Investigating novel and conventional blood biomarkers for diagnosis and prognosis of cardiovascular disorders

PhD Thesis

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List of abbreviations

ADMA	asymmetric dimethylarginine
AI	aggregation index
AUC	area under the curve
BE	base excess
BMI	body mass index
CCCK-18	caspase-cleaved cytokeratin-18
CK-18	cytokeratin-18
CPC	cerebral performance category
CPR	cardiopulmonary resuscitation
EI	elongation index
EuReCa	European Registry of Cardiac Arrest
FiO_2	fraction of inspired oxygen
HCO_3^-	serum bicarbonate
ICU	intensive care unit
IHCA	in-hospital cardiac arrest
LORCA	Laser-assisted Optical Rotational Cell Analyzer
MCV	mean corpuscular volume
OHCA	out-of-hospital cardiac arrest
$PaCO_2$	partial pressure of carbon dioxide
PaO_2	Partial pressure of oxygen
PEA	pulseless electrical activity
RBC	red blood cell
rho	Spearman's correlation coefficient
ROC	receiver operating characteristic
ROSC	return of spontaneous circulation
SAPS II	Simplified Acute Physiology Score
SDMA	symmetric dimethylarginine
SOFA	Sequential Organ Failure Assessment
t½	aggregation half-time
VF	ventricular fibrillation
VT	ventricular tachycardia
WLST	withdrawal of life-sustaining therapy
γ	threshold shear rate

1. Prologue

1.1. Biomarker research and discovery

Nowadays biomarkers are widely used for diagnosis, prognosis and follow-up of treatment in patients with cardiovascular diseases. Over the past two decades, a number of studies looking for various markers detectable in blood have been published. Biomarkers are circulating molecules that provide an insight into pathophysiological processes and aid to establish a diagnosis, refine the prognosis and guide the treatment [1]. In a broader sense, any characteristics that are measured as indicators of physiological or pathological biological processes or responses to an exposure or intervention can be listed in the group of biomarkers [2]. They can be used for diagnosis, therapy monitoring, measuring pharmacodynamic response, predictive or prognostic purposes, to ensure safety by indicating toxicity or for establishing susceptibility or risk for development of a disease [3].

A **prognostic biomarker** is used to identify the likelihood of a clinical event, disease recurrence, or progression in patients who have the disease or medical condition of interest. In addition, prognostic biomarkers are especially important for assessing the risk of a future adverse clinical event (e.g. death, poor neurological outcome), which information is crucial in the level of care decisions or estimating the length of stay in hospital and/or in intensive care units (ICU) [4].

A **diagnostic biomarker** is applied to detect or confirm the presence of a disease or condition of interest or to identify individuals with a subtype of the disease. These markers contribute to the critical determination of whether an individual has a particular medical condition for which treatment or any intervention may be indicated. Diagnostic biomarkers are often used as eligibility criteria for enrolment in a clinical trial studying a medical condition [2].

Diagnostic biomarkers should be treated separately from **susceptibility or risk biomarkers**, which are intended to indicate the potential for developing a disease or medical condition before clinical symptoms appear. These markers can mainly be applied to guide preventive strategies [5].

The **ideal biomarker** possesses high sensitivity, allowing early detection, and also sufficiently high specificity for a given disease or outcome. It is advantageous if it

can be measured easily, inexpensively, and non-invasively producing rapid, reproducible results. The source of biomarkers should be readily available, such as blood. Biomarker levels should vary rapidly in response to treatment and should support risk stratification and possess prognostic value in terms of real outcomes. Moreover, biomarker research should help to better understand underlying pathological processes in a particular medical condition and shed light on new therapeutic perspectives potentially improving outcomes [6].

1.2. Basic concepts and terms to describe diagnostic or prognostic tools

The terms of sensitivity, specificity, positive predictive value, negative predictive value describe how well diagnostic or prognostic tests indicate the true presence or absence of a disease/condition. A 2x2 contingency table illustrates the 4 possible outcomes (*Table 1.*). The test result is **true positive** if it correctly identifies the presence of the disease or condition, whereas a **false positive** result occurs when the individual is diagnosed incorrectly as a positive without the disease or condition. Conversely, a **true negative** result means a negative result which is classified correctly as being negative, and a **false negative** identifies the patient incorrectly as negative despite the true presence of the disease or condition [7].

Sensitivity is the ability of a test to correctly classify an individual with disease or condition. Sensitivity = true positives / (true positives + false negatives) x 100. The closer the value is to 100% the more sensitive the test and the lower the false negative result. Highly sensitive tests will not miss individuals with the condition, consequently, tests with high sensitivity are good for screening purposes. **Specificity** is the proportion of individuals without the disease or condition who are correctly classified as negative. Specificity = true negatives / (true negatives + false positives) x 100. The higher and closer to 100% the specificity value, the lower the probability of a false positive screening result. In contrast to sensitive procedures, tests with high specificity are rather good to confirm the suspected disease or condition. The **false positive rate** (=1 - specificity) is defined as the proportion of incorrect positive results that are in fact negative. The **false negative rate** (=1 - sensitivity) is the proportion of incorrect results that are in fact positive [7].

Table 1. *A 2x2 contingency table with four possible outcomes of the relationship between the test result and the presence of the disease.*

		Disease / condition		
		+	–	
Test	+	True positive	False positive	*Positive predictive value*
	–	False negative	True negative	*Negative predictive value*
		All with disease / condition → *Sensitivity*	All without disease / condition → *Specificity*	

When evaluating the success of a test, the **positive predictive value** is the probability that the disease or condition is present when the test is positive (= true positives / [true positives + false positives]). Similarly, the **negative predictive value** is the probability that the disease is not present when the test is negative (= true negatives / [true negatives + false negatives]). **Accuracy** means the proportion of true test results from the total number of patients for whom the test was carried out (= [true positives + true negatives] / [true positives + false positives + false negatives + true negatives]) [7].

1.3. Receiver Operating Characteristic analysis

The relationship between sensitivity and specificity of a binary classifier (e.g. presence or absence of a condition) is represented on the **Receiver Operating Characteristic** (ROC) analysis. ROC curves are widely used in biomarker research as a quantitative method to evaluate the "success" of diagnostic tests, so the diagnostic performance or accuracy of a test in discriminating patients with condition or disease from patients without it [8]. The **Area Under the Curve** (AUC) is used for quantitative determination of the overall discrimination ability of a test between two outcomes [7]. A test is effective when the curve is concentrated in the upper left corner because in this case the sensitivity and specificity are both high. If the curve is closer to the diagonal line, the test is ineffective in discriminating the outcomes. (*Figure 1.*)

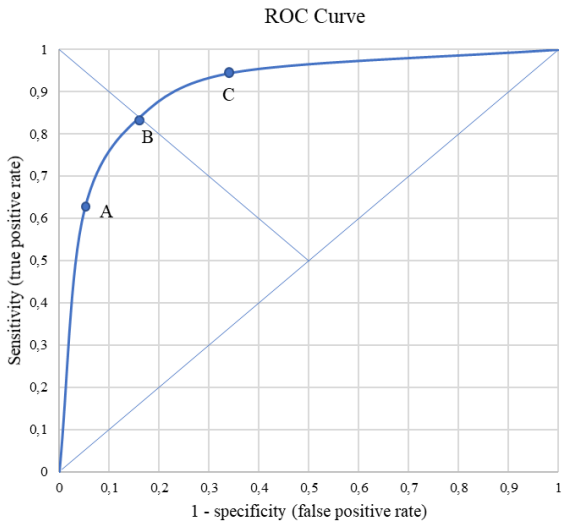

Figure 1. ROC Curve: *The three point (A, B and C) demonstrates three examples for different decision criteria. "A" means a strict decision criterion with lower sensitivity and higher specificity, "B" is a moderate decision criterion with equal sensitivity and specificity, while "C" is a milder decision criterion with high sensitivity and lower specificity. (The figure was created by the author of this thesis according to the book of Elek Dinya [9].)*

The ROC curve is formed by connecting multiple points together. Each point plotted on the ROC curve represents a sensitivity/specificity pair that was determined at a particular test threshold value. The higher the sensitivity/specificity pair values, the better the estimation of AUC. A perfect test would have an AUC value of 1.0, which means no false positives and no false negative cases, whereas a value of 0.5 suggests the test result is no better than obtained by chance alone. AUC values can be interpreted as follows: 1.0 is a perfect test, 0.9 – 0.99 is an excellent test, 0.8 – 0.89 is a good test, 0.7 – 0.79 is moderate test, 0.51 – 0.69 is a poor test, and 0.5 means no prognostic value [7,10]. To select an operating point on a ROC curve, we first need to specify the objective function that we aim to optimise.

1.4. Practical considerations of diagnostic and prognostic tools

My PhD work focused on two main groups of patients with pathological conditions involving the cardiovascular system: patients suffered cardiac arrest with successful cardiopulmonary resuscitation (CPR) and women diagnosed with early-onset preeclampsia.

Concerning the prognostication of **resuscitated patients**, it is important to have adequate **specificity** of biomarkers to avoid misjudging an individual with potential chance to recovery as having a poor prognosis. Since most prognostic tests are focused

to predict poor neurological outcome, it is desirable to possess a high specificity, which means a very-low rate of falsely pessimistic predictions potentially leading to an inappropriate withdrawal of life-sustaining therapy (WLST). There is no universal consensus on the desired level of specificity of a test for neuroprognostication after cardiac arrest. Requesting 100% specificity would decrease the sensitivity to levels where clinical utility is already equivocal, while allowing a false positive rate of 1-2% would increase the clinical relevance of the biomarker [11].

On the other hand, in **early-onset preeclampsia**, high **sensitivity** should be preferred to avoid missing the detection of an individual with potential risk for preeclampsia, even at the cost of more false positive cases, because false negative results are unequivocally more harmful than false positive results. Lower risk threshold and lower positive predictive values may be reasonable for the early detection of an individual with elevated risk for preeclampsia to ensure the opportunity to introduce an early preventive therapy (i.e. low-dose aspirin prophylaxis) [12], guide the surveillance strategy during the pregnancy and to choose the optimal time for delivery [13,14].

I. NOVEL AND CONVENTIONAL BIOMARKERS FOR POST-RESUSCITATION PROGNOSIS

2. Introduction for cardiac arrest and resuscitation research

Cardiac arrest is a profound clinical and public health challenge across the globe. The loss of effective mechanical cardiac function leads to absence of systemic blood circulation, hemodynamic collapse, typically due to sustained ventricular tachycardia (VT) or fibrillation (VF), pulseless electric activity (PEA), or asystole. Prompt cardiopulmonary resuscitation (CPR) with the early removal of the precipitating factor is essential to prevent permanent damage or death. Cardiac arrest is usually divided into out-of-hospital cardiac arrest (OHCA) and in-hospital cardiac arrest (IHCA) based on the location where it happened.

2.1. Epidemiological facts about cardiac arrest and survival

2.1.1. Out-of-hospital cardiac arrest

The European Registry of Cardiac Arrest (EuReCa), an international project of the European Resuscitation Council, provides the most comprehensive information on the epidemiology of cardiac arrest in Europe [15,16]. However, the true incidence of OHCA is not known probably because of high number of unwitnessed cardiac arrests or cases without reporting to emergency medical services. According to reported observations, the overall incidence of OHCA where CPR was attempted is 56 per 100,000 population per year. Survival to 30 days reaches approximately 8-10% in cases, who received CPR either before or on arrival of the emergency medical services and 35% of patients admitted with return of spontaneous circulation (ROSC) were discharged alive [15,16]. A recent meta-analysis reviewed the global data about OHCA patients in the past 40 years. According to their findings, the pooled incidence of ROSC among OHCA patients who received CPR worldwide is about 30%, (which finding is similar to EuReCa TWO – 33%), a fifth of the patients survive admission and 8.8% survive discharge. The pooled 1-month survival rate was 10.7%, and 7.7% of the patients survived 1 year [17].

In Hungary, the incidence of reported OHCA is about 118 per 100,000 per year, while CPRs performed annually were 79 per 100,000. Percentage survival in cases with CPR attempted (discharged from hospital alive or alive at least 30 days after the event) was about 13% according to the EuReCa ONE – registry [15].

2.1.2. In-hospital cardiac arrest

The estimated incidence of IHCA in the United States was 292,000 annually analysing data from 2008 to 2017. In 2017, 25% of IHCA patients could be discharged alive according to the American Heart Association's Get With The Guidelines-Resuscitation registry [18,19]. A meta-analysis covering the period between 1985–2018 worldwide shows a pooled survival rate after in-hospital cardiac arrest at discharge of 17.6% and the one-year survival to be 13.4% [20]. In Europe, the annual incidence of IHCA is 1.5 – 2.8 per 1,000 hospital admissions and 15 – 34% of patients survive 30 days or hospital discharge [21]. In Hungary, there is no available local cardiac arrest registry, therefore the exact epidemiological data in our country are not known [22].

2.2. Short and long-term complications after ROSC

Even after successful resuscitation, the long-term survival remains poor despite all efforts. The post-cardiac arrest syndrome is a complex pathophysiological process that develops after systemic ischaemia and the subsequent reperfusion response during CPR. The mortality after ROSC mostly results from ischaemic brain injury, myocardial dysfunction, multiple organ failure resulting from systemic ischaemia-reperfusion injury and persistent precipitating aetiology [23-25]. The early death within 3 days occurs mostly due to circulatory failure, while later death is mainly related to severe hypoxic-ischaemic encephalopathy and the subsequent WLST [26]. Death after resuscitated OHCA occurs mostly due to withdrawal of care because of neurological reasons (73%), while this can be identified as the reason for death only in 27% of the IHCA cases, where the withdrawal of care because of comorbidities, refractory hemodynamic shock and sudden cardiac death are more frequent than in OHCA [27]. The ischaemia-reperfusion injury alters different immunological and coagulation pathways, triggers endothelial injury and microcirculatory disorders, which may lead to sepsis-like syndrome and multiple organ failure [28-30].

Those patients who survive the post-resuscitation period and have been discharged from the ICU often experience long-term complications such as cognitive, emotional, physical problems, and chronic fatigue, which are often barriers of return to work and reduce the quality of life [31,32]. An adequate rehabilitation with a multidisciplinary team is needed to help to recover neurological and physical conditions

[11]. The large majority of these patients are able to live independently, continue activities of everyday life and return to work [33].

2.3. Prognostic tools and algorithm

Predicting the overall survival and neurological function of cardiac arrest survivors are amongst the biggest challenges facing the medical team. Early predictors of outcome that would support clinical decision-making are required to avoid inappropriate WLST or costly, prolonged treatment in patients with no chance of neurologically meaningful survival and to correctly guide goals-of-care conversations with family members [34].

The early determination of neurological outcome after cardiac arrest in comatose patients is an essential element of risk stratification to identify treatment strategy. Approximately half of the deaths caused by hypoxic–ischaemic brain injury result from WLST following prognostication of a poor neurological outcome [35,36]. Current guidelines recommend a multimodal approach to assess the severity of hypoxic-ischaemic brain injury combining multiple methods to reduce the risk of falsely pessimistic prediction. Prognostication of poor neurological outcome of unresponsive patients after 72 post-cardiac arrest hours is based on clinical neurological examination (no pupillary or corneal reflexes, status myoclonus), electrophysiological investigations (highly malignant electroencephalogram and bilateral absence of somatosensory evoked cortical-N20 potentials), neuroimaging (diffuse and extensive anoxic brain injury on CT/MRI) and biomarkers (high serum levels of neuron-specific enolase – NSE at 48 – 72 hours) [11].

2.3.1. Biomarkers

In the past decades, multiple biomarkers have been tested for neuroprognostication after cardiac arrest, one of them is NSE. This protein is released into the blood following the injury of neuronal and neuroendocrine cells, thus the blood values are presumed to correlate with the extent of hypoxic–ischaemic brain injury from cardiac arrest [37,38]. It is challenging to find a reliable biomarker threshold for identifying patients who will have poor outcome. The timing and the variability of techniques of the measurement influence the thresholds and cause inconsistency. False positive results of NSE levels can be caused by extracerebral sources, which may disturb the evaluation [39]. According to current recommendations, NSE is most accurate at 48–72 h after ROSC, however, the threshold values are very inconsistent at any time. The accuracy to predict

poor outcome is limited, therefore NSE usage is recommended in combination with multiple methods for neuroprognostication of comatose patients after ROSC [34,40].

Other biomarkers tested for neuroprognostication are released to the blood after injury of neuronal or glial cells and considered to reflect the severity of brain injury (e.g. S100 calcium-binding protein B, neurofilament light chain, tau protein, glial fibrillary acidic protein) [41-43].

The inconsistency of different measurement techniques and thresholds, the sometimes limited availability, and the weak evidence due to small sample size limit the general usability of biomarkers. Moreover, it is difficult to determine the proper and consistent cut-off with maximal specificity and acceptable sensitivity for poor outcome. On the other hand, biomarkers have many advantages: they provide quantitative information, not affected by the presence of sedation or paralysis; moreover, they are easy to measure with an appropriate laboratory background and can be evaluated blindly to other clinical data excluding subjective prophecy about the outcome of the patient [44]. Consequently, the investigation of traditional biomarkers from new aspects and the evaluation of novel biomarkers followed by their incorporation in prognostic algorithms are certainly justified.

2.3.2. Limitations of current prognostication

Accurate neurological prognostication is essential in cardiac arrest survivors with suspected brain injury to ensure that patients with significant potential for recovery are not destined for inappropriate care withdrawal due to falsely pessimistic prediction of the neurological outcome. Current recommendations are mostly suitable for neurological prognostication in comatose patients [11,45], and although high proportion of patients (especially after IHCA) reach acceptable neurological function in the ICU, they may suffer multiple organ failures, which may lead to death independently of their neurological status [46]. As mentioned above in IHCA the underlying comorbid conditions and haemodynamic instability drives mortality instead of neurological withdrawal of care [27]. The current algorithm is suggested to be used 24–72 hours or later after ROSC for neuroprognostication of unresponsive, comatose patients [11,45]. These facts emphasise the importance to broaden the prognostication strategy after cardiac arrest and also raise the question of whether the general conception to evaluate biomarkers reflecting exclusively neurological injury is correct. It is worth considering investigating and finding biomarkers

that could have additional information about overall survival and complete the current prognostication algorithm. Identification of reliable indicators is essential to predict the overall outcome, thereby improving understanding of the aetiology, and to guide post-resuscitation management after cardiac arrest, wherever it occurs [21].

2.4. Novel biomarkers with potential prognostic value after cardiac arrest

2.4.1. L-arginine pathway molecules

Cardiac arrest leads to endothelial dysfunction, which can play a potentially important role in the development of post-cardiac arrest syndrome. Therefore, endothelial injury and subsequent microcirculatory dysfunction are associated with poor outcomes of resuscitated patients [47]. Impaired nitric oxide synthesis is considered a major feature of a dysfunctional endothelium [48]. Nitric oxide, a pleiotropic molecule, has several intracellular effects leading to vasorelaxation, endothelial regeneration, inhibition of leukocyte chemotaxis, and platelet adhesion [49]. L-arginine pathway molecules are one of the main regulators of nitric oxide synthesis and vascular regulation, hence indicators of endothelial dysfunction. L-arginine is a substrate for nitric oxide synthase, which catalyses its two-step oxidation to nitric oxide and L-citrulline in endothelial cells, thus regulating vascular tone and cardiovascular homeostasis [50,51]. Methylarginines are the main regulators and endogenous inhibitors of nitric oxide synthase catalytic function. Asymmetric dimethylarginine (ADMA) is a direct competitor for binding to the catalytic site of nitric oxide synthase, in addition ADMA and symmetric dimethylarginine (SDMA) compete with L-arginine at the level of transport into the cell as well. ADMA has been previously described to inhibit nitric oxide formation and increase oxidative stress in vascular endothelial and smooth muscle cells [52]. The bioavailability of nitric oxide depends on the balance between L-arginine and ADMA. Consequently, the reduced L-arginine/ADMA ratio results in the inhibition of nitric oxide production (*Figure 2.*).

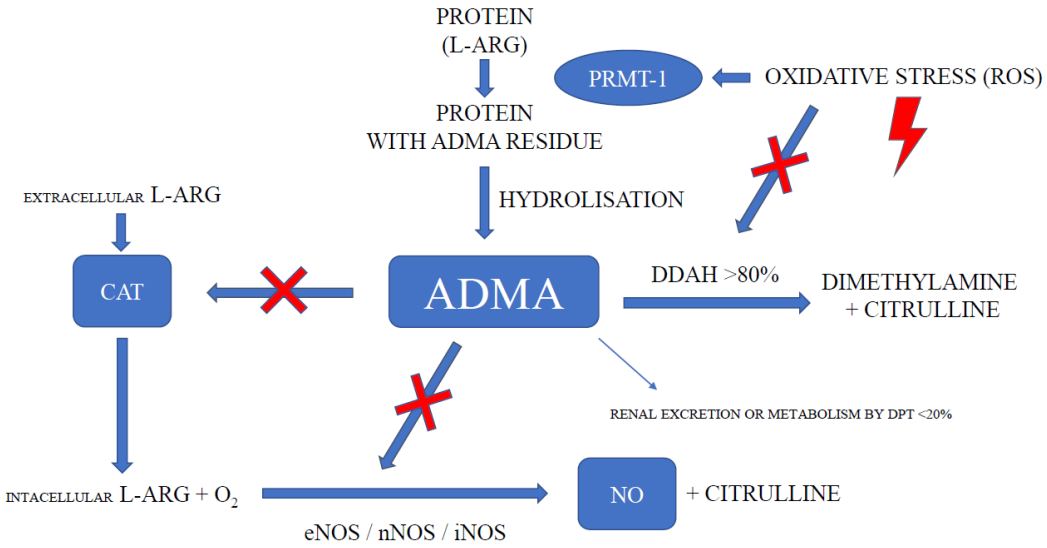

Figure 2. Generation, elimination, and degradation of ADMA. *ADMA derives from the methylation of arginine residues in proteins catalysed by PRMTs resulting in a methylated arginine derivative (protein with ADMA residue). ADMA is a competitive inhibitor of NOS. The major metabolism of ADMA occurs via degradation through the enzyme DDAH forming dimethylamine and l-citrulline or it is excreted into the urine and metabolized by the dimethylarginine pyruvate aminotransferase (DPT). Increased level of oxidative stress activates PRMT-1 and inhibits DDAH. ADMA – asymmetric dimethylarginine; CAT – cationic amino acid transporter; DDAH – dimethylarginine dimethylaminohydrolase; L-ARG – L-arginine; NO – nitric oxide; NOS – nitric oxide synthase; PRMT-1 – protein arginine N-methyltransferase; ROS – reactive oxygen species. (The figure was created by the author of this thesis according to the publication of John P. Cooke [53])*

Increased ADMA levels were observed in hypertension, hypercholesterolemia, diabetes, and atherosclerosis, and the elevated levels are associated with progression and outcome in several cardio- and cerebrovascular disorders [54,55]. Elevated circulating concentrations of L-arginine derivates have been associated with the severity of myocardial ischaemia and adverse outcomes after percutaneous coronary interventions [56-58] and ischaemic stroke [59-61]. Higher circulating ADMA levels on admission strongly associate with mortality of critically ill and septic patients [62,63].

2.4.2. Cell death and cytokeratins

Several forms of cell death have been discovered in recent years. The most well characterised and investigated form of controlled cell death is apoptosis (i.e. programmed cell death), while the uncontrolled form of cell death is generally termed necrosis [64].

Necrosis is mostly induced by sudden severe external injury, such as hypoxic insult [65]. During necrosis, the cell swells intensely, the cell membrane loses its integrity

and barrier function, cell organelles are damaged, which results in the release of the cell content into the environment upregulating various pro-inflammatory factors and inducing an inflammatory process that causes further damage in the surrounding area. Necrosis is a passive, energy-independent process and does not require gene expression changes to take place [64].

Contrary to necrosis, **apoptosis** is characterised by energy-dependent biochemical processes leading to distinct morphological alterations. Various vital physiological mechanisms occur through apoptosis such as normal cell turnover, appropriate immunological functioning, hormone-dependent atrophy, embryonic development. Moreover, apoptosis is essential to ensure the purposive eradication of damaged or defective cells [65]. This well-regulated active process ultimately results in the controlled death of the cell without spillage of its contents into the surrounding environment. The cell breaks down into intact membrane-bounded parts (i.e. apoptotic bodies) that are engulfed and degraded by intact cells of surrounding tissue or by specialised cells, thus the completion of the process is carried out without further damage caused by inflammatory response. The key step of this process is the activation of a series of cysteine-aspartic proteases (i.e. caspases), which are involved in cascade amplification of the death signal and cleavage of target proteins leading to DNA fragmentation, destruction of the nuclear and cytoskeletal proteins [66].

Cytokeratins are cytoskeletal structural proteins and members of the intermediate filament superfamily in epithelial and parenchymal cells [67]. As a consequence of cardiac arrest and ischaemic-reperfusion conditions, the systemic cell damage and subsequent apoptotic and necrotic cell death are amplified [68]. Ischemia and reperfusion cause intracellular Ca^{2+} overload and reactive oxygen species formation predisposing to mitochondrial injury [69]. The subsequent cytochrome c release [70] results in the activation of executioner caspases and cleavage of cell components including cytokeratins through complex biochemical pathways [71,72]. It has been described that cytochrome c is released into the bloodstream after closed-chest resuscitation of induced cardiac arrest caused by ventricular fibrillation in animal model [73]. During apoptosis, caspases cause the fragmentation of the cytokeratin-18 (CK-18), forming caspase-cleaved cytokeratin-18 (CCCK-18), which hence is considered to be an apoptosis-specific cell death biomarker.

On the other hand, necrotic cell death results in the release of the full-length CK-18 to the circulation (*Figure 3.*).

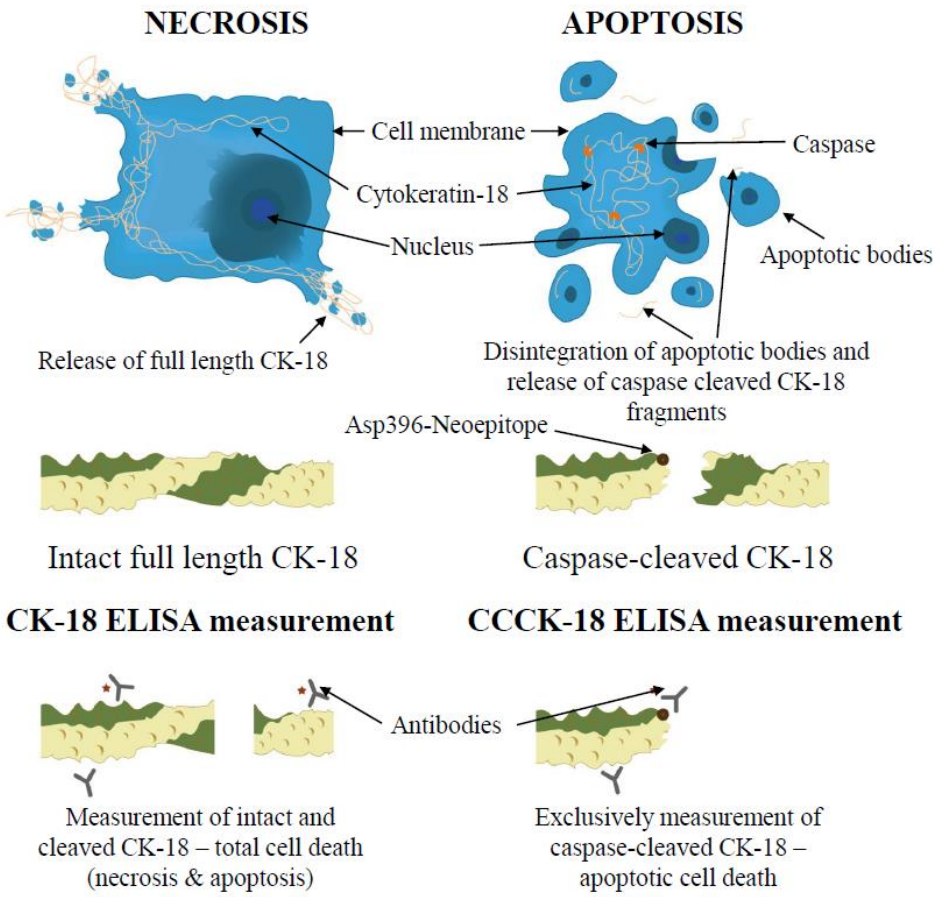

Figure 3. *Characteristics of CK-18 release during apoptosis and necrosis and their measurement process (The figure was created by the author of this thesis according to the Diapharma product brochure https://diapharma.com/dili/p10011_p10040_vlvbio1605_hepatocyte_dpg_edit_101316b/)*

Previous studies found an association between the increased levels of CK-18 and its caspase cleaved fragments and outcomes in different disorders. The increased level of CCCK-18 in septic and critically ill patients was associated with mortality in previous studies [74]. CCCK-18 concentrations are elevated in patients with acute myocardial infarction compared to stable or unstable angina patients and the marker is significantly increased at the site of coronary occlusion as compared to peripheral blood samples [75]. More studies investigated the marker in neurological disorders, such as ischemic stroke [76], intracerebral [77], and aneurysmal subarachnoid haemorrhage [78], and traumatic brain injury [79].

3. Hypothesis and objectives

Based on the above-detailed limitations of the current prognostication methods we intended to approach the post-resuscitation prognosis from the overall survival point of view. Our aim was to identify potentially promising biomarkers in the early post-resuscitation phase, which could have additional information about the overall survival of unselected resuscitated patients without focusing exclusively on their neurological status. Besides the widely investigated hypoxic-ischaemic brain injury, systemic endothelial injury and cell damage are presumably amplified as the consequence of ischaemic reperfusion injury after resuscitation. Therefore, we focused on investigating the blood levels of markers reflecting these pathological phenomena.

In addition to identifying novel biomarkers, we aimed to determine the main characteristics and alterations of NSE, conventionally used laboratory, clinical and vital parameters according to survival and neurological outcome, and to test the prognostic accuracy of widespread used prognostic scoring systems such as Simplified Acute Physiology Score (SAPS II) and Sequential Organ Failure Assessment (SOFA) severity scores in our general cohort of resuscitated patients.

3.1. L-arginine pathway molecules

As L-arginine, ADMA and SDMA were described earlier as prognostic markers in different acute and chronic cardio- and cerebrovascular disorders and critically ill patients, we assumed that the level of these markers in the peripheral blood associate with the general outcome after resuscitated cardiac arrest.

- The primary objective of our study was to investigate the alterations and kinetics of the L-arginine-nitric oxide pathway molecules with repeated sampling in the early post-resuscitation care and characterise them according to survival.
- Our secondary aim was to evaluate their distinct association patterns with prognostic scoring systems that are widespread and conventionally used in everyday critical care (SOFA and SAPS II).
- Moreover, our objective was to investigate the possible association of markers with the best neurological function reached in the ICU.

3.2. Cytokeratin-18

It seems reasonable to assume that greater tissue damage in the state of clinical death until ROSC leads to greater functional impairment, poorer survival, and more significant residual symptoms. We hypothesised that overall tissue damage as a consequence of ischaemia and reperfusion injury can be characterised by CK-18 and CCCK-18 levels. The fragments of the CK-18 cleaved by caspases can be recognised by a monoclonal antibody and in combination with the full CK-18 measurement, the predominant mode of cell death can be determined using the CCCK-18/CK-18 ratio. The lower this ratio, the more necrosis dominates the cell death processes [80].

- We hypothesised that CK-18 and CCCK-18 levels may have prognostic value in predicting mortality and functional impairment, therefore our primary aim was to characterise for the first time these marker levels and CCCK-18/CK-18 ratio in survivors compared to non-survivor subjects after resuscitation. We presumed that the dynamics of tissue damage may also have an impact on the outcome, therefore the kinetics of CK-18, CCCK-18 values may also have prognostic value.
- As these biomarkers have not been previously investigated in resuscitated patients, one of our secondary objectives was to investigate the association of CK-18, CCCK-18 values with the characteristics and circumstances of cardiac arrest and CPR. In addition, we aimed to explore the connection between the markers and prognostic scoring systems conventionally used in critical care.
- Furthermore, we aimed to reveal whether cell death parameters may be more sensitive to certain organ damage or whether CK-18, CCCK-18 levels are affected by organ failure in the post-resuscitation care and to test if there is any impact of neurological status on the marker levels.

4. Materials and methods

4.1. Study design, setting

We designed a single-centre observational cohort study adhering the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) Statement [81], in which we recruited patients prospectively from January 2018 to January 2019 in the Intensive care unit of the 1st Department of Medicine, Department of Anaesthesiology and Intensive Care and Department of Emergency Medicine at the

University of Pécs. We provided a non-stop phone hotline during the recruitment period. ICU staff contacted us by phone within 6 hours after admitting patients after successful resuscitation, which was defined as the return of spontaneous circulation (ROSC). Standard post-resuscitation care was applied for each patient in the ICU without interaction with the research team. Therapeutic hypothermia was not applied, however, the overall goal was to maintain normothermia and to prevent fever (core temperature 37.5℃) during post-resuscitation care of every patient. The total follow-up period was 30 days after cardiac arrest.

The study was approved by the Local Ethics Committee of the University of Pécs (file number: 6941 – PTE 2018.) and followed the principles of the Declaration of Helsinki for all human investigations. Informed consent for being included in the study was obtained from legal representatives or, in case the patients had regained consciousness, from the patients themselves.

4.2. Eligibility criteria

We enrolled adult (age ≥18 years) patients admitted to the ICU for post-resuscitation care regardless of the aetiology, initial rhythm, or whether it was in- or out-of-hospital cardiac arrest. We enrolled every reported patient who suffered cardiac arrest and the length of CPR took at least 2 minutes. Burned patients or patients in the early post-operative phase or with primary traumatic aetiology were excluded to avoid false pessimistic estimation of tissue damage and cell death caused by hypoxic insult.

4.3. Data collection

Collected data included general information about patients, such as age, gender, and comorbid conditions listed below:

- Chronic hypertension
- Ischaemic heart disease (previous acute myocardial infarct, percutaneous coronary angiography, documented angina pectoris or known coronary artery disease)
- Type 2 diabetes mellitus
- Heart failure
- Permanent atrial fibrillation
- Previous ischaemic stroke or transient ischemic attack

- Carotid artery stenosis
- Chronic obstructive pulmonary disease
- Peripheral artery disease
- Previous pulmonary embolism
- Previous, cured malignant disease
- Active malignant or hematologic disease

Variables that are necessary for calculating **prognostic scores (SOFA and SAPS II)** were recorded and calculated according to the worst parameters of the first 24 hours after cardiac arrest using an online calculator (https://www.mdcalc.com/).

- **SOFA:** partial pressure of oxygen (PaO_2), the fraction of inspired oxygen (FiO_2), need for mechanical ventilation, platelet count, Glasgow Coma Scale, bilirubin, mean arterial pressure, administration of vasoactive agents and their highest dose if required, creatinine, urine output.
- **SAPS II:** age, heart rate, systolic blood pressure, highest temperature in 24 hours, lowest Glasgow Coma Scale value in 24 hours, PaO_2/FiO_2, if on mechanical ventilation or continuous positive airway pressure, blood urea nitrogen, urine output, sodium, potassium, bicarbonate, bilirubin, white blood cell count, chronic disease (metastatic cancer, haematologic malignancy or AIDS), type of admission (scheduled, medical, unscheduled surgical)

We recorded the presumed **cause of cardiac arrest** in every subject, and categorised them according to the most common aetiological factors that occurred in our cohort into the following groups:

- Ischaemic heart disease
- Heart failure
- Sepsis
- Hyperkalaemia
- Aspiration
- Hypothermia
- Stroke
- Pulmonary embolism
- Pneumonia
- Unknown

The **circumstances of CPR** were also reported:

- Localisation of cardiac arrest: in- or out-of-hospital
- Resuscitation performed during nightshift or weekend
- First monitored rhythm: VT/VF, PEA, asystole
- Length of the CPR (min)
- Epinephrine requirement and dose
- Mechanical ventilation requirement within 6 hours after cardiac arrest

The following **physical parameters** were documented at enrolment:

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- Mean arterial pressure (mmHg)
- Heart rate (/min)
- Body temperature (°C)

Conventionally measured **laboratory parameters** required for routine post-resuscitation care were recorded:

- electrolytes (sodium, potassium)
- markers of renal function (creatinine, blood urea nitrogen)
- markers of hepatic function (glutamyl oxaloacetic transaminase, glutamyl pyruvic transaminase, total bilirubin, International Normalized Ratio)
- inflammatory parameters (C-reactive protein, procalcitonin)
- troponin-T, as a marker of myocardial injury
- lactic dehydrogenase, as a marker of tissue damage
- complete blood count: white blood cell count, neutrophil, haemoglobin, haematocrit, platelet count
- lactic acid, as markers of anaerobic shift and severe tissue hypoperfusion
- blood gas parameters (pH, PaO_2, partial pressure of carbon dioxide - $PaCO_2$, base excess - BE)

We examined the presence of **vital organ system failure** during the post-resuscitation period. Organ system failure was defined as follows:

- *circulation:* the patient still required vasopressor or inotropic support at 24 post-cardiac arrest hours.

- *respiration:* the patient still required positive pressure ventilation support at 24 post-cardiac arrest hours.
- *liver function:* transaminase elevations reaching or exceeding three times the normal value.
- *kidney function*: decreased urine production (<500 ml/day) or creatinine clearance below 30 ml/min.

4.4. Sample collection and processing

Blood samples were drawn from routinely provided arterial or central venous cannula into Vacutainer® EDTA-tubes (Becton Dickinson) within 6, at 24±3 and 72±3 hours after cardiac arrest. The blood samples were centrifuged within 10 minutes at 1500 g for 15 minutes. The plasma supernatant was immediately portioned out into cryo tubes and stored at −80 °C until processing.

We used the stored samples for the determination of plasma concentrations of CK-18, CCCK-18, and NSE in collaboration with the Department of Laboratory Medicine (University of Pécs, Hungary) by using enzyme-linked immunosorbent assay kit (CCCK-18, CK-18 - Shanghai YL Biotech Co., Ltd., China; NSE - FineTest, Wuhan Fine Biotech Co., Ltd., China) with the detection limit of 5.64 ng/L, 19.00 ng/L and 1.41 ng/mL, respectively. The CK-18 assay detects both uncleaved and cleaved fragments, thus it refers to total cell death (apoptosis and necrosis), while the CCCK-18 assay binds only the cleaved variant thus referring only to apoptosis (*Figure 3*).

L-arginine, ADMA, and SDMA were measured by high-performance liquid chromatography after derivatisation in collaboration with the Department of Applied Chemistry (University of Debrecen, Hungary) [82,83]

We calculated the change of the investigated markers from 6 to 24 and from 24 to 72 post-cardiac arrest hours. At each time point, derived parameters were determined: CCCK-18/CK-18 ratio to establish the dominant mode of cell death and L-arginine/ADMA ratio reflecting the nitric oxide production.

All samples were processed by the same technicians using the same equipment and blinded to all clinical data. The biomarker values were blinded to clinicians to avoid the influence on post-resuscitation care approaches or decision-making processes.

4.5. Outcomes

The follow-up period ended on the 30th day after cardiac arrest. In the study period, we evaluated our results according to three different mortality endpoints. The **primary outcomes** included mortality within 72 post-cardiac arrest hours, during ICU stay, and within 30 days.

We determined the routinely used mortality risk scores (SOFA and SAPS II), the presence of different vital organ system failure (circulatory, respiratory, liver, kidney) and neurological status as **secondary outcomes**. The neurological condition was described with the commonly used functional outcome scale in resuscitation studies, according to the Cerebral Performance Category (CPC) score (*Table 2.*) [84]. CPC contains five categories, where CPC 1 means intact brain function or minimal brain injury, CPC 2 includes patients with minor neurological disabilities, CPC 3 implies a wide range of different severe neurological disabilities, CPC 4 indicates persistent vegetative state, while CPC 5 is regarded as death or brain death [85]. To avoid false pessimistic estimation of neurological function and to get information about patients who died in ICU or before 30 days, the best neurological status (i.e. the highest CPC score) reached in the ICU was recorded instead of CPC at discharge [46]. For clarity and statistical purposes, we dichotomised patients according to good (CPC 1-3) and poor (CPC 4-5) neurological outcomes.

Table 2. *Cerebral Performance Categories* [85]

CPC 1	Good cerebral performance: conscious, alert, able to work, might have mild neurologic or psychologic deficit.
CPC 2	Moderate cerebral disability: conscious, sufficient cerebral function for independent activities of daily life. Able to work in sheltered environment.
CPC 3	Severe cerebral disability: conscious, dependent on others for daily support because of impaired brain function. Ranges from ambulatory state to severe dementia or paralysis.
CPC 4	Coma or vegetative state: any degree of coma without the presence of all brain death criteria. Unawareness, even when appearing awake (vegetative state) without interaction with the environment; may have spontaneous eye-opening and sleep/awake cycles. Cerebral unresponsiveness.
CPC 5	Death: certified brain dead (apnea, areflexia, EEG silence, etc.) or dead by traditional criteria.

4.6. Statistical analysis

Normality distribution of continuous variables was tested by Kolmogorov-Smirnov statistical test. Normally distributed variables are expressed as mean and standard deviation, non-normally distributed ones as median and interquartile range, while categorical variables are described as frequencies and percentages. Comparison of non-normally distributed data between groups was carried out using the Mann-Whitney U-test. Student T-test was applied for analysis of normally distributed data. Categorical variables were compared using the Chi-square test. Correlation analysis was performed calculating Spearman's correlation coefficient (rho). For variables with significant correlation, linear logistic regression analysis was performed, and R^2 values were reported. ROC analysis and the AUC were used to determine the most appropriate cut-off values of investigated biomarker levels for study endpoints, "z" tests were used for comparison of multiple ROC curves. Univariate binary logistic regression tests were used to evaluate association between the recorded initial variables and mortality displaying the corresponding beta values and 95% confidence intervals. Variables with a p-value ≤ 0.05 in the univariate analysis were included in the multivariable models considering the principle of multicollinearity. Multivariable logistic regression was applied to identify factors independently associated with mortality. The statistical analysis of the collected data was accomplished by IBM SPSS Statistics® 27.0 software. A p-value <0.05 was considered statistically significant.

5. Results

5.1. Characteristics of the study cohort

A total of 54 patients were enrolled with the median age of 67 [61-78] years, 48% were male. 72% of patients suffered cardiac arrest in the hospital. The initial rhythm was mainly asystole (23 patients, 43%), and around one-fourth of patients (14 patients, 26%) had PEA, while VF/VT presented among other one-fourth of patients (14 patients, 26%). In the case of three patients (5%), we had no information about the initial rhythm. Cardiac aetiology (ischaemic heart disease, heart failure) was determined as the presumed cause of cardiac arrest among almost two thirds of our patients (61%). Half of our cohort reached acceptable neurological status (CPC 1-3) during the ICU stay, while the other half suffered from coma, persistent vegetative state, or brain death (CPC 4-5) as shown in *Figure 4*. 8 patients who reached acceptable neurological function (CPC 1-3) later died

due to non-neurological reasons (multiorgan failure) in the ICU. *Figure 5.* shows the flow chart about the exact numbers of survivors at each investigated endpoint. *Table 3.* compares the characteristics of 30-day survivors and non-survivors, while characteristics according to ICU and 72-hour mortality are summarised in the appendix (*Appendix 1-2.*).

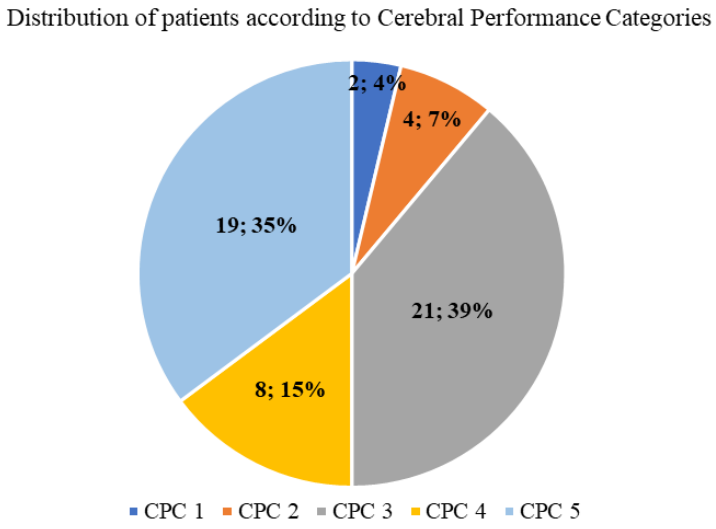

Figure 4. *Distribution of patients by neurological outcome according to best CPC reached during the ICU stay (number of patients; %).*

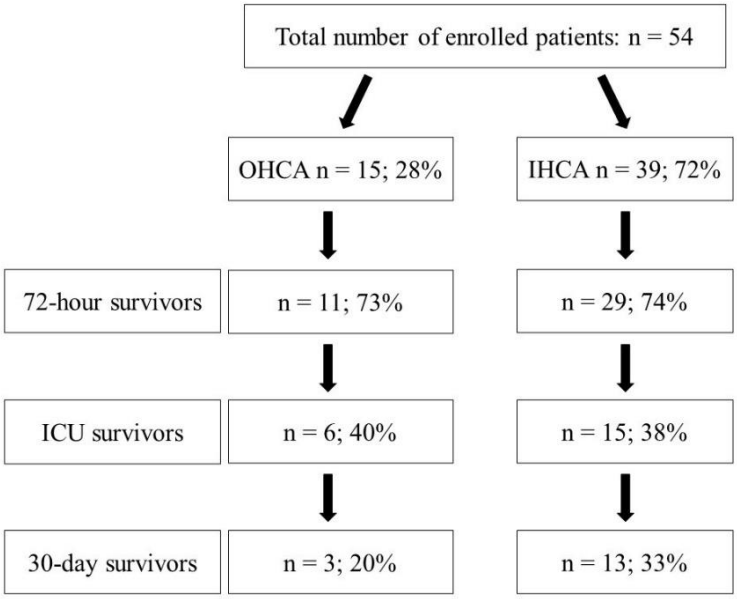

Figure 5. *Number of patients who survived 72 hours, ICU discharge, and 30 days after cardiac arrest (number of patients; %).*

Baseline patient characteristics, circumstances and presumed aetiology of cardiac arrest, characteristics of CPR, vital parameters at enrolment, and most relevant comorbid conditions of the study population are summarised in *Table 3*. We did not find statistically significant differences between **30-day non-survivors and survivors** regarding the

characteristics detailed in *Table 3*. Among 30-day survivors, the CPC category was significantly more beneficial (3.0 [2.3 – 3.0] vs. 4.5 [3.0 – 5.0]; $p < 0.001$), as expected, and significantly more favourable SAPS II and SOFA scores were observed.

Table 3. *Characteristics of the study population according to 30-day mortality*

	Survivors (n=16; 30%)	**Non-survivors (n=38; 70%)**	**p-value**
Baseline			
Age (years)	61 [58 – 76]	69 [62 – 80]	0.134
Male gender	5 (31%)	21 (55%)	0.107
Characteristics of cardiac arrest and CPR			
Localisation: in-hospital cardiac arrest	13 (81%)	26 (68%)	0.337
Resuscitation during nightshift or weekend	10 (63%)	29 (76%)	0.301
First monitored rhythm:			
• Ventricular tachycardia/fibrillation	4 (25%)	10 (26%)	0.920
• Pulseless electrical activity	3 (19%)	11 (29%)	0.435
• Asystole	8 (50%)	15 (40%)	0.475
• Unknown	1 (6%)	2 (5%)	0.885
Time of the resuscitation (min)	10 [5 – 23]	10 [5 – 20]	0.916
Patients required epinephrine	11 (69%)	34 (90%)	0.062
Dose of epinephrine (mg)	2 [0 – 3]	2 [1 – 3]	0.234
Mechanical ventilation requirement within 6 hours after cardiac arrest	15 (94%)	34 (90%)	0.621
Aetiology of cardiac arrest			
Ischaemic heart disease	6 (38%)	11 (29%)	0.537
Heart failure	6 (38%)	10 (26%)	0.411
Sepsis	1 (6%)	4 (11%)	0.621
Hyperkalaemia	1 (6%)	4 (11%)	0.621
Aspiration	1 (6%)	2 (5%)	0.885
Hypothermia	1 (6%)	1 (3%)	0.520
Stroke	0	2 (5%)	0.350
Pulmonary embolism	0	2 (5%)	0.350
Pneumonia	1 (6%)	1 (3%)	0.520
Parameters on enrolment			
Systolic blood pressure (mmHg)	115 [104 – 133]	114 [102 – 138]	0.896
Diastolic blood pressure (mmHg)	61 [55 – 69]	61 [54 – 68]	0.905
Mean arterial pressure (mmHg)	77 [71 – 91]	77 [70 – 86]	0.842
Heart rate (/min)	76 [66 – 90]	91 [70 – 105]	0.065
Body temperature (°C)	36.6±0.7	36.2±1.5	0.269
Comorbidities, previous medical history			
Hypertension	12 (75%)	27 (71%)	0.767
Ischaemic heart disease	4 (25%)	16 (42%)	0.235
Diabetes mellitus	4 (25%)	18 (47%)	0.127
Heart failure	6 (38%)	11 (29%)	0.537
Permanent atrial fibrillation	3 (19%)	7 (18%)	0.977
Stroke or transient ischaemic attack	1 (6%)	9 (24%)	0.132
Carotid artery stenosis	2 (13%)	3 (8%)	0.594

Chronic obstructive pulmonary disease	5 (31%)	3 (8%)	**0.027**
Peripheral artery disease	1 (6%)	6 (16%)	0.341
Previous pulmonary embolism	1 (6%)	2 (5%)	0.885
Previous, cured malignant disease	3 (19%)	5 (13%)	0.597
Active malignant or haematologic disease	2 (13%)	7 (18%)	0.594
Prognostic scores			
SOFA	9 ± 4	11 ± 3	**0.049**
SAPS II	64 ± 17	80 ± 14	**<0.001**

Prognostic score points were calculated concerning the worst detected value within 24 hours after cardiac arrest. Continuous data are presented as median values with interquartile range [percentiles 25–75] or mean ± standard deviation, categorical data as the number of subjects and percentages. CPR: cardiopulmonary resuscitation; SOFA: Sequential Organ Failure Assessment Score; SAPS II: Simplified Acute Physiology Score II; ICU: intensive care unit.

A total of 33 patients (61%) died in the ICU by an average of 6 (min. 1 - max. 26) days. The table in the appendix shows the population characteristics according to **ICU mortality** (*Appendix 1.*). Based on this division, significantly higher number of patients died in the ICU with ischaemic heart disease or diabetes mellitus in the past medical history, while this difference was not significant according to 30-day mortality. Significantly higher SOFA and SAPS II points were calculated among ICU non-survivors similarly to 30-day mortality.

Around one-fourth (26%) of the patients died within the first three days after ROSC. The table in the appendix demonstrates the population characteristics according to **72-hour mortality** (*Appendix 2.*). There was no statistically significant difference between 72-hour survivors (n=40; 74%) and non-survivors (n=14; 26%) regarding age, gender, cardiac arrest characteristics (e.g. in-hospital or out-of-hospital, length of the cardiopulmonary resuscitation, first monitored rhythm), suggested aetiology of cardiac arrest, vital parameters on enrolment, or comorbidities. 72-hour non-survivors had significantly higher points of SAPS II score, while SOFA scores were not significantly different according to 72-hour mortality.

To summarise patient characteristics according to three mortality endpoints, in our cohort the age, gender, and the length of the CPR, initial rhythm did not influence the survival at any investigated endpoint, and mortality was independent of whether the cardiac arrest occurred in- or out-of-hospital and whether it happened during working hours or nightshift/weekend. The mechanical ventilation and epinephrine requirement and the basic vital parameters on enrolment were also similar between survivors and non-survivors. The percentage distribution of the comorbidities, past medical history and

presumed cause of cardiac arrest also showed no significant difference between survivors and non-survivors, except for the above-detailed observations concerning mortality in ICU.

5.2. L-arginine, ADMA, SDMA

5.2.1. Biomarker levels according to 72-hour mortality

Table 4. summarises the absolute plasma levels of L-arginine, ADMA, and SDMA and their change over the first three post-cardiac arrest days between 72-hour survivors and non-survivors. Significantly higher initial ADMA levels were discovered among patients who died within 72 hours after cardiac arrest. We did not observe significant difference concerning initial ADMA levels according to the location of CPR (IHCA: 0.61 [0.46 – 0.85] vs. OHCA: 0.64 [0.45 – 0.87], p= 0.977).

Table 4. *L-arginine pathway molecules according to the 72-hour mortality*

	Survivors (n=40; 74%)	**Non-survivors (n=14; 26%)**	**p-value**
Biomarker plasma levels within 6 hours after cardiac arrest			
L-arginine (µmol/L)	33.45 [27.84 – 46.96]	46.16 [27.89 – 72.44]	0.079
ADMA (µmol/L)	**0.55 [0.45 – 0.69]**	**0.88 [0.64 – 0.97]**	**0.001**
SDMA (µmol/L)	0.93 [0.65 – 1.60]	0.93 [0.76 – 1.29]	0.969
Biomarker plasma levels 24 hours after cardiac arrest			
L-arginine (µmol/L)	38.95 [31.26 – 60.56]	45.62 [17.64 – 70.11]	0.910
ADMA (µmol/L)	0.54 [0.45 – 0.78]	0.78 [0.51 – 1.05]	0.145
SDMA (µmol/L)	1.03 [0.75 – 1.98]	1.32 [0.88 – 2.28]	0.515
Change in biomarker plasma levels from 6 to 24 hours after cardiac arrest			
ΔL-arginine (24h-6h) (µmol/L)	5.16 [-4.48 – 23.37]	-5.21 [-25.32 – 21.38]	0.234
ΔADMA (24h-6h) (µmol/L)	0.03 [-0.08 – 0.10]	-0.12 [-0.20 – 0.02]	0.079
ΔSDMA (24h-6h) (µmol/L)	0.17 [-0.02 – 0.42]	0.22 [0.02 – 0.42]	0.713

Data are presented as median values with interquartile range [percentiles 25–75]. ADMA: asymmetric dimethylarginine; SDMA: symmetric dimethylarginine.

5.2.2. Biomarker levels according to ICU mortality

Investigating the ICU mortality, none of the L-arginine pathway molecules showed significant difference between survivors or non-survivors. The initial ADMA levels tended to remain higher among ICU non-survivors, but the difference did not reach significance. The plasma ADMA levels of ICU non-survivors decreased from 6 to 24 hours, while the values of the surviving group raised by 24 hours (-0.08 [-0.16 – 0.05] µmol/L vs. 0.07 [-0,04 – 0.11] µmol/L, p= 0.024) (*Table 5.*). Subgroup analysis of IHCA patients revealed significantly decreased 6-hour L-arginine/ADMA ratio in patients who died in the ICU (*Figure 6.*).

Table 5. *L-arginine pathway molecules and their change according to ICU mortality*

	Survivors (n=21; 39%)	**Non-survivors (n=33; 61%)**	**p-value**
Biomarker plasma levels within 6 hours after cardiac arrest			
L-arginine (µmol/L)	33.17 [29.43 – 52.68]	42.25 [23.59 – 49.96]	N.S.
ADMA (µmol/L)	0.58 [0.46 – 0.66]	0.70 [0.46 – 0.89]	N.S.
SDMA (µmol/L)	0.77 [0.62 – 1.73]	0.96 [0.81 – 1.46]	N.S.
Biomarker plasma levels 24 hours after cardiac arrest			
L-arginine (µmol/L)	48.82 [31.04 – 69.06]	38.14 [23.24 – 56.77]	N.S.
ADMA (µmol/L)	0.59 [0.51 – 0.79]	0.54 [0.41 – 0.81]	N.S.
SDMA (µmol/L)	0.97 [0.74 – 2.79]	1.30 [0.85 – 1.81]	N.S.
Biomarker plasma levels 72 hours after cardiac arrest			
L-arginine (µmol/L)	61.01 [47.35 – 77.33]	53.78 [35.98 – 75.31]	N.S.
ADMA (µmol/L)	0.60 [0.50 – 0.76]	0.62 [0.45 – 0.85]	N.S.
SDMA (µmol/L)	0.90 [0.67 – 2.20]	1.48 [0.92 – 1.75]	N.S.
ΔL-arginine (24h-6h) (µmol/L)	10.81 [-2.78 – 25.16]	2.84 [-17.13 – 13.24]	N.S.
ΔADMA (24h-6h) (µmol/L)	0.07 [-0,04 – 0.11]	-0.08 [-0.16 – 0.05]	**0.024**
ΔSDMA (24h-6h) (µmol/L)	0.21 [0.003 – 0.46]	0.11 [-0.01 – 0.39]	N.S.
ΔL-arginine (72h-24h) (µmol/L)	18.98 [-6.31 – 29.75]	8.95 [-9.21 – 28.66]	N.S.
ΔADMA (72h-24h) (µmol/L)	0.01 [-0.06 – 0.14]	0.07 [-0.06 – 0.14]	N.S.
ΔSDMA (72h-24h) (µmol/L)	-0.06 [-0.37 – 0.06]	0.16 [-0.23 – 0.55]	N.S.

Data are presented as median values with interquartile range [percentiles 25–75]. ADMA: asymmetric dimethylarginine; ICU: intensive care unit; SDMA: symmetric dimethylarginine. N.S.: non-significant, p>0.05.

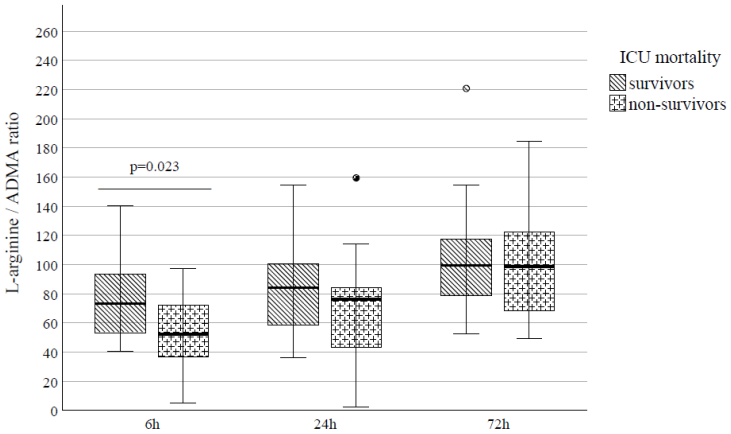

Figure 6. *L-arginine/ADMA ratio and ICU mortality in IHCA group*

5.2.3. Biomarker levels according to 30-day mortality

Analysing the kinetics of the markers according to 30-day mortality, opposite changes in ADMA concentrations were detected from 6 to 24 hours between survivors and non-survivors (-0.08 [-0.16 – 0.06] in non-survivors vs. 0.07 [-0.03 – 0.11] in

survivors, p= 0.028) similarly to the observation according to ICU mortality (*Table 6.*). In contrast, no significant alterations concerning L-arginine, SDMA levels, or their change were detectable at any investigated time point. The L-arginine/ADMA ratio slightly elevated up to 72 post-cardiac arrest hours in the total population regardless of the mortality (6 h: 66.04 ± 4.33; 24 h: 80.04 ± 5.35; 72 h: 99.99 ± 7.13; $p<0.05$) (*Figure 7.*).

Table 6. *Biomarker levels and their change according to 30-day mortality*

	Survivors (n=16; 30%)	**Non-survivors (n=38; 70%)**	**p value**
Biomarker plasma levels within 6 hours after cardiac arrest			
L-arginine (μmol/L)	33.31 [29.24 – 51.86]	41.67 [25.01 – 51.68]	N.S.
ADMA (μmol/L)	0.54 [0.46 – 0.64]	0.67 [0.44 – 0.89]	N.S.
SDMA (μmol/L)	0.77 [0.62 – 1.96]	0.95 [0.76 – 1.43]	N.S.
Biomarker plasma levels 24 hours after cardiac arrest			
L-arginine (μmol/L)	42.97 [28.61 – 69.06]	39.09 [31.26 – 57.75]	N.S.
ADMA (μmol/L)	0.59 [0.51 – 0.77]	0.54 [0.44 – 0.83]	N.S.
SDMA (μmol/L)	0.89 [0.7138 – 3.06]	1.18 [0.89 – 1.81]	N.S.
Biomarker plasma levels 72 hours after cardiac arrest			
L-arginine (μmol/L)	62.32 [54.14 – 74.25]	49.26 [36.01 – 78.26]	N.S.
ADMA (μmol/L)	0.59 [0.51 – 0.79]	0.65 [0.45 – 0.84]	N.S.
SDMA (μmol/L)	1.22 [0.72 – 2.34]	1.32 [0.74 – 1.71]	N.S.
Change in biomarker plasma levels			
ΔL-arginine (24h-6h) (μmol/L)	10.81 [-2.48 – 26.59]	2.84 [-10.41 – 17.60]	N.S.
ΔADMA (24h-6h) (μmol/L)	0.07 [-0.03 – 0.11]	-0.08 [-0.16 – 0.06]	**0.028**
ΔSDMA (24h-6h) (μmol/L)	0.17 [0.003 – 0.56]	0.14 [-0.01 – 0.41]	N.S.
ΔL-arginine (72h-24h) (μmol/L)	19.16 [4.96 – 34.88]	8.62 [-7.60 – 27.12]	N.S.
ΔADMA (72h-24h) (μmol/L)	0.04 [-0.05 – 0.16]	0.06 [-0.07 – 0.13]	N.S.
ΔSDMA (72h-24h) (μmol/L)	-0.06 [-0.32 – 0.13]	0.08 [-0.32 – 0.47]	N.S.

Data are presented as median values with interquartile range [percentiles 25–75]. ADMA: asymmetric dimethylarginine; SDMA: symmetric dimethylarginine. N.S.: non-significant, $p>0.05$.

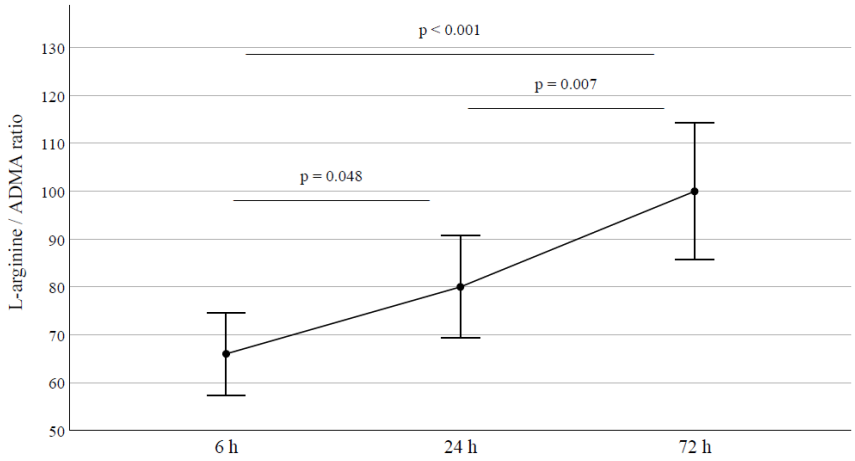

Figure 7. *The kinetics of the L-arginine/ADMA ratio in the total study population*

5.2.4. ADMA and prognostic scores

Neither SAPS II nor SOFA score showed significant difference between IHCA and OHCA subgroups. The statistical analysis revealed a significant positive correlation between the initial ADMA levels and the SAPS II score (rho=0.393, R^2=0.178, p=0.002) (*Figure 8.*).

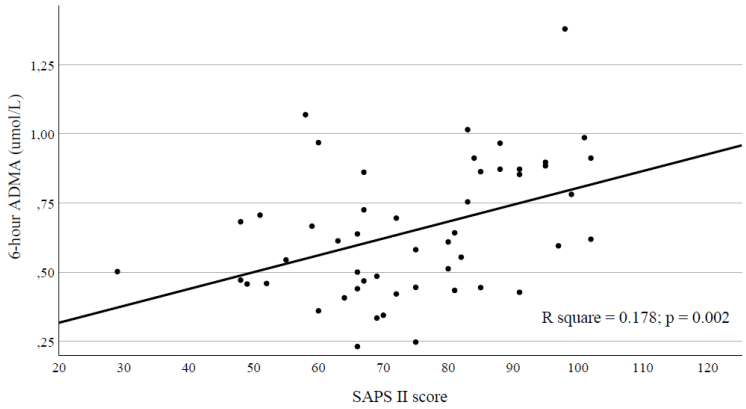

Figure 8. *Linear regression analysis of 6-hour ADMA levels and SAPS II score*

Figure 9. demonstrates the curves of combined ROC analysis of SOFA, SAPS II, and initial ADMA for 72-hour mortality. The results showed that the AUC of SAPS II and initial ADMA were comparable reflecting similar sensitivity and specificity in prediction of 72-hour mortality, in contrast SOFA provided poor prognostic information for mortality (SAPS II AUC: 0.817 [0.688 – 0.946], p<0.001; ADMA AUC: 0.789 [0.628 – 0.950], p=0.001; SOFA AUC: 0.608 [0.433 – 0.783], p=0.232)

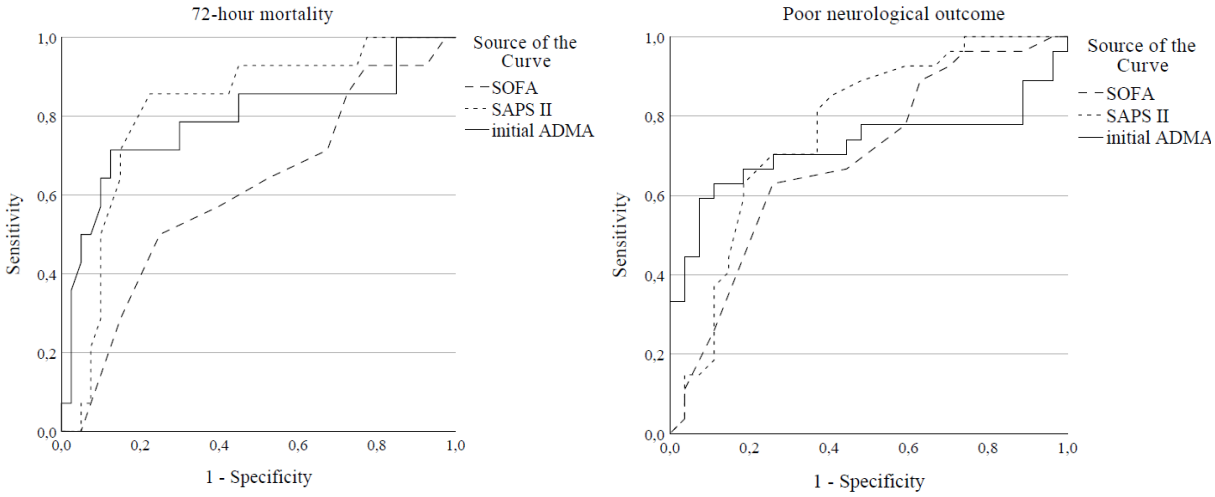

Figure 9. *ROC Curves of initial ADMA, SOFA, and SAPS II for 72-hour mortality and poor neurological outcome*

5.2.5. ADMA and neurological outcome

Significantly elevated initial ADMA levels were detected among patients with persistent vegetative state or brain death (CPC 4-5) (*Figure 10.*). ROC analysis of initial ADMA for prediction of poor neurological outcome (CPC 4-5) indicated an AUC of 0.723 [0.574 – 0.871] (p= 0.005) (*Figure 9.*). Based on the ROC analysis, the best cut-off for poor neurological outcome (CPC 4-5) was determined as >0.65 µmol/L (sensitivity: 66.7%; specificity: 81.5%). The values over 0.89 µmol/L have maximal specificity (100%) for CPC 4-5 with 33.3% sensitivity.

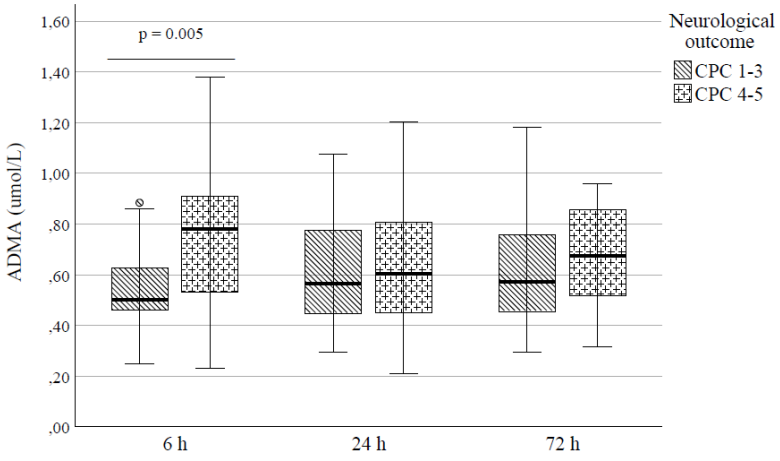

Figure 10. *ADMA levels according to acceptable (CPC 1-3) and poor (CPC 4-5) neurological outcome*

5.2.6. Independent prediction of 72-hour mortality

Based on ROC analysis, the initial ADMA level was found to be a predictor of 72-hour mortality (*Figure 9.*) with the best cut-off value of >0.81 µmol/L (sensitivity:

71.0%; specificity: 87.5%). Univariate logistic regression analyses including each variable assessed within 6 hours after cardiac arrest identified initial ADMA, serum bicarbonate (HCO_3^-), and lactate levels as significant markers for 72-hour mortality. Multivariable analysis revealed that initial ADMA (OR: 1.8 per 0.1 µmol/L increase in ADMA; 95% CI: 1.252 – 2.611; p=0.002) is an independent predictor for 72-hour mortality after cardiac arrest (*Table 7.*).

Table 7. *Univariable (a.) and multivariable (b.) regression analysis for 72-hour mortality*

a. Univariable logistic regression analysis for 72-hour mortality:

Variable	Odds ratio - Exp(B) (lower CI - upper CI)	p value
ADMA 6h (per 0.1 µmol/L increase)	1.81 (1.25 – 2.61)	**0.002**
HCO_3^- 6h (per 1 mmol/L increase)	0.89 (0.79 - 0.99)	**0.034**
Lactate 6h (per 1 mmol/L increase)	1.26 (1.06 - 1.49)	**0.008**

b. Binary logistic regression analysis for 72-hour mortality:

Model 1 - Binary Logistic Regression - Enter	B	Odds ratio - Exp(B) (lower CI - upper CI)	p value
ADMA 6h (per 0.1 µmol/L increase)	0.573	1.77 (1.23 – 2.56)	**0.002**
HCO_3^- 6h (per 1 mmol/L increase)	-0.132	0.88 (0.77 – 1.00)	0.054

Model 2 - Binary Logistic Regression - Enter	B	Odds ratio - Exp(B) (lower CI - upper CI)	p value
ADMA 6h (per 0.1 µmol/L increase)	0.488	1.63 (1.14 – 2.33)	**0.008**
Lactate 6h (per 1 mmol/L increase)	0.189	1.21 (0.99 – 1.48)	0.065

ADMA: asymmetric dimethylarginine; CI: confidence interval; HCO_3^-: bicarbonate

5.3. Markers of cell damage and death - CK-18, CCCK-18 and NSE

As there was no significant difference according to 72-hour and ICU mortality for any of the markers discussed in this subsection, results according to 30-day mortality will be explained in detail here and values related to this endpoint will be presented in the following tables and figures.

5.3.1. Biomarker levels according to mortality, neurological outcome and organ failure

Figures 11-13. demonstrate box plot diagrams of CK-18, CCCK-18, CCCK-18/CK-18 ratio, and NSE values measured at 6, 24, and 72 hours after ROSC according to 30-day mortality. None of the investigated biomarkers or their kinetics showed significant difference between survivors and non-survivors. We observed no significant alteration in the marker levels over the first three days in any patient group. Investigating

the 72-hour or ICU mortality, we did not obtain significant alterations either comparing non-survivor to survivor group, therefore these results are not illustrated.

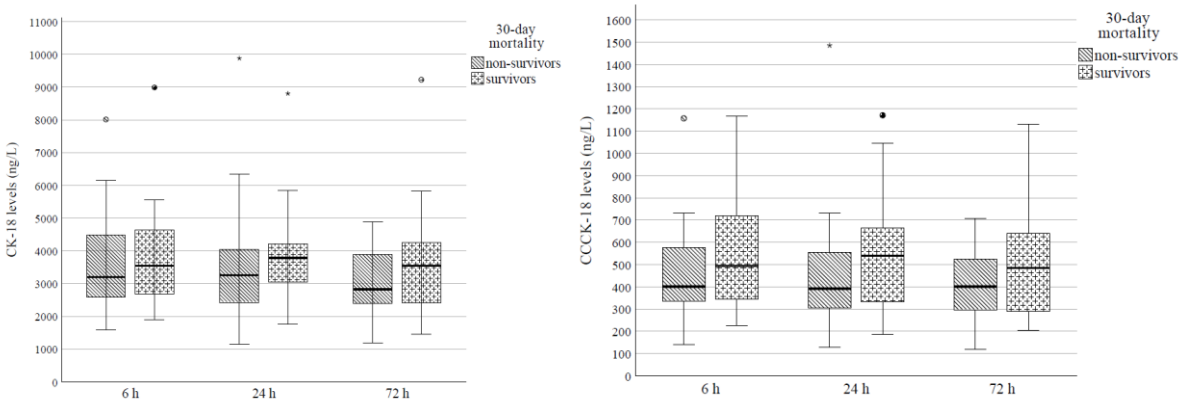

Figure 11. *CK-18 and CCCK-18 levels reflecting the total cell death according to 30-day mortality*

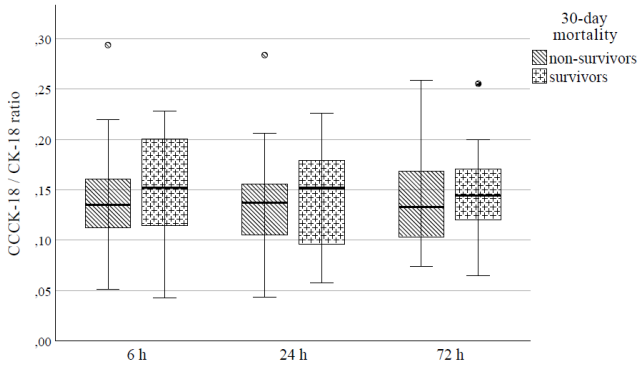

Figure 12. *CCCK-18/ CK-18 ratio reflecting the dominant mode of cell death according to 30-day mortality*

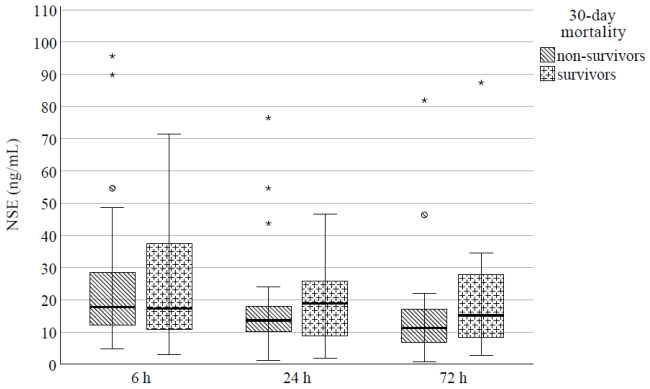

Figure 13. *NSE values reflecting the neuronal injury according to 30-day mortality.*

We could not confirm a connection between the cell death marker levels and neurological outcome either (*Figure 14.*).

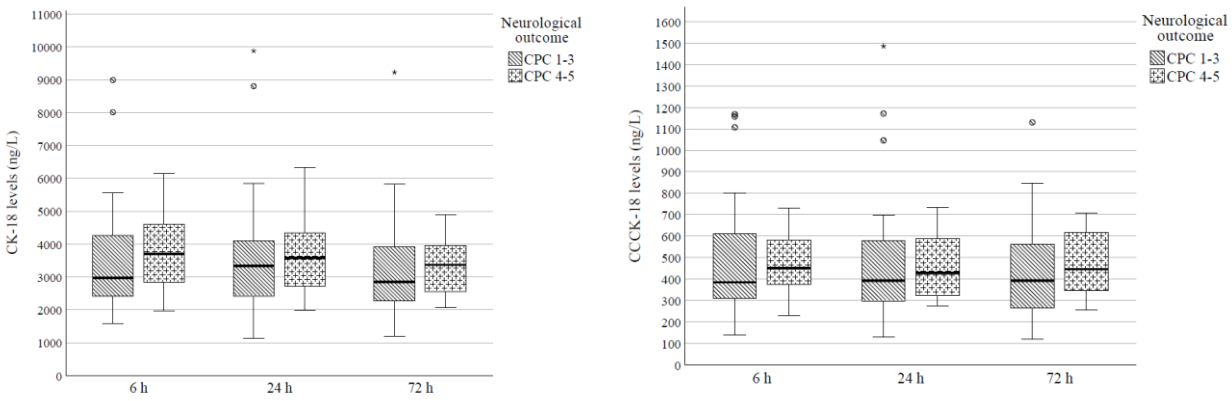

Figure 14. *CK-18 and CCCK-18 levels in the investigated time points according to neurological category (CPC 1-3 as acceptable vs. CPC 4-5 as poor outcome)*

Although the initial and 24-hour CK-18 values were not associated with the prognostic scores, the 72-hour CK-18 level already showed significant correlation with SAPS II (rho=0.581; p<0.001) and SOFA scores (rho=0.418; p=0.012). The results of linear regression analyses are illustrated in *Figure 15*. We did not observe this connection concerning CCCK-18, the marker of apoptosis.

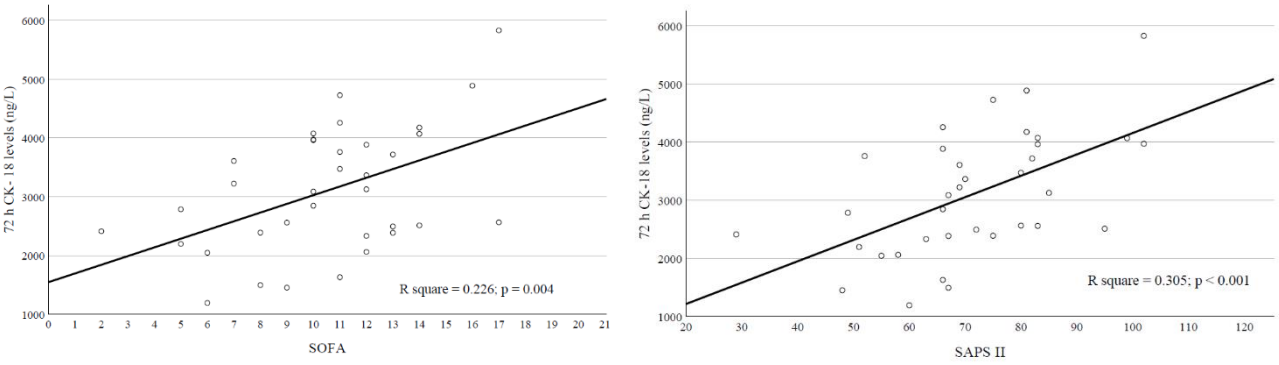

Figure 15. *Linear regression analysis of 72-hour CK-18 levels with SOFA and SAPS II score*

Our results could not confirm any association between the number of organ system failures and the extent of cell death reflected by cytokeratins (*Figure 16.*). On the other hand, subgroup analysis revealed that the CK-18 level did not decrease over the first three days after ROSC in the presence of renal failure compared to patients with intact renal function, where a decline was visible, resulting in a significant difference at 72 post-cardiac arrest hours (*Figure 17.*). The non-significant results concerning other organ failures are not illustrated.

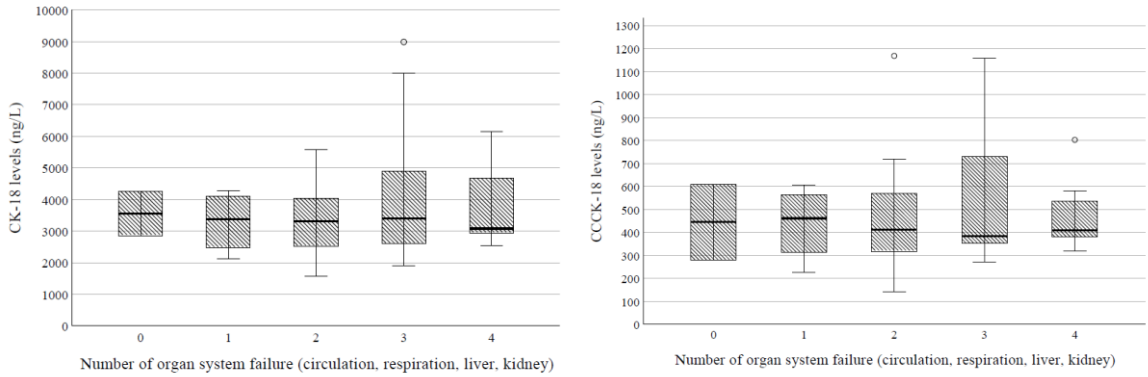

Figure 16. *CK-18 and CCCK-18 levels according to the number of organ system failure*

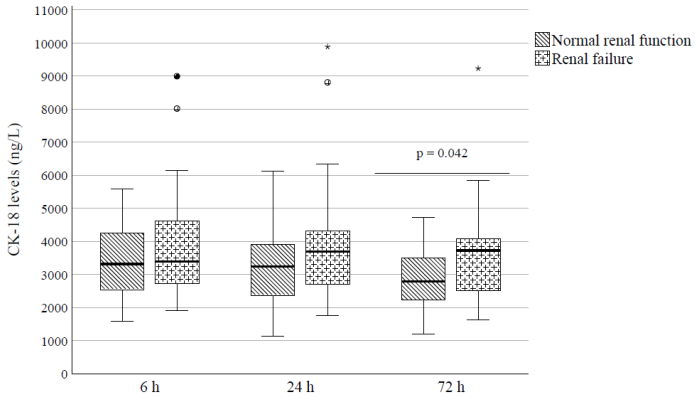

Figure 17. *CK-18 levels according to normal or impaired renal function*

To better understand which factors are influencing the circulating concentration of CK-18 and CCCK-18 cell death markers after cardiac arrest, we performed subgroup analyses concerning different aspects of CPR characteristics. According to our results, the location of cardiac arrest (whether it was an IHCA or OHCA) was not related to the circulating marker levels at any investigated time points over the first three days after ROSC (*Figure 18.*) The detected initial rhythm at the start of CPR (i.e. shockable – VF/VT or non-shockable - PEA or asystole) did not distinguish significantly the marker levels (*Figure 19.*). Length of CPR (from the detection of cardiac arrest until ROSC) being shorter < 10 min or longer ≥ 10 min did not significantly affect the marker levels either (*Figure 20.*).

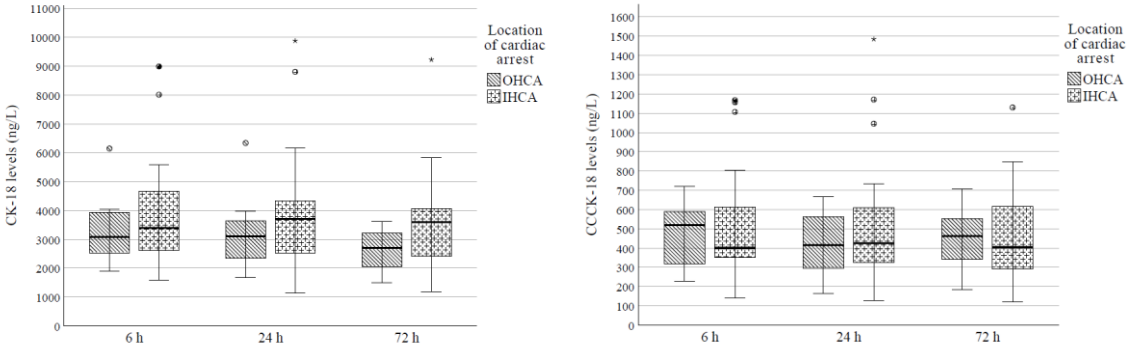

Figure 18. *CK-18 and CCCK-18 levels according to the location of cardiac arrest*

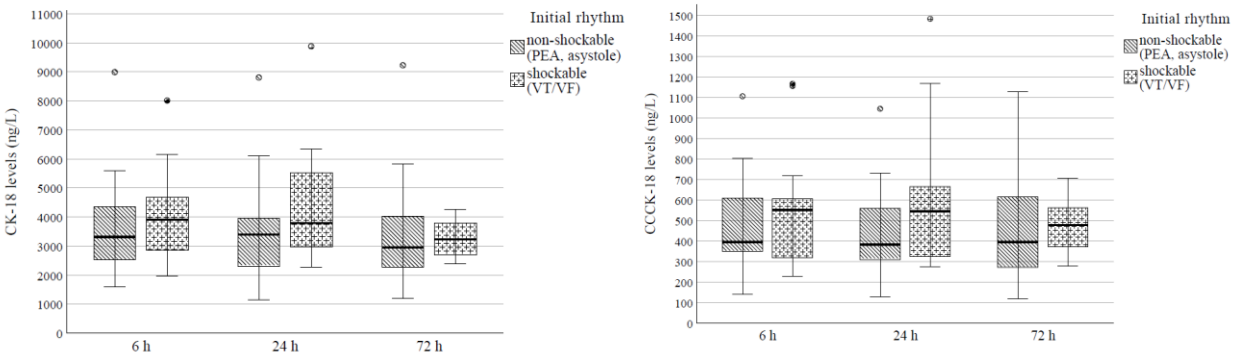

Figure 19. *CK-18 and CCCK-18 levels according to the initial rhythm*

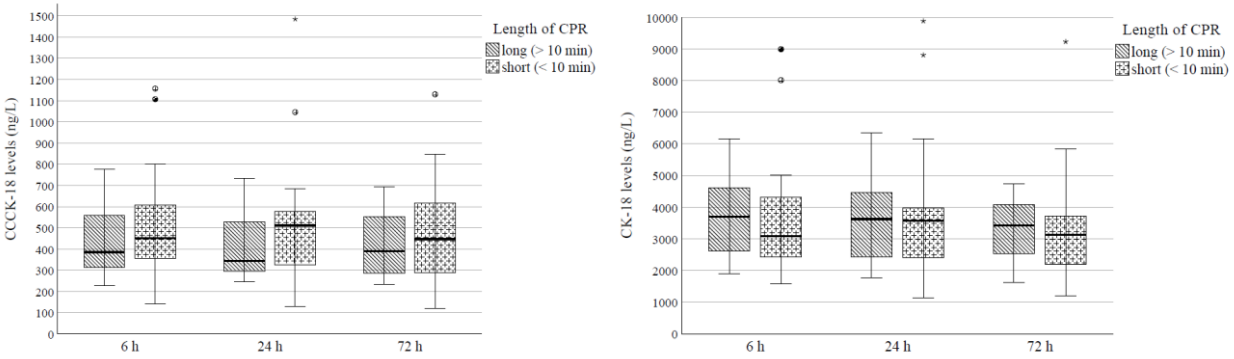

Figure 20. *CK-18 and CCCK-18 levels according to the length of CPR*

5.4. Characteristics and alterations of routine laboratory parameters

We recorded the conventionally used laboratory parameters in the first three days analysed according to 30-day mortality after cardiac arrest. The values are summarised in *Table 8*. Non-survivors had significantly elevated initial liver function parameters (glutamic oxaloacetic transaminase, glutamic pyruvic transaminase), troponin-T and lactic dehydrogenase values. Haemoglobin and haematocrit values were significantly lower among 30-day survivors after 24 and 72 post-cardiac arrest hours. White blood cell and neutrophil count were raised among non-survivors at 72 post-cardiac arrest hours. Initial and 24-hour lactate levels were higher among non-survivors (initial: 3.2 [2.1 – 4.6] vs. 7.1 [4.3 – 9.7]; p=0.005; and 24 h: 1.0 [0.8 – 1.5] vs. 1.7 [1.1 – 2.5]; p=0.008) as well and patients with more excessive initial lactate (3.6 [2.1 – 5.7] vs. 8.7 [6.0 – 10.7]; p<0.001) or 24-hour lactate levels (1.1 [0.9 – 1.7] vs. 1.8 [1.1 – 2.5]; p=0.030) were more prone to have poor (CPC4-5) neurological status (*Figure 21.*)

Table 8. *Routine laboratory parameters and 30-day mortality after cardiac arrest*

	Survivors (n=16; 30%)	**Non-survivors (n=38; 70%)**	**p value**
Laboratory parameters within 6 hours after cardiac arrest			
blood urea nitrogen (mmol/l)	6.64 [3.99 – 12.89]	10.72 [7.32 – 15.32]	NS
creatinine (umol/l)	113.00 [83.00 – 256.00]	113.00 [95.50 – 186.75]	NS
lactic dehydrogenase (IU/l)	421.00 [312.25 – 687.00]	1096.00 [642.00 – 2354.50]	**0.001**
glutamic oxaloacetic transaminase (IU/l)	27.00 [24.00 – 100.50]	98.50 [41.50 – 680.25]	**0.003**
glutamic pyruvic transaminase (IU/l)	18.00 [13.00 – 62.00]	51.00 [24.50 – 582.00]	**0.020**
total bilirubin (umol/l)	6.90 [4.45 – 18.15]	13.30 [6.25 – 23.1]	NS
Troponin-T (ng/mL)	44.98 [27.11 – 87.05]	142.30 [52.61 – 389.60]	**0.013**
INR	1.19 [1.03 – 1.28]	1.35 [1.12 – 1.76]	**0.050**
CRP (mg/l)	17.50 [2.48 – 97.20]	55.35 [7.60 – 117.43]	NS
white blood cell count (G/l)	12.45 [9.50 – 16.11]	13.95 [9.67 – 21.82]	NS
neutrophil (G/l)	9.07 [5.94 – 12.93]	11.50 [6.96 – 15.08]	NS
haemoglobin (g/l)	101.50 [92.00 – 120.00]	116.00 [97.75 – 134.50]	NS
haematocrit (%)	32.75 [29.15 – 38.00]	35.85 [29.38 – 42.00]	NS
platelet count (G/l)	267.00 [179.75 – 381.25]	236.00 [174.75 – 336.75]	NS
Laboratory parameters 24 hours after cardiac arrest			
blood urea nitrogen (mmol/l)	7.03 [4.90 – 10.32]	12.52 [7.99 – 19.32]	**0.009**
creatinine (umol/l)	109.50 [77.25 – 202.50]	145.50 [89.00 – 223.00]	NS
CRP (mg/l)	61.75 [29.53 – 90.88]	77.45 [31.78 – 129.68]	NS
white blood cell count (G/l)	10.19 [7.19 – 19.97]	12.42 [9.67 – 19.15]	NS

neutrophil (G/l)	8.23 [5.33 – 18.75]	11.12 [8.83 – 17.00]	NS
haemoglobin (g/l)	92.50 [82.50 – 115.50]	115.00 [103.50 – 133.00]	**0.015**
haematocrit (%)	28.35 [25.35 – 37.33]	34.80 [30.70 – 40.20]	**0.018**
platelet count (G/l)	237.00 [145.75 – 369.75]	267.00 [142.50 – 321.50]	NS
Laboratory parameters 72 hours after cardiac arrest			
blood urea nitrogen (mmol/l)	6.62 [5.02 – 9.08]	10.68 [6.77 – 17.93]	NS
creatinine (umol/l)	86.50 [67.00 – 143.50]	131.00 [76.00 – 237.00]	NS
CRP (mg/l)	94.85 [50.65 – 156.05]	136.70 [60.20 – 224.60]	NS
white blood cell count (G/l)	9.20 [6.82 – 12.76]	16.27 [12.91 – 20.14]	**0.002**
neutrophil (G/l)	7.77 [5.65 – 11.27]	13.06 [10.34 – 17.92]	**0.002**
haemoglobin (g/l)	91.00 [80.75 – 112.00]	111.50 [94.25 – 124.00]	**0.033**
haematocrit (%)	27.70 [25.50 – 34.18]	33.35 [28.68 – 39.00]	**0.050**
platelet count (G/l)	192.50 [109.50 – 293.25]	197.00 [126.00 – 275.50]	NS

Continuous data are presented as median value with interquartile range (percentiles 25–75), categorical data as number of subjects and percentages. (CA=cardiac arrest; CRP=C-Reactive Protein; INR=International Normalized Ratio; NS=non-significant p>0.05)

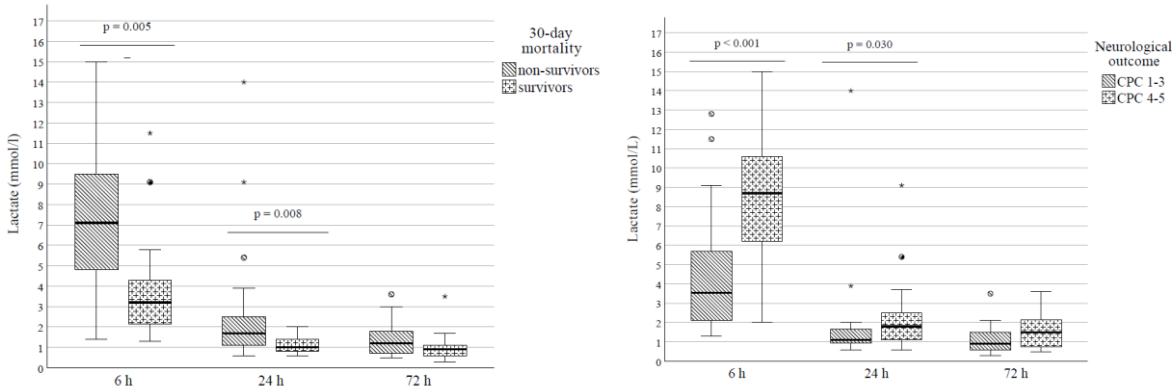

Figure 21. *Lactate levels at the investigated time points according to 30-day mortality and neurological category (CPC 1-3 as acceptable vs. CPC 4-5 as poor outcome)*

5.5. The role of prognostic scores and lactate in prediction of 30-day mortality and neurological outcome

CK-18, CCCK-18, NSE, or their change had no significant relationship with 30-day mortality revealed by univariate regression analysis. However, initial lactate seemed to predict 30-day mortality and neurological outcome. 24 and 72-hour lactate values carried mild statistical importance compared to initial lactate levels, therefore, in later statistical analyses we considered initial lactate. ROC analysis for prediction of the 30-day mortality was peformed with SOFA, SAPS II, and lactate levels. AUC values were 0.638 [0.465 – 0.811] (p=0.122) for SOFA, 0.767 [0.616 – 0.917] (p=0.003) for SAPS II and 0.753 [0.607 – 0.899] (p=0.005) for lactate, respectively. In mortality prediction,

SOFA had poor, SAPS II had moderate value, while the AUC of lactate per se was similar to SAPS II based on ROC analysis. There was no statistically significant difference comparing the AUC values (SAPS II vs. lactate p=0.892; SAPS II vs. SOFA p=0.088; lactate vs. SOFA p=0.373). (*Figure 22.*)

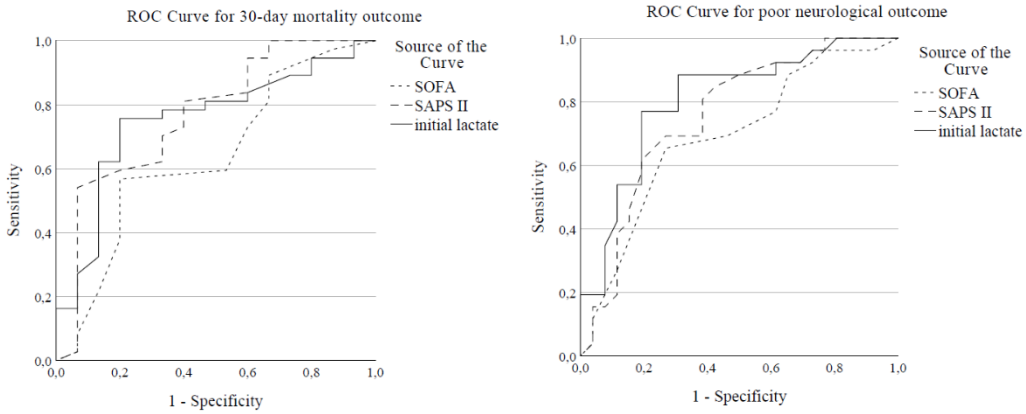

Figure 22. *ROC Curve of initial lactate, SAPS II and SOFA for 30-day mortality and poor neurological outcome (CPC 4-5)*

ROC analysis was carried out with the same variables for the prediction of poor neurological outcomes defined as CPC 4-5. AUC values were 0.689 [0.544 – 0.835] (p=0.019) for SOFA, 0.757 [0.623 – 0.891] (p=0.001) for SAPS II and 0.806 [0.685 – 0.928] (p<0.001) for lactate. Consequently, lactate per se had good predictive value for unfavourable neurological outcomes, however, the differences of the AUC values were not statistically significant (SAPS II vs. lactate p=0.356; SAPS II vs. SOFA p=0.322; lactate vs. SOFA p=0.129) (*Figure 22.*). The ROC curve of initial lactate per se indicated a cut-off as 4.90 mmol/L to predict 30-day mortality (sensitivity: 74%; specificity: 80%) and as 6.00 mmol/L (specificity: 80%, sensitivity: 84%) for poor neurological outcome (CPC 4-5).

6. Discussion

6.1. Summary of findings

Our main goal was to assess biomarkers with potential prognostic value for overall survival in general cohort of resuscitated patients regardless of aetiology and characteristics of cardiac arrest and circumstances of CPR, and without focusing exclusively on the neurological outcome. We investigated two main groups of biomarkers, the L-arginine, ADMA, and SDMA line reflecting impaired endothelial function and vascular regulation together with CK-18 and its caspase-cleaved form and

their derived parameters referring indirectly to apoptotic and necrotic cell death as the consequence of systemic cell damage due to the ischaemic insult. As to date no data is yet available concerning these biomarkers in the literature among general cohort of successfully resuscitated patients, we evaluated the absolute value and kinetics of these markers from repeated sampling on the first three days after ROSC to find the most suitable time where these parameters would have the highest prognostic value. Our results suggest that initial circulating ADMA levels may indicate more severe hypoxic insult and can predict short-term 72-hour mortality among cardiac arrest victims. However, a clear connection was missing between L-arginine pathway molecules and outcomes during the later post-resuscitation period. Although the 72-hour CK-18 values correlated with the recorded SAPS II and SOFA scores, we could not prove the prognostic value for mortality and neurological outcome either of the CK-18 and its caspase-cleaved form. Moreover, NSE was not a useful predictor of survival or neurological outcome in our cohort either. Concerning the laboratory parameters routinely used in everyday critical care, in line with previous literature, elevated level of initial lactate measured within 6 hours after cardiac arrest was found to be a promising predictor for 30-day mortality and poor neurological outcome in our unselected resuscitated cohort. As a conclusion, we would like to emphasise the need to develop a generally applicable prognostic algorithm with the integration of novel or known biomarkers that can help predict overall survival in resuscitated patients including those individuals for whom limited data are available about the circumstances of cardiac arrest and resuscitation.

6.2. L-arginine pathway molecules

One of our most important observations is that elevated initial ADMA levels measured within 6 hours after cardiac arrest independently predicted short-term mortality and it was associated with poor neurological outcomes. However, circulating ADMA concentrations measured later after cardiac arrest or their kinetics were not associated with any of the investigated endpoints. Other molecules involved in nitric oxide production and vascular regulation, such as L-arginine, SDMA and their derived parameters, such as L-arginine/ADMA ratio and the change of marker levels did not prove effective to predict mortality. ADMA is a known prognostic marker of several cardiovascular diseases [55,86], hence it may be a rational observation that ADMA predicts early death during post-resuscitation care, as it occurs rather due to cardiovascular failure and haemodynamical instability [26].

A most recent review summarises several animal and human studies investigating the process and characteristics of the metabolism of L-arginine pathway molecules in various hypoxic conditions [87]. They mention more studies where hypoxia was associated with elevated ADMA levels, without significant increase in SDMA concentrations. For example, exposure to chronic-intermittent hypobaric hypoxia results in continuous ascent of ADMA but not of SDMA levels [88]. Significantly increased ADMA serum concentrations can be obtained in patients with obstructive sleep apnoea syndrome [89]. L-arginine pathway molecules have also been suggested as potential prognostic markers for acute exacerbation of chronic obstructive pulmonary disease [90].

L-arginine pathway molecules have been investigated also in cerebrovascular disorders, such as ischaemic stroke and carotid stenosis [60,91]. Elevated plasma ADMA levels were detected in patients who suffered acute ischaemic stroke. Moreover, increasing concentrations were associated with worse outcomes. Authors suggested that the production of endothelial nitric oxide, an endogenous regulator of vasodilation in cerebral arterioles, is inhibited by increased ADMA levels, consequently leading to reduced cerebral blood flow [91]. Others also confirmed the observation that ADMA increases the arterial stiffness and tone in cerebral blood vessels resulting in cerebral hypoperfusion [92]. L-arginine pathway molecules are more increasing in the early phase of ischaemic stroke compared to patients with asymptomatic significant carotid stenosis or healthy subjects, reflecting a more pronounced endothelial dysfunction. They suggested that the elevated initial ADMA levels could be linked to the pathogenesis of endothelial cell dysfunction or could be the consequence of oxidative stress [60].

We observed a reduction of initial ADMA levels to 24 hours among patients who died within 30 days, while ADMA levels slightly increased in survivors. The difference between the initial ADMA levels of non-survivors and survivors disappeared by 24 post-cardiac arrest hours. ADMA per se might contribute to brain injury by either reducing cerebral blood flow and facilitating excitotoxic neuronal death or contributing to the activation of the thrombo-inflammatory cascade [93]. Excessively high initial ADMA may adversely affect cerebral perfusion after cardiac arrest, leading in short term to exacerbation of hypoxic-ischemic injury following resuscitation and death in the early post-resuscitation phase. On the other hand, elevated initial ADMA levels might indicate a more severe hypoxic insult or pre-existing endothelial dysfunction. In the literature, only a few studies investigated these marker levels in acute hypoxic conditions in humans.

In healthy male volunteers, normally, the mean plasma nitric oxide concentration should upgrade after acute hypoxic exposure as a consequence of diminished circulating ADMA levels [94]. Conversely, we observed a mild decrease in ADMA levels among non-survivors, while in survivors the values slightly increased by 24 hours. This finding suggests that the decrease in ADMA concentration observed on the first day after cardiac arrest in our more severe group of patients who died within 30 days may be an adaptive mechanism, presumably an attempt to counteract cerebral hypoperfusion by restriction of ADMA excess.

We observed a continuous increase of L-arginine/ADMA ratio up to 72 post-cardiac arrest hours in the total study population and determined a significantly decreased initial L-arginine/ADMA ratio in IHCA patients who died in the ICU. These findings are consistent with the observation of Molnar et al., who suggested that a temporary increase of L-arginine along with a decrease of ADMA could be a protective mechanism after ischaemic stroke [60]. Lack of L-arginine, the source of nitric oxide, could lead to more severe oxidative stress induced by hypoxic insults, which may explain that the group of IHCA patients with diminished initial L-arginine/ADMA ratio were more prone to die during ICU stay. A recent prospective observational study found that higher arginine and lower arginine/ADMA ratio measured within 24 hours after OHCA were independently associated with 90-day mortality [95]. Similarly to their findings, we could detect significantly elevated L-arginine/ADMA ratio in survivors in the IHCA group. They did not report any significant difference between survivors and non-survivors regarding ADMA levels. Contrary to their findings, L-arginine was not a prognosticator of death in our population. Moreover, this marker did not diverse between survivors and non-survivors for any of the investigated endpoints. However, their population was exclusively made up of patients who suffered cardiac arrest out of the hospital, while mostly IHCA patients composed our cohort. Importantly, the pathophysiological background and most common aetiology responsible for mortality during post-resuscitation care may vary depending on whether cardiac arrest happened inside or outside the hospital, since two-thirds of OHCA patients die due to brain injury in ICU, while multiple organ failure drives the mortality after IHCA [96].

6.3. Cell death markers: cytokeratins and NSE

To the best of our knowledge, information about plasma levels of CK-18 and its caspase-cleaved forms in patients undergoing resuscitation has not yet been reported in

the literature. The levels of CK-18 reflecting total cell death including apoptosis and necrosis were persistently elevated accompanied by decreased CCCK-18/CK-18 ratio on the first three days of post-resuscitation care, compared to populations of other studies that refer to a large extent of cell death dominantly due to necrosis [97]. We hypothesised that survival depends on the rate of cell death after cardiac arrest, but contrary to our expectations, survival was not linked to the concentrations and kinetics of CK-18, CCCK-18, or CCCK-18/CK-18 ratio, despite 72-hour CK-18 values significantly correlated with prognostic scoring systems. It is possible that mortality may rather be determined by the damage of a smaller group of cells responsible for critical function and survival, but this signal may vanish in the mass of total cell death. Survival may rather depend on the remaining functional capacity and the ability to recover than the extent of damage that the above-mentioned biomarkers indirectly represent.

The length of CPR did not affect the cell death marker levels either despite the presumption that a longer state of arrest may cause a larger extent of cell damage. On the other hand, exact information about the no-flow time i.e. the interval between cardiac arrest and the start of effective resuscitation was lacking, although this period may be just as important as the duration of resuscitation. Probably because of this reason, resuscitation time did not affect mortality or neurological outcome in our study either.

We observed similarly negative results when evaluating the relationship between other organ system failure and cell death markers, apart from renal function. While CK-18 levels showed a slowly declining trend over time in patients with normal renal function, they remained high in patients with renal insufficiency, what observation mostly accounts for the impaired renal elimination and less probably the increased release from injured renal epithelial cells [98,99]. In a previous study increased serum concentration of total CK-18 was reported in patients with chronic kidney disease stages 3-5 without significant elevation of CCCK-18 levels similar to our findings [100].

We could not prove any connection between the cell death biomarkers and neurological outcomes. Practically the peripheral blood levels of CK-18 and CCCK-18 do not refer to neuronal cell death, as cytokeratins appear mostly in epithelial cells but not in neurons, where intermediate filaments are made up of neurofilaments [101]. The source of the elevated levels of cytokeratins in other studies dealing with neurological

disorders could be the damage of epithelial cells due to other organ failure or of cells of the perineurial and arachnoid sheaths [102].

Consequently, we tested a marker that represents neuronal injury more specifically. NSE was described previously as a prognostic marker for poor neurological outcome after cardiac arrest and it has an additional role in neuroprognostication in the current guidelines [11,45]. In contrast to our expectations, there was no significant difference in our study population concerning this marker and we could not confirm the prognostic value for 30-day mortality or neurological outcome. An explanation could be the remarkable heterogeneity of our unselected population, which was mostly composed of various IHCA cases, and even the OHCA group had diverse aetiology. Only a few papers published data about the prognostic value of NSE in patients after IHCA [103]. The prognostic value of NSE was higher for OHCA than for IHCA patients in the study of Kaspar et al. [104]. They explained the difference with the higher number of confounders and mortality without hypoxic-ischemic encephalopathy in IHCA patients.

6.4. Conventionally used laboratory parameters and lactate

A number of physiological and biochemical parameters showed significant or tendentious deterioration among the deceased as expected. Interestingly, the haemoglobin levels of the survivors were beneath the documented concentrations of non-survivors. There are assumptions [105] that the physiologically optimal haematocrit is somewhat under the laboratory normal range. Evolution targeted higher than optimal levels to compensate for blood loss due to injuries. In the civilized era, where such blood loss is rare, people became relatively polyglobular. Therefore, in acute conditions - e.g. after resuscitation - a value closer to the optimal may be beneficial for survival. Presumably, the decreased viscosity due to slightly decreased haematocrit provides more favourable microcirculation at the tissue level.

Failure of tissue perfusion during cardiac arrest leads to anaerobic metabolism. Lactate is the end-product of anaerobic metabolism that can be used as a marker of cellular hypoxia and to predict mortality in critical illness [106]. Previous investigations have proved the utility of lactate as a marker for disturbances of tissue perfusion to predict survival in cardiac arrest patients [107,108]. In our cohort, elevated initial lactate levels measured within 6 hours after cardiac arrest could predict 30-day mortality and poor neurological outcome. It was reported by Grimaldi et al. that lactate levels <5.1 mmol/L

were associated with favourable outcomes at discharge from the ICU after cardiac arrest [109]. Similarly to their results, in our population the cut-off was 4.90 mmol/L to predict 30-day mortality and 6.00 mmol/L for poor neurological prognosis. A most recent publication emphasised the need for the development of a proper prognostication tool in IHCA [108]. They retrospectively investigated the predictive role of the initial lactate levels among patients who suffered IHCA and required mechanical ventilation. According to their results, elevated lactate levels were associated with mortality and the AUC indicated moderate ability to predict mortality similarly to our results. This study mainly focused on mortality, while we evaluated the prognostic role of lactate levels concerning the neurological outcome as well. According to our results elevated lactate levels measured within 6 hours after cardiac arrest could help the prediction of coma, vegetative state, or brain death.

6.5. Prognostic scoring systems and biomarkers

The reliability of widely used prognostic scoring systems (SOFA, SAPS) is equivocal because of their moderate discrimination ability in post-resuscitation care [110]. In our population, these scores had moderate prognostic value for mortality. In a study made up of OHCA patients treated with therapeutic hypothermia, Acute Physiology And Chronic Health Evaluation (APACHE) II, SAPS II, and OHCA scores had moderate accuracy to distinguish neurological outcomes and mortality, moreover, the SOFA score was described as a poor predictor in this population [111]. Bisbal et al. reported the SAPS III as less accurate in determining the in-hospital mortality of post-cardiac arrest patients compared to other general prognostic scores [112]. Significant efforts have been made in recent years to develop scoring systems that can be applied for outcome estimation of resuscitated patients [113]. Despite being promising, these emerging scores are not as widely used as classical general prognostic scores and often require missing background information about the patient or peri-arrest circumstances, so we chose conventionally used SOFA and SAPS II as outcome measures in our study.

We confirmed the association of SOFA and SAPS II with L-arginine pathway molecules. Previous research findings revealed correlations among ADMA levels, L-arginine/ADMA ratio and microvascular reactivity, the extent of organ failure, and mortality in patients with sepsis [114]. In critically ill patients ADMA correlated with SOFA score [62]. These observations are consistent with our results. Based on our findings the prognostic accuracy of SAPS II (a time-consuming assessment method) and initial

ADMA for 72-hour mortality after ROSC were comparable. We conclude that early determination of initial ADMA after ROSC may be as effective and accurate as SAPS II in the prediction of the early post-CPR mortality. Regarding the later post-resuscitation mortality prediction, initial lactate values per se had similar moderate prognostic value as SOFA and SAPS II for 30-day mortality.

72-hour CK-18 values significantly correlated with both SAPS II and SOFA scores, which suggests that the systemic cell death processes are connected to the overall functional capacity and organ function abnormalities represented by the score points. However, the overall outcomes were not related to the cell death marker concentrations. The apoptosis-specific CCCK-18 did not show any connection with SOFA or SAPS II in any investigated time point suggesting the dominant role of necrosis during post-resuscitation pathological processes.

6.6. Strengths and limitations

The **strength** of our study is its prospective nature using serial sampling instead of admission only sampling in the first three days after cardiac arrest to evaluate the kinetics and changes of multiple conventional and novel biomarkers. Our study population was made up of unselected resuscitated patients including in and out-of-hospital cardiac arrest survivors covering the widest range of resuscitated patients with different comorbidities and aetiology to explore reliable predictive markers regardless of the circumstances and aetiology of cardiac arrest. While most studies evaluated prognostication of OHCA patients, we were able to find potential prognostic markers in IHCA patients and markers which might be also used in both groups.

It is generally observed, that although patients reach acceptable neurological function during their ICU stay, they may later die as the consequence of multiple organ failure, especially after IHCA [96]. Considering this, we felt it confusing to categorise patients emerging from a comatose state into the unfavourable CPC group based on late-developing multiple organ failure. Therefore, the pure neurological outcome (regaining the consciousness) and the overall outcome (72-hour, ICU or 30-day mortality) were separately analysed, and the best achieved CPC score was recorded during the ICU stay similarly to the study of Fabio Silvio Taccone and colleagues [46]. Importantly, 8 patients (14.8% of the total population) in the CPC 1-3 group died due to non-neurological reasons

in the ICU despite acceptable neurological status (death after awakening). All of these 8 patients had sepsis and failure of two or more organ systems.

A **limitation** of our study is the low total number of enrolled patients. Concerning the high mortality rate, we could not collect enough data for long-term analysis. The study was conducted in a single centre, so local treatment strategies and guidelines could limit the generalisability of our findings. Further research is warranted to explore the prognostic value of multiple markers (including the L-arginine pathway molecules) for long-term outcome after cardiac arrest.

Probably, the no-flow time would be more valuable information than the length of the CPR until ROSC. However, the exact time of the collapse was not known in the majority of IHCA and some OHCA patients, thus it prevented us from reporting the accurate no-flow time in this cohort.

Favourable neurological outcome is usually considered as a CPC 1 or 2, while CPC 3-5 implies poor outcomes. We had low number of individuals with really good outcomes (CPC 1-2) during the 30-day follow-up period. For a statistical reason, to have similar size groups, we reclassified the CPC as follows: CPC 4-5 (vegetative state or death) as poor neurological outcome, and CPC 1-3 as good/moderate neurological outcome. Another reason of the extension of the acceptable neurological outcome categories with CPC 3 was the potential for a later neurological recovery of some patients during rehabilitation since the most improvement in cognition occurs during the first three months [115].

Prognostication of neurological outcome after cardiac arrest is based on clinical neurological examination, electrophysiological investigations, neuroimaging, and biomarkers [11,45]. However, in our study, we only focused on biomarkers in the peripheral blood, and we did not evaluate the mentioned examinations or tools for prognostication.

7. Future perspectives

Further multicentre studies with large numbers of subjects allowing long-term follow-up are needed to confirm the prognostic significance of L-arginine pathway molecules in a later (e.g. rehabilitation) phase of post-cardiac arrest patients. The investigation of L-arginine metabolism contributes to the more profound understanding of distinct pathophysiological processes in the post-resuscitation period, thus these

molecules may not only possess a prognostic role but may also open up new therapeutic approaches. Recently, there has been a growing interest in the potential therapeutic effects of arginine supplementation in cardiovascular disorders and critically ill patients [116]. Administration of external arginine or the suppression of arginase enzyme may improve the production of nitric oxide by optimization intracellular L-arginine bioavailability and balancing the arginine/ADMA ratio [55]. Elevated ADMA levels and increased ADMA/arginine ratio may help to select those individuals who could potentially benefit from supplementation [51].

The general initial hypothesis that the extent of systemic cell death associates with outcomes after cardiac arrest should not be rejected based on our findings exclusively. Presumably, several cell components and biomarkers participating in crucial biochemical pathways of different forms of cell death releasing into the circulation have not yet been investigated concerning their potential role to aid prognostication after cardiac arrest.

It is important to emphasise the demand for gaining comprehensive information about pathologically altered processes in cardiovascular disorders, such as inflammation, oxidative stress, apoptosis, or neurohumoral activation since these mechanisms play a crucial role in the development of life-threatening conditions in cardiovascular or critically ill patients as well. Therefore, it is suggested to combine multiple biomarkers reflecting the above-mentioned pathways to increase their diagnostic and prognostic value to optimise prevention, therapy, and rehabilitation [1].

8. Conclusion

Here, we investigated for the first time the prognostic value and the kinetics of the L-arginine-pathway molecules, cytokeratins (CK-18 and CCCK-18) with NSE during the early phase of successful cardiopulmonary resuscitation. Our results suggest that initial circulating ADMA levels may indicate more severe hypoxic insult and can predict 72-hour mortality among cardiac arrest victims. Although in our recent study we could not prove the prognostic value of novel cell death markers (CCCK-18, CK-18), and previously well-investigated NSE, we confirmed the role of initial lactate levels for prediction of mortality and neurological outcome in unselected resuscitated patients. At a quick glance, the simply obtainable early lactate level per se may give similarly useful information as the SAPS II and SOFA scores, which can be calculated later after admission and require more time.

II. MATERNAL HEMORHEOLOGICAL PROPERTIES IN EARLY-ONSET PREECLAMPSIA

9. Introduction for early-onset preeclampsia research

9.1. Epidemiological facts and definition

Hypertensive disorders (i.e. preeclampsia, eclampsia, HELLP syndrome, gestational hypertension, chronic hypertension and preeclampsia superimposed on chronic hypertension) affect 5–10% of pregnancies worldwide and are still one of the leading causes of maternal and perinatal morbidity and mortality [117]. Globally, each year 76 000 women and 500 000 neonates die in connection with this disorder [118]. In developed countries, preeclampsia is responsible for approximately 14% of all pregnancy-related deaths [119].

Preeclampsia is defined as gestational hypertension in previously normotensive women accompanied by new-onset proteinuria and/or maternal organ dysfunction (acute kidney injury; liver involvement; neurological, haematological complications), and/or uteroplacental dysfunction manifesting at or after 20 weeks of gestation [120]. Based on the onset time there are two subtypes of the disease. *Early-onset preeclampsia* develops before 34 weeks of gestation and has higher risks of maternal morbidity, perinatal death and severe neonatal morbidity compared to the *late-onset* form (≥ 34 weeks of gestation) [121]. According to a prospective cohort study in Hungary between 2010 and 2012, the rate of early-onset preeclampsia was 0.5% (11/2200) and 3.2% (71/2200) of late-onset preeclampsia [122].

9.2. Pathophysiology of early-onset preeclampsia

The pathophysiological explanation of *early-onset preeclampsia* is predominantly defective placentation during the first few weeks of pregnancy. Therefore, the placenta and its pathological alterations are in the centre of the disease. The exact processes in the background of this complex condition are still equivocal. The manifest maternal syndrome of preeclampsia is considered to be a result from factors arising from placentation problems leading to disturbed systemic circulation [123].

Trophoblast cells play crucial role in normal placentation. *Cytotrophoblasts* cells are stem cells since they continually differentiate into syncytiotrophoblasts during placental development. *Syncytiotrophoblast* cells form placental epithelial covering of

villi, directly communicate with the maternal endometrial vasculature and are responsible for nutrient supplementation and gas exchange at the maternal-foetal interface. In early stages of normal, physiologic pregnancy, cytotrophoblasts invade into the uterine spiral arteries. This invasion is essential to reform these spiral arteries to low resistance vessels with reduced elasticity and enlarged luminal diameter. These processes are required for proper placental blood flow, which meets the increasing demand of the foetus during pregnancy [124]. Contrary, in preeclampsia, the cytotrophoblast invasion of the maternal uterine vessels is impaired, what results in abnormal vascular remodelling and remaining smooth muscle cells in the vessel wall, which leads to increased vascular resistance and elevated pressure (*Figure 23.*).

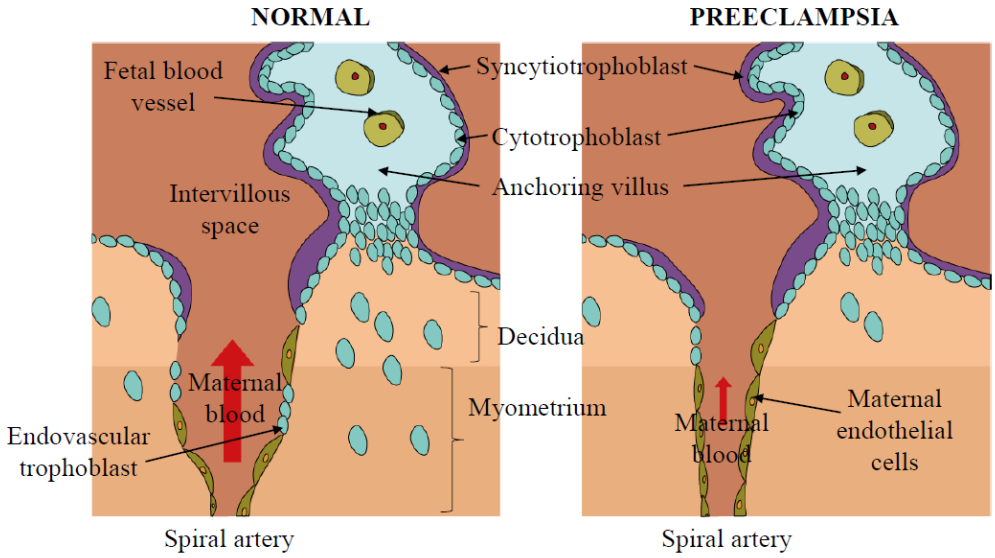

Figure 23. *Abnormal placentation and impaired vascular remodelling in preeclampsia (The figure was created by the author of this thesis according to the publication of Keith R McCrae [125])*

Uteroplacental malperfusion and defective remodelling of the spiral arteries disturb the syncytiotrophoblast function. Due to the subsequent oxidative stress, syncytiotrophoblasts release multiple factors, such as pro-inflammatory cytokines, exosomes, anti-angiogenic agents, and cell-free foetal DNA [126]. Elevated levels of soluble receptor for vascular endothelial growth factor, and the reduced levels of placental growth factor were already reported in early-onset preeclampsia [127,128]. The released factors through their antiangiogenic effects further inhibit physiological haemodilution and lead to endothelial dysfunction, first locally in the placenta but later in the systemic circulation as well. In addition, pro-inflammatory factors might contribute to pathological systemic endothelial response, characterised by increased capillary permeability, activation of the

coagulation cascade resulting in microvascular thrombosis, and sustained vascular hypertension leading to the manifestation of clinical signs and symptoms [129]. The removal of the placenta also terminates the symptoms of preeclampsia in postpartum period. Whether it is delayed, (e.g. in cases of extra-uterine pregnancies), the symptoms may persist for longer period of time until the placenta will be fully reabsorbed [130]. Consequently, the only truly effective way to save the life of both mother and foetus is to terminate the pregnancy and deliver the new-born with subsequent placenta removal [131].

9.3. Complications, short- and long-term maternal and foetal consequences

Short-term maternal consequences could be life-threatening conditions such as placental abruption, acute pulmonary oedema, respiratory distress syndrome, acute renal failure, stroke, eclampsia, multiple organ failure, and disseminated intravascular coagulation. The leading cause of maternal death is cerebral haemorrhage, which is presumably a consequence of severe hypertension [132]. Women with a history of preeclampsia are more prone to suffer from cardiovascular disease and have an increased risk for myocardial infarction, heart failure, hypertension, or stroke in later life [133]. It has been observed that the earlier the onset of preeclampsia, the more severe the condition and the higher the risk of developing subsequent cardiovascular disease [134].

Preeclampsia and its consequences affect not only pregnant women but can be lethal for the foetus as well since the placenta is not able to ensure adequate perfusion. Due to placental insufficiency 4% of the foetuses die already in utero, 25% develop intrauterine growth retardation and 27% of the neonates are premature [117]. Prematurity itself can lead to serious complications both in the perinatal period and in later childhood, consequently affecting the health of the new-born for the rest of its life. Preterm birth is associated with higher rates of infant respiratory distress syndrome, intraventricular haemorrhage, sepsis, bronchopulmonary dysplasia, and later neurodevelopmental disability in childhood [135].

9.4. Preeclampsia and hemorheology

As preeclampsia can be described as a systemic cardiovascular disease, adequate blood flow properties and favourable hemorheological characteristics are essential to maintain a kind of proper microcirculation in the placenta. The science of **hemorheology** investigates blood flow conditions, the physical properties of blood elements including

cellular and plasmatic components. The most important hemorheological parameters include haematocrit, fibrinogen level, plasma and whole blood viscosity, red blood cell (RBC) aggregation and deformability, and in broader sense the properties of white blood cells, platelets, and endothelium. Deterioration of these factors leads to impaired microcirculation and impaired tissue perfusion [136]. In our current study, we focused on microrheological parameters.

Erythrocyte aggregation means reversible rouleaux formation of RBCs mostly due to bridging action of large molecular weight proteins (fibrinogen and immunoglobulins) in plasma. There are two theories concerning the background of the RBC aggregation process. According to the "*bridging theory*" RBC aggregation is caused by macromolecules enhancing the connection of the cells, while the "*depletion layer theory*" suggests that the diminished macromolecular concentration creates an osmotic gradient between two RBCs leading to cell-cell interactions [137]. Under low shear conditions, erythrocytes first arrange into linear and then three-dimensional structures similar to a stack of coins, but with increasing shear forces the process becomes reversible leading to disaggregation of erythrocytes. The formation also occurs in vitro in a plasma-free isoosmotic environment, thus not only the components of plasma but also intracellular factors are involved in the process. Previous studies have shown that aggregation is influenced by extracellular factors such as the concentration of fibrinogen and other polymers (e.g. dextran) in the plasma and by adhesion molecules on the cell surface [137]. RBC aggregation is a determinant of blood viscosity, and its increase enhances friction between fluid plates, thus adversely affecting both macro-, and microcirculation.

Erythrocyte deformability is described as the ability of RBC to adapt to mechanical forces and so be able to cross over narrow capillaries and ensure sufficient tissue oxygenation. Normal RBCs are biconcave in shape, 7-8 μm in diameter and around 2 μm thick. In contrast, the lumen of the capillaries in human tissues can be 3-5μm in diameter. The passage of erythrocytes through such capillaries requires the above-mentioned major modification of their structure, the so-called RBC deformability i.e. their ability to change shape. Deformability is determined by the internal cell viscosity, RBC membrane properties, morphology, and surface-volume ratio. However, other extrinsic factors in the circulation also affect this property, such as serum protein concentration, pH and several drugs. Rigid RBCs are unable to deform in response to shear forces, consequently, the deformation capacity is reduced, thus higher viscosity

values can be measured, especially when higher shear stresses are applied [138] Similarly to aggregation, deformability also affects the macro- and microcirculation, but in inverse proportion. Its elevation allows RBCs in high-volume vessels to assume a streamlined shape, thereby reducing viscosity. At the capillary level, this property helps to pass through blood vessels of a much narrower lumen than their diameter.

To summarise, elevated RBC aggregation and decreased deformability can adversely affect the hemorheological properties and impair tissue perfusion.

9.4.1. Hemodynamical and hemorheological alterations in normal pregnancy

Major hemodynamical rearrangement occurs during normal pregnancy. The endothelial smooth muscle cells secrete vasorelaxant factors, what results in vasodilatation of the peripheral blood vessels, therefore total peripheral resistance and consequently blood pressure decreases. Cardiac output increases by an increase in heart rate and a rise in plasma volume. The subsequent "dilution" is compensated by slightly enhanced erythropoiesis in physiological pregnancy [139,140]. Eventually, despite compensation, *physiological haemodilution* develops with mild anaemia and diminished haematocrit. Overall, the change is still beneficial for pregnancy, as it reduces total peripheral resistance and whole blood viscosity, and it will ensure to maintain optimal uterine blood flow through the pregnancy. Constant and adequate blood pressure is important since the uteroplacental circulation is not an autoregulated but a blood pressure-dependent system [141]. However, the blood supply to the placenta does not only depend on blood pressure and vascular morphology, but also on the hemorheological properties [142].

Hemorheological changes in normal pregnancy already appear during first trimester. Physiologic haemodilution and increasing hypercoagulability are accompanied by elevated erythrocyte aggregation and decreased deformability during second trimester while plasma viscosity remains unchanged. RBC aggregation is gradually escalating through the pregnancy and its values in second and third trimester become markedly pronounced than before the pregnancy or in the first trimester. During the second trimester, a temporary reduction in erythrocyte deformability is observed, which remains significantly lower during the third trimester [143].

9.4.2. Hemodynamical and hemorheological alterations in preeclampsia

Contrary to physiologic pregnancy, early-onset preeclampsia is accompanied by reduced total blood and plasma volume, [144] depleted cardiac output, and increased vascular resistance [145], the physiological haemodilution is consequently absent. On the contrary, women with late-onset subtype possess increased cardiac output co-occurring attenuated total vascular resistance [146].

Pathologic alterations in blood rheology and impaired blood flow at the uteroplacental cross-over were already previously considered as possible triggers or consequences of preeclampsia [147]. Elevated RBC aggregation and decreased deformability were observed in some studies [147,148] and one of them reported a relationship between the presence of foetal growth restriction and increased maternal RBC aggregation [149]. However, others could not confirm alterations of RBC properties in preeclampsia [150].

Limited information is available about the peripartum and postpartum period in the early-onset form since most studies that have previously investigated hemorheological changes in preeclampsia usually did not distinguish between early- and late-onset forms, although it is nowadays an increasingly accepted fact that the pathophysiological background of the two forms may be different [120,123]. Therefore, further investigations are required to reveal the pathophysiological and prognostic significance of these parameters, especially in early-onset form, which has more severe short- and long-term maternal and foetal consequences [151].

10. Hypothesis and objectives

Our study primarily aimed to determine the hemorheological parameters at diagnosis, at delivery, and in the early postpartum stage among women diagnosed with early-onset preeclampsia compared to healthy uncomplicated pregnancies. Based on the literature on hypertensive disorders in pregnancy, we hypothesise that hemorheological alterations especially affecting RBC properties are present in early-onset preeclampsia patients, but limited information is available on rheological changes in the peripartum and early postpartum period. Therefore, our goals are to expand the knowledge about the maternal hemorheological properties in preeclampsia with repeated blood sampling from the diagnosis through the delivery until postpartum 72 hours. We intended to identify one

or more rheological parameters as potential factors giving additional information to the current diagnostic and screening method. Our secondary aims are to reveal the connection between maternal RBC properties and characteristics of pregnancy outcomes (e.g. week of gestation at birth, neonatal physical parameters).

11. Materials and methods

11.1. Subjects

13 **preeclamptic** non-smoking women admitted to the Department of Obstetrics and Gynaecology, University of Pécs were involved in this prospective, case-control study. Women with early-onset preeclampsia were selected based on the criteria as described by the International Society for the Study of Hypertension in Pregnancy: increased blood pressure (≥140 mmHg systolic or ≥90 mmHg diastolic on ≥ 2 separate occasions at least 4 hours apart within a 24 h period) that manifested before the 34th week of gestation in previously normotensive women, accompanied by one or more organ failure, such as significant proteinuria (≥30 mg/mol protein in 24-hour urine collection in the absence of urinary tract infection), acute kidney injury, liver involvement, neurological, haematological complications, uteroplacental dysfunction [120].

The maternal **control group** was made up of 24 healthy, non-smoker, age- and gestation-matched women attending the same clinic. All women were informed about the study protocol by a physician, who excluded any relevant comorbidities in the anamnestic data (e.g.: chronic hypertension, known diabetes mellitus, cardiovascular, gynaecologic disorders) and performed a physical examination. In the first period, we recruited 34 pregnant women suggested to be healthy and to have a pregnancy without complications. Finally, 10 women have been excluded due to complications during the pregnancy, the delivery, or the early postpartum period (*Figure 24.*).

In both groups, exclusion criteria were multiple pregnancy, intrauterine developmental abnormality of the foetus, chorioamnionitis, intrauterine infection, severe maternal anaemia, participation in another study, and the individual not being able to sign the informed consent. All women gave their written informed consent. The study protocol was approved by the Regional and Local Research Ethics Committee at the Medical School, University of Pecs (Reference number: 6942-PTE 2018). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

11.2. Data collection

Collected data included patient anamnestic information, comorbid conditions, symptoms of the preeclampsia, mode and time of the delivery, gestational age at birth. We recorded the laboratory parameters (electrolytes, markers of renal and hepatic function, inflammatory parameters, complete blood count) at the time of preeclampsia diagnosis and within 72 hours after the delivery and maternal physical parameters (height, bodyweight, body mass index - BMI, heart rate, systolic and diastolic blood pressure, mean arterial pressure) at enrolment.

Physical parameters of the neonates, such as birth weight, length, head circumference, shoulder width were also recorded. Neonates were characterized by the Apgar score by evaluating the new-born on five simple criteria on a scale from zero to two (Appearance, Pulse, Grimace, Activity, Respiration). Apgar score was determined right after the birth (Apgar 1) and 5 minutes later (Apgar 5).

11.3. Sample collection

In the patient group the first blood sample was drawn at the time of the preeclampsia diagnosis. Among gestational age-matched healthy non-smoking pregnant women the first sample was collected at the time of the enrolment (26-34. weeks of gestation), later discussed as “initial” values. In both group blood samples were drawn two more times: within the 1st hour after delivery and 72±3 hours later. Every time 2x6 ml of peripheral blood from antecubital veins was collected into EDTA-Vacutainer tubes. The hemorheological measurements were performed within one hour after blood sampling in the Hemorheological Laboratory of the University of Pécs under standardized conditions by the same investigator person. *Figure 24.* summarises the process of recruitment and data collection.

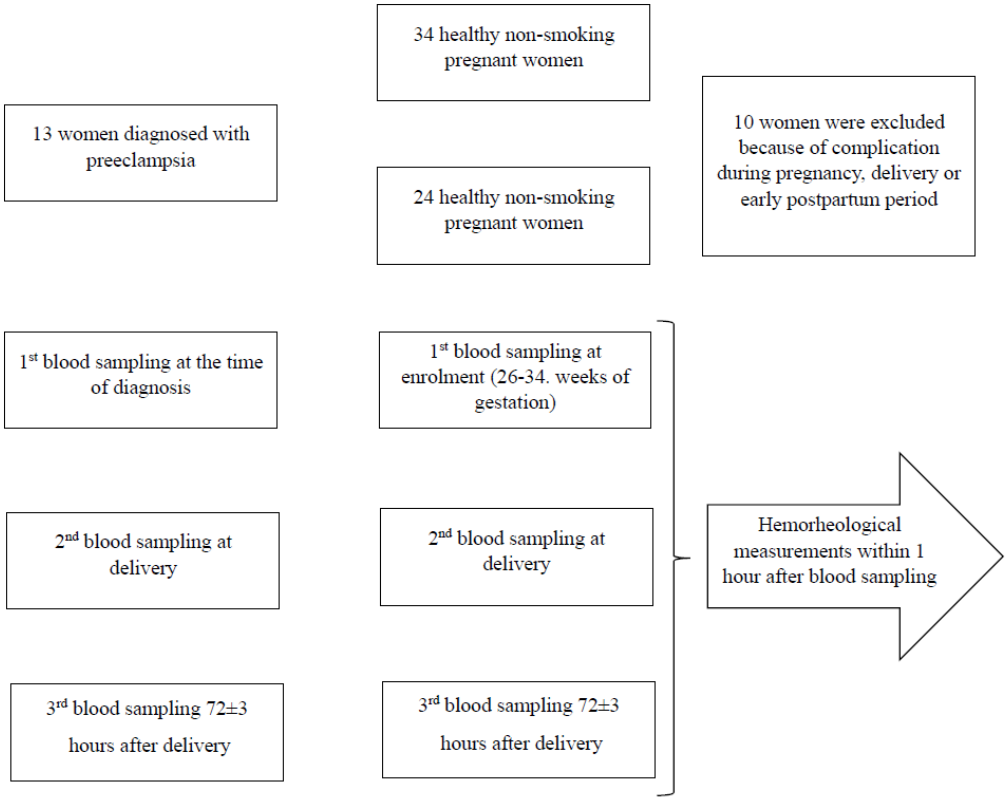

Figure 24. *Process of recruitment and data collection of women diagnosed with preeclampsia and control pregnant women*

11.4. Hemorheological measurements

RBC aggregation was determined with two different methods. **Myrenne aggregometer** (model MA-1, Myrenne GmbH, Roetgen, Germany) applies and measures the infrared light transmission through an erythrocyte suspension between a transparent plate and a cone. This technique is based upon the increase of light transmission through plasma gaps between the rouleaux aggregates or rouleaux-rouleaux complexes made up of RBCs. The cone plate system rotates the injected 30 µl blood sample for 10 seconds at high shear stress (at 600 s^{-1}), to disperse all pre-existing cell aggregates. Then the system stops instantly (M mode), or the shear is reduced to a lower 3 s^{-1} shear stress (M1 mode) to stimulate aggregation, what leads to increasing light transmission proportional to the rate of RBC aggregate formation during stasis (*M index*) or at low shear (*M1 index*). The aggregation is determined by the quantity of infrared light transmission measured by photosensors at room temperature. The two dimensionless indices (M, M1) increase with enhanced erythrocyte aggregation [152].

The **Laser-assisted Optical Rotational Cell Analyzer** (LORCA - R&R Mechatronics, Hoorn, Netherlands) determines the erythrocyte aggregation by detecting

laser backscattering from the RBC aggregates. 1 ml blood sample is injected between the outer, rotating cylinder, and the inner, static cylinder of the instrument and RBCs are disaggregated at a high shear rate (500 s−1) at 37 °C. Then the motor rapidly stops. The intensity of reflected light is measured for 120 seconds and is plotted as a function of time. The *aggregation index* (AI) and the *aggregation half-time* (t½ - which is the time required to reach half of the maximum aggregation) are calculated from the syllectogram during the first 10 seconds of the measurement. Further parameter describing red blood cell aggregation is the *threshold shear rate* (γ) which is defined as the smallest shear rate required for the complete disaggregation of RBCs [153].

In this study **erythrocyte deformability** on different shear stresses was determined by LORCA as well. 20 µl blood sample was diluted in a viscous medium called polyvinilpyrrolidon and injected between the cylinders. A laser-diode is projected through the fluid, the light diffracts on the red blood cells resulting in a diffraction pattern on a diaphragm. This will be analysed by a video camera and a computer system. As a result of the applied increasing shear stress RBCs will be elongated and the diffraction pattern is changing from circular to elliptical shape. Based on the measurements we could express RBC deformability as elongation index (EI) given at each shear stress [154].

We adhered to the guidelines proposed by the International Expert Panel for Standardization of Hemorheological Methods during the tests [155].

11.5. Statistical analysis

Statistical analysis of the collected data was evaluated by IBM SPSS Statistics® 27.0. Continuous variables are reported as mean and standard deviation or medians and interquartile ranges. Categorical variables are reported as frequencies and percentages. The Kolmogorov-Smirnov test was applied to test for normality. Comparisons of continuous non-normally distributed data between groups were carried out using the Mann-Whitney U-test. Student T-test was used for the analysis of normally distributed continuous data. The Chi-square test was applied for analysis of categorical data. Bivariate correlation analysis was performed calculating Spearman's correlation coefficient (rho). The diagnostic power of the scores and parameters was assessed using AUC of the ROC curve. The predicted probabilities were calculated from the combination of initial AI and M variables produced by binary logistic regression analysis.

12. Results

Table 9. presents the most relevant demographic and clinical data of the two groups. The mean values of maximum measured systolic and diastolic blood pressure in the preeclampsia group were 180±18 mmHg and 112±13 mmHg. All enrolled women diagnosed with preeclampsia had significant proteinuria on admission measured by sulfosalicylic acid precipitation method. 54% of neonates were male in preeclamptic pregnancies and 38% in control group. Preterm deliveries and infants with low birth weight, length, head circumference, and shoulder-width were more common and significantly more unfavourable Apgar 1, and 5 points were recorded in the preeclampsia group as expected. Only one patient of the 13 enrolled women with preeclampsia received transfusion of blood components on the fourth day after delivery.

Table 9. *Maternal and neonatal demographic and clinical data*

Baseline characteristics		Preeclampsia	Control	p value
Maternal	Age (years)	29.08 ± 2.13	30.42 ± 1.39	0.589
	Gestation age at first sampling (weeks)	29.69 ± 0.67	28.71 ± 0.48	0.240
	Systolic blood pressure at admission (mmHg)	160 [146 – 175]	120 [119 – 130]	**<0.001**
	Diastolic blood pressure at admission (mmHg)	100 [90 – 110]	80 [70 – 80]	**<0.001**
	Heart rate at admission (/min)	82 [80 – 95]	82 [77 – 88]	0.320
	Body height (m)	1.63 ± 0.06	1.67 ± 0.07	0.094
	Body weight (kg)	78 [70 – 87]	80 [70 – 86]	0.824
	BMI (kg/m^2)	30.1 [26.8 – 33.5]	28.2 [25.7 – 30.2]	0.227
	Change in body weight (kg)	10 ± 7	13 ± 4	0.132
	Length of hospital stay (day)	8 [6 – 15]	4 [4 – 5]	**<0.001**
Neonatal	Gestational age at birth (weeks)	30.23 ± 0.86	39.06 ± 0.28	**<0.001**
	Birth weight (gram)	1355.83 ± 157.69	3420.00 ± 89.04	**<0.001**
	Birth length (cm)	36.67 ± 1.64	49.76 ± 0.80	**<0.001**
	Head circumference (cm)	27.81 ± 1.01	34.24 ± 0.35	**<0.001**
	Shoulder width (cm)	28.09 ± 1.30	36.88 ± 0.57 cm	**<0.001**
	Apgar 1	7.0 [6.0 – 8.0]	9.0 [9.0 – 9.0]	**<0.001**
	Apgar 5	9.0 [8.0 – 9.0]	10.0 [10.0 – 10.0]	**<0.001**

The results were expressed as the mean value ± standard deviation of the mean or median and interquartile range. BMI: body mass index.

Analysis of the recorded routinely used laboratory parameters disclosed significantly elevated RBC count (4.29±0.12 vs. 3.91±0.05 G/L; p=0.009) and haemoglobin (126.58±2.60 vs. 117.91±1.38 g/l; p=0.003), while slightly but significantly diminished mean corpuscular volume (MCV) (84.71±2.85 vs. 87.86±4.56 fl; p=0.037) was noted at preeclampsia diagnosis compared to the values at enrolment of healthy volunteers (*Figures 25-27*). Postpartum values did not show significant differences.

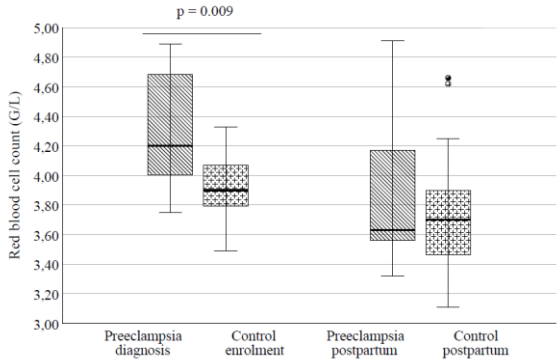

Figure 25. *Red blood cell count at diagnosis of preeclampsia and enrolment of control group and in the postpartum period (within 72 hours after the delivery).*

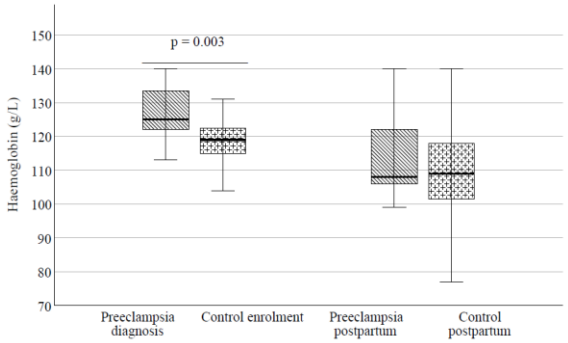

Figure 26. *Haemoglobin values at diagnosis of preeclampsia and enrolment of control group and in the postpartum period (within 72 hours after the delivery).*

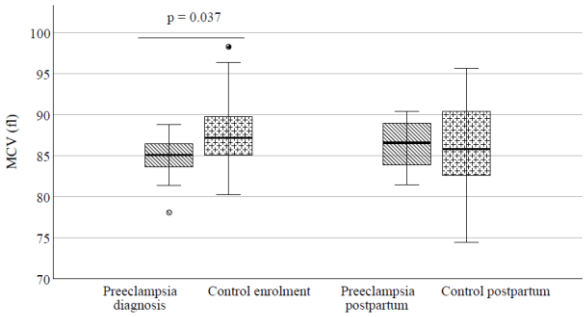

Figure 27. *MCV values at diagnosis of preeclampsia and enrolment of control group and in the postpartum period (within 72 hours after the delivery).*

12.1. RBC aggregation

RBC aggregation measured with two distinct methods was significantly enhanced in the preeclampsia group compared to healthy pregnancies. The M values measured by Myrenne aggregometer were higher in preeclampsia at diagnosis: 9.8±0.4 vs. 8.5±0.2; p=0.007 and at delivery: 10.7±0.8 vs. 8.0±0.4 (p=0.002). Within the group of healthy pregnant women, the M index raised to the 72 hours after birth compared to values at enrolment or delivery. This alteration was not observed among women with preeclampsia, where M values remained elevated throughout the investigated period (*Figure 28.*). Statistical analysis could not verify significant alterations concerning "M1" values.

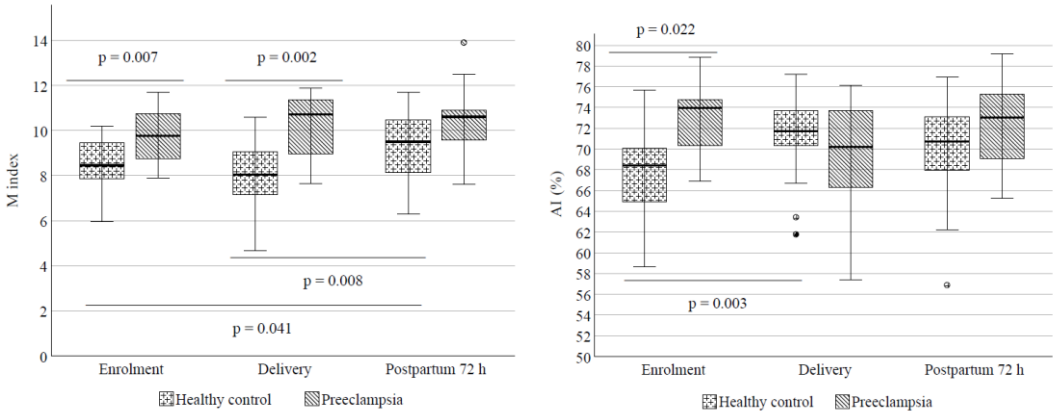

Figure 28. *M index and AI in preeclampsia and control group in the three investigated time point*

The RBC aggregation measured by LORCA was more pronounced in preeclampsia at diagnosis than among healthy women (AI: 72.9±3.5% vs. 67.5±3.9%; p<0.001) (*Figure 28.*). Within the control group, RBC aggregation significantly increased from the first blood sampling at enrolment to the time of delivery (AI: 67.5±0.8 % vs. 71.1±1.0 % p=0.003), while this increment was not visible in preeclampsia.

Positive significant correlation was observed between initial AI measured at diagnosis and the gestational age of the neonate in the preeclampsia group (R^2=0.554; p=0.006) (*Figure 29.*)

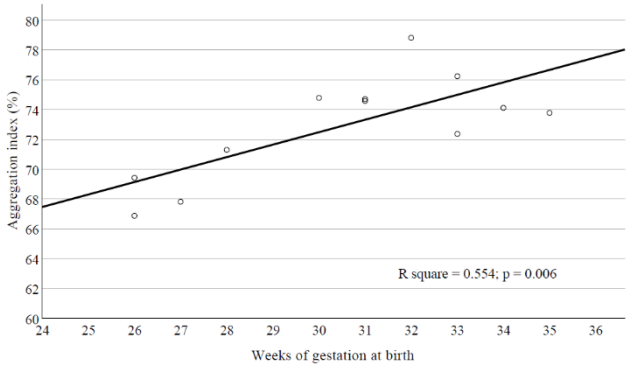

Figure 29. *Linear regression analysis of initial AI measured at diagnosis of preeclampsia and weeks of gestation at birth*

It is important to note that contrary to preeclampsia, no significant correlation or linear regression were detectable among healthy pregnant women concerning their AI value at enrolment and the gestational age at enrolment (*Figure 30.*). The initial AI values were independent from the haemoglobin values in both groups as shown in *Figure 31*.

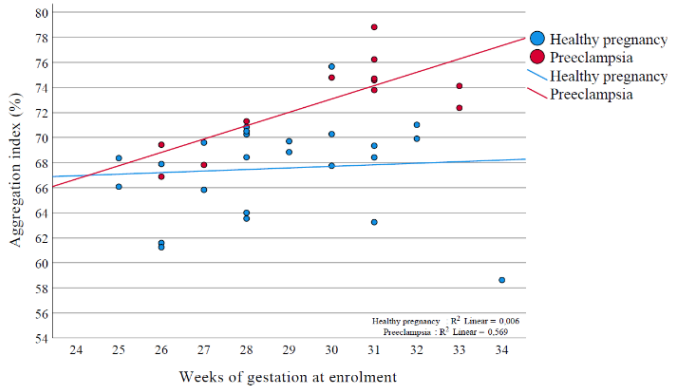

Figure 30. *Scatter plot with fit lines of AI measured at the time of preeclampsia diagnosis or healthy control enrolment by weeks of gestation at the time of inclusion in the study*

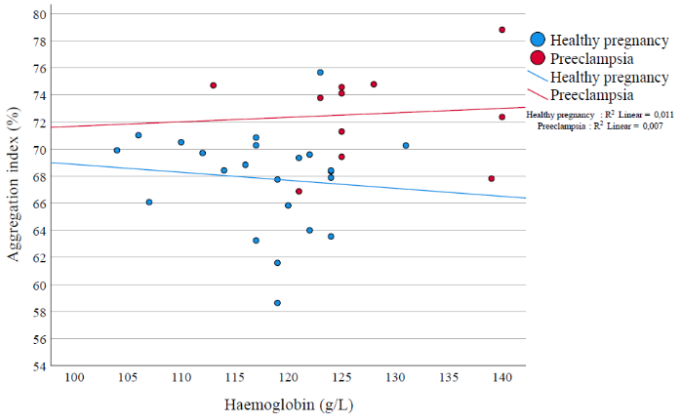

Figure 31. *Scatter plot with fit lines of AI and haemoglobin measured at the time of preeclampsia diagnosis or healthy control enrolment*

12.2. RBC deformability

In comparison between patients with preeclampsia and normal pregnant women we observed statistically significant reduced erythrocyte deformability on medium shear stresses (9.49, 5.33, 3 Pa) at diagnosis of preeclampsia compared to enrolment of healthy pregnant women (*Figure 32.*).

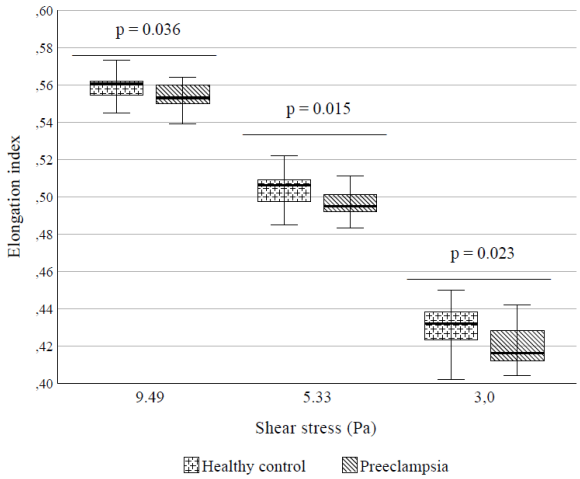

Figure 32. *Elongation index at medium shear stresses in preeclampsia and control group*

Analysing the kinetics within groups, we established significant improvement of the RBC deformability to postpartum 72 hours in women with preeclampsia compared to the values formerly measured at diagnosis or during delivery (*Table 10.*). Our results did not show any elevation in EI values in the group of healthy pregnant women. Significantly decreased elongation ability appeared at high shear stress during delivery, though 72 hours later the deformability returned to similar values as measured at enrolment (*Table 11.*).

Table 10. *Elongation index on different shear stresses in preeclampsia group*

	Time of blood sampling			p values		
Shear stress	Diagnosis (1)	Delivery (2)	Postpartum 72 h (3)	1 vs. 2	2 vs. 3	1 vs 3
EI, 30.00 Pa	0,625 ± 0,006	0,626 ± 0,006	0,626 ± 0,005	0,724	0,469	0,644
EI, 16.87 Pa	0,599 ± 0,007	0,600 ± 0,006	0,602 ± 0,006	0,606	0,109	0,189
EI, 9.49 Pa	0,554 ± 0,008	0,555 ± 0,008	0,600 ± 0,010	0,769	**0,015***	**0,048***
EI, 5.33 Pa	0,496 ± 0,009	0,499 ± 0,010	0,505 ± 0,012	1,000	**0,008***	**0,018***
EI, 3.00 Pa	0,421 ± 0,011	0,423 ± 0,012	0,431 ± 0,015	0,916	**0,007***	**0,015***
EI, 1.69 Pa	0,329 ± 0,017	0,332 ± 0,016	0,341 ± 0,019	0,901	**0,021***	**0,009***
EI, 0.95 Pa	0,226 ± 0,019	0,229 ± 0,020	0,239 ± 0,023	0,720	**0,016***	**0,018***
EI, 0.53 Pa	0,122 ± 0,023	0,126 ± 0,025	0,134 ± 0,028	0,530	**0,046***	**0,026***
EI, 0.30 Pa	0,045 ± 0,028	0,047 ± 0,018	0,053 ± 0,029	0,690	0,314	0,321

The results were expressed as the mean value ± standard deviation of the mean. *p<0.05

Table 11. *Elongation index on different shear stresses in healthy pregnant women*

	Time of blood sampling			p values		
Shear stress	Enrolment (1)	Delivery (2)	Postpartum 72 h (3)	1 vs. 2	2 vs. 3	1 vs 3
EI, 30.00 Pa	0,622 ± 0,008	0,620 ± 0,008	0,624 ± 0,007	**0,037***	**0,010***	0,231
EI, 16.87 Pa	0,600 ± 0,006	0,598 ± 0,006	0,602 ± 0,008	**0,018***	**0,032***	0,489
EI, 9.49 Pa	0,559 ± 0,007	0,557 ± 0,006	0,561 ± 0,010	**0,022***	0,076	0,693
EI, 5.33 Pa	0,504 ± 0,009	0,504 ± 0,008	0,507 ± 0,012	0,240	0,092	0,450
EI, 3.00 Pa	0,430 ± 0,011	0,430 ± 0,012	0,433 ± 0,018	0,195	0,143	0,716
EI, 1.69 Pa	0,339 ± 0,016	0,340 ± 0,017	0,344 ± 0,025	0,291	0,063	0,600
EI, 0.95 Pa	0,235 ± 0,021	0,240 ± 0,022	0,242 ± 0,030	0,891	0,373	0,516
EI, 0.53 Pa	0,130 ± 0,023	0,135 ± 0,024	0,137 ± 0,031	0,862	0,668	0,442
EI, 0.30 Pa	0,050 ± 0,029	0,055 ± 0,018	0,048 ± 0,029	0,833	0,389	0,694

The results were expressed as the mean value ± standard deviation of the mean. *p<0.05

12.3. Indicators of RBC aggregation for preeclampsia diagnosis

ROC analysis was carried out with maternal aggregation parameters in the first investigated time point to test the diagnostic power for preeclampsia. The analysis of initial AI measured at preeclampsia diagnosis or control enrolment indicated a cut-off point of 69.4% for preeclampsia with an AUC of 0.837 [0.684 – 0.990] (p=0.001), where sensitivity was 83.3% and specificity was 62.5%. ROC analysis of initial M values showed an AUC of 0.750 [0.576 – 0.924] (p=0.019) and indicated a cut-off as 8.39 (sensitivity: 90.9% and specificity: 50%) for preeclampsia. The predicted probabilities from the combination of initial AI and M variables produced by binary logistic regression analysis showed slightly increased AUC with 0.900 ([0.789 – 1.000] p<0.001) comparing the ones of AI or M value per se as classifiers for preeclampsia (*Figure 33.*).

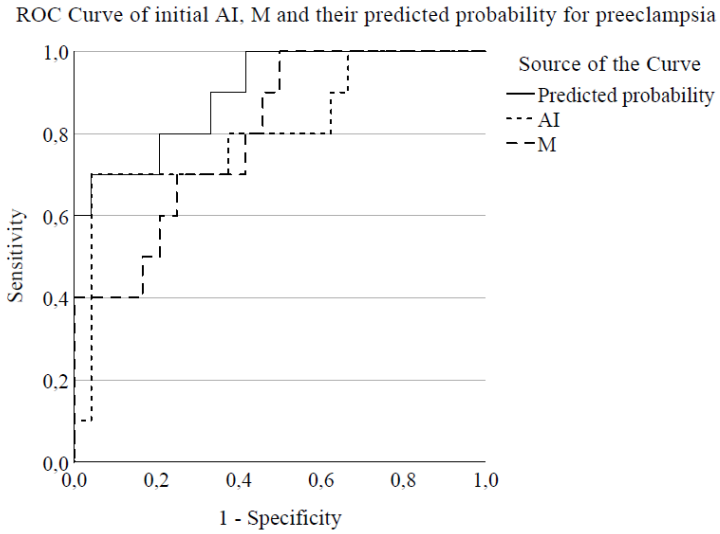

Figure 33. *ROC curve for preeclampsia comparing initial AI and M values per se measured at diagnosis or enrolment and their combination expressed as predicted probability*

13. Discussion

13.1. Summary of findings

Our research findings are intended to emphasise and confirm the role of maternal hemorheological alterations in pathophysiological processes affecting microcirculation in early-onset preeclampsia. Our investigations focused mainly on peripartum alterations of RBC properties including their aggregation and deformability. The most remarkable findings of our research are the potential diagnostic power of elevated AI and M index per se and in combination reflecting enhanced RBC aggregation with high sensitivity and acceptable specificity. We observed positive linear relationship between AI and gestational age at birth in preeclampsia, which association was missing in healthy pregnancies. Significantly reduced initial EI values were observed on medium shear stresses reflecting impaired erythrocyte deformability at diagnosis of preeclampsia. RBC deformability improved within three days after delivery in women with preeclampsia compared to the values measured at diagnosis or during delivery.

13.2. Routinely measured RBC laboratory parameters

Total blood volume, plasma volume, and erythrocyte mass are gradually increasing throughout the normal healthy pregnancy. Additionally, the volume of plasma expands proportionally more than the RBC mass, resulting in decreased haemoglobin concentrations as a consequence of physiological haemodilution [156]. In contrast with the mentioned natural processes occurring during a healthy pregnancy, we obtained slightly elevated RBC count and haemoglobin values in mothers diagnosed with early-onset preeclampsia, which results reinforce previously published findings [148]. In our preeclampsia group, MCV values at enrolment were slightly beneath the values of healthy mothers, contrary to prior investigations reporting somewhat increased MCV values in preeclampsia [148,157]. It is of note that these pre-cited study populations were composed of mothers at a later stage of pregnancy since the mean gestational age both of preeclampsia and control group were higher than our subjects. Other studies established that the erythrocytes with lower MCV values predominate in women with preeclampsia compared to mothers without pregnancy complications [158]. According to a recent publication analysing the osmotic and mechanical stability of erythrocytes, it is suggested that erythrocytes with a tendency to microcytosis accompanied by lower volume and lower haemoglobin content are osmotically more stable, and they assumed that somewhat

diminished MCV values could be a mechanism of compensatory mechanical selection, which could be beneficial in preeclampsia [159]. However, the desired range of MCV in early-onset preeclampsia remains unclear.

13.3. Erythrocyte aggregation

According to our results, elevated initial maternal AI and M values were proved to be the most promising indicators of early-onset preeclampsia with high sensitivity and acceptable specificity. Moreover, we gained more favourable AUC by their combination compared to their individual analysis. The elevated RBC aggregation at preeclampsia diagnosis compared to controls refer to impaired microcirculation in early-onset preeclampsia, which is also supported by the relationship between AI and gestational age at birth expressed in weeks. This latter association suggests that the longer the disease persists, the more deteriorated the AI values are, reflecting progressively enhancing maternal RBC aggregation in preeclampsia. It is worth mentioning that we did not observe significant correlation or linear regression within the group of healthy expectant concerning their AI value at enrolment and their gestational age at enrolment. These observations suggest that in normal pregnancy the gestational age alone did not influence AI values, this association was specific to preeclampsia. It should be noted that initial AI values in both groups were independent of haemoglobin values, so they did not affect our measurement results in either group.

Analysing the group-specific kinetics, we can conclude that the initially lower RBC aggregation tendency increased within the first 72 postpartum hours in healthy pregnant women. Contrary, in preeclampsia RBC aggregation remained continuously elevated without statistically significant alteration concerning the three investigated time points.

The RBC aggregation activity mostly depends on the plasma protein levels and intrinsic properties of the erythrocytes such as alterations affecting the cell membrane [160]. In preeclampsia rather changes of cellular factors are suggested to be responsible for pronounced aggregation propensity [161]. Increased RBC aggregation can be attributed to the reduced sialic acid content of the cell membrane that weakens repulsive forces [162] and conformational changes of the membrane [163] enhancing erythrocyte aggregation.

13.4. Erythrocyte deformability

Initial EI values at medium shear stresses were decreased at preeclampsia diagnosis compared to the values measured in normal pregnancy which observation reflects altered RBC deformability. The relatively rapid improvement in deformability values during the postpartum period within the preeclampsia group may presumably occur due to the termination of gravidity, which has maintained the disease. From this point of view, it can be hypothesised that RBC elongation ability characterised by EI may be a sensitive and early marker of the normalisation of the maternal microcirculation after delivery.

The reason for reduced RBC deformability in preeclampsia is previously attributed to the coexistence of chronic inflammation and hypoxia. These pathological phenomena are accompanied by increased concentration of free radicals inducing changes in RBC membrane properties and subsequent increase of intracellular Ca^{2+} which may are in the background of reduced elongation ability [164]. In addition, an increased Ca^{2+} pump activity leads to adenosine triphosphate depletion in RBCs resulting in poor deformability [165].

13.5. Literature and previous investigation methods

Literature available to date regarding RBC deformability and aggregation in preeclampsia seem to be in conflict. Based on some previous research, decreased elongation ability of erythrocytes was mentioned in maternal blood samples of expectants who developed preeclampsia [147,148,166]. B. Schauf et al. have discovered utero-placental hypoperfusion with subsequent RBC membrane damage resulting in decreased cell deformability in preeclampsia, which features may improve by intravenous magnesium administration [167]. Nevertheless, others could not verify any significant difference between preeclamptic and normal pregnancy concerning deformability [168,169]. Results are also diverse in terms of aggregation properties. Tranquilli et al. reported increased propensity of erythrocytes to aggregate in the maternal blood in preeclampsia [163], while other authors [150,170] did not observe remarkable alterations regarding erythrocyte aggregation. In line with our experiences, L. Heilmann et al. found statistically elevated values of haematocrit, haemoglobin, RBC aggregation and reduced RBC deformability by high shear stress in patients with severe preeclampsia suggesting that hemorheological

parameters play an important role in the microcirculation of the intervillous space of placenta [148].

Most of the studies dealing with hemorheological alterations in preeclampsia were published more than 2-3 decades ago. As preeclampsia definitions, the measurement methods and interpretation of results may have changed since then, it is necessary to re-evaluate these results. The diagnostic criteria for early-onset preeclampsia have been broadened and clarified [120], the distinction between the early- and late-onset forms of the syndrome are increasingly recognised, thus the investigation of the two entities is necessary to perform completely separately. The pathophysiological background varies in the two forms of the disease, inducing diverse clinical features. Early-onset preeclampsia is suggested to be originated mainly from defective placentation, whilst late-onset type may develop due to maternal genetic predisposition to cardiovascular and metabolic disease [123]. Hemorheological properties presumably play a critical role in the pathophysiology of both forms, but the parameters may not change to the same extent and direction. Therefore, it would be desirable, to treat the two types separately from a rheological point of view as well.

Regarding RBC aggregation, previous investigations applied mostly Myrenne aggregometer per se [147,148], while only a few reported results were carried out by LORCA instrument [150]. To the best of our knowledge, there are no data in the literature that examine the two measurement methods simultaneously in early-onset preeclampsia as we have performed in this recent study. Here, it can be seen from our report that we gained similar results in terms of RBC aggregation with the two types of measurement methods, moreover, their combination may even rise the diagnostic power.

13.6. Screening for preeclampsia

In the past decade, extensive efforts have been made to develop an efficient screening algorithm to identify high-risk patients and reduce the prevalence of preeclampsia through pharmacologic intervention administered from the early phase of the pregnancy before the onset of symptoms [12]. Currently, the best screening model in the first trimester combines multiple examination containing maternal risk factors, comorbid conditions, physical parameters, mean arterial pressure, placental growth factor level, and uterine artery pulsatility index. According to a recent recommendation, women identified at high risk should receive 150 mg acetylsalicylic acid prophylaxis daily commencing at

$11–14^{+6}$ weeks of gestation until either 36 weeks, when delivery occurs, or when preeclampsia is diagnosed [151]. Determination of factors and specific biomarkers used in current screening algorithm is effortful, time-consuming, and requires costly measurements and contribution of qualified professionals. In contrast, we investigated a cheap method with easy implementation and indicators that are quantitative, objective and can be blinded to other clinical characteristics. Hemorheological tests are practically at minimal cost, apart from the purchase of the instruments (LORCA or Myrenne aggregometer) and can be easily performed without special training. The above-detailed measurements require quite small amount of blood sample (20 μL for deformability and 1 ml for aggregation measurement by LORCA, and 30 μl for Myrenne aggregation) and provide quickly obtainable results as the implementation of the mentioned methods lasts ca. 15 min. Therefore, further investigations are suggested to reveal whether RBC aggregation and deformability parameters, especially AI and M index possess the potential for susceptibility or risk biomarkers in early stage of preeclampsia before the onset of symptoms. Moreover, their inclusion in the screening algorithm should be considered.

13.7. Strengths and limitations

The **strength** of our study is the prospective, case-control design with repeated blood sampling in the peripartum period. This design allows us to gain new insights not only about pathological but also the normal peripartum alterations of erythrocyte properties by examining kinetics within the two groups and comparing these changes between the groups. For RBC aggregation measurement, two methods were simultaneously applied: Myrenne, which measures the increase of light transmission through plasma gaps between RBC aggregates, and LORCA, which detects laser backscattering from the erythrocyte aggregates. Utilising the two instruments together provides more comprehensive information on RBC aggregation properties. We recruited exclusively mothers suffering from preeclampsia with early onset as the information about hemorheological properties in this type is still limited. Our control group was not only maternal age but also gestational age-matched to avoid erythrocyte alterations as a result of various gestational stages.

The main **limitation** of our study is the low total number of enrolled patients, although it must be admitted that it is challenging to recruit more subjects into the early-onset preeclampsia group in view of the low incidence of the disease. The study was

conducted in a single-centre, so local treatment strategies and guidelines could limit the generalisability of our findings. The follow-up period ended at 72 postpartum hours, thus we could not report data about long term maternal and neonatal consequences.

14. Future perspectives

As mentioned above the long-term follow-up involving higher number of patients with the possible expansion to a multi-centre design is worth considering to investigate the potential role of peripartum hemorheological alterations in the development of cardiovascular complications in the later life of the mother. It is also worth pondering the combination of peripartum maternal hemodynamical and echocardiographic parameters concurrently with the hemorheological measurements to gain a most comprehensive insight about macro-, and microcirculation and their relationship. Another interesting question would be to reveal the possible relationship between gestational ultrasound parameters (e.g. resistant index, and pulse index of the umbilical artery and middle cerebral artery), and maternal hemorheological properties. To complete the measurements by evaluating other physical characteristics of the erythrocytes (e.g. osmotic and mechanical stability) accompanied by morphological analysis by electron microscope would promote the interpretation of the results.

Besides the analysis of maternal blood samples, our research team plans to examine the umbilical cord blood and neonatal peripheral blood samples in the early postnatal period. These results would contribute to the prediction of foetal complications and the long-term follow-up of the neonates would hold the potential to explore the relationship with later developmental disabilities.

15. Conclusion

Enhanced erythrocyte aggregation and deteriorated deformability characterise the maternal blood samples at diagnosis of early-onset preeclampsia. Investigating the peripartum changes within groups, distinct kinetics were observable concerning the RBC parameters. RBC properties could contribute to the prognostication of early-onset PE, but further investigations are warranted to confirm the prognostic role before the onset of symptoms.

16. Summary of novel findings

16.1. Novel and conventional biomarkers for post-resuscitation prognosis

- Initial ADMA levels independently predict early death within 3 days after resuscitated cardiac arrest.
- Elevated ADMA levels are associated with persistent vegetative state or brain death during post-resuscitation care.
- Significant positive correlation was revealed between initial ADMA and SAPS II score.
- CK-18, CCCK-18 and NSE are not associated with survival or neurological outcome after cardiac arrest in our cohort of unselected resuscitated patients.
- CK-18 levels were persistently elevated with decreased CCCK-18/CK-18 ratio on the first three days referring to a large extent of cell death dominantly due to necrosis.
- 72-hour CK-18 level showed significant correlation with SAPS II and SOFA scores.
- Initial lactate level predicts 30-day mortality and poor neurological outcome and provides similarly useful information per se as SAPS II and SOFA scores.

16.2. Maternal hemorheological properties in early-onset preeclampsia

- Elevated AI and M index reflecting increased erythrocyte aggregation were proved to be the most promising indicators in maternal blood samples with high sensitivity for early-onset preeclampsia diagnosis.
- Significant positive linear relationship was found between AI and gestational age at birth in preeclampsia, which association was missing in healthy pregnancies.
- Lower EI values were observed at preeclampsia diagnosis compared to normal pregnancy reflecting deteriorated RBC deformability, which improved rapidly in postpartum period in preeclampsia group.

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20. Scientometrics

Scientific papers:

- Total: 9
- English language papers: 7

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List of publications:

First author papers upon which this thesis relies:

1. **Csiszar, Beata**; Marton, Zsolt; Riba, Janos; Csecsei, Peter; Nagy, Lajos; Toth, Kalman; Halmosi, Robert; Sandor, Barbara; Kenyeres, Peter (corresponding author); Molnar, Tihamer. L-Arginine, asymmetric and symmetric dimethylarginine for early outcome prediction in unselected cardiac arrest victims: a prospective cohort study. INTERNAL AND EMERGENCY MEDICINE (2021). Published: 03 June 2021; https://doi.org/10.1007/s11739-021-02767-z **Q1;** IF: 3.397 (2020) H-index: 47
2. **Csiszár, Beáta**; Németh, Álmos Márton, Zsolt; Riba, János; Csécsei, Péter; Molnár, Tihamér; Deres, László; Halmosi, Róbert; Tóth, Kálmán; Kenyeres, Péter. A citokeratin-18 sejthalálmarker vizsgálata sikeres cardiopulmonalis resuscitatión átesett betegpopulációban. ORVOSI HETILAP 161: 1 pp. 26-32., 7 p. (2020); **Q4**; IF: 0.540 (2020) H-index: 21

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2. **Beata Csiszar**, Gergely Galos, Simone Funke, Miklos Koppan, Matyas Meggyes, Laszlo Szereday, Peter Kenyeres, Kalman Toth, Barbara Sandor. Maternal hemorheological changes in early-onset preeclampsia. Magyar Haemorheologiai Társaság XXVII. Kongresszusa, online, 2021. április 23.
3. **Csiszár Beáta**, Márton Zsolt, Riba János, Csécsei Péter, Molnár Tihamér, Deres László, Kőszegi Tamás, Tóth Kálmán, Halmosi Róbert, Kenyeres Péter. A cytokeratin-18 prognosztikus szerepének vizsgálata posztreszuszcitációs ellátás során. Magyar Kardiológusok Társasága 2020. évi Tudományos Kongresszusa online. 2020. november 11-14.
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6. **Csiszár Beáta**, Kevey Dóra Kinga, Császár András, Vida Gabriella, Funke Simone, Szereday László, Meggyes Mátyás, Tóth Kálmán1, Sándor Barbara. Preeclampsiás édesanyák és újszülöttjeik hemoreológiai paramétereinek vizsgálata. Magyar Haemorheologiai Társaság XXVI. Kongresszusa. 2019. április 12-13. Pécs, Hotel Therapia.
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Book of abstracts and conference books, editorial work:

1. **Csiszár, Beáta;** Hankó, Csilla; Kajos, Luca Fanni; Mező, Emerencia**:** Medical Conference for PhD Students and Experts of Clinical Sciences 2021: Book of Abstracts. Pécs, Hungary: Doctoral Student Association of the University of Pécs (2021), 124 p. ISBN: 9789634296539
2. **Csiszár, B;** Hankó, Cs; Kajos, L F; Kovács, O B; Mező, E; Szabó, R; Szabó-Guth, K: IX. Interdiszciplináris Doktorandusz Konferencia 2020 Absztraktkötet: 9th Interdisciplinary Doctoral Conference 2020 Book of Abstracts. Pécs, Hungary: Pécsi Tudományegyetem Doktorandusz Önkormányzat (2020), 384 p. ISBN: 9789634295822
3. **Csiszár, Beáta**; Hankó, Csilla; Kajos, Luca Fanni; Mező, Emerencia: IX. Interdiszciplináris Doktorandusz Konferencia 2020 Tanulmánykötet; 9th Interdisciplinary Doctoral Conference 2020 Conference Book. Pécs, Magyarország: Pécsi Tudományegyetem Doktorandusz Önkormányzat (2020), 595 p. ISBN: 9789634295839
4. **Csiszár, Beáta**; Hankó, Csilla; Kajos, Luca Fanni; Mező, Emerencia: Medical Conference for PhD Students and Experts of Clinical Sciences: Book of Abstracts. Pécs, Hungary: Doctoral Student Association of the University of Pécs (2020), 83 p. ISBN: 9789634295440
5. Bódog, Ferenc; **Csiszár, Beáta**: VIII. Interdiszciplináris Doktorandusz Konferencia 2019. Absztraktkötet; 8th Interdisciplinary Doctoral Conference 2019. Book of Abstracts.

Pécs, Magyarország: Pécsi Tudományegyetem Doktorandusz Önkormányzat (2019), 185 p. ISBN: 9789634293743

6. **Csiszár, Beáta**; Bódog, Ferenc: Medical Conference for PhD Students and Experts of Clinical Sciences: Book of abstracts. Pécs, Hungary: Pécsi Tudományegyetem Doktorandusz Önkormányzat (2019), 87 p. ISBN: 9789634292913
7. **Csiszár, Beáta**; Bódog, Ferenc; Mező, Emerencia; Závodi, Bence: VIII. Interdiszciplináris Doktorandusz Konferencia 2019. Tanulmánykötet; 8th Interdisciplinary Doctoral Conference 2019. Conference Book. Pécs, Magyarország: Pécsi Tudományegyetem Doktorandusz Önkormányzat (2019) , 327 p. ISBN: 9789634293729
8. Bódog, Ferenc; **Csiszár, Beáta**; Hayden, Zsófa; Kovács, Olivér; Rácz, Tamás. VII. Interdiszciplináris Doktorandusz Konferencia 2018. Tanulmánykötet; 7th Interdisciplinary Doctoral Conference 2018. Conference Book. Pécs, Magyarország: Pécsi Tudományegyetem Doktorandusz Önkormányzat (2018), 492 p. ISBN: 9789634292111
9. Bódog, Ferenc; **Csiszár, Beáta**; Pónusz, Róbert: Medical Conference for PhD Students and Experts of Clinical Sciences Book of Abstracts. Pécs, Magyarország: Pécsi Tudományegyetem Doktorandusz Önkormányzat (2018) ISBN: 9789634292760
10. Bódog, Ferenc; **Csiszár, Beáta**; Hayden, Zsófia; Mészáros, Orsolya; Sapolov, Anatolij; Pónusz, Róbert. VII. Interdiszciplináris Doktorandusz Konferencia 2018 Absztraktötet: Digitális, bővített kiadás; 7th Interdisciplinary Doctoral Conference 2018 Book of Abstracts: Digital, extended edition. Pécs, Magyarország: Pécsi Tudományegyetem Doktorandusz Önkormányzat (2018), 207 p. ISBN: 9789634292098
11. Bódog, Ferenc; **Csiszár, Beáta**; Hegyi, Dávid; Pónusz, Róbert. DKK17-Doktoranduszok a Klinikai Kutatásokban Absztraktkötet. Pécs, Magyarország: Pécsi Tudományegyetem Doktorandusz Önkormányzat (2017), 89 p. ISBN: 9789634291602

21. Appendix

Appendix 1. *Characteristics of the study population according to mortality in the ICU*

	Survivors (n=21; 39%)	**Non-survivors (n=33; 61%)**	**p value**
Baseline			
Age (years)	63 [58 - 79]	67 [62 – 79]	0.511
Male gender	8 (38%)	18 (55%)	0.238
Characteristics of cardiac arrest and CPR			
Localisation: in-hospital cardiac arrest	15 (71%)	24 (73%)	0.917
Resuscitation during nightshift or weekend	14 (67%)	25 (76%)	0.467
First monitored rhythm:			
• Ventricular tachycardia/fibrillation	5 (24%)	9 (27%)	0.777
• Pulseless electrical activity	5 (24%)	9 (27%)	0.777
• Asystole	9 (43%)	14 (42%)	0.975
• Unknown	2 (10%)	1 (3%)	0.310
Time of the resuscitation (min)	10 [5 – 22]	10 [5 – 20]	0.862
Patients required epinephrine	15 (71%)	30 (91%)	0.061
Dose of epinephrine (mg)	2 [0 – 2.5]	2 [1 – 3.5]	0.103
Mechanical ventilation requirement within 6 hours after cardiac arrest	20 (95%)	29 (88%)	0.363
Aetiology of cardiac arrest			
Ischaemic heart disease	6 (29%)	11 (33%)	0.713
Heart failure	6 (29%)	10 (30%)	0.892
Sepsis	2 (10%)	3 (9%)	0.957
Hyperkalaemia	2 (10%)	3 (9%)	0.957
Aspiration	1 (5%)	2 (6%)	0.839
Hypothermia	1 (5%)	1 (3%)	0.743
Stroke	0	2 (6%)	0.250
Pulmonary embolism	0	2 (6%)	0.250
Pneumonia	2 (10%)	0	0.071
Parameters on enrolment			
Systolic blood pressure (mmHg)	114 [102 – 141]	114 [103 – 132]	0.927
Diastolic blood pressure (mmHg)	60 [54 – 68]	62 [57 – 69]	0.684
Mean arterial pressure (mmHg)	75 [70 – 92]	77 [71 – 86]	0.894
Heart rate (/min)	78 [69 – 94]	92 [66 – 105]	0.303
Body temperature (°C)	36.7 [36.3 – 37.2]	36.4 [36.1 – 36.7]	0.204
Comorbidities, previous medical history			
Hypertension	15 (71%)	24 (55%)	0.917
Ischaemic heart disease	4 (19%)	16 (48%)	**0.029**
Diabetes mellitus	4 (19%)	18 (55%)	**0.010**
Heart failure	6 (29%)	11 (33%)	0.713
Permanent atrial fibrillation	4 (19%)	6 (18%)	0.936
Stroke or transient ischaemic attack	2 (10%)	8 (24%)	0.175
Carotid artery stenosis	2 (10%)	3 (9%)	0.957
Chronic obstructive pulmonary disease	6 (29%)	2 (6%)	**0.023**
Peripheral artery disease	1 (5%)	6 (18%)	0.152
Previous pulmonary embolism	1 (5%)	2 (6%)	0.839

Previous, cured malignant disease	5 (24%)	3 (9%)	0.138
Active malignant or haematologic disease	4 (19%)	5 (15%)	0.708
Prognostic scores			
SOFA	9 ± 4	11 ± 3	**0.021**
SAPS II	67 ± 17	80 ± 13	**0.002**

Prognostic score points were calculated concerning the worst detected value within 24 hours after cardiac arrest. Continuous data are presented as median values with interquartile range [percentiles 25–75] or mean ± standard deviation, categorical data as the number of subjects and percentages. CPR: cardiopulmonary resuscitation; SOFA: Sequential Organ Failure Assessment Score; SAPS II: Simplified Acute Physiology Score II; ICU: intensive care unit.

Appendix 2. *Characteristics of study cohort according to 72-hour mortality*

	Survivors (n=40; 74%)	**Non-survivors (n=14; 26%)**	**p value**
Baseline			
Age (years)	66 [59 – 78]	72 [63 – 81]	0.309
Male gender	21 (53%)	5 (36%)	0.279
Characteristics of cardiac arrest and CPR			
Localisation: in-hospital cardiac arrest	29 (73%)	10 (71%)	0.939
Resuscitation during nightshift or weekend	28 (70%)	11 (79%)	0.538
First monitored rhythm:			
• Ventricular tachycardia/fibrillation	9 (23%)	5 (36%)	0.332
• Pulseless electrical activity	11 (28%)	3 (21%)	0.655
• Asystole	18 (45%)	5 (36%)	0.545
• Unknown	2 (5%)	1 (7%)	0.762
Time of the resuscitation (min)	10 [5 – 24]	8 [5 – 19]	0.842
Patients required epinephrine	32 (80%)	13 (93%)	0.451
Dose of epinephrine (mg)	2 [1-3]	2 [1-3]	0.493
Mechanical ventilation requirement within 6 hours after cardiac arrest	37 (93%)	12 (86%)	0.451
Aetiology of cardiac arrest			
Ischaemic heart disease	12 (30%)	5 (36%)	0.692
Heart failure	13 (33%)	3 (21%)	0.435
Sepsis	3 (8%)	2 (14%)	0.451
Hyperkalaemia	3 (8%)	2 (14%)	0.451
Aspiration	3 (8%)	0	0.292
Hypothermia	2 (5%)	0	0.394
Stroke	1 (3%)	1 (7%)	0.429
Pulmonary embolism	1 (3%)	1 (7%)	0.429
Pneumonia	2 (5%)	0	0.394
Parameters on enrolment			
Systolic blood pressure (mmHg)	115 [103 – 140]	113 [95 – 126]	0.667
Diastolic blood pressure (mmHg)	61 [53 – 68]	62 [57 – 69]	0.928
Mean arterial pressure (mmHg)	77 [70 – 91]	76 [71 – 84]	0.671
Heart rate (/min)	78 [65 – 99]	94 [79 – 101]	0.241
Body temperature (°C)	36.3±1.3	36.2±1.5	0.762
Comorbidities, previous medical history			

Hypertension	29 (73%)	10 (71%)	0.939
Ischaemic heart disease	13 (33%)	7 (50%)	0.243
Diabetes mellitus	19 (48%)	3 (21%)	0.088
Heart failure	14 (35%)	3 (21%)	0.347
Permanent atrial fibrillation	8 (20%)	2 (14%)	0.636
Stroke or transient ischaemic attack	8 (20%)	2 (14%)	0.636
Carotid artery stenosis	4 (10%)	1 (7%)	0.751
Chronic obstructive pulmonary disease	8 (20%)	0	0.070
Peripheral artery disease	5 (13%)	2 (14%)	0.864
Previous pulmonary embolism	2 (5%)	1 (7%)	0.763
Previous, cured malignant disease	5 (13%)	3 (21%)	0.418
Active malignant or haematologic disease	7 (18%)	2 (14%)	0.781
Prognostic scores			
SOFA	10 ± 3	12 ± 3	0.267
SAPS II	70 ± 16	87 ± 11	**<0.001**

Prognostic score points were calculated concerning the worst detected value within 24 hours after cardiac arrest. Continuous data are presented as median values with interquartile range [percentiles 25–75] or mean ± standard deviation, categorical data as the number of subjects and percentages. CPR: cardiopulmonary resuscitation; SOFA: Sequential Organ Failure Assessment Score; SAPS II: Simplified Acute Physiology Score II; ICU: intensive care unit.

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L-arginine, asymmetric and symmetric dimethylarginine for early outcome prediction in unselected cardiac arrest victims: a prospective cohort study

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Abstract

Early prediction of the mortality, neurological outcome is clinically essential after successful cardiopulmonary resuscitation. To find a prognostic marker among unselected cardiac arrest survivors, we aimed to evaluate the alterations of the L-arginine pathway molecules in the early post-resuscitation care. We prospectively enrolled adult patients after successfully resuscitated in- or out-of-hospital cardiac arrest. Blood samples were drawn within 6, 24, and 72 post-cardiac arrest hours to measure asymmetric and symmetric dimethylarginine (ADMA and SDMA) and L-arginine plasma concentrations. We recorded Sequential Organ Failure Assessment, Simplified Acute Physiology Score, and Cerebral Performance Category scores. Endpoints were 72 h, intensive care unit, and 30-day mortality. Among 54 enrolled patients [median age: 67 (61–78) years, 48% male], the initial ADMA levels were significantly elevated in those who died within 72 h [0.88 (0.64–0.97) µmol/L vs. 0.55 (0.45–0.69) µmol/L, $p = 0.001$]. Based on receiver operator characteristic analysis (AUC = 0.723; $p = 0.005$) of initial ADMA for poor neurological outcome, the best cutoff was determined as > 0.65 µmol/L (sensitivity = 66.7%; specificity = 81.5%), while for 72 h mortality (AUC = 0.789; $p = 0.001$) as > 0.81 µmol/L (sensitivity = 71.0%; specificity = 87.5%). Based on multivariate analysis, initial ADMA (OR = 1.8 per 0.1 µmol/L increment; $p = 0.002$) was an independent predictor for 72 h mortality. Increased initial ADMA predicts 72 h mortality and poor neurological outcome among unselected cardiac arrest victims.

Keywords Cardiopulmonary resuscitation · Post-resuscitation care · Asymmetric dimethylarginine · Prognostication · Cardiac arrest · Mortality

Introduction

Management of post-resuscitation care, including post-cardiac arrest syndrome, ischemic brain injury, myocardial dysfunction, and multiple organ failure (MOF) remains an unmet clinical challenge with high mortality. The 1-year survival rate after out-of-hospital cardiac arrest (OHCA) is around 8% [1] and 13% among in-hospital cardiac arrest (IHCA) patients [2]. Early and effective prediction of the mortality, neurological and functional consequences is clinically essential for the medical team to choose the optimal level of treatment, to decide about withdrawal of life-sustaining therapy, to guide goals-of-care conversations with relatives, and to improve the cost-effectiveness of care [3]. Current guidelines recommend a multimodal approach (neurological examination, electrophysiological investigations, neuroimaging, and biomarkers) for prognostication

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of neurological outcome after resuscitation from cardiac arrest [4]. The major limitation of the current algorithm is that it can be applied only to a minority of patients who remain comatose. To improve the quality of prognostication after cardiac arrest, addressing the role of extracerebral causes of death is warranted [5]. Only one-fourth of patients who suffered IHCA die due to neurological injury, while most of them may reach acceptable neurological function but suffer from MOF which may lead to death [6]. A reliable biomarker that can be used in unselected resuscitated patients would provide useful information about the general outcome and survival without focusing only on the neurological status.

Endothelial injury, microcirculatory dysfunction, and coagulopathy developing after resuscitated cardiac arrest are associated with poor outcome [7]. The nitric oxide system plays a crucial role in the regulation of vascular tone and nitric oxide is identified as a mediator of vasodilation under a variety of physiological and pathophysiological conditions, such as hypoxia and ischemia [8]. Enzymatic activities of nitric oxide synthases catalyze the two-step oxidation of L-arginine to nitric oxide and L-citrulline. Methylarginines are described as the main regulators and endogenous inhibitors of nitric oxide synthase catalytic function by competing with L-arginine for binding to the catalytic site of nitric oxide synthase (NG-monomethyl L-arginine and asymmetric dimethylarginine—ADMA) or by binding to the L-arginine membrane uptake carrier (ADMA and symmetric dimethylarginine—SDMA). ADMA has been demonstrated to inhibit nitric oxide formation and increase oxidative stress in vascular endothelial and smooth muscle cells [9].

Previous investigations found a strong association of high plasma ADMA level upon admission and mortality in critically ill patients [10]. Elevated circulating concentrations of L-arginine derivatives have been associated with progression and outcome in a variety of conditions including cardiovascular [11] and cerebrovascular disorders [12–14].

We aimed to explore, for the first time, the alterations of the L-arginine-nitric oxide pathway molecules in the early post-resuscitation care of cardiac arrest survivors, and their distinct association patterns with the prognostic scoring systems, neurological function, and outcome measures such as 72 h, intensive care unit (ICU) and 30-day mortality.

Materials and methods

Study population

This is a prospective, single-center observational study conducted from January 2018 to January 2019 in the Intensive care unit of the 1st Department of Medicine, Department of Anaesthesiology and Intensive Care and Department of Emergency Medicine at the University of Pécs. Our cohort was made up of 54 adult patients [median age: 67 (61–78) years, 48% male] who suffered IHCA or OHCA, and after successful resuscitation were admitted to the ICU for post-resuscitation care. Successful resuscitation was defined as the return of spontaneous circulation (ROSC). 23 patients admitted to the ICU of the 1st Department of Medicine, 18 patients to the Department of Anaesthesiology and Intensive Care, and 13 patients from the Department of Emergency Medicine were enrolled in our cohort. Standard post-resuscitation care was applied for each patient in the ICU without interaction with the research team. Therapeutic hypothermia was not applied during post-resuscitation care, but each patient was kept in normothermia. This report follows the STROBE Statement [15]. The study was approved by the Local Ethics Committee of the University of Pécs (file number: 6941 – PTE 2018.) and has followed the principles outlined in the Declaration of Helsinki for all human investigations. Informed consent for being included in the study was obtained from legal representatives or, in case the patients had regained consciousness, from the patients themselves.

Sample and data collection

Data collected included patient anamnestic information, comorbidities, the circumstances of cardiopulmonary resuscitation, variables that are necessary for calculating Simplified Acute Physiology Score (SAPS II) and Sequential Organ Failure Assessment (SOFA) severity scores. Mortality by 72 h after cardiac arrest, mortality occurred in the ICU and 30-day mortality, and the best neurological status was used as outcome measures. Plasma samples were collected within 6, 24, and 72 h after ROSC to determine the biomarker concentrations by high-performance liquid chromatography. Besides, laboratory and vital parameters were also assessed in the mentioned investigated time points after cardiac arrest. The SAPS II and SOFA scores were calculated according to the worst parameters of the first 24 h after cardiac arrest. The neurological outcome was measured using cerebral performance category (CPC) score, which consists of a scale of 5 levels: (1) a return to normal cerebral function and normal living, (2) disability but sufficient function for independent activities of daily living, (3) severe disability, limited cognition, inability to carry out independent existence, (4) coma and (5) brain death. CPC scores 1–3 were determined as good and 4–5 as poor neurological outcome. The best neurological status reached in the ICU was recorded using the CPC scale to avoid false pessimistic neurological classification in patients who regained consciousness after resuscitation and then died due to extracerebral causes with satisfactory neurological status during the follow-up period [16].

Biomarkers

Blood samples were drawn into Vacutainer® EDTA-tubes from resuscitated patients on admission within 6 h and 24 ± 3 and 72 ± 3 h after cardiac arrest to determine plasma concentrations of L-arginine, ADMA and SDMA. The samples were centrifuged within 10 min at 3500 rpm for 15 min. The supernatant was immediately stored in aliquot at − 80 °C until determining the L-arginine derivative concentrations at the end of the recruitment process. L-arginine, ADMA, and SDMA were measured in the plasma by high-performance liquid chromatography after derivatisation in collaboration with the Department of Applied Chemistry at the University of Debrecen, Hungary [17, 18]. We calculated the change of the investigated markers from 6 to 24 and 24–72 h. All samples were processed by the same technicians using the same equipment and blinded to all clinical data. The biomarker values were not available for clinical purposes and did not influence therapeutic approaches or the decision-making process.

Statistical analysis

Statistical analysis of the collected data was evaluated by IBM SPSS Statistics® 27.0. The Kolmogorov–Smirnov test was applied to test for normality of continuous variable distribution. Comparisons of continuous non-normally distributed data between groups were carried out using the Mann–Whitney U test. Student T test was used for analysis of normally distributed continuous data. The continuous variables are reported as medians and interquartile ranges or mean and standard deviation. Correlation analysis was performed calculating Spearman's correlation coefficient (rho). For variables with significant correlation, linear logistic regression analysis was performed, and R^2 values were reported on the figures. Receiver Operating Characteristic (ROC) analysis and the Area Under the Curve (AUC) were used to determine the most appropriate cutoff values of initial circulating ADMA levels and the investigated endpoints, especially for the evaluation of the 72 h mortality and neurological outcome based on CPC categories. Univariable binary logistic regression tests were used to evaluate associations between the recorded initial variables and 72 h mortality with corresponding beta values and 95% confidence intervals. Variables with p value ≤ 0.05 in the univariable analysis were included in the multivariable models considering the principle of multicollinearity. Multivariable logistic regression was used to identify factors independently associated with 72 h mortality. Sample size and power analysis were performed for the overall population using PS program version 3.1.2. For the sample size of $n = 54$, patients needed to detect a true difference of $d = 0.267$ in initial ADMA for 72 h mortality with 92% power, where type I error probability is $\alpha = 0.05$. A p value < 0.05 was considered statistically significant.

Results

During the 30-day follow-up, 11% of the patients reached good neurological status (CPC 1–2) and 39% had severe neurological disability (CPC 3), while half of the patients suffered from coma, vegetative state, or brain death (CPC 4–5). The biomarker levels and their change were also analyzed according to the chosen clinical endpoints. The primary endpoint was the 72 h mortality, besides, we analyzed ICU mortality, 30-day mortality and neurological outcome (CPC). Figure 1 shows the exact number of survivors during the 30-day follow-up.

72 hour mortality

Characteristics of the study group made up of 54 successfully resuscitated patients are summarized in Table 1. Around one-fourth of the patients died within the first 3 days after the ROSC. There was no statistically significant difference between survivors and non-survivors regarding age, gender, cardiac arrest characteristics (e.g., in-hospital or out-of-hospital, length of the cardiopulmonary resuscitation, and first monitored rhythm), suggested etiology of cardiac arrest, vital parameters on enrollment, or

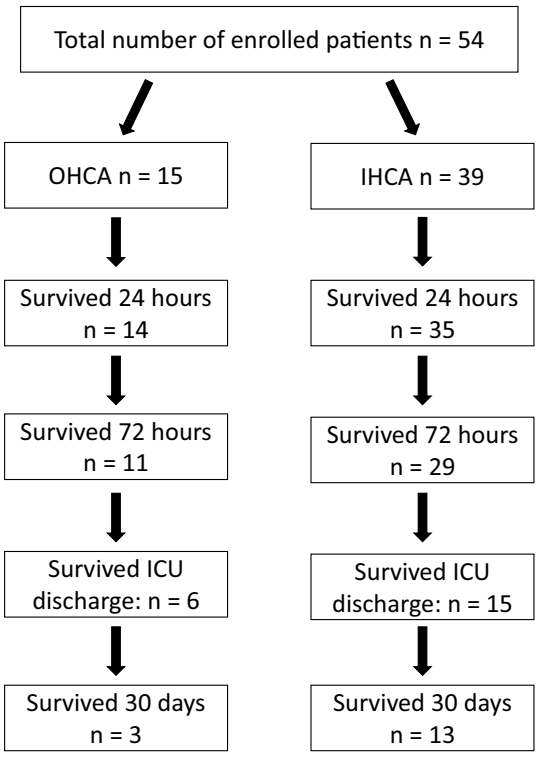

Fig. 1 Study population: Flow-chart about the exact number of survivors (*IHCA* in-hospital cardiac arrest, *OHCA* out-of-hospital cardiac arrest, *ICU* intensive care unit)

Table 1 Characteristics of the study population according to 72 h mortality

	Survivors ($n=40$; 74%)	Non-survivors ($n=14$; 26%)	p value
Baseline			
Age (years)	66 [59–78]	72 [63–81]	0.309
Male gender	21 (53%)	5 (36%)	0.279
Characteristics of the CA and the CPR			
Localisation: in-hospital CA	29 (73%)	10 (71%)	0.939
Resuscitation during nightshift or weekend	28 (70%)	11 (79%)	0.538
First monitored rhythm			
Ventricular tachycardia/fibrillation	9 (23%)	5 (36%)	0.332
Pulseless electrical activity	11 (28%)	3 (21%)	0.655
Asystole	18 (45%)	5 (36%)	0.545
Unknown	2 (5%)	1 (7%)	0.762
Time of the resuscitation (min)	10 [5–24]	8 [5–19]	0.842
Patients required epinephrine	32 (80%)	13 (93%)	0.451
Dose of epinephrine (mg)	2 [1–3]	2 [1–3]	0.493
Mechanical ventilation within 6 h after CA	37 (93%)	12 (86%)	0.451
Etiology of CA			
Ischemic heart disease	12 (30%)	5 (36%)	0.692
Heart failure	13 (33%)	3 (21%)	0.435
Sepsis	3 (8%)	2 (14%)	0.451
Hyperkalaemia	3 (8%)	2 (14%)	0.451
Aspiration	3 (8%)	0	0.292
Hypothermia	2 (5%)	0	0.394
Stroke	1 (3%)	1 (7%)	0.429
Pulmonary embolism	1 (3%)	1 (7%)	0.429
Pneumonia	2 (5%)	0	0.394
Unknown	14 (35%)	3 (21%)	0.347
Parameters on enrolment			
Systolic blood pressure (mmHg)	115 [103–140]	113 [95–126]	0.667
Diastolic blood pressure (mmHg)	61 [53–68]	62 [57–69]	0.928
Mean arterial pressure (mmHg)	77 [70–91]	76 [71–84]	0.671
Heart rate (/min)	78 [65–99]	94 [79–101]	0.241
Body temperature (°C)	36.3 ± 1.3	36.2 ± 1.5	0.762
Comorbidities, previous medical history			
Hypertension	29 (73%)	10 (71%)	0.939
Ischemic heart disease	13 (33%)	7 (50%)	0.243
Diabetes mellitus	19 (48%)	3 (21%)	0.088
Heart failure	14 (35%)	3 (21%)	0.347
Permanent atrial fibrillation	8 (20%)	2 (14%)	0.636
Stroke or transient ischemic attack	8 (20%)	2 (14%)	0.636
Carotid artery stenosis	4 (10%)	1 (7%)	0.751
Chronic obstructive pulmonary disease	8 (20%)	0	0.070
Peripheral artery disease	5 (13%)	2 (14%)	0.864
Previous pulmonary embolism	2 (5%)	1 (7%)	0.763
Previous, cured malignant disease	5 (13%)	3 (21%)	0.418
Active malignant or hematologic disease	7 (18%)	2 (14%)	0.781
Prognostic scores			
SOFA	10 ± 3	12 ± 3	0.267
SAPS II	70 ± 16	87 ± 11	< 0.001

Continuous data are presented as median values with interquartile range [percentiles 25–75] or mean ± standard deviation, categorical data as the number of subjects and percentages

CA cardiac arrest, *CPR* cardiopulmonary resuscitation, *SOFA* Sequential Organ Failure Assessment Score, *SAPS II* Simplified Acute Physiology Score II, *ICU* intensive care unit

comorbidities. 72 h non-survivors had significantly higher points of SAPS II score.

Table 2 summarizes the absolute plasma levels and the changes of L-arginine, ADMA, and SDMA between the patients who survived the 72 h after the cardiac arrest or died in this period. Significantly higher initial ADMA levels were observed among patients who died within 3 days. Comparing the initial ADMA levels between the IHCA and OHCA groups, we did not observe significant difference [IHCA: 0.61 (0.46–0.85) vs. OHCA: 0.64 (0.45–0.87), $p = 0.977$].

ICU mortality

A total of 33 patients died in the ICU (by average 6; min. 1–max. 26 days). Investigating the ICU mortality, none of the L-arginine pathway molecules showed a significant difference between survivors or non-survivors. The ADMA levels tended to remain higher among ICU non-survivors but the difference did not reach significance. The plasma ADMA levels of ICU non-survivors decreased from 6 to 24 h, while the values of the surviving group elevated by 24 h [− 0.08 (− 0.16 to 0.05) µmol/L vs. 0.07 (− 0.04 to 0.11) µmol/L, $p = 0.024$] (Suppl.-Table 1). Subgroup analysis of IHCA patients revealed significantly decreased 6 h L-arginine/ADMA ratio in patients who died in the ICU (Suppl.-Fig. 1).

30-day mortality

70% of the patients died within 30 days after cardiac arrest. Analyzing the kinetics of the markers according to 30-day mortality outcome, an opposite change was observed in ADMA level from 6 to 24 h between survivors and non-survivors similarly to the observation according to ICU mortality [− 0.08 (− 0.16 to 0.06) in non-survivors vs. 0.07 (− 0.03 to 0.11) in survivors, $p = 0.028$] (Suppl.-Table 2). In contrast, L-arginine, SDMA levels, or their change showed no significant difference in any of the clinical endpoints at any investigated time point. The L-arginine/ADMA ratio slightly elevated up to 72 post-cardiac arrest hours in the total population regardless of the mortality (6 h: 66.04 ± 4.33; 24 h: 80.04 ± 5.35; 72 h: 99.99 ± 7.13; $p < 0.05$) (Suppl.-Fig. 2).

Prognostic scores and biomarkers

The SAPS II and SOFA score had significant but moderate prognostic value for ICU mortality based on ROC analysis [SOFA AUC: 0.695 (0.537–0.853) $p = 0.020$; SAPS II AUC: 0.747 (0.602–0.891), $p = 0.003$]. Neither SAPS II nor SOFA score showed significant difference between IHCA and OHCA subgroups. The statistical analysis revealed a significant positive correlation between the initial ADMA levels and the SAPS II score (rho = 0.393, $R^2 = 0.178$, $p = 0.002$) (Suppl.-Fig. 3). The L-arginine levels per se did not show significant correlation with the investigated scores or parameters. Figure 2 demonstrates the analysis of the three parameters (SOFA, SAPS II, and initial ADMA) for 72 h mortality in a combined ROC curve. The results showed that the AUC of SAPS II and initial ADMA were comparable reflecting similar sensitivity and specificity in prediction of 72 h mortality, in contrast SOFA provided poor prognostic information for mortality [SAPS II AUC: 0.817 (0.688–0.946), $p < 0.001$; ADMA AUC: 0.789 (0.628–0.950), $p = 0.001$; SOFA AUC: 0.608 (0.433–0.783), $p = 0.232$].

Neurological outcome

The initial ADMA levels were significantly elevated among patients with poor neurological outcome (CPC 4–5)

Table 2 L-Arginine pathway molecules and their change according to the 72 h mortality

	Survivors ($n = 40$; 74%)	Non-survivors ($n = 14$; 26%)	p value
Biomarker plasma levels within 6 h after CA			
L-arginine (µmol/L)	33.45 [27.84–46.96]	46.16 [27.89–72.44]	0.079
ADMA (µmol/L)	0.55 [0.45–0.69]	0.88 [0.64–0.97]	0.001
SDMA (µmol/L)	0.93 [0.65–1.60]	0.93 [0.76–1.29]	0.969
Biomarker plasma levels 24 h after CA			
L-arginine (µmol/L)	38.95 [31.26–60.56]	45.62 [17.64–70.11]	0.910
ADMA (µmol/L)	0.54 [0.45–0.78]	0.78 [0.51–1.05]	0.145
SDMA (µmol/L)	1.03 [0.75–1.98]	1.32 [0.88–2.28]	0.515
Change in biomarker plasma levels from 6 to 24 h after CA			
ΔL-arginine (24–6 h) (µmol/L)	5.16 [− 4.48 to 23.37]	− 5.21 [− 25.32 to 21.38]	0.234
ΔADMA (24–6 h) (µmol/L)	0.03 [− 0.08 to 0.10]	− 0.12 [− 0.20 to 0.02]	0.079
ΔSDMA (24–6 h) (µmol/L)	0.17 [− 0.02 to 0.42]	0.22 [0.02–0.42]	0.713

Data are presented as median values with interquartile range [percentiles 25–75]

ADMA asymmetric dimethylarginine, *CA* cardiac arrest, *SDMA* symmetric dimethylarginine

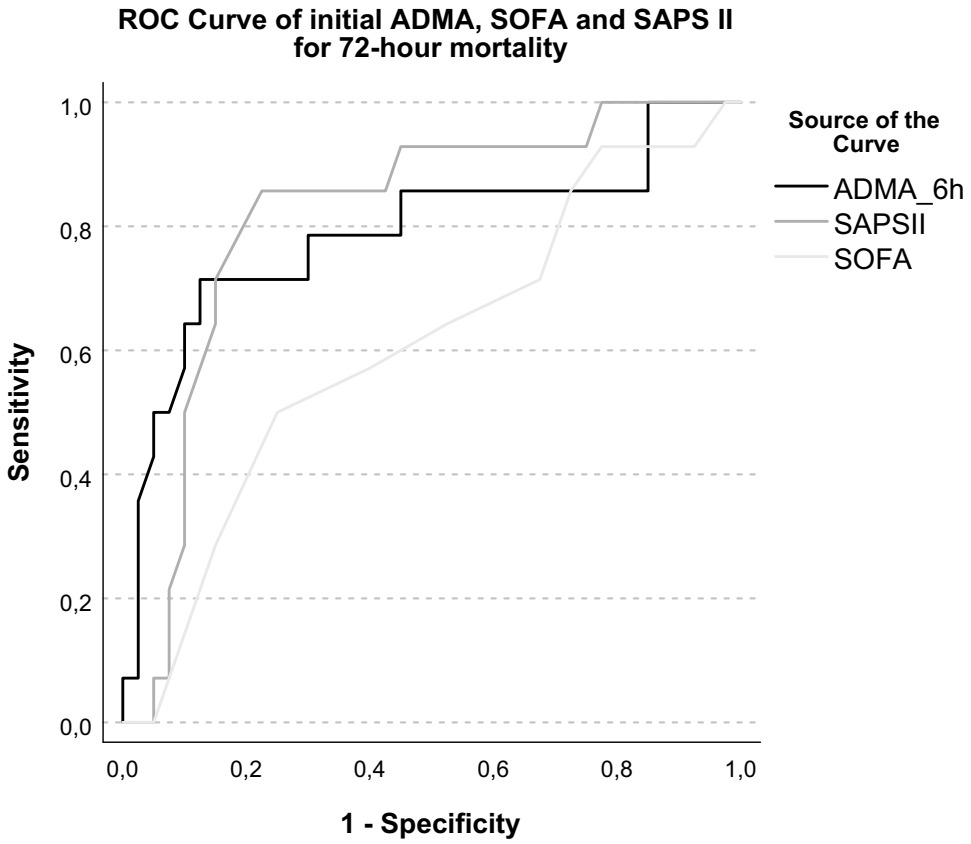

Fig. 2 ROC Curve of initial ADMA, SOFA, and SAPS II for 72-day mortality. SAPS II AUC: 0.817 [0.688–0.946], $p < 0.001$; ADMA AUC: 0.789 [0.628–0.950], $p = 0.001$; SOFA AUC: 0.608 [0.433–0.783], $p = 0.232$ (*ADMA* asymmetric dimethylarginine, *AUC* Area Under the Curve, *SAPS* Simplified Acute Physiology Score, *SOFA* Sequential Organ Failure Assessment, *ROC* Receiver Operating Characteristic)

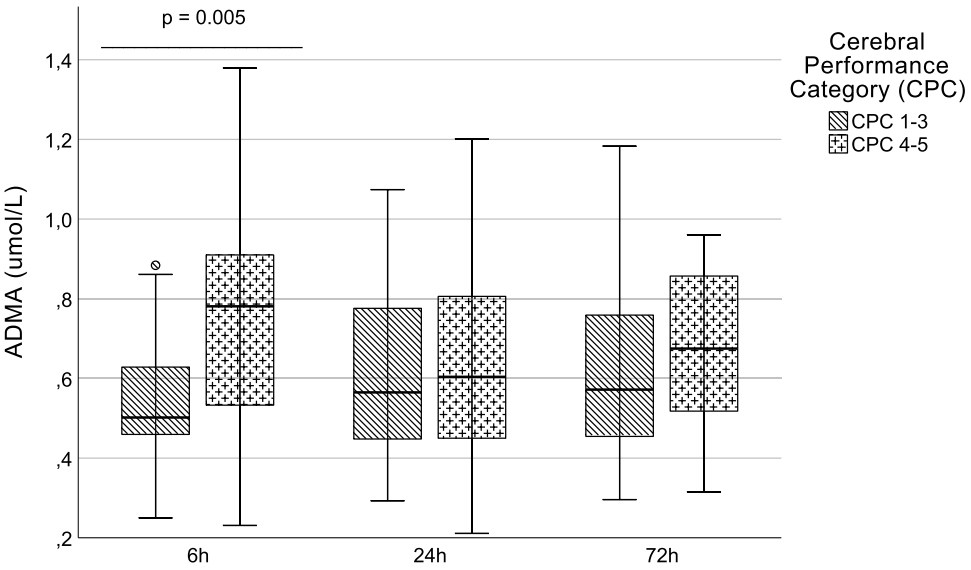

Fig. 3 ADMA and neurological outcome (max. CPC). (*ADMA* asymmetric dimethylarginine, *CPC* cerebral performance category)

(Fig. 3). ROC analysis of initial ADMA for prediction of a coma, vegetative state, or brain death (CPC 4–5) showed an AUC of 0.723 (95% CI 0.574–0.871; $p = 0.005$). Based on the ROC analysis, the best cutoff for poor neurological outcome (CPC 4–5) was determined as > 0.65 µmol/L (sensitivity: 66.7%; specificity: 81.5%).

Independent prediction of 72 h mortality

Based on ROC analysis, initial ADMA level was found to be a predictor of 72 h mortality (Fig. 2) with a best cutoff value of > 0.81 µmol/L (sensitivity: 71.0%; specificity: 87.5%). Univariable logistic regression analyses including each variable assessed within 6 h after cardiac arrest

identified initial ADMA, serum bicarbonate, and lactate levels as significant markers for 72 h mortality. Multivariable analysis revealed that initial ADMA (OR: 1.8 per 0.1 µmol/L increase in ADMA; 95% CI 1.252–2.611; $p = 0.002$) is an independent predictor for 72 h outcome after cardiac arrest (Table 3).

Discussion

To the best of our knowledge, the L-arginine pathway molecules and their change in the early post-resuscitation phase have not yet been evaluated in unselected cardiac arrest patients. Here, we investigated the prognostic value of L-arginine, ADMA, SDMA plasma levels, and kinetics in combination with other conventionally used laboratory parameters among a general, unselected population of cardiac arrest survivors including IHCA and OHCA patients. The major result of our study was the observation that initial ADMA level measured within 6 h after cardiac arrest was an independent predictor of short-term mortality and poor neurological outcome. While the 6 h ADMA levels had unequivocal prognostic value for 72 h mortality, the levels measured at either 24 and 72 h or their change did not associate with any of the investigated endpoints. Neither ICU nor 30-day mortality was predicted by any of the L-arginine pathway molecules. It was described previously that cardiovascular failure and hemodynamical instability are responsible for early death within 3 days after cardiac arrest, while later death is mainly related to neuronal injury due to severe hypoxic-ischemic encephalopathy and the subsequent withdrawal of life-sustaining therapy [19]. The different pathophysiological backgrounds of early and later post-resuscitation death may explain that ADMA, as a prognostic marker associated with the severity and mortality of many cardiovascular diseases, may also be promising for predicting mortality in the early phase of post-resuscitation care [20]. Accordingly, we could not prove the prognostic value of SDMA and L-arginine for mortality in the post-resuscitation phase.

A most recent review about the metabolism of ADMA in hypoxia summarizes the results of both animal and human studies concerning the metabolites of L-arginine pathway in various hypoxic conditions [21]. They mention more studies where ADMA levels were elevated in hypoxia, while SDMA levels did not show significant elevation. One of them found continuous increase of ADMA but not of SDMA in chronic-intermittent hypobaric hypoxia [22]. Others observed higher ADMA serum concentrations in patients with obstructive sleep apnea syndrome [23]. Furthermore, the L-arginine pathway molecules have been suggested as prognostic markers for acute exacerbation of chronic obstructive pulmonary disease [24].

Previous publications investigated the L-arginine pathway molecules in ischemic stroke [13, 25]. Plasma ADMA levels were higher in acute ischemic stroke patients compared to the control group and stroke outcome was worse in patients with increased ADMA levels than those with stable ADMA levels. They explained the observation with the mechanism that increased ADMA levels inhibit the production of endothelial nitric oxide (which plays a crucial role as an endogenous regulator of vasodilation in cerebral arterioles) consequently reducing the cerebral perfusion [25]. Other investigations also confirmed that ADMA increases the vascular tone in cerebral blood vessels and leads to cerebral hypoperfusion [26]. Molnar et al. observed that metabolites

Table 3. Univariable (a) and multivariable (b) regression analysis for 72 h mortality

a. Univariable logistic regression analysis for 72 h mortality			
Variable		Odds ratio—Exp(B) (lower CI–upper CI)	*p*
ADMA 6 h (per 0.1 µmol/L increase)		1.81 (1.25–2.61)	0.002
HCO_3^- 6 h		0.89 (0.79–0.99)	0.034
Lactate 6 h		1.26 (1.06–1.49)	0.008
b. Binary logistic regression analysis for 72 h mortality			
Model 1—Binary Logistic Regression—Enter	*B*	Odds ratio—Exp(B) (lower CI–upper CI)	*p*
ADMA 6 h (per 0.1 µmol/L increase)	0.573	1.77 (1.23–2.56)	0.002
HCO_3^- 6 h	− 0.132	0.88 (0.77–1.00)	0.054
Model 2—Binary Logistic Regression—Enter	B	Odds ratio—Exp(B) (lower CI–upper CI)	p
ADMA 6 h (per 0.1 µmol/L increase)	0.488	1.63 (1.14–2.33)	0.008
Lactate 6 h	0.189	1.21 (0.99–1.48)	0.065

ADMA asymmetric dimethylarginine, *CI* confidence interval

of the L-arginine pathway were elevated in the very acute phase of ischemic stroke indicating a more pronounced endothelial dysfunction compared with asymptomatic significant carotid stenosis or healthy subjects. They suggested that the elevated initial ADMA levels could be linked to the pathogenesis of endothelial cell dysfunction or could be the consequence of oxidative stress [13].

We observed a decrease from higher initial levels of ADMA levels up to 24 h among patients who died within 30 days after cardiac arrest, while ADMA levels slightly elevated in survivors. The discrepancy between the initial ADMA levels of non-survivors and survivors disappeared by 24 post-cardiac arrest hours. ADMA per se might contribute to brain injury by either reducing cerebral blood flow and facilitating excitotoxic neuronal death or contributing to the activation of the thrombo-inflammatory cascade [12]. Excessively high initial ADMA may adversely affect cerebral perfusion after cardiac arrest, leading in the short term to exacerbation of hypoxic-ischemic injury following resuscitation and early death in the post-resuscitation phase. On the other hand, elevated initial ADMA levels might indicate a more severe hypoxic insult or pre-existing endothelial dysfunction as discussed in the publication of Molnar et al. mentioned above. In the literature, only a few studies are investigating these marker levels in acute hypoxic conditions in humans. In healthy male volunteers, the mean plasma nitric oxide concentration was elevated after acute hypoxic exposure, which was associated with a reduction in plasma ADMA level leading to elevated plasma nitric oxide concentrations [27]. This finding suggests that the decrease in ADMA concentration observed on the first day after cardiac arrest in our more severe group of patients who died within 30 days may be an adaptive mechanism presumably counteracting cerebral hypoperfusion. Although it is important to note that the concentration of nitric oxide was not determined in our present study.

We observed a continuous increase of L-arginine/ADMA ratio up to 72 post-cardiac arrest hours in the total study population and found a significantly decreased L-arginine/ADMA ratio at 6 post-cardiac arrest hours in patients who died in the ICU after IHCA. These findings are consistent with the observation of Molnar et al., who suggested that a temporary increase of L-arginine along with a decrease of ADMA could be a protective mechanism after ischemic stroke [13]. Nitric oxide, a pleiotropic molecule, has several intracellular effects leading to vasorelaxation, endothelial regeneration, inhibition of leukocyte chemotaxis, and platelet adhesion [28]. In turn, lack of L-arginine, the source of nitric oxide, could lead to oxidative stress induced by hypoxic insults such as cardiac arrest, thus it may explain the reduced initial L-arginine/ADMA ratio in IHCA non-survivors. A most recent prospective observational study found that a higher arginine and lower arginine/ADMA ratio measured within 24 h after OHCA were independently associated with 90-day mortality [29]. Similarly to their findings, we could detect significantly elevated L-arginine/ADMA ratio in survivors in the IHCA group. They did not find significant difference between survivors and non-survivors regarding ADMA levels. Despite their findings, L-arginine was not a prognosticator of death in our population. Moreover, this marker did not show difference between survivors and non-survivors for none of the investigated endpoints. However, their population was exclusively made up of patients who suffered OHCA, while we enrolled mostly IHCA patients. Importantly, the pathophysiology and the most common conditions leading to death during post-resuscitation care could be different. Two-thirds of OHCA patients die due to brain injury in ICU, while MOF is the main cause of mortality after IHCA [6].

In this study, we recorded the best CPC reached in the ICU. It is generally observed, that despite patients reach acceptable neurological function during their ICU stay, they may later die as the consequence of MOF, especially after IHCA [6]. Considering this, we felt it confusing to categorize patients emerging from a comatose state into the unfavorable CPC group based on late-developing MOF. Therefore, the pure neurological outcome (regaining the consciousness) and the overall outcome (ICU or total mortality) should be separately analyzed. Therefore, the best achieved CPC score was recorded during the ICU stay similar to the study of Fabio Silvio Taccone and colleagues [16]. Importantly, eight patients (14.8% of the total population) in the CPC 1–3 group died due to non-neurological reasons in the ICU despite acceptable neurological status (death after awakening). All these eight patients had sepsis and failure of two or more organ systems.

The reliability of SOFA and SAPS II scores for prognostication in cardiac arrest patients remains unclear [30]. In our population, these scores had moderate prognostic value and we confirmed their association with L-arginine pathway molecules. Previous research findings revealed correlations among ADMA levels, L-arginine/ADMA ratio and microvascular reactivity, the extent of organ failure, and mortality in patients with sepsis [31]. In critically ill patients, ADMA correlated with SOFA score [10]. These observations are consistent with our results. Based on our findings, the prognostic accuracy of SAPS II (a time-consuming assessment method) and initial ADMA for 72 h mortality after ROSC were comparable. We conclude that early determination of initial ADMA after ROSC may be as effective and accurate as SAPS II in prediction of the early post-CPR mortality.

The strength of our study is its prospective nature using serial sampling in the first 3 days after cardiac arrest to evaluate the kinetics and changes of the L-arginine pathway molecules. Our study population was made up of unselected resuscitated patients including in- and out-of-hospital

cardiac arrest survivors of three different intensive care units allowing us to explore reliable predictive markers regardless of the circumstances and etiology of cardiac arrest and covering the widest range of resuscitated patients with different comorbidities and etiology. While most studies evaluated prognostication of OHCA patients, here, we were able to find potential prognostic markers in IHCA patients and markers which might be also used in both groups. The best neurological status reached in the intensive care unit was recorded to avoid false pessimistic neurological estimation of patients (especially after in-hospital cardiac arrest) reaching acceptable neurological status but dying as the consequence of hemodynamic dysfunction or MOF.

The limitation of our study is the low total number of enrolled patients. Concerning the high mortality rate, we could not collect enough data for long-term analysis. Further research is warranted to explore the prognostic value of multiple markers (including the L-arginine pathway molecules) for long-term outcome after cardiac arrest. Probably, the no-flow time (i.e., the interval between collapse and start of CPR) would be more valuable information compared to the length of the CPR until ROSC. However, the exact time of the collapse was not known in the majority of IHCA and some OHCA patients, thus it prevented us from reporting the accurate no-flow time in this cohort. To satisfy the proportional odds assumption, we reclassified the CPC as follows: CPC 4–5 (vegetative state or death) as poor neurological outcome, and CPC 1–3 as good/moderate neurological outcome, because of the low number of individuals with really good outcome (CPC 1–2) until 30-day follow-up. The reason of the extension of the acceptable neurological outcome categories with CPC 3 was the potential for a later neurological improvement of some patients during rehabilitation. The study was conducted in a single-center, so local treatment strategies and guidelines could limit the generalisability of our findings.

Conclusions

Here, we investigated for the first time the prognostic value and the kinetics of the L-arginine-pathway molecules during the early phase of successful cardiopulmonary resuscitation. Our results suggest that initial circulating ADMA level may indicate more severe hypoxic insult and can predict 72 h mortality among cardiac arrest victims.

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Author contributions PK, ZM, PC, KT, RH, and BC designed the study protocol. ZM, JR, PK, and BC enrolled the patients, collected the data and samples. LN contributed important reagents and performed the measurement. BC, TM, and BS analyzed the data. BC and TM interpreted the results, wrote the paper, and all the authors reviewed the paper prior to submission.

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Availability of data and material All data relevant to the study are included in the article or uploaded as supplementary material. No additional data are available due to data protection requirements.

Declarations

Conflict of interest The authors have no financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed. The authors declare no competing interests.

Human and animal rights statement and ethics approval The study was approved by the Local Ethics Committee of the University of Pécs (file number: 6941-PTE 2018.) and has followed the principles outlined in the Declaration of Helsinki for all human investigations.

Informed consent Informed consent for being included in the study was obtained from legal representatives or, in case, the patients had regained consciousness, from the patients themselves.

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A citokeratin-18 sejthalálmarker vizsgálata sikeres cardiopulmonalis resuscitatión átesett betegpopulációban

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Bevezetés: Citokeratin-18 (CK-18) az újraélesztés kapcsán kialakuló ischaemiás-reperfúziós károsodás kiváltotta teljes sejthalál során kerül a véráramba. Kaszpázok által hasított formája specifikus az apoptózis folyamatára. A markerek számos kórképben prognosztikus értékűnek bizonyultak. Tanulmányunkban elsőként vizsgáltuk prognosztikus értéküket reanimált betegpopulációban.
Módszer: 40, sikeresen újraélesztett betegnél határoztuk meg a sejthalálmarkerek szintjét 6 órán belül, 24 és 72 óra múlva. Ezeket összevetettük a 30 napos túléléssel, a neurológiai kimenetellel, a szervfunkciós károsodást jellemző laboratóriumi, fizikális és terápiás jellemzőkkel, valamint a reanimáció körülményeivel.
Eredmények: A reanimált betegek CK-18-plazmakoncentrációja a szakirodalomban leírt egészséges, posztoperatív és szeptikus populáció értékeinek a többszöröse volt (3842 vs. 242; 559; 1644 ng/l); a hasított és intakt CK-18 aránya alacsonyabb volt (0,14 vs. 0,58; 0,22; 0,24), ami jelentős sejtkárosodásra és a nekrózis dominanciájára utal. A markerek szintje azonban nem mutatott összefüggést a túléléssel, a neurológiai statusszal és a reanimáció körülményeivel sem. Veseelégtelenség esetén a CK-18 szintjének csökkenése elmaradt. Szignifikáns negatív korrelációt figyeltünk meg a 6 órás hemoglobin- és CK-18-szint között ($r = -0{,}400$, $p<0{,}01$), a 30 napos túlélésnek mégis az alacsonyabb hemoglobinértékek kedveztek.
Következtetés: Várakozásunkkal ellentétben a vizsgált markerek nem bírtak prognosztikus értékkel újraélesztett betegpopulációban. A kimenetelt valószínűleg nem a teljes sejtkárosodás, hanem egy kisebb, a fenti markerekkel szenzitíven nem vizsgálható kritikus szerepű sejtpopuláció károsodása, valamint a beteg tartalékkapacitásai befolyásolják.
Orv Hetil. 2020; 161(1): 26–32.

Kulcsszavak: újraélesztés, citokeratin-18, prognózis

The prognostic value of cytokeratin-18 cell death marker in cardiac arrest survivors

Introduction: Cytokeratin-18 (CK-18) is releasing into the blood during systemic cell death due to ischemia-reperfusion injury after cardiac arrest. Its caspase-cleaved form is specific to apoptosis. Previous investigations proved their prognostic value in different conditions. We firstly investigated the prognostic value of these markers after cardiac arrest.
Method: Plasma samples of 40 resuscitated patients were collected 6, 24, and 72 hours after successful resuscitation to determine the marker concentrations. We investigated the association of the markers with the 30-day mortality, neurological outcome, circumstances of the cardiac arrest, laboratory and physical parameters.
Results: Resuscitated patients had highly elevated CK-18 levels (3842 vs. 242; 559; 1644 ng/L) and decreased caspase-cleaved CK-18/CK-18 ratio (0.14 vs. 0.58; 0.22; 0.24) compared to healthy subjects, septic and postoperative

patients suggesting severe grade of cell death, mainly necrosis. Neither the marker concentrations nor their kinetics showed difference between survivors and non-survivors. They did not show association with the length of the resuscitation, the initial rhythm or the neurological outcome either. CK-18 decreased in patients with good renal function in contrast to patients with renal failure. Significant negative correlation was observed between the 6-hour cytokeratin-18 and hemoglobin concentrations ($r = -0.400$, $p<0.01$), while the 30-day survival was associated with lower hemoglobin levels.

Conclusion: Surprisingly the biomarkers did not show prognostic value among resuscitated population. The outcome is probably not determined by the complete cell damage, but the loss of a small group of cells with critical role and the reserve capacity of the patient.

Keywords: resuscitation, cytokeratin-18, prognosis

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Rövidítések

BE = (base excess) bázistöbblet; ccCK-18 = (caspase-cleaved CK-18) kaszpázok által hasított CK-18; CK-18 = (cytokeratin-18) citokeratin-18; CKD = (chronic kidney disease) krónikus vesebetegség; COPD = (chronic obstructive pulmonary disease) krónikus obstruktív tüdőbetegség; CPC = Cerebral Performance Category; CPR = cardiopulmonalis resuscitatio; CRP = C-reaktív protein; EDTA = etilén-diamin-tetraecetsav; eGFR = (estimated glomerular filtration rate) becsült glomerulusfiltrációs ráta; ELISA = (enzyme-linked immunosorbent assay) enzimhez kötött ellenanyag-vizsgálat; GOT = glutamát-oxálacetát-aminotranszferáz; GPT = glutamát-piruvát-aminotranszferáz; IHCA = (in hospital cardiac arrest) kórházon belüli szívleállás; INR = (international normalized ratio) nemzetközileg normalizált ráta; ISZB = ischaemiás szívbetegség; IQR = (interquartile range) interkvartilis tartomány; LDH = laktátdehidrogenáz; NSE = neuronspecifikus enoláz; OHCA = (out of hospital cardiac arrest) kórházon kívüli szívleállás; $PaCO_2$ = az artériás szén-dioxid parciális nyomása; PaO_2 = az artériás oxigén parciális nyomása; PCT = prokalcitonin; PEA = (pulseless electrical activity) pulzus nélküli elektromos aktivitás; S100B = (S100 calcium-binding protein B) S100 kalciumkötő fehérje B típusa; TIA = (transient ischemic attack) átmeneti ischaemiás roham

A klinikai halál állapotában a szöveti perfúzió és oxigenizáció a sejtélethez szükséges kritikus szint alá csökken. A kialakult hypoxiás periódus és az energiahiány beindítja a sejthalál folyamatát, mely a sejtet ért károsító hatástól függően két úton mehet végbe. Nekrózis esetében a stresszhatás fatális mértékű, a sejt elveszti integritását, és a környezetbe kerülő sejtalkotók gyulladásos reakciót indukálnak, mely a környezet további károsodását okozza. Az apoptózis sejtszinten szabályozott, energiaigényes folyamat, melyet a sejtet korlátozott mértékben érő károsodás indít be. A sejt intakt membránnal határolt részekre – apoptotikus testekre – esik szét, amelyeket a környező szövet sejtjei kebeleznek be és bontanak le gyulladásos reakció nélkül. Fontos elemei a kaszpázcsalád enzimei, melyek a haláljel kaszkádszerű felerősítésében és a célfehérjék hasításában vesznek részt [1].

A cytoskeletalis, intermedier filamentumokhoz tartozó citokeratin-18 (CK-18) főként epithelialis és parenchymás sejtekben expresszálódik. Apoptózis során a CK-18-at a kaszpázok több helyen hasítják, és a fragmentumok a vérben kimutathatóvá válnak. Teljes hosszúságú CK-18 nekrózis során kerül a véráramba, míg hasított formája (caspase-cleaved cytokeratin-18 – ccCK-18) kizárólag apoptózis során szabadul fel [2].

A ccCK-18 szerepét számos kórállapotban vizsgálták, többek között szeptikus betegcsoportban, akut myocardialis infarctusban, májbetegségekben, krónikus vesebetegekben [3–6]. A szérum-ccCK-18 aneurysmaruptura miatti subarachnoidealis vérzés, valamint intracerebralis haemorrhagia során is emelkedett értékeket mutatott, a 6 hónapos mortalitás és a kedvezőtlen kimenetel független prediktorának bizonyult [7, 8]. Ischaemiás strokeban a 72 óra után mért ccCK-18-értékek szignifikánsan magasabbak voltak az elhunytak körében [9].

Az újraélesztést követően gyakori szövődmény a hypoxiás-ischaemiás agykárosodás, mely tartós vegetatív állapot kialakulásához és halálhoz vezethet. Erre utaló kifejezetten kedvezőtlen prognózis esetén az életfenntartó kezelés aktív megvonására, így a terápiás erőfeszítések csökkentésére kerülhet sor, tehát nem történik új terápiás lépés bevezetése, és a létfenntartó, szervtámogató kezelés további kiterjesztését mellőzzük. A döntés súlya miatt nagyon fontos a hamisan rossz prognózis elkerülése. A legújabb irányelvek alapján a prognózis a fizikális paramétereken, elektrofiziológiai és elektroencefalográfiás, valamint agyi képalkotó eljárásokon alapul. Emellett a neuronspecifikus enoláz (NSE) és az S100B biomarkerek szerepét is felvetik, az ezekkel kapcsolatos határértékek azonban nem egyértelműek. Szükség van olyan új markerek azonosítására, melyek a prognózis helyességét tovább erősítik [10, 11]. Sikeres reanimáción átesett betegpopulációval kapcsolatban nincs szakirodalmi adat a CK-18 és ccCK-18 prediktív értékét illetően, így a markerek használhatóságát elsőként vizsgáltuk.

Hipotézisünk szerint a klinikai halál állapotában, a spontán keringés helyreállításáig bekövetkező nagyobb

szöveti károsodás nagyobb funkcionális károsodáshoz, rosszabb túléléshez és jelentősebb maradványtünetekhez vezet. A szisztémás szöveti károsodást a sejthalál mértékére utaló CK-18- és ccCK-18-szintekkel jellemezhetjük. Feltételeztük, hogy a markerek koncentrációja és kinetikája prognosztikai értékkel bírhat a resuscitatiót követő mortalitás, illetve funkcionális károsodás előrejelzésében. Vizsgálni kívántuk, hogy a CK-18-, ccCK-18-szintek milyen összefüggésben állnak a rendelkezésre álló egyéb, gyakran használt klinikai és biokémiai markerekkel, szervfunkciós paraméterekkel.

Módszer

Vizsgálatunkba 40 beteget vontunk be, akiknél kórházon belül vagy azon kívül történt minimum 2 percig tartó sikeres újraélesztés, és postresuscitatiós kezelésüket a Pécsi Tudományegyetem Klinikai Központja I. Belgyógyászati Klinikájának Belgyógyászati Intenzív Osztályán, Sürgősségi Betegellátó Osztályán vagy Aneszteziológiai és Intenzív Terápiás Intézetében kezdték meg. Kizárásra kerültek azok a betegek, akiknél reanimáció előtt jelentős szövetroncsolódás (politraumatizált, égett, korai posztoperatív betegek) vagy a beteg várható hosszú távú funkcionális állapotát eleve jelentősen rontó súlyos előzetes neurológiai kórkép állt fenn, illetve akiknél a hemodinamikai állapotot nem sikerült stabilizálni, így egynapos túlélés sem volt várható, vagy a vizsgálatba való vélelmezett beleegyezését a beteg vagy családja megtagadta. A betegút jellegéből fakadóan nem voltak reprezentálva a vélhető akutcoronaria-szindróma miatt újraélesztést követően azonnal a perkután intervenció irányába továbbított betegek sem. A vizsgálatban részt vevők terápiás hypothermiában nem részesültek, azonban a hyperthermia prevenciójára a klinikai gyakorlatnak megfelelően minden esetben fokozottan ügyeltünk.

A reanimációt követően 6 órán belül, majd 24 ± 3 és 72 ± 3 óra múlva történt vérvétel a ccCK-18, CK-18 markerek szintjének meghatározására (EDTA-val antikoagulált, a feldolgozásig –80 °C-on tárolt plazmából; 'human caspase-cleaved cytokeratin-18' [ccCK-18] és 'cytokeratin-18' [CK-18] ELISA Kit, YL Biotech Co., Ltd., Shanghai, Kína). A CK-18 ELISA Kit esetében az intakt és a hasított formájú CK-18 koncentrációja, így a teljes sejthalál mértéke határozható meg, míg ccCK-18-mérésnél csak a kaszpázok által hasított fehérjét detektáljuk. A ccCK-18/CK-18 arányból következtethetünk a nekrózis mértékére.

Rögzítésre kerültek a rutin-betegellátás során vizsgált, a reanimációt követő 6., 24. és 72. órás mintavételekhez időben legközelebb eső aznapi laborparaméterek: elektrolitok – nátrium, kálium, karbamid, kreatinin; troponin-T; a GOT-, GPT-, LDH-, INR-, CRP-, PCT-, vérkép- és vérgázértékek (pH, PaO_2, $PaCO_2$, BE), laktát. Emellett rögzítettük a reanimáció körülményeit (a CPR időhossza, az alkalmazott gyógyszerek, feltételezett etiológia, kezdeti ritmus), a beteg teljes körű anamnézisét, ismert betegségeit, az ellátás során alkalmazott gyógyszereket és beavatkozásokat, az intenzív osztályos kezelés hosszát. Vizsgálati végpontnak a 30 napon belüli halálozást tekintettük. Betegeink funkcionális és neurológiai állapotának megítéléséhez az *1. táblázatban* részletezett Cerebral Performance Category (CPC)-besorolást használtuk [12]. Az ellátás során a betegnél észlelt legjobb CPC-értéket jegyeztük fel. A vizsgálat protokollját a Regionális Kutatásetikai Bizottság jóváhagyta – engedélyszám: 6941-PTE 2018.

1. táblázat A Cerebral Performance Category-skála a neurológiai status megítélésére *P. Safar* Resuscitation after brain ischemia közleménye alapján [12]

CPC 1.	Jó agyi funkció. A tudat megtartott, a beteg éber, képes dolgozni és normális életvitelt folytatni. Esetleg minimális pszichológiai vagy neurológiai deficit áll fenn.
CPC 2.	Mérsékelt cerebralis funkciózavar. A tudat megtartott. A mindennapi önálló életvitelhez szükséges kielégítő agyi funkciók. A beteg részidőben történő munkavégzésre alkalmas speciális körülmények között.
CPC 3.	Súlyos cerebralis funkciózavar. A beteg mások segítségét igényli a mindennapokban (intézményen belül, vagy otthon rendkívüli családi segítséggel). A kategória széles tartományt ölel fel az enyhe kognitív zavartól egészen a súlyos dementiáig, paralízisig.
CPC 4.	Eszméletlen betegek (kóma vagy vegetatív állapot), akik környezetüket nem észlelik, arra nem reagálnak. Nem figyelhető meg verbális vagy pszichológiai interakció semmilyen formában. Az agyhalál minden kritériumát nem meríti ki.
CPC 5.	Agyhalál, csupán a keringés megtartott. Apnoe, areflexia, EEG-jelek hiánya stb.

EEG = elektroencefalográfia

A statisztikai értékelésnél a folytonos változók esetén a medián értékeket, valamint az interkvartilis tartományt (IQR), a kategorikus változóknál a gyakoriságot és a százalékos előfordulást tüntettük fel. A csoportok összehasonlítását a Mann–Whitney-féle nemparaméteres próbával végeztük a nem normális eloszlást mutató adatoknál. A kategorikus változók kapcsolatának kiértékelése khinégyzet-próbával történt. A folytonos változók közötti kapcsolat megítélésére a nemparaméteres Spearman-féle korrelációs koefficienst (rho), valamint az ehhez tartozó p-értékeket összegeztük. Az eltéréseket és az összefüggéseket $p<0,05$ esetén tekintettük szignifikánsnak.

Eredmények

A CPR helyszíne alapján a 40 vizsgálati alany közül 29 esetben (72,5%) kórházon belül (IHCA), 11 betegnél (27,5%) pedig kórházon kívül (OHCA) történt a reanimáció. Az esemény 31 esetben (77,5%) ügyeleti időre vagy hétvégére esett. Az iniciális ritmus 13 esetben (32,5%) volt sokkolható kamrai tachycardia vagy kamrafibrilláció, 25 betegnél (62,5%) PEA vagy asystolia,

2. táblázat | A 30 napot túlélt és 30 napon belül elhunyt betegcsoport jellemzői (medián és [IQR])

	30 napot túlélők (n = 14) – 35%	30 napon belül elhunytak (n = 26) – 65%	Szignifikancia (p-érték)
Női nem – fő (%)	10 (71,4%)	13 (50,0%)	N.S.
Életkor (év)	61 [58–77]	66 [62–79]	N.S.
CPR (perc)	10,0 [5,0–30,0]	10,0 [5,0–20,0]	N.S.
max. CPC-érték	3 [3–3]	5 [4–5]	0,001

CPC = Cerebral Performance Category; CPR = cardiopulmonalis resuscitatio; IQR = interkvartilis tartomány; N.S. = nem szignifikáns

2 esetben pedig nem sikerült ezt kiderítenünk. Rövid reanimáció (≤10 perc) 18 esetben (53%), hosszú (>10 perc) reanimáció 16 esetben (47%) történt; 6 esetben nem tudtunk megbízható információt nyerni ennek hosszáról. A százalékos adatok az ismert esetek megoszlására utalnak. Az etiológiát tekintve 52,4%-ban cardialis ok (ISZB, szívelégtelenség) miatt következett be keringés- és légzésleállás. A további okok között ionzavarok (hyperkalaemia), aspiráció, hypothermia, tüdőembolia fordult elő. A betegek egy részénél nem sikerült egyértelműen azonosítani a kiváltó tényezőt. A komorbiditást illetően magasvérnyomás-betegség (70%), ISZB (42,5%), 2-es típusú diabetes mellitus (37,5%), szívelégtelenség (32,5%), permanens pitvarfibrilláció (22,5%), stroke vagy TIA (17,5%), COPD (17,5%), perifériás artériás érbetegség (12,5%), pulmonalis embolia (7,5%) fordult elő az anamnézisben.

A *2. táblázatban* összegeztük a túlélők és az elhunytak demográfiai adatait, a resuscitatio hosszát és a neurológiai kimenetelt. A nemet és az életkort illetően nem volt szignifikáns különbség a túlélők és az elhunytak között, az újraélesztés időtartama sem különbözött a két csoportban. A CPC-skála alapján felmért neurológiai status, így a 30 nap során elért legjobb CPC-kategória a túlélőknél szignifikánsan kedvezőbb volt. Összességében az egyes kategóriákban a megoszlás a következőképpen alakult: CPC 1.: 1 eset, CPC 2.: 2 eset, CPC 3.: 16 eset, CPC 4.: 6 eset, CPC 5.: 15 eset.

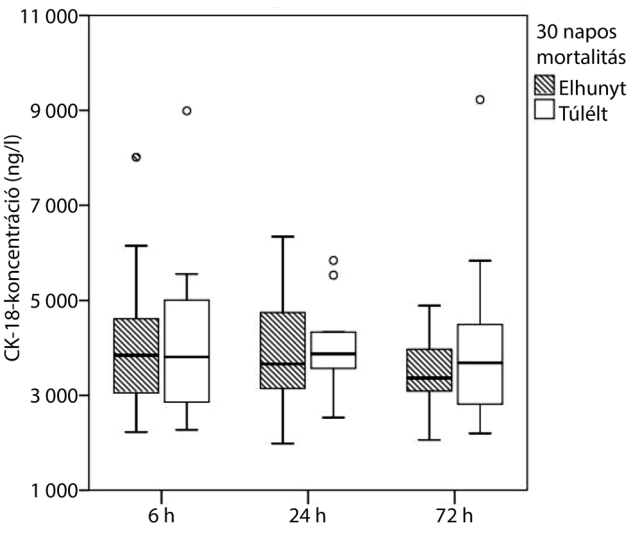
A laboratóriumi paraméterek közül az elhunytak 6. órás troponin-T-, transzaminász-, INR-, fehérvérsejt-, 24. és 72. órás hemoglobin-, valamint 6. és 24. órás laktátértékei bizonyultak szignifikánsan magasabbnak *(3. táblázat)*. Az egyes értékek más időpontokban szignifikáns különbséget nem mutattak. Az *1. és 2. ábrán* a 6 órán belül, a 24., illetve 72. órás időpontban mért CK-18- és ccCK-18-értékeket ábrázoltuk a túlélés szerinti bontásban. Az egyes csoportok között nem találtunk statisztikailag szignifikáns különbséget, és érdemi változást sem tapasztaltunk a 3 mérési időpont között. Vizsgálatunk során a maximális CPC-pontszám, így a neurológiai kimenetel sem mutatott összefüggést a biomarkerek szintjével. A reanimáció hossza sem befolyásolta a sejthalálmarkerek szintjét.

3. táblázat | A 30 napot túlélt és 30 napon belül elhunyt betegcsoport laboratóriumi jellemzői (medián és [IQR])

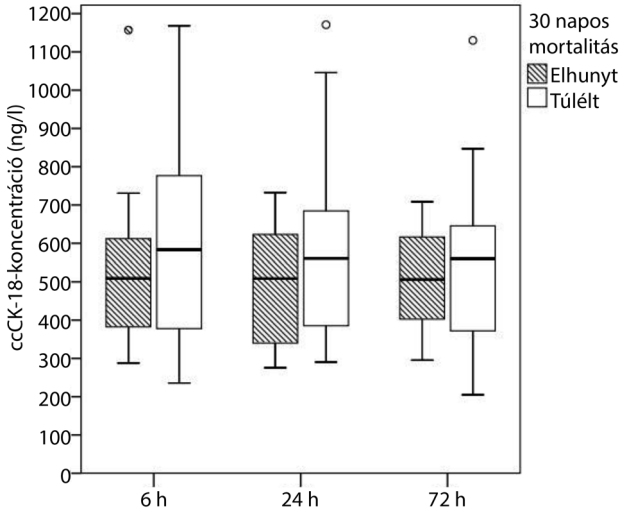
Laboratóriumi paraméterek, medián [IQR]	30 napot túlélők (n = 14) – 35%	30 napon belül elhunytak (n = 26) – 65%	Szignifikancia (p-érték)
Troponin-T (ng/l) 6 h	*45,0* [26,4–140,2]	*158,9* [50,1–441,0]	0,038
GOT (U/l) 6 h	*26,0* [23,0–93,0]	*109,0* [49,0–726,0]	0,002
GPT (U/l) 6 h	*18,0* [13,0–114,0]	*93,5* [26,5–764,5]	0,026
INR 6 h	*1,2* [1,0–1,3]	*1,4* [1,1–1,9]	0,047
Fehérvérsejtszám (Giga/l) 6 h	*11,2* [8,7–15,4]	*16,1* [10,9–23,8]	0,046
Hemoglobin (g/l) 24 h	*100,0* [84,8–120,5]	*119,0* [109,0–134,0]	0,012
Hemoglobin (g/l) 72 h	*95,5* [80,3–116,0]	*113,0* [102,0–125,0]	0,023
Hematokrit (%) 24 h	*30,0* [26,1–38,7]	*36,6* [33,4–41,9]	0,007
Hematokrit (%) 72h	*29,7* [24,7–35,9]	*37,2* [32,0–40,5]	0,025
Laktát (mmol/l) 6 h	*3,1* [2,0–5,2]	*7,7* [5,0–10,7]	0,003
Laktát (mmol/l) 24 h	*1,1* [0,8–1,5]	*1,8* [1,1–2,4]	0,027

GOT = glutamát-oxálacetát-aminotranszferáz; GPT = glutamát-piruvát-aminotranszferáz; INR = nemzetközileg normalizált ráta; IQR = interkvartilis tartomány

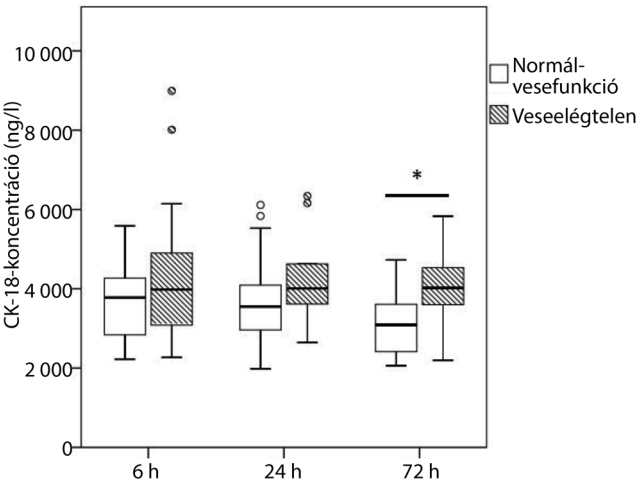
Veseelégtelenség (beszűkült vizeletprodukció [<500 ml/nap] vagy 30 ml/perc alatti eGFR) esetén a CK-18 koncentrációja változatlanul magas értéket mutatott a 72 óra során. Ép vesefunkció mellett a marker szintje csökkent, és a 72. órára szignifikánssá vált a különbség a két csoport között (veseelégtelen: 4021,0 [3535,3–



1. ábra | CK-18-koncentrációk a 3 mintavételi időpontban

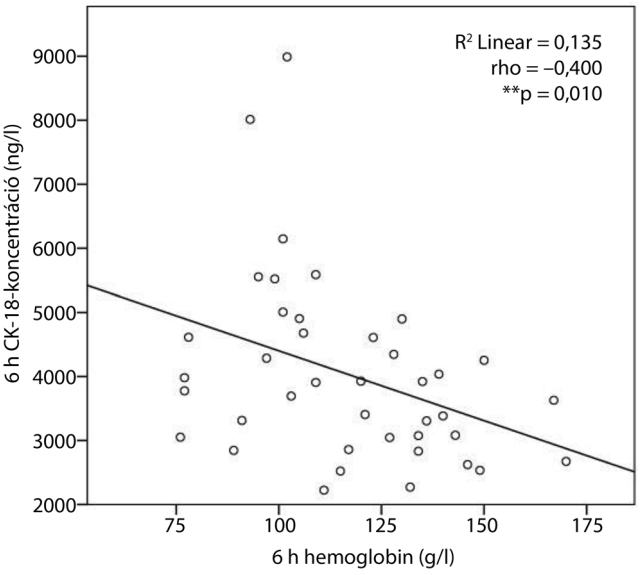


2. ábra | ccCK-18-koncentrációk a 3 mintavételi időpontban



3. ábra | CK-18-koncentrációk és vesefunkció

*p = 0,022



4. ábra | CK-18-koncentrációk és hemoglobin

4710,8] ng/l vs. 3088,0 [2401,5–3786,5] ng/l; p = 0,022) *(3. ábra)*.

A CK-18- és a hemoglobinszint között szignifikáns negatív korrelációt figyeltünk meg (rho = –0,400; p = 0,010) *(4. ábra)*.

Megbeszélés

A rövidebb reanimáció az általános vélekedés szerint jobb prognózist jelent. A várttal ellentétben vizsgálatunkban a reanimáció hossza sem a kimenetelt, sem a sejthalálmarkerek szintjét nem befolyásolta, a túlélők és az elhunytak esetében azonos átlagos reanimációs időt találtunk. Vizsgálatunkban azonban nem reprezentáltak az igen rövid (<2 perc) újraélesztések, amelyeknél a beteg gyorsan és gyakran azonnali kielégítő tudattal tér vissza (például gyorsan terminálódó ritmuszavar esetén), és definitív intenzív terápiás postresuscitatiós kezelést nem igényel. A „rövid" (<10 perc) csoporton belül csak 5 alany részesült 5 percnél rövidebb reanimációban, 13 esetben ez 5–10 perc volt. A felosztás azt sem veszi figyelembe, hogy mekkora volt a légzés-keringés megállás beállta és az újraélesztés megkezdése közt eltelt hypoxiás idő, vagy a keringésleálláshoz esetlegesen vezető megelőző hypoxia mértéke.

Rohlin és mtsai a kórházon belül történt szívmegállások esetében kapcsolatot találtak a rövidebb reanimáció és a jobb 30 napos túlélés között, viszont 30 percet meghaladó CPR esetében is a vártnál magasabb túlélési arányt figyeltek meg, így önmagában a CPR időtartama csekély prognosztikus értékkel bír [13]. A várható kimenetel nem feltétlenül a spontán keringés visszatéréséig eltelt időtől, hanem a páciensek általános állapotától, a klinikai halálhoz vezető egyéb tényezőktől függhet.

A reanimációt követően a neurológiai statust jellemző CPC-értéket 30 napig követtük, és a legjobb elért értéket vettük alapul [14]. Nem meglepő módon a túlélők szignifikánsan jobb pontszámot értek el, de még ez is alatta marad a szakirodalomban fellelhető adatoknak. Azon vizsgálatokban viszont az elbocsátáskori CPC-értéket elemezték [15]. A jobb értékek hátterében a 30 napot túlélő betegek további kezelése során elért javulás állhat, illetve a később visszaeső és elhunyt betegek gyengébb értékeinek kiszelektálódása.

Számos élettani és biokémiai paraméter mutatott a feltételezettnek megfelelő szignifikáns vagy tendenciózus romlást az elhunytak körében. A troponin-T a myocardialis sérülést, a GOT-, GPT-, INR-értékek a májfunkció károsodását, a laktát és bizonyos fokig a GOT, GPT a globális szöveti perfúzió romlását, a diffúz szövetsérülést jelezheti. A 30 napos túlélés szempontjából az alacsonyabb hemoglobinértékek tűntek kedvezőbbnek. Feltételezhetően az enyhén csökkent hemoglobin (és hematokrit) következtében csökkenő viszkozitás kedvezőbb mikrocirkulációt biztosít szöveti szinten.

Betegeink összesített CK-18- és ccCK-18-szintjei jóval magasabbnak bizonyultak a szakirodalomban leírt

4. táblázat | A 6. órás CK-18-, ccCK-18-értékek és arányuk összehasonlítva más vizsgálatban tapasztalt egészséges kontroll-normáltartománnyal, a posztoperatív és a szeptikus betegcsoport értékeivel [16] (medián és [IQR])

	A 6 órán belül mért markerszintek vizsgálatunkban	Szakirodalmi adatok [16]		
		Egészséges kontroll	Posztoperatív	Szeptikus
CK-18 (ng/l)	*3842,5* [3047,5–4662,8]	*241,9* [216,9–285,3]	*558,7* [465,6–793,0]	*1643,8* [1096,5–2633,5]
ccCK-18 (ng/l)	*532,9* [378,9–646,9]	*143,7* [134,4–168,1]	*116,0* [106,6–165,1]	*392,6* [258,4–654,5]
ccCK-18/CK-18 arány	*0,14* [0,11–0,18]	*0,58* [0,55–0,67]	*0,22* [0,18–0,25]	*0,24* [0,14–0,35]

ccCK-18 = kaszpázok által hasított CK-18; CK-18 = citokeratin-18

egészséges, posztoperatív, illetve szeptikus alanyokéihoz képest, ami nagy mértékű szöveti károsodásra utal. A ccCK-18/CK-18 arány jelentősen alacsonyabbnak mutatkozott, amiből a nekrotikus sejthalál dominanciájára következtethetünk *(4. táblázat)* [16]. A markerek nem mutattak összefüggést sem a halálozással, sem a CPC-skála szerinti neurológiai besorolással, így prognosztikus értékük az újraélesztett betegek esetében nem igazolódott. Valószínű, hogy a beteg túlélését nem a szisztémás sejthalál, hanem egy jóval kisebb, kritikus funkciójú sejtcsoport károsodása határozza meg, mely a vizsgált markereinkkel nem különíthető el a kevésbé fontos sejtek pusztulásától. Az agy a többi szövethez képest alacsony relatív tömege révén valószínűleg alig járul hozzá a markerek emelkedéséhez, az általa okozott változás kimutathatatlan a teljes változáshoz képest. Emellett megjegyzendő, hogy a neuronok nem expresszálnak citokeratinokat; az intracerebralis vérzés, illetve stroke kapcsán észlelhető ccCK-18-szint-emelkedést [7–9] valószínűleg az alapbetegség egyéb szervekre áttevődő hatása okozza.

A sejthalálmarkerek a megfigyelési időszak során magas értéken stagnáltak *(1. és 2. ábra)*. Feltételezzük, hogy a sejtek elhalása, a CK-18 és ccCK-18 felszabadulása időben elnyújtott folyamat, az eliminációval pedig egyensúlyt tartott a nekrózis okozta gyulladás, az intenzív osztályos kezelés és az egyéb szövődmények által kiváltott szekunder szövetsérülés. Az apoptózist jelző ccCK-18-szint a túlélőknél tendenciózusan magasabbnak tűnt, így valószínű, hogy az apoptotikus sejthalál a túlélés szempontjából kedvezőbb a nekrózishoz képest. Elképzelhető, hogy a túlélők körében a sejteket érő károsodás mértéke még nem érte el a fatális szintet, és lehetővé tette az energiaigényes, élettanilag kedvezőbb sejthalálút, az apoptózis beindítását, kímélve ezzel a környező sejteket. Az elhunytak körében a sejteket érő stressz valószínűleg meghaladta ezt a kritikus szintet, és a sejtek nekrózissal elhaltak; náluk kevésbé volt lehetőség az apoptotikus folyamatok beindítására.

Roth és mtsai a szérum és a vizelet CK-18-szint-emelkedését figyelték meg CKD 3–5. stádiumú veseelégtelenségben [6], ami magyarázza a *3. ábrán* látható, károsodott veseműködés mellett tapasztalt, tartósan emelkedett CK-18-szinteket. Elképzelhető az is, hogy a károsodott veseműködés miatt a CK-18 eliminációja is romlik, ami hozzájárulna ahhoz, hogy szintje a veseelégtelen csoportban magas maradjon.

A *4. ábrán* feltüntetett CK-18- és a hemoglobinszint között észlelt szignifikáns negatív korrelációból arra következtethetünk, hogy magasabb hemoglobinszint esetén kisebb mértékű sejthalál történik. A 30 napos túlélésnek viszont épp az alacsony hemoglobinszint kedvezett *(3. táblázat)*. Reanimáció során, a kritikusan meglassult véráramlás mellett a magasabb hemoglobinkoncentráció több oxigént tud biztosítani, így valószínűleg kisebb lesz a hypoxiás károsodás. Postresuscitatiós – már megfelelő hemodinamikájú – helyzetben ezzel szemben az alacsonyabb hematokritszint kedvezhet a mikrokeringésnek és a regenerációnak.

Következtetés

A sejthalálfolyamatokat tükröző CK-18 és ccCK-18 szintje jelentősen megemelkedik reanimációt követően, és a nekrózis mértéke az apoptózishoz viszonyítottan nagyobb, mint normálkörülmények között. Mindazonáltal a fenti paraméterek hasonlóan alakulnak a postresuscitatiós kezelést túlélő és a kezelés során elhunyt betegek között, és függetlennek látszanak a neurológiai károsodás mértékétől, valamint az egyéb körülírt szervfunkciós károsodásoktól is. A CK-18 és ccCK-18 sejthalálmarkerek így nem alkalmasak arra, hogy reanimáción átesett betegeknél segítsék a halálozás vagy a neurológiai károsodás prognosztikáját.

Anyagi támogatás: A cikk az Emberi Erőforrások Minisztériuma ÚNKP-18-3-I. kódszámú Új Nemzeti Kiválóság Programjának támogatásával készült.

Szerzői munkamegosztás: Cs. B.: Irodalomkutatás, a vizsgálati terv kidolgozása, engedélyek beszerzése, adat- és mintagyűjtés, adatbázis-kezelés, statisztikai kiértékelés, a kézirat elkészítése. N. Á.: Irodalomkutatás, adat- és mintagyűjtés, adatbázis-kezelés. M. Zs., R. J.: Közreműködés a protokoll kidolgozásában, értesítés beteg érkezésekor, szakmai áttekintés. Cs. P., M. T.: Közreműködés a vizsgálati terv kidolgozásában, az eredmények értékelése, szakmai áttekintés. D. L.: A minták feldolgozása. H. R.: Laboratóriumi háttér biztosítása a minták feldol-

gozásához, szakmai áttekintés. T. K.: Az eredmények értékelése, szakmai áttekintés. K. P.: A vizsgálat tervezése, az eredmények áttekintése, statisztikai és szakmai értékelés, a kézirat szövegezése. A cikk végleges változatát valamennyi szerző elolvasta és jóváhagyta.

Érdekeltségek: A szerzőknek nincsenek érdekeltségeik.

Köszönetnyilvánítás

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„Potest uti adversis numquam felicitas."
(A szerencsések nem tudják, hogy a bajjal miként bánjanak.)