Mechanical assist in cardiac arrest: Optimising circulatory support. Experimental studies.

Erik J. S. Packer

Thesis for the degree of Philosophiae Doctor (PhD)
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Scientific environment

This research project was performed at the animal facilities (Vivarium), a core facility at the Faculty of Medicine at the University of Bergen, under the auspices of the Interventional Cardiology Research group, University of Bergen, and the Department of Heart Disease, Haukeland University Hospital, Bergen, Norway.

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Abbreviations

mAP Mean aortic pressure

BIVAD Biventricular assist devices (Paper I)

BiPella Combination of left and right impeller assist devices (Paper II)

CA Cardiac arrest

CO Cardiac output

CPP Coronary perfusion pressure

CPR Cardiopulmonary resuscitation

mCVP Mean central venous pressure

ECMO Extracorporeal membrane oxygenation

ECPR Extracorporeal cardiopulmonary resuscitation

F French (0.33mm)

IHCA In-hospital cardiac arrest

mLADf Mean left descending artery flow

LVAD Left ventricular assist device

mLVP Mean left ventricular pressure

MCS Mechanical circulatory support

OHCA Out-of-hospital cardiac arrest

mPAf Mean pulmonary artery flow

ROSC Return of spontaneous circulation

RVAD Right ventricular assist device

VF Ventricular fibrillation

List of publications

Paper I:

Packer EJS, Slettom G, Solholm A, Mongstad A, Haaverstad R, Tuseth V, Grong K, Nordrehaug JE. Left versus biventricular assist devices in cardiac arrest.
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Paper II:

Packer EJS, Slettom G, Solholm A, Omdal TR, Stangeland L, Zhang L, Mongstad A, Løland K, Haaverstad R, Grong K, Nordrehaug JE, Tuseth V. Balanced biventricular assist versus extracorporeal membrane oxygenation in cardiac arrest.
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Paper III:

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ASAIO J. 2021 Jul 20
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Abstract

Introduction

Mechanical circulatory support (MCS) may be useful in cardiac arrest (CA), both in- and out- of hospital. However, efficacy and survival benefit has been difficult to evaluate compared to standard cardiopulmonary resuscitation. In three experimental studies we aimed to assess different modes of MCS during CA in providing adequate organ perfusion and systemic circulation and identify predictors of sustainable post-CA heart function.

Different theoretical assumptions were the background for analysis in the three study protocols performed as acute experiments in anaesthetized pigs:
Paper I: A major limitation to the effectiveness of a LVAD alone during CA is the lack of left ventricular (LV) filling due to minimal pulmonary circulation. We therefore wanted to assess if the combination of a left- and right ventricular assist device (BIVAD/BiPella) was beneficial as circulatory support versus a LVAD alone.
Paper II: ECMO has the potential to replace systemic circulation during CA. However, concerns have been voiced regarding retrograde flow-delivery and effect on the myocardium during circulatory collapse. Based on results from Paper I we optimized BiPella support aiming to improve and maintain acceptable coronary perfusion pressure, believing this could potentially rectify the poor outcome of BIVAD/BiPella in Paper I if successful. Thus, in Paper II we compared the efficacy of balanced biventricular circulatory assist with extracorporeal membrane oxygenation (ECMO).
Paper III: Pressure build-up in the left ventricle during cardiac arrest may be detrimental during extracorporeal cardiopulmonary resuscitation (ECPR) as indicated in Paper II. Therefore, we wished to investigate if unloading (venting) the left ventricle using add-on LVAD could be of benefit. However, the ideal flow-contributions of each assist device when combining LVAD and ECMO during ECPR in is not known. We therefore wanted to compare ECMO with standard or reduced flow and add-on LVAD versus ECMO alone. Finally, we wished to assess the contribution of add-on LVAD regarding pulmonary flow.

Materials and methods

The animal experiments were performed at the Vivarium, University of Bergen, and protocols were approved by the Norwegian Animal Research Authority or by the Norwegian Food Safety Authority.

Paper I and II were performed with percutaneous techniques. The final experiment was an open chest model.

All protocols followed a similar timeline:

1. Anaesthesia and instrumentation of the pig.
2. Baseline evaluation.
3. Induction of CA by application of a 9V DC battery to the myocardium.
4. Immediate initiation of mechanical circulatory support (MCS).
5. Three attempts of cardioversion at the end of the CA period.
6. If successful return of spontaneous circulation (ROSC) was achieved, unsupported observation (Paper II and Paper III).

Comparisons between intervention groups:

1. Haemodynamics (during and after CA).
2. Organ tissue blood flow rate (organ perfusion) and device output as calculated from fluorescent microspheres.
3. Arterial blood gases and biomarkers.
4. ROSC.
5. Sustained cardiac function post-ROSC (Paper II and Paper III).

In Paper I, twenty animals were randomized in two groups receiving circulatory support either by the Impella CP alone (LVAD) or in combination with the Impella RP (BIVAD/BiPella) during 30 minutes of CA.

In Paper II, twenty pigs were randomized to receive MCS either by BiPella or by extracorporeal membrane oxygenation (ECMO) during 40 minutes of CA. If ROSC was successful, animals were observed for 60 minutes unsupported.

In Paper III, twenty-four animals were randomized in three groups. Extracorporeal cardiopulmonary resuscitation (ECPR) in Group 1 was provided by ECMO with standard-flow and add-on Impella CP. In Group 2: ECMO with reduced flow combined with Impella CP. In Group 3, animals were supported by standard-flow ECMO alone. ECPR lasted for 60 minutes. If ROSC was successful, 180 minutes unsupported observation followed.

Results

Paper I demonstrated that BIVAD/BiPella provides superior circulatory support and perfusion for peripheral organs (including the brain) related to higher LVAD output and increased central aortic pressure compared to LVAD alone. However, myocardial perfusion was related to the pressure difference between mean aortic pressure and mean left ventricular pressure during cardiac arrest. Myocardial perfusion was inferior with BiPella resulting in significantly fewer ROSC (5/10 vs 10/10, $p = 0.033$) despite significantly higher $etCO_2$ ($p = 0.029$).

Paper II showed that balancing RVAD and LVAD to ensure acceptable coronary perfusion pressure and concomitant LVAD output was feasible, also sustaining vital organ perfusion. However, ECMO provided a more optimal systemic circulatory support. Device output and mean aortic pressure were increased with subsequent improved peripheral tissue perfusion reflected by reduction of s-lactate. In animals where sufficient myocardial perfusion pressure (mean aortic pressure – mean LV pressure > 10-15 mmHg) could not be achieved, perfusion (ml/min/g) was reduced in the subendo- and midmyocardium, averaging 0.59 ± 0.05 vs. 0.31 ± 0.07, ($p = 0.005$) and 0.91 ± 0.06 vs 0.65 ± 0.15 ($p = 0.085$), but not in the subepicardium (1.02 ± 0.07 vs 0.86 ± 0.17, $p = 0.30$) irrespective of group. These subjects also had inferior post-ROSC cardiac function.

Paper III showed that add-on LVAD improved haemodynamics compared with ECMO alone during refractory CA. Add-on LVAD could not substitute a reduced ECMO-flow. Three animals with reduced ECMO flow and adjunctive Impella support did not achieve ROSC. With ECMO alone, ROSC was obtained in all animals. However, 4/8

died post-ROSC due to development of cardiogenic shock. In the remaining 21 animals, 17 animals had sustained cardiac function at study termination 3 h after ROSC. Animals without sustained cardiac function (7/24) had reduced mAP ($p < 0.001$), CPP ($p = 0.002$) and mPAf ($p = 0.004$) during CA and ECPR.

Conclusions

Paper I: Biventricular support during cardiac arrest was associated with high intraventricular pressure in the left ventricle resulting in decreased myocardial perfusion pressure, reduced myocardial tissue blood flow rate and subsequent reduction in ROSC.

Paper II: Myocardial perfusion and sustained cardiac function were related to myocardial perfusion pressure during VF irrespective of MCS (ECMO and balanced biventricular support). Balanced biventricular support maintained lower intraventricular pressure compared to ECMO.

Paper III: Add-on LVAD improved haemodynamics compared to ECMO alone. An add-on Impella could not substitute a reduction in ECMO flow. Increased mean aortic pressure, myocardial perfusion pressure and mean pulmonary artery flow were related to sustained cardiac function and ROSC.

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1. Introduction

1.1 Background

Out-of-hospital cardiac arrest (OHCA) was reported in 3715 people in Norway in 2019. Of these, approximately 10% survived the initial 30 days. Survival rate has been stable between 2013 and 2019. In-hospital, 1002 cases of cardiac arrest (CA) were registered, with a 25% 30 day survival.[1] Approximately 60-70% of cardiac arrest cases have cardiac causes. If cardiac arrest is caused by myocardial infarction, and ST-elevation is present in the electrocardiogram (ECG), percutaneous coronary intervention (PCI) is indicated and may improve prognosis.[2,3]

During CA, circulation of blood to all organs ceases. Within minutes irreversible damage to the organs occurs.[4,5] Unless blood flow can be re-initiated or substituted and the underlying cause reversed, death is inevitable. The probability of a favourable neurological outcome decreases by the minute, and after 20 minutes of no-flow, survival with good neurological status is exceedingly rare.[6] Therefore, rapid initiation of good quality cardiopulmonary resuscitation (CPR) is crucial. Even if CPR can be initiated rapidly by competent personnel or bystanders, maintaining adequate output/circulating blood volume to ensure sufficient vital organ perfusion is a major challenge, and duration over 20 minutes is associated with poor neurological outcome.[7] Also, with OHCA transport is difficult since standard CPR may endanger paramedic staff unacceptably. External mechanical chest compressions may be useful for improving quality of CPR and enable transport, but evidence is conflicting.[8-10]

Extracorporeal membrane oxygenation (ECMO), a miniature heart-lung machine, has the potential of fully substituting systemic circulation during refractory cardiac arrest. In some major cities, extracorporeal cardiopulmonary resuscitation (ECRP) programmes using ECMO out-of-hospital have been implemented. Specialized personnel implant ECMO in the setting of refractory CA hoping to improve outcomes compared to conventional CPR.[11-15] However, most reports are small, including a few propensity matched studies. Although indicating feasibility and potential, impact on survival varies.[16-18] Also with regard to in-hospital cardiac arrest (IHCA) recent propensity matched analyses have indicated benefit,[19,20] but others have not.[21]

Systematic reviews have tried to identify predictors of survival both with OHCA and IHCA.[22,23] Some experimental studies have shown that percutaneous left ventricular assist devices may have benefits when treating patients with in-hospital cardiac arrest compared to current standard life support.[24] However, device potential may be limited during ECPR due to insufficient return of blood to the left ventricle (LV). This severely decreases device output regardless of the device's maximal output and fluid substitution.[25-27] Initial reports, including a registry from our own hospital, may indicate feasibility and potential benefit of Impella and ECMO in cardiogenic shock and cardiac arrest in daily clinical practice.[28-30] A Danish nationwide study from 2011-2020 reported a survival of 26% in refractory OHCA. ECPR was effected either by ECMO or LVAD (Impella).[31] Lately, the combination of ECMO and a left ventricular assist device (LVAD) have been investigated in limited clinical settings due to concerns regarding myocardial conservation, survival and post-resuscitation function with ECMO alone during ECPR.[29,32-34] Specifically pertaining to the retrograde blood flow of the ECMO circuit constituting increased left ventricular afterload subsequent to successful ROSC.[35,36] Although predominantly discussed in relation to cardiogenic shock, LV unloading may have a role in cardiac arrest.[37-39]

Over time the use of ECPR has escalated with successive increase in demand on economic resources and personnel. However, a review was not able to show increasing survival over a 12 year period (survival approximately 30%).[40] In 2018 the International Liaison of Committee on Resuscitation performed a systematic review assessing the use of ECPR compared to manual or mechanical resuscitation and found no overall benefit.[41] The authors report low quality of evidence across studies. This conclusion is echoed in the Extracorporeal Cardiopulmonary Resuscitation Organisation (ELSO),[42] American Heart Association (AHA),[43] and European Resuscitation Council guidelines.[44] Only one randomized trial has to date been published on the use of ECPR during refractory CA.[45] The study was terminated early with a survival of 43% in the ECPR group versus 7% in the standard treatment group. Noteworthy, this research group represent unique skills and logistics, and although providing an indication of the potential of ECPR in selected patients, such results may be difficult to reproduce routinely.

1.2 Rationale for the experimental protocols

Experimental cardiac arrest protocols using swine are important contributors to the development of new methodology and novel hypotheses, and in the initial testing of medical equipment designed for human use.[10,20,25-27,46,47] Generally, the porcine model offers both a size and similar anatomy and physiology compared to humans, albeit not identical. For instance, the pulmonary artery is directed differently compared to humans due to quadruped versus biped stance. This was particularly relevant regarding the RVAD instrumentation used in Paper I and II.

This thesis is based upon previous porcine models investigating the use of left ventricular assist devices during refractory cardiac arrest,[25-27] providing the context for the experimental research questions in Paper I. In previously published experimental models with VF induced by ischaemia (LAD-occlusion), the percutaneous left ventricular assist device (Impella 2.5) provided sufficient myocardial and cerebral perfusion during a limited period (30 minutes) of cardiac arrest. The LVAD output was hampered by suction due to limited filling of the left ventricle from the pulmonary circulation during VF. Neither fluid load during VF nor a more powerful LVAD device improved the haemodynamics or outcome substantially.[25-27]

Based on these experimental studies the protocol for Paper I compared LVAD (Impella CP) alone to the combination of the same LVAD and a novel right ventricular assist device (Impella RP) with regard to improvement of the LVAD output, haemodynamics, myocardial perfusion and ROSC rate. Paper II focused on haemodynamics, ROSC and coronary perfusion pressure (CPP) during VF and ECPR with the combination of LVAD/RVAD compared to ECMO. In Paper III the potential benefit of combining ECMO with the LVAD device was assessed. Importantly, all three protocols were designed to delineate haemodynamic effects during cardiac arrest and mechanical circulatory support in an idealized setting. Based on the gained experience with these experimental protocols, the duration of cardiac arrest was gradually increased; 30 min in Paper I, 40 min in Paper II and 60 min in Paper III.

1.3 Assist devices

1.3.1 Left ventricular assist device

The Impella CP is a 14F percutaneously implanted continuous-flow rotational pump that is capable of delivering up to 3.5 l/min. In clinical practice it is implanted through a femoral artery with the inlet of the device in the left ventricular cavity, and the outflow in the ascending aorta. It can be implanted rapidly in a cardiac catheterization laboratory (cath. lab.). See Figures 1 and 3 for overview.

1.3.2 Right ventricular assist device

The Impella RP is a continuous-flow right heart assist device, capable of delivering up to 4 l/min. It is implanted through a femoral vein with the inlet in the inferior caval vein or right atrium, and the outflow in the common pulmonary artery trunk. It can be combined with the Impella CP to provide complete support of the heart; BIVAD or BiPella (Figures 1 and 3). BiPella can be inserted and initiated within minutes after the patient arrives at a cath. lab.

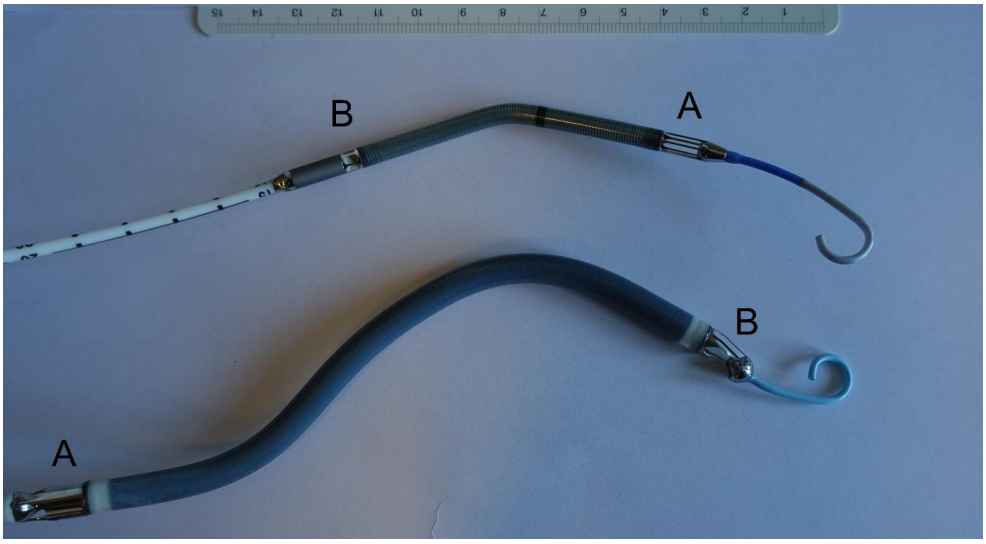

Figure 1: Image of Impella CP (top) and Impella RP (bottom) ex-vivo. A: Inlet, B: Outlet (Packer et al, ASAIO J (2018), 64(4): 489-496).

1.3.3 Extracorporeal membrane oxygenation

The extracorporeal membrane oxygenation unit (ECMO) is a miniaturized heart-lung machine which externally circulates and oxygenates blood (Figure 2). Typically, a

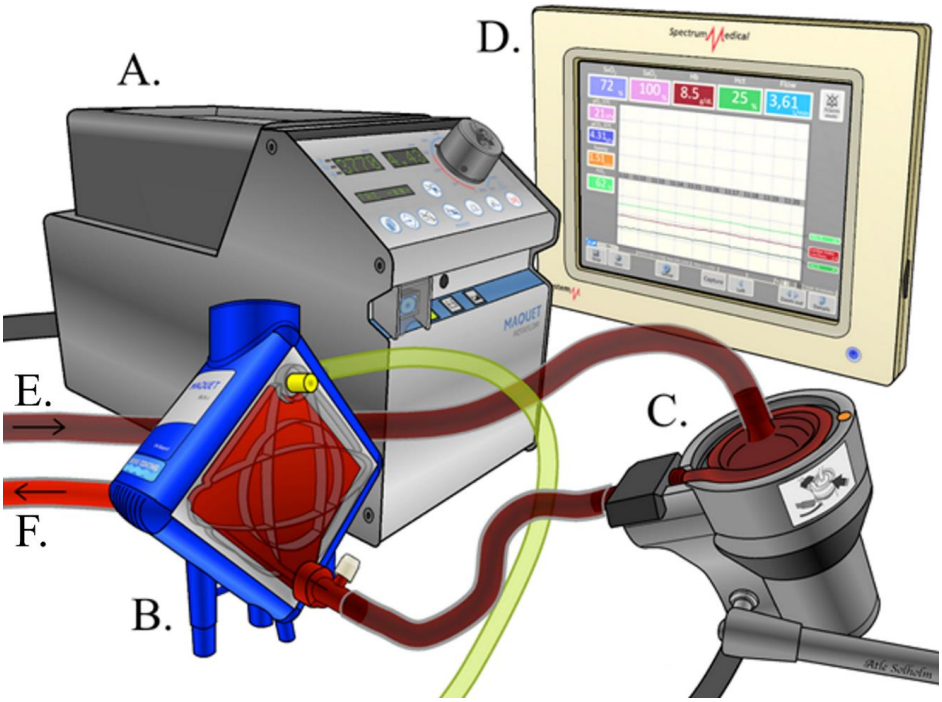

Figure 2: Rotaflow™ ECMO system
A: The Rotaflow ECMO unit, B: The Maquet PLS-i oxygenator, C: The Rotaflow centrifugal pump, D: Surveillance Monitor, E: Venous ECMO cannula (venous blood from the research animal), F: Arterial ECMO cannula (oxygenated blood to the research animal). (Artwork: A. Solholm 2021).

venous cannula is inserted in a femoral vein, and an arterial cannula is placed in a femoral artery. The venous cannula has several perforations, and the distal tip usually extends into the right atrium. Through this sheath venous blood is extracted and circulated with a centrifugal pump through an external membrane oxygenator and heat exchanger before it is returned to the body through the arterial cannula. The outflow of the arterial cannula normally extends into the common iliac artery (Figures 3 and 5) or the very distal part of the abdominal aorta. Extracorporeal cardiopulmonary resuscitation (ECPR) with ECMO can be employed both during in-hospital- and out-of-hospital cardiac arrest. With proper training ECMO may be implanted percutaneously and within minutes.

2. Aims

The aims of this thesis were to evaluate different modalities of mechanical circulatory support in providing adequate life support and organ perfusion during ventricular fibrillation. We wished to assess effectiveness and to identify predictors of sustainable post-CA heart function.

Paper I:

1. To investigate the effectiveness of the Impella CP with or without support of the Impella RP.
2. Compare outcome as measured by return of spontaneous circulation (ROSC).

Paper II:

1. Comparison of the haemodynamic efficacy and vital organ perfusion of balanced biventricular support versus extracorporeal membrane oxygenation.
2. Identification of possible prognostic markers.
3. Compare outcome as evaluated by ROSC and sustained cardiac function up to 60 minutes of post-ROSC observation.

Paper III:

1. Compare ECMO life support with or without concomitant Impella CP unloading of the left ventricle on haemodynamics and tissue perfusion.
2. Assess effects of two different ECMO flow settings with adjunctive percutaneous LVAD support and appraise potential simultaneous LV unloading.
3. Evaluate outcome by ROSC and sustained cardiac function up to 180 minutes of post-ROSC observation.

3. Methods

Experimental cardiac arrest protocols using domestic pigs are common.[48] Haemodynamic and biochemical reference values are previously described and are similar to human.[49,50] The anatomical, physiological and biochemical similarity with humans allow repeated interventions including blood sampling, electrocardiography and pressure monitoring while accommodating treatment. If animals are sized sensibly, medical equipment and drug therapies can be tested before application in human beings.[51,52] Without preclinical data, this would in many instances be ethically debatable. The porcine model may be considered the most appropriate in cardiovascular research.[53] Moderate expense, availability and ease of procurement are also of benefit.

Over time, study groups at the Department of Clinical Science have accumulated extensive experience and know-how regarding porcine models for cardiovascular surgery, anaesthetic procedures, ischaemic cardiac arrest, and the non-ischaemic cardiac arrest model developed for this thesis. Within the framework of the Vivarium and a stable research environment, animal welfare can be ensured following regulatory guidelines and at the same time enabling reasonable quality of research by sharing knowledge in translational medicine. Specifically, the anaesthetic protocol is validated and haemodynamics are consistent and predictable.[54]

The three animal studies comprising this thesis are interventional, comparing different strategies of novel character for handling circulatory arrest. For each single study a list was prepared by computerized block randomisation with five blocks of four animals, two from each experimental group (Paper I and Paper II), and with eight blocks of three animals, one from each experimental group (Paper III). A new experiment substituted an animal discarded due to technical failure during preparation and instrumentation. This routine secures a randomized but even allocation of animals to experimental groups over time, thus minimizing unwarranted influence of the learning curve in the execution of these technically demanding experiments.

Due to the principle of minimizing animal expenditure, and limited data to base a power calculation upon, groups of 8-10 were chosen. In this setting, groups of 10 have the power to detect relevant differences in the range of 10-20%. A small sample size makes interpretation of results prone to type II errors. Strict adherence to protocol with repeated measurements, sensitive evaluation with relevant markers, and a model that is as simple as possible is necessary to minimize the risk.

3.1 Animals and anaesthesia

The experimental protocols were approved by the Norwegian Animal Research Authority (Paper I, Project 6192) or by the Norwegian Food Safety Authority (Paper II and III, Project 13165). All experiments were executed in accordance with the European Communities Council Directive of 2010/63/EU.

Pigs (NOROC) of either sex weighing 50-67 kilos were acclimatised at the experimental facility in a controlled environment and fed on a standard diet. On the day preceding the experiment, 300 mg of aspirin was administered orally. Animals were fasted overnight with free access to water.

Premedication consisted of atropine (1.0 mg), diazepam (10 mg), and ketamine (20 mg /kg), administered i.m. via a butterfly cannula in the neck, and further mask ventilation for a short period with 3% isoflurane in oxygen. Two ear veins were cannulated to provide intravenous access for infusion of fluids and anaesthetic drugs. Intravenous anaesthesia was induced by loading doses of midazolam 0.3 mg/kg, fentanyl 0.02 mg/kg and sodium pentobarbital 15 mg/kg. Anaesthesia was maintained throughout the experiment with a continuous infusion of midazolam 0.3 mg/kg/h, fentanyl 0.02 mg/kg/h, and sodium pentobarbital 4 mg/kg/h. Following tracheostomy and intubation, animals were mechanically ventilated with 58% nitrous oxide and oxygen with a tidal volume set to 11 ml/kg and frequency adjustments aiming at an arterial pCO_2 of 5.0-5.5 kPa (Julian, Drägerwerk, Lübeck, Germany). The sodium pentobarbital was dissolved in the basic liquid substitution; Ringer's acetate with 20 mmol/l of potassium chloride (KCl) added, administered at 15 ml/kg/h i.v.[54] A supra-pubic urinary bladder catheter was inserted surgically. The animals were continuously monitored with ECG,

pulse oximetry and rectal temperature. The end-tidal CO_2 ($etCO_2$) was recorded from a sensor in the ventilator's connector to the endotracheal tube.

3.2 Closed chest models (Paper I and II)

3.2.1 Basic instrumentation

A 5F cannula (Radifocus Introducer II, Terumo, Leuven, Belgium) was placed in the left jugular vein for extra fluid infusion during the induced cardiac arrest. With a semi-percutaneous approach, vascular cannulas were placed in both groins by surgical incisions down to the muscular layer to facilitate arterial palpation and sheath introduction. After placement of the initial sheath, 5000 IE units of Heparin was given and repeated by the hour. Arterial blood samples were drawn including acid-base measurements (ABL800Flex, Radiometer Medical ApS, Brønshøj, Denmark) before further preparation and cannulation. Sheaths were then inserted in the right femoral vein (10F) and right femoral artery (10F) to allow instrumentation with an 8F Acunav catheter (Siemens Medical Solutions, Malvern, PA, USA) and intracardiac echocardiography (ICE) with Vivid Q (GE Vingmed Ultrasound, Horten, Norway). Blood flow rate in the left carotid artery was measured with a 3 mm probe and transit time flowmeter (T402-PB, Transonic Systems Inc., Ithaca, NY, USA). Through a 5F cannula placed in the right carotid artery, a pigtail catheter was inserted into the left ventricle for injection of fluorescent microspheres (Dye-Trak "F"; Triton technology Inc., San Diego, CA, USA). Pressures in the left ventricle, the abdominal aorta and the inferior caval vein were measured (TruWave®, Edwards Lifesciences, Irvine, CA, USA).

An 18F Cook sheath (Cook Medical Inc., Bloomington, IN, USA) was introduced into the left femoral artery after predilatation in the BiPella group over an Amplatz super stiff wire (Cook Medical Inc.). In the right femoral vein, a 23F sheath (Abiomed, Aachen, Germany) was placed over an Amplatz super stiff wire (Cook Medical Inc.) after predilatation. In animals randomized to ECMO (Maquet Rotaflow Driver, Maquet Rotaflow Console, Maquet PLS set, Rastatt, Germany), cannulation was performed under fluoroscopic guidance (Cios Alpha, Siemens, Erlangen, Germany)

using a Maquet HLS 19F sheath (Maquet Cardiopulmonary AG) for arterial inlet. A 23F cannula (Maquet Cardiopulmonary AG) was placed with the distal tip in the right atrium. After insertion of cannulas, the ECMO tubes were connected and the system put on "stand by" (Figure 3).

Up-front implantation of the assist devices was performed for several reasons. Generally, implantation of the circulatory assist is more cumbersome in swine than in humans, for instance requiring some surgery for implantation of cannulas. In Paper I the novelty of Impella RP meant there was a significant learning curve with regard to deployment. Also, since the device is designed for use in humans (biped stance versus quadruped stance) it was more challenging to implant in swine due to differences in pulmonary artery anatomy. Thus, the risk of prolonged no-flow time with deployment subsequent to induction of cardiac arrest meant that the experiments with high probability would prove unpredictable and possibly futile due to lack of standardisation. Next, up-front implantation of the mechanical circulatory support would provide idealised and standardised conditions optimal for investigating and further delineating differences in haemodynamic effects between different concepts of circulatory support.

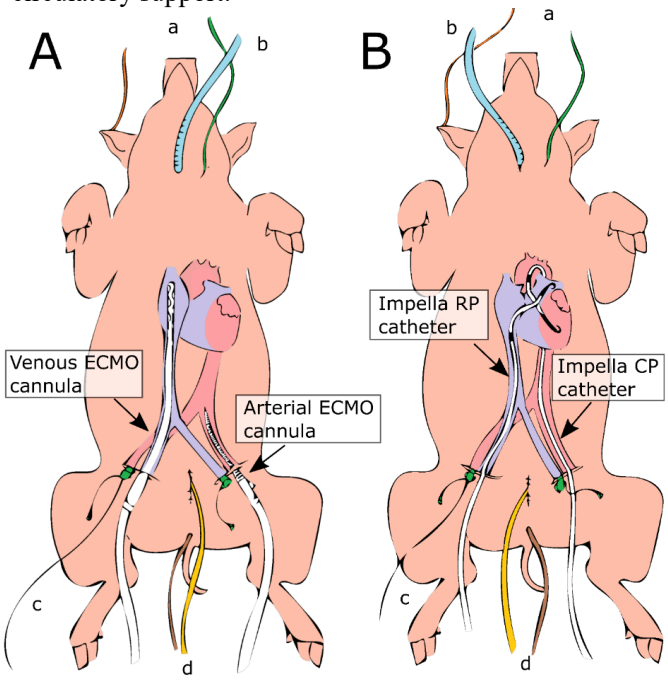

Figure 3: Experimental set-up in the closed chest model. Panel A: ECMO. Panel B: BIVAD/BiPella.
a: Tracheostomy and intubation cannula, b: Venous infusion, c: Pigtail catheter, d: Suprapubic catheter and rectal temperature probe (Packer et al, ASAIO J (2020), 66(10):1110-1119).

3.2.2 LVAD and RVAD placement

A Swan-Ganz catheter was floated into the pulmonary artery bifurcation through the 23F venous access under fluoroscopic guidance. Through the lumen of this catheter a 0.25" Amplatz super stiff wire (Cook Medical Inc.) was placed in the right pulmonary artery over which the Impella RP was introduced under fluoroscopic guidance. After calibration of the haemodynamics sensor in the inferior caval vein/right atrium, the final RVAD position was guided and verified by Intracardiac Echocardiography (ICE) (GE Vingmed Ultrasound) and fluoroscopy. The inlet of the RVAD was in the transition between the inferior caval vein and the right atrium, and the outflow was above the pulmonary valve (Figure 3). We found that ICE images of the pulmonary valve could best be achieved from the aorta and could confirm the continuous flow of the Impella RP with Doppler (Continuous wave and colour).

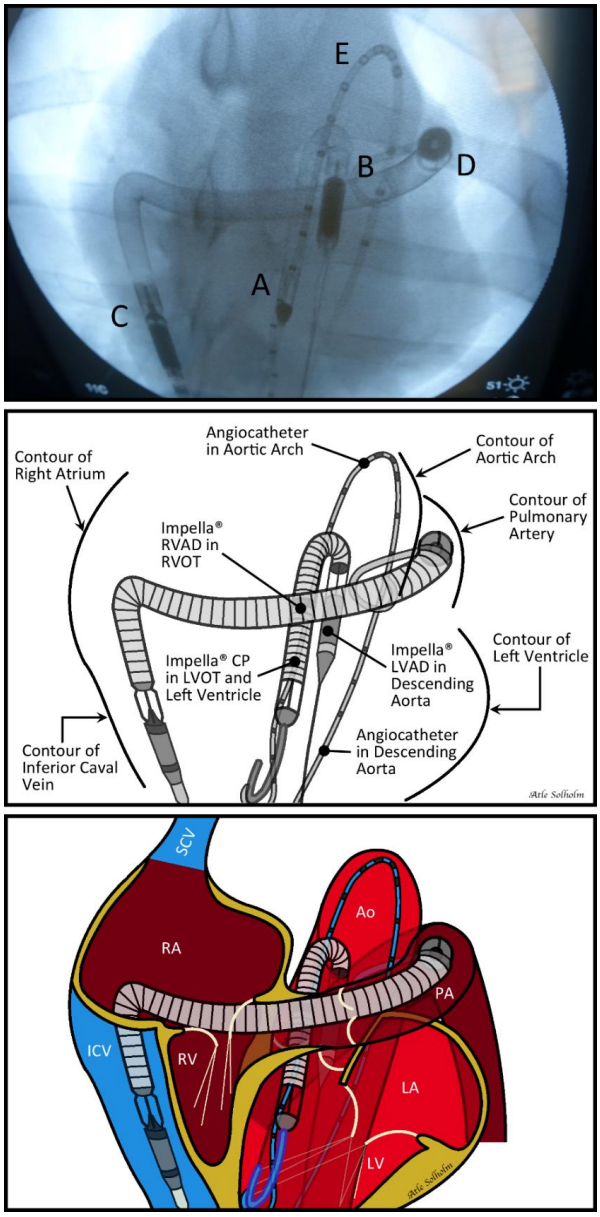

Figure 4: X-ray image of biventricular assist devices in vivo.
A: Impella CP inlet, B: Impella CP outlet, C: Impella RP inlet, D: Impella RP outlet, E: Pigtail catheter
(Top panel: Packer et al, ASAIO J (2018), 64(4): 489-496/Middle and lower panels: A. Solholm 2022).

Irrespective of randomization, but after RVAD deployment in the BIVAD group, the Impella CP was placed over a 0.013" 300 mm wire with the inlet in the left ventricular apex and the outflow in the ascending aorta. When in place, both pumps were started at lowest possible flow output (P1) to prevent thrombus formation (Figure 4) as recommended by the manufacturer.

3.3 Open chest model (Paper III)

Premedication, anaesthesia and basic instrumentation were executed in the same manner as previously described in the closed chest model (Ch. 3.2), as was cannulation of femoral arteries and veins including placement of ECMO cannulas and insertion of a pigtail catheter to left ventricle (Figure 3).

A median sternotomy and pericardiotomy had to be performed to expose the beating heart for the facilitation of transit time flowmetry measurements and epicardial echocardiography. Subsequently, the pulmonary artery trunk and the proximal left anterior descending coronary artery (LAD) could be prepared to allow placement of 16 mm and 3 mm perivascular probes connected to a transit time flowmeter (CM4000, Medistim, Oslo, Norway). Exposure of the myocardium allowed direct epicardial echocardiography, which is preferable in pigs due to interposed air-filled lungs between the thoracic wall and the heart, narrow intercostal spaces and placement and rotation of the heart that makes transthoracic echocardiography challenging.[55] Transoesophageal echocardiography can be performed,[56,57] however, the anterior location limits right heart evaluation.[58]

Through the intubation incision, a 14F arterial sheath (Cook Medical Inc., Bloomington, IN, USA) could be introduced in the left carotid artery to enable the deployment of a percutaneous LVAD, the Impella CP (Abiomed), to the left ventricle (Figure 5).

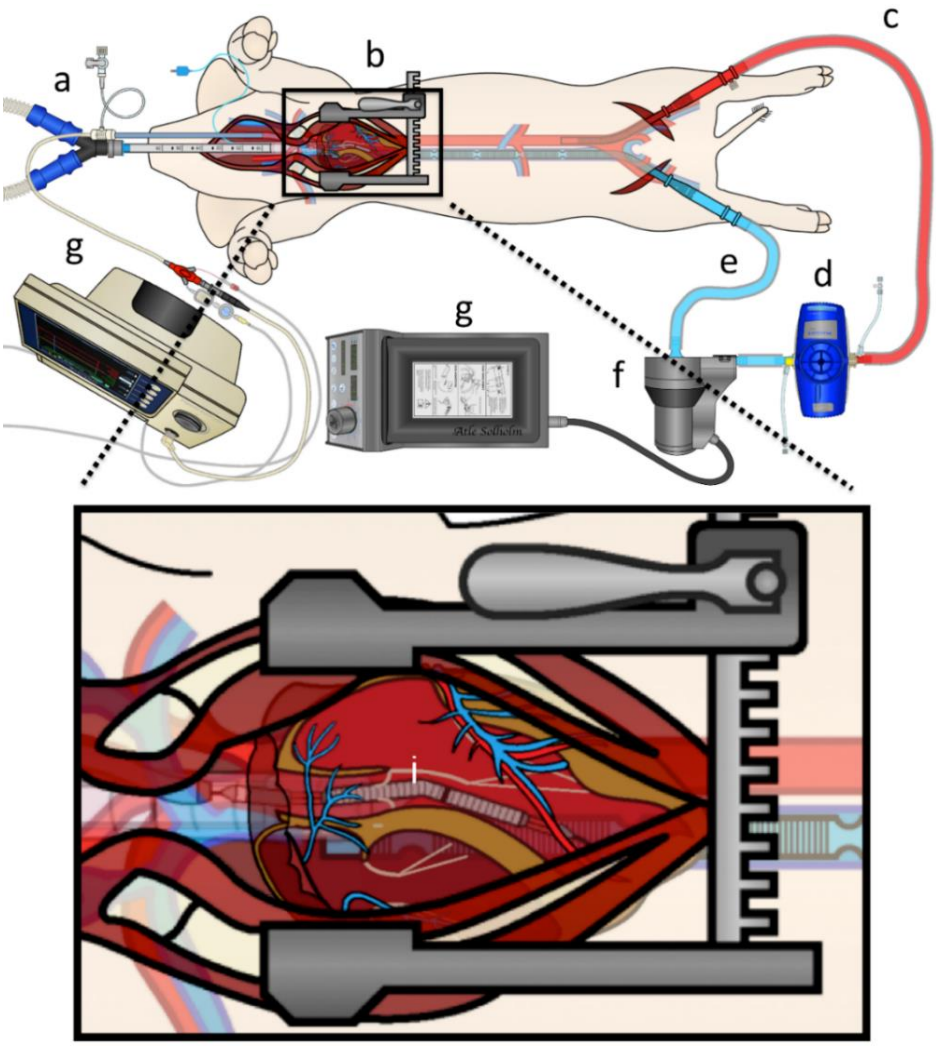

Figure 5: Experimental set-up in ECMO + LVAD animals. a: Ventilation and Impella cannulas, b: Sterno- and pericardiotomy, c: Arterial ECMO cannula, d: Oxygenator, e: Venous ECMO cannula, f: ECMO pump, g: ECMO console, h: Impella console and catheter, i: Impella CP. (Artwork: A. Solholm 2021).

3.4 Experimental protocols

After the basic instrumentation and stabilization, but before introducing the LVAD- and RVAD device and ECMO cannulas, a full registration at baseline with arterial blood gases, blood chemistry, haemodynamics, and the first microsphere injection was performed in all animals.

The mechanical circulatory support devices and ECMO cannulas were then placed (see earlier) and ventricular fibrillation was induced by applying 9V DC current for 1-2 seconds with two needle electrodes through the chest wall during fluoroscopy (Paper I and II) or directly on the epicardium (Paper III). ECPR was then immediately commenced and the ventilator set to deliver 100% O_2 according to general guidelines.[59]

In Paper I the primary endpoint was ROSC or no ROSC after 30 min of VF supported by either LVAD alone or by LVAD+RVAD. Haemodynamic variables were continuously evaluated, whereas tissue perfusion, biochemical variables were obtained at baseline and after 15 and 30 min of VF.

In Paper II successful defibrillation and ROSC after 40 min of VF supported by either LVAD+RVAD or ECMO was a primary endpoint. Sustained cardiac function after ROSC, defined as unsupported cardiac function for 60 min with mean arterial pressure (mAP) above 40 mmHg, was another endpoint. Besides evaluating haemodynamic variables including coronary perfusion pressure continuously, tissue perfusion and biochemical variables were obtained at baseline, after 20 and 40 min of VF and after 60 min of unsupported spontaneous cardiac function.

In Paper III ROSC or no ROSC was one important endpoint. Sustained cardiac function, here defined as unsupported spontaneous cardiac function for 180 min after ROSC with mAP above 40 mmHg was another. Other endpoints included haemodynamic variables (CPP, pulmonary artery flow etc.) and blood chemistry (s-lactate and s-troponin T). Haemodynamics was continuously recorded, tissue perfusion and biochemical variables were evaluated at baseline and 30, 90 and 180 min after ROSC.

In all three studies the LVAD settings were continuously adjusted to maximize output but to avoid suction alarm as read from the consol. In Paper I the RVAD settings were also maximized avoiding suction alarm. As a standard the ECMO flow was set to 72 ml/kg/min in Paper II and III, 80% of calculated cardiac output for a pig.[60] In the study group with reduced ECMO output in Paper III, flow was set to 36 ml/kg/min.

In clinical studies and practise, both feasibility, time spent to establish adequate ECPR after start of VF, mortality, morbidity and even economy are important factors that must be evaluated and considered. Although being of a translational nature, in the present experimental studies the circulation support devices were implanted up-front (Seen last paragraph 3.2.1). This thesis primarily focuses on haemodynamic potential and factors influencing organ perfusion during cardiac arrest with ECPR. In these experimental settings the frequency of successful defibrillations and sustained spontaneous cardiac function after ROSC are important markers for the degree of myocardial ischaemic injury during VF and cardiac arrest using the different modalities of ECPR. Also, due to the need for standardization, VF was triggered by a DC current, and not by an ischaemic trauma. Furthermore, a maximum of three defibrillation attempts, and no pharmacological interventions to support post-ROSC function were allowed in these protocols. Importantly, assist devices were weaned over 5-10 minutes after ROSC to help accentuate potential between-group differences in the post-ROSC observation periods (Papers II-III).

3.5 Quantification of organ perfusion and water content

3.5.1 Microsphere evaluation and water content

Microsphere injections may be considered a reference method in experimental protocols for the quantification of organ tissue blood flow rate.[61] The method is also validated in cardiac arrest.[62,63] Quantification depends upon harvesting organs after study termination, therefore is not applicable in humans. Previously, radiolabelled microspheres were utilized. Due to the radioactivity involved, this method has been supplanted by quantification of coloured or fluorescent microspheres.[64,65]

There are several prerequisites and preconditions of the method.[66,67]

1.
Microspheres need to be mixed in the central circulation before being transported by arterial blood flow to the tissues and the reference organ (an arterial catheter with a constant rate extraction pump) in numbers proportional to blood flow rate during administration. Usually, microspheres are injected into the left atrium or the left ventricle in animals with spontaneous circulation.

2.
Microspheres need to be permanently embolized and retained in the capillary bed of the different tissues.

3.
A minimum quantity of beads needs to be deposited for valid quantification of blood flow rate, and microspheres from one injection should not obstruct or influence tissue perfusion.

4.
Size and density of microspheres should be uniformly distributed in the arterial blood stream to secure proportional distribution to organs and tissues.

5.
Accurate detection and quantification is required, which is dependent upon type of microsphere. For instance, the fluorescent beads need to be chosen to avoid overlap of both the excitation- and emission spectra that could make interpretation more difficult.

In a setting of total mechanical circulatory support (i.e. cardioplegic arrest and VF), microspheres may be injected directly into the arterial cannula from the heart-lung machine or ECMO-device. With the combination of intracardiac impeller devices (BIVAD or LVAD), spheres must be injected into the left atrium or ventricle for optimal distribution. Withdrawal of a relevant reference blood sample for the evaluation of tissue perfusion during CA could be achieved in both Paper I and Paper II. However, the opposing directions of blood flow from the simultaneously running ECMO and LVAD devices during CA in Paper III create a watershed phenomenon at an unknown level in the central aorta (Figure 6).[35] The turbulent flow created by this phenomenon made the time necessary for reference blood sampling unpredictable, and we found microspheres in circulation despite prolonging the reference-sampling period in pilots. Therefore, reliable quantification of microspheres could not be achieved during cardiac arrest and ECPR. Consequently, microspheres were injected at baseline and during post-ROSC observation in Paper III, and comparisons could only be made between pigs with sustained cardiac function.

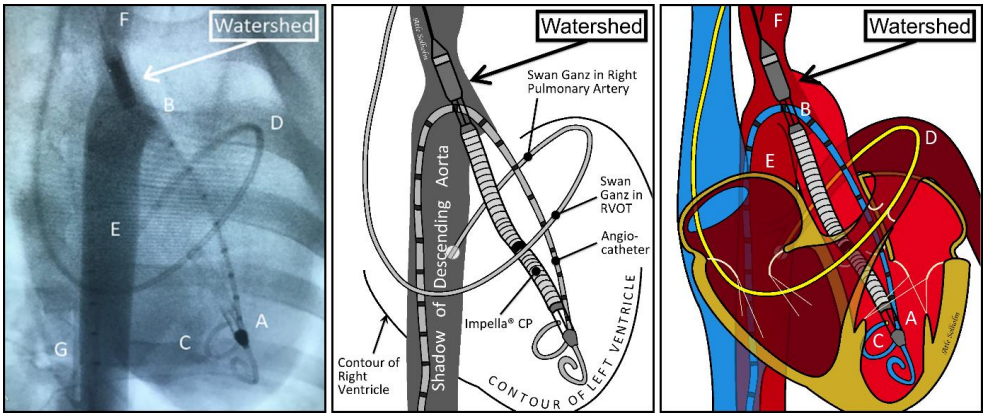

Figure 6: Watershed phenomenon created by opposing flow directions of the ECMO circuit and the Impella CP. Contrast injected into descending aorta (E) through the ECMO side-port on the arterial cannula. A: Impella CP inlet, B: Impella CP outlet, C: Pigtail catheter in the left ventricle, D: Swan-Ganz catheter, E: Descending aorta, F: Right carotid artery, G: Venous ECMO cannula. (Artwork: Left Panel: EJS. Packer 2021/Middle and Right Panels: A. Solholm 2022).

In all experiments, 15 µm fluorescent microspheres (Dye-Trak "F", Triton Technology Inc., San Diego, CA, USA) were injected to quantify organ tissue flow (perfusion).[68] In Paper I and Paper III injection of microspheres was done via a pigtail catheter in the left ventricle. In Paper II beads were injected via the pigtail catheter or a side port on the arterial ECMO cannula depending upon mode of circulatory assist. Microspheres were injected slowly over 45 seconds, and reference blood was drawn either from a sheath in the femoral- or the carotid artery starting immediately before injection and lasting for 3 minutes to accommodate for all injected microspheres to be distributed to the arterial microvascular beds and the reference blood. The microspheres were propagated to the different organs either by cardiac action, or during cardiac arrest with the help of the assist devices, before lodging in the capillary bed. Different colours (6 x 10^6 microspheres dissolved in saline solution) were injected in a randomized sequence. Each colour has distinct fluorescence- and emission spectra. In Paper I and II, microspheres were injected at baseline and twice during cardiac arrest. For Paper II, an additional injection was performed at the end of experiment. In Paper III microspheres were injected at baseline and three times during the observation period post-ROSC.

After euthanasia organ tissue was sampled from the cerebral cortex, both kidney cortices, and the ventricular- and ileum mucosa. Also, representable samples from the right ventricle and from the left ventricle (divided into subendo-, midmyo- and subepicardial wall layers) supplied by the left anterior descending artery (LAD) were obtained. The tissue- and reference blood samples were weighed, dissolved in potassium hydroxide, microspheres filtered, colours dissolved and quantified by fluorescence spectrophotometry (RF-5301PC, Shimadzu, Kyoto, Japan). Tissue blood flow rates were calculated as ml/min/gram wet weight.[69]

Water content was calculated by weight comparison before and after drying tissue samples for three weeks at 60 ^{0}C.

3.5.2 Transit time flowmetry

Transit time flowmetry is based on the principle of measuring ultrasound passing through a moving fluid and comparing the time difference between sending and receiving the sound upstream and downstream from a reflector opposite to the transmitter/receiver.[70] The methodology has been compared and validated against other available methods for quantification of flow in vitro and in vivo, and is used clinically for the evaluation of coronary bypass grafts.[71-74]

In Papers I and II, right carotid blood flow was measured using transit time flowmetry to evaluate peripheral circulation (Transonic Systems Inc.). In Paper III, pulmonary artery flow and left anterior descending artery flow were both monitored by this method (Medistim) focusing on differences between ECPR modes during VF.

3.6 Haemodynamic variables

All haemodynamic variables were monitored and recorded continuously (ACQ-7700, Data Sciences International, St. Paul, MN, USA). Pressures in the left ventricle, abdominal aorta and inferior caval vein were measured (TruWave®, Edwards Lifesciences, Irvine, CA, USA).

End-tidal CO_2 was monitored directly from the ventilator at specified times (Julian Drägerwerk, Lübeck, Germany), as previously noted (see section 3.1). $EtCO_2$ is used clinically as a marker for ventilation and circulatory support during CA [75,76] and arguably as a predictor of prognosis. [77,78] It has been shown that $etCO_2$ above a level of 1.3 kPa is critical for survival [76] and that levels between 2 and 3 kPa can be achieved by conventional CPR.[79]

3.7 Blood chemistry and biomarkers

Blood samples were acquired through the intravascular sheaths at baseline, during the VF-/ECPR period and during post-ROSC spontaneous cardiac function. Arterial blood gases, including lactate, were stored on ice for a short time and analysed (ABL800Flex, Radiometer Medical ApS, Brønshøj, Denmark). S-lactate is used clinically as a marker of tissue hypoperfusion.[80] It can also be used to monitor the quality of cardiopulmonary resuscitation,[81] and arguably as a predictor of survival in both OHCA and IHCA.[82-84] It may also be a predictor of neurological function.[85]

Serum was prepared and analysed for s-troponin T, s-glucose and s-creatinine at the Laboratory for Clinical Biochemistry, Haukeland University Hospital (Cobas, Roche Diagnostics GmbH, Mannheim, Germany). High troponin T levels is a marker of mortality in OHCA patients of both ischaemic and non- ischaemic aetiology.[86] However, it may not be possible to discern between the two based upon troponin levels,[87] nor may it be used to discriminate survival or neurological outcome.[88]

3.8 Echocardiography and macroscopic evaluation

3.8.1 Intracardiac echocardiography (Paper I and II)

Intracardiac echocardiography (ICE) was performed using an 8F catheter (ACUNAV 8F, Siemens Healthcare GmbH) introduced to the heart through the established venous and arterial sheaths. ICE has widespread use in humans. It may be utilized as guide to transseptal puncture and percutaneous closure of septal defects or with left atrial appendage closure. ICE is also used for various electrophysiological procedures and of late also in structural heart interventions.[89-91] In an experimental setting (with an

intubated pig) and in a closed chest model, ICE is a good option to evaluate heart function. ICE offers an excellent anatomical overview but lacks some of the functionality available transthoracic ultrasound in humans. However, whole heart imaging has been difficult due to limited ultrasound penetrance. [58,92] Also, strain measurements and 3-dimensional ICE are not generally available at present. Despite some limitations, ICE has been used in a variety of porcine models including electrophysiological mapping, closure of septal defects and structural heart interventions, often as part of feasibility evaluation before application in humans.[93-95]

We performed measurements according to the following protocol: Five representative and consecutive cardiac cycles (cine loops) were recorded. One RR interval was chosen for assessment. Through the left- and right ventricular outflow tract, aortic- and pulmonary orifice, pulsed- and continuous wave Doppler measurements (CWD, PWD) were obtained. Short axis images from the apical and midpapillary level of the left ventricle were acquired. Long axis views were difficult to capture in a standardized manner with ICE.

Both in Paper I and Paper II we used ICE to ensure that the Impella RP crossed the pulmonary valve. In Paper II ICE was also utilized for evaluation of left ventricular short axis dimensions.

3.8.2 Echocardiography and speckle tracking strain (Paper III)

A Vivid E9 scanner with a 2.7 – 8.0 MHz cardiac sector transducer (M4S, GE, Vingmed Ultrasound Horten, Norway) was used to acquire images and cine loops. A soft pad made of silicone was necessary to create sufficient space to the myocardium for obtaining ideal images (Figure 7).[96] From the apex two-, three- and four chamber views could be obtained for evaluation of dimensions and volumes. Further, short axis images from the apical and midpapillary level of the left ventricle were acquired. Through the left ventricular outflow tract and aortic orifice, pulsed- and continuous wave Doppler measurements (CWD, PWD) were recorded. Loops were obtained during respiratory hold at end-expirium. This minimized beat to beat haemodynamic variation. End-systolic and end-diastolic volumes and wall dimensions are presented.

End-diastole was defined as the first deflection of the QRS-complex in the ECG, end-systole as the closure of the aortic valve. Five representative and consecutive cardiac cycle (cine loops) were recorded, and one RR interval was chosen for evaluation. Analyses were performed (including ICE images) using the commercially available software, EchoPAC v 203 (GE Vingmed Ultrasound).

Speckle tracking echocardiography (STE) was performed to analyse myocardial function. The method may detect reduced myocardial function at an earlier stage than standard 2D- echocardiography.[97] GLS has been shown to be a reliable method of evaluating left ventricular function, for instance compared to ejection fraction.[98,99]
In Paper III, we analysed left ventricular function in the two-, three- and four-chamber view, reporting midwall strain using an 18-segment model in accordance to the common standard of 2D speckle tracking echocardiography of the European Association of Cardiovascular Imaging and American Society of Echocardiography.[100]

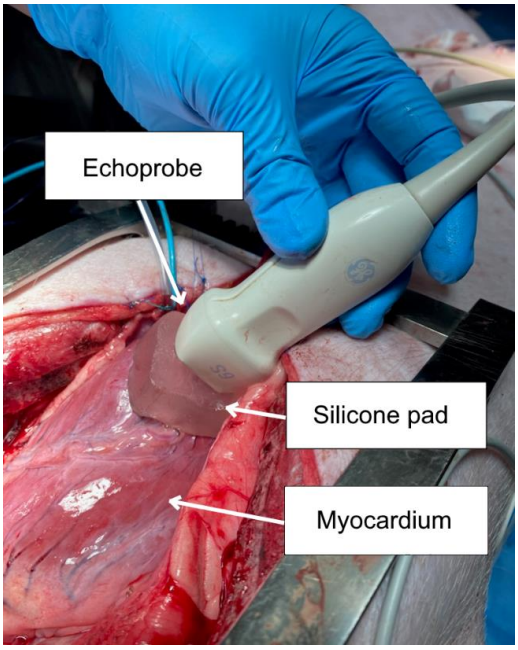

Figure 7: Epicardial echocardiography in the open chest model. Photo showing set-up. Note the silicone pad that displaces the echoprobe from the myocardium for most ideal images. (Packer et al, ASAIO J (2021), online ahead of print, PMID: 34294641).

3.8.3 Macroscopic evaluation

At necropsy, the organs were harvested for examination and sampling of tissue for other evaluation (microsphere quantification and water content measurement). Macroscopic inspection could be performed with visual comparison, and photos could be taken (Figure 12). Notably, water content could be used to directly quantify and support macroscopic evaluation.

3.9 Statistical analysis

Paper I and II: Statistical calculations were computed using IBM SPSS Statistics software (v. 23, IBM, Armonk, NY, USA). Data are expressed as the mean ± standard error of the mean or median (25%; 75%) unless otherwise stated. Baseline variables were compared by two-sample Student's t-test on data with normal distribution and with Wilcoxon–Mann–Whitney U-test on ranks if the Kolmogorov–Smirnov test for normal distribution or the Levene equal variance tests were significant. Haemodynamic and other continuous variables during VF were compared by two-way analysis of variance for repeated measurements (RM-ANOVA) with BIVAD vs. LVAD as grouping factor (p_g) and time as within-factor (p_w). If Mauchly's test of sphericity was significant ($p < 0.05$), the Greenhouse–Geisser adjustment of degrees of freedom was selected for the evaluation of main effects and interaction. A significant interaction ($p_i < 0.10$) justified new ANOVAs on simple main effects followed by post hoc comparisons of individual means with Neumann–Keuls multiple contrast tests when justified by the preceding ANOVA. Simple linear regression analysis was used to evaluate the relationship between pressure gradients and myocardial blood flow rates. Fisher's exact test was employed to compare categorical variables. Except for the interaction effect in the RM-ANOVA, a significant difference was noted when $p < 0.05$.

Paper III: Data are presented as means (SD) or median (1^{st} ; 3^{rd} quartile). Descriptive means are presented at baseline in figures. During ECPR, between-group p-values are reported immediately after induction of VF unless otherwise stated, and descriptive means (SD) were computed using the R package tidyverse. Two-sample t-test or Mann-Whitney rank sum test was used to compare animals with ROSC versus no-

ROSC and sustained cardiac function versus non-survivors. Differences were considered significant if $p<0.05$. Temporal- and between-group differences were analysed using mixed effects models. Analyses were performed using R (The R Foundation for Statistical Computing, Vienna, Austria). Mixed effects modelling was executed using the R package nlme.

4. Summary of results

4.1 Paper I

With biventricular support an increased LVAD output could be found; 3.2 ± 0.2 (SEM) vs. 2.0 ± 0.2 l/min at 15 minutes of VF, and 3.0 ± 0.2 (SEM) vs. 2.1 ± 0.1 l/min after 30 minutes (of VF) ($p_g < 0.001$). Biventricular support also increased aortic and LV pressure (Figure 8).

In the myocardium there was a transmural gradient between subendocardium and the subepicardium, with better maintained perfusion in the latter (Figure 9). There was a significant correlation between the tissue blood flow rate in the different wall layers of the left ventricular wall and the pressure difference between mean aortic pressure and left ventricular pressure (LVP_{mean} - AOP_{mean}) in the fibrillating hearts (Figure 10). We found improved myocardial perfusion in the LVAD- compared to the BIVAD group related to a significantly higher LVP_{mean} - AOP_{mean}. End-tidal CO_2 was significantly higher in the BIVAD assisted animals (Figure 8) but was inversely related to the rate of ROSC. Spontaneous circulation was achieved in all animals in the LVAD group while only 5 out of 10 in the BIVAD group ($p = 0.033$).

With biventricular support, organ perfusion quantified by fluorescent microsphere analysis was significantly higher in the kidneys and ileum. Perfusion was also borderline increased in the cerebral cortex at 15 and 30 minutes of VF: 0.33 ± 0.03 and 0.26 ± 0.04 (SEM) vs. 0.23 ± 0.03 and 0.17 ± 0.03 (SEM) ($p_g < 0.053$) compared to LVAD, however decreased in both groups over time (Table 1). S-lactate and pCO_2 were increased in LVAD animals compared to BIVAD, and pO_2 and pH were decreased. Important blood gas values are summarized in Table 2.

In summary: Biventricular support was associated with higher LVAD output and better systemic support during cardiac arrest. However, biventricular support was negatively related to increased intraventricular pressure and decreased myocardial perfusion pressure, correlating significantly to lower rates of ROSC.

Table 1
Key variables in 10 pigs with BIVAD and 10 pigs with LVAD during 30 min of ventricular fibrillation. Values are mean ± SEM or median (Quartile 1; Quartile 3).

Variable	Baseline	15 min VF	30 min VF	
		Myocardial circulation		
LV-ENDO$_{flow}$ (ml/min/g)				
BIVAD	0.92 (0.59; 1.00)	0.02 (0.01; 0.33)	0.04 (0.01; 0.44)	
LVAD	0.81 (0.74; 1.04)	0.31 (0.24; 0.53)$^{\#}$	0.29 (0.19; 0.48)	
LV-MID$_{flow}$ (ml/min/g)				
BIVAD	0.84 (0.59; 0.96)	0.27 (0.11; 0.55)	0.20 (0.08; 0.67)	
LVAD	0.83 (0.71; 1.09)	0.63 (0.54; 0.99)$^{\#}$	0.61 (0.47; 0.92)$^{\#}$	
LV-EPI$_{flow}$ (ml/min/g)				
BIVAD	0.68 (0.33; 0.82)	0.51 (0.42; 1.01)	0.46 (0.40; 1.01)	
LVAD	0.71(0.54; 0.81)	0.72 (0.49; 1.11)	0.81 (0.58; 1.03)	
RV$_{flow}$ (ml/min/g)				
BIVAD	0.60 (0.41; 0.71)	0.55 (0.49; 0.81)	0.59 (0.54; 1.03)	
LVAD	0.66 (0.63; 0.83)	0.68 (0.54; 1.08)	0.77 (0.58; 1.07)	
		General circulation		
Cardiac-/device output (L/min)				
BIVAD	5.5 ± 0.4	3.2 ± 0.2	3.0 ± 0.2	$p_w = 0.24$, $p_g < 0.001$, $p_i = 0.019$
LVAD	5.0 ± 0.3	2.0 ± 0.2	2.1 ± 0.1	
Right carotid artery$_{flow}$ (ml/min)				
BIVAD	147 ± 12	78 ± 6	73 ± 5	$p_w = 0.079$, $p_g = 0.90$, $p_i = 0.61$
LVAD	174 ± 17	81 ± 9	73 ± 9	
Cerebrum$_{flow}$ (ml/min/g)				
BIVAD	0.57 ± 0.04	0.33 ± 0.03	0.26 ± 0.04	$p_w < 0.001$, $p_g = 0.053$, $p_i = 0.55$
LVAD	0.52 ± 0.3	0.23 ± 0.03	0.17 ± 0.03	
Gastric mucosa$_{flow}$ (ml/min/g)				
BIVAD	0.21 ± 0.01	0.17 ± 0.01	0.14 ± 0.01	$p_w = 0.16$, $p_g = 0.83$, $p_i = 0.57$
LVAD	0.23 ± 0.01	0.15 ± 0.02	0.14 ± 0.02	
Ilium mucosa$_{flow}$ (ml/min/g)				
BIVAD	0.37 ± 0.05	0.26 ± 0.04	0.20 ± 0.03	$p_w = 0.007$, $p_g = 0.040$, $p_i = 0.24$
LVAD	0.31 ± 0.02	0.16 ± 0.03	0.13 ± 0.02	
Kidney$_{flow}$ (ml/min/g)				
BIVAD	2.93 ± 0.12	0.76 ± 0.15	0.56 ± 0.13	$p_w = 0.13$, $p_g = 0.023$, $p_i = 0.28$
LVAD	3.07 ± 0.23	0.35 ± 0.05	0.32 ± 0.07	

VF; ventricular fibrillation; LV- and RV- = left and right ventricle; ENDO, MID, EPI = subendo- midmyo- and subepicardium; $^{\#}$ = different from BIVAD by Mann-Whitney nonparametric two-sample tests at Baseline. Repeated mesasurement ANOVA for the VF-period. p_w, p_g and p_i = probabilities for main effects with RM-ANOVA for within and between groups and for the interaction; § = different from BIVAD at the same point in time by *post hoc* multiple contrast tests.

Table 2

Arterial blood gases and biochemical variables in 10 pigs with BIVAD and 10 pigs with LVAD before and during 30 min of ventricular fibrillation. Values are mean ± SEM.

Variable	Baseline	15 min VF	30 min VF	RM-ANOVA
pO_2 (kPa)				
BIVAD	28.1 ± 0.6	53.9 ± 3.5	53.0 ± 3.1	$p_w = 0.005$, $p_g = 0.022$, $p_i = 0.86$
LVAD[a]	26.8 ± 0.8	42.2 ± 5.6	37.4± 6.4	
pCO_2 (kPa)				
BIVAD	5.3 ± 0.1	3.9 ± 0.1	3.7 ± 0.2	$p_w = 0.52$, $p_g = 0.065$, $p_i = 0.017$
LVAD	5.1 ± 0.2	4.2 ± 0.3	4.6 ± 0.3*§	
Lactate (mmol/L)				
BIVAD	1.35 ± 0.13	3.38 ± 0.50	5.07 ± 0.62	$p_w < 0.001$, $p_g = 0.010$, $p_i = 0.50$
LVAD	1.41 ± 0.10	5.41 ± 0.39	7.38 ± 0.69	
Hb (g/dl)				
BIVAD	9.6 ± 0.5	9.1 ± 0.4	9.2 ± 0.4	$p_w = 0.16$, $p_g = 0.042$, $p_i = 0.28$
LVAD	9.3 ± 0.3	9.9 ± 0.4	10.4 ± 0.2	

VF = ventricular fibrillation; RM-ANOVA = analysis of variance for repeated measurements for the VF period. p_w, p_g and p_i = probabilities for main effects with RM-ANOVA for within and between groups and for the interaction; * = difference from 15 min VF within the same group; § = different from BIVAD at the same point in time. [a] n = 9 in the LVAD group.

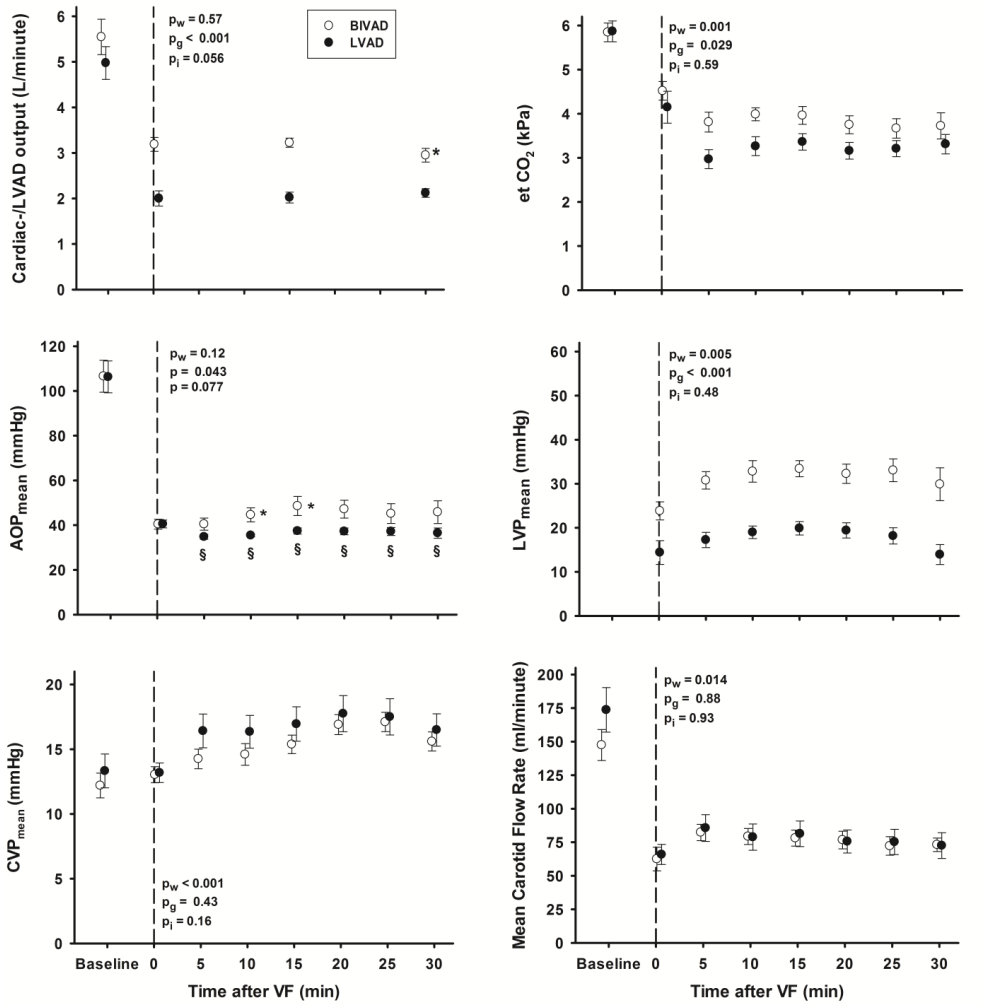

Figure 8: Haemodynamic variables during cardiac arrest, Paper I.

LVAD: Left ventricular assist device, $etCO_2$: end-tidal CO_2), AOP_{mean}: Mean aortic pressure, LVP_{mean}: Mean left ventricular pressure, CVP_{mean}: mean central venous pressure. p_g, p values for between groups; p_w, p values within groups; p_i, p values for interaction effect. (Packer et al, ASAIO J (2018), 64(4): 489-496).

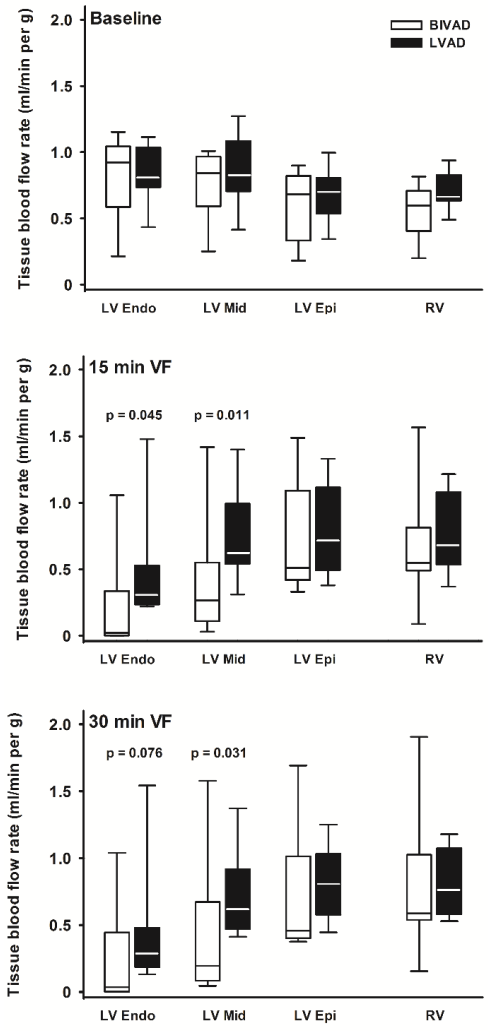

Figure 9: Myocardial blood flow rate in endo-, midmyo-, epicardium and right ventricle before and during cardiac arrest with MCS. Top panel: Baseline, flow ratio LV-ENDO versus LV-EPI >1. Bottom two panels: Cardiac arrest, flow ratio LV-ENDO versus LV-EPI <1 (Packer et al, ASAIO J (2018), 64(4): 489-496).

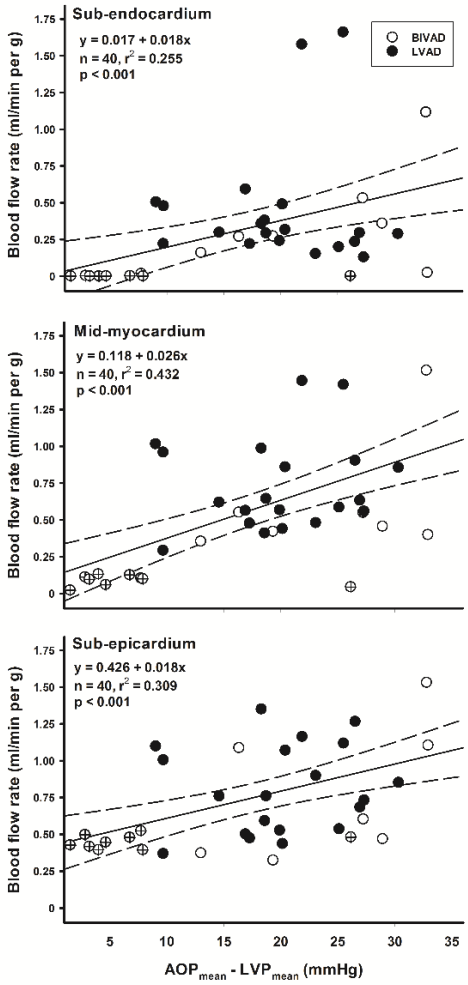

Figure 10: The relationship between myocardial/coronary perfusion pressure and blood flow rates. No animals achieved ROSC with perfusion pressure below 10 mmHg.
Symbols with "+" represent animal that did not achieve ROSC (Packer et al, ASAIO J (2018), 64(4): 489-496).

4.2 Paper II

During VF, device output, carotid flow, kidney perfusion, mean aortic pressure (AOP_{mean}) and mean left ventricular pressure (LVP_{mean}), were all significantly higher in the ECMO group (Figure 11 and Table 3), and s-lactate values were lower compared to the BiPella group (Table 4). No between-group differences were found regarding cerebral and myocardial tissue perfusion as quantified by fluorescent microspheres (Table 3).

Three vs. two animals (ECMO vs. BiPella) did not reach the pre-specified endpoint. In 15 animals with sustained cardiac function for 60 min after ROSC, left ventricular subendocardial blood flow rate averaged 0.59 ± 0.05 ml/min/g during VF compared to 0.31 ± 0.07 ml/min/g in five animals with circulatory collapse ($p = 0.005$). Corresponding values for the midmyocardium was 0.91 ± 0.06 vs. 0.65 ± 0.15 ml/min/g ($p = 0.085$) and subepicardium 1.02 ± 0.07 vs. 0.86 ± 0.17 ml/min/g ($p = 0.30$) (Table 3).

In surviving animals intracardiac echocardiography showed decreased end-diastolic short axis diameters in the BiPella group compared to ECMO, mainly related to increased heart rate with reduced filling volumes. Severe myocardial oedema in animals with poor myocardial function post-ROSC (5/20), related to CPP during VF (Figure 12) was also noted irrespective of group. Similarly, at necropsy, haemorrhagic infarction and oedema could be seen macroscopically (Figure 12). This qualitative evaluation could be confirmed and quantified by comparing water content between animals with non-sustained and sustained cardiac function in the subendocardium: 83.9 (82.2; 85.4)% vs. 81.6 (80.6; 82.5)%, $p = 0.045$.

In summary: ECMO provided a near physiological systemic circulatory support, although both methods could sustain vital organ perfusion. Myocardial perfusion and sustained cardiac function post-ROSC were related to coronary perfusion pressure during cardiac arrest irrespective of mode of assist.

Table 3
Key variables in pigs with BiPella and 10 pigs with ECMO at Baseline and during 40 min of ventricular fibrillation. Values are mean ± SEM or median (Quartile 1; Quartile 3).

Variable	Baseline	20 min VF	40 min VF	
Myocardial circulation				
LV-ENDO$_{flow}$ (ml/min/g)				
Not ROSC/-Sustained (n=5)	0.59 ± 0.07	0.27 ± 0.04	0.34 ± 0.12	
ROSC/Sustained (n=15)	0.75 ± 0.07	0.64 ± 0.05$^{\#}$	0.55 ± 0.05	
LV-MID$_{flow}$ (ml/min/g)				
Not ROSC/-Sustained (n=5)	0.54 ± 0.02	0.67 ± 0.14	0.64 ± 0.16	
ROSC/Sustained (n=15)	0.75 ± 0.07	0.90 ± 0.07	0.91 ± 0.08	
LV-EPI$_{flow}$ (ml/min/g)				
Not ROSC/-Sustained (n=5)	0.62 ± 0.04	0.86 ± 0.22	0.86 ± 0.13	
ROSC/Sustained (n=15)	0.67 ± 0.07	0.98 ± 0.07	1.07 ± 0.09	
RV$_{flow}$ (ml/min/g)				
Not ROSC/-Sustained (n=5)	0.50 (0.46;0.89)	0.77 (0.61;1.00)	1.02 (0.52;1.04)	
ROSC/Sustained (n=15)	0.36 (0.32;0.68)	0.82 (0.72;1.05)	0.83 (0.66;1.24)	
General circulation				
$etCO_2$ (kPa)				
BiPella	5.4 (5.3; 6.0)	4.3 ± 0.2	4.0 ± 0.3	$p = 0.37$
ECMO	5.3 (5.1; 6.4)	-	-	
Cardiac-/device output (L/min)				
BiPella	3.5 (3.2; 4.7)	2.6 ± 0.3	2.0 ± 0.2	$p_w < 0.14$, $p_g < 0.001$, $p_i = 0.27$
ECMO	3.8 (3.3; 5.7)	4.1 ± 0.2	4.1 ± 0.2	
Right carotid artery$_{flow}$ (ml/min)				
BiPella	159 ± 10	97 ± 6	83 ± 8	$p_w < 0.13$, $p_g = 0.001$, $p_i = 0.61$
ECMO	189 ± 23	169 ± 18	162 ± 19	
Cerebrum$_{flow}$ (ml/min/g)				
BiPella	0.35 ± 0.04	0.28 ± 0.05	0.22 ± 0.05	$p_w < 0.091$, $p_g = 0.13$, $p_i = 0.23$
ECMO	0.34 ± 0.04	0.38 ± 0.07	0.38 ± 0.08	
Gastric mucosa$_{flow}$ (ml/min/g)				
BiPella	0.21 (0.15; 0.24)	0.18 ± 0.03	0.17 ± 0.02	$p_w = 0.22$, $p_g = 0.015$, $p_i = 0.11$
ECMO	0.21 (0.19; 0.32)	0.27 ± 0.03	0.33 ± 0.05	
Ileum mucosa$_{flow}$ (ml/min/g)				
BiPella	0.32 ± 0.03	0.23 ± 0.04	0.19 ± 0.03	$p_w = 0.29$, $p_g = 0.044$, $p_i = 0.24$
ECMO	0.27 ± 0.01	0.30 ± 0.03	0.30 ± 0.02	
Kidney$_{flow}$ (ml/min/g)				
BiPella	2.42 ± 0.15	0.76 ± 0.46	0.57 ± 0.15	$p_w = 0.72$, $p_g < 0.001$, $p_i = 0.059$
ECMO	2.84 ± 0.16	2.02 ± 0.20	2.30 ± 0.30	

VF; ventricular fibrillation; LV- and RV- = left and right ventricle; ENDO, MID, EPI = subendo- midmyo- and subepicardium; $etCO_2$ = end-tidal CO_2; $^{\#}$ = different from BIVAD by Mann-Whitney nonparametric two-sample tests. Repeated measurement ANOVA for the VF-period. p_w, p_g and p_i = probabilities for main effects with RM-ANOVA for within and between groups and for the interaction.

Table 4

Arterial blood gases and biochemical variables in 10 pigs with BIVAD and 10 pigs with LVAD before and during 40 min of ventricular fibrillation. Values are mean ± SEM or median (Quartile 1; Quartile 3).

Variable	Baseline	20 min VF	40 min VF	RM-ANOVA
pO_2 (kPa)				
BiPella	26.3 (24.3; 28.2)	22.4 ± 1.2	19.1 ± 1.5	$p_w = 0.77$, $p_g = 0.023$, $p_i = 0.27$
ECMO	26.5 (24.6; 29.1)	26.3 ± 3.4	28.7 ± 3.3§	
pCO_2 (kPa)				
BiPella	4.8 ± 0.1	4.2 ± 0.2	4.2 ± 0.2	$p_w = 0.29$, $p_g < 0.001$, $p_i = 0.49$
ECMO	5.2 ± 0.2	6.7 ± 0.2	6.6 ± 0.3	
Lactate (mmol/L)				
BiPella	1.04 ± 0.08	3.81 ± 0.51	5.68 ± 0.62*	$p_w < 0.001$, $p_g < 0.001$, $p_i < 0.001$
ECMO	0.85 ± 0.06	1.05 ± 0.0§	0.98 ± 0.01§	
Hb (g/dL)				
BiPella	9.0 ± 0.1	-	9.5 (8.5; 10.0)	$p = 0.47$
ECMO	8.8 ± 0.2	-	9.1 (8.1; 9.9)	
Creatinine (mmol/L)				
BiPella	88 ± 3	-	104 ± 30	$p = 0.007$
ECMO	84 ± 3	-	89 ± 40	
Troponin T (ng/L)				
BiPella	12 ± 2	-	50 (31; 81)	$p = 0.013$
ECMO	12 ± 2	-	25 (15; 41)	

VF = ventricular fibrillation; RM-ANOVA = analysis of variance for repeated measurements for the VF period. p_w, p_g and p_i = probabilities for main effects with RM-ANOVA for within and between groups and for the interaction; * = difference from 20 min VF within the same group; § = different from BiPella at the same point in time.

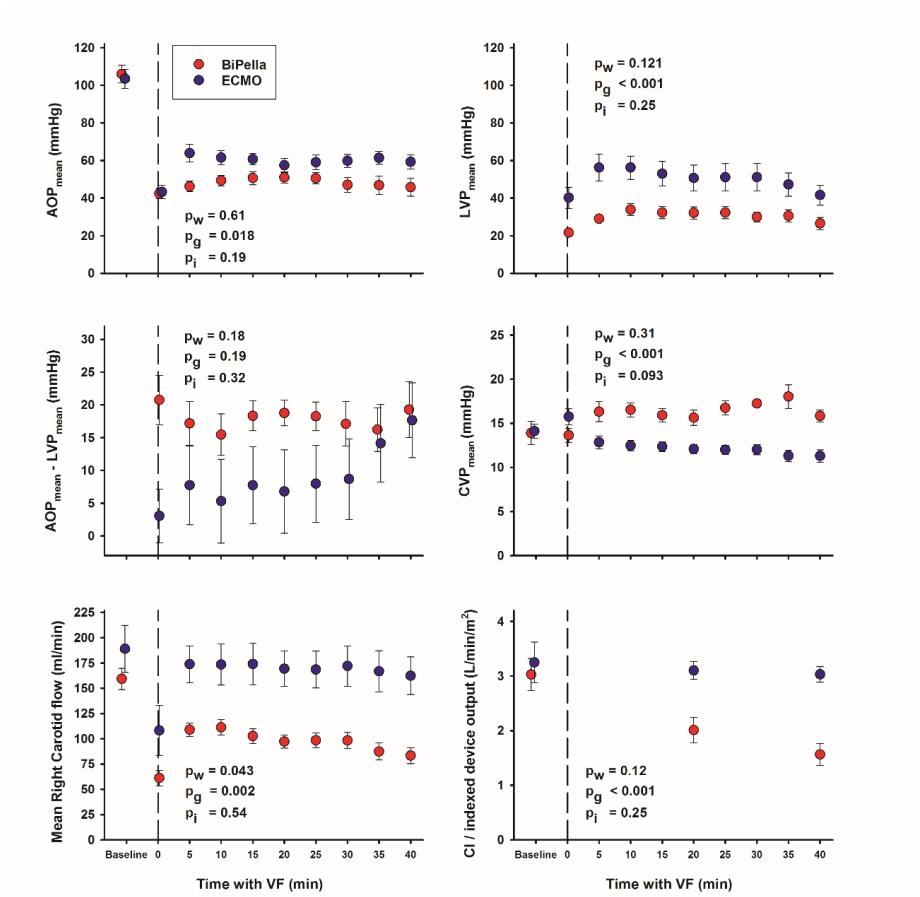

Figure 11: Haemodynamic variables during cardiac arrest, Paper II.

AOP_{mean}: Mean aortic pressure, LVP_{mean}: Mean left ventricular pressure, CVP_{mean}: mean central venous pressure. CI: Cardiac Index. p_g, p values for between groups; p_w, p values within groups; p_i, p values for interaction effect. (Packer et al, ASAIO J (2020), 66(10):1110-1119).

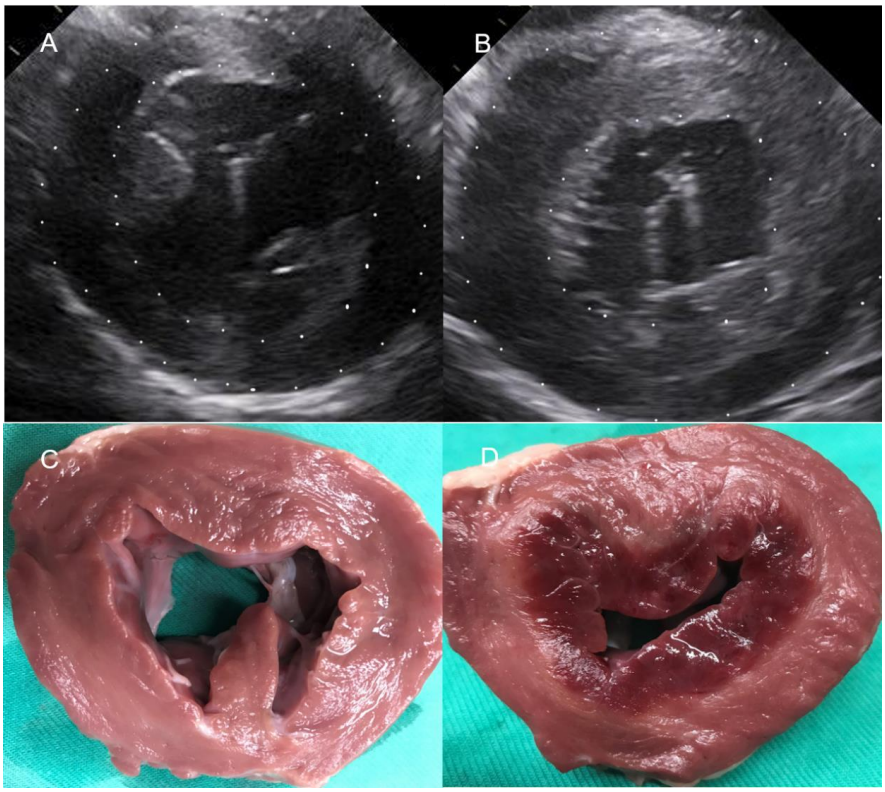

Figure 12: Cross-sectional view of the left ventricle obtained by intracardiac echocardiography from the right atrium (Upper panels A and B) and corresponding photos after necropsy (Bottom panels C and D). Left panels (A and C) depict examples of satisfactory circulatory assist. Right panel (B and D) shows the profound changes associated with severely reduced myocardial perfusion during CA. Note the massive oedema and haemorrhagic transformation of myocardium in the subendocardium (Packer et al, ASAIO J (2020), 66(10):1110-1119).

4.3 Paper III

During 60 minutes of cardiac arrest, device output, mean aortic pressure (mAP) and myocardial perfusion pressure (CPP = mAP - mean left-ventricular pressure (mLVP)) were significantly increased with Group 1 (ECMO 72 ml/kg/min + Impella) versus the other two groups (Figure 13 and Table 5). mLVP was significantly lower with Group 1 (ECMO 72 ml/kg/min + Impella) and Group 2 (ECMO 36 ml/kg/min + Impella) versus Group 3 (ECMO 72 ml/kg/min), $p = 0.013$ and $p = 0.003$ respectively. In Group 3, mean pulmonary artery flow (mPAf) was -0.06 ± 0.08 (SD) l/min versus 0.5 ± 0.7 l/min in Group 1 (ECMO 72 ml/kg/min + Impella) and 0.5 ± 0.4 l/min in Group 2 (ECMO 36 ml/kg/min + Impella) (Figure 13).

Seventeen of 24 animals had sustained cardiac function at study end, including 8/8 in Group 1. 3/8 did not achieve ROSC with Group 2. Pigs that achieved ROSC in Group 2 had sustained cardiac function at study termination (5/5). In Group 3, 8/8 achieved ROSC, but 4/8 died post-ROSC due to cardiogenic shock at 68, 102, 112 and 208 minutes, respectively. Non-survivors (7/24) had reduced mAP ($p < 0.001$), CPP ($p = 0.002$) and mPAf ($p = 0.004$) during ECPR. Summary of blood chemistry can be seen in Table 6. In survivors, left ventricular stroke volume, cardiac output and ejection fraction was not significantly different between groups. Compared to baseline, global longitudinal strain decreased to a similar extent in all groups.

In summary: Add-on LVAD improved haemodynamics compared with ECMO alone during refractory cardiac arrest (ECMO 72 ml/kg/min + Impella), but could not substitute a reduction of ECMO flow in Group 2. Increased mAP, CPP and mPAf were related to sustained cardiac function and ROSC. Increased mLVP was related poor post-ROSC cardiac function for ECMO alone.

Table 5

Haemodynamic variables at Baseline and the average of 12 registrations (every 5th min) with ongoing ECPR during 60 min of ventricular fibrillation in pigs with ECMO 72 ml/kg/min+Impella (Group 1), ECMO 36 ml/kg/min+Impella (Group 2) and ECMO 72 ml/kg/min (Group 3), eight animals in each group. Values are mean ± SD or median (Quartile 1; Quartile 3).

Variable	Baseline	Average level during 60 min VF and ECPR
Cardiac-/assist device output (L/min)		
Group 1	3.7 (3.2; 4.6)	5.0 ± 0.5
Group 2	4.3 (3.0; 7.7)	3.1 ± 0.8*#
Group 3	4.0 (2.9; 4.4)	4.2 ± 0.3*
	p = 0.72	*p < 0.001*
mAP (mmHg)		
Group 1	86 ± 11	78 ± 14
Group 2	81 ± 11	52 ± 14*
Group 3	77 ± 13	60 ± 12*
	p = 0.32	*p < 0.001*
mLVP (mmHg)		
Group 1	-	26 ± 7
Group 2	-	19 ± 14#
Group 3	-	37 ± 13
		p = 0.021
mCPP (mmHg)		
Group 1	-	52 ± 12
Group 2	-	37 ± 11*
Group 3	-	23 ± 13*
		p = 0.002
$mLAD_f$ (ml/min)		
Group 1	46 ± 10	50 ± 9
Group 2	40 ± 11	37 ± 11*
Group 3	48 ± 13	57 ± 10
	p =0.36	*p =0.003*
mPA_f (L/min)		
Group 1	2.7 ± 0.3	0.6 (0.18; 0.91)
Group 2	2.7 ± 0.8	0.4 (0.02; 0.65)
Group 3	2.8 ± 1.1	-0.07 (-0.4; -0.1)*
	p =0.97	*p =0.002*
mCVP (mmHg)		
Group 1	9.6 ± 2.1	10.6 ± 2.8
Group 2	9.3 ± 2.2	10.6 ± 2.3
Group 3	9.4 ± 2.8	9.5 ± 3.4
	P =0.97	*p =0.71*

p = probability for One-way ANOVA or Kruskal-Wallis ANOVA on Ranks; * = different from Group 1; # = different fom Group 3; mAP = mean aortic pressure; mLVP = mean left ventricular pressure; mCPP = mean coronary perfusion pressure; $mLAD_f$ = mean flow in left anterior descending coronary artery; mPA_f = mean flow in pulmonary artery; mCVP = mean central venous pressure.

Table 6
Arterial blood gases and biochemical variables at Baseline and at 30 and 60 of VF in pigs with ECMO 72 ml/kg/min+Impella (Group 1), ECMO 36 ml/kg/min+Impella (Group 2) and ECMO 72 ml/kg/min (Group 3), eight animals in each group. Values are mean ± SD or median (Quartile 1; Quartile 3).

Variable	Baseline	30 min VF	60 min VF	RM-ANOVA
pO_2 (kPa)				
Group 1	40.0 (29.2; 46.5)	42.4 ± 14.1	40.4 ± 12.2	$p_w = 0.84$, $p_g = 0.73$, $p_i = 0.50$
Group 2	58.7 (40.4; 68.0)	44.9 ± 18.9	48.3 ± 25.1	
Group 3	50.8 (17.1; 55.0)	41.1 ± 17.4	38.3 ± 19.1	
pCO_2 (kPa)				
Group 1	5.0 ± 0.4	5.1 ± 1.6	4.5 ± 1.2	$p_w = 0.055$, $p_g = 0.018$, $p_i = 0.50$
Group 2	5.0 ± 0.3	4.6 ± 1.5	4.5 ± 1.1	
Group 3	5.0 ± 0.6	6.0 ± 0.3	5.9 ± 1.0	
Lactate (mmol/L)				
Group 1	1.04 ± 0.26	1.60 ± 0.61	2.0 ± 1.00	$p_w = 0.012$, $p_g = 0.096$, $p_i = 0.36$
Group 2	0.98 ± 0.30	3.04 ± 1.65	4.45 ± 1.88	
Group 3	1.03 ± 0.43	1.83 ± 0.74	2.45 ± 2.12	
Hb (g/dL)				
Group 1	8.5 (7.8; 8.9)	8.4 ± 1.6	8.3 ± 1.6	$p_w = 0.22$, $p_g = 0.92$, $p_i = 0.65$
Group 2	8.4 (7.8; 10.1)	8.6 ± 0.8	8.1 ± 1.4	
Group 3	9.2 (8.4; 9.5)	8.6 ± 1.2	8.5 ± 1.2	
Creatinine (mmol/L)				
Group 1	84 ± 11	-	81 ± 14	
Group 2	83 ± 10	-	92 ± 14	
Group 3	84 ± 9	-	94 ± 14	
Troponin T (ng/L)				
Group 1	18 (7; 44)	-	41 (15; 96)	
Group 2	17 (11; 33)	-	54 (30; 284)	
Group 3	14 (11; 15)	-	20 (15; 23)	

VF = ventricular fibrillation; RM-ANOVA = analysis of variance for repeated measurements for the VF period. p_w, p_g and p_i = probabilities for main effects with RM-ANOVA for within and between groups and for the interaction.

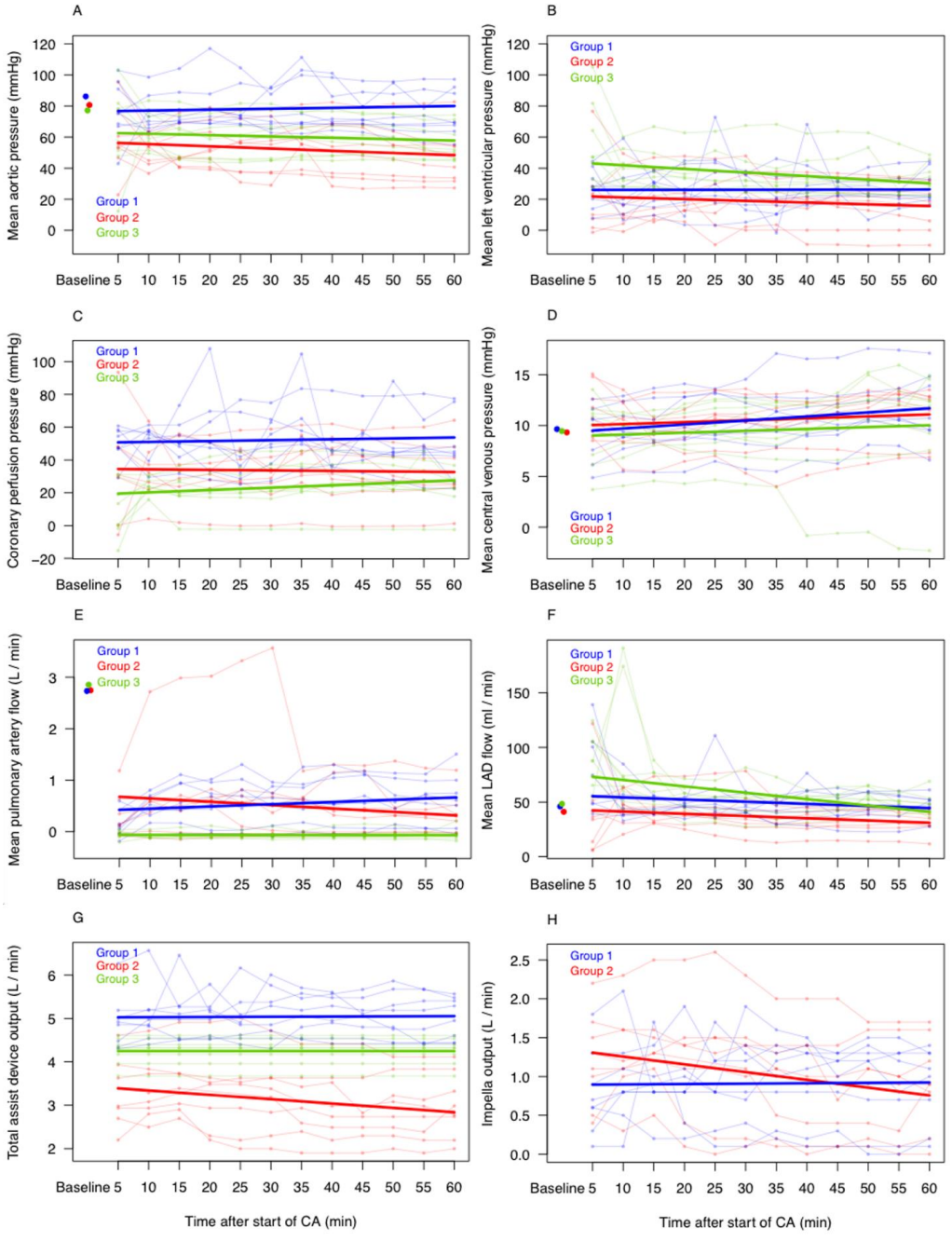

Figure 13: Haemodynamic variables during cardiac arrest, Paper III. Group 1: ECMO 72 ml/kg/min + Impella, Group 2: ECMO 36 ml/kg/min + Impella, Group 3: ECMO 72 ml/kg/min. n=8 in all groups. LAD: Left descending artery (Packer et al, ASAIO J (2021), online ahead of print, PMID: 34294641).

5. Discussion

This thesis demonstrates that improving systemic circulation by use of mechanical circulatory support (MCS) during refractory cardiac arrest may benefit peripheral organs (Paper I-III), including the brain (Paper I and II). This could be achieved by add-on RVAD in the first protocol, with ECMO in the second and with add-on LVAD in combination with standard-flow ECMO in the third. However, careful monitoring of the pressure difference between the aorta and the left ventricle, i.e. coronary perfusion pressure, seemed to indicate that increased pressure difference during VF provided myocardial protection. Coronary perfusion pressure may be optimized by unloading the left ventricular chamber (ECMO + Impella), or balancing device outputs when combining LVAD and RVAD (BiPella).

Coronary perfusion pressure was related to myocardial perfusion and ROSC rate (Paper I), subendocardial perfusion and sustained cardiac function post-ROSC (Paper II), and ROSC rate and sustained cardiac function post-ROSC (Paper III). Add-on LVAD provided a limited pulmonary artery circulation during ECPR which may benefit gas exchange post-ROSC and seemed to predict sustained cardiac function (Paper III).

5.1 Haemodynamic measurements

5.1.1 Haemodynamics

Haemodynamics during CPR was the main endpoint in all three Papers. All animals could be included in the analyses for the entirety of VF. All assist devices described in this thesis generate continuous flow output. Therefore, pressure went from cyclical to non-cyclical during CA. All pressures during assisted circulation were therefore reported as mean pressures. The myocardium is fibrillating in this state with no “systolic” or “diastolic” phase.

During MCS, mean aortic pressure (mAP) is a direct reflection of the effect of the assist device in question. With LVAD alone, mAP was 30-40 mmHg. The combination of RVAD and LVAD generated 40-50 mmHg compared to approximately 60 mmHg with ECMO alone. Low-flow ECMO and LVAD generated around 50

mmHg, whereas standard-flow ECMO and LVAD created approx. 80 mmHg. In the first two Papers, we could not relate mAP to ROSC or sustained cardiac function and survival after ROSC. The reason(s) are elusive as it is unlikely that mAP during cardiac arrest is not relevant. In Paper III, mean aortic pressure seemed to have significant impact as seen in the analysis of survivors vs. non-survivors.

Coronary perfusion pressure (CPP) is usually defined as aortic pressure minus right atrial (or central venous) pressure. Most researchers use "diastole" measurements, regardless of spontaneous circulation or compressional CPR.[62,101-104]

The mechanical effect of LV pressure and potential influence on myocardial blood flow during compressional CPR is controversial.[105] However, in a simplistic model, blood flow in the LV myocardium follows the epicardial arteries through septal- and smaller vessels towards the endocardium, before returning through the venous system to the right atrium. During cardiac arrest, increasing LV pressure might impact the subendocardial capillaries causing them to collapse since autoregulation by smooth muscle tone ceases in this setting.[101] In our models, mechanical emptying of the LV chamber during "systole" did not occur. In general, mLVP was substantially higher than mCVP (except for LVAD alone in Paper I) ranging from 15 mmHg with LVAD alone, to 60 mmHg with ECMO alone. To be noted, numerical differences between groups were small for mCVP, ranging from 8-17mmHg in all studies. Thus, we could neglect CVP from the equation and redefined coronary perfusion pressure (Coronary perfusion pressure = mAP – mLVP). Figure 10 demonstrates the relationship between coronary perfusion pressure and myocardial perfusion. This phenomenon if discussed further in chapter 5.3.2.

To our knowledge, the impact of LV pressure on the myocardial perfusion pressure (CPP) has not previously been described. It may pertain specifically to continuous-flow ECPR. It has at least one practical implication. In the catheterization laboratory in connection with PCI, it is easy and quick to obtain LV pressure, also during ECPR. A pigtail catheter is normally inserted as a part of the deployment of an Impella device. If ECMO is placed in the cath. lab., as it is in many hospitals, a pigtail catheter can easily

be placed in the LV chamber after initiation of ECPR. Hence, simultaneous monitoring of mAP and mLVP can proceed.

In summary, we found that mLVP was increased with un-balanced BIVAD in Paper I, and with ECMO alone in Paper II and III. Interestingly, mLVP seemed to decrease over time during CA with ECMO alone in both Paper II and III. Theoretically, a net backward flow through the pulmonary vasculature due to suction from the ECMO unit may be part of the explanation. This may justify further enquiry.

5.1.2 Device output

Although Impella CP has a theoretical maximum output of 3.5 l/min, it is rarely achievable in clinical practice.[106] One reason may be insufficient venous return to the left heart, even with ample fluid loading.[27] In cardiac arrest, this condition is aggravated several fold. In Paper I we could achieve a mean output of approximately 2 l/min over 30 minutes of CA. Concomitant use of the Impella RP could improve LVAD output to approximately 3 l/min.

Porcine anatomy is another potentially limiting factor. A short and acutely angled aortic arch may impact the lumen of the impeller device by narrowing it, thereby reducing device output. As a solution to this potential problem, we changed the insertion site from the femoral artery to the carotid artery in the final protocol. Also, caution is needed when crossing the aortic valve in both swine and humans so that the Impella CP does not kink in the main body during deployment. In Paper III we did not detect any impact of the impeller lumen from kinking during deployment due to change of insertion site.

The Impella RP has a higher theoretical output than the Impella CP. This may potentially create an unbalanced circulation with increased LV pressures, as was seen in Paper I. In Paper II this was compensated by decreasing Impella RP output. However, it is of considerable interest to combine the Impella RP with a more potent LVAD in the future. As a first step, it could be combined with the surgically deployed

Impella 5.0, even though this LVAD did not provide any benefit on its own compared to the Impella 2.5 in a previous protocol.[26]

The Extracorporeal Life Support Organisation (ELSO) guidelines recommend ECMO output of 60-80 ml/min/kg in adults.[42,107] Previous animal studies have indicated that elevated flow rate during cardiopulmonary bypass is associated with detrimental fluid accumulation.[108] Based on this and clinical practice at the Department of Heart Disease, ECMO output was set to 72 ml/kg/min in Paper II. This constitutes approximately 80% of estimated cardiac output in swine. The same settings were applied for standard-flow ECMO + Impella and ECMO alone subjects in Paper III.

ECMO output can normally be controlled in a stable manner at the pre-specified output unless limited by "suck-down". "Suck-down" is characterised by the centrifugal pump producing suction that collapses the inferior vena cava. This phenomenon is related to cannula size and insufficient blood volume, and results in forward failure. It can often be alleviated by temporarily reducing power of the centrifugal pump, increasing cannula size and/or correcting hypovolemia. This was not a significant issue in our protocols.

The combination of ECMO with reduced flow (36 ml/kg/min) and Impella potentially has a more physiologically directed blood flow during ECPR. A previous experimental study questioned the effect of reducing ECMO flow and compensating for decreased mAP by i.v. noradrenaline and fluids. The investigators did not find a difference with regard to reperfusion injury.[109] In our experience, we could not substitute reduced ECMO flow by increased Impella CP output. In general, the impeller could produce less than one l/min and output deteriorated over time despite fluid loading. One reason may be oppositely directed suction-forces applied to the right heart and pulmonary circulation by the assist devices. However, in a minority of the animals we could achieve a much higher LVAD output, which warrants further investigation. Also, in the standard-flow ECMO + Impella group (Group 1), add-on LVAD provided approximately one l/min. However, the device output remained stable during ECPR in contrast to Group 2 (ECMO 36 ml/kg/min + Impella). In Group 1 (ECMO 72

ml/kg/min + Impella), the combined device output could generally substitute baseline CO fully. Contrary to a previous experimental trial, in which mAP was raised from 60 mmHg to 80 mmHg by pressors and fluids produced no effect on endpoints,[110] the effect on mAP in our trial seemed linked to improved outcome.

5.1.3 Flow measurements

In Paper I, carotid flow was not significantly different between groups despite higher device output with BIVAD. In Paper II carotid flow was increased with ECMO compared to BIVAD and seemed to relate more directly to device output. However, we were not able to find significant differences in cerebral perfusion (as quantified by microspheres). This contradicts a different study group that found an association between cerebral perfusion and haemodynamics.[111] Auto-regulatory mechanisms may play a role.

With spontaneous cardiac rhythm, pulmonary artery flow is a measure of cardiac output. However, during cardiac arrest, pulmonary artery flow decreased dramatically irrespective of concomitant circulatory assist. In Paper III, pulmonary artery flow was increased in survivors during extracorporeal cardiopulmonary resuscitation and seemed related to improved outcome and sustained cardiac function post-ROSC. Further, the lack of pulmonary artery flow during CA seemed to precede ventilation issues with acute hypoxemia during post-ROSC observation which was difficult to reverse by pulmonary recruitment.

Although normally considered accurate in smaller vessels, transit time measurements of flow through the pulmonary artery trunk seemed to systematically underestimate cardiac output compared to echocardiography, or as calculated by microspheres in our model. The reasons are unclear. Underestimation of flow has been reported previously in experimental studies and ascribed to a very asymmetric flow profile during maximum flow.[112] It seems unlikely that this explanation applies in our case. As a compensatory measure, re-calibration against other quantification methods is encouraged. We were not able to do this in our protocol, and all results were therefore systematically underestimated compared to echocardiography or read-out from the

ECMO- or LVAD units for instance. Paper III still seemed to indicate the importance of pulmonary flow versus no-flow. Moreover, with no attempt at relating flow to absolute values, relative differences between groups and changes over time (trends) might still be valid.

LAD flow values seemed reasonable, and within the range that has previously been observed.[113,114] During and after CA, mean LAD flow remained at levels comparable to baseline. With suboptimal resuscitation, a compensatory surge in LAD flow is normally seen after cardioversion. This phenomenon can be noted in individual animals in Paper III.

5.1.4 Fluid loading

All animals received a basic fluid substitution of Ringer's acetate with 20 mmol/l of potassium chloride added, administered at 15 ml/kg/h i.v. In previous experimental studies, volume-loading animals during VF benefits LVAD device output to some extent and potentially also the outcome.[25-27] For this reason, we adopted the same approach in all protocols. The fluid regimen consisted of 2000 ml of Ringer's acetate during CA. The total volume administered was not changed despite prolonging the time of cardiac arrest (30, 40 and 60 minutes in Paper I, II and III, respectively). We believed LVAD output would be less dependent on fluids when combined with an RVAD or ECMO, and that proportional increase of fluids with time would in effect be detrimental. Notably, the BIVAD animals received a net surplus of 1.4 l during cardiac arrest compared to ECMO in Paper II. It cannot be ruled out that this influenced the group comparisons to some extent.

5.2 Outcomes

5.2.1 ROSC, sustained cardiac function and post-ROSC haemodynamics

There is no single outcome measure in cardiac arrest trials. ROSC is an immediate measure of outcome, whereas cardiac function and haemodynamics are relevant in the short term.[115] These markers are appropriate and feasible in experimental research, but long-term outcomes, such as cerebral function, less so.

Coronary perfusion pressure is shown to be related to ROSC in conventional CPR, with a CPP of 15 mmHg or more necessary to achieve ROSC.[116] We found a significant difference between BIVAD and LVAD in Paper I that translated into fewer ROSC (5 vs 10 animals, $p = 0.033$). We did not have the statistical power to demonstrate survival differences between groups in Paper II and III, nor was there a statistical discernible difference between groups with regard to ROSC or post-ROSC cardiac function. Although this may be a realistic conclusion, the small groups compared increase the likelihood of falsely accepting the null hypothesis.

Comparing animals that did not achieve ROSC or maintain cardiac function post-ROSC, we found a marked difference in coronary perfusion pressure and tissue blood flow rate during the VF period in the subendo- and midmyo-, but not the subepicardium in Paper II. In Paper III, higher mLVP seemed related to poor post-ROSC cardiac function for ECMO alone subjects. Increased mAP, CPP and mPAf was linked to maintained post-ROSC cardiac function and ROSC.

5.2.2 $EtCO_2$ as prognostic marker

In Paper I we compared $etCO_2$ levels between LVAD only and BIVAD. For both groups, $etCO_2$ was well above values attainable by conventional CPR, although significantly higher with BIVAD. End-tidal CO_2 correlated to device output, however, contrary to previous data,[78,79] not to survival. This was probably due to decreased myocardial perfusion in the BIVAD group during CA, related to increased mean LV pressure and decreased coronary perfusion pressure. Although systemic circulation (including pulmonary gas exchange) was more favourable in the BIVAD group, the detrimental effect on myocardial perfusion contributed to significantly fewer ROSC (se section 5.1.1 and 5.3.2).

For animals supported by ECMO, pulmonary circulation and gas exchange is bypassed, and $etCO_2$ cannot be used as a marker. Thus, in Paper II and III $etCO_2$ is not reported when ECMO was involved.

5.3 Tissue perfusion

5.3.1 Myocardium

In normal physiological conditions, myocardial perfusion is determined by a pressure gradient across the vascular bed and resistance to flow: $Q = \Delta P/R$, Q is the flow, ΔP the pressure gradient (usually defined as aortic pressure – right atrium pressure during diastole), and R is resistance. Resistance is usually constituted by vascular tone in the epicardial vessels, smooth muscle in all vasculature and wall stress during systole in which intra-myocardial vasculature is squeezed off.[101] Autoregulatory mechanisms ensure the transmural distribution of blood flow in the myocardium during spontaneous circulation, with a flow ratio between the endo- and epicardium > 1.[102] However, in CA, loss of autoregulation has been shown to occur rapidly, primarily due to loss of smooth muscle tone of the arteriolar bed with subsequent dispersion of vascular resistance.[101] In this setting, transmural perfusion is related to the driving force only. That is, the pressure difference, ΔP. With conventional CPR, a perfusion ratio between the endo- and epicardium is < 1.[102] The distribution patterns described above are depicted in Figure 9. In Paper I, we found that tissue blood flow was increased in the subepicardium compared to the subendocardium during VF. This result was consistent also in Paper II, regardless of mode of circulatory assist. In a clinical setting, often with the presence of a coronary stenosis, even very moderate, the susceptibility of the endocardium to ischaemia is aggravated and a distal to proximal distribution gradient is also introduced.[101,102]

A correlation between coronary perfusion pressure during CPR and myocardial blood flow and the relation to successful ROSC, has previously been shown.[62,104,117] A CPP below 5-10 mmHg, does not generate myocardial blood flow. Perfusion pressure of 30-40 mmHg enables flow rates of approximately 40-60% of baseline.[101] Generally, we found markedly higher myocardial blood flow rates compared to mechanical compressions, or compared to a smaller percutaneous LVAD as can be seen in Figure 9.[27,62,103]

5.3.2 Cerebral perfusion

Quantification of cerebral perfusion with microspheres has been evaluated and validated in many previous experimental protocols.[25-27,62,103] Resting blood flow rates are usually reported in the range of 0.3-0.5 ml/min/g. The blood flow rates in the present studies are within this range. Some authors report dismal blood flow of approximately 5% of baseline values during conventional CPR,[62] which may be improved by vasopressors or mechanical compressions.[103,118] Previous experimental protocols by our research group have shown that with the Impella 2.5, cerebral perfusion can be maintained at approximately 40% of baseline during 15 - 40 minutes of CA.[25-27] In Paper I, cerebral blood flow constituted around 40% of baseline values with the Impella CP and increased to approx. 50% with BIVAD ($p = 0.053$). With BiPella in the following protocol (Paper II), cerebral flow could be maintained at 65-70%. Compared to baseline values, cerebral perfusion was not reduced during cardiac arrest with ECMO-supported circulation. Although numerically different compared to BiPella, group differences were not statistically significant ($p = 0.13$).

Post-ROSC hypoperfusion in the cerebral tissue has been reported, after a possible but not compulsory shorter period of hyperemia.[118,119] In Paper II, cerebral perfusion returned to baseline values post-ROSC. In Paper III, we found that cerebral flow increased in Group 1 and 2 (add-on LVAD) for the full observation period, whereas for ECMO alone, flow remained stable compared to baseline. The interpretation of this finding is uncertain and may warrant further investigation.

5.3.3 Peripheral organs

Blood flow is centralised to the brain and heart during cardiac arrest. Flow rates are only a fraction of baseline in the kidneys during conventional CPR. With the Impella 2.5, kidney blood flow was between 10-20% of baseline,[25,27] and with the Impella CP alone, flow rates were marginally better. With BIVAD/BiPella, kidney perfusion was doubled, constituting approx. 30% of baseline. With ECMO alone, tissue blood flow rates of the kidneys were 80-90% compared to baseline. No comparisons can be made for Paper III during cardiac arrest and ECPR.

5.4 Blood chemistry and biomarkers

5.4.1 Blood gases

In Paper I, we found a significant difference between BIVAD and LVAD, with significantly lower s-lactate in the BIVAD group. In contrast to previous studies,[82] this did not correlate with the rate of ROSC. The s-lactate levels in the present studies mainly reflect systemic circulation, and do not reflect myocardial perfusion during VF as demonstrated in Paper I. S-lactate levels were low in the BIVAD group. As with $etCO_2$, s-lactate could not predict ROSC. In Paper II, BiPella animals had significantly increased s-lactate, compared to ECMO animals which had normal s-lactate levels throughout ECPR. Despite this, we did not find a significantly different rate of ROSC between groups. With low-flow ECMO and Impella CP in Paper III, s-lactate was significantly increased at the end of ECPR compared to the other groups and three animals did not achieve ROSC. In this study, myocardial perfusion was optimized compared to the previous studies, and it may be reasonable to assume that s-lactate could monitor quality of CPR more directly in this setting.

Post-ROSC, s-lactate levels and rate of clearance are related with improved outcome.[120] In Paper II, s-lactate was still increased in the BiPella group compared to ECMO at end of post-ROSC observation, but with clear tendency to normalize in survivors. In Paper III, no between-group differences could be found after 3 h post-ROSC monitoring. Presumably, observation time was too short in our models for results to be conclusive, and comparisons could only be made for survivors.

Low Hb levels may negatively influence oxygen delivery to the tissues,[121] and anaemia may be related to poorer neurological outcome and prognosis.[122,123]
In the percutaneous models we did not detect excessive bleeding during or after instrumentation. However, in Paper II, Hb was significantly reduced compared to ECMO at the end of post-ROSC observation, but not during VF. This may have been due to different fluid regimens. In ECMO subjects, the tubing system was primed with 0.6 litres of Ringer's solution, whereas BiPella animals received 2 litres of Ringer's solution during VF. Overt bleeding was not recorded, and no occult bleeding was seen at necropsy, thus haemodilution is a likely contributor. The risk of haemorrhagic

bleeding was increased in the open chest model, and three animals had to be substituted in the protocol due to excessive bleeding during instrumentation. However, no between-group differences in haemoglobin levels were noted during VF in the subjects included in the analysis.

During conventional CPR, blood flow and pulmonary gas exchange follows the same pattern as in spontaneous circulation and is regulated by compression frequency, depth and changing thoracic pressures.[124,125] The quality of CPR may be evaluated indirectly by end-tidal CO_2 as a measure of pulmonary flow and gas exchange, as previously discussed. However, in our protocols the different circulatory assist devices influenced gas exchange greatly. Notably, with ECMO alone there was no pulmonary gas exchange or pulmonary circulation at all, and pO_2 and pCO_2 is controlled by the oxygenator and sweep gas settings. With LVAD alone, BiPella or ECMO + Impella, there was some pulmonary flow. Gas exchange varied dependent upon this flow. The impact can best be seen in Paper I for pO_2 and pCO_2 during VF, and for pCO_2 and possibly SaO_2 in Paper III.

Guidelines recommend high oxygen tension during cardiac arrest.[126] However, what is sufficiently high is not without controversy. A recent experimental study compared use of 50% versus 100% oxygen but could not find any difference in brain tissue oxygen levels.[127] In Paper II, we ventilated animals with 42% oxygen throughout the study and at the same time maintained SaO_2 above 98% in all but two subjects. In this protocol we found increased arterial oxygen levels after ROSC in BiPella subjects and speculated if this could be related to improved pulmonary circulation compared to ECMO. The impact of pulmonary flow also seemed important in Paper III. We registered ventilation issues with hypoxemia and decreased SaO_2 in non-surviving ECMO animals.

Theoretically, cerebral perfusion may be impacted by the watershed phenomenon created by the opposing assist devices in case of poor pulmonary gas exchange,[35,36] which may be of relevance in Paper III. However, we did not monitor oxygen tension

specifically in the cerebrum during cardiac arrest. This would be of interest in a future protocol.

Increased arterial pCO_2 is associated with acidosis which may induce vasodilation and cause negative inotropy,[128,129] although it is not altogether obvious that it is detrimental. On the contrary, compensatory hyperventilation treatment seems related to reduced prognosis.[130,131] In the ECMO alone group, arterial pCO_2 was higher compared to the combined ECMO and Impella groups with the same sweep gas settings due to the lack of pulmonary circulation and gas exchange. The pCO_2 levels were also increased in Paper I, partly due to the above-mentioned reason. However, in this study hypercapnia could have been corrected through changes in sweep gas settings, if recognised after blood gas measurements. Notably, despite increases in pCO_2, we did not experience grave acidosis in any of our protocols.

5.4.2 Biomarkers

Troponin release may be detected after short ischaemic events (30-90 seconds) with new high sensitivity analyses as early as 15-30 minutes after the event, with peak levels measured after 3-4 hours.[132] Reflecting this, we performed troponin T analyses in the two last protocols but not in the first, as we did not expect to find interesting or measurable differences based on 30 minutes of cardiac arrest with no observation period in Paper I. In Paper II, troponin T was increased with BIVAD both at end of CA and at termination of study compared to ECMO. However, ROSC rate and post-ROSC cardiac function (sustained cardiac function) was similar between groups. In the final experimental series, troponin T increased significantly after cardiac arrest with Group 2 (ECMO 36 ml/kg/min + Impella) compared to the other two groups. Differences between groups may reflect the extent of myocardial support and ischaemia. However, release of troponin T has been shown to depend upon local plasma flow, as has wash-out.[133] This may be relevant for the differences found in Paper II and III.

S-creatinine values reflected systemic output in all papers irrespective of group, and could also be related to the tissue blood flow rates we found. Both in Paper II and in

Paper III, s-creatinine differed significantly between groups during CA, but not at study termination. This may indicate recovery, but more likely that subjects available for comparison at the final assessment were comparable due to the drop-out of individuals most affected by CA (i.e. animals that did not achieve ROSC or died during post-ROSC observation).

5.5 Echocardiography and macroscopic evaluation

5.5.1 Intracardiac echocardiography (ICE)

For dimensions and flow velocity, ICE is an acceptable option with normal values available during spontaneous circulation.[58,92] Both in Paper I and in Paper II, we used ICE to confirm that the outlet of the RVAD was distal to the pulmonary valve. Colour Doppler was useful to confirm flow from the device. In Paper II cardiac variables were obtained both at baseline and post-ROSC for survivors. Notably, long-axis measurements were difficult to obtain in a standardised manner in our experience. Short axis views could provide information of left ventricle changes including oedema associated with poor myocardial perfusion (see Figure 12).

5.5.2 Epicardial echocardiography (Paper III)

Groups at the Dep. of Clinical Science have examined different methodology for uptake of images, including strain assessment in open chest large animal models.[134] We could not find between group differences in Paper III when we compared dimensions, volumes, and function by ejection fraction in survivors. Further, we applied speckle tracking echocardiography to quantify global longitudinal strain (GLS). In contrast to other methodology, some authors have found that strain is not greatly influenced by the open chest setting, although not without controversy.[135] GLS decreased similarly between groups in Paper III.

A limitation to epicardial echocardiography is the displacement of the myocardium by the direct pressure of the silicone pad and echoprobe.[136] This introduces a systematic bias, equally applicable to all subjects regardless of group. Further, echocardiography was pre-planned to be performed at predefined time points (baseline and before

termination of experiment), and could not accommodate for animals that did not achieve ROSC, or died before final assessment (Paper II and III). In a future protocol, echocardiographic evaluation during cardiac arrest may be of interest, for instance to relate effect of LVAD with changes in left ventricular dimensions. Notably though, colour Doppler is not feasible in our experience due to extensive interference by the LVAD or RVAD. Finally, the recorded increase in heart rate toward the end of the experiment might influence uptake of images, possibly including global longitudinal strain.[137] However, the increase was noted in all subjects irrespective of group (Paper II and III).

5.5.3 Macroscopic evaluation

In all study protocols, the heart was harvested and sliced transversely for microsphere quantification. At the same time, visual inspection was possible. Clearly, poor perfusion during CA preceded profound changes to the myocardium that correlated to echocardiographic images (Figure 12) and can best be described as "stone heart". This phenomenon was noticed and first described in relation to cardiac surgery in the late sixties/early seventies,[138-140] but has later been described in relation to poor resuscitation, and also demonstrated in animal models.[141] Figure 12 shows cross-sectional images of the left ventricle with typical changes related to good and poor circulatory assist and myocardial perfusion. By comparing water content in survivors with non-survivors this qualitative evaluation could be substantiated for Paper II. Interestingly, in the same manner, water content was increased in non-survivors in Paper III further validating this assumption.

5.6 Methodological considerations

5.6.1 Animal model

Although there are sufficient similarities with human anatomy and physiology to make the use of domestic pigs as research subjects relevant, there are pitfalls. For instance, congenital heart defects, like atrial septal defects, are more common than in humans.[142] Acute pulmonary arterial hypertension is also known to occur in relation to declamping and reperfusion after cardiopulmonary bypass and may be especially

detrimental in connection with atrial septal defects.[143] We examined the heart to look for this specifically in Paper III where we experienced arterial oxygen desaturation in some subjects after re-established spontaneous circulation, albeit without finding any defects.

Another caveat with swine is the predisposition for malignant arrhythmias in relation to interventions,[144] and especially in connection with manipulation in the left ventricle. One animal in Paper III had to be replaced due to incessant VF before baseline measurements were performed. In another subject, VF was induced by wire manipulation during deployment of the LVAD. This animal was included in the study in the ECMO alone group. With the closed chest protocols, we did not experience any unwanted persisting tachycardia during model preparation. However, especially during RVAD deployment, extrasystolic beats were rife. We also recorded short episodes of VT that were self-limiting. This is also a common feature in humans.

In our experience, vessels are more fragile in young swine compared to humans. For instance, we experienced intima dissection when inserting cannulas. For this reason, we changed our approach somewhat during Paper I with regard to sheath insertion: Instead of free-dissecting and securing vessels with vessel-loops, we adopted a semi-percutaneous approach. Incisions through the hide of the animal was made to expose arteries to digital palpation, but otherwise leaving the vessels in-situ. In our experience, this could reduce vessel complications and preparation time.

Next, there are specific anatomical differences related to the quadruped compared to biped stance. This was specifically relevant with regards to the shape of the RVAD which is designed to fit the anatomy in humans. Even so, it was possible to deploy in swine. However, the process was more cumbersome with the need of echocardiographic control to make sure the outlet portion of the RVAD was distal to the pulmonary artery valve. Final confirmation of the continuous flow was done by colour Doppler. The cumbrousness of deployment excluded the possibility of a direct comparison with insertion in humans. This would have been interesting, since very little experience was available at the time of Paper I.

Anatomy was also relevant with regard to the LVAD as previously mentioned (5.1.2). In swine, the aortic arch is short and acutely angled. In Paper I and II the LVAD was inserted from the femoral artery and it was very important to pass the Impella CP through the arch and into the LV in a controlled way to avoid compacting the lumen of the impeller. This was obviated in Paper III by switching the insertion site to the left carotid artery.

Unless using mini-pigs, Landrace pigs have to be young for reasonable handling due to rapid growth. This means that animal subjects are without cardiovascular disease. Landrace pigs rarely have pre-existing epicardial collateral circulation. This in contrast to dogs, which have an extensive collateral network in the myocardium. The microcirculation including the septal branches are similar to the human heart.[53] In acute cardiac arrest models, this has to be taken into account, necessitating prudent interpretation of results.

Between Paper II and III we changed from closed chest- to an open chest model for reasons outlined in 3.3. Change of model may influence haemodynamic parameters relevant when directly comparing results between the different papers.[145]

5.6.2 Study design

Although similar in design in many respects, the length of CA arrest was altered between studies, and an observation period was added in the two last protocols. Length of MCS was chosen to be clinically relevant, but in the initial study conservative, to make sure differences were related to the intervention and not length of CA by itself. With an overly long CA period, ROSC would not be achieved in any subject and the experiment would be futile.

Weaning was performed rapidly after defibrillation regardless of clinical state in Paper II and III in order to optimally detect between-group differences. In routine clinical practice, weaning in the post-resuscitation period is guided by many variables including aetiology of pathology, organ function evaluation, echocardiographic

assessment, haemodynamic parameters (including inotropic support) and biomarkers.[146]

Comparators were the same in all experiments except for Paper I in which "sustained cardiac function" for obvious reasons could not be reported. This enables evaluation of consistency across studies, and improves judgement regarding plausibility and interpretation of results. It could be argued that the length of post-ROSC observation was too short to be meaningful. Due to the novelty of interventions studied during VF, there was little or no previous data to base assumptions upon when determining the length of circulatory assist or observation. With improvement in the modes of circulatory assist between the different protocols (and insights provided), length of MCS could be extended successively.

5.6.3 Non-ischaemia model

Arguably, myocardial ischaemia is the main cause of cardiac arrest.[1,147-150] However, we chose a non-ischaemic model for several reasons. First, we tried to reduce the number of unknown factors for matters of interpretation. Second, introducing ischaemia would complicate already intricate models. Third, we believe our model has a more general applicability. For instance, our experimental set-up should encompass drowning accidents, accidental hypothermia and arrhythmias. Fourth, our model simulates a more "ideal" resuscitation setting and may help delineate practical limits obtainable by the circulatory assist set-up for each comparison. It may be reasonable to assume that differences would be aggravated in an ischaemic model as indicated in a previous experimental model comparing ROSC rate, cardiac function and survival.[151] However, as mentioned in Paper I, the low myocardial blood flow rate values found in subjects that did not achieve ROSC corresponded to values created by ischaemia due to an acute coronary occlusion.[113] Further trials introducing ischaemia to our model would substantiate the assumption.

5.6.4 Statistical considerations

In Paper I and II, continuous haemodynamic variables were analysed using two-way repeated measurements ANOVA. In the first two papers missing data were sparse, and during cardiac arrest data was generally balanced between groups. In this case, ANOVA is robust, and output should be similar or equal with more complex modelling. In Paper III however, we chose to apply linear mixed models (LME) due to increased complexity of the protocol with three smaller study groups (8 versus 10) making statistical considerations even more vulnerable to outliers and missing data. Due to longer post-ROSC follow-up, we also had to expect more censoring (deaths). Linear mixed models are more robust in these instances taking into account all available data. Hence, in the final protocol LME was a more adequate statistical model for continuous, temporal data.[152]

6. Conclusions

Paper I:

1. BIVAD provided superior systemic haemodynamic support compared to LVAD alone during CA.
2. ROSC rate was increased with LVAD compared to BIVAD. This was related to hampered myocardial perfusion due to reduced coronary perfusion pressure with BIVAD.

Paper II:

1. Both ECMO and balanced BiPella could maintain vital organ perfusion during VF, although ECMO provided superior systemic haemodynamic conditions.
2. Coronary perfusion pressure seemed to be a predictor for ROSC and post-resuscitation cardiac function. Irrespective of group, myocardial perfusion was dependent upon adequate perfusion pressure (CPP) created by the circulatory support during cardiac arrest. No subjects with a coronary perfusion pressure < 10 mmHg survived.
3. No between-group differences could be found with regards to ROSC or sustained cardiac function.

Paper III:

1. Add-on LVAD could improve haemodynamics combined with standard-flow ECMO.
2. Add-on LVAD could not substitute a reduction of ECMO flow.
3. Increased mean aortic pressure and myocardial perfusion pressure were related to ROSC rate and sustained cardiac function. Increased mean left ventricular pressure during ventricular fibrillation preceded poor post-resuscitation heart function. Increased pulmonary artery flow may improve post-resuscitation ventilation.

General conclusions:

1. With continuous flow circulatory assist $etCO_2$ alone may not be a reliable surrogate for satisfactory resuscitation during cardiac arrest.
2. With continuous-flow circulatory assist devices it seems that coronary perfusion pressure (or myocardial perfusion pressure) is defined by the pressure difference between the aorta and the LV cavity and not the right atrium during cardiac arrest.
3. Optimization of haemodynamic support can ensure coronary perfusion pressure well above a threshold of 10-15 mmHg necessary for ROSC, and at the same time provide systemic output that may substitute spontaneous circulation.
4. In the setting of in-hospital cardiac arrest, balanced BiPella/BIVAD may be an acceptable alternative if ECMO is not available.

7. Implications and suggestions

7.1 Clinical impact

The results in this thesis are of a translational nature and may not be directly transferrable to clinical practice in humans. These data may primarily provide basis for hypothesis generation which should be taken into account when considering areas of potential clinical impact listed under. It should also be noted that the use of ECPR is extremely demanding on personnel and economic resources, limiting availability to selected affluent societies of the industrialised world with advanced health care systems.

Mean left ventricular pressure and quantification of myocardial perfusion pressure should be evaluated in clinical cardiac arrest and should be registered within the confines of a trial to evaluate clinical usefulness. In a cardiac catheterization laboratory these measurements can easily be obtained.

BiPella may be a reasonable alternative as mechanical circulatory assist during cardiac arrest in the setting of a cardiac catheterization laboratory if ECMO service is unavailable. With expected development of more powerful left ventricular assist devices, BiPella support may improve further.

The combination of standard-flow ECMO and add-on LVAD seems to be the most promising form of extracorporeal cardiopulmonary resuscitation. This combination provides superior output, and at the same time may maintain maximum coronary perfusion pressure. ECMO alone can be initiated out-of-hospital, and the LVAD can be placed once in-hospital, for instance in relation to percutaneous coronary intervention.

With in-hospital cardiac arrest, implantation of a left ventricular assist device is the fastest and least invasive initial step in the cath. lab. Subsequent escalation with ECMO or a right ventricular assist device if cardiac arrest persists is optional. Weaning from ECMO is easier with an LVAD in place.

7.2 Future research

Combining the Impella RP with a more powerful percutaneous left ventricular assist device would be an interesting future protocol. The theoretical output should be comparable to that of ECMO. Further insight is required regarding optimization of flow-settings and balancing of assist devices, potentially facilitating tailoring of circulatory support according to clinical need.

Protocols may be elaborated to include ischaemia with an acute coronary occlusion leading to VF, reperfusion and extracorporeal cardiopulmonary resuscitation and ROSC-attempts. To simulate the real world more intimately, no-flow or low-flow time before initiation of the assist devices could be incorporated. Further, circulatory support could be continued post-ROSC during potential cardiogenic shock.

Clinical studies incorporating measurements of left ventricular pressures during resuscitation should be performed to evaluate benefit in humans. Correlating invasive measurements to non-invasive methods, for instance echocardiographic evaluation, would be relevant.

Clinical cardiac arrest studies combining ECMO and add-on LVAD need to be undertaken to evaluate if this might improve survival. As a minimum, cases should be registered within the framework of national or major cardiac arrest registries.

The impact of watershed when combining ECMO and add-on LVAD during cardiac arrest with regard to cerebral perfusion needs further elucidation.

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