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Extracorporeal cardiopulmonary resuscitation, selection and candidacy

CARL-HENRIK ÖLANDER



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Abstract

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Extracorporeal cardiopulmonary resuscitation (ECPR) is an exclusive treatment using extracorporeal membrane oxygenation (ECMO) in refractory cardiac arrest (CA). Treatment is associated with risk of serious complications, including neurologic impairment and renal failure. Success of treatment is dependent on appropriate selection of candidates. The aim of this thesis was to investigate the role of end-tidal carbon dioxide (ETCO₂), as a selection criterion for ECPR. Moreover, to define and describe the potential ECPR-cohort in Sweden and investigate conditions for a national ECPR-program.

Experimental porcine models of CA were used in studies I and II. CA was induced, and cardiopulmonary resuscitation (CPR) was performed. Physiological parameters, biochemical markers and histology were evaluated in relation to ETCO₂. In study I, ECMO was started at a predetermined time of CPR. Levels of ETCO₂ during CPR was found to be associated with the extent of cerebral and renal injury following ECPR. In study II, the same model was used. However, start of ECMO was triggered by a predetermined threshold of ETCO₂ during CPR. Results suggest that ETCO₂ could be used as a marker for brain injury following ECPR.

Extract from The Swedish Cardiac Arrest Registry formed the data basis for studies III and IV. Internationally proposed selection criterion for ECPR was applied to the data. ECPR-eligible cohorts were defined and described. In study III, data on in-hospital cardiac arrests was assessed. Results showed low numbers of ECPR-eligible patients annually. Estimates of gain of ECPR suggested a limited benefit in survival and neurological outcome, if ECMO was started within 60 min. In study IV, data on out-of-hospital cardiac arrests was assessed. Low numbers of candidates suggested that only a minority of ECPR-capable hospitals in Sweden have a population base large enough to justify an ECPR-program.

In conclusion, this thesis has demonstrated a correlation between ETCO₂ during CPR and the resulting injury in brain and kidney following ECPR. It supports its use as selection criteria for ECPR candidacy. The estimated incidence of ECPR-candidates in Sweden is low. Moreover, calculations on gain of ECPR on survival and neurologic outcome is limited. Selection criteria and geography are the major determinants for ECPR-candidacy.

Keywords: Extracorporeal cardiopulmonary resuscitation (ECPR), end-tidal carbon dioxide (ETCO₂), in-hospital cardiac arrest (IHCA), out-of-hospital cardiac arrest (OHCA)

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To my family

List of Papers

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

- I. Ölander CH, Vikholm P, Schiller P, Hellgren L. End-Tidal Carbon Dioxide Impacts Brain and Kidney Injury in Experimental Cardiopulmonary Resuscitation - ECPR. *Shock* 2021 Apr 1;55(4):563-569.
- II. Ölander CH, Vikholm P, Schiller P, Lindblom R, Hellgren L. Extracorporeal Cardiopulmonary Resuscitation Guided by End-Tidal Carbon Dioxide – a Porcine Model. *J Cardiovasc Transl Res*. 2022 Apr;15(2)291-301.
- III. Ölander CH, Vikholm P, Schiller P, Hellgren L. Eligibility of extracorporeal cardiopulmonary resuscitation on in-hospital cardiac arrests in Sweden: a national registry study. *Eur Heart J Acute Cardiovasc Care* 2022 Jun 22;11(6):470-480.
- IV. Ölander CH, Vikholm P, Olovsson I, Hellgren L. The estimated incidence of candidates for extracorporeal cardiopulmonary resuscitation amongst out-of-hospital cardiac arrests in Sweden, a registry-based study. *In manuscript*

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Abbreviations

aB	arterial Blood
AED	Automated External Defibrillator
AKI	Acute Kidney Injury
ANOVA	ANalysis Of VAriance
AV O ₂ diff	Arterio-Venous Oxygen difference
CA	Cardiac Arrest
CBF	Carotid Blood Flow
CO	Cardiac Output
CO ₂	Carbon diOxide
CPC	Cerebral Performance Category
CPP	Cerebral Perfusion Pressure
CPR	Cardio-Pulmonary Resuscitation
CVP	Central Venous Pressure
DO ₂	Delivered Oxygen
ECMO	Extra-Corporeal Membrane Oxygenation
ECPR	Extracorporeal Cardio-Pulmonary Resuscitation
ELSO	Extracorporeal Life Support Organization
EMS	Emergency Medical Services
ETCO ₂	End Tidal Carbon diOxide
FiO ₂	Fraction Inspired Oxygen
HEMS	Helicopter Emergency Medical Services
ICP	Intra Cranial Pressure
IHCA	In-Hospital Cardiac Arrest
ILCOR	International Liaison Committee On Resuscitation
IQR	Intra Quartile Range
LPR	Lactate Pyruvate Ratio
MAP	Mean Arterial Pressure
mPAP	mean Pulmonary Arterial Pressure
NNT	Numbers Needed to Treat
OHCA	Out-of-Hospital Cardiac Arrest
OR	Odds Ratio
PAC	Pulmonary Artery Catheter
PCO ₂	partial Pressure of Carbon diOxide
PEEP	Positive End-Expiratory Pressure
P-IL6	Plasma Inter-Leukin-6

P-NGAL	Plasma Neutrophil Gelatinase Associated Lipocalin
PO ₂	partial Pressure of Oxygen
P-S100B	Plasma Calcium Binding Protein B
P-TNF α	Plasma Tumour Necrosis Factor alfa
PVR	Pulmonary Vascular Resistance
ROSC	Return Of Spontaneous Circulation
SaO ₂	Saturation (arterial) of Oxygen
SBE	Standard Base Excess
SCAR	Swedish Cardiac Arrest Registry
SvO ₂	Saturation (venous) of Oxygen
TOR	Termination Of Resuscitation
VO ₂	Volume of Oxygen uptake
VT	Ventricular Tachycardia
VF	Ventricular Fibrillation

Introduction

Cardiac arrest (CA) is one of the leading causes of premature death amongst the adult population. Survival after conventional cardiopulmonary resuscitation (CPR) is increasing (1). In Sweden this is thought to be due to improved public awareness and knowledge of CPR, as well as an increased presence of public automated external defibrillators (AED), considerably shortening time to first treatment (2). However, despite best possible treatment, some patients will remain in refractory CA and not achieve return of spontaneous circulation (ROSC). For a selected few of these patients, treatment with extracorporeal cardiopulmonary resuscitation (ECPR) could be an option. By incorporating a machine to the circulation which pump and oxygenate the blood, vital organ perfusion can be restored and time for definitive treatment of the cause of the CA prolonged.

ECPR treatment is a resource demanding and costly therapy with risk of serious complications but could potentially save lives otherwise lost when using CPR alone. Thus, it is understandable that numbers of ECPR treatment has rapidly increased during the last decade (3). Being a fairly novel treatment, many issues remains however to be resolved. Perhaps most importantly, which patients are suitable for treatment. Currently, there is no scientific consensus on selection criteria for ECPR, but whatever criteria are used, it inevitably affect numbers available for treatment. As for any rare treatment, too few cases per centre risks to affect the success rate (4).

It was the aim of this thesis to investigate one of the proposed selection criteria for ECPR, end-tidal carbon dioxide (ETCO₂), and to define the potential ECPR-cohort in Sweden.

Background

Extracorporeal membrane oxygenation (ECMO)

In the early -70s, extracorporeal membrane oxygenation was introduced in the United States by the cardiac surgeons Hill and Bartlett. The treatment was first successfully used on an adult patient suffering from acute respiratory distress syndrome (Hill 1971) and a 3-year-old boy with postoperative cardiogenic shock after a Mustard operation (Bartlett 1972). In 1975, Bartlett successfully treated “Baby Esperanza”, a premature suffering from infant respiratory distress syndrome, caused by aspiration of meconium at delivery. In the coming years and following decade, treatment with ECMO was implemented worldwide, predominantly and most successfully in the neonatal setting. In 1989, the first meeting of the Extracorporeal Life Support Organization (ELSO) was organized. During the H1N1-epidemic and the publication of the Caesar trial in 2009 (5), ECMO became more generally known to the public. This increased political interest in the treatment and made funding more available.

The principles of treatment are based on a machine pumping and oxygenating the blood instead of the patient's heart and lungs. Large bore venous canulae drain blood to an extracorporeal circuit, whereafter it is mechanically propelled through an oxygenator and returned to the patient by a second canula, fig 1. Initially this was achieved by using a regular heart lung machine in the operating theatre. Technological advancements have provided us with more efficient low resistance oxygenators due to the invention of poly-methyl-pentene fibres as well as centrifugal pumps and heparin-coated circuits. The equipment has decreased in size and become transportable, making implementation of treatment more easily available outside the theatre.

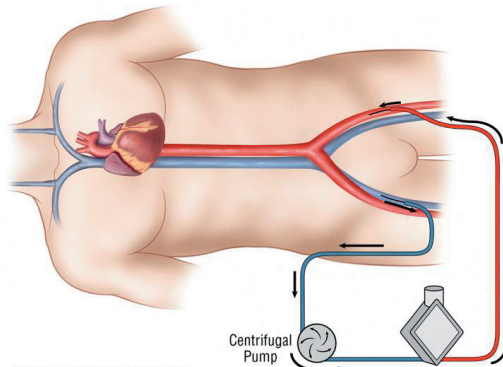


Figure 1: Schematic figure of peripheral VA-ECMO circuit with venous drainage, centrifugal pump, oxygenator and arterial return canula.

Despite technological advancements, ECMO is still encumbered with risk of serious and potentially lethal complications such as bleeding, coagulation disorders, with or without haemolysis, peripheral or central gas-/thrombi-embolism, severe infections and technical failures interrupting systemic circulation and gas exchange. Hence, must the potential benefit of treatment be carefully weighed against its risks.

Extracorporeal cardiopulmonary resuscitation (ECPR)

ECMO assisted cardiopulmonary resuscitation was first attempted by Mattox in 1976 (6) but has become more commonly used in the new millennium. Retrospective studies have shown improved survival compared to conventional CPR (7–9). The CHEER-trial from 2015, initially reported increased survival when ECPR was used in combination with hypothermia and early reperfusion, but the reported improvement was later lost (10). Over the years, ECPR survival rates remain unchanged around 30% (11).

Lately the first randomized controlled trial (12) was interrupted on interim due to ethical considerations on the superiority of ECPR to conventional CPR. However, an expert opinion from the International Liaison Committee on Resuscitation (ILCOR) state that; “ECPR may be considered if there is a potentially reversible cause of a cardiac arrest that would benefit from temporary cardiorespiratory support”, but; “there is insufficient evidence to recommend the routine use of ECPR in cardiac arrest” (13).

Unfortunately, several patients suffer permanent complications from ECPR, mainly affecting brain and renal function which renders them healthcare dependent for the rest of their lives (14,15). The difficulties thus lie

in being able to predict which patients will benefit from the treatment and for whom it will only cause long-term suffering.

Predictive factors for outcome in ECPR

In an attempt to improve accurate selection of patients suitable for ECPR, retrospective studies on outcome have been performed in search for prognostic factors of survival and neurological function (16–18). They have identified minimal no-flow time (absence of CPR), shockable rhythm (ventricular tachycardia/-fibrillation), short low-flow time (i.e., CPR), high pH and low arterial blood Lactate, as positive predictors. Advanced comorbidity is an evident negative predictor. Despite this knowledge, is the rate of survival unchanged over the years (11). Currently there is no international consensus on selection criteria for ECPR.

End-tidal carbon dioxide in cardiac arrest

The desired objective of prognostic markers is to enable assessment of the patient's physiological condition, at the time of decision to start ECPR treatment or not. This is in turn dependent on the quality and effect of the resuscitation performed during CPR.

End-tidal carbon dioxide (ETCO₂) is defined as the partial pressure of carbon dioxide (CO₂) at the end of an exhaled breath. It is regularly used to confirm accurate position of endotracheal tubes and to adjust ventilation in intubated patients. CO₂ is the product of aerobic metabolism, which is released from the tissues and via the blood stream returned to the lungs to be exhaled. The level of ETCO₂ is thus determined by the production of CO₂, cardiac output (CO) and ventilation. In the event of CA, production can be assumed to be unaltered. If ventilation is kept constant, ETCO₂ will reflect CO, i.e., the effect of CPR.

ILCOR includes ETCO₂ in their recommendations during CPR for confirmation of endotracheal position, quality of compressions and for detection of ROSC (19). They have refrained to specify a threshold of ETCO₂ when further attempts to resuscitate is no longer indicated. In summary, ETCO₂ can be considered a recognized and established measurement of use in conventional CPR.

Conditions for ECPR in- and out-of-hospital

Besides the patient's physiological status, is the location of the arrest affecting predictive factors for outcome of ECPR. If suffering CA when admitted to hospital (IHCA) you are more likely to be monitored, which increases chances of detection and rapid intervention. Resuscitation will be performed by professionals and definitive treatment of the cause of the cardiac arrest, as well as ECPR, is readily available. On the other hand, hospitalization indicates some degree of comorbidity which might decrease chances of success, or possibly even disqualify you from ECPR treatment. If you are out-of-hospital (OHCA), chances of the arrest being witnessed is lower which probably affects time of no-flow. CPR is more likely performed by lay-men and time to first defibrillation is probably delayed. Time of low-flow is prolonged as compared to IHCA, as transport to an ECPR-capable hospital is required.

Consequently, geographical location of ECPR capable hospitals, population density in different regions, infrastructure and means of transportation are also of importance when ECPR is discussed. It might be that the different conditions of IHCA and OHCA warrant different selection criteria. The benefits, risks, outcomes and cost effectiveness of ECPR should probably be analysed separately in the two groups.

The Swedish Cardiac Arrest Registry (SCAR)

There are no national directives, nor national consensus on selection criteria for ECPR in Sweden as of today. It is also not known the proportion of IHCA and OHCA that are potential ECPR candidates. There is however a national registry with both IHCA and OHCA included, containing detailed information about the patients, the resuscitation and its outcome. It is a validated registry of quality started in 1990. It is financed by the Swedish government with grants from the Swedish Resuscitation Council and today includes 98% of all CA in Sweden. It is an often-used registry for research. During 2021, nineteen articles based on data from SCAR, were published in reputable journals.

Aims

General aims

- To create a reproducible experimental model for CPR and ECPR in cardiac arrest.
- To investigate the correlation between ETCO_2 during CPR, and any possible damage in brain or renal function, following ECPR.
- To experimentally investigate the value of ETCO_2 during CPR, as a marker for start of ECPR.
- To describe and define the potential cohort of candidates for ECPR in IHCA and OHCA.
- To estimate the theoretical effect on survival and neurological outcome, if ECPR is performed.
- To investigate geographical and demographical conditions for a national ECPR program in Sweden.

Specific hypotheses

Study I

ETCO_2 during CPR, correlates to organ damage in brain and kidney following ECPR, in experimental refractory cardiac arrest.

Study II

In experimental refractory cardiac arrest, can the level of ETCO_2 during CPR be used - as a complement to time, to guide initiation of ECPR treatment. This is reflected by the degree of organ damage in brain and kidneys following ECPR.

Study III

Numbers of eligible candidates for ECPR in IHCA are few and strongly dependent on selection criteria. There is a limited gain on survival and neurologic outcome if ECPR is performed.

Study IV

Numbers of candidates for ECPR in OHCA are few and strongly dependent on selection criteria. The geographical location of the CA is decisive for the probability of ECPR being performed.

Methods

Experimental models and data source

In studies I and II, were a porcine based experimental model of refractory CA constructed. The studies were approved by Uppsala Ethical Committee on Laboratory Animal Research. Under anaesthesia was ventricular fibrillation (VF) inflicted, using electrical current. Conventional CPR was performed by means of mechanical compressions, whereafter ECPR was initiated. The model was constructed to allow optimal conditions for ECPR by preparing and cannulating the animals before induction of CA.

Cannulas were placed in the Femoral- vein and artery for the use of ECMO. For physiological monitoring, infusion of drugs and blood/urine sampling, were the Carotid-, Femoral-, Pulmonary arteries and the Jugular and Sagittal veins, as well as the lateral cerebral ventricle and urinary bladder cannulated. An ultrasonic flow-sensor was applied on the left carotid artery. During the experiment was physiological parameters recorded and blood sampled, for later measurement of levels of plasma neutrophil gelatine associated lipocalin (P-NGAL) and plasma calcium binding protein B (P-S100B). In study II was microdialysis of the brain added to the protocol. At end of the experiment the animals were euthanized, and brains and kidneys harvested for histological examination.

In study III and IV was data of witnessed IHCA and OHCA between 02-01-2015 and 30-08-2019 extracted from SCAR, after receiving ethical approval from the Swedish Ethical Review Authority. By applying internationally suggested selection criteria for ECPR on available data from SCAR, the potential ECPR cohorts could be defined. Additional data in the registry, made it possible to describe demographical and geographical properties of the cohorts.

Measurements and analytical methods

Cardiac output and thermodilution

The pulmonary artery catheter (PAC), also known as the Schwann Ganz catheter was invented in 1970. It is used for measuring pressures of the right side of the heart, filling pressures of the left atrium and cardiac output (CO). It is

inserted by venous access through the right atrium, passes the tricuspid valve and through the right ventricle, to end up with its tip placed in the pulmonary artery. It is equipped with a thermistor and an inflatable balloon at its distal end. When inflated, the filling pressures of the left atrium is reflected via the capillaries of the lung, to the pressure sensor at the tip of the PAC.

Thermodilution is based on the principle of measuring flow through the heart, i.e., CO. By injecting cold saline of a known temperature in the pulmonary artery, and measure the temperature drop over time at a known distance away, a thermodilution curve is achieved. The degree of temperature difference is directly proportional to CO, i.e., slow temperature drop indicates low CO, and a rapid temperature drop indicates high CO.

In this study was the PAC “floated” through the heart, using the balloon. Its position in the pulmonary artery was verified by the specific signature of the pressure wave form of the pulmonary artery. Three injections of cold saline were performed, CO was calculated, and the average was considered the baseline CO of the animal.

Biochemical markers

Plasma calcium binding protein B (P-S100B)

S100B is present in adipocytes, striated muscle, enteric glial cells, chondrocytes, melanocytes and heart muscle. The highest protein concentrations have been detected in astroglia cells (20). Circulating levels of S100B produced by cells outside the central nervous system is minor compared to levels seen after traumatic brain injury (21) and can be considered insignificant compared to intracranial sources (22). It is quickly eliminated due to a half-life of 30 to 90 minutes (23).

S100B is a calcium binding protein regulating intracellular levels of calcium, which enables several intracellular and extracellular activities such as differentiation, proliferation and motility (24). It is also actively excreted into the extracellular fluid. In the neuronal tissues it acts as a neurotrophic factor and is involved in tissue repair (25). S100B released within the central nervous system cannot pass the blood-brain-barrier unless its integrity is disrupted, as in trauma or ischemia (26,27).

To our knowledge there is no known lower threshold in porcine, for when S100B is to be considered pathological. Depending on methods used, the corresponding level in humans is commonly set to 0.1 ng/ml (28). Normal values have reliably been shown to exclude major central nervous system injury (29).

It was the intention of this thesis to measure the P-S100B in relation to levels of ETCO₂ during CPR and any histopathological findings in the brain following ECPR. Analyses was performed, using enzyme-linked immunosorbent assay.

Plasma neutrophil gelatine associated lipocalin (P-NGAL)

NGAL (also called human neutrophil lipocalin) is a protein expressed in neutrophils, and in low levels of epithelial cells in the prostate, kidney, respiratory and alimentary tracts (30). It has a bacteriostatic effect by sequestering iron, preventing its use and limiting bacterial growth (31). In the kidney it is excreted by the tubular cells during stressful conditions, mainly into the urine (32).

NGAL exist in monomeric and dimeric forms, of which the renal tubules mainly produce the monomeric form (33). However, the predominant isoform found in plasma in patients with acute kidney injury (AKI) (induced by cardiac surgery) are dimeric and thus more likely of neutrophilic origin (34). Most commercially available methods for analysis of NGAL do not discriminate between isomers but have still shown to be indicative of acute kidney injury (35). Furthermore, in respect to severe AKI, comparison of NGAL in urine and plasma, were found to be in favour of P-NGAL (36).

It was the intention of this thesis to measure P-NGAL in relation to levels of ETCO₂ during CPR, as well as to urinary output and any histopathological findings in the kidney following ECPR. P-NGAL was analysed using porcine specific enzyme-linked immunosorbent assay.

Histology

Tissues from kidneys and brains were fixated in formaldehyde and blocked in paraffin. Three large sections were obtained from the kidney (cranial pole, middle, and caudal pole including cortex and medulla) and from the brain three different parts including cortex (parietal, frontal and occipital), brain stem (medulla, midbrain and thalamus) and cerebellum. Sections of 4µm were stained with haematoxylin and eosin. Lesions were described, and their degree of severity and distribution were scored.

The histopathological changes in kidneys were grouped into, 1) Distension of Bowman's space, 2) Hyperaemia/pooling of blood in lumen of vessels, 3) Damage to tubular epithelium to cortex and medulla and 4) Other findings such as focal haemorrhage and fibrin in the vessels. The histopathological changes for the brains were grouped into, 1) Red neurons (ischaemic damage), 2) Neutrophil vacuolation, 3) Perivascular oedema and 4) Haemorrhages (extravasation of blood into the brain white or grey matter). Histopathological evaluations and blocking of organs were performed blindly by a veterinary pathologist, without knowledge of levels of ETCO₂ or CPR-times.

Microdialysis

Microdialysis is a method for continuous sampling of substances in the interstitial fluid and can be described as a biosensor. Starting with trials on animals

in the -70s, it plays an important role in modern neurological intensive care, for early detection of potential pathological events.

A microdialysis catheter is inserted in the tissue to mimic the function of a capillary. Chemical substances from the interstitial fluid diffuses to the circulating dialysis fluid inside the catheter, where it is collected, and the concentrations can be determined. The method has previously been used in research, to evaluate brain injury in the CPR setting (37–39).

According to recommendations by Bellander et al (40), frontal parts of the brains were used as locus for insertion of the microdialysis catheters. Urea was used as an endogenous control for microdialysis catheter performance. Substances of importance for detection of ischemia were analysed.

Glucose

Low levels can be caused by ischemia but is dependent on metabolism and must be analysed in respect to systemic levels.

Lactate

is not alone a reliable marker of ischemia but is also dependent on metabolism. To facilitate the interpretation, it is used as the numerator to pyruvate in a ratio.

Pyruvate

is produced in the anaerobic part of glycolysis and enters the cycle of Krebs, provided the presence of adequate amounts of oxygen, or it is else converted to lactate.

Lactate pyruvate ratio (LPR)

is a measurement of the mitochondrial function. Values exceeding 25, is considered pathological in humans and an early sign of ischemia.

Glycerol

constitutes an important part of the cell membrane. Increased levels are an indication of cell membrane decomposition, potentially secondary to apoptosis or ischemia.

Glutamate

is a neurotransmitter with energy dependent reabsorption. Increased levels are a sign of energy depletion in the cell, potentially caused by ischemia.

Cerebral performance category (CPC)

The Glasgow-Pittsburgh CPC-scale is widely used in research and quality assurance to assess neurologic function following CA. The scale ranges from 1 (intact cerebral function) to 5 (brain death). CPC-score 1 and 2 (moderated cerebral disability, but independent) is considered favourable outcomes by the Utstein reporting template, adopted by ILCOR (41).

Study I

Experimental protocol

The porcine model of CA described above, was used to investigate whether ETCO_2 during CPR, correlates to organ damage in brain and kidney following ECPR.

Ten animals with an average weight of 46 (41-50) kg were anesthetized and then tracheotomized. The ventilator was set to FiO_2 0.3, a PEEP of 5 cmH_2O and tidal volumes were adjusted to normo-ventilate the animals. After extensive cannulation, were the animals let to stabilize whereafter baseline measurements were performed.

CA was induced and mechanical compressions started. Sampling of blood and urine, and recordings of physiological parameters were performed according to the timetable of figure 2.

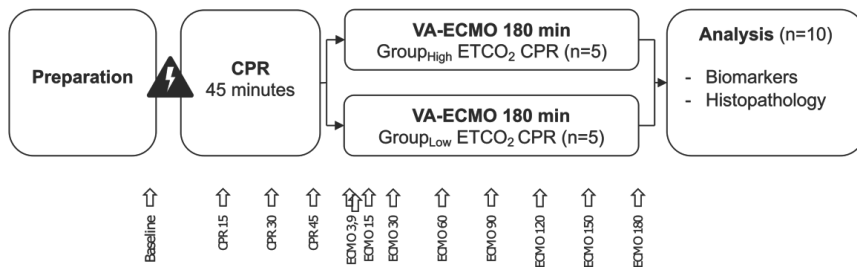


Figure 2: Graphical presentation of protocol in Study I. Arrows describe point in time for sampling. Numbers next to arrows, describe minutes after start of CPR or ECMO.

After 45 minutes of refractory CA, was the mechanical cardiac compression device stopped, and ECMO started. The blood flow was set to correspond to the measured CO at baseline.

After a total time of 225 minutes, was the experiment terminated and the animals euthanized. Kidneys and brains were harvested for histological examination.

Measurements and analyses

Physiological parameters were registered using standard equipment

Thermodilution, by means of PAC was used for calculation of CO. Quantification of NGAL and S100B was performed using porcine specific ELISA. Histological specimens from brains and kidneys were blindly analysed. Pathological changes in each slide were graded in severity from 0-4 and summarized, giving a total score for that specific area.

Statistics

The protocol stated to divide the cohort in two groups (G_{high} and G_{low}) according to the levels of ETCO_2 at the end of CPR. The level was set to 10 mmHg. Due to the low number of animals, results were presented with inter-quartile range (IQR) and a non-parametric analysis was performed.

To reduce the effect of differences in baseline between groups and influence of random variability between individuals, a repeated measurement mixed effect model was used, whereafter statistical analysis was performed with analysis of variance (ANOVA). P-values of less than 0.05 were considered statistically significant.

Study II

Experimental protocol

The same porcine model of CA was used to investigate whether the level of organ damage in brain and kidney following ECPR, is more dependent on levels of ETCO_2 during CPR, than time of CPR.

Twelve animals with an average weight of 51 (46-60) kg were anesthetized and endotracheally intubated. The ventilator was set to FiO_2 0.3, a PEEP of 5 cmH_2O and tidal volumes were adjusted to normo-ventilate the animals. After extensive cannulation, including placement of a microdialysis catheter in the left frontal cortex, were the animals let to stabilize whereafter baseline measurements were performed.

CA was induced and mechanical compressions started. In this study was CPR continued as long as ETCO_2 was measured higher than 10 mmHg. ECMO was started and mechanical compressions stopped when ETCO_2 fell below 10 mmHg for more than 1 minute. Time for start of ECMO was registered. The ECMO blood flow was set to correspond to the measured CO at baseline. ECMO treatment persisted for 180 minutes.

Sampling of blood, urine, microdialysis fluid and recordings of physiological parameters were performed according to the timetable of figure 3. After termination of the experiment were the animals euthanized and kidneys and brains were harvested for histological examination.

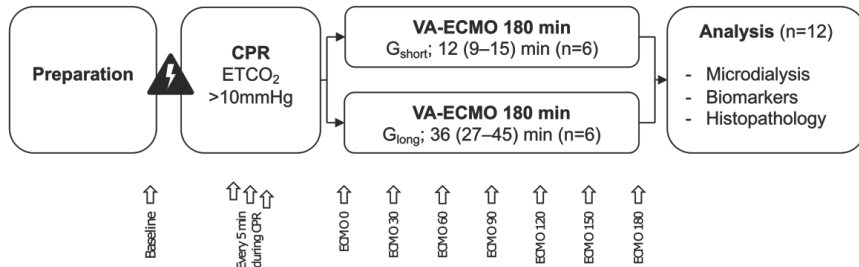


Figure 3: Graphical presentation of protocol in Study II. Arrows describe point in time for sampling. Numbers next to arrows, describe minutes after start of CPR or ECMO.

Measurements and analyses

Physiological parameters were registered using standard equipment.

Thermodilution, by means of PAC was used for calculation of CO. Quantification of NGAL and S100B was performed using porcine specific ELISA. Histological specimens from brains and kidneys were blindly analysed. Pathological changes in each slide were graded in severity from 0-4 and summarized, giving a total score for that specific area. Microdialysis fluid was analysed for glucose, lactate, pyruvate, LPR, glycerol and glutamate. Urea was used as an endogenous control.

Statistics

The protocol stated to equally divide the cohort in two groups (G_{short} and G_{long}) according to time of CPR. Six animals were assigned to each group. Due to the low number of animals, results were presented with inter-quartile range (IQR) and a non-parametric analysis was performed. To reduce the effect of differences in baseline between groups and influence of random variability between individuals, a repeated measurement mixed effect model was used, whereafter statistical analysis was performed with analysis of variance (ANOVA). P-values of less than 0.05 was considered statistically significant.

Study III

Selection of study groups

Following ethical approval, data on witnessed intra-hospital cardiac arrest (IHCA) between 02-01-2015 and 30-08-2019 was extracted from SCAR. The registry provided information on the patients (age, sex, any presence of diabetes mellitus, previous myocardial infarction, stroke or cancer), details of the CA (primary rhythm, time of no-flow and low-flow as well as time to ROSC)

and the result of CPR (hospital survival, CPC-score at discharge and 30-days mortality).

Patients with a no-flow time exceeding 5 minutes as well as patients suffering from onset of stroke during the same period of hospitalization, or if metastasized cancer was diagnosed were excluded. Due to lack of international consensus on selection criteria for ECPR, we decided on two groups of arbitrary, but commonly used selection criteria, fig 4. Group_{restrictive} included patients 18-65 years of age with a primary shockable rhythm. Group_{liberal} included patients 18-70 years of age, independent of primary rhythm and/or with any observed sign of life.

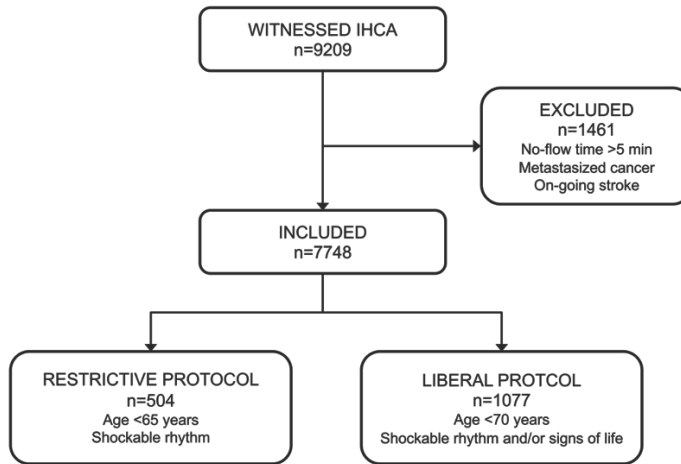


Figure 4: Flow-chart for selection of candidates for ECPR in Study III

Statistics and estimates

Descriptive data from SCAR defines the cohorts.

To calculate the estimated gain on survival and neurological function of ECPR compared to CPR, the outcome of CPR was plotted as a function of time. CPR was ended because of ROSC, or futility. The outcomes, in terms of survival and CPC-score at discharge were defined for later comparison.

Patients with ongoing CPR were considered ECPR candidates. Start of ECPR was assumed to be equivalent to ROSC in the conventionally treated group. Potential risks or advantages of ECPR were thus disregarded of. By using logistic regression for survival and CPC-score of CPR at the different time-points for start of ECPR, could the results be compared. The estimated gain of ECPR on survival and CPC-score at discharge could be calculated, and odds ratios (OR) and numbers needed to treat (NNT) be presented.

Study IV

Selection of study groups

Following ethical approval, data on witnessed out-of-hospital cardiac arrest (OHCA) between 02-01-2015 and 30-08-2019 was extracted from SCAR.

The registry provided information on the patients (age, sex), details of the CA (no-flow time, any occurrence of lay-man CPR, the identity of the responding emergency medical service (EMS) station, primary rhythm, termination of resuscitation (TOR) prior to hospital arrival, any occurrence of incipient or permanent ROSC at hospital arrival) and the result of CPR (hospital survival, CPC-score at discharge and 30-days mortality).

Patients with a no-flow time exceeding 5 minutes were excluded. As in study III, two groups of arbitrary but commonly used selection criteria were constructed, fig 5. Group_{restrictive} included patients 18-65 years of age, with a primary shockable rhythm. Group_{liberal} included patients 18-70 years of age, independent of primary rhythm. Patients with ROSC at hospital arrival were excluded.

All hospitals in Sweden with cardiothoracic surgical services were considered ECPR capable hospitals. EMS-stations with an estimated time of transportation exceeding 40 minutes were excluded. All helicopter emergency medical services (HEMS) were included.

With the purpose to explore our estimate of time of transportation, was a subgroup analysis of the region of Uppsala performed. By interrogation of EMS/HEMS journals could the geographical location of the arrest be pinpointed, and the exact time of transportation be calculated. The aetiology of the CA was verified by using information in medical records.

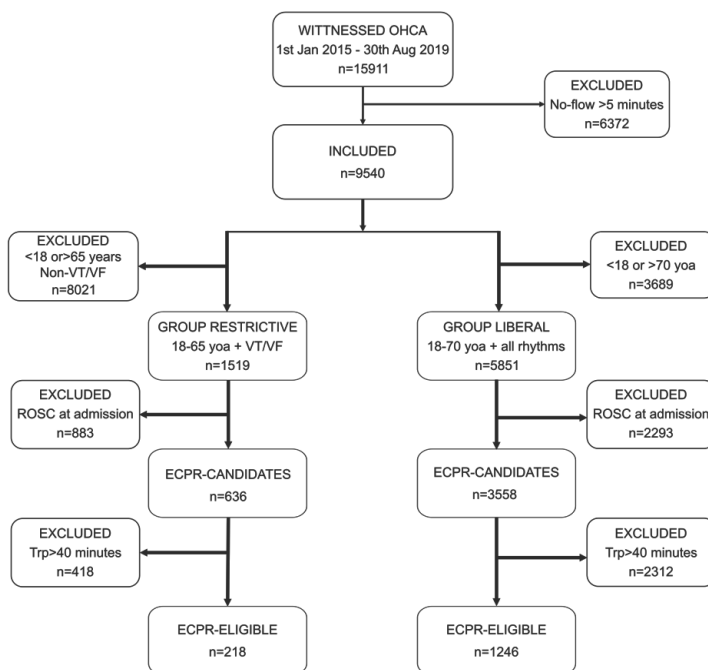


Figure 5: Flow-chart for selection of candidates for ECPR in Study IV

Calculations and estimates

Descriptive data from SCAR defines the cohorts.

Times of transport was calculated using Google maps®.

Results

Study I

All animals survived the experiment. Chance equally divided the animals to the study groups. Average ETCO_2 during CPR was 11 (IQR 10–20) mmHg in G_{high} and 5 (IQR 4–6) mmHg in G_{low} .

Brain

The most apparent finding was the correlation between the blood flow in the Carotid artery and the levels of ETCO_2 during CPR, fig 6. Flow decreased as a function of time but remained significant at end of CPR (45 minutes); 24 (IQR 20-24) mL/minute in G_{high} , and 4 (IQR 2-5) mL/minute in G_{low} , ($p < 0,01$).

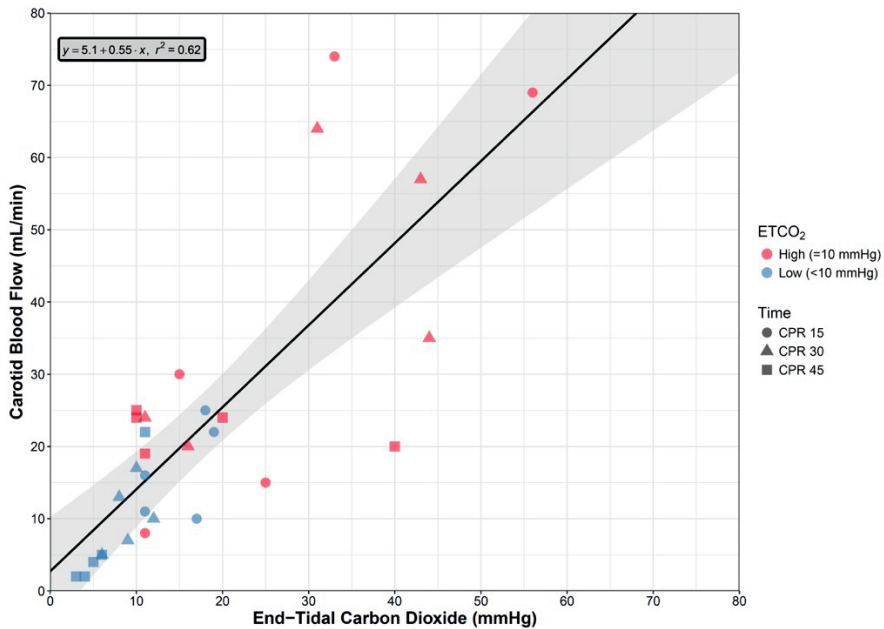


Figure 6: The correlation between carotid blood flow and ETCO_2 during CPR (I). The coefficient of determination (r^2) was 0.62.

Plasma levels of S100B were significantly different at end of ECPR with 0.5 (IQR 0.5-0.6) ng/L in G_{high} and 0.8 (IQR 0.7-0.9) ng/L in G_{low} respectively.

Histopathology displayed lesser damage in central parts of the brain but more pronounced damage in cortical areas in G_{high} . The opposite was true for G_{low} . No difference between groups was observed for the cerebellum, fig 7.

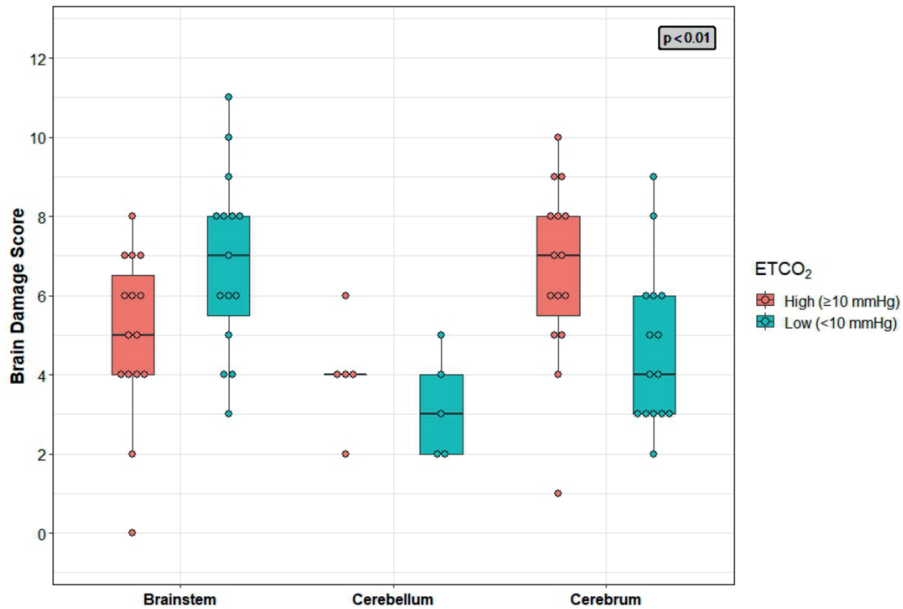


Figure 7: Boxplot of brain damage score in the different regions of the brain (I).

Kidneys

Urinary output was higher in G_{high} during the entire experiment with the largest difference during ECMO; 32 (0-220) ml/h compared to 6 (4-10) ml/h in G_{low} ($p < 0.05$). This was in analogy with plasma levels of NGAL, fig 8. Histopathological changes were observed, but there were no differences in damage score between groups.

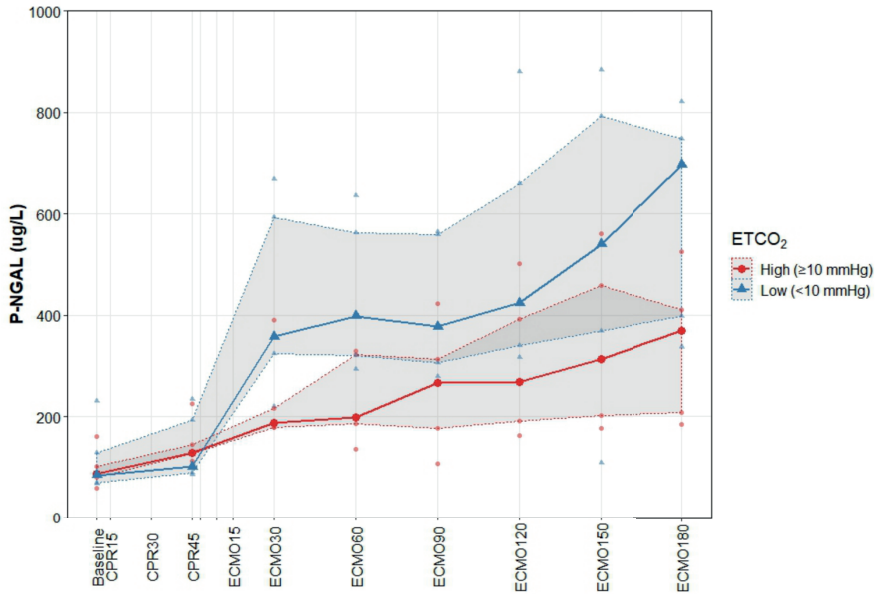


Figure 8: Levels of P-NGAL as a function of time (I). Shaded areas represent IQR.

Study II

All animals (n=12) survived the experiment and were equally divided, according to time of CPR, into G_{short} ; average 12 (9–15) minutes and G_{long} ; average 36 (27–45) minutes.

Metabolic parameters

pH was higher in G_{short} ; 7.7 (7.56-7.74) compared to G_{long} ; 7.17 (7.11-7.40) ($p<0.01$), at end of CPR, and aB-Lactate was lower; G_{short} ; 7.9 (4.3-9.1) mmol/L, G_{long} ; 14.0 (14.0-16.0) mmol/L. Differences remained but decreased during ECMO. At end of experiment was pH 7.41 (7.35-7.46) in G_{short} and 7.43 (7.4-7.47) in G_{long} ($p<0.01$). aB-Lactate was 5.1 (4.3-6.6) mmol/L in G_{short} and 7.3(5.4-8.3) mmol/L in G_{long} ($p<0.01$), fig 9.

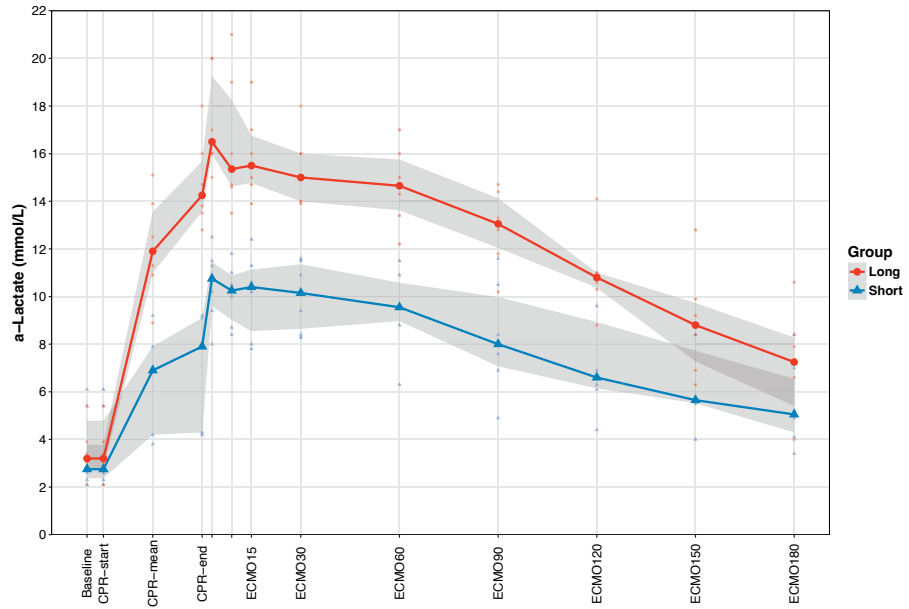


Figure 9: Levels of aB-Lactate as a function of time (II). Shaded areas represent IQR.

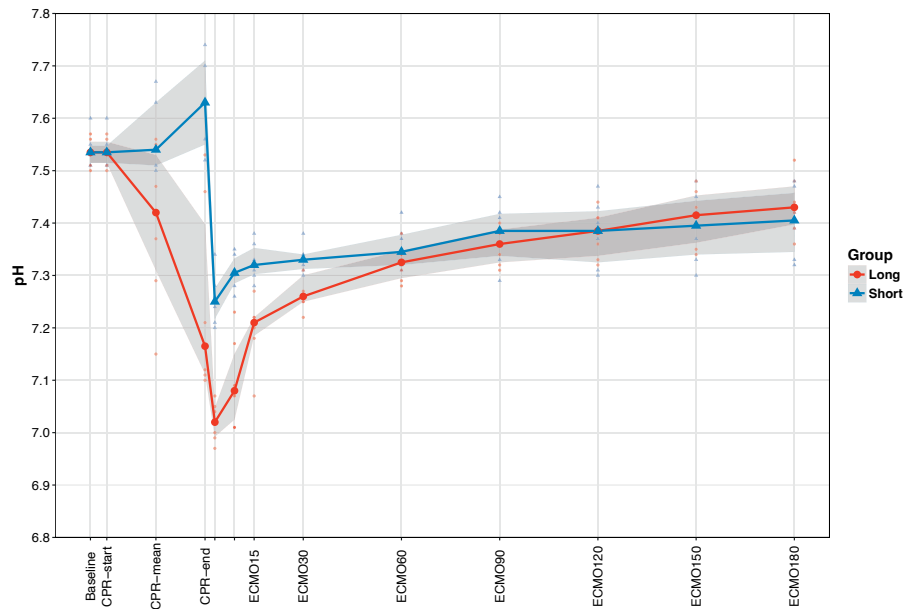


Figure 10: Levels pH as a function of time (II). Shaded areas represent IQR.

Brain

The carotid blood flow was higher at end of CPR; 27 (9.5-22) mL/minute in G_{long} and 7 (5.5-9.3) mL/minute in G_{short} ($p < 0.02$). The difference decreased during ECMO treatment. Microdialysis revealed significantly higher levels of LPR, glycerol and glutamate in G_{long} compared to G_{short} at end of CPR. The differences levelled out during ECMO in all parameters but glycerol, table 1. No differences in P-S100B or histopathology, fig 10, was observed between groups at end of experiment.

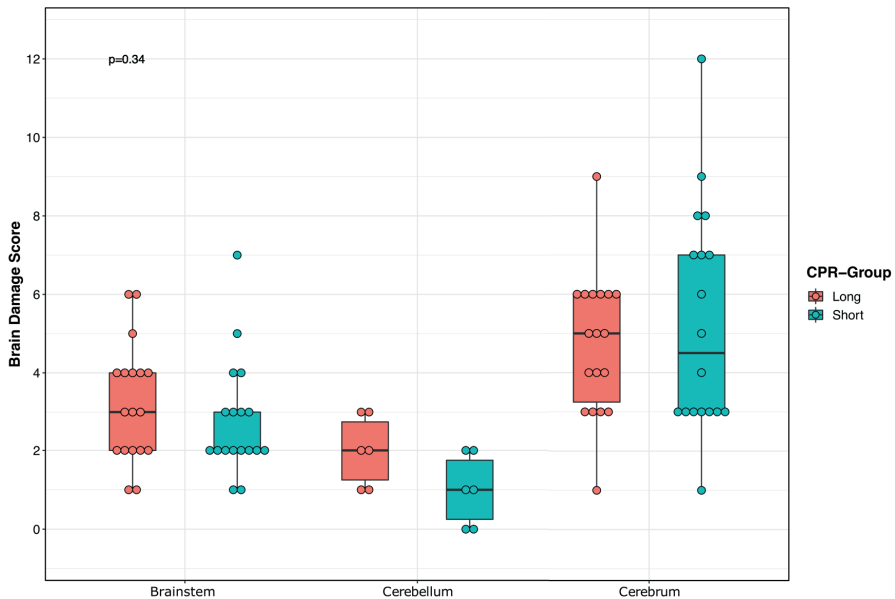


Figure 11: Boxplot of brain damage score in the different regions of the brain (II).

Kidneys

Urinary output was higher in G_{short} during ECMO but evened out as a function of time. However, a significant difference remained at end of ECMO; 230 (91-750) mL/h in G_{short} and 225 (38-315) mL/h in G_{long} ($p < 0.01$). P-NGAL was higher in G_{long} ; 3.24 (206-411) ug/L compared to 138 (97-162) ug/L in G_{short} at end of experiment ($p = 0.04$). No histopathological differences between groups were observed.

Table 1: Results of cerebral microdialysis (II). Values represent medians with IQR in parenthesis.

	Group	Baseline	P	CPR	P	ECMO	P
				End		End	
Glucose (mmol/L)	Short	0.43 (0.16-0.63)	0.03	0.40 (0.19-0.68)	0.49	0.41 (0.29-0.63)	0.28
	Long	0.71 (0.54-0.83)		0.54 (0.31-0.74)		0.18 (0.16-0.42)	
Lactate (mmol/L)	Short	1.62 (1.16-1.92)	<0.01	2.19 (1.62-2.86)	<0.01	0.85 (0.74-1.10)	0.25
	Long	2.33 (1.82-3.03)		5.14 (4.94-5.35)		0.70 (0.60-1.10)	
Pyruvate (μmol/L)	Short	0.04 (0.02-0.06)	0.45	0.04 (0.02-0.04)	0.90	0.03 (0.03-0.04)	0.68
	Long	0.05 (0.04-0.05)		0.03 (0.03-0.04)		0.03 (0.03-0.04)	
Lactate/Pyruvate	Short	38 (29-74)	0.08	60 (44-67)	<0.01	28 (21-35)	0.43
	Long	44 (40-69)		152 (129-186)		22 (15-42)	
Glycerol (μmol/L)	Short	47 (26-50)	<0.01	37 (31-42)	<0.01	23 (19-26)	<0.01
	Long	56 (52-63)		65 (59-70)		36 (31-42)	
Glutamate (μmol/L)	Short	24 (17-40)	0.12	29 (16-33)	0.02	5.4 (3.3-6.2)	0.82
	Long	55 (42-72)		71 (53-89)		4.3 (4.2-6.5)	

Study III

Numbers of ECPR candidates amongst IHCA

Out of totally 9209 witnessed IHCA, 7748 candidates remained after exclusion criteria was applied, and 504 candidates (5.5%) in Group_{restrictive} and 1077 (11.7%) in Group_{liberal}, after applying inclusion criteria. Following 20 minutes of CPR, 32 candidates (0.4%) in Group_{restrictive} and 64 (0.8%) in Group_{liberal} remained eligible for ECPR, fig 4.

Results of conventional CPR in IHCA

The median age was lower in the study groups compared to the non-selected. The prevalence of diabetes mellitus and previous myocardial infarction was similar in all groups. Stroke was less common in Group_{restrictive}. Out of all IHCA, 97% had a no-flow time of less than 2 minutes. ROSC was achieved to a very high extent in both study groups. Survival and CPC-scores at discharge were slightly higher in Group_{restrictive} compared to Group_{liberal}, table 2.

Table 2: Detailed information of CPR and outcome in the respective groups (III).

	Total (n=7748)	Restrictive (n=504)	Liberal (n=1077)
Age, median (IQR)	73 (65-81)	58 (51-62)	62 (54-67)
Male, n (%)	4803 (62)	382 (76)	756 (70)
Diabetes mellitus, n (%)	2037 (28)	113 (24)	260 (26)
Previous Myocardial Infarction, n (%)	1664 (23)	100 (21)	208 (21)
Previous stroke, n (%)	754 (10)	11 (2.3)	60 (6.0)
ROSC, n (%)	5497 (71)	463 (92)	969 (90)
Telemetry, n (%)	4994 (65)	436 (87)	878 (82)
Primary shockable rhythm, (n%)	2001 (26)	504 (100)	749 (70)
No-flow time, n (%)			
≤1 minute	7231 (93)	487 (97)	1026 (95)
≤2 minutes	7547 (97)	499 (99)	1064 (99)
Advanced airway, n (%)	3858 (51)	161 (32)	349 (33)
Mechanical CPR, n (%)	909 (12)	47 (9.6)	109 (10)
1-month survival, n (%)	2933 (38)	395 (78)	776 (72)
1-year survival, n (%)	2527 (33)	374 (74)	734 (68)
CPC 1-2, n (%)	2123 (27)	312 (62)	597 (55)

Calculated effect of ECPR on IHCA

In Group_{restrictive}, the estimated gain of survival of ECPR peaked at 20 minutes of CPR (38%) and gradually declined to be 7.9% at 60 minutes of CPR. The estimated gain of survival with preserved CPC score of 1-2 lasted till 50 minutes of CPR and was then lost. In Group_{liberal} was any gain of ECPR lost at 60 minutes of CPR. The estimated gain of survival with preserved CPC score of 1-2 was only 0.8% after 50 minutes of CPR, fig 11.

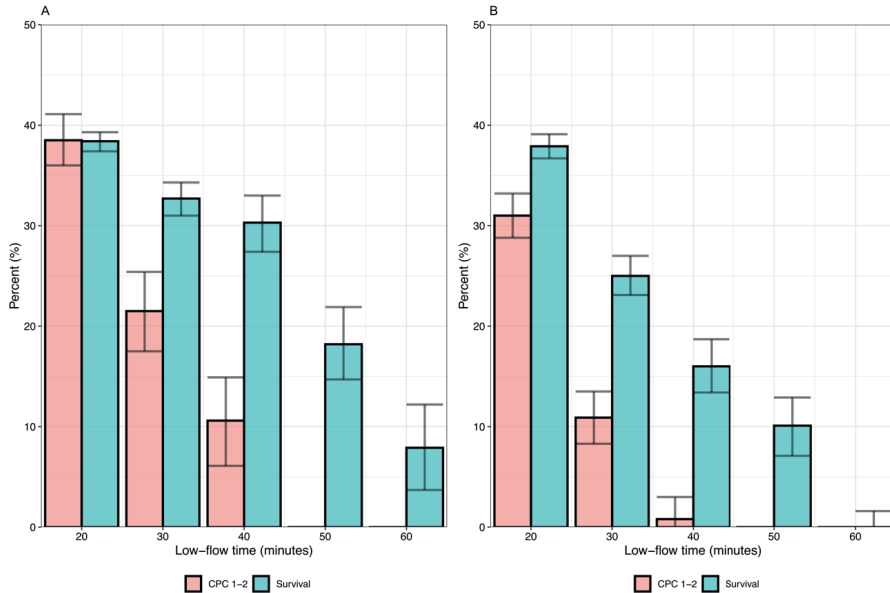


Figure 12: The estimated gain of survival and CPC-scoring when initiating ECPR at different time intervals of low-flow (III). A: Group_{restrictive}, B; Group_{liberal}.

Study IV

Numbers of ECPR candidates amongst OHCA

Out of totally 15911 witnessed OHCA, 9540 candidates remained after exclusion criteria was applied, and 1519 candidates (15.9%) in Group_{restrictive} and 5851 (61.3%) in Group_{liberal}, after applying inclusion criteria. After exclusion of patients with ROSC at hospital arrival, 636 and 3558 patients remained in Group_{restrictive} and Group_{liberal} respectively. Further exclusion of patients with estimated time of transportation exceeding 40 minutes, resulted in 218 candidates for ECPR in Group_{restrictive} and 1246 in Group_{liberal}. fig5.

Results of conventional CPR in OHCA

Median age in both study groups were 52 years, and in the non-selected group 66 (0-104) years. The majority of patients in all groups were men. The percentual occurrence of CPR performed by lay men were 60-71%, and most common in Group_{restrictive}. Prehospital termination of resuscitation (TOR) was 2% in Group_{restrictive} compared to 4% in the non-selected group and Group_{liberal}. Incipient ROSC and ROSC at hospital arrival was more common in Group_{restrictive}. Also, 30-day survival and CPC-score 1-2 at discharger was more common in Group_{restrictive}, table 3.

Table 3: Outcome of CPR in the respective groups of OHCA (IV).

	Unsorted n (%)	Restrictive n (%)	Liberal n (%)
Size of cohort	15911 (100)	1519 (100)	5851 (100)
Male	10553 (66)	1148 (76)	3818 (65)
Bystander CPR prior to EMS arrival	9606 (60)	1072 (71)	3803 (65)
TOR prior to hospital admission	634 (4)	36 (2)	232 (4)
Incipient ROSC	6992 (44)	1029 (68)	2882 (49)
ROSC at hospital arrival	5344 (34)	883 (58)	2293 (39)
CPC 1,2 at discharge from hospital	1705 (11)	535 (35)	906 (15)
30 days survival	1961 (12)	606 (40)	1036 (18)

Geographical distribution of ECPR candidates for OHCA

The regional distribution of monthly ECPR candidates is plotted in in fig 12 and varies between 0-6 candidates in Group_{restrictive} and 1-24 candidates in Group_{liberal}.

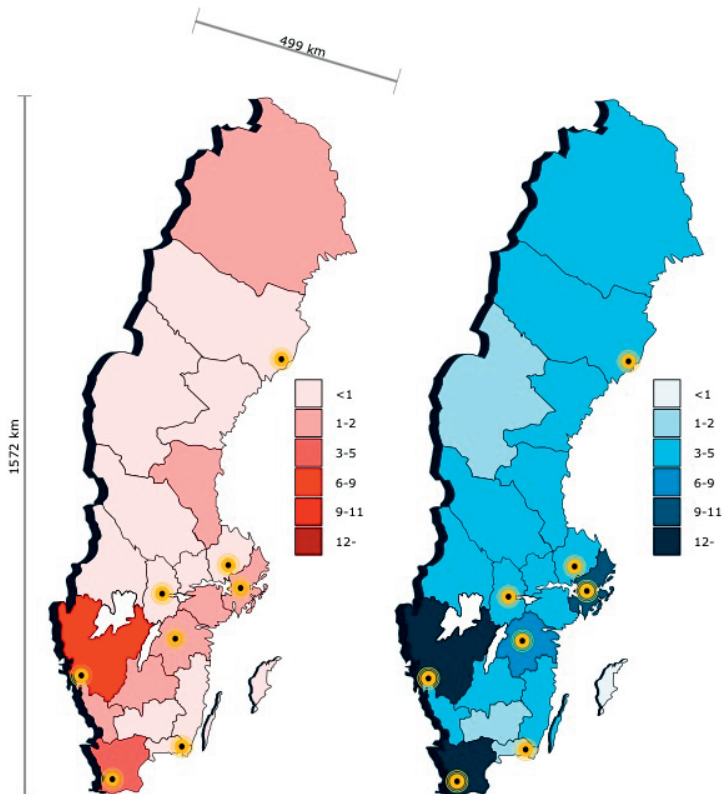


Figure 13: The monthly regional incidence of ECPR candidates in Group_{restrictive} (red) and Group_{liberal} (blue), independent of time of transportation (IV). ECPR capable hospitals are marked with yellow and black dots.

Totally, 65 EMS/HEMS stations were situated within 40 minutes of transport from an ECPR-capable hospital. ECPR-capable hospitals were serviced by 2-27 EMS/HEMS units. In Group_{restrictive}, 34% (218/636) suffered CA within 40 minutes of transportation. Corresponding figure in Group_{liberal} was 35% (1246/3558). The distribution of ECPR-candidates by individual ECPR capable hospital is described in fig 13.

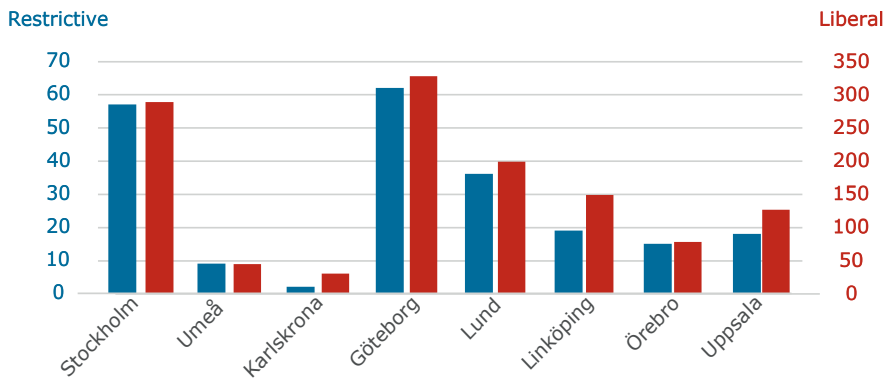


Figure 14: The distribution (n) of ECPR-candidates by individual ECPR-capable hospital amongst OHCA (IV).

Subgroup analysis of the Region of Uppsala

Out of 109 ECPR candidates presumed to be within 40 minutes of transportation to Uppsala University Hospital, 84 patients (77%) were actually within reach.

In Group_{restrictive}, 86% (19/22) of the ECPR candidates had a CA of confirmed cardiogenic aetiology and in Group_{liberal} 45% (49/109). All ECPR candidates in the Region of Uppsala during the study period are plotted in fig 14.

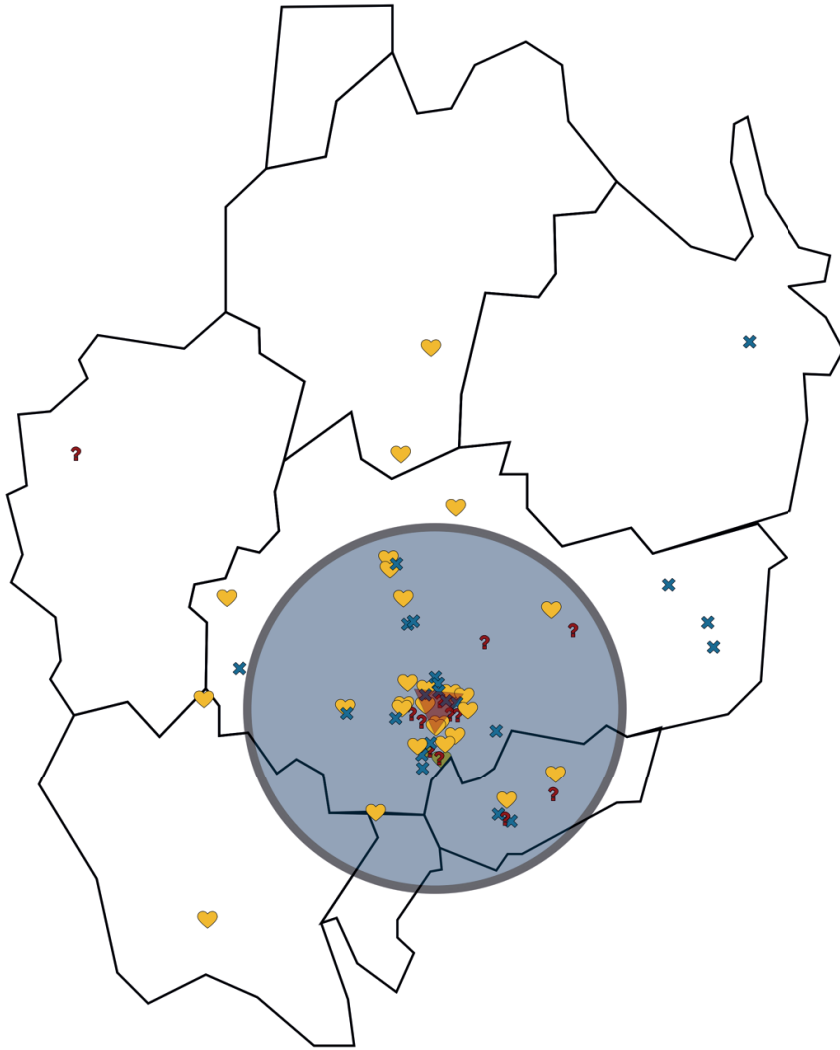


Figure 15: ECPR candidates in the Region of Uppsala during the entire study period (IV). The geographical location of each individual CA is described. The circle corresponds to 40 minutes of transport. CA of confirmed cardiogenic aetiology are marked with a heart, non-cardiogenic with a cross, and unknown with a question mark.

Discussion

We have created a reproducible experimental model of CPR and ECPR in refractory CA. Using this model, we have demonstrated a correlation between ETCO_2 during CPR and the resulting damage in brain and kidney following ECPR. The role of ETCO_2 as a marker for initiation of ECPR has been evaluated. Furthermore, an estimate of the incidence of ECPR eligible candidates amongst OHCA and IHCA in Sweden has been calculated and suggest low numbers annually. Selection criteria and geography are the major determinants for ECPR eligibility.

Animal modelling

Studying ECPR is cumbersome. It is an extremely unusual treatment with diverse criteria for selection and techniques for implementation. Treated patients present with widely varying physiological status and comorbidity. Furthermore, the uneven experience and expertise between centres will inevitably influence the results. It is a heterogenous population to study which makes it hard to reach any valid conclusions. That considered, is animal modelling an appealing and sensible substitute.

Animal modelling allows for measurement of parameters otherwise not suited for in the clinical environment, or unwanted due to the increased risks they add to already vulnerable patients. It also lets you control factors that cannot be controlled in a clinical setting, which can be of importance when interpreting the results. Obviously, animal ethics must be considered within this context.

This thesis complies with “The three R’s Principle” (Replace, Reduce, Refine) as suggested by biologists Russel and Burch. Simplified, they argue that replacing animals by using other methods must be considered. Reduction of numbers of animals, as well as maximizing information obtained per animal must be sought. Refinement of husbandry and experimental procedures, to minimize stress and pain is an obligation. The experimental protocols of this thesis have been designed accordingly.

Statistics

Data-points in study I and II are longitudinal and densely repeated on the same few individuals over time. Inference of group differences has been analysed. Several methodological approaches have been considered.

Testing at each different time point using well known methods for independent groups (e.g., Kruskal Wallis), is inefficient and risks losing features of the time course. Furthermore, missing data can result in sample bias.

Repeated measures analysis of variance could have been applied, as our data are balanced. However, it requires complete data on each subject and depends on the assumption of restrictive sphericity (i.e., equal variability of the measurements at each time point and equal correlations between every two measurements on the same individual), which is questionable for longitudinal data (42). Even though the problem can be overcome (43), we opted for analysis by means of Repeated-Measurement Mixed Effect Model. This method has been suggested to be superior to the previously mentioned. It carries the advantage of including all data, reduces the effect of interindividual difference in baseline measurements, and handles the potential random variability which is correlated to multiple testing (42,44).

The role of ET_{CO}₂ during cardiac resuscitation

ET_{CO}₂ is an established and valuable factor in use during conventional CPR (19). Several studies confirm the correlation between ET_{CO}₂ and CO (45–47). In study I, we found a strong correlation ($r^2=0.62$) between ET_{CO}₂ and carotid blood flow during CPR, a finding supported by other investigators (14,48,49). In accordance, study II revealed higher carotid blood flow in subjects enduring longer times of CPR with preserved levels of ET_{CO}₂, compared to shorter CPR with rapidly declining levels of ET_{CO}₂.

Unfortunately, ET_{CO}₂ has failed to prove predictive for survival following CPR but is predictive for ROSC (50). So far, it has mainly been used as a negative predictor due to extremely low chances of survival if ET_{CO}₂ is less than 10 mmHg at 20 minutes of CPR (51,52). For some centres, this fact has been adopted as an exclusion criteria to ECPR (53). However, the physiological situation following ROSC after CPR is very much different from restored circulation on ECMO. Mechanically restored circulation after prolonged CA is immediate and can be matched to meet the requirements of both blood flow and gas exchange. Possibly, this alters the previously known outcome. On the other hand, whether it is desirable to instantly meet the metabolic demands is not known. Theoretically this might inflict a “second hit” by causing reperfusion injuries (54–57). Further studies on this subject are highly warranted.

Measurement of ET_{CO}₂ during CPR is not without pitfalls. Pre-arrest factors of comorbidity (e.g., trauma, burns, hyperthyroidism, sepsis) as well as

the cause of the arrest (e.g., asphyxia (58)) could potentially affect levels of ETCO₂. Intra-arrest factors such as ventilatory volume per minute (i.e., rate and tidal) (59), as well as drugs used during resuscitation (e.g., vasopressors (60) and bicarbonate (61)) will also influence ETCO₂. Furthermore, technical aspects of how the measurement is performed (i.e., main-stream-, side-stream capnometry or capnography, integrity and potential dead space of the ventilatory circle, means of calibration, et cetera (62)), may raise concerns of its accuracy.

Aside from pre-arrest factors, both experimental studies of this thesis (I and II) have been equally conducted with respect to intra-arrest factors and technical aspects. It has been our purpose to design the protocols to mimic real-life scenarios. That is where the measurements are performed, and decisions eventually made. Our measurements of ETCO₂ were made continuously throughout the experiment with minimal fluctuations of readings, increasing the probability of accurate interpretation. Furthermore, interpretation of readings was made by two independent investigators. Modern research suggest that the trend of ETCO₂ during resuscitation is more indicative of outcome rather than isolated values (63,64), to which we agree.

End-point parameters in experimental studies (I and II)

Plasma calcium binding protein B (P-S100B)

The use of S100B is a part of the Scandinavian guidelines for traumatic brain injury in the adult (65,66) and is a diagnostic tool of neuro intensive care in Scandinavia.

In the experimental studies of this thesis (I and II), S100B increased above baseline in all groups, and above what is considered normal in humans. In study I, there was a significant difference between groups which remained at the end of experiment. In study II, no significant changes between groups were seen. The differences found were consistent with pathological differences of histology, although pathological findings were overall mild. Indeed, S100B has been reported to rise prior to detectable clinical or radiological findings (67). Our findings are in accordance with other porcine models of CPR (68).

The neurological status of the subjects was not clinically evaluated due to ethical limitations. However, the predictive value of S100B on chances of regaining consciousness (69), as well as neurologic function following CPR in humans (70), has been reported. Specifically, it is associated with neurological complications following ECPR (71). We find the results of S100B to be in favour of our conclusions.

Microdialysis of the brain

In study II we increased temporal resolution of any potential pathological findings in the brain. This was achieved by describing the metabolic changes, using microdialysis. We observed metabolic derangement in both groups but considerably worse in the group with longer CPR. Probably this was caused by suboptimal cerebral circulation, as suggested by the observed drop in carotid blood flow. The finding was evident by a considerable rise in the lactate-pyruvate ratio and level of glutamate at induction of CA, and is in accordance with other studies on microdialysis and CA (37,38,49).

During the course of ECMO, differences between groups were eradicated, except for glycerol. This could be interpreted as if cerebral metabolism is, at least partly, restored by extracorporeal treatment. However, some degree of tissue damage is inevitable. Differences between groups in levels of glycerol at end of ECPR, was not consistent with histological findings in the brain of study II. It is reasonable to assume they were to minor to be reflected.

Histopathology of the brain.

In study I, cerebral histological changes ranged from moderate to an extensive degree of ischemic damage in 66% of the samples. Damage score was found to be lower in the brainstem but more pronounced in the cortical areas, in subjects with higher values of ET CO_2 during CPR. The opposite was true for the group with lower values of ET CO_2 during CPR. The reasons for this injury pattern could possibly be explained by uneven distribution of cerebral blood flow during CPR (49,72,73), use of epinephrine (74), and reperfusion injury (54-57).

ECPR treatment offers a unique opportunity to regulate blood flow and optimize gas exchange to control reperfusion. This ability seems to be underutilized as of today and is unfortunately not often discussed when it comes to ECPR. Further studies focused on the dynamics of cerebral blood flow on ECPR, as well as ischemia and reperfusion injury in the different areas of the brain are needed.

Brain damage scores in study II was overall lower compared to study I. No differences between groups in study II were found. This is quite remarkable, given that the time of CPR ranged from 11-45 minutes. 11 minutes can be considered a short time of CPR. However, some subjects will already be severely deranged, while others can last until 45 minutes before deterioration starts. We believe these findings points in the direction of our conclusions.

Plasma neutrophil gelatinase associated lipocalin (P-NGAL)

In acute kidney injury (AKI), NGAL has proven to rise within two to three hours (75,76), which could be observed in both experimental studies (I and II).

In study I, we found lower levels of P-NGAL and higher urinary output in the group with higher levels of ETCO₂ during CPR. This is in accordance with findings by Prowle et al, who could also show histological derangement after ROSC in a similar porcine model (77). In study II, levels of P-NGAL were higher and urinary output were slightly lower in the group treated with longer times of CPR. Levels of P-NGAL and urinary output were consistent with each other in both studies.

Although ETCO₂ reflects systemic circulation during CPR, it cannot be compared to native circulation. The renal medulla is very sensitive to hypoxia and hypotension. Thus, it is not surprising that groups treated with longer times of CPR eventually suffer AKI, as measured by P-NGAL. On the other hand, it is evident from study I, that ETCO₂ is not to be disregarded of. Previous studies show that levels of NGAL is proportional to the severity of AKI (78). NGAL can predict AKI in the adult cardiac surgery patient, critical ill and in the transplant patient (77,79,80). Indeed, renal impairment, also in the ECPR setting, is a severe negative prognostic factor (15,81) for outcome.

Histopathology of the kidneys

Renal histopathological changes were seen in all groups. Study I failed to show any differences between groups although renal pathology was evident. In regard to levels of P-NGAL, AKI should have been present, but might also have been transient. Likely, time of CPR and hypoperfusion should have been longer for more sustainable histological changes to appear (82). The finding of equal pathologic derangement in animals treated with varying times of CPR in study II, was more in line with our hypothesis. The clinical significance or renal histopathology findings is unclear.

Summary

It is safe to say that the role of ETCO₂ during resuscitation has been thoroughly investigated and scrutinized since its introduction, and that it is still ongoing. It is unlikely that ETCO₂ will ever be the exclusive factor for selection of ECPR candidacy. However, it remains an important contributor of information, which is further supported by this thesis. Although the importance of each different biochemical marker, as well as the means for pathological evaluation and physiological measurements in the studies (I and II) can be disputed, they all point in the same direction. We think this strengthens the validity of our findings.

Eligibility and outcome of ECPR for IHCA and OHCA (III and IV)

The key to successful ECPR treatment is mainly dependent on selection of candidacy. Several determinant factors have been identified which are based on retrospective studies on outcome following ECPR. These mainly include, time of no-flow (83–85), time of low-flow (i.e., CPR) (86–90), and rhythm (i.e., shockable vs non-shockable) (16,84,91–93). Age is most often part of the selection algorithm but there is conflicting evidence on the relevant cut off value (18,92,94,95). The same is true for biochemical parameters such as aB lactate and pH (16,18). Presently, there is a trend to discard biochemical parameters in the selection process. Focus is rather shifted towards cerebral preservation, as suggested by distinguished practitioners of ECPR as Lionel Lamhaut of the SAMU, France. Their group have demonstrated that occurrence of any sign of life during CPR, is in favour of survival and neurological outcome following ECPR (96).

Most probably, no single factor can be used to decide on ECPR eligibility. Several factors are needed in order to do an overall assessment of the physiological effect of CPR. Furthermore, it is reasonable to assume that criteria differ between the out-of-hospital and in-hospital setting, in regard to the different conditions they offer (97).

At present, there are interim guidelines on selection criteria for ECPR issued by ELSO (98), but no international consensus. Inclusion and exclusion criteria differ between centres. With this in mind, we aimed to describe the ECPR eligible cohorts from a restrictive to a liberal perspective, by means of selection criteria. Even though inclusion and exclusion criteria were arbitrary selected, they are scientifically justified and recurrently used by others. To some extent we have had to conform to information available in the registry.

Protocols in study III and IV have been designed to not lose any potential ECPR candidates. Assumptions made in calculations of estimated gain of ECPR (III), has been in favour of ECPR-treatment. Results show a benefit in survival and CPC-scores, mainly during the first 30 minutes (probably before ECPR could be realistically initiated), and that it remains in some degree up till 60 minutes of CPR. Indeed, survival and neurological outcome after ECPR drops if circulation is restored on ECMO only after 60 minutes of low-flow (87,88,90). Although unknown medical contraindications, negative side effects and risks of ECPR-treatment, has been disregarded of in this models, our findings are in agreement with other authors (87,93,99).

Conventional CPR is fairly successful on CA with shockable rhythm within the first 20 minutes of resuscitation (100). During this time should ECPR probably not be attempted because of the added risks and limited gain it offers. The optimal time of transport for ECPR in the OHCA setting has been investigated and found to be 16 minutes (101). In study IV we have allowed 40

minutes of transport as the upper time limit for inclusion of patients. This means, that the actual time from CA to ECPR has been allowed to exceed 60 minutes. Unfortunately, the subgroup analysis of the region of Uppsala (IV) reveal our estimate on transportability to be incorrect in 23 % of the cases. Furthermore, all potential practical problems such as time for evaluation and decision-making, as well as availability of canulating crew and time to canulate has not been taken into account. Overall, it is reasonable to assume that our results on ECPR candidacy is overestimated.

Clinical implications

Studies I and II

The experimental studies have strengthened the previously known relationship between ETCO_2 and systemic circulation during CPR and is also indicative of a correlation with end organ damage in the brain and kidney following ECPR. If these results can be confirmed by others and prove to be valid in humans; hopefully, ETCO_2 can be attributed greater and more decisive importance in the selection process. Candidates excluded based on barely exceeded time limits, could possibly be included for treatment, if ETCO_2 could demonstrate a physiological successful CPR. Conversely, candidates with inadequate and ineffective CPR according to ETCO_2 , could contradict inclusion, despite fulfilling other criteria. Possibly, this could improve patient selection and results of ECPR. Furthermore, when the predictive values of inclusion and exclusion criteria can be determined, chances of international consensus on selection criteria increases. A uniform selection process improves the possibilities of studying ECPR in the future.

Studies III and IV

The registry studies demonstrate fairly low numbers of patients available for ECPR treatment, both for IHCA and OHCA. When considering that the success of an ECPR program is also dependent on numbers treated, only a minority of the ECPR capable hospitals have a population base large enough to justify an ECPR program. Even though a recent randomized controlled trial showed superior results of ECPR compared with CPR (12) and calculations of health-economics show a benefit of ECPR (102–105), time from CA to ECMO is undoubtedly very important. In a sparsely inhabited country like Sweden, with geographically scattered ECPR capable hospitals, time of transport is a decisive factor excluding many potential candidates. Our findings could potentially help to allocate resources and economical assets of the Swedish national health care system.

Limitations

There are several limitations to this thesis.

It is an ethical concern of keeping the numbers of animals to the minimum but still achieve the aims of the research. Nevertheless, the small sample size in the experimental studies (I and II) must be considered. In order to compensate we have used multiple investigational modalities to clarify and strengthen our results. Also, the statistical method used has been chosen accordingly.

The physiological status of the animals when arriving in the laboratory has been a factor out of our control. All animals have been treated in accordance with good animal care; however, nutritional status and fluid balance are unknown and could theoretically have affected baseline parameters.

Time of ECPR in the experimental protocol was limited and must be considered. It can be argued that three hours is too short to detect significant histological changes in the brain and kidney with our chosen methods.

Although the porcine anatomy resembles that of humans, findings cannot be directly translated into clinical practice. The clinical relevance of the findings for the subjects in these studies cannot be determined, as they were not awakened.

The retrospective nature of the registry studies (III and IV) may cause limitations; numbers of non-reported data are unknown; there is probably a higher risk of under-reporting, and/or inaccuracy of registration, if attempted resuscitation failed. Furthermore, factors not known to the investigators, contraindicating ECPR candidacy, might have been present. On the other hand, overestimation of ECPR eligibility cannot be ruled out.

Even though SCAR is a registry for CA, a primary cardiac aetiology cannot be guaranteed.

Finally, all CA should be run according to ILCOR guidelines, but deviations may have occurred that can have affected our results.

Conclusions

Study I

ETCO₂ is associated with the extent of cerebral and renal injury in mechanical CPR following experimental ECPR. Whether higher levels of ETCO₂ during CPR is more favourable, cannot be determined by this study. The finding supports the important role of ETCO₂ in the selection for ECPR candidacy.

Study II

Results suggest that ETCO₂ could be used as a marker for brain injury following ECPR and supports its use as a complement to time of CPR as an eligibility criterion for ECPR. Cerebral microdialysis indicates a time dependent risk of ischemic injury, which is restored during ECMO. No apparent histological differences of tissue damage in brains or levels of S100B in plasma were detected between groups with significant different times of CPR.

Study III

In Sweden, the number of assumed ECPR eligible patients per year among IHCA is low. The number of national ECPR candidates among IHCA is highly dependent on selection criteria. Our estimate of the gain of ECPR suggests that there could be a benefit in survival and neurological outcome if extracorporeal treatment is started within 60 min of the IHCA.

Study IV

The quantity of ECPR candidates amongst OHCA is highly dependent on selection criteria and geographics. In Sweden, 1.4-7.8% of all witnessed OHCA is estimated to be potential ECPR candidates, on average corresponding to 4-22 OHCA per month, nationwide. This suggests that only a minority of the ECPR capable hospitals have a population base of ECPR eligible patients amongst OHCA, large enough to justify an ECPR program.

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Sammanfattning på svenska

Hjärtstopp är en av de ledande orsakerna till förtida död bland den vuxna befolkningen. Även om överlevnaden med hjälp av traditionell hjärt-lung-räddning ökar, kommer vissa patienter att förbli refraktära till behandlingen och inte återfå spontan cirkulation. För ett fåtal av dessa patienter kan ECMO-assisterad hjärt-lung-räddning (ECPR) erbjudas. Behandlingen tar tunga intensivvårdsresurser i anspråk och är behäftad med allvarliga risker och komplikationer. I syfte att optimera användandet av resurser och minimera lidande för patienter, är urvalet av yttersta vikt. Tyvärr finns det för närvarande begränsad kunskap kring detta och det råder inte consensus kring optimala selektionskriterier.

Målet med avhandlingen har varit att undersöka det prediktiva värdet av ett av de förslagna selektionskriterierna; end-tidalt koldioxid (ETCO₂) samt försöka definiera den potentiella ECPR-kohorten i Sverige.

I två experimentella djurstudier (I och II) har vi kunnat påvisa en korrelation mellan ETCO₂ under hjärt-lung-räddning och graden av vävnadsskador i hjärna och njurar efter ECPR. Våra resultat stödjer användandet av ETCO₂ som ett selektionskriterium för ECPR.

Med utgångspunkt från existerande selektionskriterier, har vi med hjälp av data från Svenska Hjärtstopps Registret undersökt hur många potentiella kandidater som skulle kunna bli föremål för ECPR årligen i Sverige, både på och utanför sjukhus (III och IV). Resultaten tyder på att endast ett fåtal sjukhus i Sverige har en tillräckligt stor populationsbas för att berättiga ett ECPR-program samt att den faktiska överlevnadsvinsten troligen skulle vara begränsad. Valet av selektionskriterier och geografiska förutsättningar är avgörande faktorer för populationsbasens storlek.

References

1. Holmberg MJ, Granfeldt A, Girotra S, Donnino MW, Andersen LW. Trends in survival and introduction of the 2010 and 2015 guidelines for adult in-hospital cardiac arrest. *Resuscitation*. 2020;157:112–20.
2. <https://arsrapporter.registercentrum.se/shlr/20201103/hj%C3%A4rtstopp-p%C3%A5-sjukhus.html#resultat-f%C3%B6r-hj%C3%A4rtstopp-p%C3%A5-sjukhus>. Svenska Hjärtstoppregistrets årsrapport 2019. Accessed Jun 2021
3. <https://www.else.org/Registry/InternationalSummaryandReports/InternationalSummary.aspx>. ELSO report of 2020. Accessed Feb 2022.
4. Mosier JM, Kelsey M, Raz Y, Gunnerson KJ, Meyer R, Hypes CD, et al. Extracorporeal membrane oxygenation (ECMO) for critically ill adults in the emergency department: history, current applications, and future directions. *Crit Care*. 2015;19:431.
5. Peek GJ, Mugford M, Tiruvoipati R, Wilson A, Allen E, Thalanany MM, et al. Efficacy and economic assessment of conventional ventilatory support versus extracorporeal membrane oxygenation for severe adult respiratory failure (CESAR): a multicentre randomised controlled trial. *Lancet*. 2009;374(9698):1351–63.
6. Mattox KL, Beall ACJ. Resuscitation of the moribund patient using portable cardiopulmonary bypass. *Ann Thorac Surg*. 1976;22(5):436–42.
7. Kim SJ, Kim HJ, Lee HY, Ahn HS, Lee SW. Comparing extracorporeal cardiopulmonary resuscitation with conventional cardiopulmonary resuscitation: A meta-analysis. *Resuscitation*. 2016;103:106–16.
8. Holmberg MJ, Geri G, Wiberg S, Guerguerian AM, Donnino MW, Nolan JP, et al. Extracorporeal cardiopulmonary resuscitation for cardiac arrest: A systematic review. *Resuscitation*. 2018;131:91–100.
9. Patricio D, Peluso L, Brasseur A, Lheureux O, Belliato M, Vincent JL, et al. Comparison of extracorporeal and conventional cardiopulmonary resuscitation: a retrospective propensity score matched study. *Crit Care*. 2019;23(1):27.
10. Stub D, Bernard S, Pellegrino V, Smith K, Walker T, Sheldrake J, et al. Refractory cardiac arrest treated with mechanical CPR, hypothermia, ECMO and early reperfusion (the CHEER trial). *Resuscitation*. 2015;86:88–94.
11. Richardson ASC, Schmidt M, Bailey M, Pellegrino VA, Rycus PT, Pilcher DV. ECMO Cardio-Pulmonary Resuscitation (ECPR), trends in survival from an international multicenter cohort study over 12-years. *Resuscitation*. 2017;112:34–40.
12. Yannopoulos D, Bartos J, Raveendran G, Walser E, Connett J, Murray TA, et al. Advanced reperfusion strategies for patients with out-of-hospital cardiac arrest and refractory ventricular fibrillation (ARREST): a phase 2, single centre, open-label, randomized controlled trial. *Lancet*. 2020;396(10265):1807–16.

13. Panchal AR, Bartos JA, Cabañas JG, Donnino MW, Drennan IR, Hirsch KG, et al. Part 3: Adult Basic and Advanced Life Support: 2020 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care. *Circulation*. 2020;142(16_suppl_2):S366–468.
14. Beyea MM, Tillmann BW, Iansavichene AE, Randhawa VK, Van Aarsen K, Nagpal AD. Neurologic outcomes after extracorporeal membrane oxygenation assisted CPR for resuscitation of out-of-hospital cardiac arrest patients: A systematic review. *Resuscitation*. 2018;130:146–58.
15. Dalia AA, Lu SY, Villavicencio M, D'Alessandro D, Shelton K, Cudemus G, et al. Extracorporeal Cardiopulmonary Resuscitation: Outcomes and Complications at a Quaternary Referral Center. *J Cardiothorac Vasc Anesth*. 2020;34(5):1191–4.
16. Debaty G, Babaz V, Durand M, Gaide-Chevronnay L, Fournel E, Blancher M, et al. Prognostic factors for extracorporeal cardiopulmonary resuscitation recipients following out-of-hospital refractory cardiac arrest. A systematic review and meta-analysis. *Resuscitation*. 2017;112:1–10.
17. Michels G, Wengenmayer T, Hagl C, Dohmen C, Bottiger BW, Bauersachs J, et al. [Recommendations for extracorporeal cardiopulmonary resuscitation (eCPR): Consensus statement of DGIIN, DGK, DGTHG, DGfK, DGNI, DGAI, DIVI and GRC]. *Anaesthesist*. 2018;67(8):607–16.
18. D'Arrigo S, Cacciola S, Dennis M, Jung C, Kagawa E, Antonelli M, et al. Predictors of favorable outcome after in-hospital cardiac arrest treated with extracorporeal cardiopulmonary resuscitation: A systematic review and meta-analysis. *Resuscitation*. 2017;121:62–70.
19. Soar J, Böttiger BW, Carli P, Couper K, Deakin CD, Djärv T, et al. European Resuscitation Council Guidelines 2021: Adult advanced life support. *Resuscitation*. 2021;161:115–51.
20. Haimoto H, Hosoda S, Kato K. Differential distribution of immunoreactive S100-alpha and S100-beta proteins in normal non-nervous human tissues. *Lab Invest*. 1987;57(5):489–98.
21. Dadas A, Washington J, Marchi N, Janigro D. Improving the clinical management of traumatic brain injury through the pharmacokinetic modeling of peripheral blood biomarkers. *Fluids Barriers CNS*. 2016;13(1):21.
22. Pham N, Fazio V, Cucullo L, Teng Q, Biberthaler P, Bazarian JJ, et al. Extracranial sources of S100B do not affect serum levels. *PLoS One*. 2010;5(9):e12691.
23. da Rocha AB, Schneider RF, de Freitas GR, André C, Grivicich I, Zanoni C, et al. Role of serum S100B as a predictive marker of fatal outcome following isolated severe head injury or multitrauma in males. *Clin Chem Lab Med*. 2006;44(10):1234–42.
24. Marenholz I, Heizmann CW, Fritz G. S100 proteins in mouse and man: from evolution to function and pathology (including an update of the nomenclature). *Biochem Biophys Res Commun*. 2004;322(4):1111–22.
25. Sorci G, Riuizzi F, Arcuri C, Tubaro C, Bianchi R, Giambanco I, et al. S100B protein in tissue development, repair and regeneration. *World J Biol Chem*. 2013;4(1):1–12.
26. Kleindienst A, Toliás CM, Corwin FD, Müller C, Marmarou A, Fatouros P, et al. Assessment of cerebral S100B levels by proton magnetic resonance spectroscopy after lateral fluid-percussion injury in the rat. *J Neurosurg*. 2005;102(6):1115–21.

27. Undén J, Astrand R, Waterloo K, Ingebrigtsen T, Bellner J, Reinstrup P, et al. Clinical significance of serum S100B levels in neurointensive care. *Neurocrit Care*. 2007;6(2):94–9.
28. Janigro D, Mondello S, Posti JP, Unden J. GFAP and S100B: What You Always Wanted to Know and Never Dared to Ask. *Front Neurol*. 2022;13:835597.
29. Marchi N, Cavaglia M, Fazio V, Bhudia S, Hallene K, Janigro D. Peripheral markers of blood-brain barrier damage. *Clin Chim Acta*. 2004;342(1–2):1–12.
30. Chakraborty S, Kaur S, Guha S, Batra SK. The multifaceted roles of neutrophil gelatinase associated lipocalin (NGAL) in inflammation and cancer. *Biochim Biophys Acta*. 2012;1826(1):129–69.
31. Schmidt-Ott KM, Mori K, Li JY, Kalandadze A, Cohen DJ, Devarajan P, et al. Dual action of neutrophil gelatinase-associated lipocalin. *J Am Soc Nephrol*. 2007;18(2):407–13.
32. Cai L, Rubin J, Han W, Venge P, Xu S. The origin of multiple molecular forms in urine of HNL/NGAL. *Clin J Am Soc Nephrol*. 2010;5(12):2229–35.
33. Xu SY, Carlson M, Engström A, Garcia R, Peterson CG, Venge P. Purification and characterization of a human neutrophil lipocalin (HNL) from the secondary granules of human neutrophils. *Scand J Clin Lab Invest*. 1994;54(5):365–76.
34. Passov A, Petäjä L, Pihlajoki M, Salminen US, Suojaranta R, Vento A, et al. The origin of plasma neutrophil gelatinase-associated lipocalin in cardiac surgery. *BMC Nephrol*. 2019;20(1):182.
35. Dent CL, Ma Q, Dastrala S, Bennett M, Mitsnefes MM, Barasch J, et al. Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. *Crit Care*. 2007;11(6):R127.
36. Albert C, Zapf A, Haase M, Röver C, Pickering JW, Albert A, et al. Neutrophil Gelatinase-Associated Lipocalin Measured on Clinical Laboratory Platforms for the Prediction of Acute Kidney Injury and the Associated Need for Dialysis Therapy: A Systematic Review and Meta-analysis. *Am J Kidney Dis*. 2020;76(6):826–841.e1.
37. Reis C, Akyol O, Araujo C, Huang L, Enkhjargal B, Malaguit J, et al. Pathophysiology and the Monitoring Methods for Cardiac Arrest Associated Brain Injury. *Int J Mol Sci*. 2017;18(1).
38. Takata K, Takeda Y, Sato T, Nakatsuka H, Yokoyama M, Morita K. Effects of hypothermia for a short period on histologic outcome and extracellular glutamate concentration during and after cardiac arrest in rats. *Crit Care Med*. 2005;33(6):1340–5.
39. Pynnonen L, Falkenbach P, Kamarainen A, Lonrot K, Yli-Hankala A, Tenhunen J. Therapeutic hypothermia after cardiac arrest - cerebral perfusion and metabolism during upper and lower threshold normocapnia. *Resuscitation*. 2011;82(9):1174–9.
40. Bellander BM, Cantais E, Enblad P, Hutchinson P, Nordström CH, Robertson C, et al. Consensus meeting on microdialysis in neurointensive care. *Intensive Care Med*. 2004;30(12):2166–9.
41. Nolan JP, Berg RA, Andersen LW, Bhanji F, Chan PS, Donnino MW, et al. Cardiac Arrest and Cardiopulmonary Resuscitation Outcome Reports: Update of the Utstein Resuscitation Registry Template for In-Hospital Cardiac Arrest: A Consensus Report From a Task Force of the International Liaison Committee on Resuscitation (American Heart Association, European Resuscitation Council, Australian and New Zealand Council on Resuscitation, Heart and

- Stroke Foundation of Canada, InterAmerican Heart Foundation, Resuscitation Council of Southern Africa, Resuscitation Council of Asia). *Resuscitation*. 2019;144:166–77.
42. Guerguieva R, Krystal JH. Move over ANOVA: progress in analyzing repeated-measures data and its reflection in papers published in the Archives of General Psychiatry. *Arch Gen Psychiatry*. 2004;61(3):310–7.
 43. Keselman HJ, Algina J, Kowalchuk RK. The analysis of repeated measures designs: a review. *Br J Math Stat Psychol*. 2001;54(Pt 1):1–20.
 44. Hickey GL, Mokhles MM, Chambers DJ, Kolamunnage-Dona R. Statistical primer: performing repeated-measures analysis. *Interact Cardiovasc Thorac Surg*. 2018;26(4):539–44.
 45. Sandroni C, De Santis P, D'Arrigo S. Capnography during cardiac arrest. *Resuscitation*. 2018;132:73–7.
 46. Garnett AR, Ornato JP, Gonzalez ER, Johnson EB. End-tidal carbon dioxide monitoring during cardiopulmonary resuscitation. *JAMA*. 1987;257(4):512–5.
 47. Skulec R, Vojtisek P, Cerny V. Correlation between end-tidal carbon dioxide and the degree of compression of heart cavities measured by transthoracic echocardiography during cardiopulmonary resuscitation for out-of-hospital cardiac arrest. *Crit Care*. 29 2019;23(1):334.
 48. Lewis LM, Stothert J, Standeven J, Chandel B, Kurtz M, Fortney J. Correlation of end-tidal CO₂ to cerebral perfusion during CPR. *Ann Emerg Med*. 1992;21(9):1131–4.
 49. Ristagno G, Tang W, Sun S, Weil MH. Cerebral cortical microvascular flow during and following cardiopulmonary resuscitation after short duration of cardiac arrest. *Resuscitation*. 2008;77(2):229–34.
 50. Touma O, Davies M. The prognostic value of end tidal carbon dioxide during cardiac arrest: a systematic review. *Resuscitation*. 2013;84(11):1470–9.
 51. Levine RL, Wayne MA, Miller CC. End-tidal carbon dioxide and outcome of out-of-hospital cardiac arrest. *N Engl J Med*. 1997;337(5):301–6.
 52. Kolar M, Krizmaric M, Klemen P, Grmec S. Partial pressure of end-tidal carbon dioxide successful predicts cardiopulmonary resuscitation in the field: a prospective observational study. *Crit Care*. 2008;12(5):R115.
 53. Guidelines for indications for the use of extracorporeal life support in refractory cardiac arrest. French Ministry of Health. *Ann Fr Anesth Reanim*. 2009;28(2):182–90.
 54. Allen BS, Buckberg GD. Studies of isolated global brain ischaemia: Overview of irreversible brain injury and evolution of a new concept - redefining the time of brain death. *Eur J Cardiothorac Surg*. 2012;41(5):1132–7.
 55. Allen BS, Ko Y, Buckberg GD, Sakhal S, Tan Z. Studies of isolated global brain ischaemia: I. A new large animal model of global brain ischaemia and its baseline perfusion studies. *Eur J Cardiothorac Surg*. 2012;41(5):1138–46.
 56. Allen BS, Ko Y, Buckberg GD, Tan Z. Studies of isolated global brain ischaemia: II. Controlled reperfusion provides complete neurologic recovery following 30 min of warm ischaemia - the importance of perfusion pressure. *Eur J Cardiothorac Surg*. 2012;41(5):1147–54.
 57. Allen BS, Ko Y, Buckberg GD, Tan Z. Studies of isolated global brain ischaemia: III. Influence of pulsatile flow during cerebral perfusion and its link to consistent full neurological recovery with controlled reperfusion following 30 min of global brain ischaemia. *Eur J Cardiothorac Surg*. 2012;41(5):1155–63.
 58. Grmec S, Lah K, Tusek-Bunc K. Difference in end-tidal CO₂ between asphyxia cardiac arrest and ventricular fibrillation/pulseless ventricular tachycardia cardiac arrest in the prehospital setting. *Crit Care*. 2003;7(6):R139-144.

59. Ruiz de Gauna S, Gutiérrez JJ, Ruiz J, Leturiondo M, Azcarate I, González-Otero DM, et al. The impact of ventilation rate on end-tidal carbon dioxide level during manual cardiopulmonary resuscitation. *Resuscitation*. 2020;156:215–22.
60. Lindberg L, Liao Q, Steen S. The effects of epinephrine/norepinephrine on end-tidal carbon dioxide concentration, coronary perfusion pressure and pulmonary arterial blood flow during cardiopulmonary resuscitation. *Resuscitation*. 2000;43(2):129–40.
61. Okamoto H, Hoka S, Kawasaki T, Okuyama T, Takahashi S. Changes in end-tidal carbon dioxide tension following sodium bicarbonate administration: correlation with cardiac output and haemoglobin concentration. *Acta Anaesthesiol Scand*. 1995;39(1):79–84.
62. Walsh BK, Crotwell DN, Restrepo RD. Capnography/Capnometry during mechanical ventilation: 2011. *Respir Care*. 2011;56(4):503–9.
63. Nicholson TC, Paiva EF. Uses and pitfalls of measurement of end-tidal carbon dioxide during cardiac arrest. *Curr Opin Crit Care*. 2020;26(6):612–6.
64. Wang CH, Lu TC, Tay J, Wu CY, Wu MC, Chong KM, et al. Association between trajectories of end-tidal carbon dioxide and return of spontaneous circulation among emergency department patients with out-of-hospital cardiac arrest. *Resuscitation*. 2022;177:28–37.
65. Undén J, Ingebrigtsen T, Romner B. Scandinavian guidelines for initial management of minimal, mild and moderate head injuries in adults: an evidence and consensus-based update. *BMC Med*. 25 2013;11:50.
66. Undén L, Calcagnile O, Undén J, Reinstrup P, Bazarian J. Validation of the Scandinavian guidelines for initial management of minimal, mild and moderate traumatic brain injury in adults. *BMC Med*. 2015;13:292.
67. Marchi N, Angelov L, Masaryk T, Fazio V, Granata T, Hernandez N, et al. Seizure-promoting effect of blood-brain barrier disruption. *Epilepsia*. 2007;48(4):732–42.
68. Zhang Y, Li CS, Wu CJ, Yang J, Hang CC. Comparison of Cerebral Metabolism between Pig Ventricular Fibrillation and Asphyxial Cardiac Arrest Models. *Chin Med J (Engl)*. 2015;128(12):1643–8.
69. Hachimi-Idrissi S, Van der Auwera M, Schiettecatte J, Ebinger G, Michotte Y, Huyghens L. S-100 protein as early predictor of regaining consciousness after out of hospital cardiac arrest. *Resuscitation*. 2002;53(3):251–7.
70. Shinozaki K, Oda S, Sadahiro T, Nakamura M, Abe R, Nakada TA, et al. Serum S-100B is superior to neuron-specific enolase as an early prognostic biomarker for neurological outcome following cardiopulmonary resuscitation. *Resuscitation*. 2009;80(8):870–5.
71. Nguyen DN, Huyghens L, Wellens F, Schiettecatte J, Smits J, Vincent JL. Serum S100B protein could help to detect cerebral complications associated with extracorporeal membrane oxygenation (ECMO). *Neurocrit Care*. 2014;20(3):367–74.
72. Mikhail Kellawan J, Harrell JW, Schrauben EM, Hoffman CA, Roldan-Alzate A, Schrage WG, et al. Quantitative cerebrovascular 4D flow MRI at rest and during hypercapnia challenge. *Magn Reson Imaging*. 2016;34(4):422–8.
73. Mörtberg E, Cumming P, Wiklund L, Wall A, Rubertsson S. A PET study of regional cerebral blood flow after experimental cardiopulmonary resuscitation. *Resuscitation*. 2007;75(1):98–104.
74. Ristagno G, Tang W, Huang L, Fymat A, Chang YT, Sun S, et al. Epinephrine reduces cerebral perfusion during cardiopulmonary resuscitation. *Crit Care Med*. 2009;37(4):1408–15.

75. Kashani K, Cheungpasitporn W, Ronco C. Biomarkers of acute kidney injury: the pathway from discovery to clinical adoption. *Clin Chem Lab Med.* 2017;55(8):1074–89.
76. Bennett M, Dent CL, Ma Q, Dastrala S, Grenier F, Workman R, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. *Clin J Am Soc Nephrol.* 2008;3(3):665–73.
77. Prowle JR, Calzavacca P, Licari E, Ligabo EV, Echeverri JE, Bagshaw SM, et al. Combination of biomarkers for diagnosis of acute kidney injury after cardiopulmonary bypass. *Ren Fail.* 2015;37(3):408–16.
78. Haase M, Devarajan P, Haase-Fielitz A, Bellomo R, Cruz DN, Wagener G, et al. The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. *J Am Coll Cardiol.* 2011;57(17):1752–61.
79. de Geus HRH, Bakker J, Lesaffre EMEH, le Noble JLML. Neutrophil gelatinase-associated lipocalin at ICU admission predicts for acute kidney injury in adult patients. *Am J Respir Crit Care Med.* 2011;183(7):907–14.
80. Pianta TJ, Peake PW, Pickering JW, Kelleher M, Buckley NA, Endre ZH. Clusterin in kidney transplantation: novel biomarkers versus serum creatinine for early prediction of delayed graft function. *Transplantation.* 2015;99(1):171–9.
81. Delmas C, Zapetskaia T, Conil JM, Georges B, Vardon-Bouines F, Seguin T, et al. 3-month prognostic impact of severe acute renal failure under veno-venous ECMO support: Importance of time of onset. *J Crit Care.* 2018;44:63–71.
82. Sabbagh R, Chawla A, Tisdale B, Kwan K, Chatterjee S, Kwicien JM, et al. Renal histopathology features according to various warm ischemia times in porcine laparoscopic and open surgery model. *Can Urol Assoc J.* 2011;5(1):40–3.
83. Murakami N, Kokubu N, Nagano N, Nishida J, Nishikawa R, Nakata J, et al. Prognostic Impact of No-Flow Time on 30-Day Neurological Outcomes in Patients With Out-of-Hospital Cardiac Arrest Who Received Extracorporeal Cardiopulmonary Resuscitation. *Circ J.* 2020;84(7):1097–104.
84. Ortega-Deballon I, Hornby L, Shemie SD, Bhanji F, Guadagno E. Extracorporeal resuscitation for refractory out-of-hospital cardiac arrest in adults: A systematic review of international practices and outcomes. *Resuscitation.* 2016;101:12–20.
85. Wang J, Ma Q, Zhang H, Liu S, Zheng Y. Predictors of survival and neurologic outcome for adults with extracorporeal cardiopulmonary resuscitation: A systemic review and meta-analysis. *Medicine (Baltimore).* 2018;97(48):e13257.
86. Fagnoul D, Combes A, De Backer D. Extracorporeal cardiopulmonary resuscitation. *Curr Opin Crit Care.* 2014;20(3):259–65.
87. Otani T, Sawano H, Natsukawa T, Nakashima T, Oku H, Gon C, et al. Low-flow time is associated with a favorable neurological outcome in out-of-hospital cardiac arrest patients resuscitated with extracorporeal cardiopulmonary resuscitation. *J Crit Care.* 2018;48:15–20.
88. Wengenmayer T, Rombach S, Ramshorn F, Biever P, Bode C, Duerschmied D, et al. Influence of low-flow time on survival after extracorporeal cardiopulmonary resuscitation (eCPR). *Crit Care.* 2017;21(1):157.
89. Yukawa T, Kashiura M, Sugiyama K, Tanabe T, Hamabe Y. Neurological outcomes and duration from cardiac arrest to the initiation of extracorporeal membrane oxygenation in patients with out-of-hospital cardiac arrest: a retrospective study. *Scand J Trauma Resusc Emerg Med.* 2017;25(1):95.

90. Bartos JA, Grunau B, Carlson C, Duval S, Ripeckyj A, Kalra R, et al. Improved Survival with Extracorporeal Cardiopulmonary Resuscitation Despite Progressive Metabolic Derangement Associated with Prolonged Resuscitation. *Circulation*. 2020;17;141(11):877-886.
91. Twohig CJ, Singer B, Grier G, Finney SJ. A systematic literature review and meta-analysis of the effectiveness of extracorporeal-CPR versus conventional-CPR for adult patients in cardiac arrest. *J Intensive Care Soc*. 2019;20(4):347-57.
92. Park SB, Yang JH, Park TK, Cho YH, Sung K, Chung CR, et al. Developing a risk prediction model for survival to discharge in cardiac arrest patients who undergo extracorporeal membrane oxygenation. *Int J Cardiol*. 2014;177(3):1031-5.
93. Ko RE, Ryu JA, Cho YH, Sung K, Jeon K, Suh GY, et al. The differential neurologic prognosis of low-flow time according to the initial rhythm in patients who undergo extracorporeal cardiopulmonary resuscitation. *Resuscitation*. 2020;148:121-7.
94. Haas NL, Coute RA, Hsu CH, Cranford JA, Neumar RW. Descriptive analysis of extracorporeal cardiopulmonary resuscitation following out-of-hospital cardiac arrest-An ELSO registry study. *Resuscitation*. 2017;119:56-62.
95. Goto T, Morita S, Kitamura T, Natsukawa T, Sawano H, Hayashi Y, et al. Impact of extracorporeal cardiopulmonary resuscitation on outcomes of elderly patients who had out-of-hospital cardiac arrests: a single-centre retrospective analysis. *BMJ Open*. 2018;8(5):e019811.
96. Debaty G, Lamhaut L, Aubert R, Nicol M, Sanchez C, Chavanon O, et al. Prognostic value of signs of life throughout cardiopulmonary resuscitation for refractory out-of-hospital cardiac arrest. *Resuscitation*. 2021;162:163-70.
97. Halenarova K, Belliato M, Lunz D, Peluso L, Broman LM, Malfertheiner MV, et al. Predictors of poor outcome after extra-corporeal membrane oxygenation for refractory cardiac arrest (ECPR): A post hoc analysis of a multicenter database. *Resuscitation*. 2021;170:71-8.
98. Richardson ASC, Tonna JE, Nanjayya V, Nixon P, Abrams DC, Raman L, et al. Extracorporeal Cardiopulmonary Resuscitation in Adults. Interim Guideline Consensus Statement From the Extracorporeal Life Support Organization. *ASAIO J*. 2021;67(3):221-8.
99. Matsuyama T, Irisawa T, Yamada T, Hayakawa K, Yoshiya K, Noguchi K, et al. Impact of Low-Flow Duration on Favorable Neurological Outcomes of Extracorporeal Cardiopulmonary Resuscitation After Out-of-Hospital Cardiac Arrest: A Multicenter Prospective Study. *Circulation*. 2020;141(12):1031-3.
100. Reynolds JC, Frisch A, Rittenberger JC, Callaway CW. Duration of resuscitation efforts and functional outcome after out-of-hospital cardiac arrest: when should we change to novel therapies? *Circulation*. 2013;128(23):2488-94.
101. Kim SJ, Jung JS, Park JH, Park JS, Hong YS, Lee SW. An optimal transition time to extracorporeal cardiopulmonary resuscitation for predicting good neurological outcome in patients with out-of-hospital cardiac arrest: a propensity-matched study. *Crit Care*. 2014;18(5):535.
102. Dennis M, Zmudzki F, Burns B, Scott S, Gattas D, Reynolds C, et al. Cost effectiveness and quality of life analysis of extracorporeal cardiopulmonary resuscitation (ECPR) for refractory cardiac arrest. *Resuscitation*. 2019;139:49-56.

103. Gravesteijn BY, Schluep M, Voormolen DC, van der Burgh AC, Dos Reis Miranda D, Hoeks SE, et al. Cost-effectiveness of extracorporeal cardiopulmonary resuscitation after in-hospital cardiac arrest: A Markov decision model. *Resuscitation*. 2019;143:150–7.
104. Matsuoka Y, Goto R, Atsumi T, Morimura N, Nagao K, Tahara Y, et al. Cost-effectiveness of extracorporeal cardiopulmonary resuscitation for out-of-hospital cardiac arrest: A multi-centre prospective cohort study. *Resuscitation*. 2020;157:32–8.
105. Bharmal MI, Venturini JM, Chua RFM, Sharp WW, Beiser DG, Tabit CE, et al. Cost-utility of extracorporeal cardiopulmonary resuscitation in patients with cardiac arrest. *Resuscitation*. 2019;136:126–30.

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