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ORIGINAL RESEARCH

Cardiac Arrest in Pigs With 48 hours of Post-Resuscitation Care Induced by 2 Methods of Myocardial Infarction: A Methodological Description

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BACKGROUND: Systematic reviews have disclosed a lack of clinically relevant cardiac arrest animal models. The aim of this study was to develop a cardiac arrest model in pigs encompassing relevant cardiac arrest characteristics and clinically relevant post-resuscitation care.

METHODS AND RESULTS: We used 2 methods of myocardial infarction in conjunction with cardiac arrest. One group (n=7) had a continuous coronary occlusion, while another group (n=11) underwent balloon-deflation during arrest and resuscitation with re-inflation after return of spontaneous circulation. A sham group was included (n=6). All groups underwent 48 hours of intensive care including 24 hours of targeted temperature management. Pigs underwent invasive hemodynamic monitoring. Left ventricular function was assessed by pressure-volume measurements. The proportion of pigs with return of spontaneous circulation was 43% in the continuous infarction group and 64% in the deflation-reinflation group. In the continuous infarction group 29% survived the entire protocol while 55% survived in the deflation-reinflation group. Both cardiac arrest groups needed vasopressor and inotropic support and pressure-volume measurements showed cardiac dysfunction. During rewarming, systemic vascular resistance decreased in both cardiac arrest groups. Median [25%;75%] troponin-I 48 hours after return of spontaneous circulation, was 88 973 ng/L [53 124;99 740] in the continuous infarction group, 19 661 ng/L [10 871;23 209] in the deflation-reinflation group, and 1973 ng/L [1117;1995] in the sham group.

CONCLUSIONS: This article describes a cardiac arrest pig model with myocardial infarction, targeted temperature management, and clinically relevant post-cardiac arrest care. We demonstrate 2 methods of inducing myocardial ischemia with cardiac arrest resulting in post-cardiac arrest organ injury including cardiac dysfunction and cerebral injury.

Key Words: animal experiment ■ cardiac arrest ■ cardiopulmonary resuscitation ■ post-cardiac arrest intensive care ■ pressure-volume measurements

Recent systematic reviews have shown that cardiac arrest animal research is lacking clinically relevant features and is often judged as having high risk of bias.[1,2] Clinical relevance in the setting of cardiac arrest concerns, amongst other aspects, the induction method of cardiac arrest, basic and advanced life support, as well as post-cardiac arrest care. The most prevalent cause of out-of-hospital cardiac arrest is myocardial infarction.[3–5] Despite this, the most commonly used induction methods in cardiac arrest animal models are pacing-induced arrhythmias and asphyxia. In a review of 490 studies, myocardial

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CLINICAL PERSPECTIVE

What Is New?
- This large animal model incorporates myocardial infarction, the most prevalent etiology to cardiac arrest, realistic resuscitative efforts, and clinically relevant long-term post-cardiac arrest intensive care.
- By integrating clinically relevant measures into preclinical testing, we hope to decrease the translational gap that currently exists.

What Are the Clinical Implications?
- A more thorough testing of interventions might result in fewer futile clinical trials being conducted.

Nonstandard Abbreviations and Acronyms

AMI-Cont	cardiac arrest group with continuous myocardial infarction
AMI-Int	cardiac arrest group with reperfusion of coronary artery before cardiac arrest
CO	cardiac output
dP/dt_{max}	maximum rate of pressure development during contraction
dP/dt_{min}	minimum rate of pressure development during relaxation
ESPVR	end-systolic pressure-volume relationship
ICP	intracranial pressure
MAP	mean arterial pressure
NSE	neuron-specific enolase
PV	pressure-volume
ROSC	return of spontaneous circulation
TTM	targeted temperature management

infarction was only used in 2% of all studies.[2] The same review found, related to post-resuscitation care, that only 13% used organ-supportive measures, 4% targeted temperature management (TTM), and for the large animal models, the median post-resuscitation observation time was merely 4 hours.[2] Hence, there is an unmet need for clinically relevant large-animal cardiac arrest models to make sure that promising interventions are rigorously tested before translation into clinical studies.

There is a growing number of randomized clinical trials testing cardiac arrest interventions.[6] Despite this, only a minority of pre-clinically tested pharmacological interventions actually advance to clinical testing and experimental positive results often translate unsuccesfully.[7] If the quality of results from preclinical studies could be improved, this could increase the likelihood of translation of positive results to clinical trials. Conducting high quality, clinically relevant experimental animal research is therefore of key importance.

The aim of this study was to develop a cardiac arrest pig model encompassing clinically relevant features with regards to induction method, resuscitation, and post-cardiac arrest intensive care.

METHODS

The study was approved by the Danish Animal Experiments Inspectorate (License number: 2019-15-0201-01647) and conducted and reported in accordance with the ARRIVE[8] and Utstein-Style[9] guidelines. The data that support the findings of this study are available from the corresponding author upon reasonable request.

Animals
Female crossbred Landrace/Yorkshire/Duroc pigs (40 kg) were fasted overnight with free access to water.

Overall Study Design
Each pig underwent myocardial infarction and cardiac arrest or sham-procedure. We initiated the experiments with a continuous myocardial infarction protocol (AMI-Cont group) and later included a group with an intermediary coronary reperfusion period before cardiac arrest (AMI-Int group, see Experimental Protocol paragraph). The sham group was subjected to the same procedures and treatments as the cardiac arrest groups except balloon inflation in the left anterior descending artery, cardiac arrest, and resuscitation (see Figure 1). Following resuscitation, the animals were kept anesthetized and mechanically ventilated for 48 hours and received post-cardiac arrest care including TTM for 24 hours. Cerebral data are presented in a separate publication to allow for a clear description of the core methodology in the current paper. A priori, we decided to include data from all pigs up until the pig was euthanized, either because of it reaching the end of protocol or premature death (eg, unsuccessful resuscitation or post-cardiac arrest organ failure). Pigs were a priori excluded if they presented with consistent mean arterial blood pressures (MAP) <65 mm Hg or mean arterial pulmonary pressure >25 mm Hg at baseline. Animals were allocated to the 3 groups at the discretion of the primary investigator in a non-randomized order and there was no blinding.
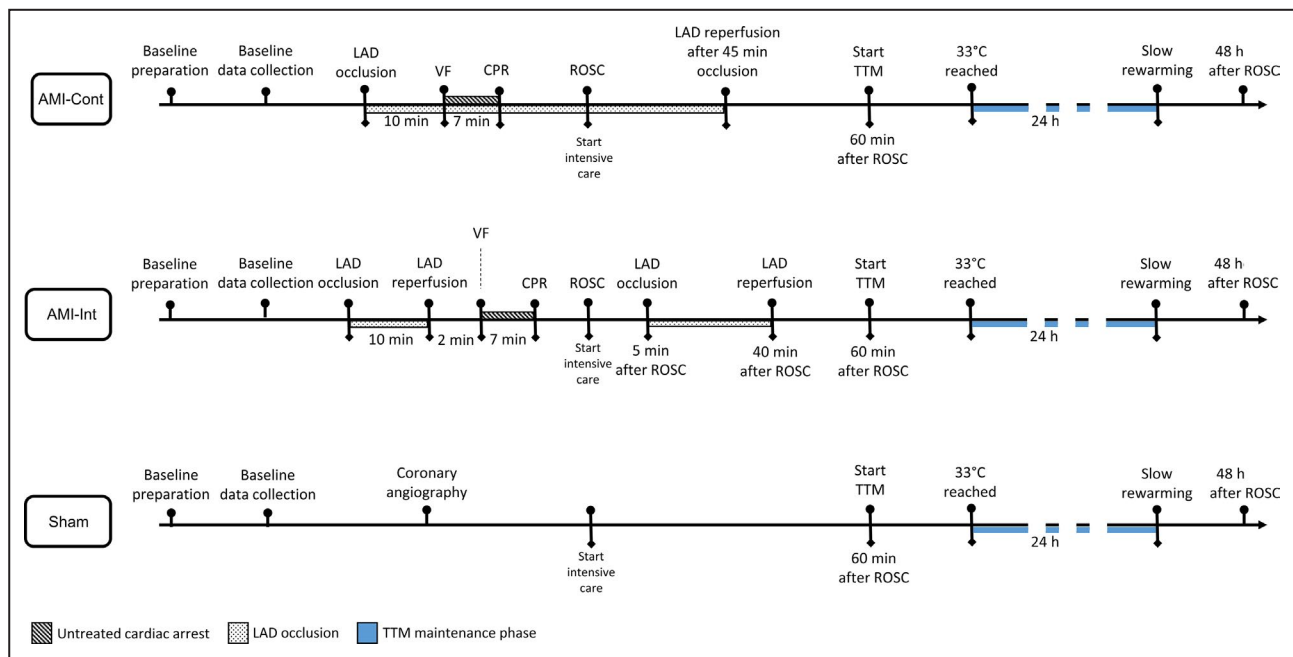
Figure 1. Timeline of the experimental protocol in each group.
AMI-Cont indicates cardiac arrest group with continuous myocardial infarction; AMI-Int, cardiac arrest group with reperfusion of coronary artery before cardiac arrest; CPR, cardiopulmonary resuscitation; LAD, left anterior descending artery; ROSC, return of spontaneous circulation; TTM, target temperature management; and VF, ventricular fibrillation.

Animal Preparation

Anesthesia was induced by ketamine (6.25 mg/kg), midazolam (0.625 mg/kg), and atropine (0.5 mg) and maintained with intravenous infusion of propofol (4.0–5.5 mg/kg per hour) and remifentanil (0.6–1.0 µg/kg per hour). See Data S1 for detailed anesthesia protocol and Figure S1 *for infusion rates*. Ringer lactate (2 mL/kg per hour) with unfractionated heparin (18 IU/kg per hour) was administered to ensure normovolemia and prevent thromboembolic events throughout the protocol. An intravenous heparin bolus was administered before myocardial infarction instrumentation (5000 IU) and an intracoronary bolus before balloon occlusions (2500 IU). An additional 10 mL/kg fluid bolus was given at baseline.

Mechanical ventilations with pressure-controlled volume guarantee (Datex Ohmeda S5, GE Healthcare, IL) were delivered with a tidal volume set at 8 mL/kg and a rate adjusted to an end-tidal CO_2 between 4.7 and 6.0 kPa. The fraction of inspired O_2 was adjusted to partial pressure of O_2 in arterial blood (PaO_2) between 20 and 25 kPa. Positive end-expiratory pressure was set to 5 cm H_2O. The fraction of inspired O_2 and positive end-expiratory pressure were adjusted per protocol to optimize oxygenation (see Table S1).

For measurement of intracranial pressure (ICP), a burr hole was made in the skull and fitted with a bolt (Bolt Kit, Raumedic AG, Helmbrechts, Germany). After puncture of the dura mater a catheter (NEUROVENT-PTO, Raumedic AG, Helmbrechts, Germany) was inserted in the right parietal lobe parenchyma and connected to a monitor (MPR2 logO Datalogger, Raumedic AG, Helmbrechts, Germany). At baseline pigs were kept at normothermia (38.5±0.5 °C). A bladder catheter with thermometer was inserted.

Monitoring and Data Collection

Throughout the experimental protocol, pigs were monitored with end-tidal CO_2, bladder temperature, urine output, saturation, one-lead electrocardiogram, heart rate, ICP, and invasive arterial blood pressure. A pulmonary artery catheter (Swan-Ganz CCOmbo catheter, Edwards Lifesciences, CA) was inserted to continuously measure pulmonary artery blood pressure, central venous pressure, cardiac output (CO), and mixed venous blood oxygen saturation. Correct catheter placement into a pulmonary artery branch was confirmed on fluoroscopy. Accumulated fluid balance was calculated as total urine output subtracted from accumulated amount of fluid treatment, anesthesia, and cardiovascular support. Systemic vascular resistance was calculated by the formula: ((MAP- central venous pressure)/CO)·80.

Pressure-volume (PV) measurements were conducted with an admittance PV-catheter (Transonic Systems Inc., NY) inserted in the left ventricle through the left carotid artery. Measurements were performed during apnea in triplicates at predetermined time points before and after cardiac arrest. Inferior vena

cava occlusion was performed to allow for both static and dynamic parameters. Data were collected with ADVantage (Transonic Systems Inc., NY) and analyzed in LabChart (ADInstruments, New Zealand). The PV system was calibrated for blood resistivity, stroke volume and heart type (normal). See Data S1 for intraobserver variability.

Arterial blood gasses (ABL 90 Flex Plus, Radiometer, Denmark), plasma EDTA and serum samples were collected at baseline and pre-defined time points after resuscitation. Blood samples were centrifuged, and supernatants stored at −80 °C for later analysis (see Data S1 for details).

After all monitoring equipment was established, a 30-minute stabilization period were performed.

Cardiac Magnetic Resonance Imaging

Hearts were excised from the pigs after 48 hours of intensive care and scanned ex-vivo on a 1.5 T magnetic resonance system (Achieva DStream 1.5T, Phillips, Netherlands). Image analysis for measurement of infarct size was performed in Horos version 3.3.6 (MD, USA). See Data S1 and Figure S2 for details.

Experimental Protocol

Myocardial Infarction and Cardiac Arrest Induction

A 6F guiding catheter was placed in the ostium of the left main coronary artery via the left carotid artery. A balloon catheter (2–3×10 mm) was placed distal to the second diagonal branch. Occlusion was confirmed by a coronary angiography. After 5 minutes of occlusion, anesthesia was discontinued, and if ventricular fibrillation (VF) had not occurred after 10 minutes, it was electrically induced by a bipolar pacing wire (9 V DC current for 1–2 seconds) in the right ventricle. Cardiac arrest was confirmed by the presence of VF on the ECG and a swift drop in MAP, and the animal was disconnected from the ventilator. In the AMI-Cont group, the left anterior descending balloon remained inflated for 45 minutes continuously, throughout the VF period, resuscitation, and early post-return of spontaneous circulation (ROSC) period. In the AMI-Int group, the balloon was deflated after 10 minutes of occlusion, and 2 minutes after deflation VF was electrically induced. The balloon catheter was re-inflated 5 minutes after return of spontaneous circulation for another 35 minutes of myocardial ischemia (see Figure 1). Occlusion or reperfusion was confirmed by coronary angiographies.

Resuscitation

After 7 minutes of untreated cardiac arrest, basic cardiopulmonary resuscitation was initiated with a 30:2 algorithm (mechanical chest compressions (LUCAS II, Jolife AB, Sweden) and bag ventilations) in accordance with European Resuscitation Council guidelines.[10] Basic cardiopulmonary resuscitation was followed by a rhythm check and subsequent biphasic 360 J defibrillation (Lifepak 20, Physio-Control, WA) if a shockable rhythm was present. Hereafter, advanced life support was started with continuous chest compressions and mechanical asynchronous ventilations. Rhythm check and subsequent shock was performed every 2 minutes. Adrenaline (0.02 mg/kg) was administered after the third and every other rhythm analysis. If in a shockable rhythm, a single bolus of amiodarone was administered after the third (5 mg/kg) and fifth (2.5 mg/kg) rhythm analyses. Advanced life support was continued until ROSC (defined as an organized rhythm with MAP >30 mm Hg lasting >1 minute) or until a total of 30 minutes of resuscitative efforts. See Data S1 for resuscitation details.

Post-Resuscitation Care

For details see Table S1. In short, the following treatment goals for vital parameters were set according to post-resuscitation care guidelines[11]: partial pressure of CO_2 in arterial blood 4.7 to 6.0 kPa (alpha-stat management), arterial blood saturation (SaO_2) 94% to 98%, MAP >65 mm Hg, CO>2.0 L/min, mixed venous blood oxygen saturation>50%, blood glucose 4 to 10 mmol/L, and potassium 3.0 to 5.5 mmol/L. Relevant fluid (4 mL/kg bolus, max 1 L/24 h), inotropic (dobutamine 0.1–15.0 µg/kg per min and adrenaline 0.01–1.00 µg/kg per min), and vasopressor (noradrenaline 0.01–1.00 µg/kg per min and terlipressin 0.2–1.0 mg) support were administered to meet set treatment goals. Within the initial 60 minutes of ROSC, if noradrenaline was inadequate at maintaining MAP>65 mm Hg, repeated adrenaline boluses (0.1 mg/bolus) were administered. Arrhythmias were treated with defibrillation or amiodarone according to the post-resuscitation protocol. If MAP dropped below 30 mm Hg, advanced life support was initiated. Potassium homeostasis was upheld by K^+ supplement and furosemide and insulin/glucose infusions. Prophylactic cefuroxime (750 mg) was administered every 8 hours following ROSC.[12] Blood glucose control was achieved with insulin and glucose administrations. All pigs received 10 mL/h enteral nutrition via feeding tube. One hour after ROSC, TTM to 33 °C was started with a surface cooling device (Arctic Sun, Medivance Inc., CO). To allow for swift cooling, neuromuscular blockade with rocuronium was established and maintained until rewarmed to 36 °C. The target temperature was maintained for 24 hours. Rewarming rate was 0.5 °C/h until normothermia (38.5 °C). Active normothermia was ensured throughout the remainder of the experimental protocol. At the end of protocol,

animals were euthanized with an intravenous injection of pentobarbital (120 mg/kg).

Statistical Analysis

Normality was evaluated by qq-plots and histograms. The majority of outcome variables were non-normally distributed. Therefore, continuous data are presented as medians with 25% and 75% quartiles. Categorical data are presented as n (%). The present study was a method development experiment of purely descriptive nature and no sample size calculation was performed. Study data were collected using REDCap electronic data capture tools hosted at Aarhus University.[13] Data management was performed in Stata 16.1 (StataCorp, TX). Figures are produced in Prism 9.0.1 (GraphPad, CA).

RESULTS

A total of 25 pigs were included in this study. One pig in the sham group was excluded because of hypotension during baseline preparation and subsequent VF during pressure-volume measurements. The remaining 24 pigs were divided as follows: AMI-Cont=7, AMI-Int=11, and sham=6 (Figure 2). Three animals underwent premature euthanasia: 1 pig in the sham group had major bleeding attributable to an accidental discontinuation of an arterial sheath at 5 hours; 1 pig in the AMI-Int group died because of a tension pneumothorax (necropsy confirmed) at 37 hours post-ROSC; and 1 pig in the AMI-Cont group died of cardiovascular collapse at the end of the protocol missing only magnetic resonance imaging data.

The median weight was 38.0 kg [37.5;38.2] in the AMI-Cont group, 40.0 kg [38.7;40.0] in the AMI-Int group, and 38.6 kg [38.0;40.0] in the sham group. The median time from intubation until balloon occlusion/sham intervention was 239 minutes [210;259] in the AMI-Cont group, 213 [206;237] in the AMI-Int group and 227 [212;240] in the Sham group.

Cardiac Arrest and Resuscitation

Table 1 displays cardiac arrest and resuscitation parameters. In the AMI-Cont group 3 of 7 (43%) pigs achieved ROSC with 7 of 11 (64%) pigs in the AMI-Int group.

Targeted Temperature Management

Bladder temperature is displayed in Table 2 and Figure S3. Time to target temperature was 163 minutes [127;164] in the AMI-Cont group, 164 [131;208] in the AMI-Int group, and 164 [141;219] in the sham group.

Cardiovascular Function

Hemodynamic parameters are displayed in Figure 3 and Table 2. Up until 24 hours post-ROSC, the sham group had higher MAP and CO in conjunction with a lower heart rate when compared with both cardiac arrest groups. In the AMI-Cont group, 3 of 3 animals received noradrenaline within the first hour after ROSC as did 5 of 7 animals in the AMI-Int group. The AMI-Cont group received a median amount of adrenaline support of 0.14 mg [0.00;7.60] during the first hour whereas the AMI-Int group received 0.02 mg [0.00;2.30]. The sham group received no cardiovascular support during the first hour.

From the onset of rewarming, a sudden drop in systemic vascular resistance in the AMI-Cont group was observed; an effect that lasted throughout the rewarming phase. This was reflected by the need for incremental doses of cardiovascular support and an increase in CO when compared with the AMI-Int group (Figure 4, for graph of infusion rates see Figure S4). The AMI-Int group sustained systemic vascular resistance at the onset of rewarming but were also in need of increased doses of cardiovascular support. Accumulated fluid balances at 48 hours after ROSC were +7.9 L [5.9;8.5] in the AMI-Cont group, +6.1 L [4.4;6.6] in the AMI-Int, and +5.1 L [4.8;5.4] in the sham group.

Left Ventricle PV Measurements

The low number of successfully resuscitated pigs in the AMI-Cont group and cardiovascular instability in this group precluded collection of dynamic parameters from all time points. The limited amount of data from the AMI-Cont group are provided in Figure S5. Figure 5 displays dynamic PV measurements for the AMI-Int and sham groups. During the first 6 hours after ROSC, end-systolic pressure-volume relationship (ESPVR) and preload recruitable stroke work x-axis intercepts were preserved relative to baseline in the AMI-Int group compared with leftward shift in the sham group. Throughout the remainder of the observation period, ESPVR slope rose slightly in the AMI-Int group with simultaneous marked rightward shift of ESPVR x-axis intercept. No major differences were observed in preload recruitable stroke work parameters during this period. Table 3 shows all static PV parameters. Ejection fraction was generally lower in the 2 cardiac arrest groups after resuscitation, but similar between all groups at 48 hours post-ROSC. The time constant of isovolumetric relaxation increased in all 3 groups during TTM, albeit to higher levels in the sham group. Maximum rate of pressure development during contraction (dP/dt_{max}) and minimum rate of pressure development during relaxation (dP/dt_{min}) were similar at baseline in all 3 groups but dP/dt_{max} was lower and dP/dt_{min} higher at the end of protocol in the 2 cardiac arrest groups compared with the sham group.
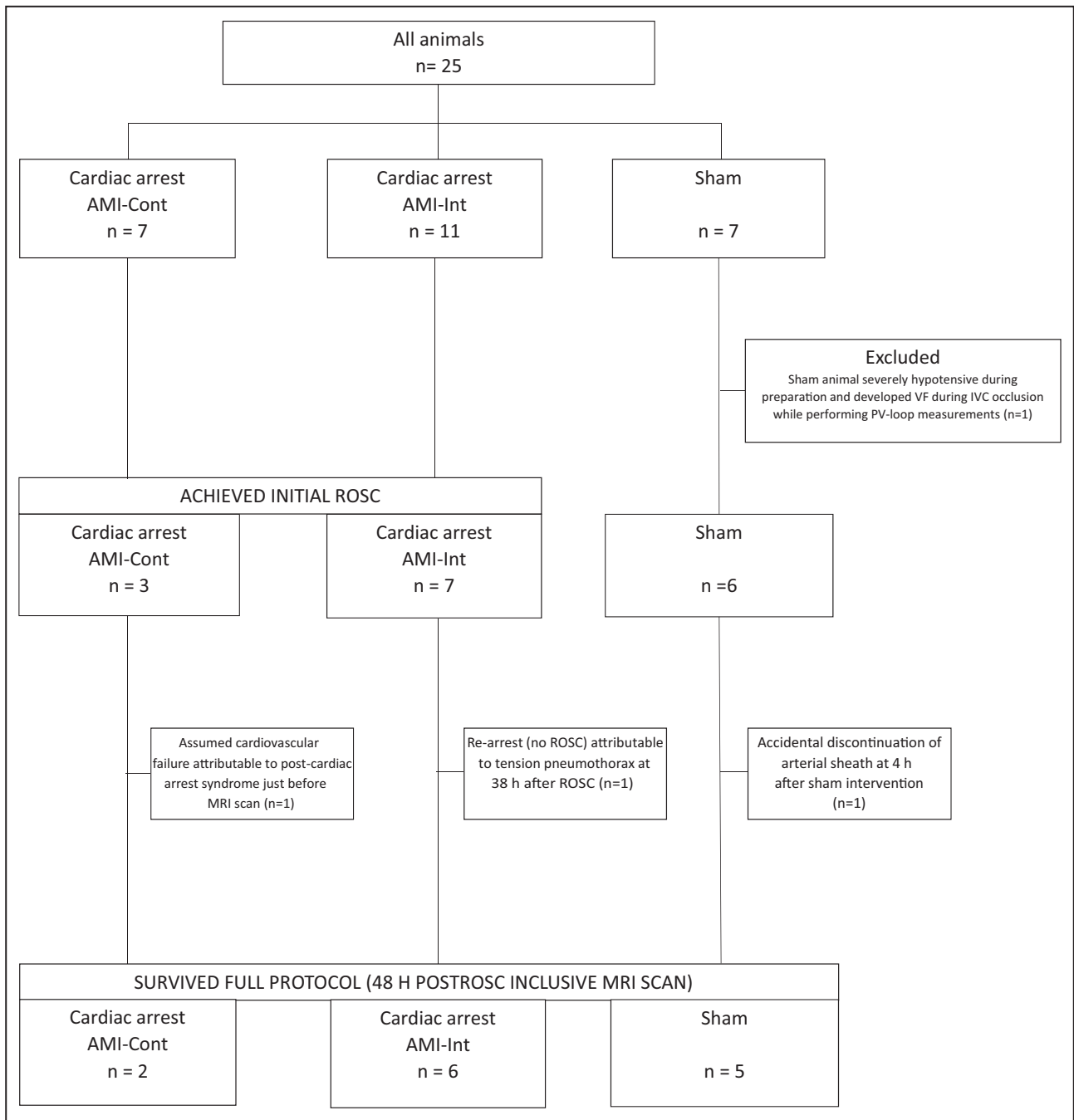
Figure 2. Flowchart of the 3 groups.
AMI-Cont indicates cardiac arrest group with continuous myocardial infarction; AMI-Int, cardiac arrest group with reperfusion of coronary artery before cardiac arrest; IVC, inferior vena cava; MRI, magnetic resonance imaging; PV, pressure-volume; ROSC, return of spontaneous circulation; and VF, ventricular fibrillation.

Cerebral Outcomes

Figure 6 displays ICP and serum neuron-specific enolase (NSE) levels. ICP levels were comparable in all 3 groups from baseline until 18 hours after ROSC. From this time point ICP rose steadily in the AMI-Cont group but not in the AMI-Int and sham group. This observation was driven by 2 out of 3 pigs in the group, which reached an ICP >50 mm Hg at 48 hours after ROSC.

The AMI-Int and sham groups, developed similarly with ICP levels <25 mm Hg with no marked deviations over time. One exception is a single pig in the AMI-Int group, in where ICP rose to 46 mm Hg 48 hours after ROSC. In all 3 groups no differences were seen in NSE levels up until 6 hours after ROSC. At 12 hours after ROSC, NSE rose considerably in the 2 cardiac arrest groups, albeit to higher levels in the AMI-Cont group.

Table 1. Resuscitation Parameters

Cardiac arrest and ALS parameters	Cardiac arrest groups	
	AMI-Cont (n=7)	AMI-Int (n=11)
VF induction method		
Electrical	7 (100)	10 (91)
Spontaneous	0 (0)	1 (9)
Presenting rhythm		
VF	7 (100)	10 (91)
Asystole	0 (0)	1 (9)
Time to VF (min)	10.8 [10.4;11.6]	12.0 [11.0;13.2]
Cumulative adrenaline dosage (mg/kg) during ALS		
All animals	0.12 [0.02;0.12]	0.04 [0.00;0.12]
ROSC animals	0.02 [0.00;0.06]	0.00 [0.00;0.04]
Cumulative amiodarone dosage (mg/kg) during ALS		
All animals	0.20 [0.19;0.20]	0.18 [0.00;0.19]
Shocks during resuscitation		
All animals	4 [4;5]	5 [2;6]
ROSC animals	4 [3;5]	2 [2;5]
No ROSC animals	5 [4;5]	7 [5;12]
Animals achieving ROSC	3 (43)	7 (64)
Time to ROSC (min)	8.4 [6.5;12.1]	4.4 [4.2;10.9]
Total shocks during post-ROSC period	0 [0;5]	2 [0;22]

Time to VF indicates duration from start occlusion to VF. Continuous data are presented as median [25%;75%]. Categorical data are presented as n (%). ALS indicates advanced life support; AMI-Cont, cardiac arrest group with continuous myocardial infarction; AMI-Int, cardiac arrest group with reperfusion of coronary artery before cardiac arrest; ROSC, return of spontaneous circulation; and VF, ventricular fibrillation.

Both cardiac arrest groups had elevated NSE levels at 48 hours when compared with the sham group. The median relative difference in NSE levels between baseline and 48 hours after ROSC were –2% [–15;6] in the sham group, 470% [213;552] in the AMI-Int group, and 2175% [490;4000] in the AMI-Cont group.

Organ Markers and Metabolism

Figure 7 shows markers of organ function and injury. Myocardial injury measured by troponin-I was higher in both cardiac arrest groups compared with sham group, with peak values occurring around 4 hours after successful resuscitation. Blood levels of troponin-I were higher during the entire post-ROSC phase in the AMI-Cont group compared with AMI-Int group. Creatinine levels after ROSC were higher in both cardiac arrest groups compared with the sham group. Elevated creatinine >100 μmol/L at 48 hours after ROSC were observed in 4 cardiac arrest animals (2 from each group) and this was associated with increased need of cardiovascular support. There was an early rise in alanine aminotransferase in the AMI-Cont group after resuscitation, however all 3 groups displayed a rise in alanine aminotransferase throughout

the maintenance phase of cooling. Only in the sham group, alanine aminotransferase levels returned to baseline at 48 hours after ROSC. Creatine kinase was markedly elevated in both cardiac arrest groups after resuscitation when compared with sham group.

Myocardial Infarct Size

Infarct size measurement were performed in 1 AMI-Cont and 3 AMI-Int animals. The AMI-Cont animal had an infarct volume relative to left ventricular volume of 4.5% while the median percentage in the AMI-Int animals were 1.4% [0.5;3.9].

Pulmonary Function

Table 4 shows parameters for pulmonary function throughout the protocol. All 3 groups demonstrated fairly similar ventilation and oxygenation parameters throughout the protocol, with a lower pressure of O_2 in arterial blood/fraction of inspired O_2 ratio at the end of protocol. This was most pronounced in the AMI-Cont group.

DISCUSSION

In this article, we provide a methodological description of a cardiac arrest model in pigs with concurrent myocardial infarction and a clinically relevant post-cardiac arrest intensive care period. We demonstrate 2 variations of myocardial infarction in conjunction with resuscitation resulting in different grades of hemodynamic instability and multi-organ injury.

Rationale for the Model

No studies offer an exact list of etiologies of cardiac arrest, however, a collection of reports show that 56% to 73% of all out-of-hospital arrests are of cardiac cause.[14–16] Angiography and autopsy studies of out-of-hospital patients with cardiac arrest have shown that between 35% and 74% of patients have myocardial infarction as the likely cause of their cardiac arrest,[3–5] and it is therefore believed to be the most prevalent cause of out-of-hospital cardiac arrest. We used 2 methods of inducing myocardial injury. The AMI-Cont method has previously been used for testing intra-cardiac arrest interventions that might affect ROSC rates.[17,18] If applied in conjunction with a long intensive care period, it is suitable for testing of interventions with a cardiac protective scope as seen in the 2 prior studies of myocardial infarction–induced cardiac arrest with long post-cardiac arrest intensive care periods.[19,20] The AMI-Int group on the other hand, demonstrated less myocardial damage with the added benefit of increased ROSC rates compared with the AMI-Cont group. Based on this and combined with a less severe

Table 2. Temperature, Hemoglobin, Urine Output, and Metabolic Parameters

TTM phases	Group	Baseline	1 h Start TTM	4 h Target temp.	12 h Maintenance phase	18 h	24 h	30 h Rewarming	36 h	42 h Active normothermia	48 h
Temperature, (°C)	AMI-Cont	38.3 [37.9:39.0]	39.2 [39.1:39.2]	32.7 [32.6:32.7]	33.1 [32.8:33.3]	32.9 [32.9:33.2]	33.0 [32.9:33.1]	33.9 [33.8:34.1]	37.0 [37.0:37.0]	37.9 [37.9:38.0]	38.0 [37.9:38.0]
	AMI-Int	38.5 [38.0:38.6]	39.2 [38.3:39.6]	33.8 [32.7:35.4]	33.0 [32.8:33.1]	33.1 [33.0:33.1]	33.0 [32.9:33.1]	33.5 [33.2:33.9]	37.0 [36.8:37.2]	38.0 [37.6:38.5]	38.2 [38.2:38.3]
	Sham	38.8 [37.9:39.1]	38.6 [38.2:39.3]	32.7 [32.5:33.3]	33.1 [33.0:33.1]	32.9 [32.9:33.0]	32.9 [32.8:33.0]	33.8 [33.7:34.0]	37.0 [36.7:37.1]	38.3 [38.3:38.4]	38.1 [38.1:38.2]
SvO_2 (%)	AMI-Cont	58 [51:66]	53 [47:57]	66 [61:67]	53 [52:66]	63 [55:67]	59 [55:60]	67 [23:69]	50 [49:61]	64 [58:66]	47 [35:61]
	AMI-Int	54 [51:57]	41 [41:62]	57 [54:68]	58 [56:69]	62 [57:70]	64 [57:70]	63 [52:66]	61 [33:67]	40 [30:57]	43 [35:49]
	Sham	56 [48:61]	56 [51:62]	66 [62:73]	68 [68:71]	70 [60:74]	66 [63:70]	63 [61:68]	65 [48:68]	56 [45:59]	47 [40:57]
Hemoglobin (mmol/L)	AMI-Cont	5.6 [5.5:5.8]	6.8 [6.2:7.4]	7.0 [6.6:7.3]	6.3 [6.2:6.6]	6.2 [6.0:6.7]	5.3 [5.1:6.8]	6.0 [5.5:6.4]	5.4 [4.2:6.0]	5.4 [4.1:5.9]	4.8 [3.6:5.2]
	AMI-Int	5.8 [4.9:6.0]	6.3 [6.0:7.6]	6.2 [5.7:7.5]	5.7 [5.2:6.6]	6.2 [5.6:6.9]	6.2 [5.9:7.3]	6.8 [5.9:7.1]	5.7 [5.3:6.3]	5.4 [5.2:5.4]	4.8 [4.4:5.3]
	Sham	5.4 [5.1:5.9]	5.5 [5.2:5.7]	6.2 [6.0:6.3]	6.7 [6.2:6.8]	6.8 [6.3:7.3]	6.1 [6.1:7.1]	6.0 [6.0:6.7]	5.7 [5.6:6.3]	5.5 [5.3:5.6]	5.5 [4.9:5.8]
Urine output (mL/kg per h)	AMI-Cont	0.5 [0.3:0.8]	1.2 [0.9:1.5]	0.9 [0.7:1.0]	1.5 [0.5:1.7]	0.6 [0.3:2.5]	0.7 [0.3:0.9]	0.5 [0.1:1.3]	0.9 [0.8:1.4]	1.1 [0.7:2.8]	1.0 [0.9:5.2]
	AMI-Int	0.7 [0.6:0.9]	0.9 [0.9:1.5]	1.0 [0.7:3.4]	1.8 [1.4:2.4]	1.6 [0.5:1.8]	0.6 [0.5:1.6]	0.4 [0.3:1.6]	0.7 [0.4:1.5]	0.8 [0.7:1.4]	1.3 [1.0:1.9]
	Sham	0.5 [0.4:0.9]	0.9 [0.5:1.0]	0.8 [0.3:2.9]	1.7 [1.0:2.0]	0.6 [0.5:1.5]	1.6 [0.6:2.1]	1.8 [0.9:2.1]	0.9 [0.6:1.2]	0.5 [0.5:0.6]	1.6 [1.3:1.7]
Blood-Lactate (mmol/L)	AMI-Cont	0.6 [0.5:1.0]	7.2 [5.4:11.9]	4.8 [2.5:11.8]	0.9 [0.6:4.4]	4.2 [0.6:6.5]	0.9 [0.8:1.1]	1.7 [0.6:7.4]	1.3 [0.4:3.7]	2.6 [0.5:2.8]	1.1 [0.6:2.4]
	AMI-Int	0.8 [0.7:0.9]	5.8 [5.2:11.0]	2.0 [1.0:6.1]	0.9 [0.6:1.3]	1.7 [0.6:7.5]	1.2 [0.8:2.8]	1.0 [0.6:3.4]	1.0 [0.6:5.0]	1.2 [0.4:2.5]	0.8 [0.4:1.2]
	Sham	0.6 [0.5:0.6]	0.6 [0.5:0.6]	0.4 [0.4:0.5]	0.5 [0.4:0.5]	0.4 [0.3:0.7]	0.4 [0.4:0.4]	0.4 [0.3:0.5]	0.4 [0.4:0.5]	0.4 [0.4:0.5]	0.4 [0.3:0.4]
Blood-Glucose (mmol/L)	AMI-Cont	5.7 [5.1:6.1]	13.6 [10.3:15.1]	8.5 [5.2:10.6]	9.3 [6.5:11.0]	9.4 [6.4:12.0]	8.3 [8.0:9.0]	11.9 [8.0:14.9]	6.8 [5.9:10.3]	7.1 [5.5:8.3]	6.0 [4.1:7.1]
	AMI-Int	5.8 [5.5:6.4]	7.3 [7.1:12.2]	4.8 [4.2:6.0]	7.5 [5.2:8.4]	8.1 [7.5:10.1]	9.1 [6.8:12.0]	7.2 [7.1:9.0]	7.8 [6.6:14.7]	5.1 [4.7:7.1]	4.1 [3.7:4.7]
	Sham	5.5 [5.3:5.6]	5.4 [5.4:5.6]	6.3 [5.0:6.7]	6.7 [6.0:7.7]	6.7 [6.5:7.0]	7.8 [5.8:8.0]	6.0 [5.6:6.4]	5.2 [5.0:5.6]	5.0 [4.5:5.5]	4.7 [4.4:5.1]
Cumulative insulin infusion (IU)	AMI-Cont	...	0 [0:0]	10 [4:10]	10 [4:16]	10 [8:20]	16 [14:30]	20 [14:40]	30 [20:50]	40 [20:54]	40 [20:54]
	AMI-Int	...	0 [0:6]	6 [0:10]	6 [0:10]	6 [0:10]	6 [4:12]	10 [6:18]	10 [8:22]	10 [8:36]	10 [8:16]
	Sham	...	0 [0:0]	0 [0:0]	0 [0:0]	0 [0:0]	0 [0:0]	0 [0:0]	0 [0:0]	0 [0:0]	0 [0:0]

Data presented as median [25%;75%]. Time points after baseline represents hours since return of spontaneous circulation/sham intervention. Conversion factor for mmol/L to g/dL is ≈ 18. The animal from the AMI-Int group that suffered a pneumothorax has missing data from the last data point. The 42 hour time point post-resuscitation is carried forward to the 48 hour data point. AMI-Int indicates cardiac arrest group with reperfusion of coronary artery before cardiac arrest; IU international units; SvO_2, mixed venous blood oxygen saturation; and TTM, target temperature management.
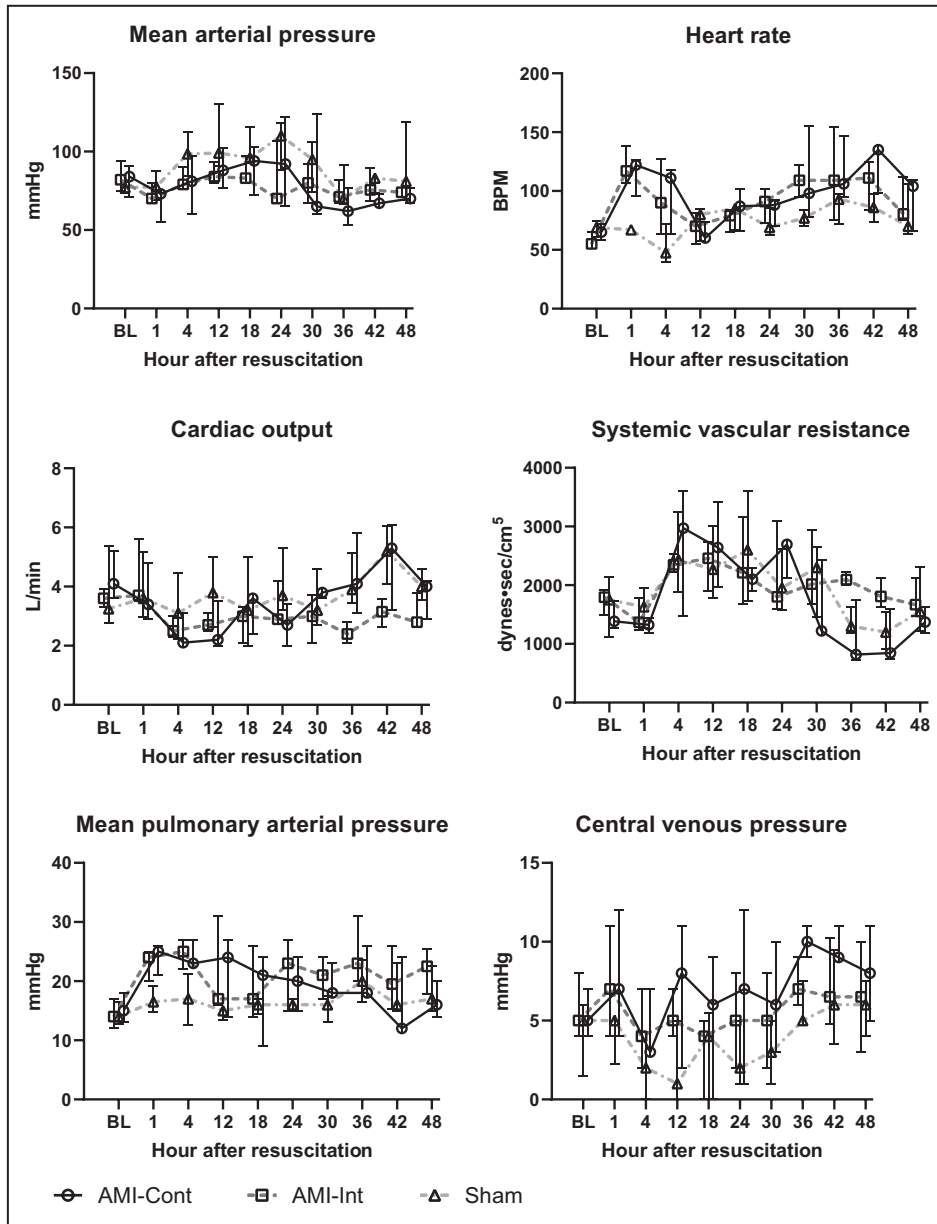
Figure 3. Hemodynamic data at baseline and after successful resuscitation.
AMI-Cont indicates cardiac arrest group with continuous myocardial infarction; AMI-Int, cardiac arrest group with reperfusion of coronary artery before cardiac arrest; and BPM indicates beats per minute.

post-cardiac arrest syndrome phenotype makes it suitable for potential advancement into a survival model.

The majority of patients with cardiac arrest receives post-resuscitation care for extended periods in the intensive care unit.[21] A number of myocardial infarction-induced cardiac arrest studies have been conducted on pigs with long survival but with short intensive care periods. Observation periods range between 24,[22–26] 72,[27,28] or 96 hours.[29–31] The longest period of post-cardiac arrest intensive care was 4 hours.[27–32] A consequence of awakening the animals shortly after resuscitation is that animals, which achieve initial

ROSC but are cardiovascularly unstable in the early post-resuscitation phase might die during the observation period in the pen without an exact cause of death. In fact, 2 animals in our study (1 from each cardiac arrest group) received our maximum noradrenaline dosage (1 μg/kg per minute) and adrenaline support within the first 2 hours of ROSC, but both pigs survived until 48 hours with normal hemodynamic status, no inotropic support and in need of only low dose noradrenaline (<0.1 μg/kg per min).

The primary cause of death in successfully resuscitated out-of-hospital patients with cardiac arrest is
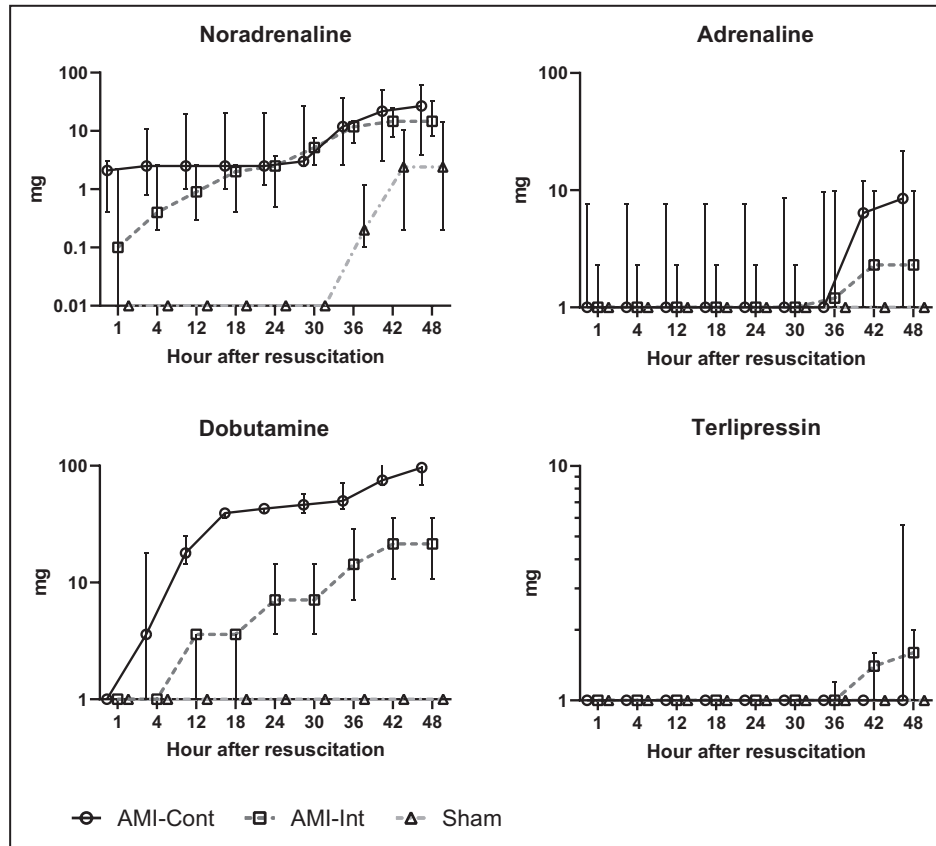
Figure 4. Cumulative cardiovascular support.
Data presented as median [25%;75%] cumulative amounts of noradrenaline, adrenaline, dobutamine, and terlipressin. The y-axis is presented on log 10 scale. The animal from the AMI-Int group that suffered a pneumothorax has missing data from the last 2 data points. The 36-hour time point post-resuscitation is carried forward to the 42- and 48-hour data points. AMI-Cont indicates cardiac arrest group with continuous myocardial infarction; and AMI-Int, cardiac arrest group with reperfusion of coronary artery before cardiac arrest.

neurological injury which is why it is often included as an end point in cardiac arrest animal experiments.[7,33] Systemic ischemia-reperfusion injury results in multiorgan injury or even failure and by taking animals of supportive care in the early phase after resuscitation, the animals are of risk of dying from perhaps preventable causes (eg, arrhythmias, cardiovascular collapse, acute renal injury, acute respiratory distress, etc.).[34] A longer intensive care period makes organ supportive measures possible that might help animals survive until neurological examination similar to patients.

Cardiovascular Function

In the AMI-Int group, the rightward shifted ESPVR and preload recruitable stroke work x-axis intercepts when compared with sham animals, in conjunction with a lower ejection fraction, lower dP/dt_{max} despite cardiovascular support all point towards decreased systolic function during the first 6 hours. Although there was a slight increase in the ESPVR slope in the AMI-Int group

during the remainder of the intensive care period, the simultaneous marked rightward shift of ESPVR x-axis intercept indicates continuous depressed systolic function.[35] This is underlined by lower dP/dt_{max} at 48 hours in both cardiac arrest groups when compared with the sham group. The effects of hypothermia on left ventricular function as observed in the sham group showed decreased diastolic function (increased time constant of isovolumetric relaxation and higher dP/dt_{min} values) with simultaneous leftward shift in ESPVR, which confirms earlier findings.[36,37] The leftward ESPVR shift was, however, restored to baseline levels during the maintenance and rewarming phases. The effect of hypothermia on diastolic function as measured by time constant of isovolumetric relaxation was abolished in both cardiac arrest groups, because of the increased catecholaminergic stimulus provided by the cardiovascular support.[38] Diastolic dysfunction because of cardiac arrest is signified by the higher dP/dt_{min} values at 48 hours in both cardiac arrest groups compared with the sham group. Cardiac dysfunction is a main
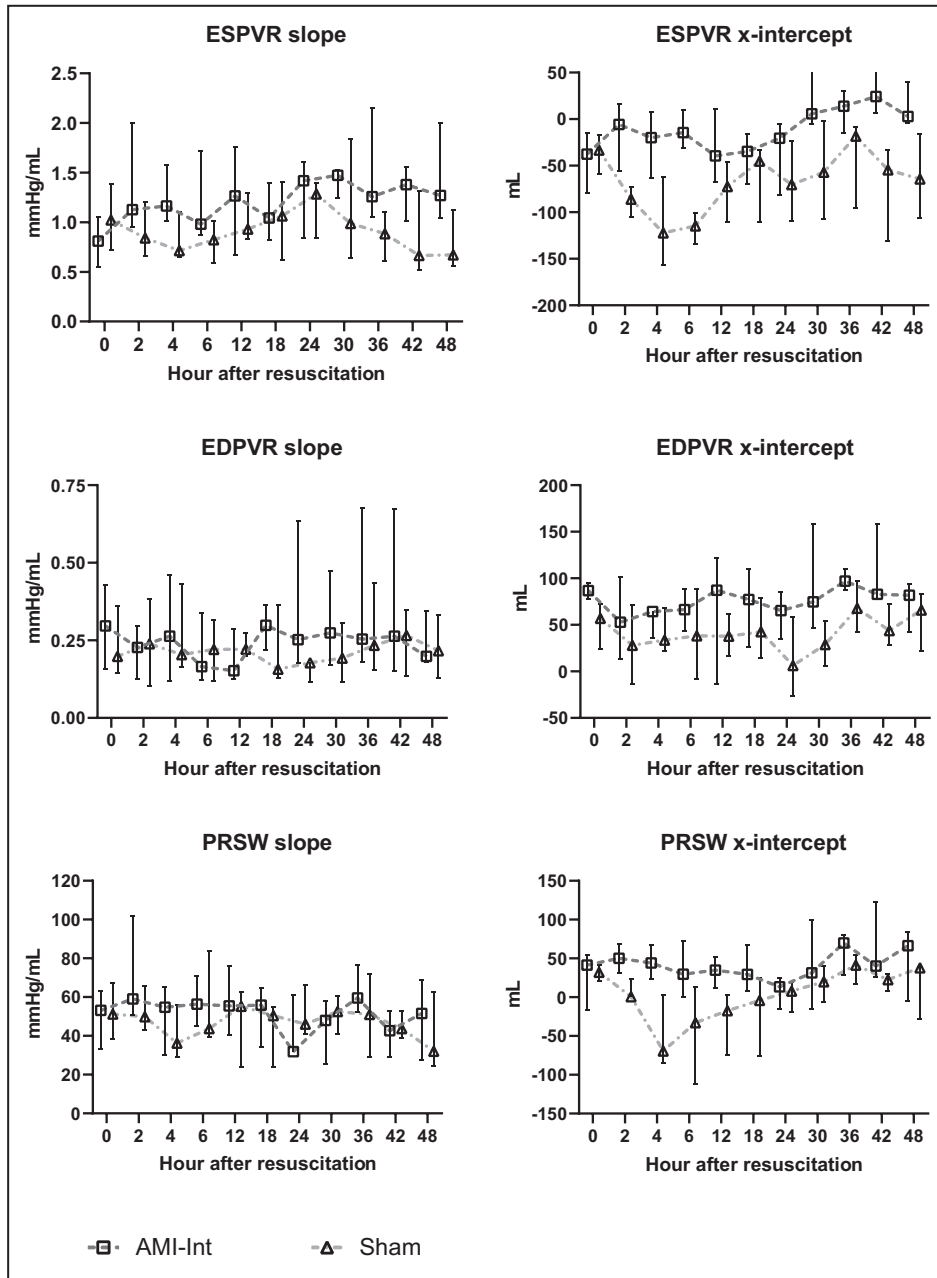
Figure 5. Dynamic pressure-volume measurements.
Data presented as median [25%;75%]. AMI-Int, cardiac arrest group with reperfusion of coronary artery before cardiac arrest; EDPVR, end-diastolic pressure volume relationship; ESPVR, end-systolic pressure volume relationship; and PRSW, preload-recruitable stroke work.

component of the post-cardiac arrest syndrome,[34] and especially in a model of myocardial infarction as used in this study. Both troponin-I levels and magnetic resonance data confirm that a myocardial infarction was indeed induced in both cardiac arrest groups. Prior studies with a reperfusion-reocclusion approach to myocardial infarction induction have reported infarct sizes of 6% to 10% relative to left ventricular mass.[29–31] The smaller infarct sizes in our model can be assigned to the lower sensitivity of a non-contrast magnetic

resonance scan relative to conventional methods for infarct size measurements.

During rewarming, we observed a marked decrease in blood pressure with preserved CO in the cardiac arrest groups, which were treated mainly by incremental doses of vasopressors. Previous studies in pigs with ≥24 hours of TTM to 33 °C have all employed a method of electrically induced cardiac arrest without myocardial ischemia, and none of the studies have demonstrated a similar hemodynamic instability during
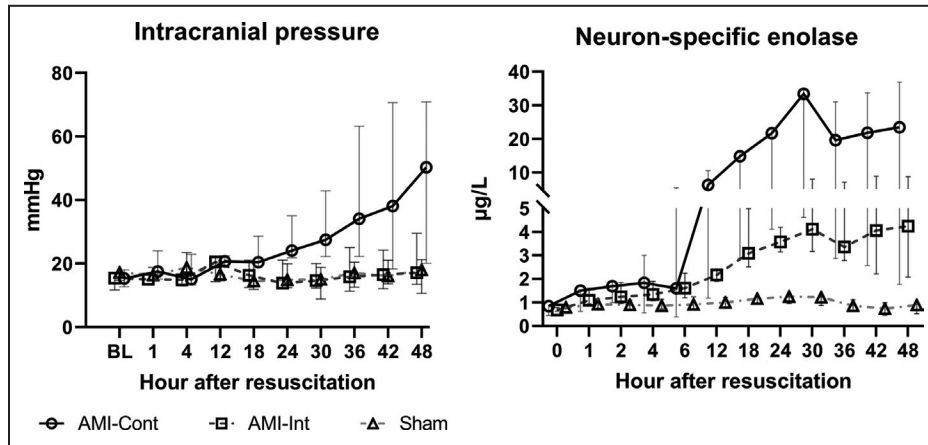
Table 3. Static PV Parameters

TTM phases	Group	Baseline	2 h	4 h	6 h	12 h	18 h	24 h	30 h	36 h	42 h	48 h
			Cooling phase	Target temp.	Maintenance phase				Rewarming		Active normothermia	
Pes (mm Hg)	AMI-Cont	97 [84:128]	96 [67:97]	96 [90:102]	106 [88:108]	98 [83:112]	122 [92:133]	136 [89:148]	…	…	…	107 [97:109]
	AMI-Int	94 [79:98]	97 [95:110]	86 [82:102]	103 [100:106]	105 [99:110]	103 [93:126]	97 [92:126]	110 [93:113]	106 [98:107]	100 [98:102]	99 [97:102]
	Sham	95 [77:102]	104 [98:150]	127 [109:151]	133 [120:148]	118 [111:141]	119 [114:138]	137 [112:140]	111 [105:116]	107 [103:110]	117 [100:130]	110 [95:112]
Ped (mm Hg)	AMI-Cont	14 [9:20]	17 [12:19]	19 [19:20]	19 [18:21]	20 [18:22]	21 [20:24]	22 [21:26]	…	…	…	26 [20:36]
	AMI-Int	12 [10:17]	13 [9:16]	11 [8:14]	13 [11:16]	10 [5:16]	11 [7:17]	21 [13:21]	15 [12:22]	20 [17:23]	19 [17:22]	17 [17:22]
	Sham	17 [16:19]	17 [16:24]	19 [14:20]	15 [15:18]	13 [12:19]	15 [13:17]	14 [13:17]	17 [16:18]	20 [19:23]	22 [19:24]	24 [20:25]
EF (%)	AMI-Cont	53 [46:60]	41 [21:66]	36 [30:42]	35 [25:49]	29 [29:30]	28 [23:53]	29 [24:44]	…	…	…	30 [27:40]
	AMI-Int	42 [34:46]	34 [30:47]	34 [25:41]	28 [21:40]	40 [30:48]	39 [33:48]	34 [32:39]	29 [26:31]	23 [22:37]	22 [18:38]	32 [29:33]
	Sham	44 [34:45]	48 [46:53]	50 [43:52]	52 [51:56]	45 [42:48]	38 [35:42]	48 [46:52]	39 [38:48]	39 [27:52]	32 [29:39]	30 [27:31]
E_a	AMI-Cont	2 [1:2]	3 [3:5]	3 [3:4]	4 [3:5]	3 [3:4]	4 [4:4]	4 [4:4]	…	…	…	2 [2:2]
	AMI-Int	1 [1:2]	3 [2:4]	3 [2:4]	3 [2:3]	3 [2:3]	3 [2:5]	3 [2:4]	3 [2:4]	2 [2:4]	4 [3:4]	3 [2:3]
	Sham	2 [2:2]	2 [2:2]	2 [2:3]	3 [2:3]	3 [3:3]	3 [2:3]	3 [2:4]	2 [2:3]	2 [2:3]	3 [2:4]	3 [2:4]
dP/dt_{max} (mm Hg/s)	AMI-Cont	1535 [1404:1822]	1888 [1725:5405]	2485 [1890:3081]	2087 [1446:3365]	1661 [1333:1988]	1897 [1480:2781]	1922 [1036:2526]	…	…	…	1101 [873:2849]
	AMI-Int	1409 [1218:1632]	2123 [1873:3477]	1777 [1286:3507]	1421 [1368:1611]	2203 [1898:2483]	2855 [2737:3110]	3413 [2335:3868]	2460 [1776:3445]	1270 [1142:2822]	1101 [985:1287]	1167 [1134:1349]
	Sham	1271 [1025:1488]	2220 [1536:3053]	2215 [1771:3628]	2608 [2490:3626]	3765 [3305:4126]	2788 [1540:3396]	2662 [2606:2699]	2041 [1913:2113]	2026 [1602:2304]	1349 [1173:1800]	1361 [1340:1385]
dP/dt_{min} (mm Hg/s)	AMI-Cont	−2377 [−2943:−1910]	−1678 [−1959:−1652]	−1872 [−2108:−1635]	−1411 [−1597:−935]	−863 [−1052:−674]	−910 [−1641:−837]	−927 [−1603:−742]	…	…	…	−1732 [−2074:−1616]
	AMI-Int	−2048 [−2446:−1864]	−1792 [−1904:−1644]	−1173 [−1373:−667]	−960 [−1472:−654]	−1291 [−1389:−964]	−1336 [−1539:−1079]	−1237 [−1710:−1084]	−1741 [−1952:−1484]	−1643 [−1733:−1640]	−1804 [−1818:−1720]	−1653 [−1894:−1459]
	Sham	−2060 [−2682:−1676]	−2101 [−2635:−1379]	−1258 [−1640:−792]	−1663 [−1824:−1392]	−1685 [−1999:−1509]	−1779 [−1962:−1382]	−1870 [−1885:−1600]	−1357 [−1448:−1251]	−1952 [−2539:−1406]	−2595 [−3078:−2137]	−2239 [−2905:−2209]
Tau	AMI-Cont	34 [32:38]	38 [17:47]	52 [41:64]	85 [45:92]	99 [75:123]	114 [59:120]	111 [65:118]	…	…	…	54 [43:64]
	AMI-Int	34 [31:37]	36 [31:37]	78 [34:93]	85 [82:118]	83 [65:126]	73 [67:92]	62 [55:93]	52 [44:62]	46 [44:48]	47 [43:47]	50 [49:50]
	Sham	38 [33:40]	51 [46:66]	125 [111:152]	81 [80:143]	99 [84:100]	84 [79:88]	72 [67:75]	74 [74:82]	51 [39:63]	43 [38:50]	54 [44:54]

Data presented as median [25%;75%]. Time points after baseline represents hours since return of spontaneous circulation/sham intervention. The missing data in the AMI-Cont group at 30, 36, and 42 hours are attributable to the cardiovascular instability making it impossible to perform PV measurements. AMI-Cont indicates cardiac arrest group with continuous myocardial infarction; dP/dt_{max}, maximum rate of pressure development during contraction; dP/dt_{min}, minimum rate of pressure development during relaxation; E_a, aortic elastance; EF%, ejection fraction; Ped, end-diastolic pressure; Pes, end-systolic pressure; PV, pressure-volume; and Tau, time constant of isovolumic relaxation.

Figure 6. Cerebral outcomes.
Data presented as median [25%;75%]. AMI-Cont indicates cardiac arrest group with continuous myocardial infarction; and AMI-Int, cardiac arrest group with reperfusion of coronary artery before cardiac arrest.

rewarming.[39–41] The reasons for the sudden drop in systemic vascular resistance need further investigations but one possible explanation is that our model may represent a more severe phenotype of the post-cardiac arrest syndrome than previous investigations.

Cerebral Injury

Based on the NSE results, both cardiac arrest models included neuronal injury. The markedly higher NSE levels seen in the AMI-Cont group could be attributable to the 2 pigs in this group who had ICPs that were
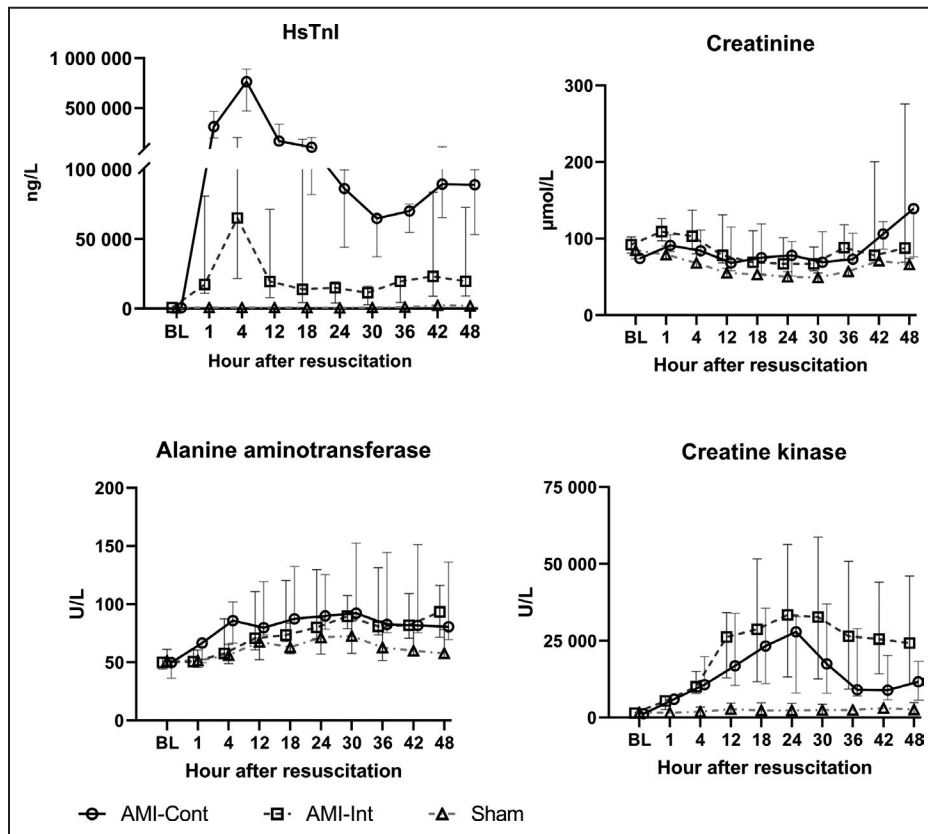
Figure 7. Organ markers.
Data presented as median [25%;75%]. HsTnI indicates high-sensitivity analysis of troponin-I; and U/L, units of catalytic activity per liter.

Table 4. Pulmonary function

TTM phases	Group	Baseline	1 h	4 h	12 h	18 h	24 h	30 h	36 h	42 h	48 h
			Start TTM	Target temp.	Maintenance phase			Rewarming		Active normothermia	
PaO$_2$/FiO$_2$ ratio (mm Hg)	AMI-Cont	501 [472:515]	319 [214:338]	363 [324:417]	403 [258:484]	495 [392:557]	496 [450:537]	355 [184:542]	347 [225:370]	305 [174:387]	137 [126:513]
	AMI-Int	476 [470:504]	348 [199:404]	431 [319:541]	415 [389:549]	512 [220:565]	409 [288:557]	381 [306:547]	312 [219:392]	304 [176:386]	396 [197:466]
	Sham	448 [424:470]	420 [397:486]	528 [488:536]	513 [482:519]	543 [517:588]	509 [483:561]	481 [481:485]	381 [372:439]	363 [348:404]	346 [296:357]
FiO$_2$ (%)	AMI-Cont	29 [27:34]	48 [26:48]	34 [26:34]	30 [29:34]	30 [26:31]	26 [25:28]	30 [26:71]	32 [27:50]	33 [32:47]	64 [26:70]
	AMI-Int	34 [33:34]	46 [39:68]	32 [29:38]	30 [27:34]	28 [28:44]	29 [25:39]	34 [24:38]	35 [29:40]	42 [31:68]	43 [28:59]
	Sham	34 [27:34]	28 [26:34]	27 [24:29]	26 [25:27]	25 [24:27]	24 [23:27]	24 [23:29]	28 [26:29]	27 [26:28]	30 [26:30]
Respiratory rate (%)	AMI-Cont	21 [18:21]	25 [22:27]	18 [17:19]	18 [17:18]	18 [17:21]	18 [16:19]	20 [16:22]	20 [14:22]	22 [16:24]	21 [15:21]
	AMI-Int	19 [17:21]	25 [23:28]	17 [15:18]	18 [15:19]	18 [16:22]	20 [13:23]	16 [14:19]	20 [15:25]	18 [14:24]	17 [15:23]
	Sham	18 [16:20]	18 [17:20]	15 [13:15]	18 [16:20]	15 [15:17]	16 [16:17]	16 [15:16]	17 [16:19]	19 [18:19]	17 [17:18]
PaCO$_2$ (kPa)	AMI-Cont	5.2 [5.1:5.5]	5.1 [4.9:6.7]	6.3 [5.8:6.4]	5.4 [5.4:5.5]	5.3 [5.1:5.4]	4.9 [4.9:5.6]	5.2 [4.7:6.3]	5.4 [5.2:5.4]	5.2 [5.0:5.4]	5.6 [4.8:5.9]
	AMI-Int	5.3 [4.9:5.5]	5.5 [5.1:5.8]	5.3 [5.1:5.8]	5.4 [4.9:5.6]	5.5 [4.4:5.8]	5.3 [4.9:5.5]	5.2 [5.1:5.5]	5.8 [5.7:6.2]	5.5 [5.1:5.9]	5.0 [4.7:5.3]
	Sham	5.2 [5.0:5.5]	5.1 [5.0:5.5]	5.7 [5.3:6.2]	5.3 [5.1:5.4]	5.2 [5.0:5.2]	5.2 [5.2:5.5]	5.6 [5.4:5.6]	5.7 [5.2:5.7]	5.1 [4.9:5.5]	5.7 [5.5:5.7]
Peak pressure (cmH$_2$O)	AMI-Cont	18 [17:18]	26 [22:28]	21 [21:23]	20 [19:21]	21 [20:21]	20 [19:21]	21 [19:21]	24 [19:26]	23 [21:27]	23 [23:24]
	AMI-Int	19 [17:20]	24 [23:27]	23 [20:25]	21 [19:22]	22 [20:36]	23 [19:29]	21 [18:30]	23 [21:27]	24 [22:33]	23 [23:33]
	Sham	18 [17:20]	19 [17:20]	19 [18:21]	20 [19:23]	19 [18:21]	19 [18:21]	18 [18:22]	20 [18:22]	22 [19:23]	22 [19:22]
PEEP (cmH$_2$O)	AMI-Cont	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:6]
	AMI-Int	5 [5:5]	5 [5:8]	5 [5:5]	5 [5:5]	5 [5:7]	5 [5:5]	5 [5:5]	5 [5:5]	6 [5:7]	5 [5:7]
	Sham	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]	5 [5:5]

Data presented as median[25;75]. Time points after baseline represents hours since return of spontaneous circulation. AMI-Cont indicates cardiac arrest group with reperfusion of coronary artery before cardiac arrest; FiO$_2$ indicates fraction of inspired O$_2$; PaCO$_2$, partial pressure of CO$_2$ in arterial blood; PaO$_2$, pressure of O$_2$ in arterial blood; PEEP, positive end-expiratory pressure; and TTM, targeted temperature management.

incompatible with a good neurological outcome. The AMI-Cont group had longer resuscitation times when compared with the AMI-Int group, which might have inflicted more cerebral injury. Furthermore, the greater cardiovascular instability and increased vasopressor usage in the AMI-Cont group, might have added secondary neurological injury. The relative and absolute increase in NSE values in the AMI-Int group is comparable with previous investigations of a similar myocardial infarction induced cardiac arrest models with intermittent reperfusion.[28,29]

Methodological Considerations

Although striving to adhere to the clinical setting, some aspects of the described model may be considered less clinically relevant: (1) VF was electrically induced, and (2) the myocardial infarction size was relatively small. We chose electrically induced VF to standardize the myocardial infarction period more uniformly to the cardiac arrest period. Furthermore, it is well known that spontaneously induced VF (even without underlying ischemia) elevates the defibrillation threshold relative to electrically induced VF.[42,43] A larger myocardial infarction would have led to a higher proportion of spontaneously induced VF episodes but undoubtedly also more hemodynamically unstable pigs in the post-cardiac arrest phase. This could have been counteracted by using extra-corporeal membrane oxygenation in conjunction with conventional cardiopulmonary resuscitation. Previous investigations of cardiac arrest pig models with concomitant myocardial infarction have used extra-corporeal membrane oxygenation, however, all with relatively shorter post-ROSC observation periods.[44–46] Although the use of extra-corporeal membrane oxygenation is increasing, extracorporeal cardiopulmonary resuscitation is reserved to a small percentage of selected cardiac arrest cases, and our model serves as a general cardiac arrest model.

With regards to TTM, we chose a 33 °C strategy as this was recommended by international guidelines at the initiation of the experiments,[11] and this has been the most common strategy in previous porcine cardiac arrest models.[39–41]

Limitations

Although we believe clinical relevance to be important for a better translation of results from the experimental to the clinical setting, one has to consider the increased variability introduced by intensive care, long-term anesthesia, and mechanical ventilation. These models do not necessarily represent many of the complexities observed in patients with cardiac arrest (out-of/in-hospital, witnessed/non-witnessed, shockable/non-shockable rhythm, etc.) and, in general this is difficult to obtain in an experimental setup. Furthermore, we only used female,

young, and healthy animals without common comorbidities (eg, congestive heart failure, diabetes mellitus and renal disease), nor did the animals receive the chronic medications adhering to these diseases.[47] The low number of animals in each group is also a limitation but given the aim of the study, we wanted to include enough animals to allow a sufficient description of the methodology.

CONCLUSIONS

This study successfully describes the development of a cardiac arrest pig model, with myocardial infarction, TTM, and clinically relevant post-cardiac arrest care. We demonstrate 2 different methods of inducing myocardial ischemia together with cardiac arrest which results in post-cardiac arrest organ injury including cardiac dysfunction and cerebral injury.

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Anesthetic protocol and pre-cardiac arrest handling

The animals were sedated with midazolam before transportation from the pen to the experimental laboratory. At arrival, premedication with ketamine (6.25 mg/kg; S-ketamine, Pfizer, NY, USA), midazolam (0.625 mg/kg; Midazolam, Hameln Pharmaceuticals ltd., UK), and atropine (0.5 mg; Atropin, SAD amgros I/S, Denmark) were delivered by intramuscular injection. Anesthesia was inducted with a dose of ketamine (6.25 mg/kg) and midazolam (0.625 mg/kg), injected in an ear vein catheter. Anesthesia was maintained with intravenous (i.v.) infusion of propofol (4.0-5.5 mg/kg/hr, Propolipid, Fresenius Kabi, Germany) and remifentanil (0.6-1 µg/kg/hr, Fresenius Kabi, Germany). A single 250 µg fentanyl bolus (Fentanyl, B. Braun, Germany) was administered prior to the surgical procedures to ensure sufficient analgesia. If signs of insufficient sedation (shivering, reflexes, tachycardia, increased $ETCO_2$, decreased SvO_2) occurred at any point during the experimental protocol, boluses of propofol (30 mg) and/or remifentanil (300 µg) were administered. If bolus frequency exceeded two per hour, infusion rates of propofol and remifentanil were increased by 25%. If max dosages of anesthetics were insufficient to maintain stable anesthesia a bolus of midazolam (10 mg) was administered. If bolus frequency of midazolam exceeded 2 per hour, a continuous infusion of midazolam (25 mg/hr) was added.

Blood potassium < 4.0 mmol/L detected pre-cardiac arrest was treated with i.v. potassium supplement. The supplemental amount was calculated from the formula ([4.0 mmol/L]-[actual K^+]*[body weight])/1 L and infused by a maximum rate of 10 mmol/hr.

Intraobserver variability of pressure-volume measurements

To assess intraobserver variability in dynamic PV-measurement, slopes and x-intercept from ESPVR, EDPVR and PRSW relationships were reanalyzed in three randomly selected animals (17 different measurements). Analysis variability for the dynamic parameters were assessed by Bland-Altman plots and the mean absolute difference for each parameter with 95% confidence intervals were as follows: ESPVR slope 0.05 (-0.01;0.11), ESPVR x-intercept 6.78 (-0.63;14.19), EDPVR slope 0.05 (0.01;0.09), EDPVR x-intercept 7.28 (0.81;13.76), PRSW slope 4.51 (-3.13;12.16) and PRSW x-intercept -24.76 (-68.55;19.04).

Blood sample handling and analysis

Serum samples were left to clot for 20 minutes before centrifuged at 4°C and 1,850 G for 9 minutes. The supernatants were stored at -80°C. High sensitivity analysis of troponin-I (TnI) were analyzed by immunochemical reaction (ADVIA Centaur XPT, Siemens Healthineers, Erlangen, Germany). Neuron-specific enolase was measured by an electrochemiluminescent immuno assay (ECLIA) using Cobas 8000 (e602) (Roche Diagnostics GmbH, Mannheim, Germany). Plasma samples were centrifuged at 4°C and 1,500 g for 20 minutes immediately after collection and stored at -80°C. Creatine kinase (CK), creatinine, and alanine aminotransferase (ALT) were determined from plasma samples according to standard procedures (Siemens Diagnostics® Clinical Methods for ADVIA 1,800). All analyses were performed using an auto analyzer (ADVIA 1,800, Siemens Healthineers, Erlangen, Germany). Intra-assay variation was in all instances below 3% coefficient of variability and inter-assay variation was below 4% coefficient of variability.

Resuscitation

Chest compressions were delivered at a frequency of 100 min^{-1} and a 50%/50% duty cycle allowing full passive recoil between compressions. The LUCAS piston was placed in the median line of the thorax with the inferior part of the piston just above the xiphoid process. The anterior defibrillator pad (Quik-Combo, Physio-Control, WA, USA) was placed on the anterior-lateral left hemi-thorax (corresponding to apex of the heart) and the posterior pad was placed on the posterior-lateral right hemi-thorax with the medial side juxta-positioned with the thoracic column. Mechanical ventilations during resuscitation was delivered in volume-controlled mode with FiO_2 100 %, tidal volume 8 ml/kg, respiratory frequency at 10 per min, and PEEP 0 CM H_2O. Upon ROSC, the ventilator was switched back to pressure-controlled with volume guarantee.

MR protocol

Three-dimensional T1-weighted and T2-weighted imaging covering the entire heart was done. For the T1w images, a 3D spoiled gradient echo sequence with isotropic voxel size of 0.7 mm; TR/TE = 12 ms/5.3 ms; flip angle = 50°; 6 averages, and scan duration 29:54 minutes was used. For the T2w images, a 3D Fast Spin Echo (3DVIEW) sequence with isotropic voxel size of 0.7 mm; Echo Train Length = 141; TR/TE = 2,000 ms/327 ms; 6 averages, and scan duration 42:26 minutes was applied. During images analysis, for each 0.7 mm slice the left ventricle myocardium area and infarct area was measured to calculate left ventricular myocardium volume, infarct size volume, and the ratio of infarct volume relative to left ventricular volume.

Table S1. Post-cardiac arrest intensive care protocol.

Treatment goals;

- PaCO$_2$ 4,6-6.0 kPa
- SaO$_2$ 94-98 %
- MAP   >65 mmHg
- Cardiac output   >2.0 L/min
- SvO$_2$ >50 %
- Blood (B)-glucose   4-10 mmol/L
- B-[K$^+$]   3.0-5.5 mmol/L
- Urine output   >1 ml/kg/hr

CNS	
Treatment goal	Interventions
• Avoid seizures	Continued sedation with propofol and remifentanil as per anesthesia protocol (see *Data S1*). If convulsions; 1. Propofol bolus 1 mg/kg 2. Midazolam 0.3 mg/kg

RESPIRATION	
Treatment goal	Interventions
• PaCO$_2$ = 4.7-6.0 kPa • SaO$_2$ = 94-98 %	Initial ventilator settings; • Pressure controlled with volume guarantee (PCV-VG) • Tidal volume = 8 ml/kg • Respiratory rate = 10-35 per min (adjusted to PaCO$_2$) • FiO$_2$ = 0.30 • PEEP = 5 cm H$_2$O A sustained (>10 min) decrease in blood oxygen levels (SaO$_2$) was intervened by first suctioning the tracheal tube. If insufficient, increase FiO$_2$ (10 %) and PEEP (2 cm H$_2$O), respectively, starting with the value furthest from initial values; if equidistant starting with PEEP. A sustained increase in oxygen levels was intervened on conversely. Lowest PEEP level at 5 cm H$_2$O.

FiO$_2$ (%)	0.4	0.5	0.6	0.7	0.8	0.9	1.0
PEEP (cm H$_2$O)	5	7	9	11	13	15	16-24

CARDIOVASCULAR	
Treatment goal	Interventions
• MAP > 65 mmHg • SvO$_2$ > 50 % • Cardiac output (CO) > 2 L/min	Immediately upon ROSC, a 10 ml/kg unheparinized fluid bolus was given. <u>Hemodynamic</u> ↓CO/SvO$_2$

|	

• Cardioversion to sinus rhythm	1. Fluid bolus (4 ml/kg)
	  1.1. Increase in SvO_2/CO → repeat
	  1.2. No effect and/or target not reached → dobutamine
	  1.3. Maximum fluid = 1 L/24 hours post ROSC
	2. Dobutamine; 0.1-15.0 µg/kg/min
	  2.1. Increased dosage until target SvO_2/CO reached
	N.B. if SvO_2 drops below 50 % but CO>2.5 L/min give fluid bolus to effect but do not start dobutamine.
	
	↓*MAP*
	1. Noradrenaline; 0.01-1.00 µg/kg/min
	  1.1. Increased dosage until target MAP reached
	2. Terlipressin
	  2.1. Start if vasoplegic despite max noradrenaline and normal cardiac output/SvO_2.
	  2.2. Bolus treatment until effect (bolus size = 0.2 mg, max 1 mg)
	3. Within initial 60 min of ROSC if noradrenaline is insufficient, repeat adrenaline boluses (0.01-0.1 mg) titrated to reach target MAP.
	4. If MAP <65 mmHg despite max dosage of noradrenaline and terlipressin, start adrenaline infusion 0.01-1.00 µg/kg/min.
	
	Arrhythmia
	1. Major cardiovascular instability;
	  1.1. Ventricular fibrillation/pulseless VT; 3 stacked shocks, 360 J with rhythm checks in between, if no effect start CPR.
	  1.2. Asystole/PEA; start CPR as per guidelines, i.e. cycles of 2 min of chest compressions and mechanical ventilation with rhythm check in between and adrenaline and/or amiodarone as recommended.
	2. Cardiovascular stable;
	  2.1. Persistent ventricular tachycardia (VT); 3 stacked synchronized shocks 360 J with rhythm checks in between, if insufficient or if intermittent VT 300 mg amiodarone i.v. repeated until cardioversion (max 1,200 mg/24 hr). If max dose reached give 1 mg/kg lidocaine and if necessary start cont. infusion 1 mg/min
	  2.2. Supraventricular tachycardia with hemodynamic instability and/or HR>120; 3 synchronized shocks 360 J, if insufficient 300 mg amiodarone i.v. repeated every hour until cardioversion
	Amiodarone is administered over 10 min to avoid hypotension. Maximum amiodarone dose = 1200 mg/24 hr.

GASTROINTESTINAL

Intervention
10 ml/hr enteral nutrition via feeding tube started post-ROSC (equivalent to 3 drops per minute.)

RENAL

Treatment goal	Interventions

• Urine output > 1 ml/kg/hr
• B-[K^+] 3.0-5.5 mmol/L
	• Basal i.v. fluid replacement with Ringer's lactate 2 ml/kg/hr. If [Na^+] falls below 137 mmol/L both basal and bolus fluid treatment is exchanged with isotonic NaCl.
	• If hypokalemia KCl is mixed with the basal fluid replacement. Total supplement given calculated by [3.5 mmol/L]-[actual K^+]*[body weight]
	• If hyperkalemia;
	  ○ 10 mg furosemide to effect, if insufficient →
	  ○ 10 IU rapid acting insulin mixed in 50 ml 50 % glucose administered over 5 min.
	  ○ If hyperkalemia and arrhythmia, 5 mmol Ca^{2+}.

INFECTION

Intervention
750 mg Cefuroxim every 8 hours post ROSC.

COAGULATION

Treatment goal	Intervention
Prevent thrombo-embolic events	Unfractionated heparin 18 IU/kg/hr (in addition 10,000 IU in total administered during cardiac catheterization).

ENDOCRINOLOGY

Treatment goal	Intervention
• B-[glucose] 4-10 mmol/L	<table><tr><td>Blood glucose (mmol/L)</td><td>Insulin dose (rapid acting)</td></tr><tr><td>10-12</td><td>4 IE</td></tr><tr><td>12-16</td><td>6 IE</td></tr><tr><td>16-20</td><td>8 IE</td></tr><tr><td>>20</td><td>10 IE</td></tr><tr><td colspan="2">Dose repeated after minimum 1 hr NB; watch for hypoglycemia during rewarming</td></tr></table> If blood glucose < 4 mmol/L, a bolus of 20 ml 50 % glucose solution is administered over 5 min with blood glucose control after 15 min.

Figure S1. Infusion rates of propofol, remifentanil, and midazolam throughout the experimental protocol.
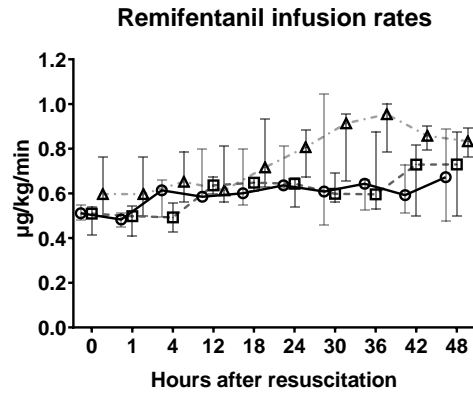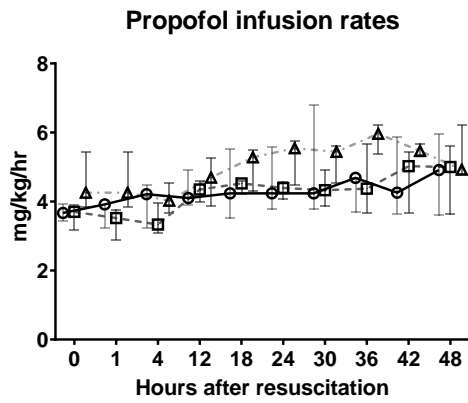
Propofol infusion rates

Remifentanil infusion rates

Midazolam infusion rates

Group size at each timepoint

Time	0	1	4	12	18	24	30	36	42	48
AMI-Cont (n)	7	3	3	3	3	3	3	3	3	3
AMI-Int (n)	11	7	7	7	7	7	7	7	6	6
Sham (n)	6	6	6	5	5	5	5	5	5	5

AMI-Cont   AMI-Int   Sham
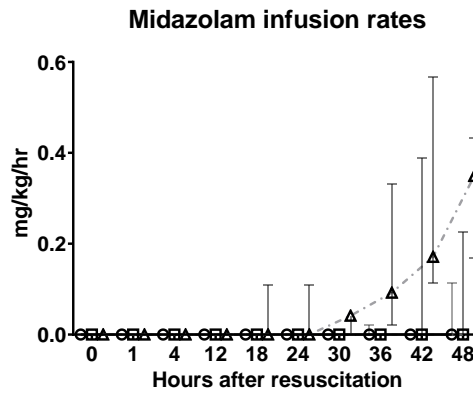
Figure S2. Mid-ventricular T2-weighted MR scans of excised hearts approximately 48 hours after myocardial infarction and cardiac arrest. A) short-axis, B) long-axis, and C) short-axis with measurements of left ventricular size (in green), left ventricular lumen including papillary muscles (in yellow), and infarct area (in orange).
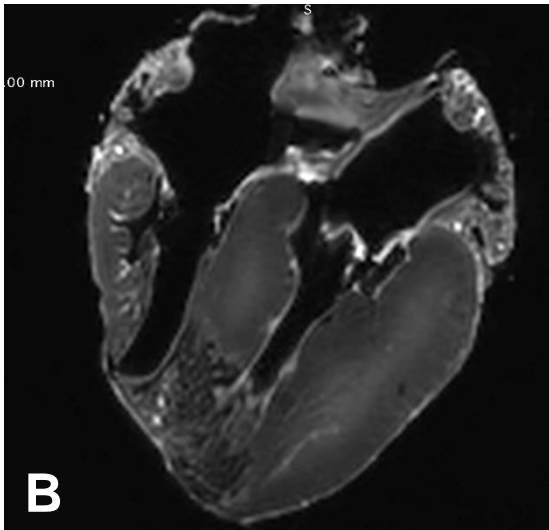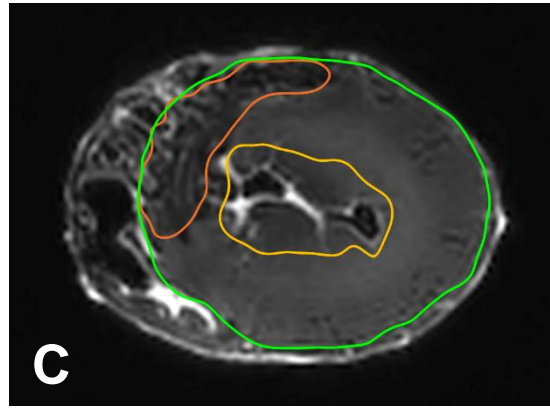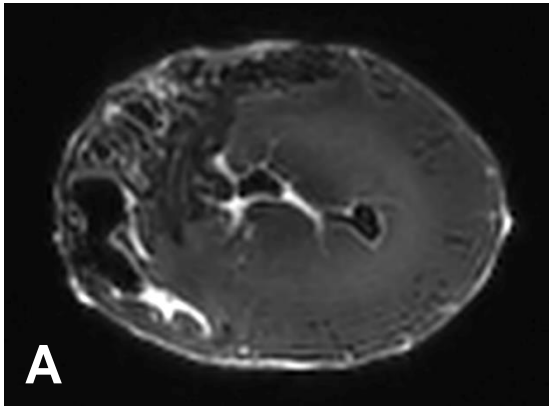
Mid-ventricular T2-weighted MR scans of excised hearts approximately 48 hours after myocardial infarction and cardiac arrest. A) short-axis, B) long-axis, and C) short-axis with measurements of left ventricular size (in green), left ventricular lumen including papillary muscles (in yellow), and infarct area (in orange).

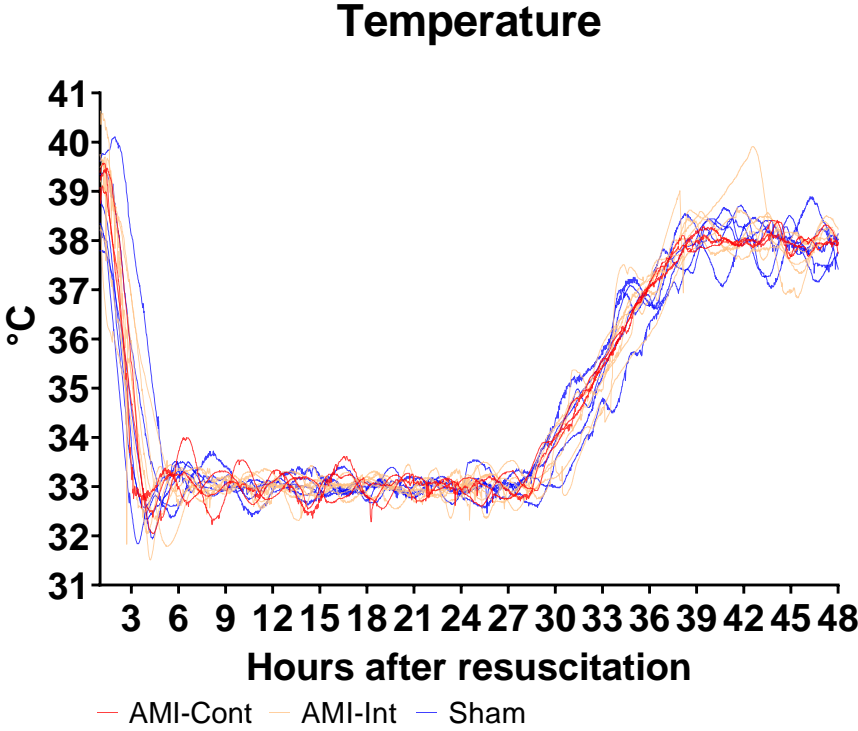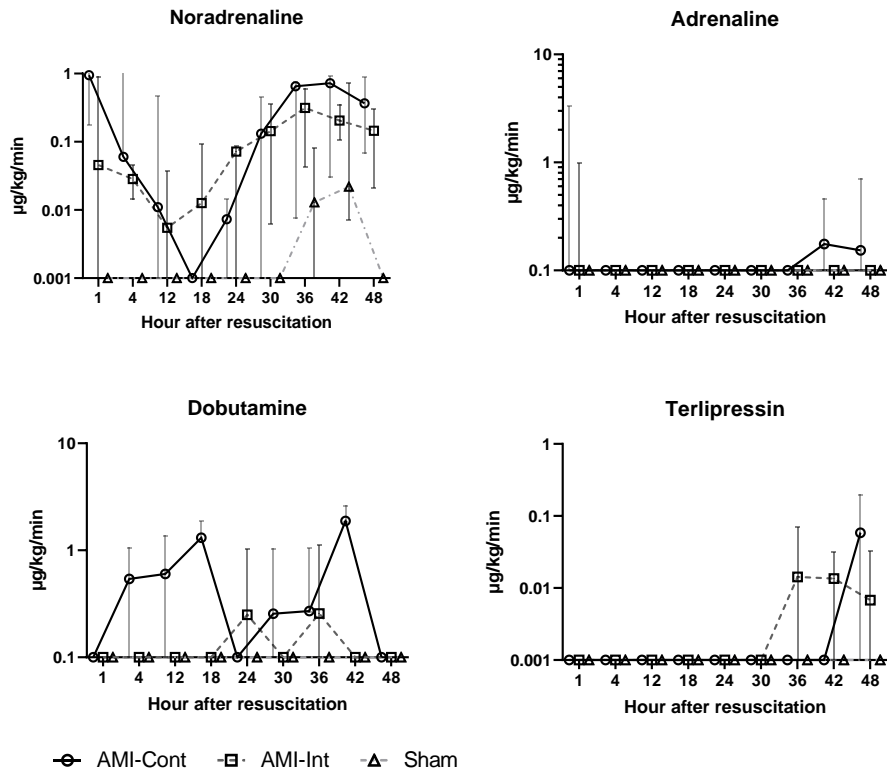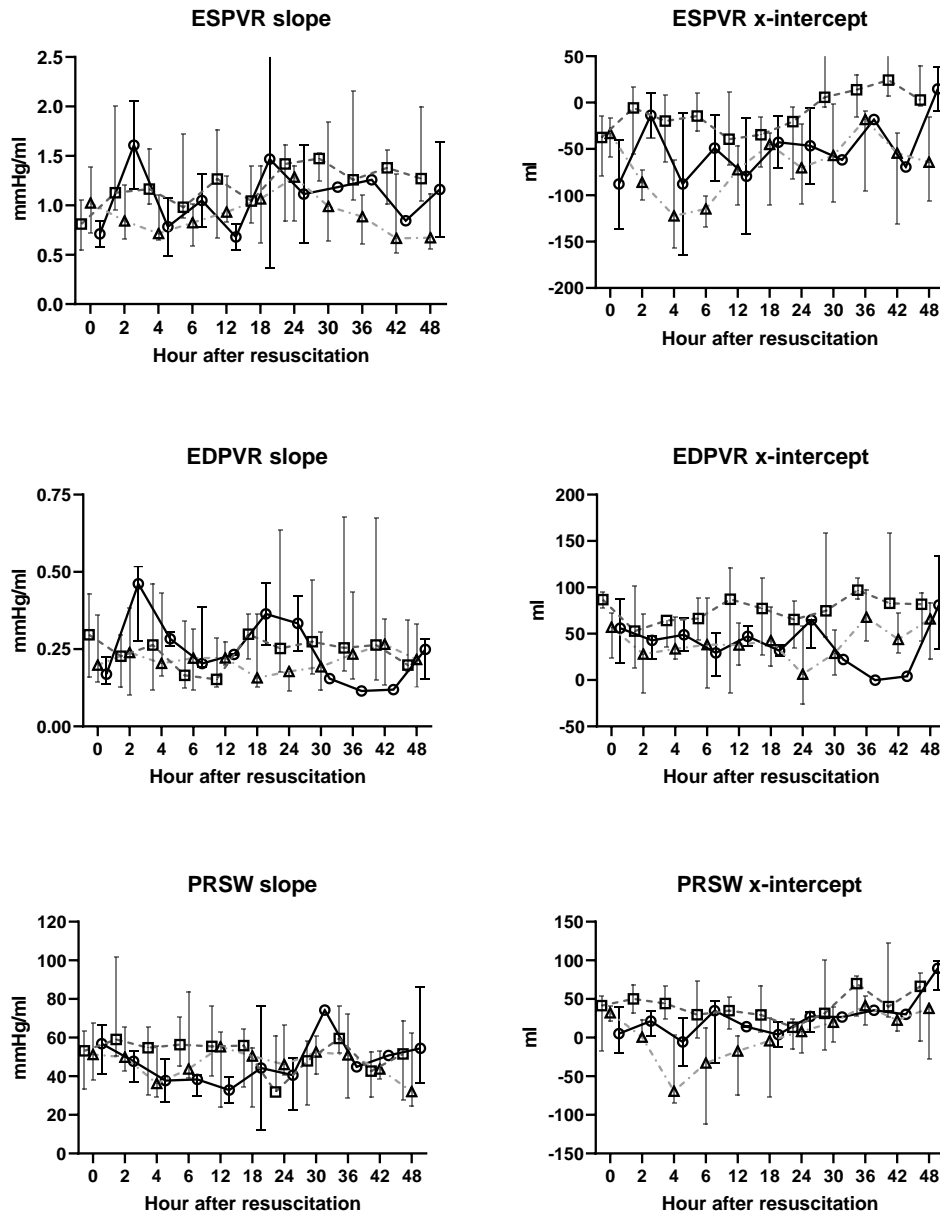**Figure S3. Temperature for each individual animal in the three groups during the intensive care period.**

Temperature

Figure S4. Data presented as median [25%;75%]. The y-axis is presented on log 10 scale.

Group size at each timepoint

Time	0	1	4	12	18	24	30	36	42	48
AMI-Cont (n)	7	3	3	3	3	3	3	3	3	3
AMI-Int (n)	11	7	7	7	7	7	7	7	6	6
Sham (n)	6	6	6	5	5	5	5	5	5	5

Figure S5. Dynamic PV-measurements for all three groups. At 30-42 hours after resuscitation only data from one animal in the AMI-Cont group exists, because PV measurements were impossible to perform due to cardiovascular instability in the remaining animals. ESPVR: end-systolic pressure-volume relationship, EDPVR: end-diatolic pressure-volume relationship, PRSW: preload-recruitable stroke work.