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Review

Perfusion

Microvascular perfusion in cardiac arrest: a review of microcirculatory imaging studies

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Abstract

Cardiac arrest represents a leading cause of mortality and morbidity in developed countries. Extracorporeal cardiopulmonary resuscitation (ECPR) increases the chances for a beneficial outcome in victims of refractory cardiac arrest. However, ECPR and post-cardiac arrest care are affected by high mortality rates due to multi-organ failure syndrome, which is closely related to microcirculatory disorders. Therefore, microcirculation represents a key target for therapeutic interventions in post-cardiac arrest patients. However, the evaluation of tissue microcirculatory perfusion is still demanding to perform. Novel videomicroscopic technologies (Orthogonal polarization spectral, Sidestream dark field and Incident dark field imaging) might offer a promising way to perform bedside microcirculatory assessment and therapy monitoring. This review aims to summarise the recent body of knowledge on videomicroscopic imaging in a cardiac arrest setting and to discuss the impact of extracorporeal reperfusion and other therapeutic modalities on microcirculation.

Keywords

microcirculation; videoimaging technology; sidestream dark field; orthogonal polarization spectral; incident dark field imaging; cardiac arrest; resuscitation; CPR; ECPR; extracorporeal CPR

Introduction

Cardiac arrest belongs to the major causes of mortality in developed countries and, in survivors, it is associated with a high incidence of neurocognitive impairment.[1] New treatment approaches, like extracorporeal life support (ECLS) utilised for extracorporeal cardiopulmonary resuscitation (ECPR), became a potential rescue therapy for victims of cardiac arrest (CA) who do not reach a return of spontaneous circulation (ROSC) after receiving appropriate advanced life support.[2] However, the parameters guiding the haemodynamic management, including the setting of ECLS during ECPR in cardiac arrest patients, are not entirely clear.[3] Serum lactate is an indicator of peripheral perfusion quality and a potential predictor of the outcome in this regard.[4] There are, however, some limitations of serum lactate evaluation, namely, a delay in its elevation and also the risk of non-specificity.[5] To directly assess peripheral microcirculation, novel methods of bedside microcirculatory imaging have been introduced: Orthogonal Polarization Spectral (OPS) and Sidestream Dark Field (SDF) imaging and, currently, Incident Dark Field (IDF) imaging. Not only can these methods reveal insufficient peripheral perfusion, they may, moreover, become a prognostic tool in stating the risk of multi-organ failure and mortality.[6,7]

The aim of this review is to summarise the available body of knowledge concerning the utilization of these recent and promising methods of microcirculatory imaging in a cardiac arrest setting. We will not only state the background of microcirculatory changes during cardiac arrest, resuscitation and post-resuscitation period,

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but we will also discuss the ffect of ECLS in this setting and potential bias of other therapeutic modalities.

Technology review

The direct observation of microcirculation has been enabled by the development of bedside in vivo videomicroscopy technologies - Orthogonal Polarization Spectral (OPS), Sidestream Dark Field (SDF) imaging and Incident Dark Field (IDF) imaging. The obtained videoimages are evaluated semi-automatically or automatically and the following parameters of microcirculation are set: total and perfused vessel density, proportion of perfused vessels, microvascular flow index and heterogeneity index.

OPS (Cytometrics Inc., Philadelphia, PA, USA), based on the use of polarised light of a specific wavelength (550 nanometres), was the first technology utilised for bedside microcirculatory imaging. Once the light is emitted on the investigated surface, it is either absorbed in the haemoglobin of red blood cells or depolarised and reflected back to the analyser. Thus, the image of red blood cells moving within microvessels is captured.[8] OPS imaging was validated in several animal and human studies through comparison with intravital microscopy - a "gold standard" method for microcirculatory assessment.

SDF, incorporated in the Microscan Video Microscope (Microvision Medical, Amsterdam, The Netherlands), introduced several innovations (e.g. a disposable cup covering the probe, higher image quality and reduced image blurring) which made SDF an effective and widely used device.[9,10] Both methods, OPS and SDF imaging, require further semi-automatic offline analysis of the captured videoimages (see below).

Incident Dark Field (IDF) imaging represents the third generation of the videomicroscopic technology incorporated in a CytoCam (Braedius Medical B.V., Huizen, The Netherlands).[11] The CytoCam consists of a small pen-like probe and a computer unit. It allows synchronization of emitted light and image capturing, automated focusing and, also, full digitalization of the record. Moreover, IDF provides better focus and contrast of the captured images and higher image resolution than SDF imaging (the CytoCam-IDF detected 30% more capillaries in the same location than SDF).[11,12] Immediate, direct, automatic evaluation of the microcirculatory images represents the key innovation in the CytoCam-IDF; however, the obtained microcirculatory parameters are probably not fully comparable with semi-automatic analysis.[13]

Off-line, semi-automatic image analysis is essential for OPS and SDF imaging. Several scoring systems were developed for the quantification of the microcirculatory variables, however, the following parameters were suggested by a published consensus:[14] total vessel density (TVD), perfused vessel density (PVD), proportion of perfu ed vessels (PPV), microvascular flow index (MFI) and the heterogeneity index. All parameters are calculated separately for small vessels (microvessels of diameter ⩽20 μm) and other vessels identified in the video-image (an example of a stabilised image with identified microvessels is shown in Figure 1). TVD is estimated as the ratio of the total vessel length in the image and the area selected as a region of interest. To evaluate the PVD and PPV, vessels are manually classified according to microcirculation as perfused (the blood flow in the vessel is hyperdynamic, continuous or sluggish) or not perfused (with intermittent flow or no-flow, respectively). For MFI quantification, the image is divided into four quadrants and the circulation in each quadrant is expressed in ordinal scale: 4 – hyperdynamic flow, 3 – continuous flow, 2 – sluggish flow, 1 – intermittent flow, 0 – no flow (examples of different microvascular blood flow categories of sublingual microcirculation in a pig model are shown in the supplementary video files, which are available online with this article, http://journals.sagepub.com/doi/full/10.1177/0267659117723455). MFI represents the average score of all quadrants. Finally, the heterogeneity index can be calculated by measuring the MFI in three to five images per site: the difference between the highest MFI minus the lowest MFI divided by the mean flow velocity. These parameters are suitable for all accessible tissues, except for the intestinal mucosa where modified parameters were suggested.[15]

Videomicroscopic techniques have enabled non-invasive, real-time visualization of microcirculation at the bedside and, due to their non-invasive and simple use, they have been employed in many medical fields, including critical care.[16,17]

There are, nevertheless, some limitations: all techniques are sensitive to movement and pressure artefacts, are not suitable for the direct measurement of high blood flow velocities and are applicable only on suitable tissue surfaces (e.g. accessible mucosa of oral cavity, conjunctiva, intestinal stoma or thin capsule of solid organs). Moreover, OPS and SDF imaging still require time-consuming manual offline analysis, with subjective semi-quantitative assessment of the microflow (even though there have been several attempts for the development of rapid fully automatic analyses software[16]). To minimise the risk of biased results with these limiting factors, consensual criteria for image acquisition and analysis have been published.[14,15,17] Several studies evaluating reproducibility of microcirculatory measures show sufficient reproducibility and low inter- and intra-observer variability.[18,19]

Literature review

Microcirculation in cardiac arrest and cardiopulmonary resuscitation

There is only scarce knowledge on microcirculation in a cardiac arrest setting, mostly from animal experimental research. During CA and CPR, microcirculation is

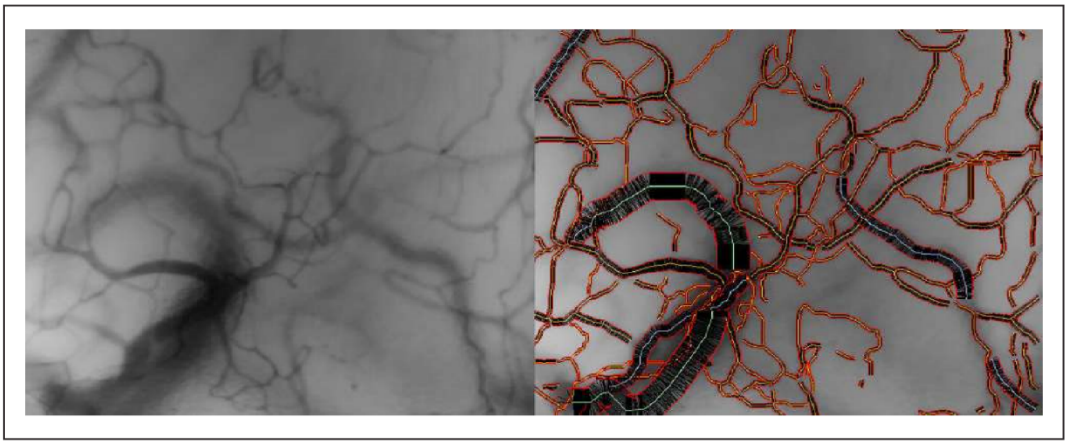

Figure 1. Stabilized videofile captured by Sidestream dark field imaging device and processed by AVA 3.1 (Microvision Medical, Amsterdam, the Netherlands). Vessels are detected on the right: small vessels up to 20 µm are highlighted by red lines, other vessels by green and blue lines.

strongly affected by lacking or grossly deteriorated systemic blood flow, but, due to its local and systemic regulatory pathways, microcirculation might exhibit different reactivity in comparison to systemic circulation.

Microcirculation in CA and CPR has been described by a few animal experimental studies in a porcine model[20-22] (describing serial changes of sublingual microcirculatory parameters) and a single human case report.[23] These studies showed the delayed reaction of the microcirculation to cardiac stun and only partial restoration of microcirculation during CPR (up to 59-85% of the pre-arrest values[21]). In successfully resuscitated animals, the microflow improved after ROSC to within 20% of the baseline values. Interestingly, microcirculatory monitoring during CPR predicted the success of resuscitation, with high sensitivity and specificity.[20]

All these studies evaluated the correlation between microcirculatory and systemic circulatory parameters, presenting conflicting results (either strong or no correlation). Nevertheless, the loss of haemodynamic coherence between micro- and macro-circulation, which probably results from complex local and systemic regulatory mechanisms, has been reported in other critical diseases.[22,24-26]

Cerebral microcirculation during CA, short duration CPR and after achieving ROSC has been presented by Ristagno et al. in a pig model.[25] They documented the close relationship between blood flow in the cerebral microvessels to the macrocirculatory parameters. Cerebral ischaemia (as expressed by cerebral tissue partial pressure of CO_2) was also in progress during CPR whereas micro and macro-circulation were partially restored. After reaching ROSC, cerebral ischaemia was reversed within a 3-minute delay, corre ponding to microcirculatory recovery.

Microcirculation after the return of spontaneous circulation

Sublingual microcirculation after ROSC does not reach the pre-arrest values and this deterioration is connected to post-resuscitation "sepsis-like syndrome". Interestingly, sublingual microcirculatory alterations might predict the outcome of post-cardiac arrest patients.

On the contrary, the cerebral cortical microcirculation was (in a rat model) reported to be fully restored despite signs of neuronal death.

After reaching ROSC, the sublingual microcirculation is significantly improved, but still does not reach the pre-arrest state. Conspicuously, the parameters that reflect microvascular flow (PVD and MFI) stay altered in post-CA subjects.[7,26-30] These changes were reported to appear early after CA and to resolve within 2 days.[29] Similar alterations of microcirculation were reported on the mucosa of the small intestine and the bulbar conjunctiva in animal studies.[7,27]

The underlining mechanism of such microcirculatory dysfunction might be the post-resuscitation "sepsis-like" syndrome, a systemic inflammatory reaction triggered by a whole body ischaemia and reperfusion. Its link to the sublingual microcirculation was suggested by several studies. Two animal studies showed a tight correlation of microcirculatory changes with myocardial dysfunction and serum cytokine levels.[7,27] Furthermore, an interesting human observational study compared the sublingual microcirculation of post-CA patients to those with sepsis and healthy control subjects. The MFI of post-CA patients were significantly altered 6 hours after intensive care unit (ICU) admission and were even lower than the MFI of septic patients.[28] The longer the hypoperfusion during CA and CPR lasted, the worse the microcirculatory

deterioration occurred aft r ROSC.[7] Importantly, all these microcirculatory alterations were independent of the systemic circulation.

Being affected by a systemic inflammatory reaction, sublingual microflow assessment might offer a useful tool to evaluate post-resuscitation disease severity and, thus, also contribute to prognostication.[31] Van Genderen et al. followed 25 adult patients after out-of-hospital cardiac arrest treated by mild therapeutic hypothermia. In the first 4 hours after ICU admission (before the onset of mild therapeutic hypothermia), they observed a significant difference between ICU survivors and non-survivors in sublingual microcirculatory parameters (MFI, PVD, PPV) whereas, during hypothermia, both groups did not differ. Further on, these parameters improved after rewarming in survivors and remained low in non-survivors.[26] Similar results were also reported regarding the neurologic outcome[28] and in a paediatric population.[30]

However, changes of sublingual microcirculation probably cannot be automatically extrapolated to other tissues. This was documented by the results of a rat model study where cerebral cortical microcirculation recovered completely after ROSC and stayed preserved in the early post-resuscitation phase (up to 6 hours) though high inflammatory mediator levels and signs of neuronal death were present.[31-33]

Microcirculation after extracorporeal reperfusion and experiences from ECMO-treated cardiogenic shock

The body of evidence on microcirculatory function after reperfusion with ECPR is still lacking. A single pilot study in post-cardiac arrest patients who experienced reperfusion with veno-arterial ECMO (VA ECMO) suggests that ECLS might support the microcirculation effectively. Similar results have been shown, however, by studies in patients with myocardial infarction suffering from cardiogenic shock: microcirculatory dysfunction was, to some extent, resolved by extracorporeal circulatory support and the remaining alteration of sublingual microcirculatory parameters during VA ECMO was found to be a predictor of ICU mortality.

The effect of sustained spontaneous or added artificial pulsatility provided by intra-aortic balloon counterpulsation (IABP) in VA ECMO-treated patients remains controversial.

Despite the growing body of evidence on the microcirculation during cardiopulmonary bypass, studies on the microcirculation during ECMO are scarce and on ECPR are still scarcer. A recent pilot study with 12 patients after refractory CA, who were treated with ECPR, suggested that ECLS might provide an adequate microcirculatory perfusion regardless of su tained or diminished spontaneous pulsatility.[32]

Patients dependent on ECLS were examined with SDF an average of 32 hours after cardiac arrest. They had lower PPV in comparison to healthy control subjects, but other microcirculatory parameters did not actually differ significantly from healthy controls. A subgroup of 5 patients with low-pulsatile or non-pulsatile blood flow (i.e. pulse pressure below 15 mmHg) had similar microcirculatory variables to those patients with spontaneously pulsatile blood flow. The pulsatile and low/non-pulsatile groups did not differ regarding global haemodynamic parameters, therapeutic intervention including vasopressors and inotropes or ECMO setting.

Studies focused on the microcirculation in ECMO-treated patients with cardiogenic shock documented a rapid reaction of microvascular flow after ECMO setting changes[33] and pointed out a close relationship between systemic haemodynamic parameters and sublingual microcirculation.[34] Similar to post-arrest patients, sublingual microcirculatory parameters might also predict the outcome in ECMO-treated subjects: in a study with 24 VA ECMO patients suffering cardiogenic shock, sublingual microcirculatory parameters were significantly lower in ICU non-survivors than in survivors during the whole time on ECMO. Moreover, PVD for all vessels (in the first 24 hours on ECMO support) was found as a predictor of ICU survival, with a high sensitivity and specificity. Importantly, microcirculatory parameters did not change significantly over time during ECMO treatment and, in survivors, weaning only caused a non-significant decrease in microcirculatory parameters.[35]

The introduction of an IABP for circulatory support in cardiogenic shock is no longer routinely recommended, based on an IABP SHOCK II study[36,37] where a significant proportion of patients also suffered cardiac arrest.[5] Nevertheless, an IABP may still be a therapy option when used in combination with VA ECMO.[38] Studying the microcirculation in this setting might bring important arguments to evaluate the benefits of such an approach. So far, however, no clear evidence of a beneficial effect of IABP on the microcirculation in VA ECMO patients has been given.[39-41]

The effects of different therapeutic interventions on the microcirculation

As already mentioned, the microcirculation is regulated by complex local and systemic pathways. Therefore, the effect of other treatment modalities (medication, infusion administration, target temperature management, etc.) on the microcirculation cannot be omitted and has to be studied carefully.

Sympathomimetics are frequently used during CPR in post-cardiac arrest care and in the treatment of post-cardiac arrest syndrome. In critically ill patients, inotropes have a mostly vasodilatory effect on splanchnic

and sublingual microcirculation, whereas vasopressors are associated with microcirculatory deterioration. The effect of vasoactive drugs may be dose dependent.[41] In patients with cardiogenic shock, despite a consistently positive effect on systemic circulation, dobutamine did not change the sublingual microcirculation, norepinephrine showed an adverse effect and enoximone administration led to a significant increase of the perfused capillary density (PVD).[42]

Epinephrine boluses during CPR might have an adverse effect on cerebral cortical microcirculation and tissue oxygenation, which diminishes within 10 minutes after ROSC.[43] A similar effect was reported after vasopressin.[44]

Nitroglycerine in continuous infusion favourably affected the microcirculation in patients suffering heart failure (which is typically associated with altered sublingual microcirculation).[45-48]

Sedatives are commonly used in post-cardiac arrest patients, as well as **anticonvulsants and analgesia.** These medications have been tested with respect to their impact on systemic haemodynamics, but their effect on the microcirculation still remains mostly unclear.[47] A detailed analysis of this issue is beyond the scope of the current review; let us mention only some widely used agents: propofol seems to have an organ-specific and a dose-dependent effect on the microcirculation[48] with a proven adverse effect on the sublingual microcirculation[49] and a possible beneficial effect on the liver and intestinal microcirculation.[50] Ketamine and midazolam, on the other hand, decreased the microcirculation in both the liver and distal ileum in an experimental rat setting[50]

Fluids administration is an essential part of shock treatment and has a direct impact on the mean arterial pressure and cardiac output. Fluids have, to a certain extent, a positive effect on the microcirculation (by means of systemic haemodynamic improvement, blood viscosity reduction or local vasodilatation), which was demonstrated in septic or hypovolemic shock.[51,52] The appropriate timing of such fluid-resuscitation seems to be crucial.[53] On the other hand, excessive amounts of fluid may result in haemodilution and the development of tissue oedema, which both reduce tissue oxygenation.[24] Thus, the assessment of the sublingual microcirculation might serve as a non-invasive indicator for fluid requirements.[54]

Blood transfusions may help to improve tissue oxygenation through increasing the oxygen-binding capacity of the blood; on the other hand, it may have a negative effect on the microcirculation (transfused erythrocytes have low deformability, low binding capacity for oxygen and, moreover, their ability to bind nitric oxide is increased, which may contribute to peripheral vasoconstriction).[51] Blood transfusion was reported to improve capillary density whereas the microcirculatory flow in the capillaries remained unchanged.[55]

Target temperature management (TTM) of adult patients after CA with initial shockable or non-shockable rhythms has been recently revised.[56] Few studies involving the effect of TTM on the sublingual microcirculation brought rather conflicting results when targeting the temperature to 32-34°C. Some studies suggested impaired sublingual microcirculation during TTM.[26,57] However, stronger evidence proved that there is no difference in the sublingual microcirculation between patients treated by TTM 32-34°C or normothermia.[58] On the contrary, TTM might have a beneficial effect on cerebral cortical microcirculation as suggested in an experimental rat model (where, however, the direct applicability to human victims was not assumed).[59] The difference of vascular reactivity between the cerebral and peripheral microcirculation might explain the neuroprotective effect of mild therapeutic hypothermia.

Artificial lung ventilation is provided to most post-cardiac arrest patients within the first 24 hours after ROSC. Mechanical ventilation (MV) and, especially, positive end-expiratory pressure are associated with an alteration in the splanchnic microcirculation. Nevertheless, the sublingual microcirculation was found unchanged in critically ill patients with positive end-expiratory pressure artificial lung ventilation.[60]

Heart rhythm disorders that may not alter systemic haemodynamics (such as atrial fibrillation) may, however, significantly affect the peripheral microcirculation. One study showed how elective direct current cardioversion significantly improved the microcirculation in patients with atrial fibrillation.[61] Similarly, cardiac resynchronization therapy was reported to improve the sublingual microcirculation in patients with heart failure.[62]

Research utilization of videomicroscopic imaging in basic research or in pharmacological studies elucidates the underlining mechanisms of microcirculatory changes and enhances the development of new treatment strategies for CA victims, such as the administration of sildenafil,[63] arginase inhibitors[64] or pharmacologically induced hypothermia.[65]

Discussion

Novel technical improvements in videomicroscopic imaging transformed this formerly cumbersome technology requiring time-consuming off-line analysis into a fully useful on-line tool. Direct observation of the microcirculation in a CA setting confirmed findings from other fields of intensive care that, to some extent, the microcirculation is dependent on systemic circulation, but global haemodynamics is only a prerequisite of functioning peripheral perfusion, which is further affected by many other mechanisms. During CPR, the microcirculation is partially restored, but approaches

the pre-arrest state only aft r full restoration of stable circulation (i.e. ROSC), whereas reperfusion with ECMO might provide sufficient microcirculatory blood flow per se. The post-cardiac arrest period is associated with sublingual microcirculatory disorder, closely related to the post-resuscitation "sepsis-like" syndrome. In post-cardiac arrest patients, the microcirculation is further affected by target temperature management and the administration of routinely used medication. Interestingly, the state of the sublingual microcirculation in the early post-cardiac arrest phase (or after the initiation of VA ECMO during ECPR) might be linked to the outcome. Of note, sublingual microcirculatory changes in a CA setting cannot be automatically extrapolated to other organs, as studies of cerebral cortical microcirculation in a rat CA model suggest.[25,59]

These findings are in line with other results of microcirculatory imaging in critically ill patients: dissociation of microcirculation and systemic circulation was observed in sepsis,[66] cardiogenic shock and heart failure[67-70] and in patients undergoing on-pump cardiac surgery.[71,72] In these studies, sublingual microcirculatory alterations were strongly associated with patient outcome.[5,72,73] Furthermore, den Uil et al., in their studies, suggested that patients whose sublingual microcirculation improved during treatment administration had better prognoses than those whose sublingual microcirculation remained impaired.[6,42]

Despite these encouraging findings, understanding the "meaning" of microcirculatory changes remains a real challenge, not only in a CA setting. We need not only randomised studies to prove the link between the sublingual microcirculation and the outcome, but research should also focus on the role of particular treatment options and pathophysiological states and on confirmation of the applicability of videomicroscopic methods in emergency care conditions.

Even though the recent body of knowledge on the sublingual microcirculation in a CA setting is mostly represented by small observational studies or animal-model experimental research, the promising results suggest that videoimaging of the sublingual area might become not only a powerful research tool, but also a way of standard bedside monitoring in CA victims, with a potential direct impact on therapy. However, there are many questions to be answered first.

Conclusion

Improved by recent innovations, videomicroscopic technologies might become a useful bedside tool to evaluate peripheral perfusion in many clinical settings, including prolonged CPR, ECPR and post-resuscitation care. Future research should extend the recent body of knowledge.

Declaration of Conflicting Interests

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2 Attachment file NO 2

MLCEK M, BELOHLAVEK J, HUPTYCH M, BOUCEK T, BELZA T, LACKO S, KRUPICKOVA P, HRACHOVINA M, POPKOVA M, NEUZIL P AND KITTNAR O. Head-up tilt rapidly compromises hemodynamics in healthy anesthetized swine. *Physiol Res* 2016; 64 Suppl 5: S677-683.

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Summary

The aims were to explore the effect of head-up tilt (HUT) to 30 and 60 degrees on hemodynamics and tissue oxygenation in anesthetized healthy swine. The data serve as a reference for a study of resuscitation efficacy at HUT such as during transport. Nine healthy swine (49±4 kg) were anesthetized and multiple sensors including myocardial pressure-volume loops catheter, carotid flow probe, blood pressure catheters, near infrared spectroscopy (NIRS) tissue oximetry and mixed venous oximetry (SVO2) catheter were introduced and parameters continuously recorded. Experimental protocol consisted of baseline in supine position (15 min), 30 degrees HUT (15 min), recovery at supine position (15 min) and 60 degrees HUT (5 min). Vacuum mattress was used for body fixation during tilts. We found that 30 and 60 degrees inclination led to significant immediate reduction in hemodynamic and oximetry parameters. Mean arterial pressure (mm Hg) decreased from 98 at baseline to 53 and 39, respectively. Carotid blood flow dropped to 47 % and 22 % of baseline values, end diastolic volume to 49 % and 53 % and stroke volume to 47 % and 45 % of baseline. SVO2 and tissue oximetry decreased by 17 and 21 percentage points. The values are means. In conclusions, within minutes, both 30 and 60 degrees head-up tilting is poorly tolerated in anesthetized swine. Significant differences among individual animals exist.

Key words

Head-up tilt • Hemodynamics • Pressure-volume loop / Cardiac output • Swine • Oximetry

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Introduction

Maintaining adequate perfusion during cardiopulmonary resuscitation (CPR) effort remains a challenge. Furthermore, during CPR it is sometimes necessary to transport the patient in head-up tilted position such as inside the buildings (climbing the stairs or transport in space-restricted elevators). At present, it is not known how harmful such tilting is or conversely, whether it can be beneficial as recently suggested by Debaty *et al.* (2015).

Head-up tilting is a challenge to hemodynamics even in healthy subjects. Full power of several compensatory mechanisms needs to be engaged to maintain peripheral blood flow (Rowel 1993). These include vasoconstriction – both arterial and venous, increased myocardial contractility, baroreceptor reflex and its orthostatic adjustment, muscular and diaphragmatic pump (Miller *et al.* 2005) local myogenic reactions and more (Stewart 2012). Normal blood volume, normal oxygen carrying capacity and intact intrinsic vascular structure are other prerequisites of orthostatic tolerance.

Under the conditions of cardiac arrest, hypoxia, acidosis and subsequent CPR with multiple drugs

administration including general anesthetics many of the abovementioned mechanism can be seriously compromised. Nevertheless, available knowledge regarding tilting during CPR is scarce. Recently, the interesting results were reported by Debaty *et al.* (2015). In a porcine CPR at 30 degrees HUT, an increased cerebral perfusion was observed despite a decrease in systemic arterial pressure and carotid blood flow by about half compared to horizontal position.

In the present study, the effect of head-up tilting to 30 and 60 degrees in healthy anesthetized swine fixed in vacuum mattress was explored in the setup similar to that of ongoing resuscitation. In order to differentiate between the effects of tilting and the effects that are secondary to cardiac arrest and subsequent CPR healthy animals with spontaneous rhythm were observed in this study. Swine is a commonly used biomodel in biomedical research including resuscitation studies. While there are similarities with human, the concern also exists that there could be differences in the response to orthostatic stress. Based on published data it was expected that 30 degrees tilt would be tolerated and 60 degrees would severely compromise hemodynamics.

Methods

Preparation and anesthesia

The study was approved by institutional care and use committee and was performed in accordance with European Guidelines on Laboratory Animal Care. Nine healthy swine (48±4.2 kg) were used. Following the premedication with midazolam 0.3 mg/kg and ketamine 20 mg/kg i.m. the marginal ear vein access was secured and animal transported to the lab. Here, initial propofol bolus (2 mg/kg) was administered and animal was orotracheally intubated and connected to mechanical ventilator. Tidal volume was set to 8 ml/kg and respiratory rate was adjusted to keep end-tidal CO_2 (etCO2) between 38-42 mm Hg. Anesthesia was maintained with continuous i.v. propofol (6-8 mg/kg.h) midazolam (0.1-0.2 mg/kg.h) and morphin (0.1-0.2 mg/kg.h). Heparin bolus (100 IU/kg) was given initially followed by continuous administration of 50 IU/kg.h so that activated clotting time (ACT) was maintained over 200 s. Normal saline was administered at 500 ml/h until central venous pressure (CVP) was 5-6 mm Hg. Saline drip was then continued at 5 ml/kg.h to maintain fluids balance. Multiple intravascular sheaths were inserted percutaneously for later placement of measurement catheters.

Monitored parameters

ECG, pulse oximetry (SPO2), rectal temperature and etCO2 were monitored noninvasively by patient monitor (LifeScope TR, Nihon Kohden, Japan). Regional tissue oximetry (rSO2) was monitored by NIRS oximetry (INVOS Oximeter, Covidien, USA) in forehead and right thigh. Pulmonary artery catheter (CCO V, Edwards Lifesciences, USA) was placed into pulmonary artery *via* femoral vein. It provided continuous cardiac output (CCO), mixed venous saturation (SVO2) and pulmonary artery pressure measurement. High fidelity pressure-volume catheter (type 7F VSL, Scisense-Transonic, USA) was inserted *via* the left carotid artery into the left ventricle under fluoroscopy control and pressure-volume data were obtained by admittance method (Raghavan *et al.* 2004). Blood pressure was measured directly *via* catheters placed in right femoral artery, carotid artery, pulmonary artery and superior vena cava by means of fluid-filled transducers (Truwave, Edwards Lifesciences, USA) placed at the heart level. Transient ultrasound flow probe (PSB, Transonic, USA) was implanted around right carotid artery to provide continuous volumetric flow measurement. Intracranial pressure (ICP) sensor (Neurovent-P, Raumedic, Germany) was inserted into parietal cortex *via* a burr hole 2 cm laterally from the midline. Measured parameters were sampled at 400 Hz by Powerlab A/D converter and continuously recorded to personal computer running Labchart Pro software (AD Instruments, USA).

Positioning

After all the systems were in place, animals were immobilized in resuscitation vacuum mattress that was shaped around the body and fixed to the motorized tilting bed. Additionally, the front legs were stretched above head and tied to the tilting bed. Care was taken that flushed pressure sensors were placed so that they would remain at the heart level during the whole protocol including tilting.

The protocol

The protocol is summarized in Figure 1. After minimum of 15 min stabilization the baseline values were recorded and the animals were tilted to 30 degrees (head-up) for 15 min. Then the position was resumed to horizontal and 15 min hemodynamic stabilization followed. Subsequent 60 degrees tilt lasted for 5 min. The time to position was 40 and 60 s, respectively. The tilt was interrupted prematurely in case of hemodynamic

intolerance defined as a decline in mean arterial pressure below 40 mm Hg or rSO2 below 40 % or carotid flow below 50 ml/min for longer than 2 min.

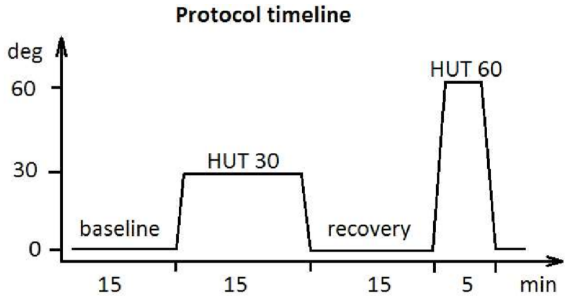

Fig. 1. Study protocol outline – Sequence of protocol phases. Horizontal axis represents sequence and duration of individual phases in minutes. HUT 30, HUT 60 – head-up tilt to 30 (60) degrees. Vertical axis represents degree of head-up inclination (deg).

Statistics

Shapiro-Wilk normality test was used to prove the normal distribution of analyzed parameters. Thus statistical analysis was performed by analysis of variance (ANOVA) for repeated measurements and paired t-test.

Results

Baseline

After anesthesia induction and stabilization, the baseline physiological parameters attained normal values that are summarized in Table 1. Significant interindividual variation was observed in carotid blood flow that ranged from 207 to 510 ml/min. This variability correlated neither with body size nor with cardiac output.

Head-up tilting

The results are summarized in Table 1 and Figure 2. Tilting to 30 degrees rapidly compromised hemodynamics and tissue oxygenation. Arterial pressure, carotid blood flow, left venticular stroke volume and end diastolic volume fell to about 50 % of respective baseline values. Intracranial pressure decreased by 16 mm Hg, however, after eliminating the effect of hydrostatic pressure drop secondary to sensor elevation, the ICP reduction was 4.5 mm Hg. Hydrostatic pressure was calculated as sin(30 deg) x 30 cm (distance between right atrium and ICP sensor), that is 15 cm H_2O or 11.5 mm Hg. Then, the calculated cerebral perfusion pressure (mean arterial pressure minus intracranial pressure) decreased from 79.3±12.8 at horizontal position to 37.6±19.6 at HUT 30. Central venous pressure changed from 6.8 to 1.8 mm Hg suggesting reduced preload. At the same time, the ejection fraction remained unchanged. Tissue oxygenation and mixed venous oxygenation decreased to 46.5 % and 31.5 %, respectively (i.e. 74 % and 64 % of their baseline values) demonstrating an increased O2 extraction partially compensating reduced oxygen delivery.

Table 1. Averaged hemodynamic and oximetry results for baseline (0 DEG), 30 and 60 degrees head-up tilt (HUT 30 and HUT 60).

Parameter	0 DEG		HUT 30		HUT 60		p values		n
(unit)	mean	SD	mean	SD	mean	SD	0 vs. 30	30 vs. 60	
MAP (mm Hg)	98.22	13.52	52.67	18.81	38.88	9.28	0.001	0.015	9
CAR (ml/min)	358.86	108.58	140.29	55.08	53.29	33.27	0.001	0.015	7
SV (ml)	88.88	22.36	41.75	14.59	40.00	16.53	0.001	n.s.	8
EDV (ml)	157.38	33.00	76.38	33.87	82.63	53.25	0.001	n.s.	8
EF (%)	56.75	9.72	57.63	16.04	56.50	17.80	n.s.	n.s.	8
CVP (mm Hg)	6.89	1.54	1.83	2.00	−5.63	4.84	0.001	0.001	9
ICP (mm Hg)	18.29	7.20	2.29	7.52	−7.43	8.10	0.001	0.001	7
SVO2 (%)	49.71	7.06	31.57	12.27	26.57	10.03	0.001	n.s.	7
rSO2 (%)	64.00	11.10	46.52	7.82	44.00	7.18	0.013	n.s.	6

MAP – mean arterial pressure, CAR – carotid blood flow, SV – left ventricular stroke volume, EDV – left ventricular end-diastolic volume, EF – left ventricular ejection fraction, CVP – central venous pressure, ICP – intracranial pressure, SVO2 – mixed venous blood saturation, rSO2 – regional oxygen saturation (forehead), n – number of samples

Tilting to 60 degrees compared to 30 degrees resulted in dramatically reduced carotid blood flow 90±109 ml/min vs. 404±164 ml/min (22 % of baseline), further reduced CVP (−5.6 mm Hg) and mean arterial pressure 39 mm Hg (40 % of resting values). However, other parameters (EDV, SV, SVO2, rSO2) did not differ significantly between 30 and 60 degrees tilt. Left ventricular ejection fraction again remained unchanged.

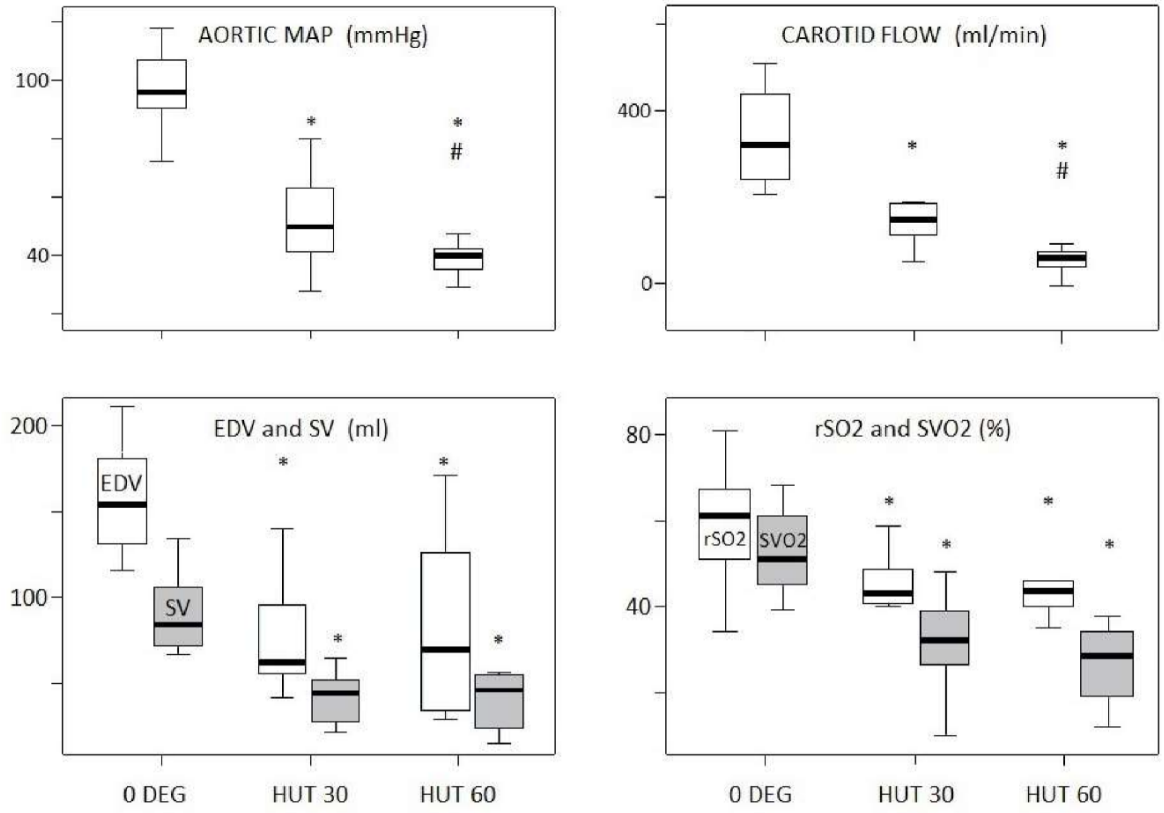

Fig. 2. Hemodynamic and oximetry parameters at baseline (0 DEG) 30 and 60 degrees head-up tilt. (HUT 30, HUT 60). MAP – mean arterial pressure, CAR – carotid blood flow, EDV – left ventricular end-diastolic volume (white), SV – left ventricular stroke volume (gray), rSO2 – regional oxygen saturation (white), SVO2 – mixed venous blood saturation (gray). * $p<0.001$ vs. 0 deg. # $p<0.015$ vs. HUT 30. Box plots: inside the box there are 50 % of values, thick line represents median. Top (bottom) 25 % of values are above (below) the box as indicated by vertical lines.

A representative time course of hemodynamic parameters changes after both tilts is presented in Figure 3. The time to the position was 40 s and 60 s and already during this period the monitored parameters rapidly decreased near to the minimal values in each position. Then, in most animals, the hemodynamics stabilized throughout the rest of HUT period and little hemodynamic compensation was observed if any. Oximetry parameters declined less rapidly than hemodynamic ones. With the return to horizontal position, all the parameters recovered promptly excluding stroke volume that required more than 10 min to return to the baseline.

Intolerance

Two and four animals did not tolerate tilting to 30 and 60 degrees, respectively and thus the HUT maneuver was interrupted.

Complications

All the animals survived the study and there were no complications. However, tilting to 60 degrees represented a challenge to securing all the monitoring systems in place so that the data recording was reliable. Despite the effort to prevent sensors misplacement some signals had to be excluded from further processing for poor recording quality.

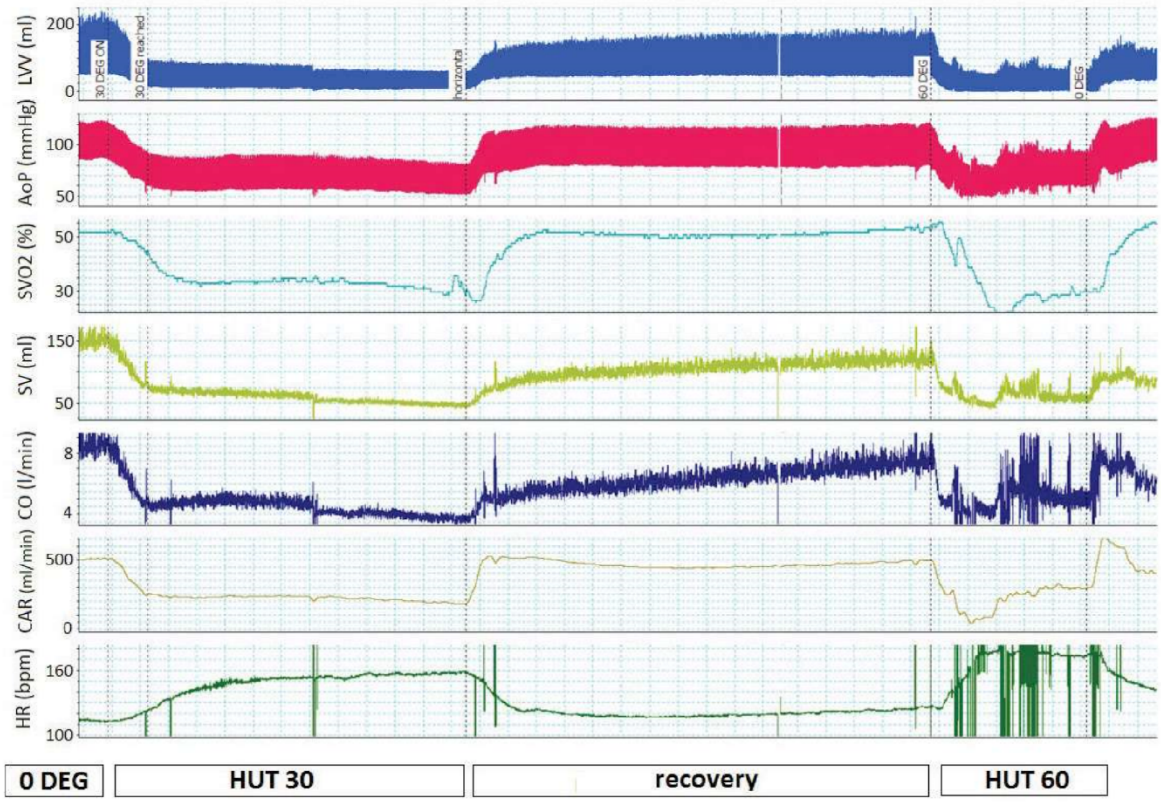

Fig. 3. Sample continuous data traces from one animal. Horizontal axis represents the protocol timeline. 0 DEG – baseline at horizontal position, HUT 30 and HUT 60 – 30 and 60 degrees head-up tilt. LVV left ventricular volume, AoP – aortic blood pressure, SVO2 – mixed venous blood saturation, CO – cardiac output, CAR – carotid blood flow, HR – heart rate

Discussion

While it was expected that head-up tilting to 60 degrees will compromise and possibly collapse hemodynamics, it was surprising to find that already 30 degrees elevation has an immediate significant adverse effect that was even not tolerated by some animals. Hemodynamic destabilization in our setup was thus greater than in other studies exploring head-up tilting in pigs. In our study, the 30 degrees HUT in anesthetized swine resulted in about 50 % decrease in mean arterial pressure, carotid blood flow, end diastolic volume and stroke volume immediately during tilting (see Fig. 3). Peripheral NIRS oximetry (rSO2) dropped to saturation values at around 40 % which indicated tissue hypoperfusion while normal values are near 60 %. In a study by Klopfenstein a 20 degrees HUT resulted only in moderate decrease in cardiac output (−20 % of baseline) and mean arterial pressure (−10 % of baseline) (Klopfenstein *et al.* 1998). In 30 degrees tilt with capnoperitoneum, Junghans *et al.* (2006) reported similar changes in hemodynamics to Klopfenstein study: cardiac output was reduced by 24 %, mean arterial pressure by 6 % and stroke volume by 30 %. Our results are more similar the resuscitation study reported by Debaty *et al.* (2015). In 30 degrees tilt during cardiac arrest treated by automated chest compressions they found considerable decrease in arterial pressure (roughly 50 %) and carotid flow (more than 50 %). Despite this they have seen improved brain tissue perfusion at 30 degrees HUT compared horizontal position, presumably due to improved venous return from brain circulation.

There could be several reasons for markedly compromised hemodynamics in our study compared to others.

General anesthesia. Propofol, an i.v. anesthetic used in the study, is known to inhibit hemodynamics by various effects (contractility, arterial vasoconstriction, venous tone). It has been suggested that in clinically relevant doses venous depression might be the most pronounced effect of propofol (Muzi *et al.* 1992). This would be in line with our results of reduced end-diastolic

volume but preserved ejection fraction and increased arterial resistance. In other studies, inhalation anesthetics are commonly administered, but these would certainly not be used during CPR.

Venous return restriction. The animal fixation by means of vacuum mattress could interfere with the venous return. Though rather homogenous pressure is expected, it cannot be excluded that the amount of pressure the mattress exerts on the body and the location of this pressure could result in variable chest and abdominal compression. The effect could then range from beneficiary centralization of venous blood or an "autotransfusion" effect to adverse blood pooling in subdiaphragmatic region, specifically if combined with tilting. In laparoscopic patients it has been reported, that intra-abdominal pressure increase by 12 mm Hg combined with 30 degrees HUT reduces cardiac output by 35 % and stroke volume by 25 % (Junghans *et al.* 2006). In present study, there were no signs of consistent changes in central venous pressure and/or airway pressure following the mattress inflation. However, no direct information was recorded concerning the pressure or flow in subdiaphragmatic region.

Animal species. It can be argued that swine posture is naturally horizontal resulting in poor tolerance to more vertical position; however, we consider this a less likely reason. The results of other studies demonstrate reasonable tolerance of anesthetized pigs to tilting. Also, the elevation to 30 degrees represents a position that in swine can physiologically occur during daily life activities.

Fluid depletion. Hypovolemia was unlikely in the current study since enough infusions were administered so that CVP was always above 6 mm Hg prior to baseline measurement. However, in human medicine, some degree of hypovolemia can be rather common.

Several hypotheses/conclusions can be drawn from our observations. Even in the absence of cardiac arrest hence in the absence of severe metabolic imbalance, head-up tilting of anesthetized swine in vacuum mattress can result in a critical reduction of hemodynamic parameters. This seems to be a consequence of reduced preload since ventricular filling and central venous pressure were markedly reduced while vascular resistance increased. Despite hemodynamic changes, myocardial perfusion remained preserved or well compensated since ejection fraction changed neither at 30 nor at 60 degrees tilt. Regarding the brain, decreased cerebral perfusion pressure and regional tissue oxygenation in forehead would suggest limited cerebral perfusion. However, it is still possible that preserved autoregulation in cerebral bloodstream and improved venous return could keep cerebral microcirculation preserved.

Whether similar effects can be seen in humans during standard out-of-hospital CPR remains to be elucidated. In fully conscious healthy human volunteers, a rapid passive tilting to 30 degrees provokes almost 20 % decrease in stroke volume that is explained by blood pooling in dependent body regions (Toska and Walloe 2002). Thus it is quite likely that under CPR conditions preload restriction will be a very important determinant of hemodynamics and any situation limiting venous return such as hypovolemia, blood pooling and impeded vasoreactivity should be addressed promptly.

Limitations

There are several limitations that deserve attention and further investigation. First, no robust markers of cerebral tissue perfusion adequacy such as electrophysiology were employed. Second, the need for global anesthesia in the doses relevant for miniinvasive procedures did introduce effects that may not apply to typical clinical scenarios. Particularly the use of propofol might have influenced the results, specifically *via* vascular reactivity. Another anesthetic such as midazolam might provide more relevant data. Additionally, despite a great care the tilting did interfere with the accuracy of data recording since some moves such those of internal organs within body cavities could not have been avoided. This might result in greater data variability than expected. Finally, significant interindividual variability was noticed with some animals not tolerating the tilt. This might confound some of the observations. The study was neither aimed nor powerful enough for any subgroup analysis.

Conclusions

Head-up position significantly compromises hemodynamics in healthy anesthetized swine restrained in vacuum mattress, probably due to preload limitation. Some of the findings might apply to human resuscitation pathophysiology.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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Abbreviations

AoP – aortic blood pressure, CAR – carotid blood flow, CPR – cardiopulmonary resuscitation, CVP – central venous pressure, EDV – end diastolic volume, EF – ejection fraction, HR – heart rate, HUT – head-up tilt, ICP – intracranial pressure, LVV – left ventricular volume, MAP – mean arterial pressure, NIRS – near infrared spectroscopy, rSO2 – regional tissue saturation, SVO2 – mixed venous blood saturation, SV – stroke volume.

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3 Attachment File NO 3

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Microcirculatory blood flow during cardiac arrest and cardiopulmonary resuscitation does not correlate with global hemodynamics: an experimental study

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Abstract

Background: Current research highlights the role of microcirculatory disorders in post-cardiac arrest patients. Affected microcirculation shows not only dissociation from systemic hemodynamics but also strong connection to outcome of these patients. However, only few studies evaluated microcirculation directly during cardiac arrest (CA) and cardiopulmonary resuscitation (CPR). The aim of our experimental study in a porcine model was to describe sublingual microcirculatory changes during CA and CPR using recent videomicroscopic technology and provide a comparison to parameters of global hemodynamics.

Methods: Cardiac arrest was induced in 18 female pigs (50 ± 3 kg). After 3 min without treatment, 5 min of mechanical CPR followed. Continuous hemodynamic monitoring including systemic blood pressure and carotid blood flow was performed and blood lactate was measured at the end of baseline and CPR. Sublingual microcirculation was assessed by the Sidestream Dark Field (SDF) technology during baseline, CA and CPR. Following microcirculatory parameters were assessed off-line separately for capillaries (≤20 μm) and other vessels: total and perfused vessel density (TVD, PVD), proportion of perfused vessels (PPV), microvascular flow index (MFI) and heterogeneity index (HI).

Results: In comparison to baseline the CA small vessel microcirculation was only partially preserved: TVD 15.64 (13.59–18.48) significantly decreased to 12.51 (10.57–13.98) mm/mm^2, PVD 15.57 (13.56–17.80) to 5.53 (4.17–6.60) mm/mm^2, PPV 99.64 (98.05–100.00) to 38.97 (27.60–46.29) %, MFI 3.00 (3.00–3.08) to 1.29 (1.08–1.58) and HI increased from 0.08 (0.00–0.23) to 1.5 (0.71–2.00), $p = 0.0003$ for TVD and <0.0001 for others, respectively. Microcirculation during ongoing CPR in small vessels reached 59–85 % of the baseline values: TVD 13.33 (12.11–15.11) mm/mm^2, PVD 9.34 (7.34–11.52) mm/mm^2, PPV 72.34 (54.31–87.87) %, MFI 2.04 (1.58–2.42), HI 0.65 (0.41–1.07). The correlation between microcirculation and global hemodynamic parameters as well as to lactate was only weak to moderate (i.e. Spearman's ρ 0.02–0.51) and after adjustment for multiple correlations it was non-significant.

Conclusions: Sublingual microcirculatory parameters did not correlate with global hemodynamic parameters during simulated porcine model of CA and CPR. SDF imaging provides additional information about tissue perfusion in the course of CPR.

Keywords: Microcirculation, Sidestream dark field imaging, Cardiac arrest, Cardiopulmonary resuscitation, Animal model, Sublingual area, Microscopy camera technology

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Background

Microcirculatory blood flow plays pivotal role in oxygen and nutrient supplementation of tissues and therefore represents the key target for treatment in critically ill patients. Currently, much effort has been taken to elucidate prevalence and consequences of microcirculatory disorders also in victims of cardiac arrest (CA), which is one of the leading causes of adulthood fatality in developed countries [1, 2]. In post-cardiac arrest patients a discordance between systemic blood flow and tissue perfusion has been reported [3, 4], and a connection between microcirculation and patients' outcome has also been suggested [3, 5–7]. However, only few studies have evaluated microcirculation during CA and cardiopulmonary resuscitation (CPR) [8–11]. Despite the fact that maintaining adequate cerebral perfusion is crucial goal of CPR, evaluation of cerebral perfusion during CPR, i.e. for research purposes, is still demanding [11]. In contrast, due to recent technological advancements, sublingual microcirculation with its potential relationship to outcome is easy to assess with a simple bedside tool [12]. However, evaluation of sublingual microcirculatory parameters with the strongest relation to outcome [13, 14] during CPR is still lacking.

Therefore, we conducted our experimental study to target changes of peripheral microcirculatory blood flow during CA and mechanical CPR in a porcine model of cardiac arrest. We presumed that microcirculatory parameters would dynamically change during CA and CPR and we expected that the microcirculation would significantly decline from the baseline during CPR. Furthermore, we aimed to compare microcirculatory parameters during CPR with variables of systemic hemodynamics: we hypothesized, that the microcirculatory parameters would not correlate both to mean arterial blood pressure and to carotid blood flow as a surrogate marker of macrocirculatory brain perfusion. Finally, we correlated microcirculation to lactate levels as a widely used surrogate for tissue perfusion and adjusted to variables possibly affecting the relationship, i.e., body temperature and hemoglobin levels.

Methods

Ethics section

The study protocol was approved by the Charles University First Medical School Institutional Animal Care and Use Committee and performed at the Animal Laboratory, Department of Physiology, First Medical School, Charles University in Prague, in accordance with Act No 246/1992 as amended, Collection of Laws, Czech Republic, which is harmonized with EU Directives 86/609/EEC as amended, 2007/526/ES, 2010/63/EU.

Anesthesia and study protocol

Eighteen healthy crossbred female pigs, four to 5 months old, mean body weight 50 ± 3 kg, were used in this study. After 24 h of fasting, the animal was premedicated with midazolam (0.3 mg/kg intramuscular) and ketamine (20 mg/kg intramuscular). Then a catheter access was established into the marginal ear vein. Anesthesia was initiated by propofol bolus (2 mg/kg) and the animal was orotracheally intubated; mechanical ventilation was started using volume controlled ventilation with tidal volumes of 8 mL/kg and respiratory rate adjusted to keep end-tidal CO_2 between 4.5 and 5.6 kPa. Anesthesia was continued with continuous intravenous infusion of propofol (6–8 mg/kg/h), midazolam (0.1–0.2 mg/kg/h) and morphine (0.1–0.2 mg/kg/h); the depth of anesthesia was regularly assessed by photoreaction and corneal reflex. After initial heparin bolus (100 IU/kg) continuous administration of unfractionated heparin (50 IU/kg/h) followed to maintain activated clotting time over 200 s. Normal saline was given intravenously in continuous infusion (starting at 500 mL/h and followed by 150–200 mL/h) to reach and maintain central venous pressure between 3 and 7 mmHg [15]. Multiple intravascular sheaths were inserted percutaneously to enable further placement of measuring catheters (see "Data acquisition" section).

After the completion of invasive procedures and a minimum of 15 min of stabilization, baseline values were obtained. Thereafter, CA was commenced by induction of ventricular fibrillation (VF) using programmed right ventricular stimulation as described previously [16]; mechanical ventilation was stopped during CA period. As shown in the protocol outline (Fig. 1), after 3 min of untreated CA, mechanical CPR was started using the LUCAS chest compression device (The LUCAS® Chest Compression System, Physio-Control Inc., Redmond, CA, USA) along with mechanical ventilation (FiO_2 100 %). The compression rate was 100 per minute [1]. The mechanical CPR was continued for 5 min.

After finishing above described procedures, experimental animals were subjected to further advanced protocol, which is not covered in this report. At the end of the advanced protocol, animals have been euthanized by morphine and propofol overdose followed by intravenous potassium chloride 1 mmol/kg.

Data acquisition

Real-time mean arterial blood pressure (ABP) and central venous pressure (CVP) were obtained by means of fluid-filled transducers (Truwave, Edwards Lifesciences, USA) placed at the hearth level connected to the catheters placed in a femoral artery and superior vena cava. Carotid blood flow velocity (CBF) measurement was

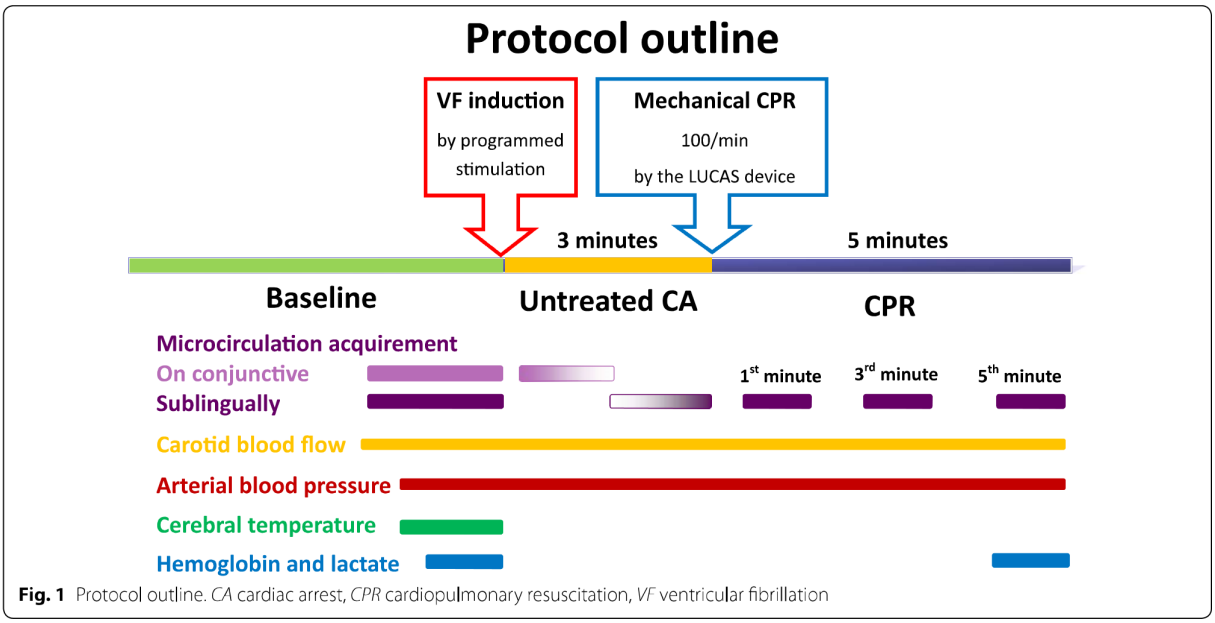

Fig. 1 Protocol outline. *CA* cardiac arrest, *CPR* cardiopulmonary resuscitation, *VF* ventricular fibrillation

performed using transient ultrasound flow probe (PSB, Transonic, USA) which was surgically implanted around right common carotid artery. Continuous cardiac output (CO), pulmonary artery pressure (PAP) and pulmonary wedge pressure were measured by means of pulmonary artery catheter (CCO V, Edwards Lifesciences, USA) placed into pulmonary artery via femoral vein. The temperatures were measured by Data Dogger Nanodac (Eurotherm, Faraday Cl, Worthing, BN13 3PL, GBR). Thermocouples (K-type, diameter 0.6 mm) were inserted into cerebral parietal cortex via a burr hole 2 cm laterally from the midline. All measured parameters were sampled 400 Hz by Powerlab A/D converter and continuously recorded to PC running Labchart Pro software (AD Instruments, USA). The parameters were analyzed offline; peripheral vascular resistance (PVR) was counted from the obtained parameters and mean values of ABP and CBF for 1-min-long intervals were counted. Except for ABP and CBF we provide only baseline values of the hemodynamic parameters because of very unreliable values provided during ongoing CPR.

Hemoglobin (HGB) and lactate levels in the blood samples from femoral artery were obtained at the end of the baseline and CPR periods using bedside analyzer (ABL90 FLEX, Radiometer Medical ApS, Brønshøj, DNK).

Microcirculatory assessment

For microcirculatory monitoring we employed current videomicroscopic approach, Sidestream Dark Field (SDF) imaging (MicroScan, Microvision Medical, Amsterdam, NLD). The principle of the SDF spectroscopy has already been described [17], in brief: hand handled video microscope emits stroboscopic green light of specific wavelength, which is absorbed and immediately emitted back by hemoglobin of red blood cells. The image of red blood cells moving within microvessels is transmitted back through the microscope to the camera. Thus noninvasive real-time images of microcirculation were obtained.

The microcirculation measurements during the experiment were performed manually by a single investigator (PK) on the conjunctivae and sublingual mucosa of the experimental animal. Due to presumed dynamic changes of microcirculation and short protocol time (3 min of CA and 5 min of CPR) we obtained video sequences of 6 s duration instead of suggested 20 s similarly to other studies [8, 18–20]. Except for this difference, both acquisition and subsequent analysis of the video images followed the published consensus criteria [21]. At least five video sequences from different parts of the sublingual mucosa were acquired per these time points: during baseline, in the last 90 s of CA and during CPR. We intended to perform also conjunctivae microcirculatory acquisition, because of close anatomical relation of the conjunctival blood supply to cerebral circulation. The aim was to capture five sequences of conjunctival microcirculation in these time points: in baseline, during the first 90 s of CA and during CPR. Conjunctival and sublingual mucosae were regularly washed by saline to remove secretion and to prevent drying.

Images were stored and analyzed off-line. Video images of insufficient quality were excluded and three images per time point were selected as follows: we chose three random images from the baseline period; in the CA we chose the first image, the middle one and the last one; from the CPR period we chose one random image from the first, the third and the fifth minute. In case that there were not three images of appropriate quality per time point, minimum of two images was set sufficient for analysis. The video images were blinded and analyzed in a random order by a single investigator (PK) blinded to data on animal and time period in which the videos were recorded. The analysis was performed using dedicated software (AVA—Automated Vascular Analysis 3.1, Microvision Medical). The following parameters were acquired: total vessel density (TVD), perfused vessel density (PVD), proportion of perfused vessels (PPV), microvascular flow index (MFI) and heterogeneity index (HI). All these indexes were calculated separately for small vessels (microvessels of diameter ≤20 μm) and other vessels (>20 μm). TVD was estimated as the ratio of total vessel length in selected region of interest and its area. To evaluate the PVD and PPV, vessels were classified according to the microcirculation as perfused (the blood flow in the vessel was hyperdynamic, continuous or sluggish) or not perfused (with intermittent flow or no-flow). For the MFI quantification the image was divided into four quadrants and the circulation in each quadrant was expressed in ordinal scale: 4—hyperdynamic flow, 3—continuous flow, 2—sluggish flow, 1—intermittent flow, 0—no flow. MFI was the average score of all quadrants. Heterogeneity index was calculated per each time point in every animal as follows: maximum MFI minus minimum MFI divided by the mean MFI.

Statistical analysis

Normality of the data was tested by Shapiro–Wilk test. Parametric data are presented as mean (±sample standard deviation, SSD), the non-parametric data are expressed as median (the first and the third quartile). To test the hypothesis of different microcirculatory parameters during baseline, CA and CPR we used Friedman test. The post hoc analysis was performed using Wilcoxon test with the Bonferroni correction for multiple comparisons. Parametric data from baseline and CPR were compared by paired t test. The correlation of microcirculatory data to ABP, CBF, temperature, lactate and HGB level was performed with the Spearman's Rank Correlation Coefficient (Spearman's ρ), Bonferroni correction for multiple correlations was applied. p value of ≤0.05 was considered statistically significant. Statistical analyses were performed with MedCalc Statistical Software version 16.4.3 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2016).

Results

Eighteen animals were included into the analysis and data acquisition was performed. We succeeded to obtain sufficient quality videoimages of sublingual microcirculation for analysis. However, to acquire conjunctival microcirculation during CA required longer period than preset (i.e., up to 130 s after CA onset), moreover, during CPR the images of conjunctival microcirculation were of poor quality not sufficient for the analysis, therefore we excluded conjunctival microcirculation from the further analysis.

Baseline characteristics

All animals were stable during baseline, mean ABP was 86.2 (±11.0) mmHg, CBF 292.5 (±69.7) mL/min, PWP 8.9 (±2.6) mmHg, PAP 18.3 (±3.7) mmHg and mean CVP was 5.6 (±2.7) mmHg, CO 5.20 (±0.97) L/min and PVR 15.9 (±3.1) mmHg × min/L. There were certain differences in the body temperature of the animals, mean cerebral temperature was 38.7 (±1.3) °C. The animals had normal blood hemoglobin 8.29 (±1.11) g/dL and low arterial lactate level of 0.90 (±0.22) mmol/L (detailed experimental data are presented in Additional file 1).

Parameters of systemic hemodynamics during CA and CPR

ABP dropped immediately after cardiac arrest onset as well as CBF, which reached no flow within 2–32 s after the induction of ventricular fibrillation. Both ABP and CBF showed temporary elevations, which occurred directly related to gasping. Mean ABP of CA period was 25.6 (±9.0) mmHg and mean CBF was 9.7 (±5.2) mL/min.

The onset of CPR resulted in the initial reperfusion overflow and further continuous decline of ABP and CBF. Mean values for the CPR period were: 48.3 (±14.9) mmHg for mean ABP and 142.7 (±27.5) mL/min for CBF. Lactate level reached 3.40 (±0.72) mmol/L. All values including hemoglobin are summarized in Table 1.

Microcirculation during CA and CPR

As expected, sublingual small vessel microcirculation (of the vessels ≤20 μm) deteriorated during CA, however, it was still partially preserved after 90 s of arrested circulation in contrast to global hemodynamics. Compared to baseline values, medians of CA parameters significantly decreased [TVD 15.64 (13.59–18.48) to 12.51 (10.57–13.98) mm/mm^2, PVD 15.57 (13.56–17.80) to 5.53 (4.17–6.60) mm/mm^2, PPV 99.64 (98.05–100.00) to 38.97 (27.60–46.29) %, MFI 3.00 (3.00–3.08) to 1.29 (1.08–1.58); see Table 2; Fig. 2 for p values]. The microcirculation was also significantly heterogeneous [HI increased from 0.08 (0.00–0.23) to 1.5 (0.71–2.00), $p < 0.0001$, Table 2; Fig. 2].

Table 1 Hemodynamic parameters, temperature, hemoglobin and lactate levels during baseline and cardiopulmonary resuscitation (CPR)

	Baseline	CPR	p value
Mean arterial blood pressure (mmHg)	86.2 ± 11.0	48.3 ± 14.9	<0.00001
Carotid blood flow (mL/min)	292.5 ± 69.7	142.7 ± 27.5	<0.00001
Pulmonary wedge pressure (mmHg)	8.9 ± 2.6		
Central venous pressure (mmHg)	5.6 ± 2.7		
Pulmonary artery pressure (mmHg)	18.3 ± 3.7		
Cardiac output (L/min)	5.20 ± 0.97		
Peripheral vascular resistance (mmHg × min/L)	15.9 ± 3.1		
Cerebral temperature (°C)	38.7 ± 1.3		
Hemoglobin femoral artery (g/dL)	8.29 ± 1.11	11.05 ± 1.30	<0.00001
Lactate femoral artery (mmol/L)	0.90 ± 0.22	3.40 ± 0.72	<0.00001

The data are given as mean ± SD

Sublingual microcirculation was partially restored during CPR: median of capillary density and microflow reached 56–85 % of the pre-arrest values [TVD 13.33 (12.11–15.11) mm/mm^2, PVD 9.34 (7.34–11.52) mm/mm^2, PPV 72.34 (54.31–87.87) %, MFI 2.04 (1.58–2.42)] and HI was 0.65 (0.41–1.07). Total capillary density as well as capillary MFI increased only non-significantly from CA ($p = 0.06$), whereas we found significant increase of capillary PVD and PPV ($p < 0.0001$ and $p = 0.048$; see Fig. 2).

Microcirculation of the other vessels (medium and large vessels of diameter >20 µm) showed different reactivity: the microcirculatory blood flow was generally preserved and vessel density alterations were not significant up to the third minute of CA. Moreover, restoration of microcirculation in other vessels was almost complete during CPR (see Table 2).

Correlation of microcirculatory variables to other parameters

Using Spearman's correlation coefficient we found only weak to moderate correlation between microcirculatory variables and baseline mean ABP or CBF. With Bonferroni adjustment for multiple correlations, however, the correlation was non-significant in both cases (see Table 3). Moreover, the CPR data (mean ABP and CBF) also did not correlate with microcirculation (see Table 4).

Furthermore, microcirculation showed only weak correlation to the baseline cerebral temperature, which was within physiologic ranges. Hemoglobin levels as well as lactate levels showed no correlation to the microcirculation.

Discussion

In our study we demonstrated sublingual microcirculatory changes in a porcine model of cardiac arrest. Small vessel microcirculation responded rapidly during CA and CPR, however, in contrast to arterial blood pressure and carotid blood flow, it was partially preserved during CA and later during CPR median values reached 56–85 % of the pre-arrest state. Interestingly, changes of microcirculation in medium and large vessels (with diameter larger

Table 2 Sublingual microcirculatory parameters during baseline, cardiac arrest (CA) and cardiopulmonary resuscitation (CPR)

	Baseline	CA	CPR	p value of Friedman test
Small vessels (≤20 µm)				
TVD (mm/mm^2)	15.64 (13.59–18.48)	12.51 (10.57–13.98)	13.33 (12.11–15.11)	0.00005
PVD (mm/mm^2)	15.57 (13.56–17.80)	5.53 (4.17–6.60)	9.34 (7.34–11.52)	<0.00001
PPV (%)	99.64 (98.05–100.00)	38.97 (27.60–46.29)	72.34 (54.31–87.87)	<0.00001
MFI	3.00 (3.00–3.08)	1.29 (1.08–1.58)	2.04 (1.58–2.42)	<0.00001
HI	0.08 (0.00–0.23)	1.5 (0.71–2.00)	0.65 (0.41–1.07)	<0.00001
Other vessels (>20 µm)				
TVD (mm/mm^2)	0.41 (0.24–0.85)	0.21 (0.03–0.63)	0.47 (0.35–0.64)	0.14 (NS)
PVD (mm/mm^2)	0.41 (0.24–0.85)	0.13 (0.01–0.38)	0.43 (0.35–0.64)	0.0005
PPV (%)	100.00 (100.00–100.00)	59.26 (50.00–100.00)	100.00 (95.04–100.00)	0.0005
MFI	3.00 (3.00–3.08)	2.06 (1.64–2.67)	2.8 (2.75–3.00)	<0.00001
HI	0.00 (0.00–0.08)	0.05 (0.00–0.41)	0.12 (0.00–0.18)	0.27 (NS)

Data are given as medians (the first and the third quartile)

TVD total vessel density, *PVD* perfused vessel density, *PPV* proportion of perfused vessels, *MFI* microvascular flow index, *HI* heterogeneity index, *NS* non-significant

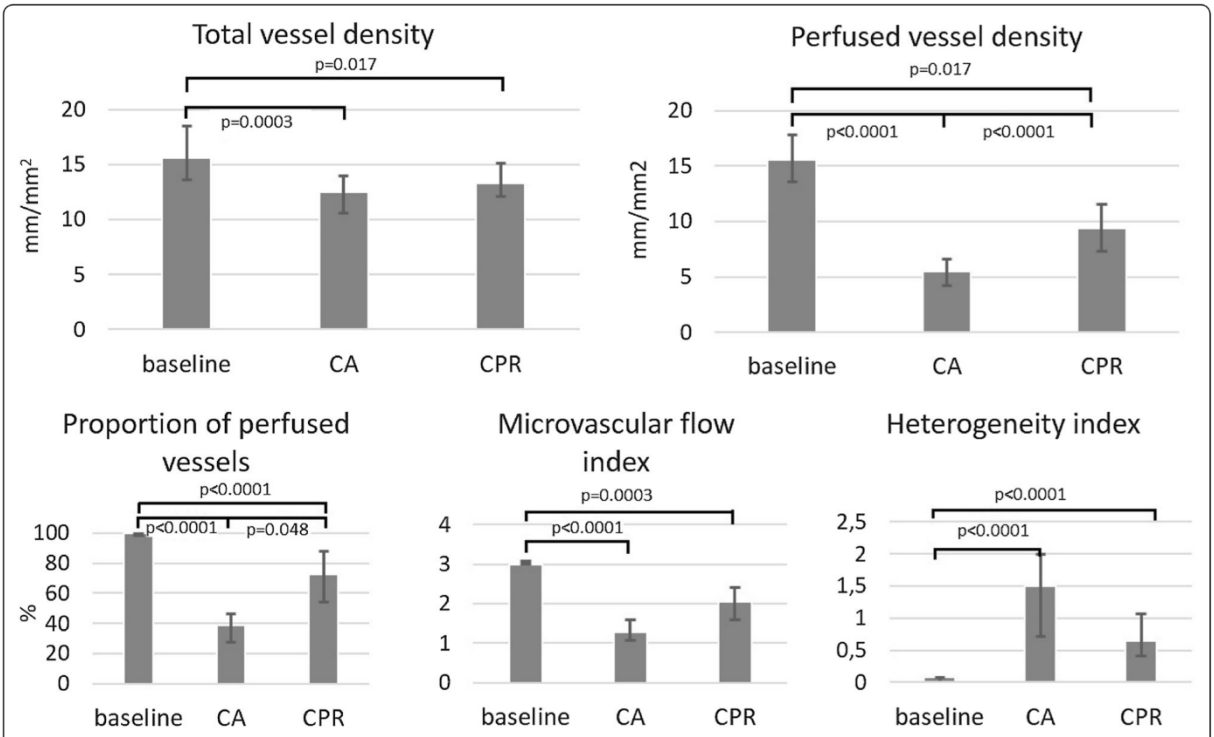

Fig. 2 Sublingual microcirculatory parameters during baseline, cardiac arrest (CA) and cardiopulmonary resuscitation (CPR). The parameters of small vessels (≤20 µm). p values are indicated in the *graph*

Table 3 Correlation coefficient (Spearman's ρ) of microcirculatory and other measured variables during baseline, n = 18

	ABP	CBF	Lactate	HGB	Cerebral T
Small vessels (≤20 µm)					
TVD (mm/mm²)	0.07	−0.46	0.29	0.15	0.11
PVD (mm/mm²)	0.06	−0.40	0.24	0.16	0.09
PPV (%)	−0.32	0.22	−0.28	−0.41	−0.03
MFI	0.05	0.18	−0.22	−0.25	−0.19
HI	−0.09	0.20	−0.29	−0.15	−0.32
Other vessels (>20 µm)					
TVD (mm/mm²)	−0.33	0.24	−0.05	−0.30	−0.03
PVD (mm/mm²)	−0.34	0.27	−0.05	−0.30	−0.07
PPV (%)	0.15	0.43	0.47	0.33	0.23
MFI	0.23	0.25	−0.13	−0.02	−0.06
HI	0.10	0.26	−0.39	−0.13	−0.18

After Bonferroni adjustment for multiple correlations all correlations are non-significant

TVD total vessel density, *PVD* perfused vessel density, *PPV* proportion of perfused vessels, *MFI* microvascular flow index, *ABP* mean arterial blood pressure, *CBF* carotid blood flow, *HGB* hemoglobin level, *T* temperature

than 20 µm) were even milder. Mean arterial blood pressure and carotid blood flow did not correlate to microcirculatory variables in this setting.

Microcirculation in cardiac arrest has been previously described by Fries et al. [8, 22], who demonstrated close relationship between global hemodynamics and microcirculatory flow (MFI) in two experimental studies in a porcine model of cardiac arrest and resuscitation (15 and 9 animals included). In our study, we showed not only changes of sublingual MFI but also course of sublingual vascular density (TVD, PVD), proportion of perfused vessels (PPV) and heterogeneity index. From these parameters, the most important is the perfused vessel density (PVD), which reflects the density of vessels as well as their perfusion. PVD was also found to be related to patients' outcome in several studies of cardiogenic shock and post-cardiac arrest syndrome in humans [6, 13, 14].

Several studies have already dealt with the correlation of microcirculation to systemic hemodynamics, albeit with conflicting results [23]. In contrast to Fries and coworkers, who presented strong correlation between microcirculatory and macrocirculatory hemodynamics, we did not prove such correlation in our setting. However, Fries et al. used different methods: they correlated minimal coronary perfusion pressure (pressure gradient between the minimal aortic and coincident right atrial pressure) and end-tidal CO_2 with MFI by the means of

Table 4 Correlation coefficient (Spearman's ρ) of microcirculatory and other measured variables during CPR, n = 18

	ABP	CBF	Lactate	HGB
Small vessels (≤20 µm)				
TVD (mm/mm^2)	0.20	0.09	−0.23	−0.03
PVD (mm/mm^2)	−0.08	0.06	−0.05	0.09
PPV (%)	−0.23	0.12	−0.05	0.15
MFI	−0.22	0.10	−0.02	0.15
HI	0.20	−0.46	0.08	−0.03
Other vessels (>20 µm)				
TVD (mm/mm^2)	0.12	−0.02	0.06	0.16
PVD (mm/mm^2)	0.09	−0.01	0.08	0.12
PPV (%)	−0.20	0.03	0.11	−0.51
MFI	−0.27	−0.05	−0.34	0.10
HI	0.27	0.10	0.36	−0.12

After Bonferroni adjustment for multiple correlations all correlations are non-significant

TVD total vessel density, *PVD* perfused vessel density, *PPV* proportion of perfused vessels, *MFI* microvascular flow index, *ABP* mean arterial blood pressure, *CBF* carotid blood flow, *HGB* hemoglobin level

linear correlations using the Pearson's correlation coefficient. Our findings are in agreement with reports during cardiogenic and septic shock [3, 4, 14, 23].

We did not prove any significant correlation between microcirculation and CBF, as a surrogate of macrocirculatory cerebral perfusion in our model. This finding is in line with recent body of knowledge regarding early post-cardiac arrest cerebral microcirculation: Secher and coworkers showed that post-cardiac arrest microcirculation is preserved despite inflammatory mediator release and signs of neuronal damage [24]. Therefore, the sublingual microcirculation, which is strongly affectable by inflammatory mediators, might provide better prognostic tool than cerebral microcirculation [11, 24].

During cardiac arrest, ABP dropped immediately and the CBF stopped within 32 s after the onset of ventricular fibrillation. In contrast, sublingual microcirculatory blood flow was partially preserved up to the third minute of cardiac arrest (35–80 % of the baseline values), which might be explained as a persistence of pressure gradient at the level of microcirculation. Nevertheless, there are some other possible explanations: during the video-images recording, mostly in the third minute of untreated cardiac arrest, rapid blood flow after the gasping with a duration of several seconds occurred. Ristagno et al. demonstrated that gasping during cardiac arrest produced significant carotid blood flow which averaged approximately 59 % of pre-cardiac arrest state [25]. As we could not avoid gasping in our animals, the microcirculatory flow in CA might have been a result of gasping-induced carotid blood flow. Moreover, another possible explanation of microcirculatory blood flow during CPR might be reversal of the blood flow. It was reported that reversal carotid blood flow occurred during manual CPR in decompression phase [26]. While we did not notice reversal carotid flow, it was technically not feasible in our setting to determine forward versus reversal blood flow or trace the direct impact of gasping on the microflow during CA and CPR.

We did not find significant correlation between baseline microcirculatory variables and cerebral temperature. Previously, it has been reported that therapeutic hypothermia impairs microcirculation [6, 27], nevertheless, fluctuations of body temperature within physiologic range might not have any impact on microcirculation. Lactate levels were only moderately elevated at the end of the CPR, which corresponds with a relatively short period of CA and CPR. However, this level might be also underestimated because of tissue hypoperfusion that disabled lactate transportation to central bloodstream. In such cases, the lactate value may fail to indicate the extent of circulatory impairment and provide an indirect evidence for a paramount importance of a bedside tool for the microcirculatory assessment. Hemoglobin levels have also significantly risen at the end of the CPR period. We do not have a clear clue to this rise, however, we speculate, that the change in capillary permeability and loss of the fluid into the interstitial space could have been responsible.

Our study demonstrated a feasibility of a noninvasive assessment of microcirculation in an animal model of cardiac arrest and resuscitation. Using hand-handled bedside SDF videomicroscopy we simulated realistic scenario and showed that the microcirculatory assessment could provide additional information about tissue perfusion. Finding of no correlation between macro and microcirculation does not mean the uselessness of current hemodynamic monitoring, but emphasizes the need for direct microcirculatory evaluation.

There are several limitations of our study: The length of the video images for microcirculatory assessment was only 6 s (compared to 20 s in most studies), which could influence the evaluation and results of microcirculatory analysis. Another limitation was, that the video sequences in animals were not captured directly at the same time during cardiac arrest, which might have caused time shift leading to inaccuracy caused by dynamic ongoing changes of microcirculation. During the CPR period the movement or pressure artifacts could not be fully avoided; although we excluded those images which did not meet the consensus criteria on image quality [21], still some of the images could be affected by these artefacts. Furthermore, for the correlation, we used mean

values of the CPR data, however, blood pressure as well as carotid blood flow was influenced by the reperfusion overflow in the first minutes of CPR, which might have influenced the results. Also the influence of the administered drugs has to be mentioned, as many of them might affect the microcirculation. Heparin, beyond its anticoagulation effect, has also been reported several times to have an anti-inflammatory action. Protective effect of heparin on microcirculation in various pathologic states has been reported [28, 29] and therefore heparin may lead to underestimation of real severity of microcirculatory deterioration. On the other hand, anesthesia maintained by propofol has been reported to reduce capillary blood flow in human [30]. There are also statistical limitations of our study: the number of included animals was not power calculated for the purposes of this report, but for the purposes of other advanced protocol, which we mentioned in the study protocol section. Nevertheless, the number of animals corresponds with other similar studies [8]. Finally, the correlation of microcirculation to other variables was adjusted by Bonferroni correction for multiple correlations, which might lead to rejection of true correlation [31]. However, we did not prove any strong correlation between sublingual microcirculation and other variables even without Bonferroni adjustment, which enables us to summarize that no correlation was found.

Conclusions

In our porcine model of cardiac arrest and resuscitation, sublingual microcirculation showed rapid decrease during the CA and slight increase in the course of CPR; CPR microcirculation reached 59–85 % of the baseline values. Microcirculatory parameters did not correlate with the global hemodynamic parameters. Non-invasive sublingual microcirculatory imaging might provide additional information about tissue perfusion in the course of CPR.

Additional file

Additional file 1. Detailed data supporting conclusions of our article.

Abbreviations

ABP: mean arterial blood pressure; CA: cardiac arrest; CBF: carotid blood flow; CO: cardiac output; CPR: cardiopulmonary resuscitation; FA: femoral artery; HGB: hemoglobin level; MFI: microvascular flow index; PA: pulmonary artery; PAP: pulmonary artery pressure; PPV: proportion of perfused vessels; PVD: perfused vessel density; PVR: peripheral vascular resistance; PWP: pulmonary wedge pressure; SDF: sidestream dark field; T: temperature; TVD: total vessel density.

Authors' contributions

PK stated the hypothesis, performed microcirculatory recording and off-line blinded analysis, statistically evaluated the data and drafted the manuscript. JB conceived the study, designed the study protocol, participated on the study performance and substantially contributed to the manuscript. MM performed the main procedures during the study and contributed to the interpretation of the results. ZM conceived and supervised the microcirculatory monitoring and analysis. MH cooperated in data acquisition, statistical analysis and data interpretation. TB and SL assisted the study procedures and data acquisition. MC, PN, OK and AL consulted study design and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials section

The dataset supporting the conclusions of this article is included within the article and an Additional file 1.

Competing interests

The authors declare that they have no competing interests.

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4 Attachment file NO 4

KRUPICKOVA P, HUPTYCH M, MORMANOVA Z, BOUCEK T, BELZA T, SMID O, KRAL A, SKALICKA H, LINHART A AND BELOHLAVEK J. Effect of pulsatility on microcirculation in patients treated with extracorporeal cardiopulmonary resuscitation: A pilot study. *Asaio j* 2016. doi:10.1097/mat.0000000000000492.

Effect of Pulsatility on Microcirculation in Patients Treated with Extracorporeal Cardiopulmonary Resuscitation: A Pilot Study

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The effect of pulsatile blood flow on microcirculation during extracorporeal cardiopulmonary resuscitation (ECPR) is not elucidated; therefore, we designed an observational study comparing sublingual microcirculation in patients with refractory cardiac arrest (CA) with spontaneously pulsatile or low/nonpulsatile blood flow after treatment with ECPR. Microcirculation was assessed with Sidestream Dark Field technology in 12 patients with CA who were treated with ECPR and 12 healthy control subjects. Microcirculatory images were analyzed offline in a blinded fashion, and consensual parameters were determined for the vessels ≤20 μm. The patients' data, including actual hemodynamic parameters, were documented. Pulsatile blood flow was defined by a pulse pressure (PP) ≥ 15 mm Hg. Compared with the healthy volunteers, the patients who were treated with ECPR exhibited a significantly lower proportion of perfused capillaries (PPC); other microcirculatory parameters did not differ. The groups of patients with pulsatile (n = 7) *versus* low/nonpulsatile (n = 5) blood flow did not differ in regards to the collected data and hemodynamic variables (except for the PP and ejection fraction of the left ventricle) as well as microcirculatory parameters. In conclusion, microcirculation appeared to be effectively supported by ECPR in our group of patients with CA with the exception of the PPC. We found only nonsignificant contribution of spontaneous pulsatility to extracorporeal membrane oxygenation-generated microcirculatory blood flow. *ASAIO Journal* 2016; XX:00–00.

Key Words: extracorporeal CPR, ECMO, microcirculation, SDF, pulsatile blood flow

Extracorporeal cardiopulmonary resuscitation (ECPR), *i.e.*, venoarterial extracorporeal membrane oxygenation (ECMO) implanted during refractory cardiac arrest (CA), has the potential to improve outcomes in patients with CA who do not achieve the return of spontaneous circulation (ROSC) with standard cardiopulmonary resuscitation (CPR).[1,2] However, remaining high mortality and poor neurologic outcomes[3] have facilitated the need for new approaches and developments. Whether efforts to sustain pulsatility in ECPR settings should be undertaken is one of the pertinent topics in this field, and there is ongoing controversy regarding the simultaneous use of ECMO and intra-aortic balloon counterpulsation (IABP).[4,5] One of the issues that are relevant to ECMO and IABP utilization is the effect of generated pulsatility on microcirculation, which represents an important endpoint of any form of circulatory support.

However, the current body of knowledge regarding the effect of combined ECMO and IABP therapy on microcirculation presents conflicting results.[6–8] Recently published study of the effect of IABP on cerebral blood flow in ECMO-treated patients revealed that the IABP effect is dependent on residual spontaneous cardiac function.[9] Presence and the different level of the residual spontaneous pulsatility may influence the microcirculation in ECMO-treated patients, and therefore, it might be the key to understand the divergence of the results from patients treated by combined IABP–ECMO therapy. Furthermore, the residual pulsatility level or its absence might become a potential auxiliary parameter for indication of this combined therapy. To elucidate, whether the level of spontaneous pulsatility in addition to ECMO flow is the determinative parameter for microcirculatory flow, we designed a study involving patients who were treated with ECPR and divided into a pulsatile (P) and a low/nonpulsatile (L/N) group. We defined pulsatility as a pulse pressure (PP) of 15 mm Hg or higher as previously suggested.[10] We hypothesized that in an ECPR setting, the microcirculatory parameters of patients with pulsatile blood flow would be improved compared with those of patients in a low/nonpulsatile status. Subsequently, we compared the microcirculatory parameters of our patients to healthy volunteers.

Methods

Study Design

This single-center pilot observational study was conducted as a noninvasive microcirculatory substudy of an ongoing

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randomized trial.[11] Both the main study and the microcirculatory substudy have been approved by the institutional review board of the General University Hospital and First Faculty of Medicine, Charles University in Prague. The main study is registered under ClinicalTrials.gov identifier: NCT01511666. A data safety monitoring board regularly reviews the data on the patients' outcomes and complications. Written informed consent for the study participation and data analysis was obtained for every participant either from the next of kin (in cases of sedated or unconscious patients who did not regain consciousness) or from patients who regained consciousness later on.

We included patients who were treated with a hyperinvasive approach including extracorporeal life support, *i.e.*, patients who suffered refractory out-of-hospital CA, did not achieve ROSC on the scene, and were transported to the hospital while receiving CPR and were provided ECPR. Another inclusion criterion was the availability of a specialist for Sidestream Dark Field (SDF) microcirculation measurement within the first 72 hours after CA. The patients were treated according to a standardized protocol that has been published in detail previously and describes the procedures for on-scene advanced life support provision, randomization, transport, insertion of the percutaneous femoro-femoral veno-arterial ECMO, angiography, eventual percutaneous coronary interventions and subsequent standardized therapy, investigations, and further complex intensive care including target temperature management.[11]

A control group of healthy volunteers was recruited from among the hospital staff.

Data Collection

The following individual data were collected: age, sex, time from CA onset (collapse) to ECMO setup, time from CA to microcirculatory measurement, etiology of the CA, baseline ejection fraction, temperature management, total on-ECMO time, neurologic performance at discharge measured using the Glasgow–Pittsburgh Cerebral Performance Categories (CPC) scale,[12] and hospitalization outcomes (discharge + destination and death + cause of death). Hemodynamic monitoring of the patients was performed with routine invasive catheters. Blood pressure (BP) was measured using an arterial catheter (fluid-filled transducer Truwave, Edwards Lifesciences LLC, Irvine, CA), and PP was calculated as the difference between the systolic and diastolic BPs. The criterion for pulsatile blood flow was a PP of 15 mm Hg or higher.[10] All hemodynamic data, including the central venous pressure (measured using a fluid-filled transducer placed in a central vein; Truwave, Edwards Lifesciences LLC), heart rate, and oxygen saturation, were recorded as actual trend values during the microcirculatory monitoring. The core body temperature was measured using a urinary catheter. We also collected data from bedside measurements of the venous hemoglobin level, lactate level, and arterial acid-base balance (ABL90 FLEX, Radiometer Medical ApS, Brønshøj, Denmark) at the time closest to the time of the microcirculatory assessment; we recorded data on the actual ECMO setting (blood flow and rumps per minute) and administered vasopressors and inotropes, *i.e.*, noradrenaline and dobutamine.

Microcirculatory Assessment

Microcirculation was assessed using SDF technology (Microscan, Microvision Medical, Amsterdam, The Netherlands).[13] Microcirculatory measurements were obtained after admission to the intensive care unit as soon as a microcirculatory measurement specialist was available. All measurements were obtained within 72 hours of CA. The measurements were performed alternately by two trained specialists (P.K. and T.B.). The principles of SDF microcirculatory monitoring have been described previously.[14]

The microcirculation measurements were performed manually on the sublingual mucosa, which was regularly washed with normal saline to remove the saliva and prevent drying. At least five, 20 second video sequences from different parts of the sublingual mucosa were acquired.

The images were stored, and three images of appropriate quality per patient were analyzed offline by an outcome-blinded single investigator (P.K.) using dedicated software (Automated Vascular Analysis 3.1, Microvision Medical). The following parameters were recorded for the capillaries (*i.e.*, microvessels with diameters ≤ 20 μm): total and perfused capillary densities (TCD and PCD, respectively), proportion of perfused capillaries (PPC), the microvascular flow index (MFI), and the heterogeneity index (HI). The TCD was estimated as the ratio of the total capillary length in the selected region of interest to the area of that region. To evaluate the PCD and PPC, the vessels were classified according to microcirculation as perfused (*i.e.*, the blood flow was hyperdynamic, continuous, or sluggish) or nonperfused (*i.e.*, intermittent flow and nonflow). For the MFI quantification, the image was divided into four quadrants, and the blood flow in each quadrant was expressed on the following ordinal scale: 4, hyperdynamic; 3, continuous; 2, sluggish; 1, intermittent; and 0, no flow. The MFI was taken as the average score across all quadrants. The HI was counted from the three measurements from each patient as follows: the lowest MFI was subtracted from the highest MFI, and the result was divided by the mean MFI. Examples of the SDF imaging of the sublingual mucosa in our study groups are presented in **Figure 1** and as Videos 1–3 (Supplemental Digital Content, http://links.lww.com/ASAIO/A121, http://links.lww.com/ASAIO/A122, and http://links.lww.com/ASAIO/A123).

Statistical Analysis

The data were tested for normality with the Shapiro–Wilk normality test. The parametric data are presented as the means (standard error of the means, SEM), and the nonparametric data are provided as the medians (first-third quartile). In the comparisons of the patients and the healthy control subjects, normally distributed data were examined with t-tests, and non-normally distributed data were examined with Mann–Whitney *U* tests. The Mann–Whitney *U* test was also used to compare the P and L/N groups of patients because of the small numbers of patients in each group. Bonferroni corrections for multiple comparisons were applied in all cases to evaluate the significance of the microcirculatory results, and an overall $p \leq 0.05$ defined a significant result. Categorical variables were compared with Fisher's exact test.

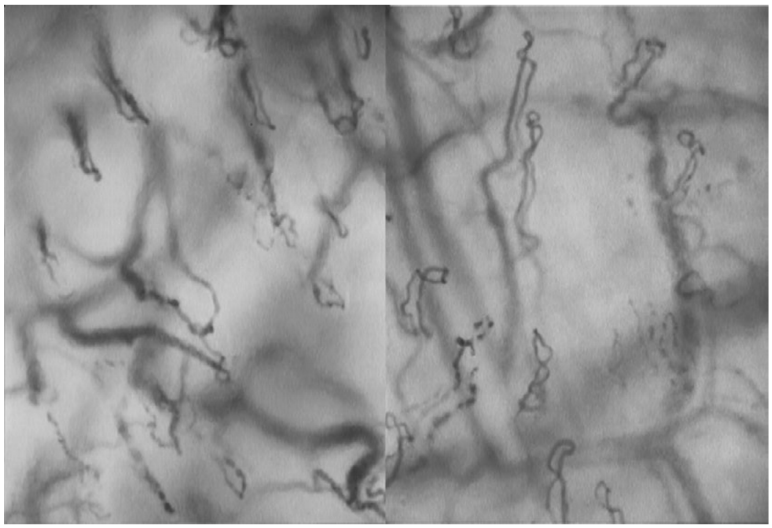

Figure 1. An example of Sidestream Dark Field microcirculatory imaging: Sublingual microcirculations in patients from the L/NP group (left) and P group (right). P, pulsatile; L/NP, low/nonpulsatile.

Results

Enrollment of Patients and Healthy Control Subjects

During the period from July 31, 2013 to April 18, 2016, 15 patients who were treated with ECPR after CA were initially included. Three patients were later excluded from the analysis for insufficient microcirculatory video image quality, the development of fulminant sepsis within a few hours of microcirculatory measurement, or IABP insertion. The other data from the excluded patients did not differ from those of the remaining patients. We enrolled 10 men and 2 women with a mean age of 53 (SEM 11) years and a mean time of ECMO implantation after collapse of 64 (SEM 23) minutes. The CA etiology was primarily acute myocardial infarction (nine patients), and three patients had myocarditis. Five patients were treated with target temperature management to 33°C, and the rest were treated to 36°C, but the actual temperature during microcirculatory monitoring was 36.4°C (35.9–36.5). Favorable outcomes (CPC 1–2) occurred in eight patients. Microcirculation was acquired 31 (SEM 18) hours after CA. The patients' data are summarized in **Table 1** and Table S1 (Supplemental Digital Content, http://links.lww.com/ASAIO/A124).

Twelve healthy volunteers were included in the control subjects group; with the exception of the history of mild hypertension and paroxysmal atrial fibrillation, none of the control subjects had any significant medical history of cardiovascular disease.

Except for PPC, which was significantly higher in the healthy volunteers (97.56% *vs.* 91.42%, $p = 0.007$), the microcirculatory variables differed only nonsignificantly between the control subjects group and patients (**Table 1**).

Pulsatile Versus Low/Nonpulsatile Blood Flow

In our study, seven patients (58%) were included in the P group (mean PP 30.6, SEM 11.5 mm Hg) and 5 patients (42%)

Table 1. Patient Data

	Patients	Control Subjects	*p*
Included	12	12	
Men	10	10	
Mean age (years)	53 (11)	46 (16)	
Pulsatile blood flow (PP ≥15 mm Hg)	7	-	
Outcome			
CPC scale 1–2	8	-	
CPC scale 4–5	4	-	
Mean time from CA to microcirculatory measurement (hours)	31 (18)	-	
TCD (mm/mm^2)	8.93 (6.65–10.19)	9.93 (8.82–12.20)	0.14
PCD (mm/mm^2)	8.13 (6.52–9.11)	9.59 (8.60–11.79)	0.03
PPC (%)	92.14 (86.29–96.57)	97.56 (96.56–98.97)	0.007*
MFI	2.71 (2.54–3.00)	3.00 (2.92–3.00)	0.03
HI	0.15 (0.00–0.20)	0.03 (0.00–0.05)	0.04

The microcirculatory variables describe capillaries (vessels with diameters ≤ 20 μm). The data are given as the mean (SEM), median (first-third quartile), or total numbers.

*Significant after applying the Bonferroni adjustment.

CA, cardiac arrest; CPC, Glasgow-Pittsburgh Cerebral Performance Categories; HI, heterogeneity index; MFI, microvascular flow index; PCD, perfused capillary density; PP, pulse pressure; PPC, proportion of perfused capillaries; SEM, standard error of the mean; TCD, total capillary density.

in the L/N group (mean PP 6.6, SEM 3.9 mm Hg). As expected, the L/N group exhibited a lower ejection fraction of the left ventricle at baseline, *i.e.*, after the implantation of ECMO, but there were no significant differences in age, sex, etiology, target temperature management, time to ECMO setup, time to microcirculatory measurement, neurologic and hospitalization outcome, vasopressor or inotrope use, core temperature, and acid-base balance or hemoglobin and lactate levels between the two groups (**Table 2**).

The microcirculatory variables did not differ significantly between the P and L/N groups. The capillary densities (TCD and PCD) tended toward higher values in the L/N group (9.72 *vs.* 6.66 mm/mm^2, $p = 0.25$ and 8.71 *vs.* 6.52 mm/mm^2, $p = 0.19$), whereas the PPC and the MFI tended toward higher values in the P group. However, all of these differences were statistically nonsignificant (**Table 2** and **Figure 2**).

Discussion

The microcirculation of our 12 patients with post-CA who were treated with ECPR was characterized by significantly lower PPC compared with the control subjects group of healthy volunteers. However, there were no differences in the TCD and PCD, the microcirculatory blood flow, or the heterogeneity of microcirculation. Concerning the sustained pulsatility during ECPR, we found no significant differences in microcirculation between the pulsatile *versus* low/nonpulsatile blood flow groups.

The level of microcirculatory compensation on ECMO support was surprisingly high in our patients, which might have several explanations. First, although the deterioration of sublingual microvascular variables (MFI and PVD) in post-CA setting has repeatedly been documented in patients with spontaneous circulation,[15,16] this temporary deterioration has been reported to diminish within 48 hours after CA,[17] which corresponds to the time of microcirculatory measurement in our patients who were reperfused by ECMO. This fact suggests that ECMO reperfusion in refractory CA may provide microcirculatory reperfusion characteristics similar to those of patients who attain ROSC. Consequently, ECMO is likely to attain reasonable microcirculatory variables in patients with post-CA (*i.e.*, regardless of the residual heart function). Den Uil *et al.*[18] demonstrated that mechanical circulatory support (*e.g.*, ECMO) in patients with cardiogenic shock improves and stabilizes the microvascular tissue perfusion; similarly, Lam and coworkers[19] documented that percutaneous left ventricular assist devices are responsible for the normalization of microcirculation in patients after ST-elevation myocardial infarction. Our small pilot study population does not allow us to draw any robust conclusions, but our results suggest that ECMO support may assure adequate microcirculatory flow in severely compromised patients.

The main goal of our pilot study was to compare microcirculation in patients with sustained pulsatile (P group) and low/nonpulsatile (L/N) blood flow groups, and we found no significant difference. Petroni *et al.*[8] similarly reported no changes in the microcirculatory variables in the case of added pulsatility after the stopping and restarting of IABP support in 12 patients who were treated with ECMO. On the other hand, we observed some trends in the sublingual microcirculation that

Table 2. Comparison of the P and L/NP Groups of Patients

	All Patients (N = 12)	P Group (N = 7)	L/N Group (N = 5)	*p*
TCD (mm/mm^2)	8.93 (6.65–10.19)	6.66 (6.39–9.84)	9.72 (8.41–10.17)	0.25
PCD (mm/mm^2)	8.13 (6.52–9.11)	6.52 (6.34–8.66)	8.71 (8.00–9.24)	0.19
PPC (%)	92.14 (86.29–96.57)	94.24 (88.62–98.21)	89.77 (84.43–93.69)	0.19
MFI	2.71 (2.54–3.00)	2.75 (2.67–3.00)	2.42 (2.42–2.83)	0.22
HI	0.15 (0.00–0.20)	0.09 (0–0.19)	0.18 (0.11–0.21)	0.42
Systolic BP (mm Hg)	87.6 (18.0)	93.9 (21.6)	78.8 (4.8)	0.19
Diastolic BP (mm Hg)	67.0 (13.3)	62.3 (16.6)	72.2 (4.1)	0.52
PP (mm Hg)	20.6 (15.2)	30.6 (11.5)	6.6 (3.9)	0.006
Ejection fraction of the left ventricle at baseline (%)	15.8 (10.3)	21.8 (9.1)	6.3 (4.3)	0.01
Mean arterial pressure (mm Hg)	73.5 (13.2)	72.4 (17.4)	75.0 (3.9)	0.81
Central venous pressure (mm Hg)	10.5 (3.9)	9.9 (4.1)	11.5 (6.1)	0.45
Pulse rate	81.7 (20.7)	87.7 (12.9)	72.0 (29.0)	0.25
Saturation O_2 (%)	100.0 (97.0–100.0)	100.0 (99.5–100.0)	97.3 (95.0–99.6)	0.22
ECMO flow (L/min)	4.63 (1.40)	4.28 (1.74)	5.13 (0.60)	0.42
ECMO (rumps per minute)	3740 (1304)	3284 (968)	4059 (470)	0.14
Core temperature (in the urinary bladder, °C)	36.40 (35.90–36.53)	36.50 (35.90–36.55)	36.30 (35.90–36.50)	0.75
Venous lactate (mmol/L)	3.10 (2.58–6.18)	2.70 (2.55–3.55)	5.90 (3.20–7.00)	0.29
Arterial pH	7.33 (0.06)	7.34 (0.06)	7.33 (0.06)	0.41
Arterial base excess (mmol/L)	–3.00 (–4.70 to 3.05)	4.80 (0.75–11.95)	–4.55 (–6.30 to –1.92)	NA
Hemoglobin (mg/dl)	9.70 (9.20–11.25)	9.40 (9.25–10.20)	11.70 (9.20–12.60)	0.33
Vasopressor therapy (intravenous noradrenaline)	10 (83%)	5 (71%)	5 (100%)	0.47
Inotropic therapy (intravenous dobutamine)	4 (33%)	3 (43%)	1 (20%)	0.58
Survival to discharge	7 (58%)	5 (71%)	2 (40%)	0.56
Favorable neurological outcome (CPC score 1–2)	8 (67%)	6 (86%)	2 (40%)	0.22
Time from CA to microcirculatory assessment (hours)	31 (18)	34 (19)	27 (17)	0.68

Pulsatile blood flow was defined as a PP ≥ 15 mm Hg. The microcirculatory variables describe capillaries (vessels with diameters ≤ 20 μm). The data are provided as the mean (SEM), median (first and third quartiles), or total number (percentage). NA: not analyzed because of insufficient data for statistical analysis.

BP, blood pressure; CA, cardiac arrest; HI, heterogeneity index; L/N, low/nonpulsatile group; MFI, microvascular flow index; P, pulsatile group; PCD, perfused capillary density; PP, pulse pressure; PPC, proportion of perfused capillaries; SEM, standard error of the mean; TCD, total capillary density.

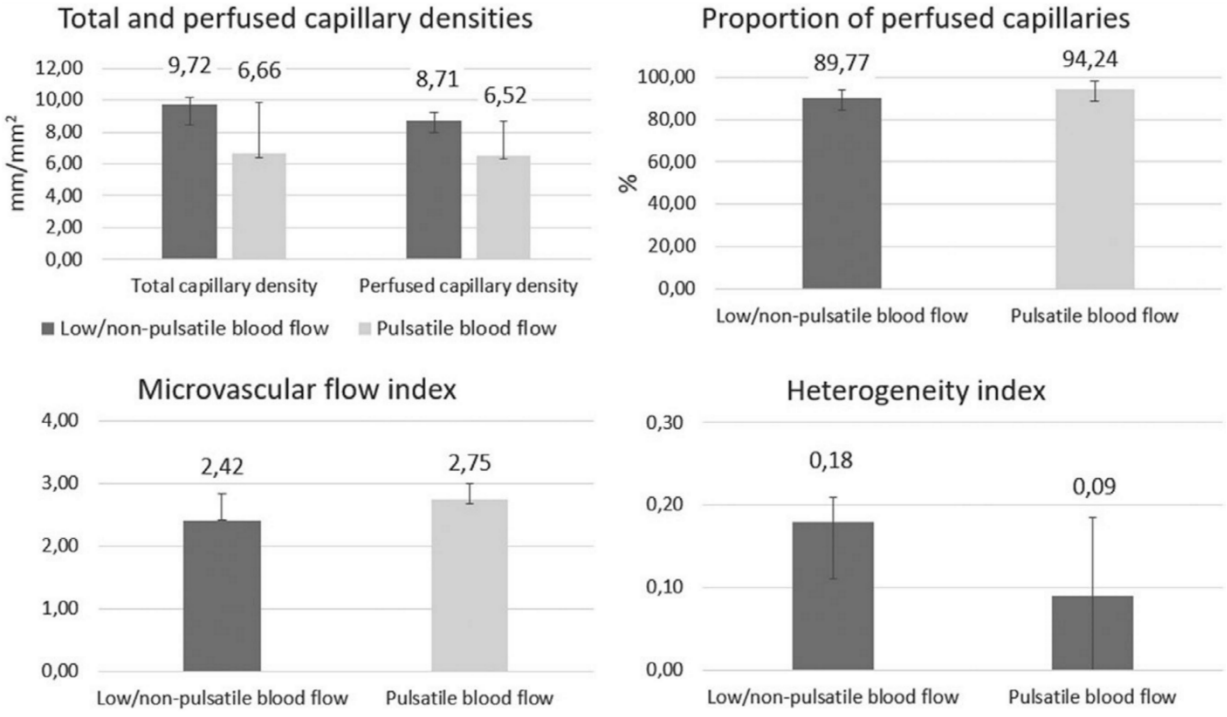

Figure 2. The differences in the microcirculatory parameters between the P and L/NP groups. The microcirculatory parameters of the small vessels (≤20 μm) are presented. The data are given as the median (the first and third quartiles are indicated by the error bars), and the differences between the groups are nonsignificant in all cases.

did not reach significance in our small study group: P group exhibited slightly higher MFI and PPC and lower HI. Surprisingly, the P group had also (nonsignificantly) lower capillary densities than the L/N group, where the TCD and PCD values were almost approaching the control subjects group (**Table 2**). This result might have been biased by different hemoglobin levels, which was nonsignificantly decreased in our P group (9.40 [9.25 – 10.20] *vs.* 11.70 [9.20 – 12.60], $p = 0.33$). Previous studies reported that hemoglobin level correlated with vessel density,[20] and amelioration of the vessel density (but not MFI) was observed after administration of blood transfusion.[21] Concerning other potential biasing factors as the use of vasoactive drugs and temperature management, we found no difference between our two groups. Moreover, the recent body of knowledge on the use of vasoactive drugs in the shock therapy did not prove beneficial effect on microcirculation above the mean arterial pressure of 65 mm Hg.[22] Similarly, no effects of temperature management on sublingual microcirculation in patients with post-CA have been reported.[16] Noticeable (but nonsignificant) is also the difference between the P and L/N groups in the discharge survival rate (71% *vs.* 40%, $p = 0.56$) and the favorable neurologic outcome (86% *vs.* 40%, $p = 0.22$; **Table 2**). The high survival rate and outcome of our selected patients are affected by the delay of the microcirculatory measurement after CA, which omitted those CA victims who died very early, and also by patients who were excluded from study because of the septic shock. Previous studies documented the link between patients' outcome and sublingual microcirculation[23]; however, our study was not aimed at this phenomenon.

Our current results actually disproved our hypothesis. We found no significant differences in the microcirculatory variables between the P and L/N groups of patients who were treated with ECPR. Furthermore, considering the comparisons with the healthy control subjects, our results suggest that ECMO may adequately restore the microcirculation in patients with refractory CA. Based on microcirculatory assessments, our results are in agreement with those of others[8] and may also support the opinion that routine insertion of an IABP to sustain pulsatility in low/nonpulsatile ECPR settings is probably not beneficial. However, the results of our pilot analysis require confirmation in a larger patient population.

There are several important limitations to our study. First, our study group was small and heterogeneous. Even though we found no statistical difference between the P and L/N groups in baseline characteristics (with exception of PP and ejection fraction of the left ventricle; **Table 2**), still this observational study included patients with different comorbidities, etiology of CA, duration of CPR, severity of cardiac dysfunction, and different therapy. Of note, because of logistic reasons, the microcirculatory measurements were taken at different intervals after CA (mean 31 hours, SEM 18 hours), which might have influenced the actual states of the recorded microcirculation. To divide our patients into P and L/N groups, we used the PP threshold value of 15 mm Hg as suggested by Undar and colleagues.[10] However, a general definition of pulsatile blood flow is lacking, and the parameters of sufficient pulsatility are still the subject of discussion in terms of maintaining adequate perfusion and avoiding complications related to continuous blood flow.[24] Finally, sublingual SDF imaging technology was used for the microcirculatory assessment. This measurement is dependent on the acquisition technique (*i.e.*, there are risks of pressure and movement artifacts) and the quality of

the semiautomatic evaluation. Nevertheless, the employment of the consensus criteria for microcirculatory assessments, as used in our study, has been reported to result in high inter- and intra-individual reliabilities.[25]

Conclusions

In this pilot study of the effects of spontaneous pulsatility on the microcirculation of patients with refractory CA, who were treated with ECPR, we did not observe any significant differences in microcirculation between the patients in the P and L/NP blood flow groups. Our results suggest that ECMO might provide sufficient microcirculatory blood flow in these patients. However, further research with larger patient populations is warranted.

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