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Tissue microcirculation in cardiac arrest setting

– impact of various methods of circulatory support

Tkáňová mikrocirkulace při srdeční zástavě

– vliv různých druhů oběhových podpor

Dissertation thesis

Supervising tutor:
Doc. MUDr. Jan Bělohlávek PhD.

Praha, 2018

Declaration:

I hereby declare that I wrote this dissertation thesis independently and that I cited all sources correctly. This dissertation contains no material that has been submitted previously for the award of any other academic degree or diploma.

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1 Introduction

1.1 Motivation

Cardiac arrest is one of the leading causes of mortality in the developed countries (Lippert F. K. et al., 2010), moreover, in survivors it is associated with a long hospitalization period and a high risk of cerebral ischemic complications. Therefore cardiac arrest represents significant socio-economic problem and in the Czech Republic the research targeted on cardiac arrest belongs to the National Priorities of Research, Development and Innovations (Research Development and Innovation Council, 2012).

The main goal of cardiopulmonary resuscitation and post-resuscitation care in cardiac arrest victims is to provide and ensure sufficient and stable blood circulation to supply tissues and organs with oxygen and nutrients. The monitoring of the effectiveness of such care, if feasible to be performed, is routinely restricted on monitoring of parameters of systemic blood circulation or indirect and non-specific indicators of the quality of peripheral microcirculation (serum lactate, diuresis etc.) (Dunser M. W. et al., 2013). However, it has been repeatedly documented that, alike patients suffering shock, the cardiac arrest victims may exhibit microcirculatory disorder despite satisfactory systemic circulation (Elbers P. W. et al., 2010, Koopmans M. et al., 2015, Omar Y. G. et al., 2013, Van Genderen M. E. et al., 2012). Such microcirculatory impairment may lead to underlying organ hypoxia and contribute to a post-cardiac arrest syndrome severity, a neurologic damage and to a development of multiorgan dysfunction syndrome. Therefore, microcirculatory monitoring and treatment strategies focused on microcirculation might improve the prognoses of cardiac arrest victims (De Backer D. and Durand A., 2014).

Evaluation of microcirculation has been enabled among others by the recent videomicroscopic methods: Orthogonal Polarization Spectral (OPS), Sidestream Dark Field (SDF), and Cytocam - Incident Dark Field (IDF) microscopy. These methods have proven their sensitivity to microcirculatory impairment in critical illness and many other states (i.e. in sepsis (Ince C., 2005), shock (Jung C. et al., 2015, Petroni T. et al., 2014, Tachon G. et al., 2014) or during cardiopulmonary bypass (Elbers P. W. et al., 2011, Koning N. J. et al., 2012, O'neil M. P. et al., 2012)). Furthermore, using these devices a link between microcirculation and patient´s outcome has been repeatedly documented (Den Uil C. A. et al., 2010, Fries M., Tang W., et al., 2006, Kara A. et al., 2016). Due to

their complete non-invasiveness, bed-side utilization, easy usage and low operating costs these devices might become an effective and useful tools for evaluating the microcirculation in cardiac arrest victims.

Despite these encouraging findings, there is only limited knowledge on microcirculatory changes during cardiac arrest and cardiopulmonary resuscitation; moreover, the information on microcirculatory changes in patients rescued by extracorporeal cardiopulmonary resuscitation is even scarcer.

1.2 Aims and main results

This dissertation thesis **"Tissue microcirculation in a pig model of cardiac arrest – the effect of different types of circulatory support"** was designed to study microcirculatory changes in cardiac arrest and under conventional and extracorporeal cardiopulmonary resuscitation in adults. It focused on sublingual microcirculatory changes visualized and evaluated by a recent non-invasive bedside videomicroscopic technology.

In a porcine model of cardiac arrest I demonstrated a decrease of the microcirculatory blood flow in cardiac arrest, which was improved after the resuscitation onset and the microcirculatory parameters during conventional cardiopulmonary resuscitation reached cca 59 to 85% of the prearrest values. The mean arterial blood pressure and carotid blood flow (as a surrogate marker of cerebral perfusion) and lactate (as a marker of peripheral perfusion) did not correlate or showed only weak correlation to microcirculatory parameters.

Microcirculation was also studied in adult cardiac arrest patients who did not achieve return of spontaneous circulation after prolonged conventional cardiopulmonary resuscitation and were finally rescued by extracorporeal membrane oxygenation. In an observational study I documented that microcirculatory parameters of the above mentioned patients significantly differed from the microcirculation of (age and sex matched) healthy volunteers, nevertheless, this difference was surprisingly small in total numbers. Preserved spontaneous pulsatility did not provide further improvement of microcirculation in patients under extracorporeal cardiopulmonary resuscitation in our study.

1.3 Structure

The chapter 2 (“State of the art”) briefly summarizes incidence, etiology, manifestation, therapy and prognoses of cardiac arrest (chapter 2.1) and overviews recent knowledge on microcirculatory architecture and its regulation (chapter 2.2). Further on, it discusses methods for microcirculatory evaluation with a special focus on videomicroscopic imaging technologies (chapter 2.3) and summarizes knowledge on microcirculatory changes in cardiac arrest, resuscitation and early post-resuscitation care (chapter 2.4 and attachment file NO 1).

Aims and hypotheses of this dissertation thesis will be declared in the chapter 3 and 4 respectively.

The main body of this thesis will be divided into two separate parts, which will cover methods and results of two separate studies: first, an experimental study on microcirculatory changes in a porcine model of cardiac arrest and resuscitation (chapter 5.1 and attachment files NO 2 and 3) and second, a clinical study on microcirculatory changes in adult patients suffering cardiac arrest, who were rescued and treated by extracorporeal membrane oxygenation (chapter 5.2 and attachment file NO 4).

The results of the above studies will be discussed in a perspective of current knowledge in the chapter 6.

2 State of the art

2.1 Cardiac arrest

2.1.1 Definition

Cardiac arrest (CA) is a pathophysiologic state defined as "the cessation of cardiac mechanical activity as confirmed by the absence of signs of circulation" (Jacobs I. et al., 2004). There are significant differences in etiology, incidence, therapy and prognoses between the in-hospital CA (IHCA) and out-of-hospital CA (OHCA) in adults, therefore these two groups are usually evaluated and studied separately.

2.1.2 Incidence

The incidence of adulthood cardiac arrest (and especially of IHCA) shows significant regional variability amongst countries, regions and even health-care providers, which can be partially explained by the population or hospital characteristics (Kolte D. et al., 2015). The average incidence of OHCA per 100,000 population per year is about 111 patients in the USA, 84 patients in the Europe and 224 patients in the Czech Republic (American Heart Association Inc, 2016, Grasner J. T. et al., 2016). The IHCA incidence is usually described as the incidence per number of hospital admissions and in the literature it is reported to range from one to five IHCA cases per 1,000 hospital admission (Sandroni C. et al., 2007). In the USA it reaches cca 209,000 events per year (American Heart Association Inc, 2016).

2.1.3 Etiology and manifestation

The etiology of CA is very heterogeneous. In the OHCA group the CA is dominantly of medical cause (in cca 91% of all cases (Grasner J. T. et al., 2016)), most of which is of a cardiac origin (caused by an ischemic cardiac disease, non-atherosclerotic disease of coronary arteries, cardiomyopathies, valvular heart disease, infiltrative and inflammatory myocardial disease, congenital heart disease or primary electrical abnormities). Other medical causes (non-traumatic bleeding, pulmonary embolism, malignancy, lung disease, suffocation, SIDS etc.) and trauma are infrequent (Engdahl J. et al., 2002). The causes of IHCA are also mostly related to a cardiac disease (with similar etiology to OHCA with a

higher incidence of rare diseases e.g. cardiac tamponade, amyloid hearth disease, post-transplantation acute hearth failure, pacemaker dysfunction etc.), followed by the frequent non-cardiac etiology (pulmonary embolism, generalized hypoxia in association with medical intervention and other lung diseases, aortic dissection, intoxication and adverse drug reactions, gastrointestinal bleeding, sepsis etc.) (Wallmuller C. et al., 2012). Previous respiratory problems, deterioration of mental status and hemodynamic instability are reported to occur several hours prior to the IHCA (Sandroni C. et al., 2007). In contrast, the OHCA is typically manifested by a sudden collapse.

2.1.4 Treatment

Despite the heterogeneous etiology, the treatment of CA (the life support) has to be initiated immediately and in the OHCA it is mostly initiated by laic witnesses. Therefore the guidelines for the basic life support have been simplified into a so called "chain of survival" (see Figure 1), which summarizes the vital steps for successful resuscitation:

- early recognition of CA and call for the emergency services;
- early initiation of the bystander cardiopulmonary resuscitation (CRP);
- early defibrillation by an automated external defibrillator;
- advanced life support provided by trained specialists and standardized post-resuscitation care (Perkins G. D. et al., 2015).

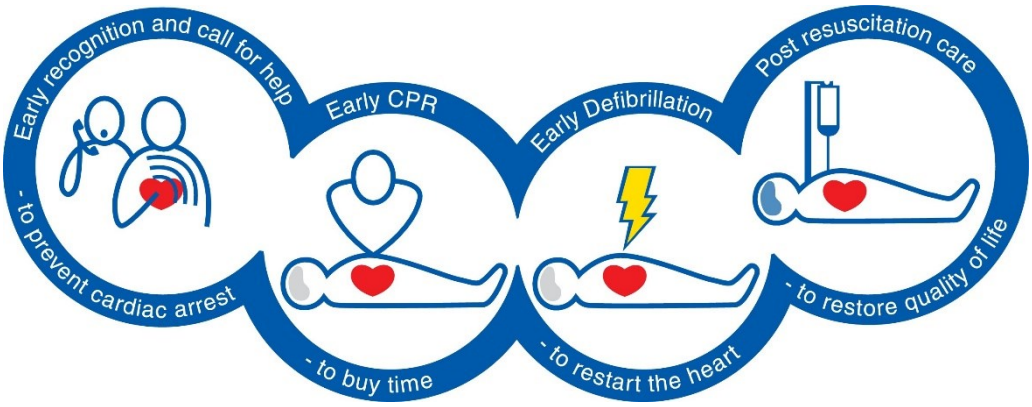

Figure 1: The chain of survival.

(reproduced from (Monsieurs K. G. et al., 2015)

The guidelines for the CPR and the post-resuscitation care are regularly revised and published by the International Liaison Committee on Resuscitation, followed by its member organizations i.e. European Resuscitation Council (Monsieurs K. G. et al., 2015).

A detailed description of the CPR, the advanced life support and the post-resuscitation care is beyond the scope of this manuscript; let me, however, mention the most important points related to this thesis:

The vital role in the CA treatment plays restitution of the arrested circulation and organ reperfusion. Until restoration of the spontaneous hearth beat and effective spontaneous circulation the systemic blood flow has to be supported by other means - in the terms of basic and advanced life support primarily by chest compressions. The compressions shall be provided manually; the use of mechanical chest compression devices shall be restricted on cases when providing sustained manual compressions is impractical or unsafe. The chest compressions shall be initiated as soon as possible in a collapsed unresponsive individual without normal breathing and continued with only brief intermissions for defibrillation attempts (in the case of a shockable hearth rhythm). The compressions shall be delivered onto the lower half of the sternum, into a depth of at least five but not more than six centimeters. The frequency shall range between 100 and 120 compressions per minute (Perkins G. D. et al., 2015).

In special circumstances an optional reperfusion with veno-arterial extracorporeal membrane oxygenation (VA ECMO) has been introduced (Belohlavek J. et al., 2012, Haas N. L. et al., 2017, Thiagarajan R. R. et al., 2009). This extracorporeal CPR (ECPR) increased chances for beneficial outcome for those patients with potentially reversible cause of CA, who did not gain ROSC after conventional CPR (Jung C. et al., 2016). However, parameters which shall indicate ECPR onset and guide the therapy are still not clear and there is an urgent call for the further research (Kim S. J. et al., 2016, Perkins G. D. et al., 2015). One of the pertinent topics in this field is also the role and applicability of added pulsatility. The added pulsatility generated by intra-aortic balloon contrapulsation (IABP) was not found beneficial for patients suffering cardiogenic shock (Thiele H. et al., 2012), nevertheless it might still be an option for patients rescued by ECPR.

2.1.5 Prognoses

The survival rate of the CA victims remains poor despite ongoing advancements of medical care, emergency services and laic education. In average only cca 10% of OHCA patients (those, in whom the CPR was started) and only 31% of patients delivered to hospital (with ROSC or ongoing CPR) survive at least 30 days after CA or to the hospital

discharge (Grasner J. T. et al., 2016). In IHCA the survival reaches cca 24% (American Heart Association Inc, 2016).

2.2 Microcirculation and its regulation

Microcirculation represents the actual "endpoint" of the circulatory system and therefore it may serve as a potential target for monitoring of the patient´s circulatory stability and effect of delivered circulatory support (Dunser M. W. et al., 2013): it has been shown that persistent alteration of peripheral microcirculation in critically ill patients may predict unfavorable outcome with a high sensitivity and specificity (Den Uil C. A. et al., 2010, Fries M., Tang W., et al., 2006, Kara A. et al., 2016). In contrary, those patients, whose peripheral microcirculation improved during the treatment administration, showed satisfactory survival with a good neurological outcome (Den Uil C. A. et al., 2010, Van Genderen M. E. et al., 2012). Understanding the changes and regulation of microcirculation may therefore be crucial in the care of critically ill patients, which is true also for the victims of cardiac arrest.

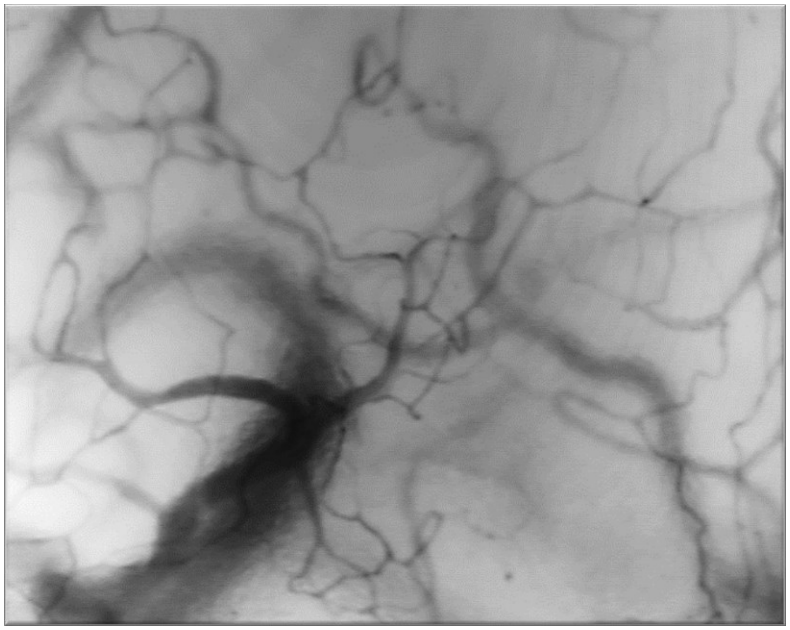

Figure 2: Branching system of the microcirculation. The image was captured by SDF imaging technology (Microvision Medical, Amsterdam, The Netherlands).

Microcirculation is adapted to transmit oxygen and nutrients from the bloodstream to tissues. Nevertheless, in various organs the microcirculatory network serves also other purposes (such as thermoregulation in the skin or blood filtration in the kidneys). The

local function of the microcirculation is vital for the local microcirculatory architecture and regulation. Microcirculation consists of a branching system of arterioles, capillaries and venules of diameter from cca 8 μm in the narrow capillaries to 100 μm (Massey M. J. and Shapiro N. I., 2016). The capillaries not only connect arterial and venous part of the circulation, but they form a complex system with a number of interconnections, parallel branches and thoroughfare channels (see Figure 2).

Microvascular blood flow in organs is relatively independent of the systemic blood pressure to satisfy the fluctuating metabolic demands of the tissues. There has been identified large number of regulatory pathways (such as flow-mediated, metabolic, autonomic neural, myogenic or hormonal regulation), which influence microcirculation under physiologic and pathophysiologic circumstances. These pathways lead to enhancement or reduction of the local microcirculatory blood flow by releasing endothelial derived factors (e.g. nitric oxide, prostacyclin, endothelin-1 or thromboxane A2) that migrate to the underlying smooth muscle and elicit its relaxation or contraction respectively. Nevertheless, the endothelial derived factors may have also other paracrine effects on the smooth muscle cells of the vascular walls or surrounding parenchymal tissue such as hypertrophy, fibrosis, proliferation, apoptosis, thrombosis or enhancement of inflammation (Gutterman D. D. et al., 2016). Importantly, the mediators as well as their impact on vascular tone (dilation or relaxation) may vary within organs and tissues, moreover heterogeneity of a mediator effect was observed even along the length of a single vessel (Penny W. F. et al., 1995). Further on, smooth muscle cell contraction might be also transmitted via electrical coupling of the neighbor cells.

To help us understand such a complex system of microcirculatory regulation many theoretical models of microvascular hemodynamics and cellular responses have been created (Secomb T. W., 2008). However, the interactions amongst regulatory pathways and the complex function of the microcirculation have not been completely elucidated.

The key effector in the microcirculatory regulation, as mentioned above, is represented by smooth muscle lawyer in the arteriolar wall. According to an important principle, which might be expressed by the Poiseuille´s law, even minor changes of the vessel diameter will affect the volume flow significantly:

(1) $$Q = (\pi \Delta P r^4)/(8L \eta),$$

where Q represents the volume flow rate, ΔP is the pressure drop, r expresses the radius and L the length of the examined segment and η is the blood viscosity.

Thus the smooth muscle lawyers of the feeding and terminal arterioles control blood influx into the daughter capillaries through their dilation or contraction and affect the number of perfused capillaries. The opening of the capillary network affects the functional surface area for the oxygen and nutrients exchange as well as the distances for their diffusion. (Segal S. S., 2005).

Another important effectors of the microflow regulation are pericytes - contractile cells associated with capillaries and post-capillary venules (Hirschi K. K. and D'amore P. A., 1996). Pericytes have been found to modulate diameter of capillaries in vitro and their impact on regulation of capillary blood flow is the subject of current research (Hamilton N. B. et al., 2010).

2.2.1 Microcirculatory assessment

The peripheral microcirculation has been traditionally evaluated by (mostly indirect and non-specific) laboratory and clinical methods such as assessment of serum lactate and diuresis, capillary refill time etc. A growing interest in microcirculatory evaluation brought almost a revolution of technologies and methods for microcirculatory evaluation; still, only a limited number of methods is suitable for microcirculatory monitoring at the bedside (Dunser M. W. et al., 2013). The detailed description of the technologies for microcirculatory monitoring and their comparison have been published before (De Backer D. and Durand A., 2014, Eriksson S N. J., Sturesson C, 2014, Tafner P. F. A. et al., 2017). A brief summary of the most important methods, along with their strong points and major limitations, is listed below.

2.2.2 NIRS

Near infrared spectroscopy (NIRS) is able to measure fractions of oxy- and deoxy-hemoglobin in the tissue and therefore enables regional tissue oxygen saturation (StO2) assessment. The near-infrared light penetrates few centimeters into the tissue and therefore NIRS is frequently employed to measure cerebral or muscle (on thenar eminence) StO2. NIRS may be used in combination with dynamic tests (i.e. vascular

occlusion test to assess microvascular reserve). Nevertheless, NIRS measurement is strongly affected by the variation of structures under the measuring probe, therefore it is used to assess the course of microcirculatory changes in an individual rather than to compare two subjects. Moreover it cannot distinguish arterial, venous or capillary perfusion.

2.2.3 Laser Doppler flowmetry

Laser Doppler flowmetry (or Laser Doppler perfusion imaging), which is based on the Doppler shift, measures an average blood perfusion in cca 1 mm^3 of the examined tissue. It has been employed in examination of the skin pathologies as well as in functional tests. The flowmetry cannot distinguish perfusion of larger vessels (and blood flow shunting through arterio-venous shunts) from capillary perfusion.

2.2.4 Assessment of partial oxygen pressure

Partial oxygen pressure assessment may serve for indirect evaluation of tissue perfusion. Continuous non-invasive measurement is performed by transcutaneous sensors (PtcO2) and it can be utilized not only to track the changes of perfusion (oxygenation) in time, but also to evaluate perfusion quality by oxygen challenge test in subjects with normal lung function. (A temporal increase of inhaled FiO2 will lead to parallel elevation of PtcO2. The lack of PtcO2 elevation may suggest altered tissue perfusion.)

2.2.5 Videomicroscopic imaging

Videomicroscopic imaging technologies enable direct real-time observation of the microcirculation and represent recent means for microcirculatory monitoring. The gold standard method of microcirculatory assessment was declared the intravital microscopy, which allows visualization of microvessels and circulating cells (such as red and white blood cells and platelets). After coupling with dyes it enables also visualization of vessels without cells (filled only by the plasma), direct measurement of glycocalyx lawyer as well as measurement of the oxygen tension, nitric oxide or reactive oxygen species. However, the use of dyes is restricted only for the animal experimental research. In human research the intravital microscopy may be employed only in the form of “nail-fold microscopy”, as the thickness of nail-fold capillaries allows transillumination. The major limitation of the nail-fold microscopy is, nevertheless, its sensitivity to room temperature and

vasopressors, which prevents the use of this modality in critically ill (Salgado D. R. et al., 2010).

The first videomicroscopic method suitable for the direct bedside use, the Orthogonal Polarization Spectral (OPS) imaging, has been introduced in 1999 by Cytometrics, Inc., Philadelphia, PA, USA. It uses polarized light of specific wavelength (550 nanometers), which is emitted to the investigated surface. Most of the light is reflected back remaining polarized, but the light which penetrates deeply into the tissue is depolarized by multiple scattering events and is finally either absorbed in the hemoglobin of red blood cells or reflected back to the analyzer. The analyzer is orthogonal (90 degrees) to the polarizer and thus it is used to eliminate the surface reflection and to let the depolarized light in to the videocamera. This way the image of red blood cells moving within microvessels is captured, which allows observation of the microcirculation on accessible surfaces (e.g. on oral mucosa). OPS imaging was validated in several animal and human studies through comparison with the intravital microscopy (Groner W. et al., 1999).

Figure 3: The MicroScan. (reproduced from MicroVision Medical http://www.microvisionmedical.com/)

Sidestream Dark Field (SDF) imaging, based on LED-technology and incorporated in Microscan Video Microscope (Microvision Medical, Amsterdam, The Netherlands; see Figure 3) has since 2007 represented an improved technique to visualize microcirculation. In SDF modality a sensing light guide is surrounded by concentrically placed LED light-emitting diodes; thus separation of optical paths eliminates direct surface reflections. The probe, covered by a disposable cap, is placed directly on tissue surface. This placement enables the light to penetrate deeper into tissue, where it is again either absorbed by

hemoglobin or reflected back to the camera (see Figure 4). Both methods, OPS and SDF imaging, provide similar quantitative data regarding vessel diameters and velocities of red blood cells. However, SDF imaging allows higher image quality and reduces image blurring (Eriksson S N. J., Sturesson C, 2014, Goedhart P. T. et al., 2007). On the other hand, both methods require further off-line analysis of the captured video-images (see below).

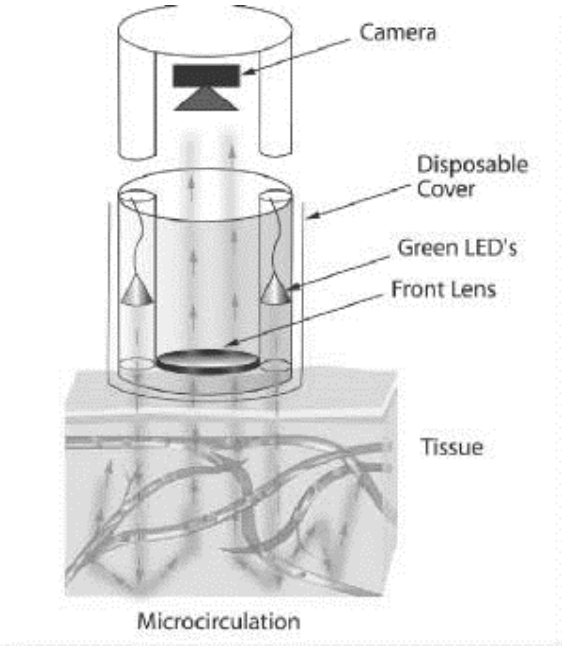

Figure 4: The technology of MicroScan camera (reproduced from MicroVision Medical http://www.microvisionmedical.com/).

Recent innovation of microcirculatory imaging, Incident Dark Field (IDF) imaging represents the third generation of videomicroscopic technology. It was presented in 2015 in the form of CytoCam device (Aykut G. et al., 2015), which consists of a small pen-like probe covered by a disposable cap and a computer unit. The probe is equipped with a Led-diode-based light emitter and videocamera with the high resolution lens system. Computer unit of CytoCam allows synchronization of the emitted light and image capturing, automated focusing and also full digitalization of the record by means of a high density microchip. Full digitalization enables immediate direct automatic evaluation of the obtained microcirculatory images, which is the key innovation. However the parameters obtained from the automatic evaluation are not fully comparable with those from the manual analysis (Carsetti A. et al., 2016). In a comparative study in healthy

volunteers the IDF method showed better focus and contrast of the captured images and it provided higher image resolution in comparison to SDF imaging (the CytoCam-IDF detected 30% more capillaries in the same location than the SDF) (Aykut G. et al., 2015, Gilbert-Kawai E. et al., 2016).

For the off-line image analysis, which is essential for OPS and SDF microcirculatory evaluation, a dedicated software for semiautomatic analysis is used. Various scores for microcirculatory analysis have been developed, but the published consensus (De Backer D. et al., 2007) suggested assessment of the following parameters: total vessel density (TVD), perfused vessel density (PVD), proportion of perfused vessels (PPV), the microvascular flow index (MFI) and the heterogeneity index (HI). All these indexes are calculated separately for small vessels (microvessels of diameter ≤ 20 µm) and other vessels (> 20 µm) (an example of a stabilized picture with identified microvessels is showed in Figure 5).

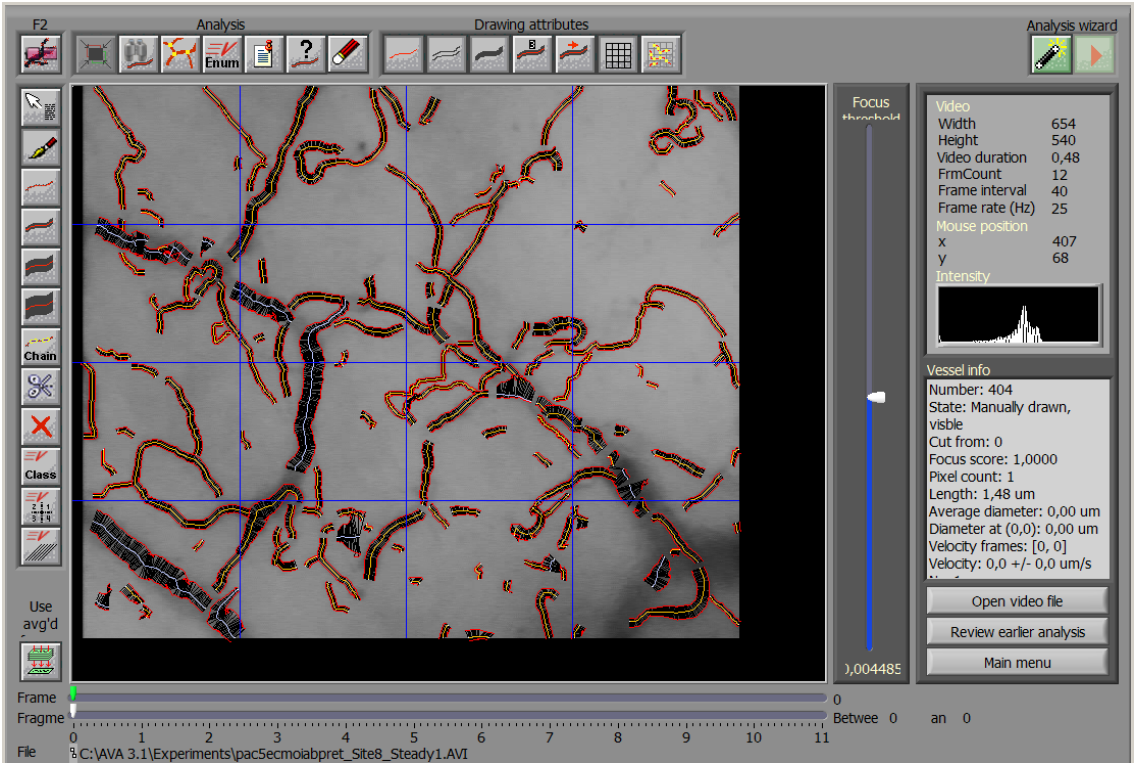

Figure 5: Off-line analysis of a videoimage captured by SDF imaging: vessels are detected and indicated by yellow (capillaries and small vessels under 20 µm) or blue lines (other vessels larger than 20 µm). Performed by AVA 3.1 software (Microvision Medical, Amsterdam, The Netherlands).

TVD is estimated as the ratio of total vessel length in the image and area selected as a region of interest. To evaluate the PVD and PPV, vessels are classified according to the 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 the 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. MFI represents the average score of all quadrants. Finally, the heterogeneity index is calculated after measuring the MFI in three to five images per site. The difference between the highest MFI minus the lowest MFI and divided by the mean MFI gives heterogeneity index. These parameters describe particularly the sublingual region, but they are suitable for other regions of interest too. An exception creates the intestinal mucosa, for which modified parameters were suggested (Lehmann C. et al., 2014).

Currently, the second international consensus on sublingual microcirculatory assessment (Ince C. et al., 2018) has been published, suggesting several modifications of the former guidelines (i.e. omiting hyperdynamic flow in the semiquantitative blood flow evaluation score) and routine use of image-quality score.

Videomicroscopic techniques have enabled real-time visualization of the microcirculation at the bedside and due to their noninvasive and simple use they have been employed in many medical fields including critical care, i.e. in patients with hemorrhagic shock (Tachon G. et al., 2014), sepsis (Pranskunas A. et al., 2012), in neonatology (Top A. P. et al., 2012), dermatology (Szczesny G. et al., 2001), transplantation medicine (Hardwicke J. T. et al., 2014), surgery (Bansch P. et al., 2014), obstetrics (Cornette J. et al., 2014) and in both experimental and clinical cardiac arrest (Fries M., Tang W., et al., 2006, Krupickova P., Huptych M., et al., 2016, Krupickova P., Mlcek M., et al., 2016, Van Genderen M. E. et al., 2012).

However, there are inevitably some limitations: OPS, SDF and IDF imaging techniques are sensitive to movement and pressure artefacts, are unable to measure high blood flow velocities and are applicable only to suitable tissue surfaces (accessible mucosa of oral cavity, conjunctiva, intestinal stoma or thin capsule of solid organs). Another important limitation represents the time consuming manual offline analysis in OPS and SDF technology, which includes also semiquantitative assessment of microflow (however, there have been attempts for development of simplified scoring system (Naumann D. N.

et al., 2016) or rapid and fully automatically analyzing software (Bezemer R. et al., 2011)). To reduce the risk of biasing results with limiting factors, the consensual criteria for image acquisition and analysis have been published (De Backer D. et al., 2007, Ince C. et al., 2018, Lehmann C. et al., 2014) and several studies evaluating reproducibility of microcirculatory measures showed sufficient reproducibility with low inter- and intra-observer variability in consensual parameters (Hubble S. M. et al., 2009, Petersen S. M. et al., 2014).

2.3 Videomicroscopy in cardiac arrest setting

We have summarized and published a detailed literature review of videomicroscopic studies, which discusses recent body of knowledge on the microcirculatory changes during CA, resuscitation (including ECPR) and post-cardiac arrest care. For the manuscript please see the Attachment file No 1 (Krupickova P. et al., 2017).

In brief, during CA and CPR sublingual microcirculation is significantly deteriorated along with systemic blood flow, but due to its local and systemic regulatory pathways microcirculation might exhibit different reactivity in comparison to systemic circulation. After achieving ROSC sublingual microcirculation ameliorates, but it does not reach the prearrest state despite satisfactory systemic hemodynamics. This microcirculatory impairment is connected to post-resuscitation “sepsis like” syndrome and its severity might be predictive of the outcome in post-cardiac arrest patients. Interestingly, the cerebral cortical microcirculation was (in a rat model) reported to be fully restored despite signs of neuronal death.

The information on microcirculatory function after reperfusion with ECPR is still lacking; however, in patients with myocardial infarction suffering from cardiogenic shock the microcirculatory dysfunction is to some extent resolved by ECMO and the remaining alteration of sublingual microcirculatory parameters has been found a potential predictor of mortality.

2.4 Conclusion of the analysis

Based on the above analysis, cardiac arrest remains with its significant morbidity and mortality an important socio-economic problem. Current research suggests that

microcirculatory disorder in critically ill patients may be connected with patients´ outcome which is also true for the victims of cardiac arrest. It has been documented that amelioration of microcirculatory alterations was connected with better prognoses. Therefore, microcirculation represents potential target for clinical state and therapy effect monitoring in cardiac arrest victims.

Direct microcirculatory monitoring at the bedside has been enabled by the non-invasive tools, e.g. videomicroscopic imaging techniques, which were successfully employed in patients under CPR as well as in post-cardiac arrest patients. Moreover, a connection between parameters of semiautomatic analysis of microcirculation and prognoses (with sufficient sensitivity and specificity) has been documented.

However, there is still only limited body of knowledge on the microcirculatory function in cardiac arrest setting, which is true also for the role and impact of circulatory supports. The course of microcirculatory changes during cardiac arrest and CPR has been already described in a porcine model, however only for microvascular flow, whereas changes of microvascular density and other parameters have not been described before. Similarly, the course of microcirculatory changes during ECPR, including impact of pulsatility on microcirculation have not been stated before.

Therefore, I decided to focus on microcirculatory changes in cardiac arrest setting, especially on the effect of circulatory supports (mechanical chest compressions and veno-arterial ECMO) on microcirculation. I chose to investigate microcirculation by the Sidestream Dark Field imaging, which represented the most innovative videomicroscopic technology at the time of the research onset (2013).

3 Aims

Based on the above stated analysis and based on the characteristics of the selected measuring technology (Sidestream Dark Field imaging) I specified and defined the following goals:

1. to asses changes of microcirculatory parameters during CA and CPR
    1.1. to state clinical hypotheses
    1.2. to find a suitable model of cardiac arrest
    1.3. to design a protocol of an experiment
    1.4. to verify feasibility of SDF imaging during CPR
    1.5. to perform experiments in order to test the hypotheses
    1.6. to analyze the obtained data, set and discuss the results and to formulate conclusions

2. to evaluate the microcirculation in patients rescued by ECPR
    2.1. to state clinical hypotheses
    2.2. to design a protocol of a clinical study
    2.3. to verify feasibility of microcirculatory assessment in ECPR patients
    2.4. to perform the measurements and collect the data
    2.5. to analyze the obtained data, set and discuss the results and to formulate conclusions

4 Clinical hypotheses

1.1. Parameters of peripheral microcirculation do change over time during cardiac arrest and cardiopulmonary resuscitation.

1.2. Parameters of peripheral microcirculation do not correlate to parameters of systemic blood flow (mean arterial pressure or carotid blood flow) during cardiac arrest and cardiopulmonary resuscitation.

2.1. There are significant differences in parameters of microcirculation between patients rescued by extracorporeal cardiopulmonary resuscitation and healthy volunteers.

2.2. There are significant differences in parameters of microcirculation between patients rescued by extracorporeal cardiopulmonary resuscitation with spontaneously pulsatile blood flow (pulse pressure over 15 mmHg) versus patients with low pulsatile or non-pulsatile blood flow.

5 Methods and results

The defined hypotheses created the background for two studies, which were in detail published before (Attachment file No 3 (Krupickova P., Mlcek M., et al., 2016), Attachment file No 4 (Krupickova P., Huptych M., et al., 2016)) and which are in brief described below. An important part of our current research was performed in a pig experimental model. The detailed description of our model including preparation and procedures has been published as well in a separate manuscript (Attachment file No 2 (Mlcek M. et al., 2016)).

5.1 Changes of microcirculatory parameters during CA and CPR

To evaluate the microcirculatory changes during CA and CPR and in order to study the correlation between microcirculation and systemic circulation we arranged an experimental study in a porcine model of CA. We hypothesized that microcirculation will change during the study protocol and that it will not directly correlate to parameters of systemic circulation.

5.1.1 Model of cardiac arrest

Due to the ethical reasons and to the technical feasibility the studies in CA and CPR are often conducted in an animal model. A suitable animal model in this regard is a domestic pig with its anatomical and physiological characteristics analogous to humans. Such analogy is particularly important in experiments that are employing medical technologies (such as mechanical chest compressor system); moreover, it enables subsequent interpretation of the results and their comparison with the clinical data in humans.

Therefore, we designed a study on microcirculation in a pig model of refractory CA. The experiments were conducted in cooperation with the research team of the Cardiac Electrophysiology Experimental Laboratory of the Institute of Physiology, The First Faculty of Medicine, Charles University in Prague. To fulfil the criteria of the humane use of animals in scientific research (the three Rs) we combined several research goals together and minimized the number of the used animals. The study was conducted with the approval of the First Faculty of Medicine Animal Care and Use Committee, in

accordance with Act No 246/1992 Coll., on the protection of animals against cruelty. For the detailed description of the animal preparation and measurement methods please see the attached manuscript (Attachment file No 2 (Mlcek M. et al., 2016)).

5.1.2 Experimental protocol

We designed the following experimental protocol: In an anesthetized pig, after previous invasive procedures and subsequent 15 minutes of stabilization, we induced ventricular fibrillation (VF) by the means of right ventricular stimulation. The untreated CA lasted for 3 minutes and was followed by 5 minutes of mechanical cardiopulmonary resuscitation using LUCAS device (The LUCAS® Chest Compression System, Physio-Control Inc, Redmond, CA, USA).

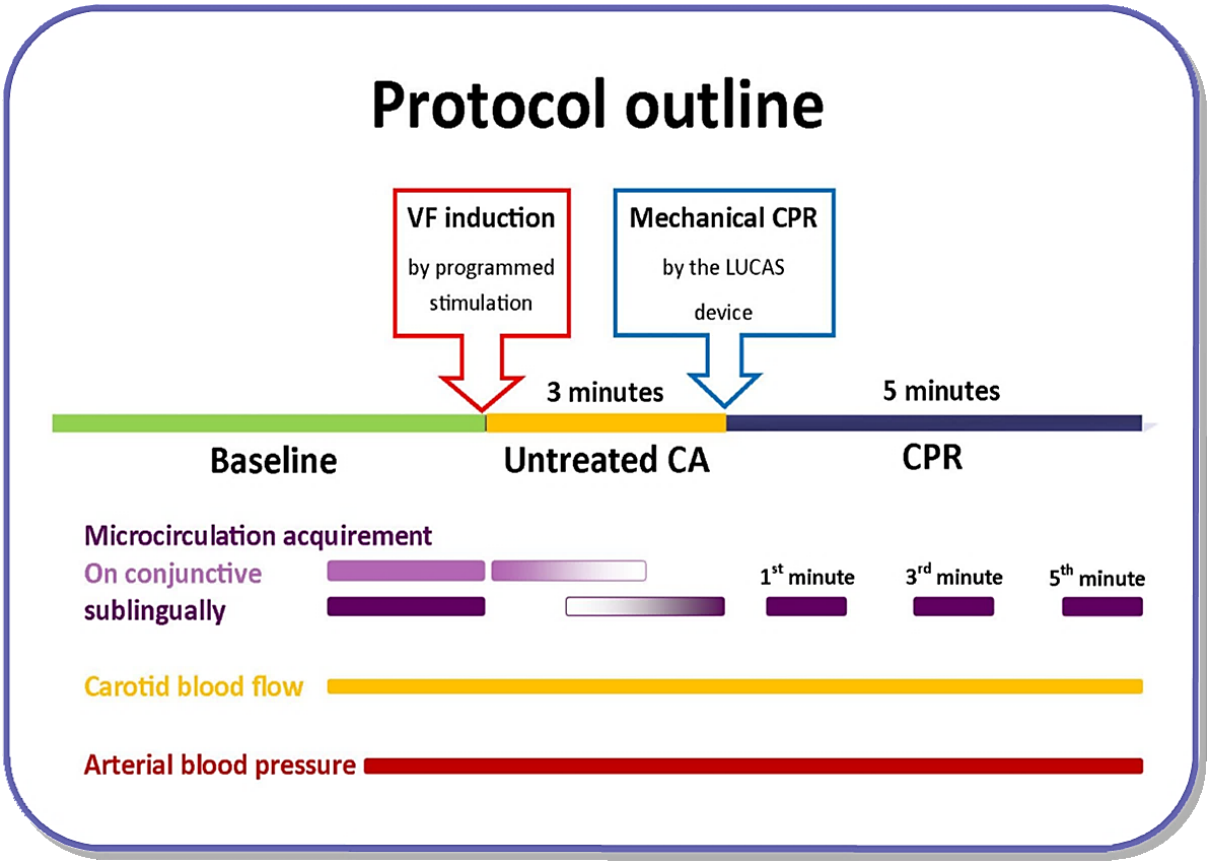

Figure 6: A protocol of the experimental study in a porcine model of cardiac arrest (reproduced from (Krupickova P., Mlcek M., et al., 2016).

Microcirculation was assessed by the SDF microscope sublingually (which is the widely used approach of microcirculatory evaluation, see chapter 2.3.) and on conjunctivae (to

evaluate peripheral perfusion supplied from the internal carotid and thus close to the cerebral microcirculation).

According to the recommendations we aimed to obtain 5 video-images of 6 second-duration from different parts of the monitored area (sublingual mucosa or conjunctiva) in the following time points:

1. in the baseline;
2. in the first and the second minute of CA on the conjunctiva and during the second and the third minute of CA sublingually;
3. in the first, the third and the fifth minute of CPR on the both sites.

See the protocol schema in Figure 6.

Continuous thorough hemodynamic monitoring including carotid blood flow (CBF) and mean arterial blood pressure (ABP) was performed during the whole experiment. Blood samples for the assessment of blood lactate, hemoglobin and acid-base balance were taken at the end of the baseline and the CPR and analyzed by a bedside analyzer. Further on, we monitored cerebral and peripheral temperature by thermocouples inserted into the brain and an artery.

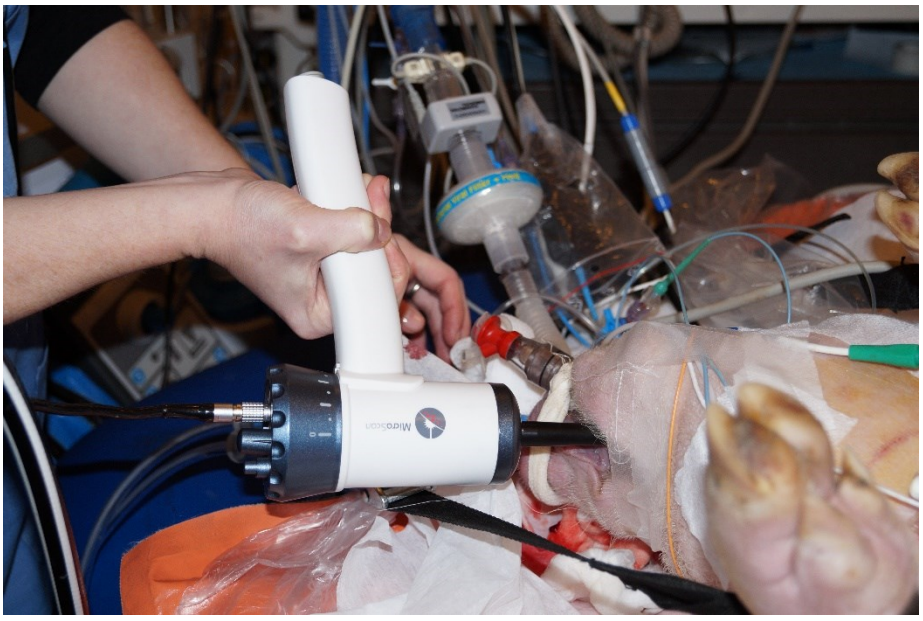

Figure 7: On scene: microcirculatory monitoring during experiment in a pig.

After finishing of the above described protocol, the experimental animal was subjected to a further advanced protocol, which is not covered in this thesis. At the end of the advanced experiment, the animal was euthanized.

The obtained video-images from microcirculatory monitoring were stored and analyzed offline. During the analysis the images were controlled for the image quality and only images of sufficient quality were evaluated (Massey M. J. et al., 2013, Massey M. J. and Shapiro N. I., 2016). Three images per time point were randomly selected for the evaluation. In case of dynamically changing data (CA and CPR data) we selected the first image, the middle one and the last one (i.e. one random image from the first, the third and the fifth minute of the CPR interval). In case that there were not three images of appropriate quality per time point, minimum of two images was set sufficient for analysis. Subsequently, the chosen images were blinded and evaluated according to the recommendations (see chapter 2.3.). The following microcirculatory indexes were set separately for small vessels (capillaries and vessels of diameter $\leq$ 20 μm) and other vessels: TVD, PVD, PPV, MFI and HI. The results were analyzed by the predefined statistical methods. For the correlation of microcirculation to systemic hemodynamics we used the actual values of carotid blood flow during the microcirculatory monitoring and values of the mean arterial blood pressure averaged in the one-minute intervals. Further on, we correlated microcirculatory parameters to lactate as a surrogate marker for the peripheral perfusion.

5.1.3 Statistical methods

The number of included animals was power calculated for the purposes of the advanced protocol, but it was used also in our microcirculatory sub-study, because the number of animals corresponded well with other similar studies (Fries M., Weil M. H., et al., 2006). To test the normality of the obtained data we used Shapiro–Wilk test (Ghasemi A. and Zahediasl S., 2012). Parametric data were presented as mean (± sample standard deviation, SSD), the non-parametric data were expressed as median (the first and the third quartile).

In order to test the No 1.1. hypothesis we used Friedman test (we were testing the difference amongst baseline, CA and CPR microcirculatory values). The post hoc analysis was performed by Wilcoxon test with the Bonferroni correction for multiple

comparisons. Parametric data from baseline and CPR (i.e. ABP, CBF and lactate level) were compared by the paired t test.

To test the correlation of microcirculatory data to ABP, CBF, temperature, lactate and HGB level we employed the Spearman's Rank Correlation Coefficient (Spearman's ρ), Bonferroni correction for multiple correlations was applied (Curtin F. and Schulz P., 1998).

The 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).

5.1.4 Experiments and results

A total number of 18 healthy female crossbred pigs of the weight 50 ± 3 kg was used in this study. The baseline characteristics as well as the changes of the monitored variables are summarized in the Table 1. Microcirculation was monitored by a single investigator according to the experimental protocol, however, the conjunctival microcirculatory data were of a very poor quality during the CPR. Therefore, only sublingual microcirculation was evaluated.

Sublingual microcirculation of the small vessels was significantly deteriorated during the CA. Nevertheless, the microcirculation did not stun during the second and the third minute of CA in contrast to systemic circulatory variables. After initiation of CPR the microcirculation was ameliorated and reached 59–85 % of the baseline values. In other vessels (with diameter above 20 μm) the total vessel density remained preserved, but the microflow changes were similar to the small vessels (see Table 2, Figure 8 and Figure 9).

After correlation of microcirculation to global hemodynamic variables we found only weak to moderate (i.e. Spearman's ρ 0.02–0.51) correlation between microcirculation and mean arterial blood pressure, carotid blood flow or lactate. Moreover, this correlation remained non-significant after adjustment for multiple correlations.

The detailed description of this study and its results have been published in an attached manuscript (Attachment file No 3 (Krupickova P., Mlcek M., et al., 2016)).

Table 1: Hemodynamic parameters, temperature, hemoglobin and lactate levels during baseline and cardiopulmonary resuscitation (CPR). Data are given as mean ± SSD. *(reproduced from (Krupickova P., Mlcek M., et al., 2016).*

	Baseline	CPR	p-value of paired t-test
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 x 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

Table 2: Median values of the microcirculatory variables during baseline, cardiac arrest (CA) and cardiopulmonary resuscitation (CPR) in the porcine model of cardiac arrest: total and perfused vessel density (TVD, PVD), proportion of perfused vessels (PPV), microvascular flow index (MFI) and heterogeneity index (HI). (reproduced from (Krupickova P., Mlcek M., et al., 2016).

	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
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

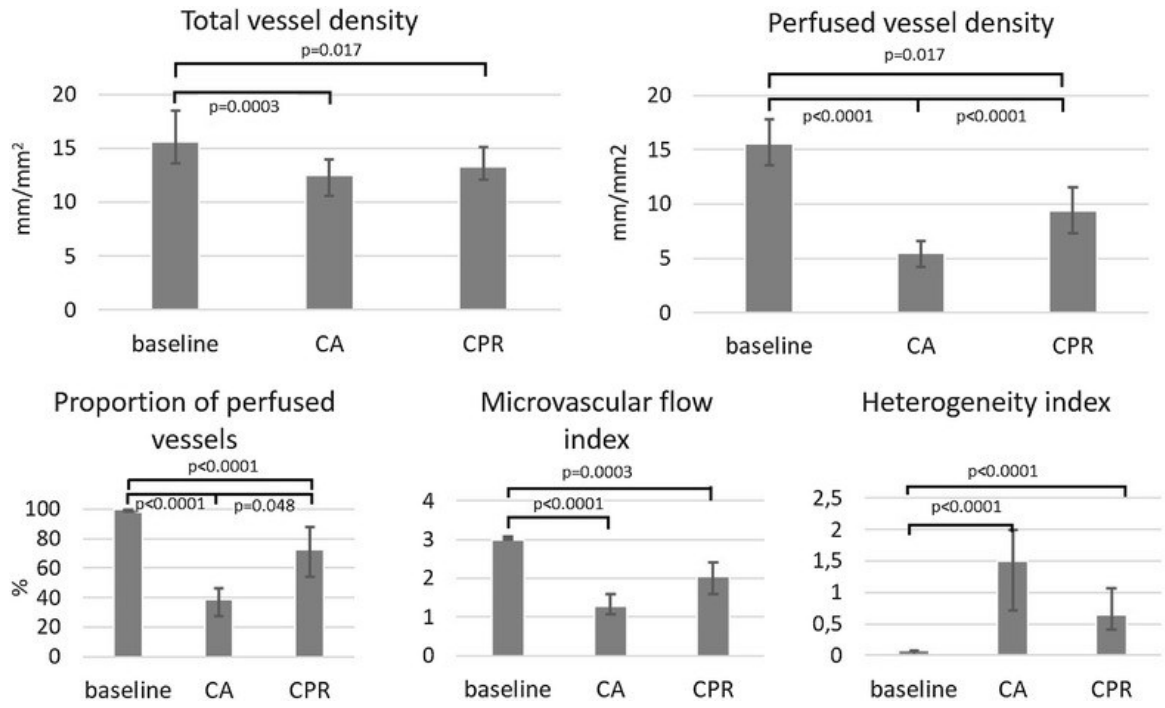

Figure 8: Sublingual microcirculatory parameters (of vessels with diameter ≤ 20 µm) during baseline, cardiac arrest (CA) and cardiopulmonary resuscitation (CPR). P values are indicated in the graph *(reproduced from (Krupickova P., Mlcek M., et al., 2016).*

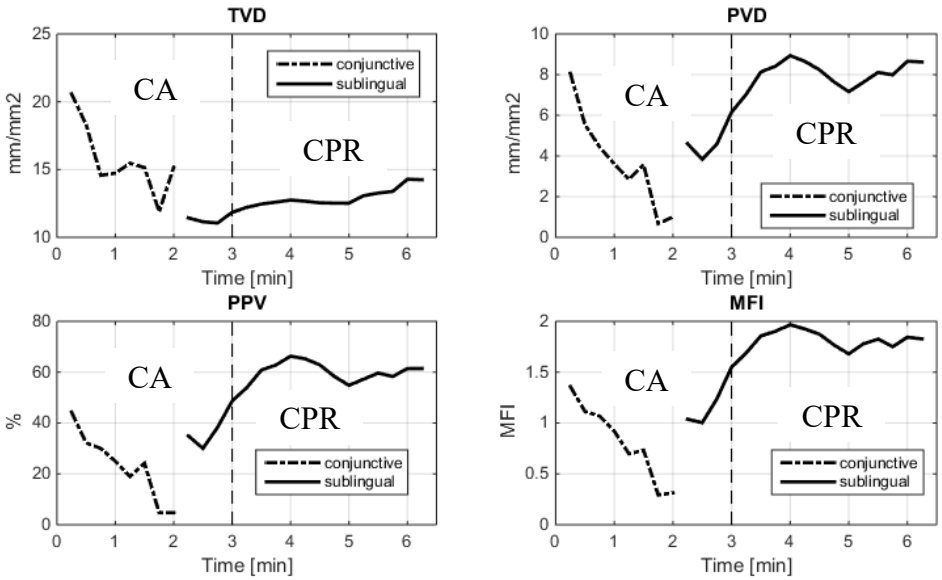

Figure 9: Time series of the microcirculatory variables in our porcine model of cardiac arrest (CA) and cardiopulmonary resuscitation (CPR): total and perfused vessel density (TVD, PVD), proportion of perfused vessels (PPV), microvascular flow index (MFI).

5.2 Microcirculation in patients rescued by ECPR

There is only scarce data on the microcirculatory function in patients under refractory CA rescued by ECPR. Therefore we designed an observational study in cooperation with the 2nd Department of Cardiovascular Medicine, General University Hospital and The First Faculty of Medicine, Charles University in Prague, Czech Republic. We hypothesized that there would be a significant difference in sublingual microcirculatory parameters between the adult ECPR patients and healthy (age and sex matched) control volunteers. Further on, we hypothesized that there would be a significant difference in the sublingual microcirculatory parameters between the subgroup of the ECPR patients with preserved hearth function as expressed by spontaneously pulsatile blood flow (defined as pulse pressure over 15 mm Hg) and the subgroup of ECPR patients with no or low blood flow pulsatility.

5.2.1 Study design and patients´ recruitment

Our study was arranged as a single-center non-invasive microcirculatory substudy of an ongoing randomized trial - "Prague OHCA" (Belohlavek J. et al., 2012). Both the main study and the microcirculatory substudy were approved by the Institutional Review Board of the General University Hospital and First Faculty of Medicine, Charles University in Prague. The main randomized trial was registered under ClinicalTrials.gov identifier: NCT01511666. Written informed consent was obtained for every participant either from the next of kin (in sedated or unconscious patients who did not regain consciousness) or from patients who regained consciousness later on.

Inclusion criteria for the patients were following:

- adult OHCA victim randomized into the hyperinvasive branch of the Prague OHCA study (see below);
- availability of a specialist for microcirculatory monitoring.

We included adult patients after refractory out-of-hospital CA, who did not achieve ROSC on the scene and who were subsequently randomized into the hyperinvasive branch of the "Prague OHCA" trial and rescued by ECPR. These patients were treated according to a standardized protocol that was published in detail previously and described the procedures for on-scene advanced life support provision, randomization, transport to the hospital under ongoing CPR, insertion of the percutaneous femoro-femoral VA

ECMO and ECPR onset, angiography, eventual percutaneous coronary interventions and subsequent standardized therapy, investigations and further complex intensive care including target temperature management (Belohlavek J. et al., 2012).

Additional exclusion criteria for this microcirculatory substudy were: ongoing sepsis, severe orofacial trauma or bleeding from oral cavity.

A control group of healthy subjects was recruited amongst hospital and faculty staff. The recruited volunteers were chosen to match by sex and age to the patients´ group. Volunteers with ongoing pregnancy or medical history of severe (i.e. cardiovascular) disease were not included.

5.2.2 Study protocol

In the included patients and healthy volunteers we measured sublingual microcirculation using SDF technology. We performed at least 5 measurements from the different parts of sublingual area, each of 20s duration. The video-images of microcirculation were screened for the image quality, stored and evaluated offline in a blinded fashion (see chapter 2.3) by a single investigator (PK).

In healthy volunteers we took the medical history. In patients we recorded following individual data: age, sex, the time of collapse, time from the collapse (CA onset) to ECMO implantation and to microcirculatory measurement, the etiology of CA and the ejection fraction of the left ventricle evaluated after the intensive care department admission. Further on, in patients we recorded core body temperature (via urinary catheter) and actual trend values of routine hemodynamic parameters in the time of microcirculatory monitoring (heart rate, oxygen saturation and invasive arterial and central venous blood pressure measured by hemodynamic catheters connected to fluid-filled transducer Truwave, Edwards Lifesciences LLC, Irvine, CA, USA). Pulse pressure was calculated from the arterial pressure as the difference between systolic and diastolic pressure. We collected data on therapy administration (including administration of noradrenaline, dobutamine or other vasoactive agent) and actual ECMO setting. The patients´ venous lactate, parameters of acid-base balance and hemoglobin were analyzed by a bedside analyzer (ABL90 FLEX, Radiometer Medical ApS, Brønshøj, Denmark).

The patients were followed and the data about their neurological performance (evaluated at discharge by Glasgow-Pittsburgh Cerebral Performance Categories – CPC scale) and clinical outcome were added.

5.2.3 Statistical methods

We tested the data for normality by Shapiro-Wilk normality test (Ghasemi A. and Zahediasl S., 2012). The parametric data are presented as the means (standard error of the means), and the non-parametric data are provided as the medians (first - third quartile).

To test the difference between the patients and the healthy controls the parametric data were examined by t-test and non-parametric data were examined by Mann-Whitney U tests. The Mann-Whitney U test was also used to test the difference between the groups of patients with pulsatile blood flow (P group) versus low or non-pulsatile blood flow (L/N group), because of the small numbers of patients in each group. Categorical variables were tested by Fisher´s exact test.

We applied Bonferroni corrections for multiple comparisons to evaluate the significance of the microcirculatory results. The overall $p \leq 0.05$ was defined a significant result.

5.2.4 Results

During the period from July 31, 2013 to April 18, 2016 we included fifteen patients, three of them were excluded from the analysis because of the development of fulminant sepsis or insufficient quality of microcirculatory video-images. Twelve healthy volunteers were included to serve as a control healthy group. The results were published in a pilot study, which creates an attachment to this thesis (see Attachment file No 4 (Krupickova P., Huptych M., et al., 2016)).

From the April 18, 2016 to the February 27, 2017 we performed further recruitment of the patients. The additional analysis showed the following results:

A total number of 15 patients were measured by SDF device 29 ± 17 hours post CA; 12 healthy volunteers (matched by age and sex to patients) were included.

There was a difference in proportion of perfused small vessels (90.6 (84.7 – 95.4) versus 97.5 (96.6 – 99.0) %, $p = 0.006$) and microvascular flow index of small vessels (2.67 (2.42 – 2.92) versus 3.00 (2.92 – 3.00), $p = 0.007$) between patients and healthy controls, other parameters did not differ (see Figure 10).

Patient groups (P group versus L/N group) did not differ neither regarding microcirculatory parameters nor in other followed variables (except for pulse pressure and ejection fraction of the left ventricle, see Figure 11 and Table 3).

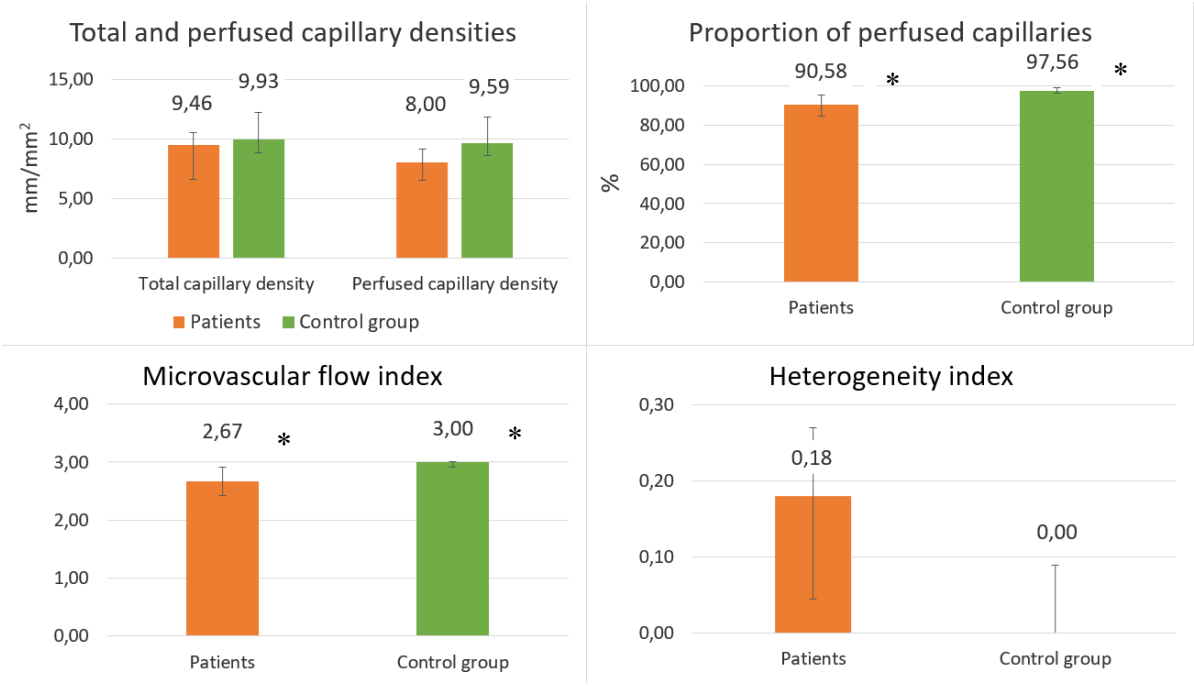

*Figure 10: Microcirculatory variables in post-cardiac arrest patients versus healthy control subjects. * indicates a significant difference ($p \leq 0.05$).*

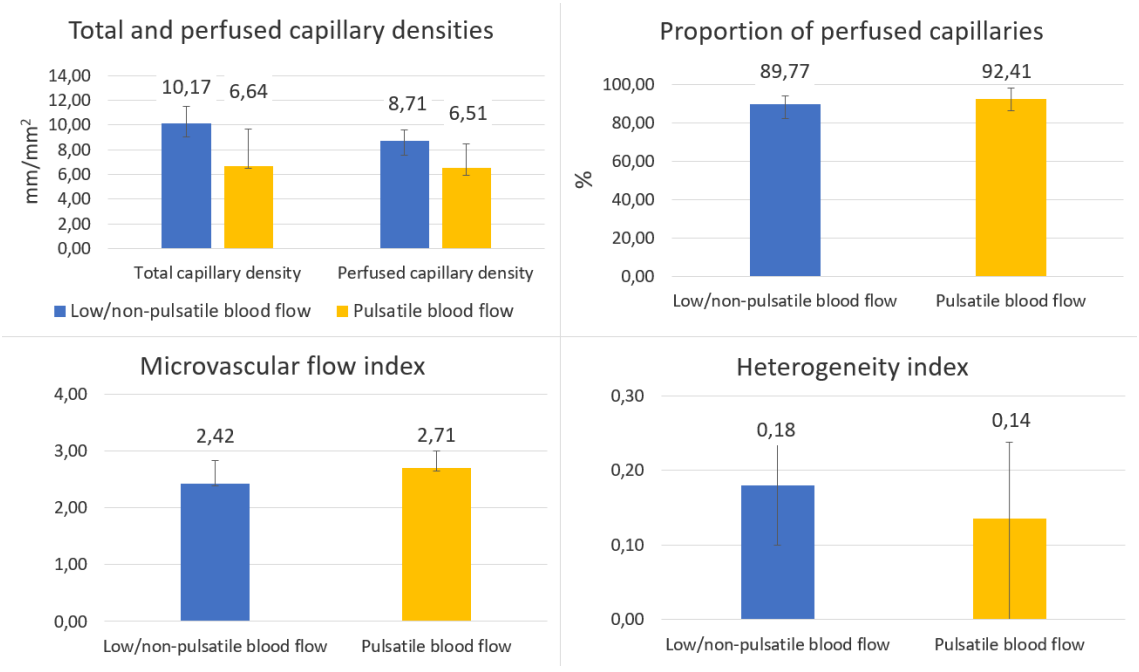

Figure 11: Microcirculatory variables in post-cardiac arrest patients with pulsatile (pulse pressure above 15 mmHg) versus low/non pulsatile blood flow.

Table 3: Comparison of the 2 sub-groups of patients: with pulsatile blood flow (P group – pulse pressure above 15 mmHg) versus low/non pulsatile blood flow (L/N group). The data are provided as the mean (standard error of the mean), median (first and third quartile) or total number (percentage). P value characterizes the level of significance of the difference between L/N and P group (evaluated by Mann-Whitney U test).

	All patients (N = 15)	**L/N group** (N = 7)	**P group** (N = 8)	**P value** Of Mann Whitney U test
Systolic blood pressure (mmHg)	89.3 (17.1)	82.7 (10.2)	95.0 (20.1)	0.18
Diastolic blood pressure (mmHg)	69.7 (15.2)	76.6 (12.6)	63.6 (15.4)	0.18
Mean arterial pressure (mmHg)	76.1 (14.1)	79.0 (11.5)	73.5 (16.4)	0.68
Pulse pressure (mmHg)	19.6 (15.4)	6.1 (4.4)	31.4 (10.9)	0.001
Ejection fraction of the left ventricle at baseline (%)	16.1 (11.8)	6.3 (4.3)	21.8 (9.1)	0.008
Central venous pressure (mmHg)	10.5 (3.7)	11.8 (3.3)	9.6 (3.9)	0.31
Pulse rate	80.7 (21.0)	72.0 (26.3)	88.4 (12.1)	0.15
Saturation O2 (%)	100.0 (99.0 - 100.0)	99.5 (95.0 – 100.0)	100.0 (99.8 - 100.0)	0.81
ECMO flow (L/min)	4.47 (1.41)	5.01 (0.58)	4.01 (1.78)	0.22
ECMO – rumps per minute	3645 (1175)	3882 (558)	3249 (901)	0.15
Core temperature (in the urinary bladder, °C)	36.10 (35.83 - 36.50)	36.10 (34.63 – 36.45)	36.20 (35.88 –36.53)	0.60
Venous lactate (mmol/L)	4.00 (2.65 – 7.10)	5.90 (3.60 – 7.10)	2.85 (2.58 – 6.03)	0.42
Arterial pH	7.35 (0.08)	7.33 (0.07)	7.37 (0.09)	0.65
Hemoglobin (mg/dL)	9.30 (9.20 – 10.75)	9.20 (9.15 – 12.15)	9.35 (9.28 – 10.10)	0.95
Vasopressor therapy (intravenous noradrenaline)	12 (80 %)	7 (100 %)	5 (63 %)	0.20

Inotropic therapy (intravenous dobutamine)	6 (40 %)	2 (29 %)	4 (50 %)	0.61
Survival to discharge	7 (47 %)	2 (29 %)	5 (63 %)	0.31
Favorable neurological outcome (CPC score 1-2)	8 (53 %)	2 (29 %)	6 (75 %)	0.13
Time from cardiac arrest to microcirculatory assessment (hours)	29 (17)	23 (16)	34 (18)	0.30

6 Discussion

In the first part of this dissertation thesis, I documented microcirculatory changes during CA and CPR in an experimental pig model, where circulatory support was provided by mechanized chest compressions. The microcirculatory blood flow dropped significantly during CA, however, in contrast to systemic circulation, it remained still partially sustained during the second and the third minute of CA. After initiation of cardiopulmonary resuscitation the microcirculatory parameters of small vessels reached 59–85 % of the baseline values. The correlation between microcirculatory parameters and mean arterial blood pressure, carotid blood flow and lactate was weak to moderate and after correction for multiple correlations it was insignificant.

During the CA the microcirculatory parameters decreased (to cca 35 % - 80% of the baseline values), however the microflow did not cease completely in the most animals even up to three minutes after the onset of cardiac stun, while the systemic blood pressure and carotid blood flow dropped to zero rapidly after the onset of ventricular fibrillation. The residual microcirculatory blood flow might be explained by the persistence of pressure gradient at the level of microcirculation. However, there are other explanations too. Most of our animals exhibited gasping during the untreated cardiac arrest and the temporary restoration of blood flow has been described after gasping (Ristagno G. et al., 2007). Further on, one might speculate that the SDF imaging recorded the reversal blood flow in the sublingual area, which is however improbable as we did not notice reversal flow in carotid artery.

The onset of mechanical CPR led to a rapid improvement of systemic circulation, however only two of the five microcirculatory parameters (of the small vessels with the diameter up to 20 μm) improved significantly (the perfused vessel density and proportion of perfused vessels), which was likely due to the preserved microflow during the CA.

Further on, I demonstrated lack of correlation between microcirculatory and systemic circulatory variables in our experimental model. Previous studies in a pig model of cardiac arrest conducted by Fries at al. showed, in contrary, a positive correlation between micro- and macro – circulation (Fries M., Tang W., et al., 2006, Fries M., Weil M. H., et al., 2006). This contradiction might be explained by the different choice of parameters of systemic circulation for the correlation testing (Fries et al. used coronary perfusion

pressure and end-tidal CO2). However, the loss of hemodynamic coherence between microcirculation and global circulation reflects the microcirculatory autoregulation, which is affected by the metabolic demands. Moreover, this micro- versus macro-hemodynamic difference has been documented in many other pathophysiological states, including critically ill patients and their animal models (i.e. in post-cardiac arrest subjects) (Jung C. and Kelm M., 2015, Van Genderen M. E. et al., 2012).

In the second part, I assessed the sublingual microcirculation in patients who suffered refractory CA and were subsequently rescued by ECPR. I found significantly lower proportion of perfused vessels and microcirculatory flow index in critically ill patients on ECMO support in comparison with healthy control subjects, but other parameters did not differ significantly. I did not confirm the hypothesis suggesting differences in microcirculatory parameters between patients´ group with spontaneously pulsatile versus those with low/ non-pulsatile blood flow.

The patients after prolonged conventional CPR, who were rescued by ECPR and at the time of microcirculatory monitoring were still under VA-ECMO support, had significantly lower PPV and MFI of small vessels in comparison to the healthy control subjects. Surprisingly, this difference was rather small (only up to 11 % difference between the two groups) and I did not prove significant differences in other microcirculatory parameters (TVD, PVD, HI). This microcirculatory "compensation" might correspond with microcirculatory changes in patients, who gained return of spontaneous circulation after conventional CPR: a deterioration of sublingual microcirculation, which was described in post-cardiac arrest subjects (Koopmans M. et al., 2015, Van Genderen M. E. et al., 2012), has been reported to diminish within 48 hours after CA (Donadello K. et al., 2011). Another explanation might indicate that ECMO assures sufficient microcirculatory function in severely compromised patients. Such speculation was also supported by the results of den Uil and colleagues, who demonstrated improvement and stabilization of microcirculatory variables by mechanical circulatory supports in patients with cardiogenic shock (Den Uil C. A. et al., 2009). Similarly, Lam et al. documented amelioration of microcirculatory parameters after implantation of percutaneous left ventricular assist devices in patients after ST-elevation myocardial infarction (Lam K. et al., 2009).

A secondary goal of this clinical study was to test the difference between sub-groups of patients with spontaneously pulsatile blood flow versus those with low or non-pulsatile circulation. The "pulsatile circulation" was in this case set as a pulse pressure (a difference between systolic and diastolic blood pressure) 15 mmHg or higher (Undar A. et al., 1999). The pulsatile blood flow corresponded with sustained residual hearth function. I found no significant differences regarding microcirculation in these two sub-groups, which might indicate that spontaneous pulsatility might play only minor role in microvascular regulation and is counteracted by other regulatory paths.

Despite interesting findings of these two studies, there were several limitations to be mentioned. First, despite that the both studies included number of subjects, which was comparable with similar studies in this field, the both studies were rather small. The experimental study was conducted in an animal model, which may not be fully comparable with human subjects, however, pig model of cardiac arrest as well as the analogy of microcirculatory function to humans has been described and used before (Gutierrez K. et al., 2015, Tiefenbacher C. P. et al., 2000). Further on, patient population included in the clinical study was heterogeneous regarding comorbidities, etiology of cardiac arrest and its duration, administered therapy and time delay between CA onset and microcirculatory measurement, which might had influenced the mechanism of microcirculatory regulation. Also data on systemic circulatory variables in healthy control subjects were lacking, as the healthy controls were not monitored by invasive catheters.
There have to be mentioned also the limitations directly connected to the SDF technology – we were not able to monitor conjunctival microcirculation during CPR in sufficient quality and due to technical problems (conjunctivae got injured easily) we decided exclude conjunctival images from the final analysis in the experimental study. The sublingual mucosa, as a part of the gastrointestinal tract, manifests different microcirculatory reactivity in comparison to cerebral or cardiac microcirculation. However, it has been repeatedly documented that in critically ill patients sublingual microcirculatory alteration might be predictive of outcome with high sensitivity and specificity (Buijs E. A. et al., 2014, Den Uil C. A. et al., 2010, Fries M., Tang W., et al., 2006). And finally, the microcirculatory measurements were performed according to the recommendations and all images were screened for signs of pressure or movement

artefacts ahead of their off-line evaluation, still, I might not fully avoid these artifacts, especially during ongoing CPR.

The results of this thesis are in line with the generally accepted model that the microcirculation is to some extent dependent on the systemic hemodynamics, but it is further modulated by the means of complex regulatory pathways (De Backer D. et al., 2010, Den Uil C. A. et al., 2008). Thus this thesis highlights the importance of microcirculatory monitoring in victims of cardiac arrest: further knowledge of microcirculatory function along with the deeper understanding of the treatment effects on microcirculation might be the key to the therapy optimization and to improvement of the cardiac arrest victims´ prognoses.

7 Conclusion

This thesis not only summarizes the recent body of knowledge on microcirculatory videomicroscopic imaging in cardiac arrest setting, moreover it studies microcirculation under different modes of circulatory support – either provided by mechanical chest compressor or extracorporeal membrane oxygenation.

I met all aims of this dissertation thesis: the experimental pig model was designed, in which I confirmed the hypotheses that microcirculatory parameters change during the cardiac arrest and resuscitation and that microcirculation in cardiac arrest setting does not correlate to systemic hemodynamic variables. I demonstrated that mechanical CPR in subjects with arrested circulation improved microcirculatory parameters; nevertheless, in some parameters this improvement was only insignificant and the microcirculatory parameters reached 59–85 % of the pre-arrest values.

Further on, I studied microcirculation in cardiac arrest patients who did not achieve return of spontaneous circulation after prolonged conventional CPR and were finally rescued by extracorporeal CPR. I confirmed hypothesis that microcirculation in patients rescued and supported by ECMO significantly differs from healthy volunteers. However, this difference was surprisingly small and might indicate that extracorporeal resuscitation supports circulation effectively. Moreover, the preserved pulsatility did not provide further improvement of microcirculation in our patients.

The results of this dissertation thesis contribute to the knowledge of microcirculatory pathophysiology in cardiac arrest setting and imply the importance of microcirculatory monitoring in this field.

8 Abstract

Introduction: This dissertation thesis aims to describe microcirculatory changes in cardiac arrest setting and to assess the impact of circulatory supports (i.e. mechanical chest compressions and extracorporeal membrane oxygenation (ECMO)) on tissue microcirculation.

Methods and results: Two separate studies were designed. Microcirculation was monitored sublingually by a recent Sidestream Dark Field (SDF) technique and its parameters were evaluated offline, separately for small (of diameter ≤ 20μm) and other vessels.

In order to monitor microcirculation during cardiac arrest (CA) and resuscitation (CPR) an experimental pig model was used; eighteen pigs were commenced to 3 minutes of untreated CA and subsequent 5 minutes of mechanical CPR. During CA the microcirculatory parameters deteriorated, in CPR they improved and reached 59 – 85 % of the prearrest values. The microcirculatory variables correlated neither to parameters of systemic circulation (mean arterial blood pressure and carotid blood flow) nor to lactate.

In the second, clinical, study the sublingual microcirculation was monitored 29 (± 17) hours after the CA onset in 15 patients, who were after unsuccessful conventional CPR rescued by ECMO. In comparison to healthy (sex and age matched) volunteers, the patients showed mild but significant reduction of proportion of perfused vessels and microvascular flow index, but other microcirculatory indexes did not differ significantly. Microcirculation did not correlate to systemic hemodynamics. Further on, we documented only insignificant microcirculatory difference between the subgroup of patients with spontaneously pulsatile blood flow (pulse pressure above 15 mmHg) versus those with low or non-pulsatile blood flow.

Conclusion: This dissertation thesis not only summarizes recent body of knowledge on microcirculatory video-imaging in CA setting, but it describes also microcirculatory changes during CA and CPR in a porcine model and demonstrates surprisingly tight compensation of the microcirculation in ECPR patients. Our results support the opinion that microcirculation in CA victims might be affected independently of the systemic hemodynamics.

Key words: microcirculation, cardiac arrest, Sidestream Dark Field imaging, CPR, ECMO

9 Abstrakt

Úvod: Cílem této dizertační práce je popsat změny mikrocirkulace, ke kterým dochází při srdeční zástavě, a zkoumat vliv jednotlivých oběhových podpor v léčbě srdeční zástavy (tj. mechanizované srdeční masáže a oběhové podpory mimotělní membránovou oxygenací (ECMO)) na periferní tkáňovou mikrocirkulaci.

Metody a výsledky: Byly navrženy 2 samostatné studie. Mikrocirkulace byla měřena sublingválně metodou Sidestream Dark Field (SDF) imaging a její parametry byly vyhodnoceny zvlášť pro malé cévy (s průměrem ≤ 20 μm) a ostatní cévy.

Pro monitoring změn mikrocirkulace během srdeční zástavy (SZ) a resuscitace (KPR) jsme využili experimentální prasečí model. U 18 prasat jsme navodili 3 minuty trvající neléčenou SZ následovanou 5 minutami mechanizované KPR. Parametry mikrocirkulace se postupně zhoršovaly během neléčené SZ a po zahájení KPR vystoupaly na 59 - 85% oproti klidovému stavu. Parametry mikrocirkulace nekorelovaly s parametry systémové cirkulace (středním arteriálním tlakem a průtokem krve v karotidě) ani s laktátem.

Druhá, klinická, studie sledovala mikrocirkulaci u pacientů po SZ, kteří byli po neúspěšné konvenční KPR resuscitováni pomocí ECMO. Zahrnuto bylo 15 pacientů 29 (± 17) hod od kolapsu a 12 zdravých dobrovolníků (pohlavím a věkem srovnatelných s pacienty). Pacienti měli v porovnání s kontrolami významně nižší poměr prokrvených kapilár a index mikrovaskulárního průtoku, ale ostatní mikrocirkulační parametry se významně nelišily. Mikrocirkulace nekorelovala se systémovým oběhem. Rovněž nebyl prokázán rozdíl mikrocirkulace u podskupiny pacientů se spontánně pulzatilním krevním tokem (systolicko-diastolickým rozdílem nad 15 mmHg) v porovnání s podskupinou s nízce pulzatilním/ nepulzatilním krevním tokem.

Závěr: Tato disertační práce nejenom shrnuje současné znalosti o mikrocirkulaci při SZ hodnocenou pomocí videomikroskopických metod, ale také samostatně studuje změny mikrocirkulace během SZ a KPR u prasečího modelu a demonstruje překvapivě dobrou kompenzaci mikrocirkulace u pacientů po reperfuzi pomocí ECMO. Výsledky dizertační práce potvrzují, že změny mikrocirkulace během SZ jsou relativně nezávislé na systémové hemodynamice.

Klíčová slova: mikrocirkulace, srdeční zástava, Sidestream Dark Field, KPR, ECMO

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11 List of abbreviations

ABP	Arterial blood pressure
CA	Cardiac arrest
CBF	Carotid blood flow
CPR	Cardiopulmonary resuscitation
ECMO	Extracorporeal membrane oxygenation
ECPR	Extracorporeal cardiopulmonary resuscitation
HGB	Hemoglobin
HI	Heterogeneity index
IDF	Incident Dark Field
IHCA	In hospital cardiac arrest
MFI	Microvascular flow index
NIRS	Near Infrared Spectroscopy
NO	Number
OHCA	Out of hospital cardiac arrest
OPS	Orthogonal Polarization Spectral
PPV	Proportion of perfused vessels
PtcO2	Percutaneous partial oxygen pressure
PVD	Perfused vessel density
ROSC	Return of spontaneous circulation
SDF	Sidestream Dark Field
StO2	Regional tissue oxygen saturation
TVD	Total vessel density
VA ECMO	Veno – arterial extracorporeal membrane oxygenation
VF	Ventricular fibrillation