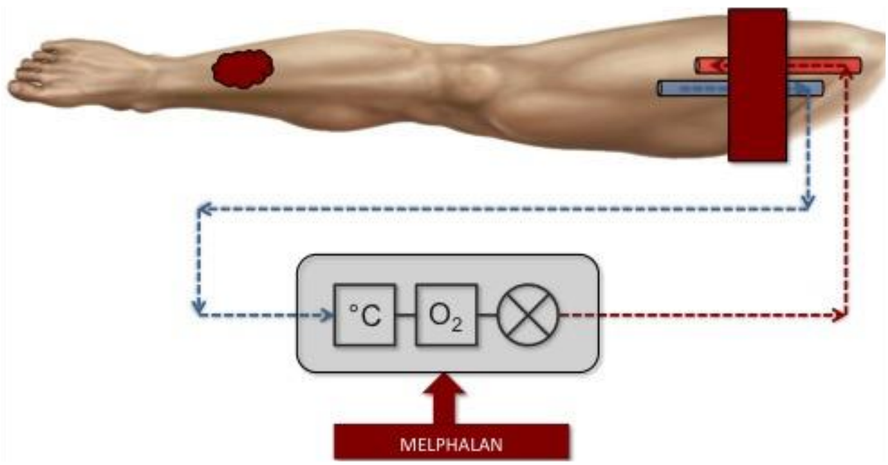

Figure 6. Schematic representation of vascular access for ILP. Photo courtesy of Dr. Roger Olofsson Bagge, Sahlgrenska University hospital, Gothenburg

Figure 7. Femoral arterial- and venous cannulation approach combined with mechanical isolation of collaterals, left leg ILP. Photo: Photo courtesy of Dr. Roger Olofsson Bagge, Sahlgrenska University hospital, Gothenburg. Patient consent given for publication.

1.7.3 Chemotherapy used in ILP

Chemotherapeutic agents are grouped based on their mechanisms of action and the specific cellular processes they target. For ILP, the alkylating agent melphalan is most commonly used, and in some patients, it is also combined with the cytokine tumor necrosis factor-alpha (TNF-α)[51].

1.7.3.1 Melphalan

Melphalan is an antineoplastic agent that functions as a bifunctional alkylating agent that attaches to the DNA molecules of cells, causing damage by preventing the separation of DNA strands and inhibiting cell division. This interference with DNA structure prevents replication, ultimately leading to the destruction of rapidly dividing cells[52]. The most commonly used dose of melphalan for ILP is 13mg/L limb volume in upper limb, and 10mg/L limb volume in lower limb[53]. Systemic side effects in the event of leakage include bone marrow suppression, which may result in fever, nausea, vomiting and diarrhea, as well as anemia, leukopenia, and thrombocytopenia[52, 54].

1.7.3.2 Tumor necrosis factor alpha

TNF-α is a versatile cytokine with important roles in cell survival, apoptosis, inflammation, and the immune system. It serves as a key mediator, for instance, in septic shock[55]. TNF-α is also able to trigger leakage of erythrocytes and lymphocytes, resulting in hemorrhagic necrosis[56]. It increases permeability and damages the vascular lining on tumor-associated blood vessels. Consequently, this leads to an indirect effect with an increase in concentration of melphalan within the tumor, but also to a direct effect with destruction of tumor blood vessels[55]. This direct and indirect antitumor effect of systemically administered TNF-α in patients with cancer have proven ineffective due to dose-limiting toxicity at lower concentrations than required for antitumor effects. However, the concentrations needed can be administrated during ILP[57]. It has been shown that TNF-α works synergistically with chemotherapeutic drugs significantly enhancing response rates when combined with melphalan, especially for patients with sarcoma and bulky tumors[57, 58]. At Sahlgrenska University hospital, we currently use TNF-α (1mg) in combination with melphalan for ILP procedures treating sarcomas, repeated procedures, and ILPs targeting bulky tumors[57].

1.7.4 Leakage monitoring

A significant concern during ILP is the risk of inadequate limb isolation, resulting in the leakage of cytostatic agents into the systemic circulation. Achieving total isolation of the limb is not always feasible due to anatomical variations, arteriosclerosis, and technical obsticals[59]. As an example, leakage through veins in the bone marrow during ILP has been showed to occur[60].

Consequently, systemic leakage may sometimes be inevitable, therefore, monitoring is crucial and is often performed using a technique with radioactively labelled albumin. The highest permissible threshold for leakage is considered to be 10%, as exceeding this limit may result in systemic toxicity due to the elevated levels of cytostatic agents[59]. A minor calibration dose (10 MBq) of technetium-99m labeled human serum albumin is systemically administered to the patient, and a precordial scintillation probe is positioned over the heart and calibrated. Following rigorously isolation of the extremity and the initiation of ECC, with stable circulation and no apparent leakage, a tenfold dose (100 MBq) of technetium-99m labeled human serum albumin is administrated into the extremity/ECC circuit[61]. Detection of undesired leakage from the ILP circuit into the systemic circulation is achieved by observing an elevation in the radioactive counts. The extent of this leakage is measured and presented as a percentage, along with a display of the remaining time until the leakage reaches 10%. Leakage can also be monitored, though not as precisely, by observing the volume level in the venous reservoir, and this we mainly use to monitor leakage of blood from the systemic circulation to the perfusion circuit.

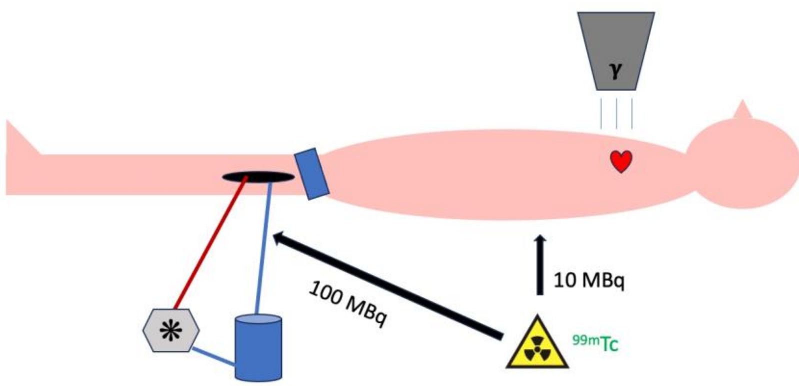

Figure 8. Leakage monitoring during ILP. Precordial scintillation probe placed over the heart, calibrated with small dose (10MBq) technetium-99m labeled human serum albumin in the systemic circulation, detecting potential leakage from the extremity (which is injected with the big dose of 100MBq) to the systemic circulation. Photo: Produced by Anna Corderfeldt Keiller.

1.7.5 Toxicity

Regional toxicity is a frequently encountered side effect of ILP, appearing in varying degrees of severity in the treated limb. However, severe regional toxicities are relatively infrequent[62]. The most dominating side effects include erythema, edema, blister formation, nail shedding, and a tingling sensation in the skin. These symptoms typically arise between two and five days following the perfusion, reaching their peak around 20 days later, and then gradually diminish after approximately two to three months[63, 64]. While severe reactions are rare, potential reactions include extensive epidermolysis, compartment syndrome, reactions necessitating amputation, and in some instances severe and irreversible paresis has also been documented[65]. Predictive factors for regional toxicity include high doses of melphalan, longer perfusion time (120 vs. 90 minutes), advanced age, higher perfusion temperature (> 40°C), and being female[62, 66].

1.7.6 Wieberdink grading

The Wieberdink grading system is commonly used to assess regional toxicity after ILP. Originating from research in the early 1980s, this grading system aimed to determine the optimal dose of cytostatic agents during ILP by evaluating the balance between treatment response and associated side effects. The optimal dosage of a cytostatic drug was defined as the highest amount tolerated with an acceptable level of risk[67]. Research on regional toxicity following ILP reveals that about 3% of treated patients have a severe reaction with lasting effects (grade IV-V), whereas nearly 80% of patients are evaluated to have mild to moderate reactions (grade II-III)[62].

Grade I	No subjective or objective evidence of reaction
Grade II	Slight erythema and/or edema
Grade III	Considerable erythema and/or edema with some blistering; Slightly disturbed motility
Grade IV	Extensive epidermolysis and/or obvious damage to the deep tissue causing definite functional disturbances; threatening or manifest compartment syndrome
Grade V	Severe reaction which may necessitate amputation

Table 1. Acute regional toxicity grading system, according to Wieberdink et al.[67]

1.7.7 Cancer treatment response

Clinical response after ILP is evaluated at 3 months follow-up using the WHO criteria for reporting results of cancer treatment[68]. The definition of complete response (CR) is when no lesions are clinically detectable. Partial response (PR) is characterized by a clinical reduction exceeding 50% in the total tumor burden measured by the number of lesions or their diameter. A progressive disease (PD) is defined by either an increase in lesions of more than 25% or the appearance of new lesions. Stable disease (SD) is characterized by the absence of meeting any of the criteria above. Overall response (ORR) is CR and PR together[68].

Figure 9. A patient with a bulky melanoma tumor on the left leg. Tumor before ILP (left) and 6 weeks after ILP treatment (right). Photo courtesy of Dr. Jan Mattsson, Sahlgrenska University hospital, Gothenburg. Patient consent given for publication.

1.8 Heart surgery, CPB and the brain

Postoperative neurological side effects became a concern soon after the heart-lung machine's introduction in the 1950s. Despite successful surgeries, some patients experienced impaired cognitive function. These side effects ranged from severe outcomes like stroke or coma to milder forms such as delirium and neurocognitive decline[69]

Procedures that involve opening the heart, like aortic or mitral valve replacements, are considered to carry an increased risk of neurological impact compared to coronary artery bypass grafting (CABG). The elevated risk is linked to a higher probability of embolization arising from vegetations, thrombi, and the formation of gaseous bubbles during surgery[69]. Approximately 60% of patients undergoing cardiac surgery experience early cognitive dysfunction or delirium within the first week, often linked to perioperative medications like narcotics, benzodiazepines, or anesthetics. Despite this, after 12 months, 20-40% may still show persistent cognitive decline[70].

Approximately 3% of patients experience serious perioperative neurological injuries, such as stroke, transient ischemic attack, or coma after heart surgery. Risk factors include age, the presence of vascular disease, and diabetes. Neurological events after surgery are also significantly more common in women than in men, as well as an increased 30-day mortality compared to men[71]. Despite patients being older and more medically compromised at the time of surgery, the incidence of surgical-related deaths during heart surgery is decreasing[70, 72].

1.8.1 Etiology of postoperative cognitive decline

The cognitive decline observed after heart surgery with CPB is believed to be a multifactorial phenomenon with various contributing factors, some of which still present an unresolved challenge. Hypoperfusion, hypooxygenation, and cerebral embolism involving clots, air, or fragments leading to small infarcts are all potential causes during CPB, adversely affecting the brain[73]. Additionally, CPB triggers a substantial systemic inflammatory response due to blood interaction with foreign surfaces within the circuit. The surgical trauma itself causes tissue damage, leading to the release of inflammatory mediators which further exacerbates the inflammatory response. Upon this, the organ ischemia/reperfusion cause additional tissue damage and trigger inflammation. Collectively, these processes promote the activation of the complement cascade and pro-inflammatory mediators[74-76]. Together, this inflammatory response can lead to the disruption of tight junctions in the blood-brain barrier (BBB), increasing permeability and following neuroinflammation[77]. In a considerable number of patients, these conditions progress to a systemic inflammatory response syndrome marked by fever, organ dysfunction, and cardiovascular instability[74] as well as cytokine-induced sickness behavior such as difficulty concentrating, depression and lethargy[78]. This response is the body's attempt to maintain a stable internal environment and facilitate healing despite the trauma induced by thoracic surgery and CPB. However, in severe immunological reactions, this may lead to cognitive impairment[79]. The complex interaction of these mechanisms highlights the intricate nature of cognitive outcomes in cardiac surgery with CPB.

1.9 Biomarkers

A biomarker is defined as a “characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention”[80]. The optimal biomarker is specific to the disease, not present in healthy individuals or other diseases. It captures a crucial aspect of the disease, reflects its severity, predicts prognosis, and indicates treatment effectiveness through changes in concentration[81]. Ideally, the biomarker should demonstrate feasibility in terms of both collection and analysis, while remaining cost-effective[75].

1.9.1 Neuronal biomarkers

Biomarkers indicating central nervous system (CNS) injury emerge following damage to neurons and glial cells. Neuronal biomarkers can be measured in various matrices, including cerebrospinal fluid (CSF) and blood. The biomarkers can enter the bloodstream through two mechanisms: a local increase in concentration within the CNS, facilitating molecular diffusion across the BBB into systemic circulation, or through BBB disruption, allowing unregulated passage of these substances into the bloodstream[82]. In the domain of cardiac surgery and postoperative decline, several biomarkers have garnered attention. These include the S100B protein, neuron-specific enolase, glial fibrillary acidic protein (GFAP), tau protein, metalloproteases, ubiquitin C-terminal hydroxylase-L1, and neurofilaments, foremost neurofilament light (NfL)[75]. The increase in neurological biomarkers in plasma after surgery suggests that a neuronal injury has taken place[83].

1.9.1.1 Neurofilament light

Neurofilament light chain (NfL) is a biomarker of injury to large caliber myelinated axons[84] and is a neuronal cytoplasmic protein providing structural stability to neurons. During normal conditions, it's released in small amounts, increasing as age advances. Increased levels of NfL in both CSF and blood are associated with the degree of axonal damage observed in a range of neurological conditions, including inflammatory, neurodegenerative, traumatic, and cerebrovascular diseases[85]. Axons in the peripheral nervous system also release NfL upon damage, observed in conditions like vasculitis and neuropathies such as Guillain-Barré[86, 87]. As the most abundant and soluble subunit of neurofilaments, NfL is reliably measurable in CSF and blood. NfL levels peak 7-10 days after neuroaxonal injury and gradually return to baseline between 200-400 days[88].

1.9.1.2 Total tau

Total tau is a biomarker of injury to thin nonmyelinated axons[84]. It serves as a crucial microtubule-associated protein, playing an important role in stabilizing the axonal cytoskeleton and aiding in the transport of vesicles within neuronal synapses[89] as well as in maintaining axonal integrity[83]. Notably, increased t-tau levels have been observed in patients with cognitive impairment following cardiopulmonary revascularization, suggesting a potential correlation between increase in biomarkers associated with cognitive decline after heart surgery and those seen in Alzheimer's disease[90]. Elevated t-tau levels in plasma have also been identified in patients with peripheral nerve diseases such as inflammatory polyneuropathies[87]. T-tau exhibits a rapid increase within an hour after neuroaxonal injury and has a blood half-life of approximately 10 hours[91]. Phosphorylated tau (p-tau217) represents a specific form of tau protein consisting of 217 amino acids. It demonstrates promising levels of diagnostic accuracy and sensitivity, positioning it as one of the most promising emerging biomarkers for Alzheimer's disease[92].

1.9.1.3 Glial fibrillary acidic protein

GFAP is the primary intermediate filament protein in mature astrocytes, essential for maintaining the structural integrity and resilience of the astrocyte cytoskeleton[93]. Injury to astroglial cells may lead to release of GFAP extracellular due to astrogliosis by proliferation and/or hypertrophy[84]. GFAP participates in crucial CNS processes, including cell motility and migration[94, 95], and is a biomarker highly expressed in neuroinflammation[96]. Unlike NfL and tau, which serve as biomarkers for axonal injury in both central and peripheral nerves, GFAP is an astrocytic protein with specific expression in the CNS. It acts as a traditional neuronal marker that exhibits stronger expression in the elderly brain and becomes activated in response to brain trauma or CNS degeneration[95]. After injury, GFAP reaches its peak at 20 hours and gradually decreases over the subsequent 72 hours[97].

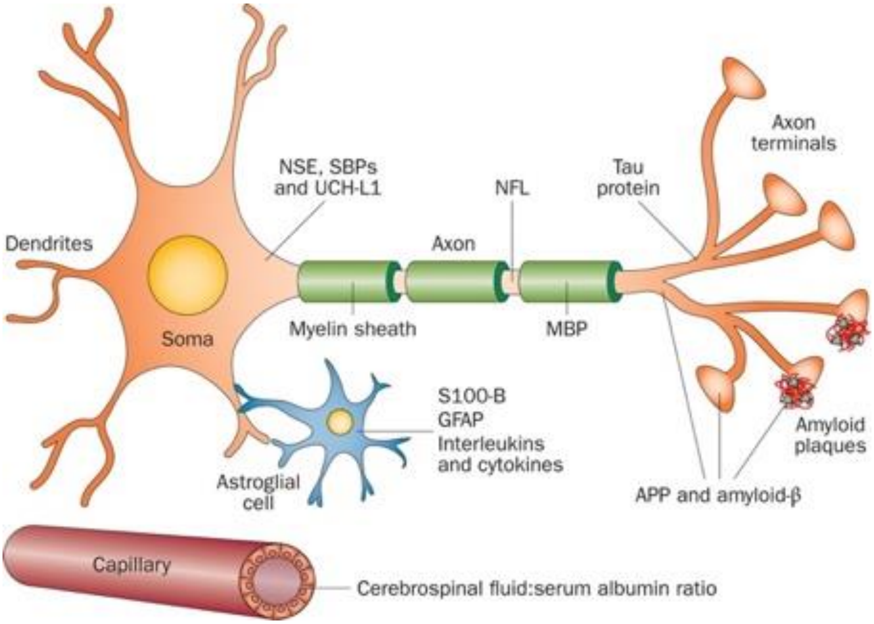

Figure 10. Illustrating a neuron showing expression sites of neuronal biomarkers following injury. Photo with permission from Springer Nature[84].

1.9.2 Single molecule array

Single Molecule Array (Simoa) is an advanced ultrasensitive technology for biomarker analysis. It has a high analytical sensitivity, allowing precise quantification of various biomarkers in blood across the range observed in diseases and physiological conditions. This technology has also shown a strong correlation between blood and CSF levels for the neuronal biomarkers studied in this thesis, eliminating the necessity for CSF collection via lumbar puncture[98-100]. Simoa is based on digital immunoassays and works by trapping individual molecules and measuring them one by one, providing enhanced sensitivity compared to traditional immunoassays. It has been applied in various research areas, including neuroscience, to detect biomarkers associated with neurodegenerative diseases, traumatic brain injury, and other neurological conditions[101].

Figure 11. Anna Corderfeldt Keiller and main supervisor Roger Olofsson Bagge during an Isolated limb perfusion procedure. Photo courtesy of Roger Olofsson Bagge.

2 AIM

The overall aim of this thesis was to explore physiological responses and alternative approaches in extracorporeal circulation to increase understanding of procedure related side effects. The thesis is based on four studies, each of which are designed and conducted with the intention of providing new physiological insights and refine perfusion techniques, ultimately leading to enhanced patient outcomes.

I. To investigate the possibility of replacing an erythrocyte-based prime solution with a crystalloid-based prime solution without a negative effect on oxygen delivery to the tissues during isolated limb perfusion.

II. To measure and describe oxygen metabolism, specifically oxygen delivery, consumption, and extraction, in an isolated extremity during isolated limb perfusion. Secondly, to investigate whether invasive oxygenation measurement during ILP correlates to non-invasive near-infrared spectroscopy.

III. To investigate if neuronal biomarkers are measurable and altered in an isolated extremity during isolated limb perfusion. Secondly, to correlate postoperative regional toxicity, sensitivity, and muscle strength with the biomarker levels.

IV. To evaluate the effect of a high or a standard cardiopulmonary bypass flow rate on biomarker release patterns associated with brain injury in patients undergoing heart surgery. Secondly, to compare the rate of postoperative delirium between these groups. Thirdly, to investigate the relationship between age, sex, cardiopulmonary bypass time, non-invasive near-infrared spectroscopy, oxygen delivery, and oxygen extraction with the level of biomarkers.

3 PATIENTS AND METHODS

Recruiting patients for clinical studies can be a challenging task, especially during pandemics and when involving patients undergoing invasive procedures, especially the relatively rarely conducted ILP procedures. The common denominator has therefore been the lengthy recruitment period. A heartfelt and humble thank you, to all the patients who chose to participate in the studies and contributed to the research results and new insights.

3.1 Overview

Paper	Study design	Subjects	Number included	Outcome measure
I	Randomized controlled trial	ILP patients	21	Lactate level
II	Explorative study	ILP patients	39	Oxygen parameters
III	Feasibility trial	ILP patients	18	Neuronal biomarkers
IV	Randomized controlled trial	Cardiac surgery patients	40	Neuronal biomarkers

Table 2. Overview of papers included in the thesis. ILP: Isolated limb perfusion.

3.2 Paper I

3.2.1 Study design

Paper I was a single-center, non-blinded, non-inferiority, randomized controlled clinical trial. The study protocol was reviewed and approved by the Regional ethical board in Gothenburg, Sweden, in March 2017 (protocol number: 1145-16), and registered at ClinicalTrials.gov (id: NCT03073304).

3.2.2 Inclusion, exclusion criteria

Patients scheduled for ILP at Sahlgrenska University hospital were screened. Twenty-two patients were assessed for eligibility. Inclusion criteria were patients scheduled for ILP, age ≥18 years and written informed consent. After providing oral and written information, 21 patients accepted to participate and were randomized into two groups, either to the control group (erythrocyte-based prime solution) or to the intervention group (crystalloid-based prime solution).

3.2.3 Experimental protocol

A schematic of the experimental protocol is presented in Figure 12. In the control group the priming solution contained a total of 700 mL, divided into 250 mL of Ringer-acetate, 250 mL packed red blood cells, 100 ml Tribonat, 100 ml Albumin 200g/L and 2500 U heparin. In the intervention group the priming solution contained a total of 700 mL, divided into 500 mL of Ringer-acetate, 100 ml Tribonat, 100 ml Albumin 200g/L and 2500 U heparin. Samples were taken from the prime solutions in both groups before ECC start for a lactate baseline value. NIRS for regional oxygenation was measured on both extremities on the lateral side of tibia from anesthesia induction until 10 minutes after the extremity isolation was released. Blood gases were continuously measured and monitored from both the arterial- and venous line (Terumo CDI 500) during ECC along with continuously measured hemodynamics. In addition, blood gas samples were drawn from radial artery, ECC (venous and arterial), and by puncture of femoral vein, as shown in Figure 12. Samples were analyzed with the I-STAT system (Abbott, Princeton, NJ, USA). Apart from the description of interventions performed, patient care, anesthesia, surgery, and postoperative care were conducted following standard clinical procedures.

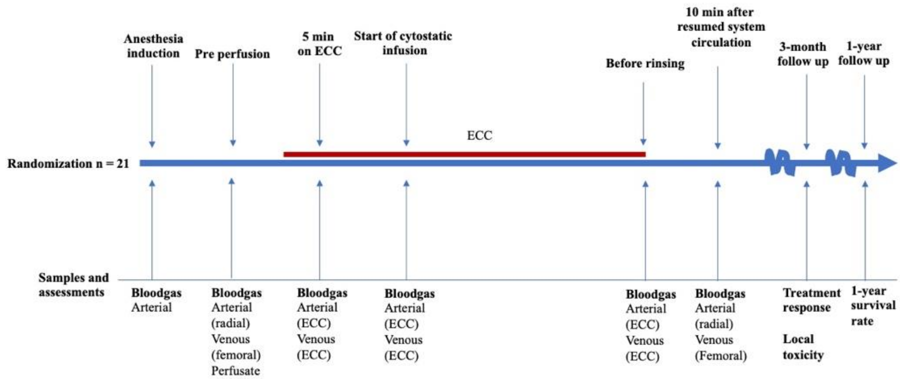

Figure 12. Flowchart of blood sampling and assessments during study I. ECC: extracorporeal circulation.

3.2.4 Outcome measures

The main outcome measure was the lactate level during ILP. Additionally, we assessed regional central venous oxygen saturation, arterial saturation, DO_2, VO_2, O_2ER, as well as response rate, regional toxicity, complications, and 1-year survival as secondary outcome measures.

3.3 Paper II

3.3.1 Study design

Paper II was a prospective explorative study derived from paper I and III. Approved by the Regional Ethical Board in Gothenburg, Sweden, in March 2017 (protocol number: 1145-16), and registered at ClinicalTrials.gov (id: NCT03073304) and the Swedish Ethical Board, Sweden in march 2019 (protocol number: 2019-01046) and registered at ClinicalTrials.gov (id: NCT04460053).

3.3.2 Inclusion, exclusion criteria

Patients scheduled for ILP, with written and informed consent, derived from paper I and III were included in this study. Exclusion criteria were included patients with a leakage of more than 0 ml blood from the systemic circulation to the isolated extremity, and a leakage of >10% chemotherapeutic agents from the extremity to the system circulation. Thirty-nine patients were eligible for the study and were included.

3.3.3 Experimental protocol

A schematic of the experimental protocol is presented in Figure 13. NIRS for regional oxygenation was measured on both extremities on the lateral side of tibia/ulna from anesthesia induction until 10 minutes after the extremity isolation was released. Blood gas was continuously measured and monitored from both arterial- and venous line (Terumo CDI 500) during ECC along with continuously measured hemodynamics. In addition, blood gas samples were drawn from the radial artery, ECC (venous and arterial), and by puncture of femoral/brachial vein, shown in Figure 13. Samples were analyzed with the I-STAT system (Abbott, Princeton, NJ, USA). Apart from the description of interventions performed, patient care, anesthesia, surgery, and postoperative care were conducted following standard clinical procedures.

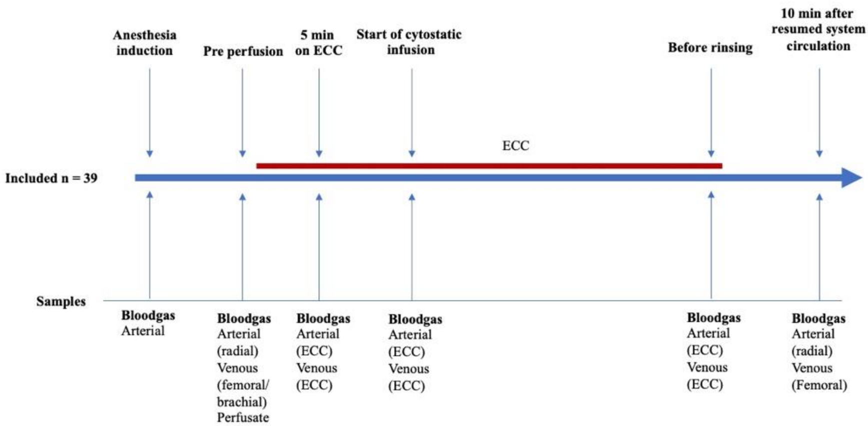

Figure 13. Flowchart of blood sampling during study II. ECC: extracorporeal circulation.

3.3.4 Oxygen calculations

The ECC pump flow rate (Q) L/min and the data from the blood gas samples were incorporated into the equation (Figure 14) for calculating CaO_2, CvO_2, DO_2, VO_2, and O_2ER. The results were then presented in relation to the surface area of the extremity for DO_2i, VO_2i.

$$CaO_2 = (1.36 \text{ x Hb (g/L)}) \text{ x } (SaO_2 (\%) \text{ x } 0.01) + (0.23 \text{ x } PaO_2 \text{ (kPa)})$$

$$CvO_2 = (1.36 \text{ x Hb (g/L)}) \text{ x } (SvO_2 (\%) \text{ x } 0.01) + (0.23 \text{ x } PvO_2 \text{ (kPa)})$$

$$DO_2^i = Q \text{ (L/min) x } CaO_2/BSA^{extremity}$$

$$VO_2^i = Q \text{ (L/min) x } (CaO_2\text{-}CvO_2)/ BSA^{extremity}$$

$$O_2ER = VO_2/DO_2$$

Figure 14. Oxygen equation in liter and kilopascal (kPa).

3.3.5 Outcome measures

The primary outcome was the description of DO_2i, VO_2i and O_2ER during ILP. The secondary outcome measure was the correlation and agreement between non-invasive NIRS and invasive muscular oxygenation measurement in an isolated extremity during ILP.

3.4 Paper III

3.4.1 Study design

Paper III was a single-center prospective feasibility trial. The study protocol was reviewed and approved by Swedish Ethical Board (protocol number: 2019-01046) in March 2019. The trial was registered at ClinicalTrials.gov (NCT04460053). Permission to store blood samples were approved by Biobank Sverige (document number: PS-0499-5-001).

3.4.2 Inclusion, exclusion criteria

Patients, of age ≥18 years, scheduled for ILP at Sahlgrenska University hospital, Gothenburg were eligible for inclusion. After providing oral and written information and time for consideration, eighteen patients consented in participating and were included in the study.

3.4.3 Experimental protocol

A schematic of the experimental protocol is presented in Figure 15. Baseline biomarkers were collected the day before surgery as well as the preoperative physiotherapist assessment, which also was performed 30 days after surgery. Near-infrared spectroscopy (NIRS) was placed on the lateral side of the tibia/ulna, to measure regional oxygenation on both extremities, from induction until 10 minutes after the extremity isolation was released. Blood gases were continuously measured and monitored from both arterial- and venous line (Terumo CDI 500) during ECC along with continuously measured hemodynamics. In addition, blood gas samples were drawn from radial artery, ECC (venous and arterial), and by puncture of femoral/brachial vein. Blood gas samples were analyzed with the I-STAT system (Abbott, Princeton, NJ, USA). Apart from the description of interventions performed, patient care, anesthesia, surgery, and postoperative care were conducted following standard clinical procedures.

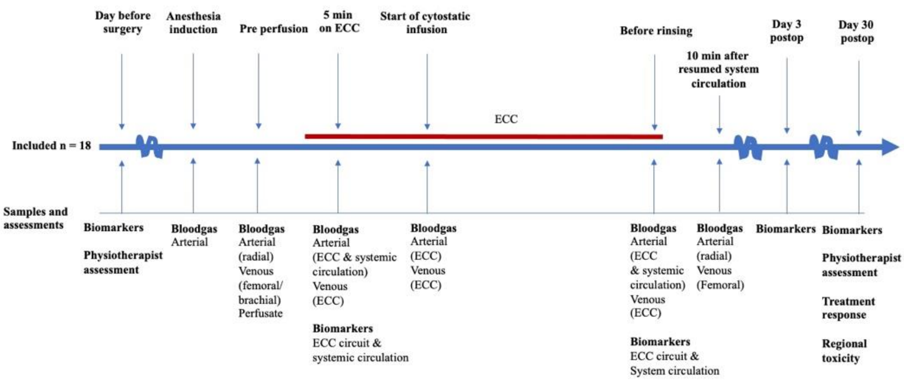

Figure 15. Flowchart of blood sampling and assessments during study III. ECC: extracorporeal circulation.

3.4.4 Outcome measures

The primary outcome was the level of GFAP, NfL and t-tau during and after ILP. Secondary outcome was correlations between these biomarker levels and postoperative regional toxicity measured according to the Wieberdink scale, alterations in muscle strength measured in Newton meter, sensitivity alterations manually assessed by physiotherapist with blunt and sharp objects, measured on a 0-2 grade scale (0 = no sensitivity, 1 = reduced sensitivity, 2 = full sensitivity).

3.5 Paper IV

3.5.1 Study design

Paper IV was a sub-study of the single-center, randomized controlled clinical trial ICAROX2 (Impact of cardiopulmonary bypass flow on renal oxygenation, blood flow and tubular injury) trial by Wijk et al. The study protocol was reviewed and approved by the Swedish Ethical Board (protocol number: 2019-01423) in June 2019, and registered at ClinicalTrials.gov (NCT04084301). Permission to store blood samples were approved by Biobank Sverige (document number: PS-0531-5-002).

3.5.2 Inclusion, exclusion criteria

Patients scheduled for elective heart surgery, with an anticipated duration on cardiopulmonary bypass (CPB) exceeding 60 minutes, underwent eligibility screening. After receiving oral and written information, a total of 101 patients in the main study and 40 patients in this sub-study agreed to participate and were enrolled. Patients were randomized 1:1 to undergo heart surgery with either a standard CPB target flow rate (2.4L/min/m^2) or a high CPB (2.9L/min/m^2) target flow rate. Inclusion criteria were patients ≥18 years scheduled for open cardiac surgery with CPB, LVEF ≥30 %, eGFR ≥30 ml/min. Exclusion criteria were emergency surgery, advanced grown-up congenital heart disease corrections, cardiac transplantation, previous cerebral infarction, verified with computed tomography or magnetic resonance imaging, body mass index > 32 kg/m^2 and the use of hypothermia < 32 °C during CPB.

3.5.3 Experimental protocol

A schematic of the experimental protocol is presented in Figure 16. Prior to anesthetic induction, all patients received NIRS electrodes above the frontal lobes for measuring cerebral oxygenation. Anesthesia was administered according to our clinical routine, involving induction with propofol and fentanyl, followed by sevoflurane-based maintenance, except during CPB where propofol was used. Patients were continuously hemodynamic monitored and during ECC arterial blood gas monitoring was performed continuously (Terumo CDI 500, Ann Arbor, USA). By the CPB patient data management system Connect manager (LivaNova, Munich, Germany) oxygen calculations were saved automatically 3 times/minute. Manually drawn blood gases were analyzed when not on CPB (Siemens rapidPoint 500, Erlangen, Germany).

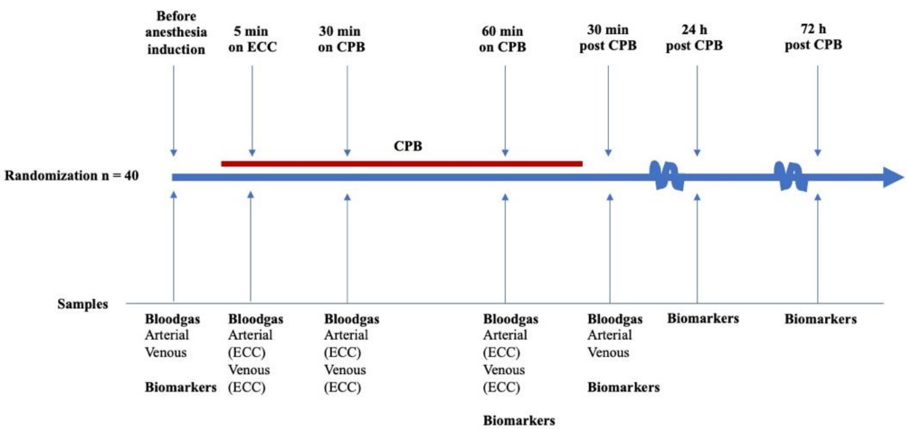

Figure 16. Flowchart of blood sampling and assessments during study IV. ECC: extracorporeal circulation.

3.5.4 Outcome measures

The primary outcome was the release pattern of the neuronal biomarkers GFAP, NfL, t-tau and p-tau217. As secondary outcome the rate of postoperative delirium was evaluated using the Nursing Delirium Screening Scale (NuDESC), as well as the correlation between age, sex, CPB time, NIRS, DO_2i, O_2ER, and biomarker levels.

3.6 Biomarker analysis (paper III-IV)

Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes for plasma extraction and centrifuged within 20–60 minutes. The plasma was then separated, divided into aliquots, and stored at −80°C until biochemical analysis. Concentrations of NfL, t-tau, p-tau217 and GFAP were quantified in plasma using ultrasensitive Single molecule array (Simoa) technology and commercially available kits, performed on an HD-X instrument (Quanterix, Billerica, MA, USA). In paper IV, plasma UGOT p-tau217 was measured following the method by Gonzalez-Ortiz et al[92]. All samples were analyzed in a single run at the Clinical Neurochemistry Laboratory, University of Gothenburg, Mölndal, Sweden, by certified laboratory technicians who were blinded to clinical data, using a single batch of reagents for each assay. The intra-assay coefficient of variation was less than 10% for all assays.

3.7 ECC set up

3.7.1 Paper I-III

The ECC circuit consists of a pediatric Affinity Pixie hollow fiber oxygenator and a 1.2 L hard-shell reservoir coated with non-heparin Cortiva Balance Biosurface (Medtronic, Minneapolis, MN, USA) and a ¼-inch perfusion custom circuit (Sorin, Mirandola, Italy). The circuit was assembled on a an ECC LivaNova S5 (LivaNova, Munich, Germany) roller pump which provides temperature monitoring, line pressure monitoring, level sensor and bubble monitoring. The water heater, an Heto HMT 200 (Heto-Holten A/S, Allerod, Denmark), was set to 41–42 °C to warm the prime solution during recirculation before starting the perfusion.

3.7.2 Paper IV

The cardiopulmonary system, consisting of the Liva Nova S5 roller pump from LivaNova (Munich, Germany), featured custom-made tubing primarily pre-connected (3/8-inch for arterial line and ½-inch for venous line), an Inspire 8 oxygenator with arterial filter, bubble detector, and hard-shell reservoir from Mirandola, Italy. It was primed with 1300 mL of Ringer-Acetate from Fresenius Kabi AB (Uppsala, Sweden). Cannula sizes were selected within safety limits provided by the manufacturer, with special attention to high-pressure drops. The target flow rate remained constant throughout the CPB period, except for adjustments during aortic cross-clamp placement and removal, or when lower flow rates were necessary due to surgical requirements. Additional Ringer-Acetate was infused if needed to maintain the flow rate; no albumin or starch solutions were administered during CPB. Blood transfusions were provided to maintain hematocrit levels above 24%. Mean arterial pressure was regulated within the range of 60 – 80 mmHg using norepinephrine or sodium nitroprusside as required. Throughout CPB, the target pO_2 was set at 20 kPa, and the target pCO_2 ranged between 5.0 – 6.0 kPa.

3.8 Statistical analysis

Data was analyzed using SPSS statistics (IBM, Armonk, NY) version 22, 27, 28, R stats version 3.6.3, rmcorr version 0.4.3 (Vienna, Austria) and SAS software version 9.4 (SAS Institute Inc., Cary, NC, US). In all four papers a probability level (p-value) of less than 0.05 was considered to indicate statistical significance. An overview of used statistical methods are shown in Table 3.

Paper	Primary endpoint	Secondary endpoint	Additional
I	Student's t-test	Student's t-test Mann-Whitney U-test Fisher's exact test	
II	Student's t-test One-way ANOVA Paired samples t-test	Repeated-measures correlation Bland and Altman plot Limits of agreement	Shapiro-Wilk test
III	Paired samples t-test Wilcoxon signed-rank test	Kruskal-Wallis test Pearson correlation	Shapiro-Wilk test
IV	Mixed models for repeated measures	Student's t-test Fisher's exact test Wilcoxon Signed Rank test	Bonferroni-Holm Lognormal distribution

Table 3. Overview flowchart for statistical tests used in Paper I-IV. Overview for statistical test used in Paper I-IV.

3.8.1 Sample size Paper I and IV

In Paper I, the sample size was determined through a power analysis using lactate levels during perfusion from a cohort comprising all patients who underwent ILP at Sahlgrenska University Hospital in 2016, a total of 28 patients. The mean value in this cohort was 3.0 with a standard deviation of 0.7. Non-inferiority was defined as a lactate level of 4.0 or lower, with an alpha value of 0.05 and a power of 80%. A sample size of 9 patients per group was deemed necessary to detect differences in lactate levels. However, 22 patients were estimated to be needed to compensate for potential dropouts.

In Paper IV, the sample size was calculated on a previous study examining oxygen delivery during different CPB flow rates[102]. Where a CPB flow rate at 2.4L/min/m^2 delivered a mean DO_2i of 322 ± 45 and a CPB flow rate at 2.7L/min/m^2 delivered a mean DO_2i of 374 ± 32 a sample size of 12 patients per group was deemed necessary to detect differences in DO_2i (with an alpha of 0.05 and power of 0.80), 20 patients were included to account for potential dropout.

4 RESULTS

4.1 Paper I

4.1.1 Patients

An overview of the clinical trial flowchart for Paper I is presented in Figure 17. Twenty-one patients were included. Three patients were excluded from the final analysis. One patient due to high systemic leakage and the decision not to proceed with ILP was made. Only two patients underwent upper extremity ILP and were both randomized into the control group. However, a decision was made to exclude them due to anatomical differences between upper and lower extremities, particularly regarding blood volume in relation to circuit size, prime volume, and expected hemodilution. The final analysis contained 18 patients, 9 patients in each group.

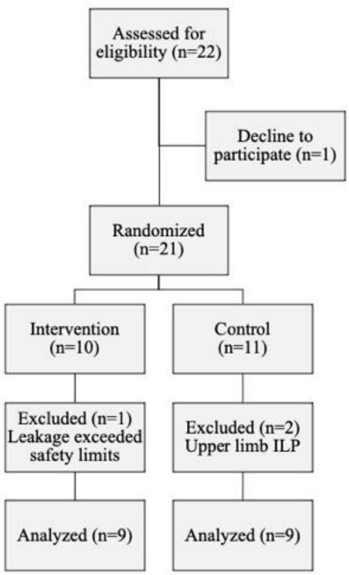

Figure 17. CONSORT diagram showing inclusion paper I.

The median age of the participants were 73 (range 35-85) years, and the male/female ratio was 11/7. Patient demographics and baseline characteristics are detailed in Table 4.

Baseline characteristics	**Intervention group (n = 9)**	**Control group (n = 9)**
Age (median, range)	68 ± 46	75 ± 34
Male (%)	7 (78%)	4 (44%)
Weight (kg)	80 ± 12	76 ± 13
Height (cm)	174 ± 7	173 ± 11
Body surface area (m^2)	1.93 ± 0.12	1.90 ± 0.22
Hemoglobin (g/L)	142 ± 18	145 ± 9
Hematocrit (%)	40 ± 6	42 ± 5
Creatinine (µmmol/L)	87 ± 11	71 ± 10
Blood volume in extremity (ml)	564 ± 88	532 ± 98
Lactate (mmol/L)	1.0 ± 0.3	1.2 ± 0.7
Reg. ScvO2 in femoral vein (%)	79 ± 7	77 ± 13
Oxygen extraction (%)	21 ± 7	24 ± 14
No co-morbidity (%)	5 (56%)	5 (56%)
Diabetes (%)	1 (11%)	0 (0%)
Heart failure (%)	2 (22%)	1 (11%)
Other co-morbidity (%)	1 (11%)	3 (33%)

Table 4. Baseline characteristics Paper I. Mean and standard deviation, median and range, or number and percentage (%). Reg. $ScvO_2$: Regional central venous oxygen saturation. With permission by Taylor & Francis in International Journal of Hyperthermia on 27 Aug 2019, available https://doi.org/10.1080/02656736.2019.

4.1.2 Lactate

In Table 5 and Figure 18 the level of lactate (mmol/L) is shown between groups. In the prime solution before perfusion, the lactate value was significantly higher in the control group (8.1 ± 1.8 vs. 0.01 ± 0.0, p = .001), this significant difference remained during perfusion with a mean lactate level of 3.6 ± 0.7 in the control group vs. 1.6 ± 0.4 in the intervention group (p = .001).

Treatment characteristics	Intervention group (n = 9)	Control group (n = 9)	p-value
ECC time (minutes)	92 ± 12	102 ± 15	.13
Blood waste (ml)	339 ± 365	261 ± 404	.67
Lactate prime solution (mmol/L)	0.01 ± 0.0	8.1 ± 1.8	.001***
Pumpflow ECC (L/min)	0.74 ± 0.27	0.77 ± 0.18	.82
Hemoglobin on ECC (g/L)	79 ± 15	98 ± 15	.02*
Hematocrit on ECC (%)	24 ± 6	30 ± 5	.58
Lactate on ECC (mmol/L)	1.6 ± 0.4	3.6 ± 0.7	.001***

Table 5. Treatment characteristics Paper I. Mean and standard deviation. Student's t-tests were used to compare groups. p <0.05, p <0.01**, p <0.001***. ECC: extracorporeal circulation. With permission by Taylor & Francis in International Journal of Hyperthermia on 27 Aug 2019, available https://doi.org/10.1080/02656736.2019.1640900*

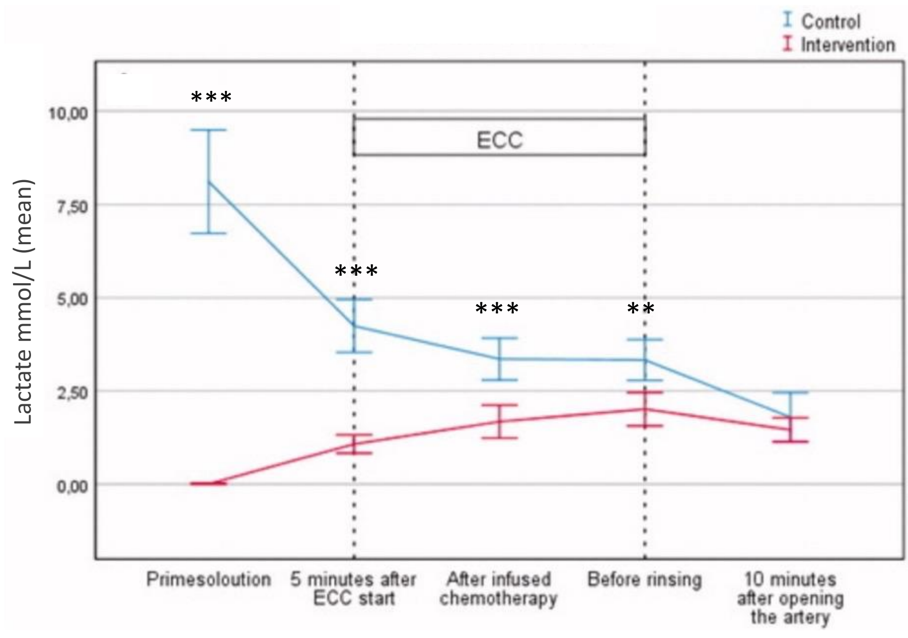

Figure 18. Lactate level during isolated limb perfusion Paper I. ECC: Extracorporeal circulation between dotted lines. p <0.05, p <0.01**, p <0.001***. With permission by Taylor & Francis in International Journal of Hyperthermia on 27 Aug 2019, available https://doi.org/10.1080/02656736.2019.164*

4.1.3 Oxygen parameters

Figure 19 presents the DO_2 during perfusion, initially showing, at 5-minutes on ECC, a significant difference between the groups, with 124 ± 49 mL/min in the control group vs. 72 ± 33 mL/min in the intervention group ($p = .019$). The following two measurements during the perfusion showed no difference between groups. Further, no differences were found in mean, seen over the entire perfusion, in DO_2, VO_2, O_2ER, regional central venous oxygen saturation or arterial saturation (Table 6).

Treatment characteristics	Intervention group (n = 9)	Control group (n = 9)	p-value
SaO2 on ECC (%)	99 ± 0.6	100 ± 0.3	.50
Regional ScvO2 on ECC (%)	83 ± 10	91 ± 4	.07
Arterial oxygen content (ml)	11 ± 2	14 ± 2	.027*
Venous oxygen content (ml)	9 ± 3	12 ± 2	.017*
Delivered oxygen on ECC (ml/min)	90 ± 49	108 ± 38	.39
Oxygen consumption on ECC (ml/min)	14 ± 2	14 ± 5	.85
Oxygen extraction (%)	22 ± 11	14 ± 4	.62
INVOS on treated extremity (%)	76 ± 14	81 ± 11	.42
INVOS on not treated extremity (%)	72 ± 10	66 ± 12	.25

Table 6. Treatment characteristics Paper I. Mean and standard deviation. Student's t-tests were used to compare groups. p <0.05, p <0.01**, p <0.001***. ECC: extracorporeal circulation; SaO2: arterial oxygen saturation; ScvO2: central venous oxygen saturation. With permission by Taylor & Francis in International Journal of Hyperthermia on 27 Aug 2019, available https://doi.org/10.1080/02656736.2019.1640900*

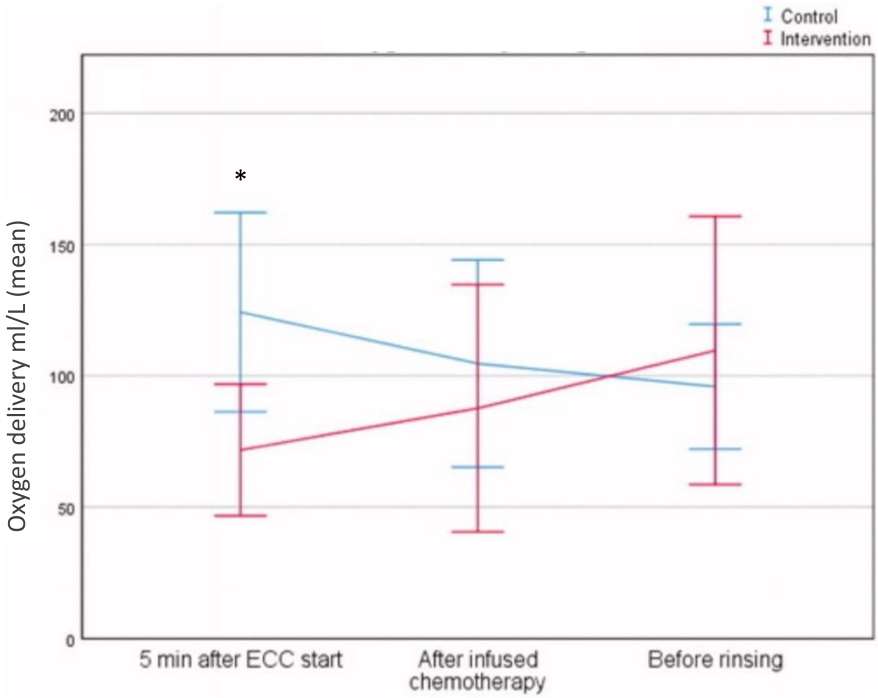

Figure 19. Oxygen delivery (mL/min) during extracorporeal circulation during isolated limb perfusion, Paper I. ECC: Extracorporeal circulation. p <0.05, p <0.01**, p <0.001***. With permission by Taylor & Francis in International Journal of Hyperthermia on 27 Aug 2019, available https://doi.org/10.1080/02656736.2019.1640900*

4.1.4 Response, toxicity, survival

There was no statistically significant differences in response rate, survival, or local toxicity, assessed 3 months postoperatively using the Wieberdink classification (grades I to V) (Table 7).

Follow-up characteristics	Intervention group (n = 9)	Control group (n = 9)	p-value
Wieberdink toxicity (grade I–II) (%)	6 (67%)	5 (56%)	.63
Wieberdink toxicity (grade III) (%)	3 (33%)	3 (33%)	1.0
Wieberdink toxicity (grade IV) (%)	0 (0%)	1 (11%)	.30
Complications, 3 months (%)	1 (11%)	2 (22%)	.52
1-year survival (%)	7 (78%)	6 (67%)	.60
Overall response rate (%)	9 (100%)	7 (78%)	.13
Complete response (%)	5 (56%)	4 (44%)	.64
Partial response (%)	4 (44%)	3 (33%)	.63
Stable disease (%)	0 (0%)	0 (0%)	–
Progressive disease (%)	0 (0%)	2 (22%)	.13

Table 7. Follow-up characteristics Paper I. Number and percentage (%). Fisher's exact test for categorical data were used to compare groups. p <0.05, p <0.01**, p <0.001***. Complications included erysipelas, leg ulcers, advanced leg edema. With permission by Taylor & Francis in International Journal of Hyperthermia on 27 Aug 2019, available https://doi.org/10.1080/02656736.2019.1640900*

4.2 Paper II

4.2.1 Patients

An overview of included patients for paper II is presented in Figure 20. Overall, 39 patients derived from Paper I and III were included, 18 patients were excluded due to exceeding the exclusion criteria of leakage more than 0 mL from the systemic circulation to the extremity, and one patient was excluded due to leakage of chemotherapeutic agents to the systemic circulation of >10%. The final analysis included 20 patients.

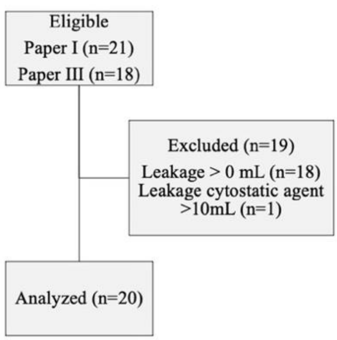

Figure 20. Diagram showing patient inclusion paper II.

The median age and of the participants was 74 (range 22 – 83) years, and the male/female ratio was 15/5. The treated extremity leg/arm ratio was 14/6. Patient demographics and baseline characteristics are detailed in Table 8.

Baseline characteristic	**Leg and Arm (n=20)**	**Leg (n=14)**	**Arm (n=6)**
Male, n (%)	15 (75%)	10 (71%)	5 (83%)
Median age, years (range)	74 (22–83)	74 (29–81)	67 (22–83)
Height, cm	175 ± 11	174 ± 12	177 ± 10
Weight, kg	80 ± 17	83 ± 15	73 ± 20
Extremity surface area, m^2	0.28 ± 0.10	0.33 ± 0.50	0.14 ± 0.21
Hemoglobin, g/L	135 ± 17	138 ± 16	126 ± 18
Hematocrit, %	39 ± 6	40 ± 5	37 ± 6
Creatinine, µmol/L	81 ± 12	79 ± 7	83 ± 13
Lactate, mmol/L	1.04 ± 0.2	1.04 ± 0.2	1.02 ± 0.3
Blood volume in extremity,	498 ± 172	587 ± 109	289 ± 83
PaO_2, kPa	26± 21	22 ± 14	35 ± 31
SaO_2, %	98 ± 1	98 ± 2	98 ± 1
SveO2 in femoral/brachial	76 ± 14	71 ± 13	88 ± 6
Oxygen extraction, %	25 ± 14	30 ± 14	14 ± 7
NIRS-treated extremity, %	69 ± 13	65 ± 13	77 ± 10
NIRS not treated extremity, %	67 ± 13	66 ± 14	70 ± 11
No-comorbidity, n (%)	6 (30%)	4 (29%)	2 (33%)
Diabetes, n (%)	5 (25%)	4 (29%)	1 (17%)
Hypertension, n (%)	11 (55%)	8 (57%)	3 (50%)
Hyperlipidemia, n (%)	2 (10%)	2 (14%)	0 (0%)
Heart failure, n (%)	2 (10%)	2 (14%)	0 (0%)
Other comorbidity, n (%)	6 (30%)	5 (36%)	1 (17%)

Table 8. Baseline characteristics Paper II. Mean and standard deviation, median range, and numbers (%). PaO2: partial pressure of oxygen, SaO2: oxygen saturation, SveO2: Venous extremity oxygen saturation, NIRS: near infrared spectroscopy. Other comorbidity: Ulcerative colitis, Chron's disease, rheumatoid arthritis, stroke, and hyperthyroidism. With permission by Sage in Perfusion on 16 May 2022, available https://doi.org/10.1177/02676591221093201

4.2.2 Oxygen parameters

For all observations, the mean DO_2i was 190 ± 59 ml/min/m^2, and VO_2i was 35 ± 8 ml/min/m^2, resulting in an O_2ER of 21 ± 8%. There was a significant difference in VO_2i between the legs and arms (38 ± 8 vs 29 ± 7 ml/min/m^2, *p*=. 02), Table 9.

Treatment characteristics	Leg and Arm (n=20)	Leg (n=14)	Arm (n=6)	p-value
Pump flow (L/min)	0.53 ± 0.2	0.64 ± 0.12	0.28 ± 0.07	<0.001***
Hemoglobin (g/L)	68 ± 15	67 ± 16	70 ± 13	0.71
Hematocrit (%)	21 ± 5	20 ± 6	21 ± 3	0.75
Lactate (mmol/L)	3.0 ± 1.3	2.5 ± 1.1	4.3 ± 0.8	<0.01**
SveO2 (femoral/brachial vein) (%)	85 ± 8	83 ± 8	89 ± 4	0.17
DO_2i (ml/min/m2)	190 ± 59	189 ± 62	192 ± 58	0.93
VO_2i (ml/min/m2)	35 ± 8	38 ± 8	29 ± 7	0.02 *
O_2ER (%)	21 ± 8	23 ± 8	16 ± 5	0.81
NIRS-treated extremity (%)	76 ± 11	73 ± 11	84 ± 9	0.06
NIRS not treated extremity (%)	69 ± 12	68 ± 13	73 ± 8	0.40

Table 9. Intraoperative characteristics during ECC Paper II. Mean and standard deviation. Student's t-test to examine differences between upper and lower limb. p <0.05, p <0.01**, p <0.001***. SveO2: venous extremity oxygen saturation, DO_2i: indexed oxygen delivery, VO_2i: Oxygen consumption, O_2ER: oxygen extraction, NIRS: Near infrared spectroscopy (NIRS). With permission by Sage in Perfusion on 16 May 2022, available https://doi.org/10.1177/02676591221093201*

4.2.3 Changes in oxygen parameters

DO_2i decreased over time (in legs from 209 ± 65 to 180 ± 66 ml/min/m^2, *p* <.01 and in arms from 252 ± 72 to 150 ± 57 ml/min/m^2, *p* <.01), and an increase in O_2ER was seen in upper limbs, with the highest value of 18% (*p* =.03). VO_2i remained unchanged thru the perfusion. An increase in $SveO_2$ was seen in lower limbs (Table 10).

Parameters	Extremity	After induction	Before isolation	ECC start	Chemo infusion	Before rinse	Circulation resumed	ANOVA *p*-Value
Hb (g/L)	Leg	125±11	127±11	67±18***	67±18***	67±18***	117±12***	<0.001***
	Arm	113±8	111±7	74±9**	74±9**	74±9**	109±9	<0.01**
Lactate (mmol/L)	Leg	1.0±0.2	1.1±0.2	2.0±1.6*	2.6±1.1***	2.9±1.0***	1.8±0.6***	<0.001***
	Arm	1.0±0.3	1.1±0.3	5.0±0.7***	4.0±1.0**	3.9±1.6**	1.7±0.5*	<0.001***
Pump flow (L/min)	Leg	-	-	0.72±0.1	0.61±0.1**	0.58±0.2**	-	<0.001***
	Arm	-	-	0.36±0.1	0.26±0.1*	0.22±0.1*	-	<0.01**
DO_2i (mL/min/m2)	Leg	-	-	209±65	178±67**	180±66*	-	<0.01**
	Arm	-	-	252±72	174±62*	150±57**	-	<0.01**
VO_2i (mL/min/m2)	Leg	-	-	39±10	38±9	38±7	-	0.79
	Arm	-	-	33±6	28±9	26±10	-	0.12
O_2ER (%)	Leg	-	30±14	20±7	24±10	24±9	22±12	0.21
	Arm	-	13±7	14±4*	16±6	18±6	11±6*	0.03*
$SveO_2$ (%)	Leg	-	71±13	86±6**	82±10*	82±10*	80±12	0.01*
	Arm	-	88±6	90±4	89±6	86±6	89±5	0.30
NIRS treated (%)	Leg	65±13	64±13	72±11	73±11	75±11	72±12	0.21
	Arm	77±10	76±10	87±9	83±10	81±10	80±16	0.19
NIRS not treated (%)	Leg	66±14	67±14	67±12	68±13	68±14	66±11	0.12
	Arm	70±11	71±11	72±8	73±8	74±9	70±8	0.41

Table 10. Characteristics during ILP, paper II. Blue area illustrate parameters measured during extracorporeal circulation. One way ANOVA repeated measure ("before isolation" through "system circulation"). Mean and standard deviation. and paired-samples t-test for significant ANOVA parameters. $p < 0.05$, $p < 0.01$**, $p < 0.001$***. Hb: hemoglobin, SveO2: venous extremity oxygen saturation, DO_2i: oxygen delivery, VO_2: oxygen consumption, O_2ER: oxygen extraction, NIRS: Near infrared spectroscopy (NIRS). With permission by Sage in Perfusion on 16 May 2022, available https://doi.org/10.1177/02676591221093201*

4.2.4 Oxygen delivery threshold

When plotting the perfusion in the context of relationship between oxygen delivery and oxygen extraction, it was observed that maintaining a DO_2i above 170 ml/min/m^2 consistently kept the oxygen extraction below 30% across all observations (Figure 21).

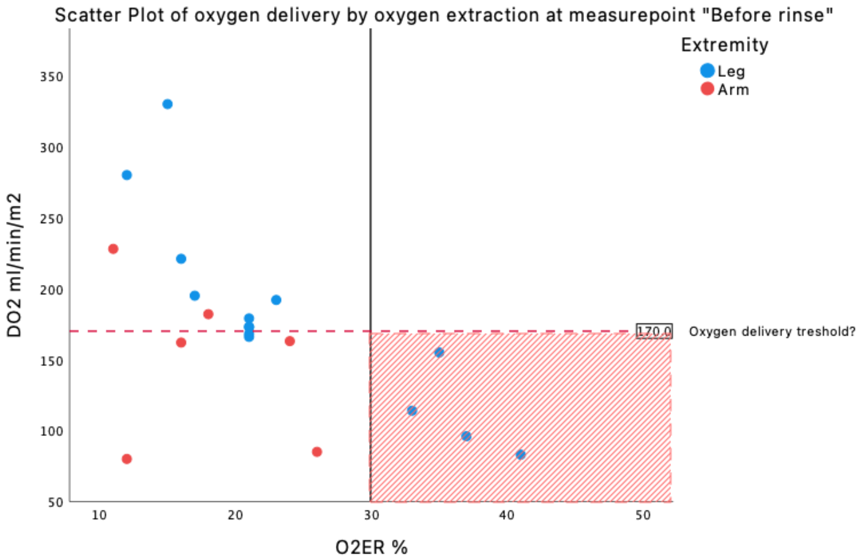

Figure 21. Scatter plot describing oxygen delivery by oxygen extraction at the end of perfusion in paper II. Red marked area shows perfusions which are above 30% of O_2ER and below DO_2i of 170ml/min/m^2, all observations in red area were legs. Oxygen delivery threshold? With permission by Sage in Perfusion on 16 May 2022, available https://doi.org/10.1177/02676591221093201

4.2.5 NIRS

All observations showed higher NIRS values during the perfusion compared to the systemically perfused extremity, and NIRS values did not significantly alter during the perfusion (Table 10). Significant correlation was detected between NIRS values and $SveO_2$, r_{rm}=0.568 (p <.001, 95% CI 0.397 – 0.701) (Figure 22). However, the limit of agreement between the two methods was too high (32.5%) for the methods to function interchangeable (Bland–Altman plot mean: 80.13±9.0, bias $SveO_2$ and NIRS: 8.26 ± 13.0, limit of agreement: −17.28–33.09).

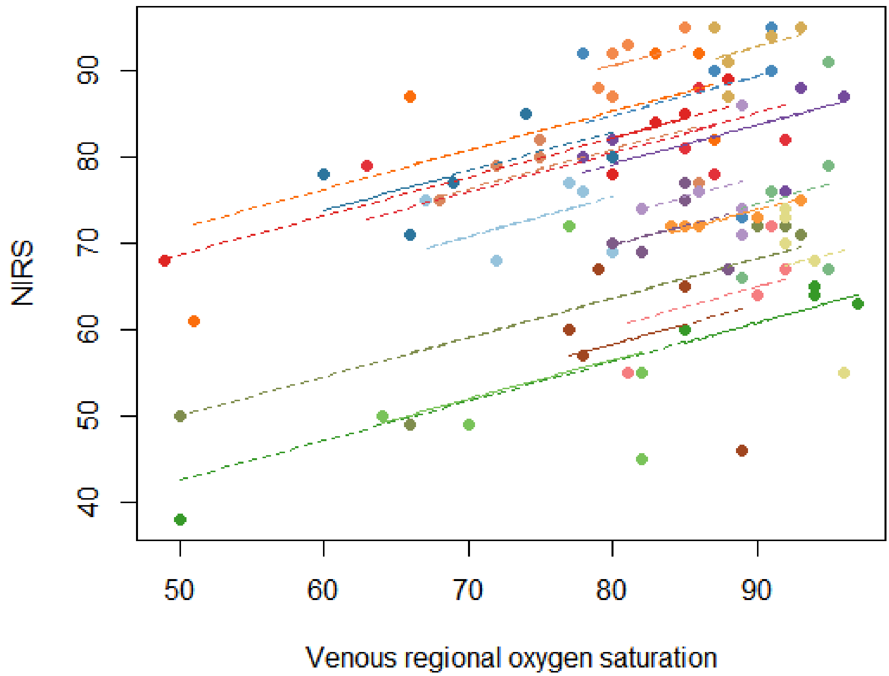

Figure 22. Repeated- measures correlation coefficient between $SveO_2$ and rSO_2 by NIRS, Paper II. The repeated- measures correlation coefficients (r_{rm}), representing the strength of the linear association between $SveO_2$ and NIRS. The inclinations between subjects are the same due to repeated measure total correlation coefficient 0.568 and length of the line represents the range for each individual three measures. NIRS: near infrared spectroscopy. With permission by Sage in Perfusion on 16 May 2022, available https://doi.org/10.1177/02676591221093201

4.3 Paper III

4.3.1 Patients

An overview of included patients for paper III is presented in Figure 23. Overall, 18 patients scheduled for ILP were included. One patient experienced a preoperative stroke (<30 days prior to ILP) and was excluded from the study due to the potential impact on biomarker levels. The final analysis included 17 patients.

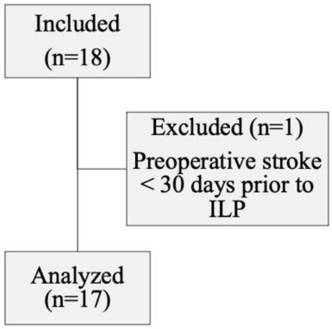

Figure 23. Diagram showing patient inclusion paper III.

The median age of the participants was 72 (range 29-89) years, the male/female ratio was 12/5. The treated extremity leg/arm ratio was 12/5 Patient demographics and baseline characteristics are detailed in Table 11.

Patient characteristics	**Leg and arm (n = 17)**	**Leg (n = 12)**	**Arm (n = 5)**
Male n, (%)	12 (71%)	8 (67%)	4 (80%)
Age years, median (range)	72 (29–89)	72 (29–89)	74 (54–89)
Total Body Surface Area m2, mean	1.97 ± 0.2	2.00 ± 0.2	1.90 ± 0.3
GFAP ng/L, median (percentiles)	160 (85–229)	145 (82–222)	197 (81–344)
NfL ng/L, median (percentiles)	19 (14–27)	18 (13–26)	24 (16–46)
Tau ng/L, median (percentiles)	1.9 (1.2–3.0)	1.8 (1.2–2.8)	1.9 (1.0–4.0)
Hemoglobin g/L, mean (SD)	125 ± 16	124 ± 18	126 ± 15
No-comorbidity n, (%)	3 (18%)	2 (17%)	1 (20%)
Diabetes n, (%)	6 (35%)	4 (33%)	2 (40%)
Hypertension n, (%)	9 (53%)	5 (42%)	4 (80%)
Hyperlipidemia n, (%)	3 (18%)	3 (25%)	0 (0%)
Heart failure or heart surgery n, (%)	5 (29%)	4 (33%)	1 (20%)
Other co-morbidity,a (%)	12 (71%)	9 (75%)	1 (20%)

Table 11. Patient demographics and baseline characteristics Paper III. Mean and standard deviation, median and percentiles or range, or numbers (%). a) Other co-morbidity: Rheumatoid arthritis (n = 1), Parkinson´s disease (n = 1), bladder cancer (n = 1), prostate cancer (n= 1), breast cancer (n = 1), meningioma (n = 1), depression (n = 2), hip- and/or knee replacement (n = 3), asthma (n = 1). With permission by Sage in Perfusion on 7 November 2023, available https://doi.org/10.1177/02676591231213506

4.3.2 Biomarkers in extremity vs. systemic circulation

Following the circulation of the cytostatic agent for 60 minutes in the isolated extremity, significantly increased median levels of both NfL and t-tau were found in the treated extremity compared to the levels found in the systemic circulation (Figure 24).

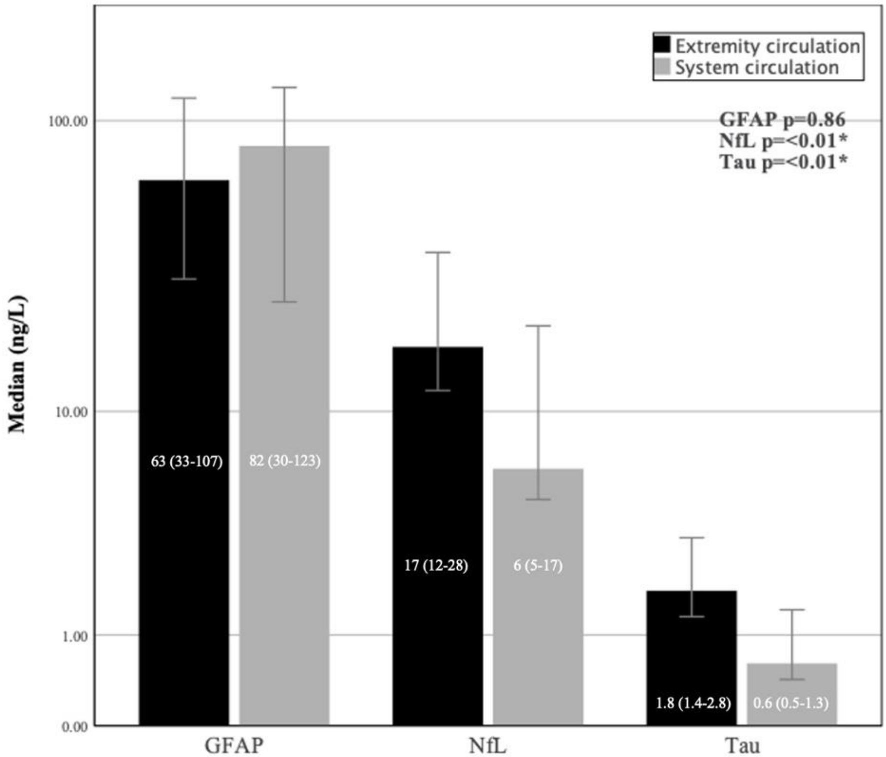

Figure 24. Clustered bar chart of median and IQR biomarkers in the extremity circulation compared to the systemic circulation at end of ILP. Logarithmic scale. System circulation is compensated for same hemodilution as perfusate due to priming volume in ECC. Wilcoxon signed ranks test. Error bars: CI 95%. p <0.05, p <0.01**, p <0.001***. With permission by Sage in Perfusion on 7 November 2023, available https://doi.org/10.1177/02676591231213506s*

4.3.3 Biomarkers during ECC in extremity

In the treated extremity, there was a significantly percentual rise observed from the start to the end of perfusion in both NfL and t-tau levels (Figure 25).

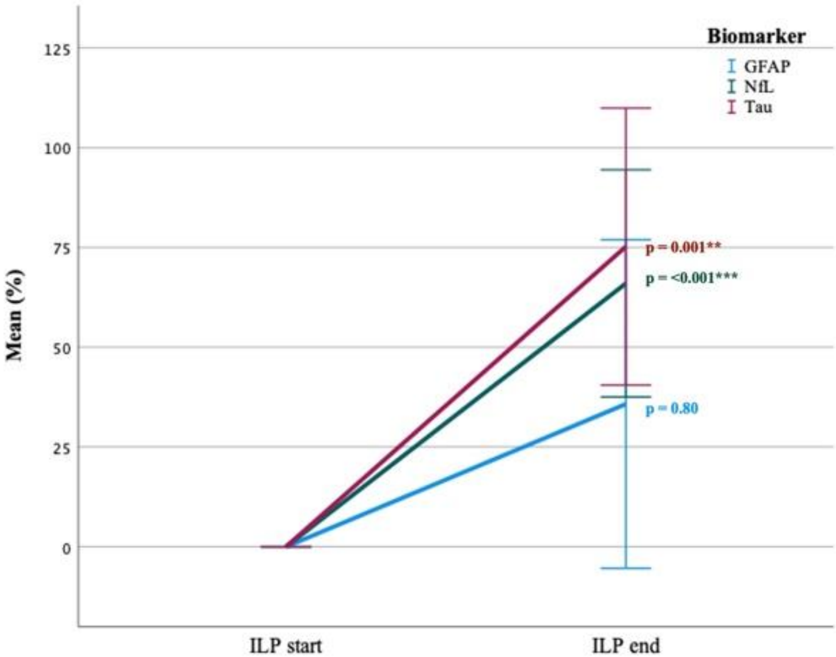

Figure 25. Multiple line of biomarkers mean change (%) from ILP start to ILP end in the isolated extremity. Error bars: 95% CI. Mean delta (%) of biomarkers between ILP start and ILP end. Paired samples T-test. p <0.05, p <0.01**, p <0.001***. With permission by Sage in Perfusion on 7 November 2023, available https://doi.org/10.1177/02676591231213506*

4.3.4 Biomarkers and toxicity reaction

Evaluating postoperative regional toxicity based on the Wieberdink grading scale revealed ten patients with grade II, six patients with grade III, and one patient with grade IV. When testing differences between toxicity grade II and III/IV concerning biomarker levels, no significant difference was seen.

4.3.5 Biomarkers, muscle strength and sensitivity

Muscle strength and sensitivity were evaluated by a physiotherapist the day before surgery and at follow-up visit on day 30. A significant reduction in overall muscle strength was seen postoperative (632 (424–779) vs. 518 (366–603) Nm, p = .01), compared to preoperative. No significant difference noted in either deep or shallow sensitivity between the pre- and postoperative assessments. Additionally, no significant correlation was observed between biomarker levels and changes in muscle strength or sensitivity between the pre- and postoperative assessments, except for a significant correlation identified between GFAP release and heightened sensitivity (p = .03)

4.4 Paper IV

4.4.1 Patients

An overview of the clinical trial flowchart for Paper IV is presented in Figure 27. In total, 40 elective patients scheduled for open-heart surgery were enrolled and randomized in this sub-study. One patient was excluded due to a rupture of the right ventricle necessitating re-do surgery, leaving 39 patients who completed the study and were analyzed.

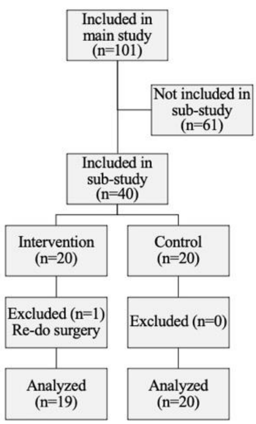

Figure 26. CONSORT diagram showing inclusion paper IV.

The median age of the participants was 72 (range 32 – 84) and the male/female ratio was 30/9. The most common surgery was a single aortic valve replacement (AVR) 33%, followed by single mitral valve repair (MVR) 28%. Patient demographics and baseline characteristics are detailed in Table 12.

Patient characteristics	**Standard flow (n=20)**	**High flow (n=19)**
Age *years,* median (min-max)	71 (32-82)	72 (44-84)
Male *n* (%)	14 (70)	16 (84)
Weight *kg*, mean SD	78 ± 13	84 ± 13
Height *cm*, mean SD	175 ± 9	177 ± 9
BSA Dubois m^2, mean SD	1.93 ± 0.20	2.01 ± 0.19
Creatinine *µmol/L*, mean SD	80 ± 18	89 ± 24
Hematocrit *%*, mean SD	36 ± 5	37 ± 4
Hemoglobin *g/L*, mean SD	120 ± 16	125 ± 14
NfL *ng/L*, median (IQR)	22.1 (12.8; 35.3)	23.8 (13.5; 38.6)
GFAP *ng/L*, median (IQR)	212 (155; 312)	235 (172; 307)
T-tau *ng/L*, median (IQR)	10.5 (8.5; 12.6)	10.7 (9.3; 13.3)
P-tau217 *ng/L*, median (IQR)	3.6 (2.4; 4.6)	5.2 (3.6; 7.1)
NIRS Right *%*, mean SD	66 ± 10	70 ± 13
NIRS Left *%*, mean SD	65 ± 11	68 ± 12
CABG *n*, (%)	0	3 (16)
AVR *n*, (%)	7 (35)	6 (32)
MVR *n*, (%)	7 (35)	4 (21)
Combination valve *n*, (%)	3 (15)	0
CABG + valve replacement *n*, (%)	2 (10)	5 (26)
Supracoronary graft *n*, (%)	1 (5)	1 (5)

Table 12. Patient baseline characteristics. Mean and standard deviation, median and min-max or IQR (25th-75th percentile), numbers and percentage. Aortic valve replacement (AVR), body surface area (BSA), coronary artery bypass grafting (CABG), glial fibrillary acidic protein (GFAP), mitral valve replacement (MVR), near-infrared spectroscopy (NIRS), neurofilament light (NfL).

4.4.2 Biomarker release pattern

Treatment characteristics show an significantly increased CPB flow rate (p<.001), DO_2i (p<.001), SvO_2 (p=.02) and per operative diuresis (p=.047) in the high CPB flow rate group.

All biomarkers exhibited a significant increase ($p<.001$) from the baseline to the highest value of respective biomarkers during the study, varying according to biomarker dynamics. The most marked increases were found for t-tau 598% and p-tau217 330%. However, when comparing the biomarkers between the two flow rate groups no significant difference was found between the two groups at any of the measured time point during the study (Figure 28 A-D).

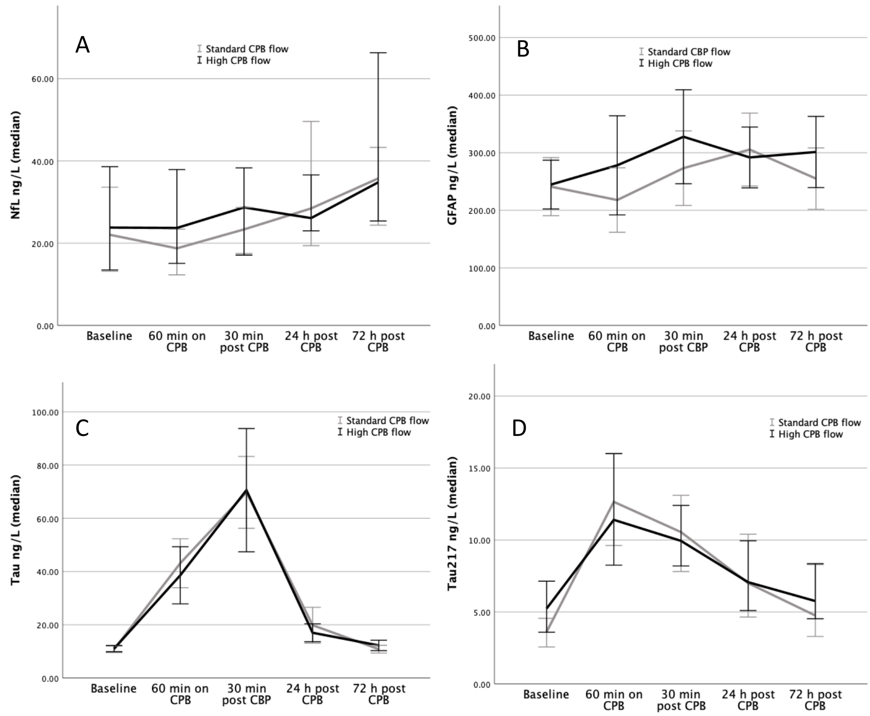

Figure 27 A-D. Multiple line of median biomarker release (ng/L) for randomized CPB flow rate groups during study period. Error bars: 95% CI. Cardiopulmonary bypass (CPB), glial fibrillary acidic protein (GFAP), neurofilament light (NfL).

4.4.3 Biomarkers and patient/treatment characteristics

Relationships were found between several baseline and treatment characteristics and levels of biomarker release. For instance, patients over 72 years of age showed increased levels of NfL (p=.020) and t-tau (p=.009). Additionally, women exhibited lower t-tau levels (p<.001) compared to men, even after adjusting for age. The median CPB time in the study was 117 minutes, and p-tau217 was found significantly increased (p=.002) if the CPB duration exceeded 117 minutes. When the DO2i during CPB was >330ml/min/m^2, there was a significant reduction in t-tau release (p=.004). Moreover, NIRS values ≥ 60% during CPB were linked to lower levels of p-tau (p=.017).

4.4.4 Postoperative delirium

Four patients (20%) in the standard CPB flow rate group were identified with postoperative delirium in the ICU, compared to none of the patients in the high CPB flow rate group (p=.11). When comparing patients with postoperative delirium to those without, NfL was seen significantly increased (p=.007) in the delirium group. Further, the NIRS levels during CPB were significantly lower in the delirium patients (53 vs. 70 %, p=.003).

5 DISCUSSION

The objective of this thesis was to explore physiological responses and alternative approaches during extracorporeal circulation in order to increase understanding of the side effects associated with the procedure. The thesis is based on four studies, even though they contain certain limitations, each study is examining unique aspects surrounding extracorporeal circulation. Papers I and II present a comprehensive investigation into the oxygen requirements of an isolated extremity during ILP. In Paper III, we introduced and validated a novel method for identifying signs of peripheral nerve injury during ILP. Furthermore, Paper IV demonstrated the release pattern of biomarkers associated with brain injury between two different CPB flow rates during heart surgery.

5.1 Ethical considerations

All studies were approved by the Regional or national ethical review board. All participants included received both written and oral information about the studies, with opportunity for questions and discussion.

In Paper I and IV the patients were randomized to either an intervention or to a control group where no intervention was done. There is always a slight risk that the intervention could have unforeseen effects on the patient, as it deviates from the clinical routine. The study protocols approved by the ethical board emphasize how these risks should be minimized, primarily through increased and more frequent monitoring of the patients' vital parameters. In Paper I, the intervention involved excluding blood from the priming solution during ILP, which potentially could compromise the oxygen delivery to the tissue to a certain degree. Continuous monitoring was conducted to ensure that the patient was not exposed to borderline oxygenation throughout the perfusion.

Also, worth noting is that an extremity does not harbor sensitive vital organs, which for example allows certain orthopedic extremity procedures to be conducted in a bloodless field, where the circulation to the extremity can be halted for up to 90 minutes without causing irreversible tissue damage[103]. In Paper IV, cardiopulmonary bypass flow rate was set to 2.9L/min/m^2 in the intervention group, which approximately is a 20 % increase compared to clinical routine. With an increased flow rate, it became necessary in some cases to enlarge the size of the arterial cannula to prevent excessive pressure drops across it. This adjustment posed an increased risk during aortic cannulation, as it required a slightly larger hole to be created in the ascending aorta.

Paper II-III were exploratory studies without interventions, except for taking additional samples, a total of approximal 70 mL for every patient, which deviates from clinical routine.

5.2 Paper I

5.2.1 Methodological considerations

The decision to conduct a randomized study was evident; we wanted to determine if a crystalloid-based solution was at least as effective as a blood-based prime solution in delivering oxygen to the tissue. Our desire to avoid the addition of blood and subsequent transfusion to the patient was motivated primarily by the negative side-effects associated with blood transfusion[104]. We chose to focus on lactate as the primary endpoint and calculated the power based on a patient cohort (28 patients) from the previous year's ILP cases. Regrettably, what we overlooked at that time, was the elevated lactate levels in stored donor blood, which in our study was ranging from 5 to 11 mmol/L. In retrospect, it might have been wiser to use SvO_2 or O_2ER as our primary endpoint. However, the secondary aims included these variables, showing no difference between the groups, strengthening the study's primary result. Another methodological reflection was that we should have reported the indexed values for DO_2 and VO_2 (extremity's BSA) to make it more comprehensible, as research primarily presents indexed oxygen parameters. A leg surface area is approximately 18% of total BSA[26], so for an estimation of how the DO_2 and VO_2 values in Paper 1 correspond to DO_2i and VO_2i, these formulas could be employed for approximation:

$$DO_2 i = \frac{DO_2}{BSA x 0.18} \qquad VO_2 i = \frac{VO_2}{BSA x 0.18}$$

5.2.2 Main findings

The primary finding was that there was no difference in lactate levels between the groups during perfusion. A lactate level of ≤4.0 mmol/L was achieved for all included patients, with the highest measured lactate level during ECC in the intervention group being 2.9 mmol/L. The secondary endpoints, revealing no difference between groups regarding DO_2, VO_2, O_2ER or regional SvO_2, strengthens the conclusion that ILP of the lower extremity, with or without packed red blood cells, resulted in comparable perfusion quality concerning meeting the tissues oxygen requirements.

5.2.3 Strengths and limitations

Strengthening the study is the randomization, showing consistent results supporting, in both primary and secondary endpoints, the safety of using a crystalloid solution to meet tissue oxygen requirements during ILP. Limitations include the small sample size; however, it is a power-calculated sample and represents a typical group of ILP patients. Ideally, we should have stratified the randomization between arms and legs to ensure an equal representation of both. Only two arms were included, and both were randomized into the control group. Subsequently, these two arms were excluded from the analysis, and the results now exclusively relate to ILP on lower extremities. We acknowledge the concerns associated with post-randomization exclusion and need to transparently address its implications. Our approach for the acceptability of the exclusion of arms was to mitigate the risk of overgeneralization. Drawing conclusions without analyzing arms in the intervention group, could lead to erroneous extrapolation, compromising study reliability, external validity and, more importantly, patient safety.

5.3 Paper II

5.3.1 Methodological considerations

This study was an prospective explorative study, including 39 patients from Papers I and III. Due to the stringent exclusion criteria of leakage ≤0 ml blood from the systemic circulation to the isolated extremity, 18 patients were excluded. This strict threshold was necessary to ensure that the measurement conditions remained consistent throughout the perfusion. The oxygen calculation formula include pump flow, saturation, and hemoglobin. To calculate extremity oxygen metabolism, the environment could not be uncontrollably influenced by fluctuations of hemoglobin from the systemic circulation to the extremity. During ILP, leakage typically occurs in about every other patient. Therefore, the expected exclusion of almost 50% of the patients due to leakage from the systemic circulation to the extremity was anticipated. Since the study aimed to map oxygen delivery, consumption, and extraction and describe oxygen metabolism in the extremity, the absence of leakage was necessary to make the calculations feasible. The circumstances created enabled us to draw general conclusions that strengthens the external validation of the study concerning oxygen metabolism in an isolated extremity during ILP.

To assess the level of agreement between the significant correlation $SveO_2$ and INVOS we used the Bland-Altman plot[105, 106] and our predefined acceptable between – method error was 30% based on Critchley and Odor's research on comparing cardiac output measurements[107, 108]. Acceptance criteria may vary depending on the context of the study and practical implications. Of course, aiming for a small spread within the limit of agreement is desirable. However, upon reflection, our predefined acceptable error may have been set too narrowly, considering the possible context and implications of the measurement, along with the significant correlation observed between the two methods.

5.3.2 Main findings

The primary aim was to measure and characterize oxygen metabolism in an isolated extremity during ILP, secondarily, to explore whether rSO_2, assessed using the NIRS technique, correlated and could be used interchangeably with invasive measurements of muscular oxygenation during ILP. (i.e., blood gas sampling). After calculating and demonstrating DO_2i, VO_2i and O_2ER during perfusion in all patients, we found that if DO_2i was kept above 170 ml/min/m^2, O_2ER consistently was below 30% in all observations. A significant correlation was also observed between rSO_2 values and $SveO_2$, with only a moderate agreement between the two methods (error 32.5%).

The results contributed to a comprehensive understanding of an extremity's oxygen requirements. This offers the potential of using goal-directed oxygen delivery to the extremity tissue. Our findings revealed that DO_2i were nearly identical for both arms and legs during perfusion. However, O_2ER and VO_2i

were lower in arms compared to legs, suggesting a lower metabolic demand in arms than in legs. This was further supported by the gradual increase in lactate values in legs during perfusion (highest value 2.9 ± 1.0 mmol/L). Despite this slight increase in lactate, O_2ER never surpasses 24 ± 9 % during the perfusion. This O_2ER increase, despite higher NIRS saturation on ECC perfused extremities, could possibly be attributed to the elevated temperature (40°C) which often aligns with increased oxygen metabolism[109].

5.3.3 Strengths and limitations

This study, to our knowledge, is the first to measure and describe extremity indexed DO_2, VO_2, and O_2ER in an isolated extremity. Limitation include the variation of prime solutions administered to arms and legs. Another limitation is the relatively small sample size; yet our measurements are conducted within subjects and compared to their initial values. This, combined with the inclusion of only patients with no leakage, enhances the validity of the findings despite the small sample size and variations in prime solutions.

5.4 Paper III

5.4.1 Methodological considerations

This single-center feasibility trial included 18 patients scheduled for ILP. The ILP treatment is a so called "National highly specialized care" in Sweden. Which means that Sahlgrenska University hospital, Gothenburg is the only center performing ILP. Normally, for patients who have traveled a long distance, the follow-up assessment is overseen by physicians from their local hospital, assuming everything proceeds as expected. We therefore focused our inclusion on prioritizing patients referred from nearby hospitals, with the aim of minimizing the need for extensive travel for the patients for the follow-up at 1 month. The enrollment of patients therefore proceeded at a slow pace. Normally, there are 20–25 ILP procedures conducted annually, involving patients from across Sweden and the Nordic countries. After including only 10 nearby patients during the first year, we decided to remove the assessment by the physiotherapist at the follow-up visit at our facility. Resulting in that only 10 out of 17 patients were assessed by a physiotherapist. Sample collections were arranged to take place at the patients' local hospitals. The treatment response and regional toxicity were assessed at the patient's local hospital and were reported back to us.

5.4.2 Main findings

In this study, the aim was to examine potential alterations in biomarker expression during ILP in an isolated extremity. Secondarily, we perused whether the extent of biomarker release aligns with regional toxicity, alterations in muscle strength, and/or postoperative sensitivity. The study revealed a significant increase, more than twice, in NfL and tau levels in the isolated extremity compared to the systemic circulation at the end of perfusion. Also, NfL and t-tau levels exhibited an approximately 70% significant increase between start and end of the perfusion in the isolated extremity. GFAP is specifically recognized as a biomarker associated exclusively with changes in the central nervous system[95], unlike NfL and Tau, which also indicate effects on the peripheral nervous system[87]. Samples taken during perfusion did not indicate an impact on biomarker release in the central nervous system during ILP, however, the postoperative samplings in the system circulation on day 3 and day 30 showed a significant increase in both NfL and GFAP release, suggesting that ILP also affects the central nervous system.

For the secondary endpoints, no significant correlation was observed between biomarker levels and regional toxicity, muscle strength, or sensitivity. However, the physiotherapist's assessment indicated a significant decrease in muscle strength postoperatively. Which might be contributed to postoperative swelling and immobilization. To establish a potential correlation, a larger study population might have been necessary, or the use of alternative assessment tools, such as electroneuronography, which could potentially detect more subtle alterations.

5.4.3 Strength and limitations

The use of the ultrasensitive Single Molecule Array (Simoa) and the expertise of researchers at the Clinical Neurochemistry Laboratory at the University of Gothenburg for biomarker analysis gave great strength to this study, given the highly reliable nature of the test results. Additionally, to the best of our knowledge, this study represents the first demonstration that isolated peripheral nerves exposed to chemotherapy during ILP exhibit measurable signs of damage, detectable in blood.

This feasibility study was conducted with a small study population, focusing on addressing and answering the primary endpoint. Additionally, it explored highly interesting secondary endpoints. However, due to the limited sample size, definitive conclusions should not be drawn from the secondary outcomes.

5.5 Paper IV

5.5.1 Methodological considerations

Paper IV was a sub-study of the randomized controlled trial ICAROX2 by Wijk et al. The initial 40 patients out of a total of 101 included patients were part of this study, were this sub-study aimed on examining biomarkers for brain injury and oxygen parameters, while the main study examined the kidney, between two different CPB flow rates. A clear advantage of the study setup was the collaborative effort of multiple clinical researchers involved in screening patients for eligibility, providing information, facilitating inclusion, sample collection, and managing the clinical procedures, which reduced the impact of the pandemic, ensuring that the pace of inclusion remained reasonably unaffected, and each study outcome was explored using the same patients. However, the primary outcome in this sub-study was the biomarker release pattern, and even though increased biomarker levels indicate brain injury, we did not deeply evaluate the clinical postoperative outcome of the patient in this study. Meaning, that the study could not determine whether the levels of biomarkers had clinical relevance in the patients cognitive outcome, which of course would have been desirable, but also expensive.

5.5.2 Main findings

The main objective of the study was to evaluate the effects of high or standard CPB flow rate on biomarker release pattern for brain injury. No significant differences were found between the groups in the biomarker release pattern at any of the measured timepoints. This finding was contrary to our hypothesis, where we expected that higher oxygen delivery would decrease the level of biomarker release. The lack of differences in biomarker levels between the groups may be attributed to the ability of the standard CPB flow rate to exceed the recommended oxygen delivery threshold[110]. Regarding the secondary outcome, we found that age, male gender, $DO_2i < 330$ ml/min/m^2, and NIRS levels < 60% were all associated with elevated biomarker levels. These findings enhance our understanding of potential risk factors that adversely may affect the brain. Interestingly, delirium was detected in 20% of the patients randomized to the standard CPB flow rate group, compared to 0% in the high flow group, although no statistical difference was noted between the groups.

5.5.3 Strengths and limitations

The study's strengths lie in the extensive range of neuronal biomarkers sampled, the high specificity and sensitivity of Simoa technology for biomarker analysis, and the rigorous standardization of CPB procedures and sample collection. However, a limitation was that advanced pre- and postoperative neurocognitive examinations were not conducted, nor was brain imaging performed. Meaning that the study provided no insights concerning associations between the release pattern of biomarkers for brain injury after cardiac surgery and neurocognitive function or structural brain injury.

6 CONCLUSIONS

I. A crystalloid-based prime solution in isolated lower limb perfusions sufficiently meets the tissue's oxygen needs.

II. An oxygen delivery above 170 ml/min/m^2 during isolated limb perfusion meets the tissue's oxygen demand in both upper and lower limbs.

III. Near-infrared spectroscopy used for measuring regional oxygen saturation in extremities during ILP, show a significant correlation with invasively measured venous oxygen saturation. However, the two methods do not sufficiently agree to be used interchangeably.

IV. Isolated peripheral nerves exposed to chemotherapy during ILP exhibit measurable signs of damage, detectable in blood.

V. The release pattern of biomarkers associated with brain injury do not significantly differ between a standard or a 20% increased CPB flow rate during cardiac surgery.

VI. Relations were found between increased levels of neuronal biomarkers and age, male gender, DO_2i < 330 ml/min/m^2, and NIRS levels < 60% after heart surgery.

7 FUTURE PERSPECTIVES

Without extracorporeal circulation, many of the surgical procedures routinely performed worldwide today would not have been possible. Since the birth of the heart-lung machine 70 years ago, diligent research has made significant progress, reducing the risks and side effects of the treatment. Despite this, there are still many relatively unexplored areas within ECC that still needs to be explored further, such as the interaction between the brain and ECC, immunological effects of ECC, optimal oxygen requirements during ECC, the vulnerability of the kidney during ECC, and more.

With this thesis, we have sought to address some of these areas. While our results provided valuable insights, they also stimulate additional curiosity and encourage further research with larger populations and even more conclusive aims and endpoints.

Here are some of our ongoing thoughts and perspective on interesting future research: Why do some patients experience severe side effects from ECC, while others tolerate it better? In Paper I, we observed trends indicating that a prime solution without blood led to better treatment response and increased one-year survival. This has prompted us to initiate a larger-scale retrospective study to further investigate this phenomenon.

Now that we know what the tissue requires for DO_2i during ILP (Paper II), could optimized perfusion potentially lead to reduced side effects during ILP? Or could we now possibly, with the help of peripherally measurable neuronal biomarkers, identify patient factors that predict more severe regional toxicity after ILP (Paper III)?

If increased oxygen delivery to the body and brain from CPB does not reduce the biomarker levels for brain injury (Paper IV), the burning question remains of how to identify the phenomenon that leads to such a significant proportion of patients experiencing varying degrees of cognitive dysfunction after cardiac surgery with CPB.

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EPILOGUE

Being granted the chance to pursue a doctorate in this field, which deeply fascinates me, has been a genuine privilege. The education has proven to be an incredibly enriching journey. Beginning with stumbling steps, with research questions and designs that I would likely approach differently today, is an insight that somewhat demonstrates personal advancement during the doctoral studies. But also, acquiring greater knowledge has not entirely come for free and without strain. Extensive sampling 24/7, endless analysis, a multitude of incomprehensible statistics, considerable frustration including a crashed computer leading to re-writing weeks of work, and many, many "redo, do it right" has also been part of the journey. However, thanks to my highly positive, encouraging, incredibly knowledgeable, and always available supervisor Roger, I have persevered and successfully completed this task. It feels incredibly fulfilling, and I am actually also a bit proud that I persisted and finally reached this milestone. My deepest gratitude to all my colleagues, the clinic, my friends, and my loving supportive family. Thank you for your consistent support, encouragement, and above all, for your patience during times I have been mentally preoccupied and unavailable for you. Without you, none of this would have been possible!

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