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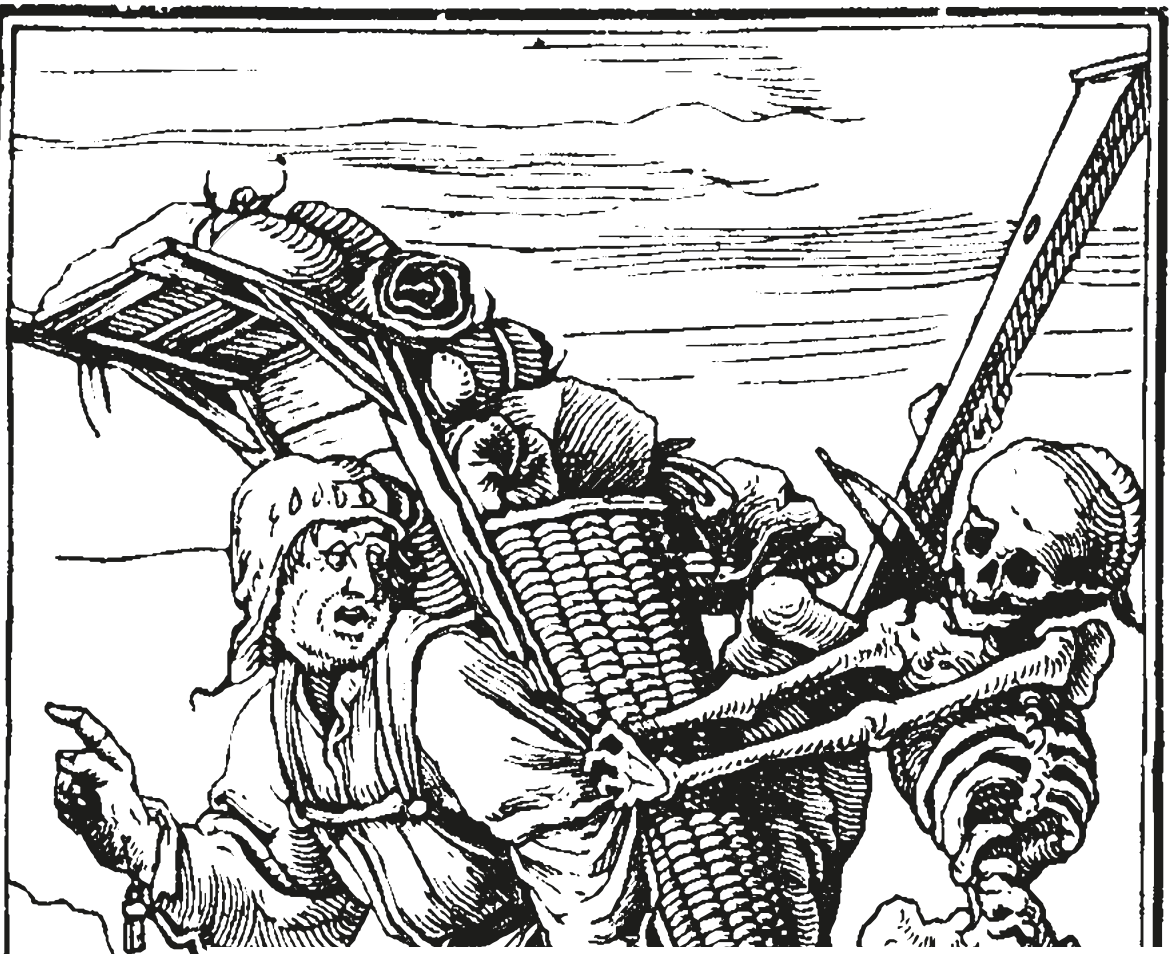

Carbon dioxide, oxygen, and serum biomarkers after out-of-hospital cardiac arrest

FLORIAN EBNER
DEPARTMENT OF CLINICAL SCIENCES | LUND UNIVERSITY

Carbon dioxide, oxygen, and serum biomarkers
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Florian Ebner

LUND
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DOCTORAL DISSERTATION

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Faculty opponent
Markus Skrifvars

Supervisor
Niklas Nielsen

Co-Supervisors
Tobias Cronberg, Susann Ullén & Johan Undén

Organization LUND UNIVERSITY Department of clinical sciences, Anaesthesiology and Intensive care Author(s) Florian Ebner	Document name Doctoral Dissertation Date of issue May 8th 2020 Sponsoring organization

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Carbon dioxide, oxygen, and serum biomarkers after out-of-hospital cardiac arrest

Abstract

Cardiac arrest is the aprupt loss of cardiac function and circulation, follwed by the loss of consciousness and breathing. Most patients succumb before admission to hospital and survivors frequently suffer from anoxic-ischemic brain injury. The number of patients who survive with good neurological outcome, is low.

In this thesis, we investigated the association of abnormal arterial partial pressures of carbon dioxide and oxygen in the phase following the return of spontaneous circulation (ROSC) with neurological outcome at hospital discharge or at follow-up 6 months after out-of hospital cardiac arrest (OHCA).

We also investigated the association of abnormal arterial partial pressures of carbon dioxide and oxygen in the phase following ROSC with a brain specific serum biomarker of neurological injury, as a sensitive surrogate marker for anoxic-ischemic brain injury.

In a final analysis, we investigated the biomarkers of neurological injury, i.e., glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal hydrolase-L1 (UCH-L1) measured at 24, 48 and 72 hours after out-of-hospital cardiac arrest and their predictive accuracy for neurological outcome at 6-month follow-up.

Exposure to abnormal arterial partial pressures of carbon dioxide and oxygen was common in resuscitated patients after OHCA, but we did not find an independent association with poor neurological outcome. Abnormal arterial partial pressures of carbon dioxide and oxygen were also not associated with peak levels of the serum biomarker tau at 48 and 72 hours, after OHCA.

Serum GFAP, UCH-L1 and their combination (GFAP+UCH-L1) predicted neurological outcome after OHCA with high accuracy over all measuring points. Overall predictive accuracy of GFAP+UCH-L1 was superior compared to neuron specific enolase (NSE), the serum biomarker presently used in clinical practice

Key words
Arterial pressure of carbon dioxide, arterial pressure of oxygen, out-of-hospital cardiac arrest, anoxic-ischemic brain injury, serum biomarkers of neurological injury.

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If at first you don't succeed try, try and try again!

Robert the Bruce, King of Scots

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I. Ebner F, Harmon MBA, Aneman A, Cronberg T, Friberg H, Hassager C, Juffermans N, Kjaergaard J, Kuiper M, Mattsson N, Pelosi P, Ullen S, Unden J, Wise MP, Nielsen N, Carbon dioxide dynamics in relation to neurological outcome in resuscitated out-of-hospital cardiac arrest patients: an exploratory Target Temperature Management Trial substudy, Crit. Care 22(1) (2018) 196.

II. Ebner F, Ullen S, Aneman A, Cronberg T, Mattsson N, Friberg H, Hassager C, Kjaergaard J, Kuiper M, Pelosi P, Unden J, Wise MP, Wetterslev J, Nielsen N, Associations between partial pressure of oxygen and neurological outcome in out-of-hospital cardiac arrest patients: an explorative analysis of a randomized trial, Crit. Care 23(1) (2019) 30.

III. Ebner F, Riker RR, Haxhija Z, Seder DB, May TL, Ullén S, Stammet P, Hirsch K, Forsberg S, Dupont A, Friberg H, McPherson JA, Søreide E, Dankiewicz J, Cronberg T, Nielsen N, The association of partial pressure of oxygen and carbon dioxide with neurological outcome after out-of-hospital cardiac arrest: an explorative International Cardiac Arrest Registry 2.0 study, Submitted for publication.

IV. Ebner F, Moseby-Knappe M, Mattsson N, Lilja G, Dragancea I, Undén J, Friberg H, Erlinge D, Kjaergaard J, Hassager C, Wise MP, Kuiper M, Stammet P, Wanscher M, Horn J, Ullén S, Cronberg T, Nielsen N, Serum GFAP and UCH-L1 for the prediction of neurological outcome in comatose cardiac arrest patients, Submitted for publication.

Abbreviations

ALS	Advanced life support
AUC	Area under the curve
BBB	Blood brain barrier
BLS	Basic life support
CBF	Cerebral blood flow
CDO_2	Cerebral oxygen delivery
CNS	Central nervous system
CPC	Cerebral performance category
CPR	Cardiopulmonary resuscitation
ECG	Electrocardiogram
EEG	Electroencephalogram
ERC	European resuscitation council
FiO_2	Fraction of inspired oxygen
GFAP	Glial fibrillary acidic protein
GCS	Glasgow coma scale
IHCA	In-hospital cardiac arrest
ILCOR	International liaison committee of resuscitation
INTCAR	International cardiac arrest registry
kPa	Kilopascal
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NFL	Neurofilament light
NSE	Neuron specific enolase
OHCA	Out-of-hospital cardiac arrest
$PaCO_2$	Arterial partial pressure of carbon dioxide
PaO_2	Arterial partial pressure of oxygen
PCAS	Post cardiac arrest syndrome
PEA	Pulseless electric activity
ROC	Receiver operating characteristics
ROSC	Return of spontaneous circulation
SSEP	Somatosensory evoked potentials
TTM	Targeted temperature management
UCH-L1	Ubiquitin C-terminal hydrolase-L1
VF	Ventricular fibrillation
VT	Ventricular tachycardia

1 Cardiac arrest - general aspects

1.1 Cardiac arrest

For most higher life forms, the cessation of blood flow to the brain, caused by either insufficient or suspended cardiac function or brain pathology is the end of life, it is death. Without intervention, cardiac arrest inevitably leads to death and in many cases, it is the expected, final stage of aging. Despite a relatively immediate loss of consciousness, the process leading to cardiac death is initially stepwise and has been divided into three distinct phases: electrical, circulatory and metabolic.[1] The possibility to reverse this condition diminishes rapidly over time and is most likely to be achieved in the electrical stage, during the first 5 minutes after cardiac arrest, by early defibrillation in combination with cardio pulmonary resuscitation (CPR). Continuous, manual CPR is paramount in the second phase, to diminish the decline in survival.[2] For every minute that CPR is delayed, the chance of survival falls by 7% to 10% and if more than 10 minutes have passed without CPR, survival with a good outcome is increasingly unlikely.[3,4]

1.2 Types of cardiac arrest

Cardiac arrest is commonly categorized by the location, into in-hospital cardiac arrest, defining a cardiac arrest that occurs within a hospital (during a hospital admission) (IHCA), and out-of-hospital cardiac arrest (OHCA), a term used for all cardiac arrests not occurring during a hospital admission. The two entities are not entirely comparable, due to considerable differences in age, etiology, bystander efforts and bystander proficiency, and first rhythm.[5-7] IHCA is more frequently associated with better 30-day survival, compared to OHCA.[8] Cardiac arrest can further be divided, according to etiology, into cardiac (for instance, myocardial ischemia or primary arrhythmia) and non-cardiac (for instance, drowning, trauma or primary cerebral events). Approximately 18 to 25% of all cardiac arrests are of non-cardiac etiology.[9-11]

A common clinical categorization of cardiac arrest of cardiac origin is by the first rhythm, subdivided into four categories; 1. Asystole, 2. Non-perfusing ventricular tachycardia (VT), 3. Ventricular fibrillation (VF) and 4. Pulseless electric activity (PEA). This categorization can further be specified by the circumstances of the cardiac arrest; witnessed (yes/no) and bystander CPR (yes/no). In support of this method of indexing, the Utstein-Style Guidelines for uniform reporting of out-of-hospital cardiac arrest were developed.[12]

The investigations conducted in the scope of this thesis will focus on the outcome of patients with OHCA of cardiac origin and presumed cardiac origin.

1.3 Primary cardiac arrest rhythms

VF describes an electrical disorganization with uncoordinated myocardial twitching leading to total forward failure of the heart, pulselessness and rapid loss of consciousness. The electrocardiogram (ECG) shows turbulent, disorganized electrical activity of the heart in such a way that the recorded electrocardiographic deflections continuously change in shape, magnitude and direction.[13] VF is the primary rhythm in cardiac arrests in around 20 to 25% of cases.[14-16] VT is a regular wide complex heart rhythm commonly exceeding 100 beats per minute in the ECG. It is not a constitutional cardiac arrest rhythm but in cases with a fast tachycardia or a preexisting heart disease, circulatory collapse can occur causing cardiac arrest. VF and VT are defined as shockable rhythms, since depolarizing the myocardium simultaneously with an electrical shock using a defibrillator may abort the arrhythmia. Non shockable rhythms are PEA and asystole. In PEA, pulseless, insufficient perfusion, or no perfusion at all is present despite electrical activity on the ECG consistent with a regular heart rhythm. Asystole is defined by the absence of a pulse, with no circulation and undetectable electrical activity on the ECG. PEA and asystole represent the majority of first rhythms detected in cardiac arrest.[15] However, some studies suggest that in many cases asystole developed from VF and represents a later stage in the cardiac arrest,[17] and is therefore associated with poorer outcome.[15]

1.4 Epidemiology

The OHCA incidence in adults with emergency medical service (EMS) treatment is variable between countries. For all of Europe, the EuReCa two study reports an average of 56 (27 to 91) patients per 100000 population per year.[18] Higher average numbers,

73 per 100000 population per year, are reported from the ROC registry in the United states.[19] In both regions, the numbers of EMS resuscitated OHCA patients have increased during the last decade, from 38 to 56 in Europe and 54 to 73 per 100000 population per year in the United States.[20,21] Overall 30-day survival or survival to discharge has also improved over the last decade in the United States from an average of 8% to 11%,[14,19] while survival figures have been stable in Europe with an average of 10 to 11%.[22,23] However, survival to hospital discharge varies considerably between countries in Europe (Figure 1). Patients with shockable first rhythm experienced a significantly better outcome (survival to discharge) in recent and previous studies with 21.2 % (previously 21.2%) in Europe and 29.5% (previously 17.7%) in the United States.[14,20,21,23] During the last 40 years, VF as first rhythm has declined from 60% to 20% as primary rhythm, while PEA and asystole have become the predominant rhythms. The significantly poorer prognosis associated with PEA and asystole, represents a new challenge for post cardiac arrest care.[24,25] However, the reasons for stagnant or improved survival rates, despite increasing numbers of patients with primary rhythms associated with poorer prognosis, is not entirely evident. It may arise from initiatives to reduce the no-flow time of the cardiac arrest, like increased basic life support training in the communities and dispatcher-assisted CPR, which is reflected in the numbers of patients receiving bystander CPR, that has increased from approximately 26% in 1992 to over 70% in 2013.[26,27] The introduction of early bystander defibrillation in the recent decade has also been shown to be associated with good outcome after OHCA.[28,29] Although the effectiveness of automated CPR devices has not been conclusively proven, the increased availability of these devices in the prehospital setting, may also have contributed to improved outcomes.[30]

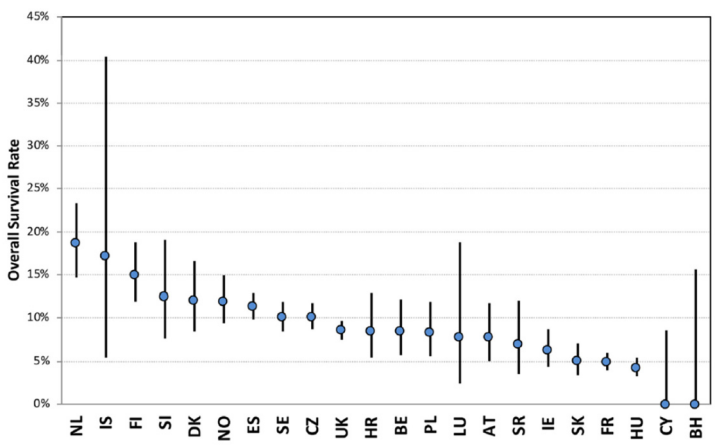

Figure 1. Survival to hospital discharge after OHCA in 21 European countries. Gräsner et al. Resuscitation 2020 148, 218 - 226. © 2020 Elsevier B.V. Reprinted with permission.

1.5 Cardiopulmonary resuscitation

> When the heart is diseased its work is imperfectly performed: the vessels proceeding from the heart become inactive, so that you cannot feel them… . If the heart trembles, has little power and sinks, the disease is advancing and death is near.

This translated passage from Ebers Papyrus, approximately 1500 BC, might be one of the earliest descriptions of the vanishing pulse, and probably the onset of ventricular fibrillation in a cardiac arrest situation.[31] After circulation has ceased, the body is in a no perfusion state until resuscitation efforts are commenced. The earliest documented recommendation to do so, dates back to 1740, when the Paris Academy of Sciences recommended mouth to mouth resuscitation for drowning victims. However, this resuscitation method was far from novel, since it had been used since biblical times by midwifes to resuscitate newborns.[32] It took another 150 years until 1891 when Dr. Friedrich Maass performed the first unequivocally documented chest compressions in a human to successfully counter a chloroform induced cardiac arrest in a child.[33] Ventilation and chest compressions are still the cornerstones of modern CPR and have been endorsed by the American Heart Association (AHA) since 1963.[32] Although, anecdotal reports exist describing the resuscitation of hens in 1775 and a 3-year-old girl in 1788 using an electrical current from a Leyden jar,[34] the first documented closed chest alternating current (AC) defibrillation in a laboratory animal was accomplished by Ferris and co-workers in 1936. It was not until 1956 that the first closed chest defibrillation in a human, also done with an AC device, was performed.[35,36] Portable defibrillators were successfully employed in the mid 1960´s and successively introduced in the prehospital resuscitation guidelines, followed by automated defibrillation devices (AED) to be used by laypeople with the start of the 21th century.[32,37]

1.5.1 The chain of survival

Since the first modern CPR guidelines of 1963, several resuscitation organizations have been established in different countries and continents to educate and refine CPR measures. In order to coordinate these efforts, a worldwide governing body, the International Liaison Committee on Resuscitation (ILCOR) was formed in 1992, reviewing and coordinating CPR guidelines in a 5-year cycle.[38]

The five links of the modern out-of-hospital chain of survival developed by the American Heart Association and the four link chain of the European Resuscitation Council (ERC) guidelines (Figure 2) are similar and both focus on the recognition of cardiac arrest and activation of the emergency response systems, i.e., early CPR with an

emphasis on chest compressions, rapid defibrillation and basic and advanced emergency medical services providing advanced life support and post resuscitation care.

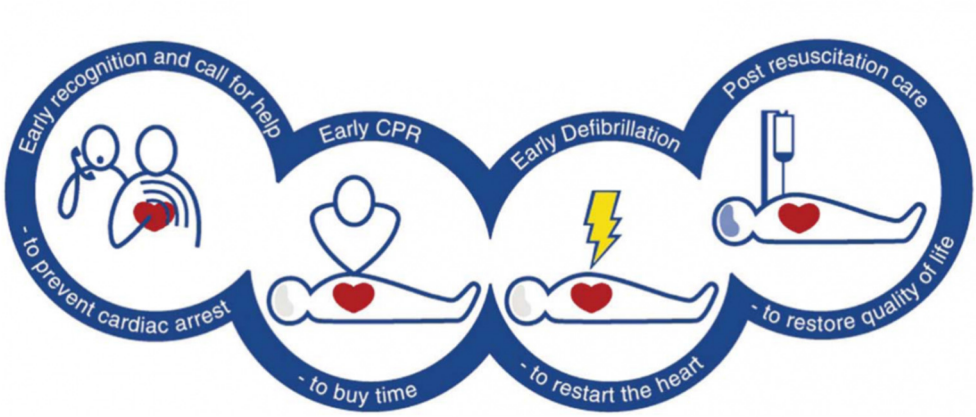

Figure 2. The ERC Chain of survival. © European Resuscitation Council – www.erc.edu (http://www.erc.edu/) - 2019_NGL_005. Reprinted with permission.

The primary goal of out-of-hospital cardiac arrest care is to keep the no-flow time as short as possible, by applying the basic concepts of CPR. In recent years a paradigm shift has occurred, from focusing on the maintenance of the airway as the first step, thereby delaying the provision of chest compressions, to chest compression first, changing the Basic Life Support (BLS) algorithm from ABC - Airway – Breathing – Circulation to CAB – Circulation – Airway –Breathing, since type of cardiac rhythm on presentation, and whether the victim received any chest compressions were highly predictive of outcome.[39,40] Advanced Life Support (ALS) expands the concept of BLS with the early use of cardiac monitoring devices and defibrillation when appropriate, airway devices to maintain ventilation, and the administration of medication. Even here, the emphasis has shifted from airway maintenance towards continuous uninterrupted high-quality chest compressions and early defibrillation. The 2015 ERC ALS guidelines (Figure 3) introduce, but not generally recommend the use of automated compression devices since evidence of mortality or morbidity benefits are lacking.[41] The 2015 guidelines also acknowledge the role of extra corporal life support techniques in selected patients.[42]

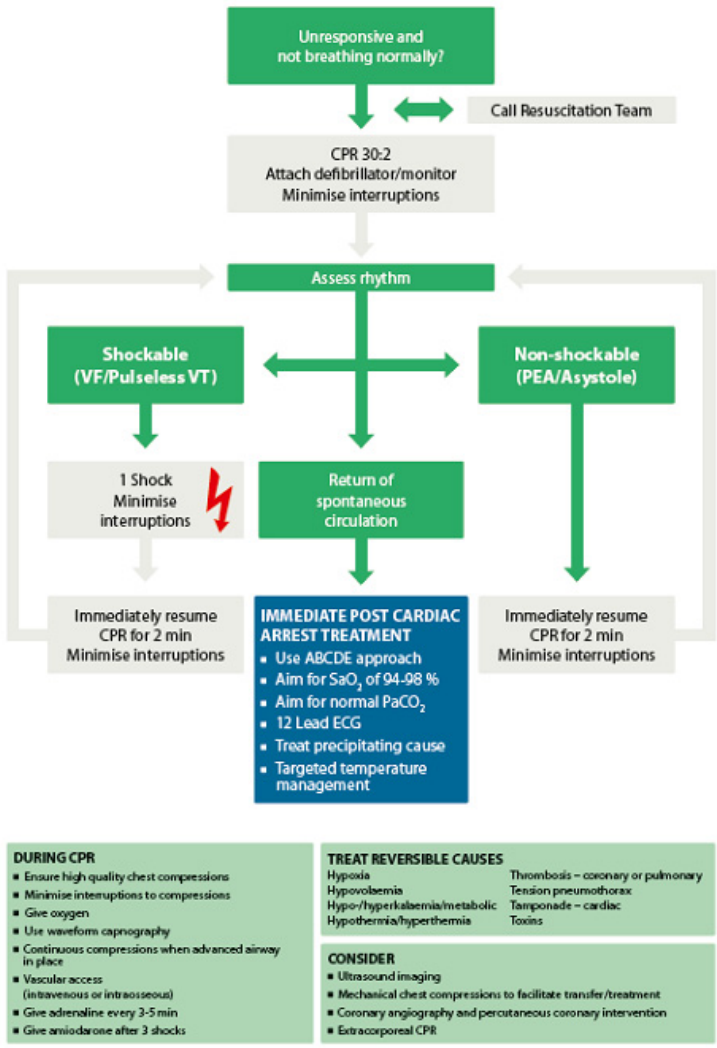

Figure 3. Advanced life support algorithm. CPR= cardiopulmonary resuscitation; VF/Pulseless VT= ventricular fibrillation/pulseless ventricular tachycardia; PEA= pulseless electrical activity; ABCDE= Airway, Breathing Circulation, Disability, Exposure; SaO_2= oxygen saturation; $PaCO_2$= partial pressure carbon dioxide in arterial blood; ECG= electrocardiogram. © European Resuscitation Council – www.erc.edu (http://www.erc.edu/) - 2019_NGL_005. Reprinted with permission.

1.5.2 Drugs during resuscitation

Drugs have traditionally been employed in an attempt to improve outcome after CPR, but convincing evidence is lacking and dosing and time of administration is largely based on preclinical trials and expert opinion. Epinephrine with its alpha and beta receptor stimulating properties is the drug primarily used during CPR. It increases vascular tonus and coronary perfusion as well as cardiac output, but also the risk for dysrhythmias, cardiac oxygen consumption (VO_2) and possibly for thrombosis. Studies evaluating the impact of different doses of epinephrine or combinations of epinephrine with other vasoactive drugs or with placebo have so far shown an improvement in ROSC, survival to admission and survival to discharge, but no improvement in favorable long-term neurological outcome.[43,44] Currently, the use of the standard dose epinephrine (1mg) every 3-5 minutes during CPR is recommended.[45] Other vasoactive drugs like vasopressin have not been shown to be superior to epinephrine and are no longer recommended as first line treatment. Atropine, a vagolytic drug previously employed during CPR is no longer recommended for asystole or pulseless electrical activity. Amiodarone, a membrane stabilizing antiarrhythmic, can be given once if shock resistant VF occurs during CPR. A second-hand antiarrhythmic drug, lidocaine, is recommended if amiodarone is unavailable. Amiodarone has shown improved survival to hospital admission, but no antiarrhythmic drug has so far shown an association with increased survival to hospital discharge.[42] Magnesium and calcium as well as buffers and fibrinolytic therapy are not routinely recommended during CPR and reserved for special circumstances like life threatening hyper- or hypokalemia, hypocalcemia, cardiac arrest due to tricyclic overdose or suspected pulmonary embolism.[42,46]

1.6 Ceasing resuscitation efforts

Despite extensive research and technical developments to sustain life preserving functions, survival to discharge after OHCA is still an exception.[29] The CPR providers are confronted with insufficient information regarding the circumstances, the cardiac arrest mechanism and the premorbid condition of the patient and therefore making appropriate decisions, with proper consideration of patient autonomy and medical benefit, is ethically challenging, in this situation.

The European resuscitation council gives general advice for the decision to withdraw CPR in the prehospital setting: 1. the safety of the provider can no longer be sufficiently assured, 2. there is obvious mortal injury or irreversible death, 3. a valid and relevant advance directive becomes available, 4. there is other strong evidence that further CPR

would be against patient's values and preferences or is considered 'futile' and 5. asystole for more than 20 min despite ongoing ALS, in the absence of a reversible cause.[47] However, more detailed clinical guidelines are lacking and the treating clinician has to tailor the decision to cease CPR for every individual case.

1.7 Post cardiac arrest syndrome

Data varies across different countries, but survival to hospital admission is commonly significantly higher in OHCA patients (~23%) than survival to discharge (~7.6%).[4,29] The principal morbidity and mortality in patients who achieved ROSC after OHCA, is due to cardiac and cerebral dysfunction caused by the preceding whole-body ischemia during the no-flow state before CPR and the low-flow state during CPR. This condition, which was initially called post resuscitation disease and later renamed post cardiac arrest syndrome (PCAS), is comprised of four principle features:[48-50]

1. Brain injury, the most prevalent manifestation of the PCAS elicited by ischemia, reperfusion, formation of free radicals, lipid oxidation, disrupted electrolyte homeostasis and cell death, resulting in coma, seizures, vegetative state and persistent neurological impairment.
2. Post cardiac arrest myocardial dysfunction, mainly due to stunning, whereas myocardial infarction is less common. Symptoms include signs of systolic as well as diastolic dysfunction, dysrhythmias and cardiovascular collapse, all of which result in a low output state and hypoperfusion. In many cases, the global cardiac dysfunction is transient and a full recovery can occur.
3. Systemic ischemia/reperfusion response, a comprehensive pansystemic reaction apparent within the first hours after ROSC, that elicits widespread inflammation, endothelial activation and activation of immunological and coagulation pathways after the onset of reperfusion. Common symptoms are vascular volume depletion, impaired vasotonus, multi organ failure and increased susceptibility to infections.
4. Persistent precipitating pathology that contributed to or caused the cardiac arrest and complicates PCAS, like acute coronary syndrome, pulmonary embolism, pulmonary diseases or various toxidromes.

Depending on the pre-arrest condition of the patient, duration of the ischemic insult and the etiology of the cardiac arrest, the different components vary in contribution to the resulting PCAS.

1.8 Post cardiac arrest care

After ROSC, patients are often critically ill and need extensive supportive care, interventions and monitoring in intensive care units. Unconscious patients require intubation and ventilation to ensure adequate gas exchange as well as hemodynamic support with fluids, inotropes, vasopressors and possibly circulatory support devices. Seizures occur frequently after cerebral anoxic-ischemic injury and therefore electroencephalography (EEG) monitoring is, besides ventilatory, circulatory and metabolic monitoring, substantial in post cardiac arrest care.[7] In addition to supportive measures, the causes of the cardiac arrest have to be investigated and treated if possible.

The revised version of the 2015 ERC guidelines outline two focus points for the post cardiac arrest phase; firstly, stabilization of physiological parameters and secondly, treatment of etiological or aggravating factors. Physiological factors that might alter outcome are ventilation, mean blood pressure, blood glucose, temperature control and electrolyte balance. The optimal targets are unknown, but the ERC provides guiding values: arterial oxygen saturation (SaO_2) 94–98%, the avoidance of hypovolemia, a mean arterial pressure with a target of urine output of 1ml/kg/h and decrease in plasma lactate concentration, blood glucose <10 mmol/l and avoidance of hypoglycemia and a plasma potassium (K^+) of 4.0–4.5 mmol/l.[51] Mild induced hypothermia to target levels of 32°C–34°C has been associated with a delay of the processes that lead to cell death during and after cardiac arrest. Following initial trials that showed beneficial outcomes, its use was recommended by the governing bodies in the United States (AHA), Europe (ERC) and internationally (ILCOR).[52,53] However, after subsequent analyses and the Targeted Temperature Management (TTM)-trial showing no difference in mortality or neurological long-term outcome between two temperature groups (33°C and 36°C) the quality of evidence behind mild induced hypothermia (33°C) was reduced from strong to low.[27,54] The 2015 ERC guidelines recommend a constant target temperature management (TTM) between 32°C and 36°C and the avoidance of pyrexia (≥37.6°C) which is common within 48 hours after cardiac arrest and associated with poor outcome.[51] The question whether mild induced hypothermia is beneficial for the OHCA patient or whether the avoidance of pyrexia is sufficient, remains unanswered and is currently being investigated in the ongoing TTM-2 trial (NCT02908308).

Seizures, clinically overt or nonconvulsive, including electrographic status epilepticus (ESE) are common features after ROSC and have been associated with poor outcome.[55] Seizures increase the cerebral metabolic rate and potentially exacerbate the brain injury sustained during cardiac arrest.[51,56] Due to sedation and muscle relaxation, diagnosis might be difficult. Therefore, intermittent EEG to detect epileptic activity and continuous EEG monitoring with a diagnosed status epilepticus as well as

pharmacological treatment is advised by the ERC.[51] Whether systematic detection and treatment of electrographic epileptic activity improves outcome is not known.[51] Although malignant EEG patterns after OHCA do not necessarily exclude good outcome.[57] However, highly malignant EEG patterns have been shown to be reliably associated with poor outcome.[58] Prophylactic seizure treatment has not been sufficiently studied and is currently not recommended.[51]

A frequent cause of OHCA is the acute coronary syndrome (ACS). Acute revascularization, medically or preferably via percutaneous coronary intervention, is indicated for patients with ST-segment elevation or a new post-arrest left bundle branch block (LBBB) on a 12 lead ECG after ROSC.[51] It is worth noting, that randomized trials are still unavailable and the recommendation is based on observational studies. Whether or not acute coronary intervention is indicated for non ST-segment elevation OHCA of cardiac origin, is still controversial, with some studies suggesting a trend towards better outcome for this group,[59,60] while others, including one randomized trial, were not able to confirm this finding.[61,62] Further trials investigating this question are ongoing.[63]

1.9 Prognostication

Despite successful resuscitation and admission to ICU the majority of OHCA patients experience neurological injury ranging from minor memory deficits to vegetative state and brain death. Prognostication of neurological outcome has been central to post cardiac arrest care in order to avoid prolonged treatment in patients with a futile prognosis and to avoid termination of care in patients that will benefit from prolonged treatment. Prognostication methods have been investigated since the mid 1970´s when Willoughby and Snyder showed that absent response to pain or pupillary light reflexes, and absent oculocephalic responses were closely associated with dismal prognosis for neurologic functioning.[64,65] Although, prognostication methods have been refined since then, a careful clinical neurological examination remains the foundation for prognostication of the comatose patient after OHCA.[66] The use of mild induced hypothermia and its interference with clinical examination as well as its potential to prolong drug effects has raised concern for the reliability of clinical prognostication tests developed in the era before mild induced hypothermia was introduced. To avoid false outcome prediction, a conservative multimodal prognostication model not earlier than 72 hours after ROSC, or rewarming, if the patient has been treated with mild induced hypothermia, is advised (Figure 4).[51] However, few prognostication studies report blinding of the treatment team, increasing the risk of a "self-fulfilling prophecy"

and reducing the quality of evidence in the studies and subsequently the prognostication guidelines.[67,68]

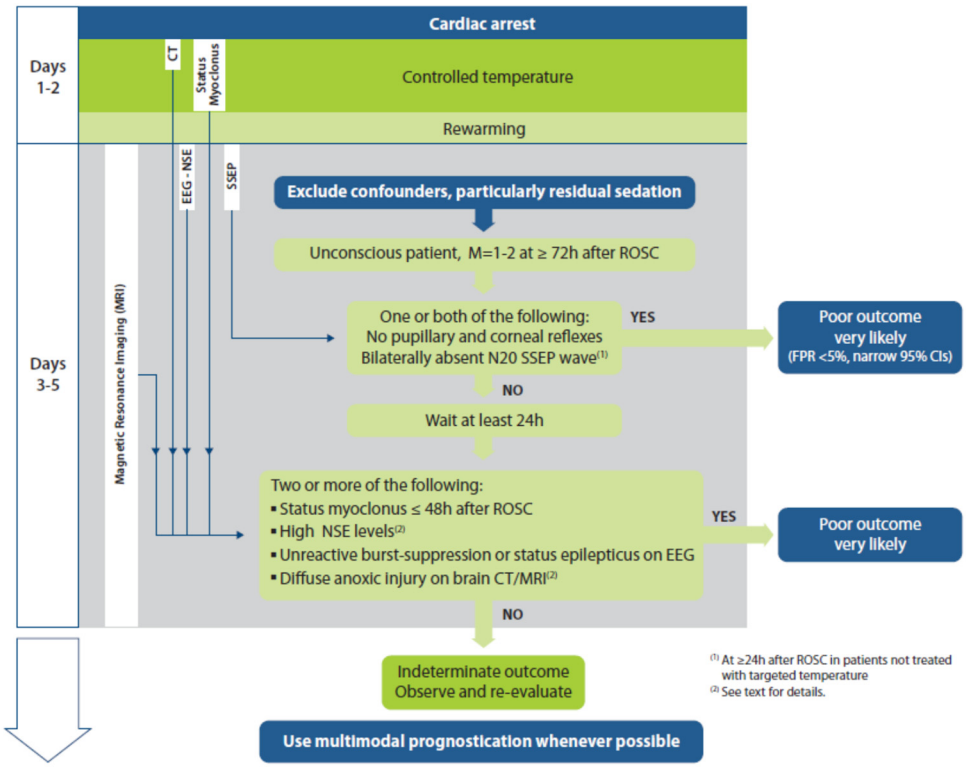

Figure 4. Prognostication strategy algorithm for comatose patients after cardiac arrest. SSEP= somatosensory evoked potentials, ROSC= return of spontaneous circulation, NSE= neuron specific enolase, h= hours, FPR= false positive rate, CT= computed tomography, MRI= magnetic resonance imaging, EEG= electroencephalogram. © European Resuscitation Council – www.erc.edu (http://www.erc.edu/) - 2019_NGL_005. Reprinted with permission.

1.9.1 Demographic background parameters

Important factors that have been identified predicting neurological outcome are the time to ROSC, whether the first rhythm was shockable (VF/VT) or not (Asytole/PEA) and whether bystander CPR was performed or not. Other factors identified are whether the OHCA was witnessed or not and neurological function on arrival to hospital. Commonly, old age and pre-arrest functional status and morbidity are also associated with neurological outcome.[69-71] However, none of the identified parameters have proven robust enough to predict outcome in individual cases.

1.9.2 Clinical neurological examination

As previously mentioned, the clinical neurological examination remains one of the cornerstones of prognostication, but a requirement for achieving a reliable result is that all sedative or muscle relaxing drugs are cleared and the patient has reached normal temperature. Clinical neurological examination for prognostication purposes is not recommended before 72 hours after ROSC. The absence of Pupillary Light Reflex (PLR) predicts poor outcome with 0% false positive rate (FPR), but with a low sensitivity of 18 to 24%. The bilateral absence of corneal reflexes (CR) shows a similar result with a slightly lower specificity (FPR 4–5%) and sensitivities 29% to 34%. An absent or extensor motor response to pain (Glasgow coma scale–motor response ≤2) has a high sensitivity (74%) for prediction of poor outcome, however, the FPR is also high 27%. Myoclonic jerks within 72 hours after ROSC are inconsistently associated with poor neurological outcome, whereas status myoclonus starting within 48 hours from ROSC was highly associated with poor outcome FPR 0–0.5%, sensitivity 15–16%.[51,72]

1.9.3 Neurophysiological examination and neuroimaging

Two principal neurophysiological methods are routinely employed in clinical practice, the EEG and somatosensory evoked potentials (SSEP). The EEG is the recording of the cortical electrical activity and can be conducted as a multichannel EEG with a large number of scalp electrodes over a period of 20 to 30 minutes or as a continuous EEG recording. In the ICU, amplitude integrated EEGs (aEEG) with fewer electrodes are commonly used for continuous recordings. The aEEG provides information over hours or days but has been associated with a somewhat decreased diagnostic yield.[73] SSEP tests the integrity of the pathway from the peripheral sensory nervous system to the sensory cortex, running via the medial lemniscal pathway in the dorsal column of the spinal cord to the brainstem and thalamus.[72]

The EEG has been used to identify patterns associated with poor outcome. Suppressed background without discharges, suppressed background with continuous periodic discharges and burst-suppression background with or without discharges have been identified as highly malignant and to predict poor outcome reliably, with 50% sensitivity and 100% specificity.[58] In less Malignant patterns, e.g., abundant periodic discharges or discontinuous background or low voltage background, sporadic patients survived with good outcome, especially if only one isolated malignant feature was present.[58] TTM treatment does not affect the quality of the EEG significantly, whereas residual sedatives may compromise EEG interpretation.

SSEP is relatively easy used at the bedside and the absence of the cortical N20 signal normally created in the somatosensory cortex has been shown to be a reliable predictor of poor outcome with very high specificity (0–2% FPR) but low to moderate sensitivity, since a preserved N20 response does not necessarily correspond with a good outcome.[74,75] SSEP results are reliable regardless of TTM treatment or moderate sedation.[67] However, high doses of barbiturates and remifentanil have been shown to influence results.[76,77] Other neurophysiological methods, e.g., bispectral index (BIS), ordinarily used to monitor anaesthesia depth have been studied as outcome predictors after OHCA but are not in routine use.[78]

Computed tomography (CT) and magnetic resonance imaging (MRI) have been evaluated for prognostication of outcome following OHCA and are used in clinical practice. Global cerebral oedema after OHCA is an indicator of hypoxic ischemic brain injury. Formal evaluation guidelines for CT scans after OHCA are lacking as well as recommendations concerning timing of a CT scan after OHCA. A structured way of assessment is the ratio of grey matter Hounsfield unit (HU) values to white matter HU values (GWR), measured in three different structures, basal ganglia, centrum semiovale, and high convexity. Poor outcome was predicted with 0% FPR and sensitivities ranging from 28% to 76%. However, even without formal GWR measurement, visual detection of generalised oedema on brain CT, by radiologists, predicted poor neurological outcome with 97.6% specificity and 14.4% sensitivity within 24 hours of ROSC.[67]

MRI provides more detailed and structured information on the extent of the hypoxic ischemic brain injury sustained during OHCA. Current guidelines suggest MRI imaging within 2 to 5 days, in patients who remain comatose and CT scanning did not reveal significant injury.[51] The whole brain median apparent diffusion coefficient (ADC) values after cardiac arrest were significant predictors of poor outcome, with a specificity of 100% and a sensitivity of 41%.[79] Nevertheless, it is important to point out that the existing MRI studies are often retrospective and that for severely unstable patients, MRI is not feasible which might constitute a bias in MRI investigations.

1.9.4 Serum biomarkers of neurological injury

The outcome prognostic potential of several serum biomarkers of neurological injury has been studied during the last decade.[80] Neuron specific enolase (NSE), released by neurons after injury and S100 calcium binding protein B (S100B), an astroglial protein, are the most frequently investigated serum biomarkers. NSE at 24, 48 and 72 hours after OHCA is recommended as part of the multimodal prognostication strategy endorsed by the ERC.[51] NSE has been shown to predict poor neurological outcome with a FPR <5% at 61, 46, and 35 ng/ml at 24, 48 and 72 hours, respectively, from ROSC with a corresponding sensitivity of 24%, 59%, and 63%. However, due the high variability of the assessment methods, current guidelines do not state a specific cut-off for the onset of poor outcome. NSE is not exclusive to central nervous system (CNS) and falsely increased values can be obtained due to hemolysis or neuroendocrine tumours.[81,82] In order to minimize the risk of false positive values, sampling at multiple time points is recommended in the current guidelines.[67] NSE in congruence with all other serum biomarkers of neurological injury, lacks specificity for individual brain regions and is rather reflective of the degree of global injury.[68]

1.10 Outcome

Overall survival to discharge after OHCA has been stable around 7–11 % and the majority of patients discharged from hospital are discharged with good neurological function.[29,83,84] However, neither the optimal time when to assess neurological outcome nor the modality with which to assess neurological outcome, has been established.[67] Assessment at hospital discharge provides an early assessment of the patient, little loss to follow-up and is less demanding on resources than assessment at a later time-point.[85] However, discharge criteria differ between hospitals and the limited time between the OHCA and follow-up at hospital discharge might not reflect the long-term outcome of the patient. A more reliable assessment is possible at 30 days, but patient improvement has been reported after this period.[86] Commonly, long-term follow-up is done at 6 months after OHCA but several studies have proposed a 90-day period to follow-up while others point out that over 20% of patients require more than 12 months to return to work indicating the need for a 1-year follow-up.[85,87] The Cerebral Performance Category (CPC) scale, adapted from the Glasgow Outcome scale for traumatic head injury, is part of the Utstein–style reporting and has until recently been the standard for post resuscitation outcome measurement.[85] The CPC divides patients into 5 categories: CPC 1 – patient is able to work and has no or minor neurological deficit, CPC 2 – the patient has sustained a moderate disability but has sufficient

cerebral capacity for independent activities of daily life, can work in a sheltered environment, CPC 3 – the patient is conscious but has severe cerebral disabilities and dependent on others for daily support, CPC 4 – vegetative state or coma, the patient is not conscious, CPC 5 – the patient is brain dead or certified dead by conventional methods.[88,89] Commonly, CPC 1 and 2 are regarded as good outcome and CPC 3 to 5 as poor outcome. In many studies using CPC as an endpoint, the outcome has been dichotomized accordingly. Assessment of CPC can be determined by face to face assessment, by proxy or by patient chart review. Other assessment instruments, most noteworthy, the modified Rankin Scale (mRS) and the Glasgow Outcome Scale Extended (GOSE), have also been employed to assess neurological outcome after OHCA. However, all of them are based on the patient's neurologic improvements, and may not reflect the recovery of other outcomes such as health related quality of life (HRQoL) and emotional issues.[72] Due to the limitations of CPC, mRS and GOSE, a core outcome set for cardiac arrest (COSCA) for reporting of effectiveness studies on cardiac arrest has been developed. COSCA includes structured data on survival, neurological outcome (mRS) and a set of HRQoL measuring scales, and has been endorsed by ILCOR, in order to facilitate meaningful comparison across studies over time.[90]

2 Cardiac arrest - special aspects

2.1 The brain during cardiac arrest

The majority of animal life on earth depends on breathing and circulation to secure oxygen uptake, oxygen delivery to peripheral tissues and the evacuation of carbon dioxide. The cessation of either, breathing or circulation for more than a short period initiates a process of cellular demise in the organism. In case of a cardiac arrest, circulation as well as oxygen delivery and carbon dioxide removal come to a standstill causing global anoxia and ischemia. Commonly, this event is defined as death.[91]

The brain has a very limited anaerobic capacity and almost immediately after the onset of cardiac arrest, intracellular acidosis develops, mitochondrial oxidative phosphorylation stops, adenosine triphosphate (ATP) is depleted, and lactate accumulates.[92] This stepwise pathophysiological process associated with cardiac arrest has been described in detail in animal models as a process beginning with the loss of consciousness 5 to 10 seconds after the circulation ceases. If circulation and oxygen supply are not restored immediately, EEG patterns become isoelectric within the following 10 to 40 seconds.[93] Arterial partial pressure of oxygen (PaO_2) diminishes to 0 kilopascal (kPa) after 2 minutes and ATP levels decline rapidly to 25% to 30% of baseline after only 4 minutes.[94] The proceeding depletion of ATP disrupts the ionic cellular homeostasis and causes K^+ efflux from the cell into the interstitium and an influx of extracellular ions like Na^+ and Cl^- together with water into the cell, entailing cellular swelling and brain edema.[95] 5 minutes after cardiac arrest, the blood brain barrier (BBB) permeability increases allowing an uncontrolled influx of serum proteins and electrolytes into the brain, aggravating the formation of brain edema.[95] After ion pumps fail to operate, calcium (Ca^{2+}) successively accumulates in the cytosol and exerts additional cytotoxic effects, leading to mitochondrial dysfunction, free radical production, lipolysis and production of free fatty acids (FFAs) expediting structural neuronal demise and increased BBB permeability.[96] Finally, the cessation of ATP production in the mitochondria results in cell death and necrosis.[94]

CPR is the attempt to reestablish blood circulation, but even during optimal CPR, the cardiac output is not better than one third of pre-arrest values and the activation of

blood coagulation and the formation of thrombi in the CNS impedes the already limited capillary perfusion and accelerates the development of brain edema.[97] If ROSC is achieved, reperfusion of the brain activates pro-inflammatory factors and generates reactive oxygen species (ROS) with the potential to aggravate the preexisting cerebral injury.[98]

Contrary to popular belief, and as outlined above, cerebral death is not immediate but a gradual process.[99] The reversibility of the process is limited. However, since multiple factors associated with positive neurological outcome after cardiac arrest have been identified it is not impossible.[83]

2.2 Oxygen and the brain

The initial appearance of oxygen in the earth´s atmosphere was a critical moment for cellular life as we know it today and displaced, the until then dominant anaerobic species, almost completely. Since the Great Oxygenation Event (GOE) roughly 2,4 billion years ago, oxygen has become critical to sustain life. Most organisms have adapted to oxidative metabolism and the increasing levels of oxygen in the atmosphere.[100-102] Today's atmospheric oxygen levels have been stable for the last 0.8 million years.[103]

Despite its weight of roughly 1.4 kg (2% of body weight), the adult human brain receives approximately 15% of the total cardiac output and stands for 20% of the total body oxygen consumption.[104] Due to its limited anaerobic capacity, adequate brain function is largely dependent on a delicate balance between cerebral oxygen demand and supply, in the different regions of the CNS, which is regulated by cerebral blood flow (CBF). CBF in combination with arterial oxygen content (CaO_2) determines cerebral oxygen delivery (CDO_2). Different brain tissues have varying oxygen demands and in case of CDO_2 perturbation, a hierarchy of neuronal vulnerability is evident, identifying the hippocampus, neocortex and the cerebellum as the most susceptible structures for damage.[105,106]

The lack of sufficient PaO_2 in the blood is defined as hypoxemia and leads subsequently to a lack of oxygen in the tissues, known as hypoxia.[107] The absence of oxygen in the blood or in the tissue is defined as anoxemia or anoxia, respectively. Hypoxic cells are unable utilize oxidative phosphorylation in the mitochondria and are forced to resort to anaerobic glycolysis for ATP production. Anaerobic glycolysis is a short lived and less efficient rescue mechanism to stave off the onset of further cell decay and cellular death, if the supply of oxygen is not reinstated.[108] Hypoxemia, begins at a threshold

PaO_2 of 7.9 kPa and has been shown to cause vasodilatation and increased CBF.[109] In a canine model, a decrease in PaO_2 from 6.6 to 3.3 kPa had the potential to double CBF.[110] The mechanisms behind the hypoxia induced cerebral vasodilatation are not entirely clear, but explanatory models exist, showing that nitrite (NO_2^-) reduction to nitric oxide (NO) and ATP release by erythrocytes increases, in addition to a hypoxemia-induced increase in cerebral adenosine liberation and endothelial NO production.[111] Other factors possibly involved are a cAMP and cGMP mediated decrease in smooth muscle Ca^{2+} sensitivity and a prostaglandin mediated hypoxic vasodilatation.[111]

Excessive amounts of inspired oxygen cause hyperoxemia which leads to tissue hyperoxia. Increasing the fraction of inspired oxygen (FiO_2) by breathing 100% oxygen has been shown to decrease mean CBF in excess of 25% for the duration of exposure.[112-114] The mechanism behind the hyperoxemia induced CBF decrease remains unclear, but various mediators and mechanisms have been suggested including increased effects of serotonin, nitric oxide synthase inhibition, inhibition of endothelial prostaglandin synthesis and increased leukotriene production.[110]

2.3 Oxygen and the injured brain

Severe hypoxia sustained during the no and minimal flow period of the cardiac arrest is the principal cause for brain damage after cardiac arrest.[67] However, healthy human subjects have been, after slow acclimatization, exposed to PaO_2 as low as 2.55 kPa at 8400m altitude, without persistent neurological damage.[115] In animal experiments EEG readings were normal at PaO_2 as low as 2.6 kPa.[116] CDO_2 in these hypoxic states depends on the hypoxia associated vascular system dilation and a high cardiac output to maintain the required CBF.[116] These circumstances are not present during or right after a cardiac arrest. Thus, hypoxic brain damage due to insufficient CDO_2 is likely to occur at higher PaO_2 levels, but a precise threshold for hypoxic damage in cardiac arrest patients has so far not been determined and is likely to be highly individual.

In the injured brain after cardiac arrest, the connection between PaO_2 and CBF can be disrupted as a study by Sundgreen et al. showed. In this study, the cerebral autoregulation in 13 out of 18 examined cardiac arrest survivors was impaired 170 – 1413 min after a cardiac arrest.[117] In a more recent investigation Voicu et al. found in their groups of cardiac arrest patients , that CBF was higher in non-survivors than in survivors, suggesting an impaired autoregulation in the brain after cardiac arrest in certain groups of OHCA patients.[118] The initial ischemic phase of cardiac arrest is followed by reperfusion of the brain, entailing activation of pro-inflammatory

mechanisms and oxidative damage due to expression of ROS.[119] The impaired autoregulation after ischemia, might expose injured parts of the brain to a higher than normal oxygen content even under normobaric, normoxic conditions. In this instance, iatrogenic hyperoxemia would further increase ROS formation and lipid oxidation and cause extended neuronal damage.[96,119]

2.4 Carbon dioxide and the brain

Carbon dioxide (CO_2) is a colorless gas and occurs currently in the earth´s atmosphere at approximately 400 parts per million (ppm). Most CO_2 arises from natural sources like volcanos, geysers, dissolving carbonated minerals and anthropogenic sources.[120] The concentration of CO_2 in earth´s atmosphere has been as high as 4000 ppm during the Cambrian period and as low as 190 ppm at the end of one of the more recent ice ages 130000 years ago. Until 1750 the concentration of CO_2 in the air had been constant for 10000 years at around 280ppm. For the last 250 years the atmospheric CO_2 content has been rising at approximately 1-2 ppm/year to today´s value.[121]

In aerobic organisms, CO_2 is an end product of carbohydrate, protein and lipid metabolization which is released into the surrounding environment. In humans, CO_2 is transported from the tissues via the venous blood stream as dissolved gas (10%), bicarbonate (HCO_3^-) (60%) or bound to proteins (30%) to the lungs where it crosses the blood-air barrier and is exhaled into the atmosphere.[122] The normal range of partial pressure of CO_2 in central venous blood is approximately 5.5–6.8 kPa while arterial blood has a CO_2 ($PaCO_2$) range of approximately 4.5–6.0 kPa.[123,124]

In the human body, CO_2 has profound effects on the acid-base status and there is a close relationship between HCO_3^- concentration and arterial pressure of carbon dioxide ($PaCO_2$) in order to preserve the optimal pH in the blood and tissues.[122] In the human brain, the $PaCO_2$ impacts on the tension of cerebral blood vessels and alters CBF. $PaCO_2$ above the normal range, hypercapnemia, causes vasodilatation and increases CBF, while $PaCO_2$ below the normal range, hypocapnemia, constricts cerebral vessels and reduces CBF.[125] Between 2.6 and 10.6 kPa the increase or decrease in CBF is around 15% – 30% per kPa change in $PaCO_2$.[126] The mechanism by which CO_2 affects cerebral vessels is not fully understood, but it is probably due to the modulation of K^+ channels via a CO_2 regulated increase or decrease in the concentration of hydrogen ions, leading to a change in intracellular Ca^{2+} concentration in vascular cells, and hence causing vasodilatation or vasoconstriction respectively.[127]

2.5 Carbon dioxide and the injured brain

The effects of increased and reduced $PaCO_2$ in the brain after injury caused by trauma or anoxia-ischemia have been investigated in several studies.[128-134] Hypocapnemia reduces intracranial pressure and has traditionally been used to manage acute intracranial hypertension to avoid imminent pressure related brain damage or brainstem herniation. However, the evidence for an outcome benefit of this practice is limited.[132] The long term exposure to hypocapnemia is likely to cause secondary brain injury due to cerebral vasoconstriction, reduced CBF and reduced CDO_2.[132] Mild hypercapnemia has been associated with increased CBF, anticonvulsive, antioxidant and anti-inflammatory characteristics,[135,136] and may be beneficial to alleviate the impact of anoxic-ischemic brain injury that occurs during cardiac arrest,[134,137] while general hypercapnemia after cardiac arrest has been associated with good as well as poor outcome.[138,139] A possible explanation for the hypothesized protective properties of hypercapnemia might be the coupling between cerebral CO_2 tension and pH in the CNS. The activity of glutamate receptors and voltage gated Ca^{2+} channels, which are vital elements in neurotransmission and thus, activity and oxygen consumption in the CNS, are strongly dependent on pH. A falling pH in the CNS might therefore dampen neurotransmission, prevent convulsions and reduce metabolic neuronal activity.[135,137,140] The mitigation of cerebral hypoperfusion by increased $PaCO_2$ levels in the post-cardiac arrest period and the entailed increase in CBF as well as CDO_2 is another hypothesized mechanism for improved outcome.[137,141]

2.6 Carbon dioxide and the modulation of inflammation

Protective ventilation strategies, tolerating higher $PaCO_2$ levels have in some studies shown better outcomes compared to more aggressive strategies. The mechanism of action of this permissive hypercapnia strategy, initially credited to lower tidal volumes, is not entirely clear since other factors like a direct protective mechanism by an increased $PaCO_2$ or an indirect effect by CO_2 induced respiratory acidosis are possible.[142] Several preclinical studies have demonstrated protective properties of hypercapnemia and acidosis in *in vivo* and *in vitro* lung injury models and in myocardial ischemia models.[143-146] In models of anoxic-ischemic brain injury hypercapnia and acidosis have also shown protective properties by delaying neuronal apoptosis and attenuating the effects of reoxygenation injury.[147,148] Other preclinical studies have shown that metabolic acidosis has similar protective properties in lung injury and

ischemic brain injury, suggesting that acidosis *per se* might be protective.[149-151] Furthermore, in preclinical studies, changes in CO_2 levels have been shown to directly alter gene expression via the NF-κB pathway,[152] and elevated $PaCO_2$ is associated with suppression of pro-inflammatory cytokines in a number of settings.[153]

2.7 Carbon dioxide, temperature and blood gas interpretation

Two principal methods are employed clinically for blood gas analysis, alpha-stat and pH-stat. The alpha-stat method analyses blood gases at 37°C and aims to maintain the patient´s pH at 7.40 and $PaCO_2$ at 5.3 kPa, regardless of the patient´s core temperature, while the pH-stat method employs a strategy of maintaining the pH of 7.40 and a $PaCO_2$ of 5.3 kPa at the actual core temperature of the patient. In normothermia, both methods should produce similar results, but since CO_2 solubility in plasma changes inversely to changes in temperature, measurements can differ in-between the two blood gas management strategies when the patient temperature changes.[154]

The alpha-stat hypothesis postulates that intracellular enzymatic activity functions best at neutral pH, which is 6.8 at 37°C. The intracellular pH is maintained during shifts in body core temperature due to a buffer system which is available in sufficient quantities and has the right chemical characteristics. Intracellular imidazole has been identified as the most appropriate buffer and therefore, the alpha refers to the degree of protonation of the imidazole groups of intracellular proteins, which is 0.55 at neutral pH. In order to keep the pH gradient over cell membranes (approximately 0.6 pH at 37°C) and thus, the intracellular alpha constant, the extracellular partial pressure of carbon dioxide has to be constant as well, but remains unknown to the clinician when employing the alpha-stat strategy.[155]

The pH changes with 0.015 units per °C change in temperature. The aim of pH-stat hypothesis is to keep the pH at 7.4 at the patient core temperature and since the solubility of CO_2 increases with lower temperatures which entails a rise in pH, CO_2 has to be added to counteract the metabolic alkalosis in the hypothermic patient when the pH-stat method is used.[156]

Neither of the two strategies has proven to be superior over the other, but each of the two methods has its own specific risks.[157] During alpha-stat acid-base management, the coupling between $PaCO_2$ and CBF remains intact and CBF is regulated by the cerebral metabolic rate of oxygen ($CMRO_2$). However, alpha-stat might lead to increased cerebral vasotonus and cerebral ischemia due to hypocapnemia. pH-stat uncouples

CBF and $CMRO_2$ and exposes the patient to "luxury" perfusion that might cause increased intracranial pressure and a theoretically increased risk for cerebral embolism.[156]

2.8 Serum biomarkers of neurological injury

2.8.1 Definition

The Biomarker Definition Working Group has provided a general definition of a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".[158] During recent decades, a category of biomarkers, defined as molecular biomarkers, have increasingly been used for disease diagnostics, monitoring and treatment. Molecular biomarkers can be specific cells, molecules, DNA, RNA, or hormones. Molecular biomarkers must be objectively measurable and should preferably be easily accessible, cheap to analyze, stable *ex vivo,* proportional to the severity of the injury, predictive, and validated for the underlying condition.[159] So far, no molecular biomarker fulfills all of these criteria.

2.8.2 Validity of molecular biomarkers

There are multiple diagnostic tests reporting the performance of biomarkers.[160] When analyzing a molecular biomarker, three features should be tested: analytical validity, clinical validity, and clinical utility. Analytical validity is the markers ability to measure what it is supposed to measure, clinical validity expresses the association with other clinical information or future outcome measures and clinical utility tells us if the marker improves efficiency, without compromising patient outcome.[161] A common measure of clinical validity for binary outcomes is diagnostic accuracy, which can be expressed in diagnostic sensitivity, the proportion of the diseased correctly classified as such (true positive rate (TP)), and in its counterpart diagnostic specificity (true negative rate (TN)), the proportion of the patients who do not have the target condition correctly classified as such.[161] An ideal test would show 100% sensitivity (0% false negatives (FN)) and 100% specificity (0% false positives (FP)), but in practice this is often not the case and therefore, the most critical metric has to be prioritized. In cardiac arrest, specificity should be as high as possible since false positive predictions may lead to erroneously pessimistic prognoses.[162] An alternative measure of diagnostic accuracy if both sensitivity and specificity are diagnostically important is the Youden´s J index,

representing the sum of sensitivity and specificity minus one, [TP/(TP+FN)] + [FP/(TN+FP)] -1. In a perfect test where there are no false positives or false negatives, the Youden´s J index is 1. If the test does not accurately exclude false negatives or false positives, the Youden´s J index would be closer to 0.[163] A graphical description of the performance of a test can be provided by a receiver operating characteristic (ROC) curve depicting the true positive rate against the false positive rate (1-specificity). The larger the area under the curve, the higher the accuracy of the test to discriminate between patient outcomes.[164] Analytical validity is generally evaluated using measures such as test precision, reliability, accuracy, sensitivity, and specificity and comparing the performance of the new test with the "gold standard" of care. There are no stipulated procedures to establish the clinical utility of a test or biomarker. Subjective measures such as how well clinicians appreciate the information gained by the new analysis method, provide an opportunity to measure clinical utility, as well as assessment via a randomized controlled trial, to establish whether or not, the benefits of the new method are superior to the standard of care.[165]

2.8.3 Prognostic serum biomarkers of neurological injury

Serum biomarkers of neurological injury (serum biomarkers), measurable in the peripheral blood, have traditionally been used in the prognostication of neurological diseases, traumatic brain injury or neurological vascular insults. Several of these biomarkers have also been studied as prognostic tools after ischemic brain injury due to cardiac arrest. Currently, only one serum biomarker, neuron specific enolase (NSE) is recommended in combination with other prognostication modalities for outcome prognostication in the ERC prognostication strategy algorithm.[51] None of the studied serum biomarkers have shown sufficient robustness to predict outcome singularly. Furthermore, the threshold values for poor outcome vary, mostly due to different levels of misclassification regarded acceptable and also due to the use of heterogeneous measurement techniques.[51]

As previously described, determining cut-off values for a test prognosticating outcome after OHCA is challenging, since common test evaluation methods, for example the Youden´s J statistic, often determine the optimal balance between specificity and sensitivity. However, in OHCA patients, this trade off would cause a large number of false positive results, and in the worst case, increase the risk for fatal erroneous prognoses, if the outcome prediction of the test applied is less than perfect. In current guidelines, there are neither recommendations for cut-off values nor for an acceptable rate of false positives. Although, a threshold of 0% false positive rate is desirable, it seems at present not feasible. Therefore, a sensible approach would be to allow for a

false positive rate of less than 5% and to compensate for this lack of accuracy by using the serum biomarker test only in combination with other modalities shown to have high predictive accuracy for neurological outcome after OHCA.

The appearance of serum biomarkers in the peripheral blood after the anoxic-ischemic CNS insult of the OHCA differs. Figure 5 depicts the appearance of a selection of serum biomarkers after ROSC.

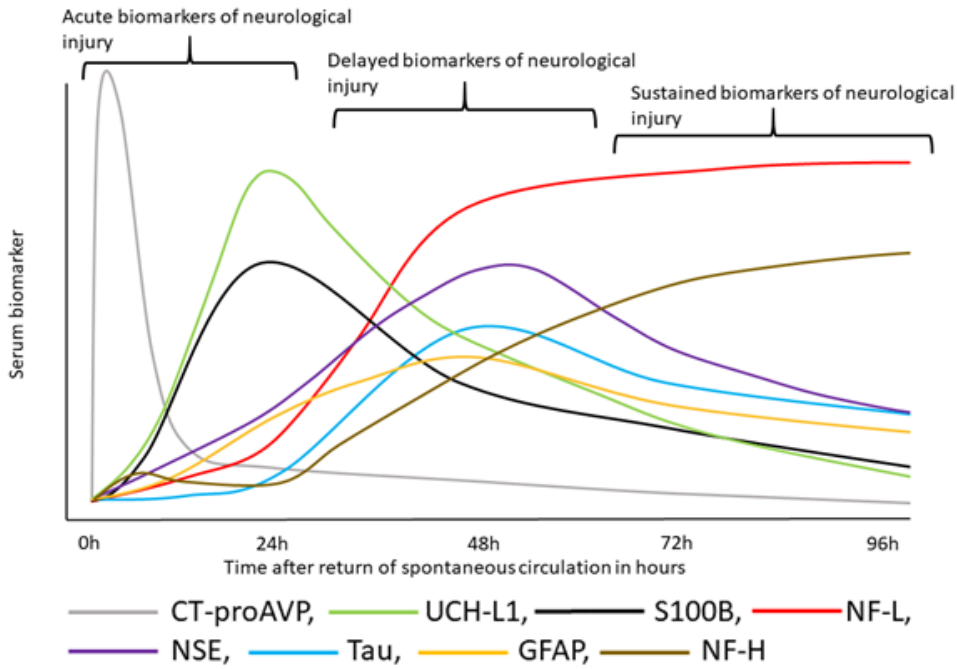

Figure 5. Schematic temporal profile of biomarkers of neurological injury after OHCA.

2.8.4 Acute serum biomarkers of neurological injury

S100B

S100 is a multigenic family of low molecular weight (9-14 kilodalton (kDa)) calcium binding proteins with S-100B as an intracellular calcium binding protein subtype, found most abundant in glial and Schwann cells of the nervous system, but also in melanocytes, chondrocytes and adipocytes.[166,167] In a study of 200 adults, serum levels in healthy individuals were around 0.05 µg/l with no significant difference between age or sex.[168] S100B is secreted by astrocytes and it acts as a intracellular regulator and an

extracellular signalling substance.[169] S100B does not cross the BBB and increased levels of S100B are indicative of glial cell damage and a presumed increased permeability of the BBB.[170,171] In cardiac arrest patients, elevated serum S100B is predictive of poor outcome, regardless of temperature management.[172] However, the optimal cut-off values vary from 0.12 µg/l to 0.80 µg/l.[80] The plasma half-life (t½) of S100B is 30 minutes and peak levels are reached at approximately 24 hours after cardiac arrest. Sustained high levels of S100B reflect the presence of ongoing neuropathological conditions, such as brain tumors or neurodegenerative diseases.[80] Despite its favorable bio-kinetic profile and being an outcome predictor for poor neurological outcome after cardiac arrest, S100B did not improve the robustness of a multimodal prognostication model.[173]

CT-proAVP (copeptin)

CT-proAVP is the C-terminal fragment of the pro-vasopressin hormone with a weight of 5 kDa and a plasma t½ of less than 30 minutes.[174] CT-proAVP is released from the hypothalamus via the posterior pituitary gland upon hemodynamic or osmotic stimuli.[175] In contrast to the in vitro unstable vasopressin, CT-proAVP is stable in vitro and can be analyzed with a commercial kit.[176] As a serum biomarker, it has been studied as a predictor of outcome in traumatic brain injury, stroke and sepsis. A prospective study (n= 84) by Annborn et al. found that CT-proAVP peaked at admission to hospital and was predictive of poor neurological outcome. Mild induced hypothermia did not interfere with the significance of the results. At a cut off value of >35.4 pmol/l, chosen from the ROC curve, sensitivity for poor neurological outcome was 76.9% while specificity was 77.5%. A cut-off value with 100% specificity for poor outcome resulted in reduced sensitivity of 25.9%.[176] Similar results were presented in a study by Broessner et al.[177] A higher cut off level of 217.9 pmol/l, chosen by Ostadal et al. showed that CT-pro-AVP had a 78.6% sensitivity and 75% specificity for 30 days survival without significant neurological dysfunction.[178] Despite the robustness of the results of the CT-proAVP studies, Annborn et al. point out that validation of the findings in larger studies is warranted.[176]

Ubiquitin C-terminal hydrolase L1

Ubiquitin C-terminal hydrolase L1 (UCH-L1), a 26 kDa neuronal deubiquitinase, with a plasma t½ of 6 to 12 hours, is found in neurons and neuroendocrine cells in vertebrates, constituting an estimated 5–10% of cytoplasmic proteins.[179] Smaller amounts of the protein are also found in human oocytes and spermatogonia, but UCH-L1 concentration is approximately 50 times higher in the brain than in other tissues.[180] UCH-L1 has been studied as a neuron derived serum biomarker for neurodegenerative diseases, seizures, traumatic and hypoxic brain injury, and increased serum levels have

been found indicative for a suspected breach of BBB integrity.[181,182] UCH-L1 has also been shown to aid the neuronal and functional recovery after ischemia in an animal model.[183] In a deep hypothermic cardiac arrest model including 19 piglets, UCH-L1 serum levels were found to correlate with the number of apoptotic neurons and to predict neuronal apoptosis with a sensitivity of 85% and specificity of 57%.[184] A prospective explorative pediatric investigation, including 19 cardiac arrest patients and 43 healthy controls from age 1 week to 17 years of age (mean 6.1±6.7 years) showed that UCH-L1 was not associated with age or core temperature and was predictive of poor outcome after median 59 (IQR 54.5–65) hours post cardiac arrest but not after median 10.5 (IQR 5.5–17) hours.[185] UCH-L1 has not been studied in adult cardiac arrest.

2.8.5 Delayed serum biomarkers of neurological injury

Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP) is a 50 kDa monomeric intermediate-filament component of the astrocytic cytoskeleton, exclusively expressed in the CNS with a plasma t½ of 24 to 48 hours.[186] Elevated serum GFAP as a marker for a transformation of BBB integrity has been found to predict neurological outcome in patients with traumatic brain injury.[187] Kaneko et al. showed that the presence of detectable serum GFAP at 12, 24, and 48h after ROSC was predictive of poor neurological outcome with 100% specificity and 51.9%, 63.6%, and 47.8% sensitivity, respectively, in patients not exposed to mild induced hypothermia (n= 32).[186] In patients treated with mild induced hypothermia (n= 12), elevated serum GFAP had 100% specificity for poor neurological outcome and 37.5%, 62.5%, and 75.0% sensitivity, respectively, at the measured time-points.[186] In a larger study (n=125), Larsson et al. found that GFAP, measured at 48, 72 and 96 hours after OHCA, was at 100% specificity less sensitive than NSE and S100B, measured at the same time-points and at the same specificity. In addition, they showed, that adding GFAP to a model containing NSE and S100B did not further improve prediction of neurological outcome.[188] In a study by Helwig et al., prospectively including 100 patients after cardiac arrest, GFAP levels at 48 hours revealed a sensitivity of 60.7% and a specificity of 66.7% to predict a poor functional outcome at 4 weeks post cardiac arrest. The authors identified a cut off level >0.08 µg/l as indicative of poor neurological outcome and levels >3 µg/l to be consistent with brain damage on brain imaging.[189]

Neuron specific enolase

Neuron specific enolase (NSE) is an isoenzyme of the glycolytic enzyme, enolase, with a weight of 78 kDa.[190] NSE is specific for neurons and peripheral neuroendocrine cells but is also found in red blood cells.[191] Increased levels of NSE may be detected with proliferation of neuroendocrine tumors, hemolysis and when neuronal injury occurs. In healthy individuals, mean NSE serum concentration originating from red blood cells is around 10 ng/ml with no gender or age effect.[171] Plasma t½ is 24 to 30 hours.[80] NSE has been extensively evaluated as an outcome predictor after cardiac arrest in several large cohorts, and levels >33 ng/ml within 72 hours after cardiac arrest were identified as a threshold value for poor neurological outcome in patients not treated with induced mild hypothermia.[192] This threshold was incorporated in guidelines but other thresholds have been identified and more recent guidelines do not recommend a distinct threshold for the onset of poor outcome.[51] The predictive value of NSE is not significantly affected by target temperature management at either 33°C or 36°C.[82] NSE is part of a suggested multimodal prognostication strategy, and high values at 48 and 72 hours are supportive of a poor outcome.[51] The possible superiority of serial NSE measurements in predicting neurological outcome over a single value has been investigated but results are varying.[193,194] A prospective cohort study found a 100% specificity for poor neurological outcome at a cut off level for NSE of 97 ng/mL determined by ROC, at a sensitivity of 49%.[195] Currently, NSE is the only recommended serum biomarker by the ERC to aid prognostication after cardiac arrest, but uniform analysis methods and cut-off levels are still lacking.[196]

Serum tau

Tau is a microtubule stabilizing protein predominantly localized in neurons, but also expressed at low levels in astrocytes and oligodendrocytes with different isoforms and a weight between 48 and 67 kDa.[197,198] Elevated serum tau concentrations can be found in patients with neurodegenerative disease and traumatic brain injury and plasma t½ between 10 to 24 hours have been reported.[80,199-201] A pilot study by Mortberg et al. investigating serum tau levels in induced mild hypothermia treated cardiac arrest patients, identified tau levels at 48 (>30 pg/ml) and 96 hours (>27 pg/ml) after cardiac arrest, to be predictive of poor outcome with the 96 hour serum tau value showing a specificity of 93% at a sensitivity of 71%.[202] In a prospective cohort study (n= 689), Mattsson et al. found increased serum tau values to be significantly associated with poor outcome after out of hospital cardiac arrest. The 72-hour serum tau levels showed a specificity of 98% with a sensitivity of 66% at a cut-off level of 11.2 ng/l. Tau was also found to be more robust to hemolysis than NSE, and targeted temperature management did not affect the outcome predictive accuracy of tau.[201]

2.8.6 Sustained serum biomarkers of neurological injury

Neurofilament

Neurofilaments (NFs) are CNS specific heteropolymers composed of four subunits NF-Light (NF-L) (68 kDa), NF-Medium (NF-M) (145 kDa), NF-Heavy (NF-H) (200 kDa) and α-internexin or peripherin (66 kDa), mainly found in axons and dendrites.[203,204] After neuroaxial injury, NFs are released into the extracellular space and subsequently into the bloodstream. Little is known about the kinetics of NFs, but the plasma t½ is likely to be several days.[205,206] Rundgren et al., found that NF-H levels at 2 and 36 hours after OHCA were significantly elevated in patients with poor outcome compared to patients with good outcome. Receiver-operating characteristic (ROC) analyses for the prediction of CPC 1-2 versus CPC 3-5 at 6-months follow-up showed an area under the ROC (AUROC) curve at 2 hours of 0.72 and at 36 hours of 0.71. However, due to a considerable overlap between the good and poor outcome groups, the authors were unable to propose a clinically relevant cut-off level.[207] A prospective observational cohort study (n= 85) showed that NF-L levels were increased in patients with poor outcomes at 5 of 5 measured time-points (within 2 hours after admission and day 2, 3, 5 and 7). NF-L levels on day 5 and 7 were the most predictive of poor outcome, and ROC NF-L analysis on day 7 predicted poor outcome at a cut-off of 252 pg/ml with a specificity of 100% and a sensitivity of 94%, AUROC was 0.994.[208] A prospective study evaluating data from the TTM-trial by Moseby-Knappe et al. found that NF-L was significantly increased at 24, 48 and 72 hours in patients with poor outcome after cardiac arrest and was, in a ROC analysis superior to serum tau, NSE and S100.[162] A 24-hour cut off level at 478 pg/ml had high specificity (98%) and sensitivity (69%) for poor neurological outcome in this study. Additionally, NF-L levels differentiated between various degrees of neurologic function, offering opportunities for a more nuanced assessment.[162]

2.9 Oxygen and serum biomarkers of neurological injury

To the author´s knowledge, only one study has investigated the effects of PaO_2 on serum biomarkers of neurological injury after OHCA. In a randomized pilot trial by Jakkula et al. investigating the effects of PaO_2 on 48-hour serum NSE concentration, resuscitated and unconscious OHCA patients were assigned to normoxemia (PaO_2 10–15 kPa) or moderate hyperoxemia (PaO_2 20–25 kPa). Serum NSE concentration after

48 hours did not differ between the two groups.[209] Studies investigating the effects of hypoxemia after OHCA on serum biomarkers of neurological injury are lacking.

2.10 Carbon dioxide and serum biomarkers of neurological injury

The association of hypercapnemia and NSE levels has been investigated in a pilot study by Eastwood et al., comparing targeted therapeutic mild hypercapnemia ($PaCO_2$ 6.7–7.3 kPa) to normocapnemia ($PaCO_2$ 4.7–6.0 kPa), and showing an association with reduced NSE levels at 24, 48 and 72 hours after ROSC.[137] The results of this study are further investigated by an ongoing, larger randomized trial.[210] Jakkula et al. randomly assigned 123 resuscitated OHCA patients to low-normal ($PaCO_2$ 4.5–4.7 kPa) and high-normal ($PaCO_2$ 5.8–6.0 kPa) $PaCO_2$ groups with NSE levels 48 hours after OHCA as endpoint. The authors of this pilot study were not able to show a significant difference in the median 48-hour NSE concentration between the groups.[209] The association between hypocapnemia and serum biomarkers of neurological injury after OHCA has, to the author´s knowledge, not been studied.

2.11 Measurement of neurological outcome

Cerebral performance category (CPC), is widely used to assess neurological outcome in patients successfully resuscitated after a cardiac arrest and its design targets this group of patients.[211] CPC can be determined by face to face assessment, by proxy or by patient chart review.[212] The patients are divided into 5 categories: CPC 1 – patient is able to work and has no or a minor neurological deficit, CPC 2 – the patient has sustained a moderate disability but has sufficient cerebral capacity for independent activities of daily life, can work in sheltered environment, CPC 3 – the patient is conscious but has severe cerebral disabilities and is dependent on others for daily support, CPC 4 – vegetative state or coma, the patient is not conscious, CPC 5 – the patient is brain dead or certified dead by conventional methods. Commonly, CPC 1 and 2 are seen as good outcome and CPC 3 to 5 as poor outcome. In many studies using CPC as an endpoint, the outcome has been dichotomized accordingly.[213] However, CPC 1 to 3 has also been defined as good outcome.[74] Generally, the CPC offers a relatively efficient approach to assess cardiac arrest outcomes, but has shown significant variability in inter- and intra-reviewer agreement, depending on the source of information (discharge summary

versus complete hospital record) and patient outcome as well as shortcomings in discrimination between mild and moderate neurological injury.[214-216] CPC at discharge has shown to be a surrogate marker for long-term outcome,[88,213] but due to the nature of the CPC scale, assessment at discharge has been considered less accurate than a later assessment.[215]

The modified Rankin Scale (mRS), originally introduced for the outcome assessment of patients after ischemic stroke, is similar to the CPC scale but more focused on functional domains.[217] Patient assessment is commonly administered by face to face interview with the patient or a proxy, but telephone interviews have also shown good reliability.[218] Frequently, "death" is added to the mRS scale in cardiac arrest studies resulting in seven categories starting with mRS Grade 0 – No symptoms at all, Grade 1 – No significant disability despite symptoms: able to carry out all usual duties and activities, Grade 2 – Slight disability: unable to carry out previous activities but able to look after own affairs, Grade 3 – Moderate disability: requiring some help, but able to walk without assistance, Grade 4 – Moderate severe disability: unable to walk without assistance, and unable to attend to own bodily needs without assistance, Grade 5 – Severe disability: bedridden, incontinent and requiring constant nursing care, Grade 6 – Death. The mRS scale has shown a substantial inter-rater variability that increased when assessments were made by raters from different professions.[219] Mass training has shown potential to improve rater consistency.[220]

The Glasgow Outcome Scale Extended (GOSE) was developed from the Glasgow Outcome Scale (GOS) in order to increase sensitivity in detecting the remaining neurological and mental handicap in traumatic brain injury survivors.[89,221] GOSE assessment consists of a structured interview with the patient or a proxy by face to face or telephone interview. The GOSE scale categorizes outcome into eight levels from 1 – Death, 2 – Vegetative state, 3 – Lower severe disability, 4 – Upper severe disability, 5 – Lower moderate disability, 6 – Upper moderate disability, 7 –Lower good recovery, 8 – Upper good recovery. The GOSE has shown satisfactory interrater reliability.[222]

Discharge disposition is commonly divided into 6 categories: Discharge to home with no services, discharge to home with home healthcare, discharge to acute rehabilitation facility, discharge to skilled nursing facility, discharge to long term acute care facility, and discharge to hospice. Discharge disposition is prone to influences not related to patient status, like insurance, location and family situation. It is also not repeatable, and thus, suspect as a useful outcome measure.[212]

A reliable outcome measure after cardiac arrest is vital for clinical studies, mRS and CPC show significant inter-rater variability and only a fair relationship with each other, while discharge disposition shows a poor relationship with both CPC and mRS.[212]

Reasons for this variation among measures are how these global measures weigh different domains of function, CPC focuses on mental function while mRS assesses functional parameters as well as structures, and activity and participation. GOSE is more nuanced and shows good reliability and validity in non–cardiac arrest cohorts but has not been thoroughly evaluated in the cardiac arrest population.[85] The CPC served as the "gold standard" of measuring outcome after cardiac arrest but its inherent limitations have resulted in the use of mRS and GOSE in current effectiveness trials.[223,224] However, as pointed out earlier, the information gained by mRS and GOSE is also restricted. Therefore, scales measuring health-related quality of life (HRQoL) like the EuroQol EQ-5D5L, HUI3 or SF-36v2 have recently been introduced, in addition to neurological outcome scales. The core outcome set for cardiac arrest (COSCA) ILCOR advisory statement of 2018 recommends assessment of neurological function with mRS and measurement of HRQoL with at least one of the three measurement tools at 90 days and at periodic intervals up to 1 year after cardiac arrest.[90]

3 Aims of the thesis

- To describe the dynamic course of PaO_2 and $PaCO_2$ after stable ROSC in OHCA patients.
- To study the association between different exposure modalities of PaO_2 and $PaCO_2$ and neurological outcome in patients after OHCA.
- To identify a numerical threshold for PaO_2 and $PaCO_2$ for the onset of the association with poor neurological outcome.
- To investigate the association between PaO_2 and $PaCO_2$ and the peak levels of the serum biomarker tau after OHCA.
- To describe the release dynamics of the serum biomarkers of neurological injury GFAP and UCH-L1 after OHCA.
- To investigate the accuracy of GFAP and UCH-L1 to predict neurological outcome in OHCA patients.
- To compare the accuracy of GFAP and UCH-L1 to predict neurological outcome after OHCA with the outcome predictive accuracy of NSE.

4 Methods

4.1 The Utstein-Style, uniform reporting of cardiac arrest data

In 1991 a Task Force of the American Heart Association, the European Resuscitation Council, the Heart and Stroke Foundation of Canada, and the Australian Resuscitation Council proposed a uniform reporting of OHCA data, called the Utstein-Style with reference to the Utstein Abby, Norway where the task force meeting was held.[12] Since then, a standardized reporting style has also been introduced for IHCA and for pediatric cardiac arrest.[225,226] The data reporting in the registries in this thesis was conducted according to the Utstein-Style template. However, in a recent validation study, the Utstein factors explained only 51% of the variation in survival to hospital discharge in an international material evaluating OHCA registries from 12 countries, for the period 1st of January 2006 through 31st of December 2011. The authors concluded that modifiable Utstein Factors should be targeted to improve survival but that the observed variability in outcome is incompletely explained by the Utstein-style templates and that further studies are needed to identify the ideal constituents of cardiac arrest data registration.[227]

4.2 Registries

4.2.1 The TTM database and the TTM-trial

The TTM database is an electronic web-based case record form, designed for the multicenter, randomized, parallel-group, assessor-blinded, monitored, and investigator-initiated clinical TTM-trial, evaluating a possible difference in mortality, neurologic function, and safety with a target temperature management at 33°C (TTM33) compared with 36°C (TTM36) in OHCA patients following sustained ROSC. Patient data for the registry was collected prospectively and anonymized by site

personnel under the supervision of the trial site investigator. Patients were enrolled from November 2010 to January 2013 and data was collected in five phases.

Phase 1 (hospital admission to start of intervention): Patients were randomly assigned to the intervention group. Pre-randomization baseline characteristics including a complete blood gas analysis were collected.

Phase 2 intervention period (start of intervention to end of intervention): Patients were sedated and mechanically ventilated in both allocation groups. Temperature management treatment for 24 hours was performed. Patients were rewarmed to a core temperature of 37°C during the following eight hours. Arterial blood gases were sampled at admission to hospital, at start of intervention and after 4, 12, 20, 28, 32, and 36 hours after start of intervention.

Phase 3 (from end of intervention period to 72 hours after end of intervention period): after rewarming, sedation was stopped or tapered. Normothermia of 37°C+/-0.5°C was maintained until 72 hours from cardiac arrest in both groups, if the patient remained in the ICU. Extubation was possible during this time. Neurological assessment was performed by blinded physicians at 72 hours or later, after the end of the intervention period.

Phase 4 (72 hours after end of intervention period to 28 days after OHCA): Neurological status, according to CPC, and survival were evaluated every day in the intensive care unit and/or at day 1, 2, 3, 4, 5, 6, 7, 14, 21 and 28, and/or at hospital discharge, whichever came first.

Phase 5 (day 28 to end of trial): Mortality and neurological status were evaluated after 90 and 180 days. Survival was followed to the end of trial.

TTM trial and database inclusion criteria

Adult (≥18 years of age), unconscious (GCS <8) OHCA patients, with stable ROSC (>20min) and OHCA of a presumed cardiac cause.

TTM trial and database exclusion criteria

Pregnancy, known bleeding diathesis (not medically induced), suspected or confirmed acute intracranial bleeding or stroke, unwitnessed arrest with initial rhythm asystole, temperature <30°C on admission, treatment limitations including do-not-resuscitate order, disease before the cardiac arrest making 180-day survival unlikely, known pre-arrest CPC 3 or 4, >4 hours from ROSC to screening, persistent cardiogenic shock with a systolic blood pressure <80 mmHg in spite of volume loading/vasopressors/inotropes or mechanical assistance.[228]

TTM-trial outcome

The primary outcome of the TTM-trial was all-cause mortality until the end of the trial, defined as 180 days after the last participant was included (survival analysis). Secondary outcomes were a composite outcome of all-cause mortality and poor neurologic function according to CPC at hospital discharge and at 180 days, all-cause mortality at hospital discharge and at 180 days, neurologic function at hospital discharge and at 180 days, quality of life at 180 days, best neurologic outcome during the trial period, and safety measures.[27]

TTM-trial follow-up and excluded patients

Of the 476 patients assigned into the TTM33 group, 3 were withdrawn or excluded from the trial while 8 of the 474 patients in the TTM36 group were excluded or withdrawn, which left a total of 939 patients for the final analysis. Of the 939 patients analyzed for survival until the end of the trial, a total of 6 were not analyzed for neurological function. Patient follow-up evaluation was performed by occupational therapists/research nurses blinded to the intervention allocation. Survival was followed to the end of the trial.[27]

4.2.2 The TTM-trial and serum biomarkers of neurological injury

29 out of 36 centers participated in the collection of serum biomarkers with a total of 819 patients included. According to a pre-specified protocol, blood samples were collected at 24, 48 and 72 hours after OHCA. 717 patients had at least one biomarker measurement at 24, 48 or 72 hours after OHCA. 102 patients had no measurement due to early death (n= 37), lost to follow-up (n= 4), withdrawn from TTM-trial (n= 1), problems with aliquoting (n= 15) and due to transfers and missing sampling (n= 45). After collection, the blood samples were processed at the respective collection sites, aliquoted and frozen to -80°C before shipment to the Integrated Biobank of Luxembourg for storage. Serum biomarker analysis was performed by certified technicians without access to clinical information. All analyses were conducted after completion of the TTM-trial and had therefore no influence on a possible direct WLST decision.

4.2.3 The International Cardiac Arrest Registry 2.0 database

The International Cardiac Arrest Registry (INTCAR) 2.0 database was designed as a collaborative effort between the North-American Neurocritical Care Society and a European network that arose from the Hypothermia Network Registry. 22 centers in

Europe and North America included adult (≥18 years of age), IHCA and OHCA patients from 2008 to 2018, admitted to their respective intensive care units. No formal exclusion criteria were specified, and treatment of the patients was at the discretion of the participating center. The centers were responsible for registration. 108 main data points, several with subgroups, were collected per patient. Besides the main INTCAR 2.0 basic data set including patient and cardiac arrest characteristics, treatment methods and prognostication, the database also collected information regarding imaging, hemodynamics, cardiology studies and procedures, EEG and seizures, and neurological short term and long term functional outcome as well as treatment methods and complications. In the INTCAR 2.0 database, the exposure to hyperoxemia (PaO_2 >40 kPa), hypoxemia (PaO_2 <8.0 kPa), hypercapnemia ($PaCO_2$ >6.7 kPa) or hypocapnemia ($PaCO_2$ <4.0 kPa) during the first 24 hours after OHCA was recorded in a dichotomous fashion (yes/no). The highest and lowest PaO_2 and the lowest $PaCO_2$ were documented numerically. 2162 OHCA patients were collected in the database. CPC was evaluated by healthcare professionals at the respective centers at hospital discharge. Long-term outcomes were collected around 6 months after presentation, by face-to-face interview, by telephone interview or by medical records.

4.3 Ethics

The investigations in this doctoral thesis have been approved by the ethical review board in Lund, Sweden.

Regional Ethical Review Board Lund, Sweden, Protocol 2007/7 Dnr 2007/272 for the INTCAR 2.0 Registry analysis (paper III)

Regional Ethical Review Board Lund, Sweden, Protocol 2009/6 Dnr 2009/324 for the TTM trial database analysis (papers I, II and IV)

4.4 Studies, objectives, design and methods

4.4.1 Paper I

Objective and design

The objective of this explorative post-hoc study of the TTM-trial was to describe the evolution of $PaCO_2$ in serial measurements at 8 predefined time points during the first

37 hours after OHCA, and to explore the association of $PaCO_2$ with neurological outcome in OHCA patients. We also investigated the interaction between mild hypercapnia and targeted temperature management in relation to neurological outcome as well as the association between our $PaCO_2$ multivariate models and peak levels of the serum biomarker of neurological injury, tau, at 48 or 72 hours after OHCA. Tau was chosen for our analyses due to its superior performance over other serum biomarkers used for prognostication of neurological outcome in cardiac arrest patients.

Patients

939 comatose (GCS <8), adult (≥18 years of age) OHCA patients derived from the two TTM-trial temperature assignment groups. 689 patients of the same cohort, previously investigated by Mattsson et al. were included in our secondary serum biomarker analysis.[201]

Methods

After pooling of the two temperature assignment groups into one cohort, the patients were divided into groups according to the maximum or minimum $PaCO_2$ exposure. We *a priori* defined the groups as hypercapnemia ($PaCO_2$ >6.0 kPa) and hypocapnemia ($PaCO_2$ <4.5 kPa). The group of patients not exposed to hypercapnemia or hypocapnemia were defined as normocapnemia ($PaCO_2$ 4.5–6.0 kPa). The primary analyses investigated the association of the maximum and minimum (extreme value) $PaCO_2$ exposure groups with neurological outcome. Secondary analyses evaluated the association with poor neurological outcome in a time weighted mean $PaCO_2$ exposure group and a maximum $PaCO_2$ amplitude analysis. We investigated the association of the time weighted mean $PaCO_2$ exposure with outcome over the first four (early exposure) and all sampling points (total exposure). We also configured the time weighted mean $PaCO_2$ groups to resemble a previous therapeutic targeted mild hypercapnia (TTMH) analysis by Eastwood et al., comparing two $PaCO_2$ target groups (4.6–6.0 kPa and 6.7–7.30 kPa).[137] In an univariable analysis, we investigated the association between maximum $PaCO_2$ and lowest pH with neurological outcome separately and combined. Furthermore, we tested the association of peak levels of serum tau at 48 or 72 hours with our $PaCO_2$ multivariate models. The main outcome was neurological function at 6-month follow-up, according to CPC scale with CPC 1 and 2 regarded as good outcome and CPC 3 to 5 as poor. For the serum biomarker analysis peak serum tau at 48 or 72 hours was the main outcome.

4.4.2 Paper II

Objective and design

The objective of this post-hoc study of the TTM-trial was to describe the evolution of PaO_2 in serial measurements, at predefined time-points during the first 37 hours after OHCA, and to explore the association of PaO_2 with long-term neurological outcome of OHCA patients. We also investigated a possible cut-off point for the onset of the association of hyperoxemia and poor neurological outcome, in support of a previous study by Roberts et al.[229] Similar to paper I, we investigated the association of peak serum tau levels with our PaO_2 multivariate models.

Patients

939 comatose (GCS <8), adult (≥18 years of age) OHCA patients previously randomized into two temperature arms and included in the TTM-trial. For the serum biomarker analysis, we analyzed the same cohort of 689 patients as in paper I.

Methods

We pooled the patients of the two temperature groups into one group and thereafter divided the patients according to their maximum and minimum PaO_2 exposure. In keeping with previous analyses,[229,230] we defined the groups as hypoxemia (<8.0 kPa), normoxemia (8.0–40.0 kPa) and hyperoxemia (>40kPa). Our primary analyses investigated the association of the maximum and minimum (extreme value) exposure groups with neurological outcome at 6-month follow-up. For our secondary analyses we designed a regression model with cut-off points across increasing PaO_2 values. We furthermore investigated the association of time weighted mean PaO_2 over all measuring points (total exposure) and the first 4 measuring points (early exposure) with neurological outcome, and the association of PaO_2 with peak serum tau. The main outcome was poor neurological outcome according to CPC, dichotomized into good (CPC 1 and 2) and poor (CPC 3 to 5) at 6-month follow-up. For the serum tau analysis, we used peak serum tau at 48 or 72 hours as outcome.

4.4.3 Paper III

Objective and design

This study was an analysis of prospectively collected data of the INTCAR 2.0 database. The objective was to investigate the association between exposure to extreme PaO_2 and $PaCO_2$ values in comatose adult OHCA patients. We also investigated cut-off points for the onset of the association with poor outcome across increasing and decreasing

PaO_2 levels as well as decreasing $PaCO_2$ levels. Furthermore, we analyzed combinations of PaO_2 and $PaCO_2$, that may, according to previous to previous findings, be associated with beneficial or detrimental outcome. The INTCAR 2.0 database analysis followed up and substantiated the results from paper I and II.

Patients

2162 adult (≥18 years of age) OHCA patients included in the INTCAR 2.0 database.

Methods

For the primary analyses, patients were allocated to groups according to their extreme value exposure; hyperoxemia (PaO_2 >40 kPa), hypoxemia (PaO_2 <8 kPa) hypercapnemia ($PaCO_2$ >6.7 kPa) and hypocapnemia ($PaCO_2$ <4 kPa). The groups of patients not exposed to extreme PaO_2 or $PaCO_2$ values were defined as normoxemia (PaO_2 8–40 kPa) and normocapnemia ($PaCO_2$ 4.0–6.7 kPa), respectively. The association with poor neurological outcome was evaluated with logistic regression models. For our secondary analyses we created regression models across ascending and descending PaO_2 levels and descending $PaCO_2$ levels to investigate possible threshold values associated with the onset of poor neurological outcome. In a further analysis we created two exposure combination groups, 1. hypercapnemia combined with normoxemia and 2. hypocapnemia combined with hyperoxemia and investigated their association with poor outcome.

4.4.4 Paper IV

Objective and design

This study was an analysis of serum biomarkers of neurological injury prospectively collected during the TTM-trial. We described the release dynamics of the serum biomarkers GFAP and UCH-L1 at 24, 48 and 72 hours after OHCA and investigated their accuracy to predict outcome. The outcome predictive accuracy of GFAP, UCH-L1 and their combination (GFAP+UCH-L1) was compared to that of NSE. We aimed in particular to compare the accuracy to predict outcome at high specificity (100% to 95%). The outcome parameter employed was CPC at 6-month follow-up, dichotomized into good (CPC 1 and 2) and poor (CPC 3 to 5).

Patients

29 of 36 TTM-trial sites participated in the collection of serum biomarker samples and samples were collected from 819 patients.

Methods

The processed serum biomarker blood samples collected during the TTM-trial were stored at the Integrated Biobank of Luxembourg. In November 2018, the samples were transferred to the United States. GFAP and UCH-L1 were analyzed in co-operation with Banyan Biomarkers, Inc., employing a commercially available in vitro diagnostic chemiluminescent enzyme-linked immunosorbent assay (Banyan BTI©, Banyan Biomarkers, Inc., San Diego, CA). All analyses were performed by board-certified laboratory technicians blinded to all clinical data. We analyzed the overall release dynamics over our measuring points at 24, 48 and 72 hours after OHCA, and separately in the good and poor outcome cohort. The predictive accuracy for poor outcome of GFAP, UCH-L1 and GFAP+UCH-L1 was tested with receiver-operating characteristics (ROC) analysis, by calculating the area under the ROC curve (AUROC). The outcome predictive performance of NSE in the same cohort had previously been analyzed by Stammet et al.[82] In order to investigate the accuracy of GFAP, UCH-L1, GFAP+UCH-L1 and NSE to predict outcome at high specificities, we calculated a partial AUROC for specificities from 100% to 95% only (Figure 6). The maximum partial AUROC (area of the dashed line in figure 6) was normed to 1.0. The results of the partial AUROC of GFAP, UCH-L1 and GFAP+UCH-L1 were compared to the partial AUROC of NSE in a subsequent analysis. To investigate the improvement of outcome predictive accuracy, we tested different models adding GFAP+UCH-L1 to clinical information (age, sex, time to ROSC, bystander CPR [yes/no], initial rhythm shockable [yes/no] and serum lactate on admission), and neurological examination (bilaterally absent corneal reflexes and bilaterally absent pupillary reflexes). Serum samples were tested for hemolysis using the Roche haemolysis index with measurements at 600 and 570 nm, and a haemolysis index (≥500 ng/ml of haemoglobin) being regarded as positive.

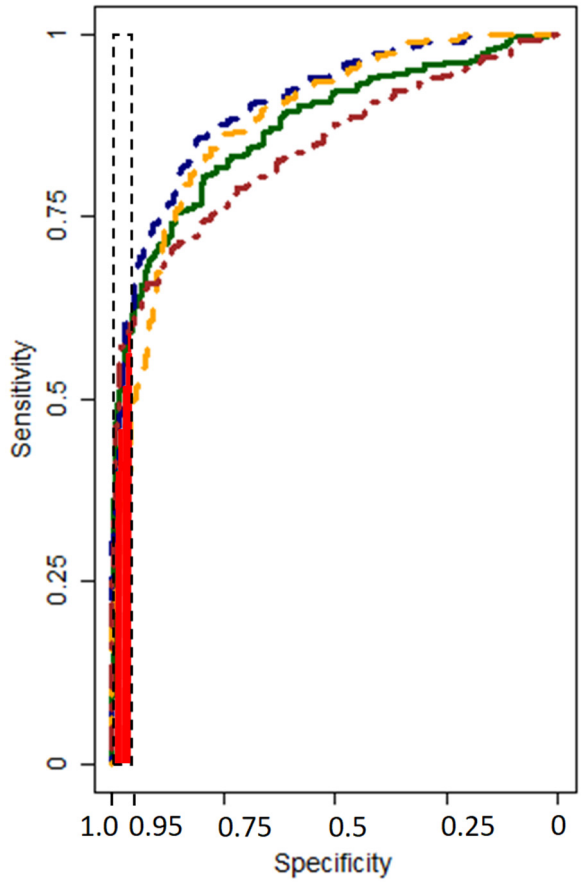

Figure 6. Example of the partial AUROC, used to compare prognostic serum biomarker accuracy at specificities from 100-95%. The area of the dashed line depicts maximum partial AUROC at specificities 100-95%. The red area under the ROC curve depicts the partial AUROC at 100-95% for the biomarker depicted by the blue ROC curve.

4.5 Statistics

For all of our analyses, null hypothesis testing was performed using two tailed tests and a two-sided significance level of P <0.05 was considered statistically significant. Statistical analyses were conducted using IBM SPSS statistics for Windows (version 22.0, Armonk NY), R Studio (v. 1.1.456 The R Foundation for Statistical Computing) and R: A language and environment for statistical Computing (version 3.3.3 R Foundation for Statistical Computing, Vienna, Austria).[231] The R package mice was used for multiple imputations.[232]

Missing data.

Missingness of the PaO_2 and $PaCO_2$ data in the TTM-trial database was assumed at random (MAR). MAR implies a systematic relationship between the missing and the observed data but not between the missing data and the propensity for missing. Testing for the assumption of MAR is so far not possible since it would require testing the non-existing data against the existing.[233] Therefore, MAR was assumed plausible on the basis of the evaluation of the existing data and expert opinion. In contrast to MAR, missing completely at random (MCAR), assuming that there is no relationship between missing data and any values in the data set, missing or observed, can be tested for, if feasible with Little´s MCAR test. However, Little´s MCAR test might have low power in small sample sizes and overestimates trivial differences in very large samples.[234] Nevertheless, missing data regardless of MCAR or MAR can be compensated for, using imputation techniques for example single value imputation or multiple imputations.

Imputation of missing data points

For the analyses in the TTM database we used multiple imputations, avoiding the risks inherent to single imputations techniques, e.g., the overestimation of the preciseness of the data which may increase the risk for a type I error.[235] We created an imputation model that consisted of data derived from independent variables included in our analysis model and auxiliary data available on the same and matching patients. Multiple datasets with varying values were generated and assessed. In a final step, the estimates from the regression models and summary measures for each imputed sample were pooled into one estimate with 95% confidence intervals (CI) according to the Rubin Rule, reflecting the inability to obtain the precise missing value.[236] In the INTCAR 2.0 database, we used the last observation carried forward (LOCF) method to impute missing long-term outcome data in a secondary analysis. This method is regarded inferior to multiple imputations and prone to bias. However, due to the limited number of data points available, its use was feasible in the INTCAR 2.0 analysis.[237,238]

Descriptive statistics

Normally distributed data was presented as mean with the corresponding standard deviation (SD), while not normally distributed measures were presented as median and interquartile range (IQR). Categorical data were presented as numbers and valid percent.

Regression models

For the analysis of the association with a binary dependent variable, logistic regression analysis was used. Linear regression models were employed to analyze dependent

continuous variables. Our regression models included a number of *a priori* defined, and in the context of OHCA, relevant co-variables (specified in the respective papers). The number of variables was chosen in keeping with the "one in ten" rule in order to avoid overfitting and increased bias.[239] Goodness of fit was tested using the Hosmer-Lemeshow test. The results of our regression analyses were presented as odds ratios, 95% CIs and P-values.

Bivariate associations

The relationship between variables from not normally distributed samples were investigated using non-parametrical tests (Mann-Whitney U or Kruskal-Wallis H). The null hypothesis was rejected when the resulting P-values were less than the significance level of 0.05.

Diagnostic performance testing

Serum biomarker data was due to skewness $\log^{10}$ transformed before analysis. To establish the diagnostic performance for poor outcome for the different serum biomarkers or combined biomarker models investigated in paper IV, receiver operating characteristic (ROC) curves were calculated by plotting the false positive rate (1-specificity) against the true positive rate at different serum biomarker level cut-points. The area under the ROC curve (AUROC), ranging from 0 to 1.0 was used to establish the discriminative accuracy for detecting poor neurological outcome of the serum biomarkers or the combined models (Figure 7). The closer to 1.0 (maximum discrimination) the AUROC of the investigated serum biomarker or combined model was, the better the overall discrimination between poor and good outcome. So far, there are no established definitions for the discriminatory accuracy of a ROC, but as general rule of thumb, the following guidelines were used: ROC= 0.5: no discrimination (equals tossing a coin as discriminator), 0.5< ROC <0.7= poor discrimination, 0.7≤ ROC <0.8= acceptable discrimination, 0.8≤ ROC <0.9= excellent discrimination, ROC ≥0.9= outstanding discrimination.[164] A test yielding an AUROC <0.5 performs worse than chance or the result might be an indicator for an analysis error.

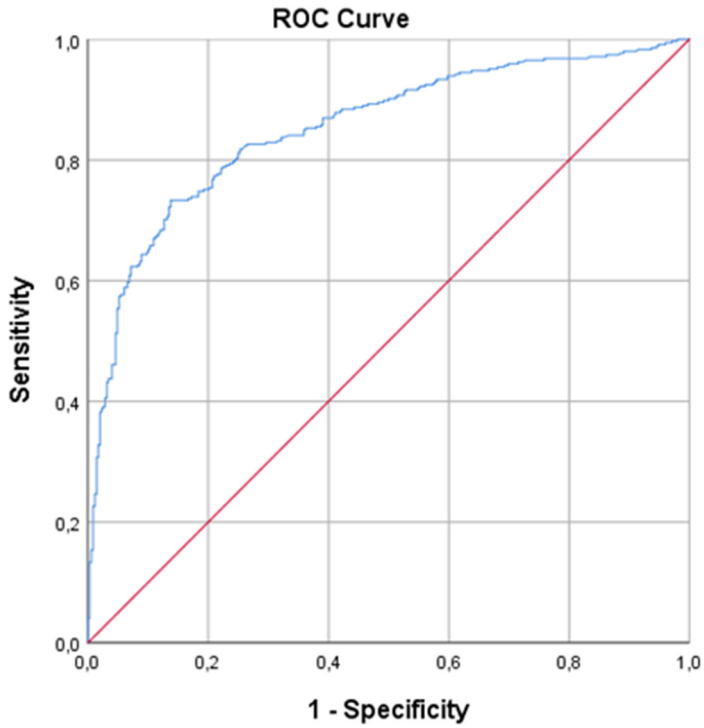

Figure 7. Plot of sensitivity versus 1-specificity for possible cut-points of a serum biomarker discriminating between good and poor outcome with an AUROC of 0.85 (outlined by the blue line). The red line depicts the 0.5 AUROC.

Model selection criterion

In paper IV we used the Akaike Information Criterion (AIC) to compare models in the same investigated group, to reach a balance between an overly simplistic and an overly complex model fit (underfitting vs overfitting) of the models employed.[240] A difference ≥2 in AIC between models was considered significant and the model with the lowest AIC was regarded as the best representation of the underlying model structure and therefore the most preferable.

5 Results

The detailed description of all results are presented in the attached original papers.

5.1 Paper I, II and III

5.1.1 Patient and outcome characteristics in paper I, II and III

In paper I and II of this doctoral thesis, we analyzed data of 939 OHCA patients included in the TTM-trial database. Our objective was to analyze a homogenous group of OHCA patients, therefore, we decided not to include patients who demised before the end of the intervention period. This group of 62 patients was the largest excluded group, followed by patients with no outcome data (n= 6) and no PaO_2 or $PaCO_2$ data (n= 2). The cohort finally included in our analyses comprised of 869 patients.

We revisited the analysis of PaO_2 and $PaCO_2$ in paper III, in a different group of OHCA patients collected in 22 ICUs in North America and Europe in the INTCAR 2.0 database. From this database we were able to include 2135 of 2162 OHCA patients. 27 patients were conscious on arrival to hospital and were therefore excluded. The baseline characteristics of the TTM-trial cohort and the INTCAR 2.0 cohort were to some degree different from each other and are presented in table 1. Survival and neurological outcome data of the TTM-trial cohort (paper I and II) and the INTAR 2.0 cohort (paper III) are presented in table 2. It is of importance, to point out that the primary outcome in the TTM-trial cohort was neurological outcome at 6-month follow-up, whereas we used neurological outcome at discharge as primary outcome measure in the INTCAR 2.0 cohort.

In the INTCAR 2.0 database, the documentation of PaO_2, $PaCO_2$, treatment, patient and cardiac arrest data was less precise compared to the TTM database, as described under section 4.4. The definition of $PaCO_2$ values differs somewhat between paper I and paper III (see section 4.4.1 and 4.4.3).

Table 1. Baseline characteristics of patients included in paper I and II, and paper III		
Demographic characteristic	Paper III n= 2135	Paper I and II n= 869
Age in years, mean (SD)	61.1 (15.9)	63.9 (12.2)
Male sex, n (%)	1432 (67.1)	707 (81.4)
Medical history		
Chronic heart failure n (%)	367 (17.2)	55 (6.3)
COPD n (%)	344 (16.1)	86 (9.9)
Cerebrovascular disease n (%)	196 (9.2)	69 (8.0)
Arterial hypertension n (%)	N.A.	347 (40.1)
Diabetes mellitus n (%)	521 (24.4)	128 (14.8)
Cardiac arrest characteristic		
Witnessed cardiac arrest n (%)	1591 (75.6)	783 (90.1)
Bystander CPR n (%)	1385 (65.5)	638 (73.4)
Bystander defibrillation n (%)	123 (5.8)	84 (9.7)
Initial rhythm shockable n (%)	1022 (50.0)	710 (81.7).
Time to ROSC, mean (SD)	30.9 (22.5)	30.4 (21.7)
Characteristic on arrival		
Sedated on arrival, n (%)	437 (21.7)	254 (29.4)
GCS Motor 1, n (%)	1544 (79.4)	443 (51.3)
Circulatory shock on admission, n (%)	902 (44.2)	111 (12.8)
Admission pH, median (IQR)	7.2 (7.1–7.3)	7.2 (7.1–7.3)

n= number, SD= standard deviation, IQR= interquartile range, %= percent, CPR= cardiopulmonary resuscitation, ROSC= return of spontaneous circulation, COPD= chronic obstructive pulmonary disease, GCS= Glasgow coma scale, all % are presented as valid percent.

Table 2. Outcome comparison of the cohorts included in paper I and II, and Paper III		
	Paper III n= 2135*	Paper I and II n= 869†
Good outcome (%)	28.9	50.6
Poor outcome (%)	71.1	49.4
Alive (%)	38.8	55.8
Dead (%)	61.2	44.2

n= number, %= percent, * neurological outcome and survival evaluated at discharge from hospital, †neurological outcome and survival at 6-month follow-up.

5.1.2 $PaCO_2$ analyses, exposure characteristics

In paper I, exposure to abnormal $PaCO_2$ values at some point within the first 37 hours after OHCA was common, 685 of 869 (79%) patients were hypercapnemic, 516 of 869 (59%) patients were hypocapnemic, 371 (43%) patients were both hypocapnemic and hypercapnemic, and 39 (4%) patients were normocapnemic throughout. Exposure to extreme $PaCO_2$ values occurred predominantly early after OHCA (Figure 8). In paper III exposure patterns were less prominent; 670 (34.5%) patients experienced hypercapnemia, 458 (23.6%) hypocapnemia and 222 (11.4%) both.

5.1.3 $PaCO_2$, primary analyses

In paper I and paper III we investigated the association between extreme $PaCO_2$ values and neurological outcome. The primary analyses in both studies were the association of the exposure to $PaCO_2$ over or under our threshold values and neurological outcome. In none of these analyses were we able to show a statistically significant association with neurological outcome. However, the point estimates for hypercapnemia exposure indicated a lower probability for poor neurological outcome, while the point estimates for hypocapnemia indicated a higher probability or no difference between the groups, for poor neurological outcome (Table 3).

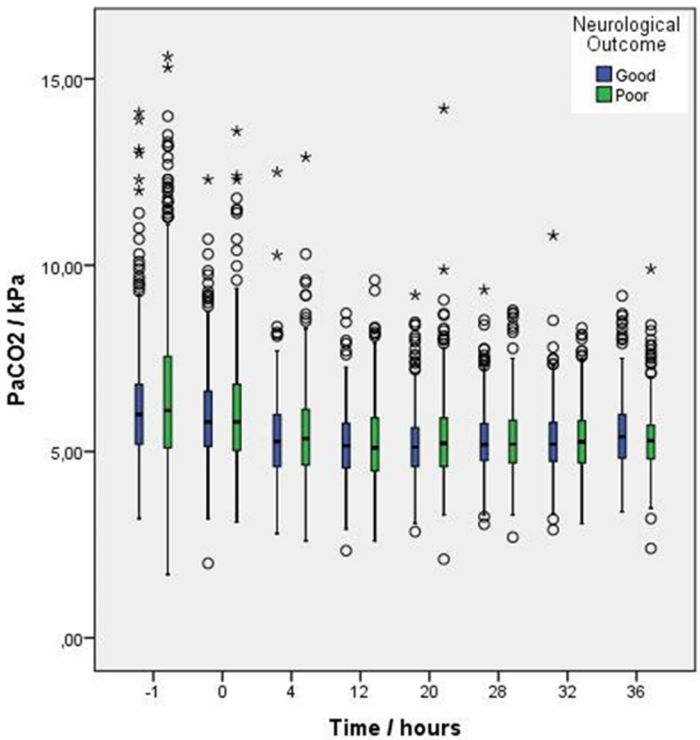

Figure 8. $PaCO_2$ over the first 37 hours after ROSC in the TTM-trial cohort in paper I (n= 869), divided into good (blue boxplots) and poor outcome (green boxplots). Presented as boxplots with median and 25% quartiles from median, and range. o= outliers, *= extreme outliers

Table 3. Associations between $PaCO_2$ and neurological outcome in paper I and paper III						
	Paper III n= 2135			Paper I n= 869		
Analysis	OR	95% CI	P-value	OR	95% CI	P-value
Hypercapnemia versus normocapnemia	0.89	0.64–1.24	0.49	0.70	0.44–1.11	0.13
Hypercapnemia versus no-hypercapnemia	0.86	0.64–1.15	0.31	0.80	0.51–1.22	0.31
Hypocapnemia versus normocapnemia	1.28	0.90–1.83	0.18	0.96	0.64–1.45	0.85
Hypocapnemia versus no-hypocapnemia	1.23	0.91–1.66	0.18	1.04	0.72–1.49	0.82

n= number, OR= odds ratio, 95% CI= 95% confidence interval, $PaCO_2$= arterial pressure of carbon dioxide.

5.1.4 $PaCO_2$, secondary analyses

In paper I, we investigated the association of time weighted mean $PaCO_2$ values for all sampling points (total exposure) and for the first 4 sampling points (early exposure). We did not find a significant association with neurological outcome either in the total exposure group (OR 1.09, 95% CI 0.83–1.42; P= 0.53) or the early exposure group (OR 0.99, 95% CI 0.81–1.22; P= 0.96). The maximum $PaCO_2$ difference was also not independently associated with neurological outcome (OR 1.04, 95% CI 0.91–1.18; P= 0.56).

The time-weighted mean $PaCO_2$ cohort was also configured to create the two groups for the TTMH analysis in paper I. However, to increase robustness in our analyses we increased the range of the groups to 4.5–6.0 kPa and 6.0–7.3 kPa. We were not able to show a difference in neurological outcome between the groups (OR 1.01, 95% CI 0.60–1.67; P= 0.98). In a further analysis we subdivided the TTMH groups according to target temperature (33 °C and 36 °C), but again, we were not able to show a significant difference between the temperature groups (OR 0.96, 95% CI 0.68–1.35; P= 0.83). There was also no difference in peak serum tau levels between the groups (OR 0.75, 95% CI 0.43–1.28; P= 0.29)

In a univariable analysis in paper I, we investigated maximum $PaCO_2$ and lowest pH separately. Both variables were significantly associated with poor neurological outcome (OR 1.17, 95% CI 1.06–1.28; P <0.001 and 0.03, 95% CI 0.01–0.09; P <0.001, respectively). In a model including both variables, only lowest pH maintained an independent association with neurological outcome (OR 0.02, 95% CI 0.05–0.11; P <0.001) whereas the association between maximum $PaCO_2$ and poor neurological outcome lost statistical significance (OR 0.97, 95% CI 0.86–1.09; P= 0.62).

Of the 689 patients in the serum tau analysis in paper I, 64 met our criteria for exclusion and 36 had missing peak serum tau levels at 48 or 72 hours. The multivariable analysis

of the remaining 589 patients showed no association between $PaCO_2$ and serum tau in our models (P= 0.12–1.00).

In paper III, we investigated the combination of hypercapnemia with normoxemia and the combination of hyperoxemia with hypocapnemia and found no statistically significant association with neurological outcome. However, the point estimate in the group exposed to hypocapnemia with hyperoxemia indicated a higher probability for poor neurological outcome (OR 1.67, 95% CI 0.89–3.14; P= 0.11), whereas the group exposed to normoxemia with hypercapnemia indicated no difference (OR 0.96, 95% CI 0.63–1.48; P= 0.86).

The analysis across decreasing $PaCO_2$ cut-off points did not reveal a threshold for the onset of poor neurological outcome (Figure 9).

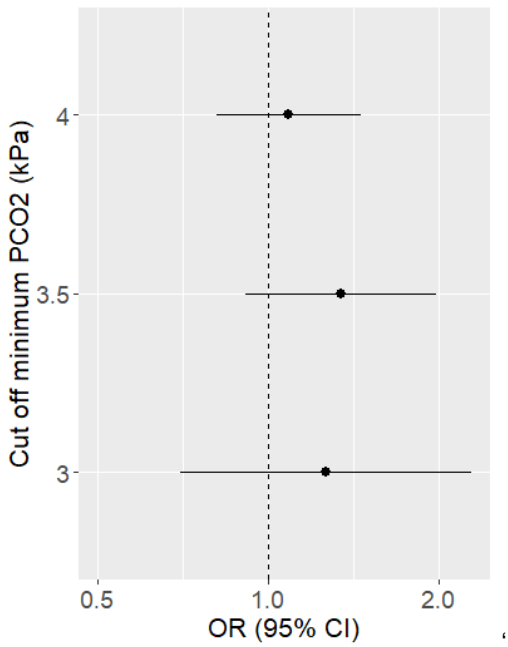

Figure 9. Forest plot showing the adjusted ORs (bullet points) with 95% CI (horizontal lines) for poor neurological outcome (CPC 3-5) across descending $PaCO_2$ cut-off points (c). ORs and CIs are presented on a logarithmic scale. OR above 1.0 indicates worse outcome under the $PaCO_2$ threshold. OR= Odds ratio, 95% CI= 95% confidence interval, CPC= cerebral performance category, $PaCO_2$= arterial partial pressure of carbon dioxide.

5.1.5 PaO_2 analyses, exposure characteristics

The analyses in paper II and III showed that exposure to extreme PaO_2 values at some time point after OHCA was a common occurrence. In paper II, 199 of 869 (22.9%)

patients were exposed to hyperoxemia, 112 (12.9%) were exposed to hypoxemia, 11 (1.3%) experienced both, hyper- and hypoxemia, and 569 (65.5%) were not exposed to extreme PaO_2 values. In paper III 357 (18.7%) patients were exposed to hyperoxemia, 343 (17.9%) patients to hypoxemia and 76 (3.9%) to both. The analyses in paper II also showed that hyperoxemia exposure occurred predominantly early, with 197 of 199 patients in this group exposed to hyperoxemia within the first 5 h after admission to hospital (Figure 10).

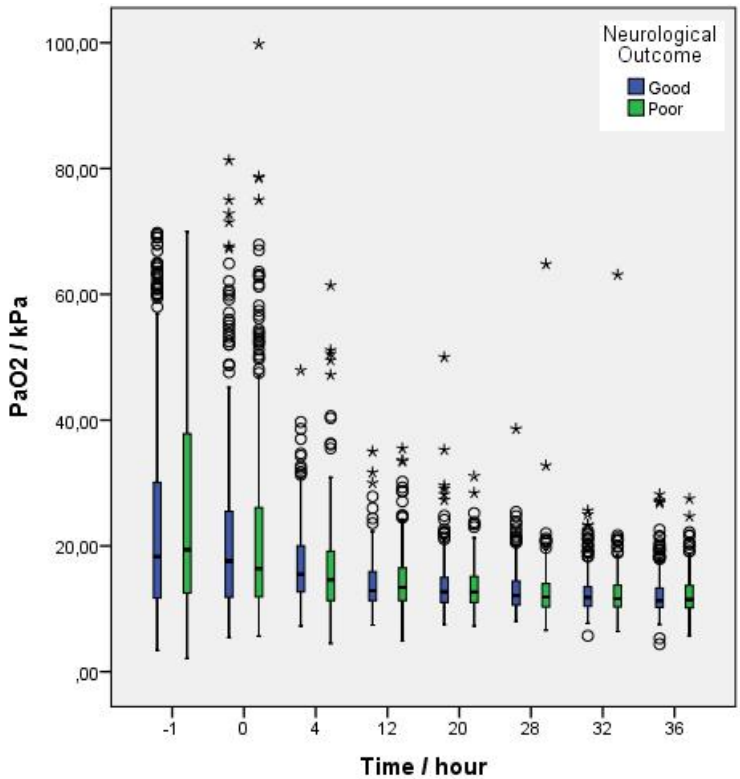

Figure 10. PaO_2 over the first 37 hours after ROSC in the TTM-trial cohort (n= 869), divided into good (blue boxplots) and poor outcome (green boxplots). Presented as boxplots with median and 25% quartiles from median, and range. o= outliers, *= extreme outliers

5.1.6 PaO_2, primary analyses

In paper II and III, we investigated the association between exposure to single extreme PaO_2 values and outcome at 6-month follow-up. We did not find an independent association between exposure to hyperoxemia or hypoxemia and poor neurological

outcome. However, in both studies, the point estimates indicate a lesser probability for poor outcome in the group not exposed to hyperoxemia (Table 4).

Table 4. Associations between PaO_2 and neurological outcome in the analyses in paper II and III

	Paper III analyses		n= 2135	Paper II analyses		n= 869
Analysis	OR	95% CI	P-Value	OR	95% CI	P-Value
Hyperoxemia versus normoxemia	1.33	0.92–1.92	0.13	1.24	0.81–1.89	0.31
Hyperoxemia versus no-hyperoxemia	1.25	0.88–1.17	0.22	1.28	0.86–1.91	0.22
Hypoxemia versus normoxemia	1.26	0.87–1.82	0.22	1.06	0.60–1.85	0.85
Hypoxemia versus no-hypoxemia	1.15	0.81–1.64	0.44	1.13	0.66–1.91	0.65

n= number, OR= odds ratio, 95% CI= 95% confidence interval, PaO_2= arterial pressure of oxygen.

5.1.7 PaO_2, secondary analyses

We investigated possible cut-off values for the onset of poor neurological outcome across increasing PaO_2 ranges in paper II and expanded this investigation in paper III with analyses across increasing PaO_2 and decreasing PaO_2 ranges. Neither in paper II nor in paper III were we able to show cut-off values which were independently associated with poor neurological outcome. Figure 11 shows the cut-off analyses across different ranges of PaO_2, from paper III.

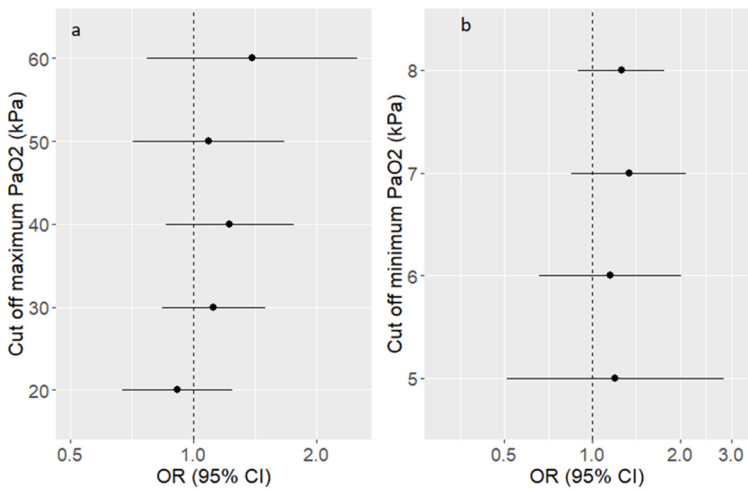

Figure 11. Forest plot showing ORs with 95% CI for poor neurological outcome (CPC 3-5) across ascending PaO_2 cut-off points (a), descending PaO_2 cut-off points (b). For (a), OR above 1.0 indicates worse outcome above the PaO_2 threshold. For (b) OR above 1.0 indicates worse outcome under the PaO_2 threshold.

The time weighted mean PaO_2 analyses conducted in paper II did not show an association with neurological outcome over all measuring points (total exposure) (OR 1.03, 95% CI 0.97–1.09; P= 0.375) or over the first 4 measuring points (early exposure) (OR 1.02, 95% CI 0.98–1.05; P= 0.288).

Of the 689 patients in the serum tau analysis in paper II, 64 met our exclusion criteria and 36 had missing peak serum tau levels at 48 or 72 hours after OHCA, leaving 589 patients for analysis. We did not find statistically significant associations between our PaO_2 multivariable models and highest serum tau at either 48 or 72 hours after ROSC (P= 0.20–0.69).

5.2 Paper IV

In paper IV we described the release dynamics of two serum biomarkers of neurological injury, GFAP and UCH-L1, and investigated the accuracy of GFAP, UCH-L1 and their combination (GFAP+UCH-L1) in predicting long-term neurological outcome. GFAP and UCH-L1 were sampled at 24, 48 and 72 hours after OHCA in a cohort of 819 patients collected at 29 hospitals during the TTM-trial. 717 patients had at least one serum biomarker value registered at the different time-points and were included in the final analysis. We also compared the accuracy of GFAP, UCH-L1 and GFAP+UCH-L1 in predicting neurological outcome with that of NSE.

Median UCH-L1 reached maximum values at 24 hours and decreased over the 48 and 72 hours measuring points, whereas median GFAP reached maximum value after 48 hours (Table 5).

Table 5. Median GFAP and UCH-L1 levels after OHCA

All patients (median, IQR)	pg/ml	All patients (median, IQR)	pg/ml
UCH-L1 24h	433 (209-1266)	GFAP 24h	53 (25-127)
UCH-L1 48h	353 (180-1242)	GFAP 48h	59 (27-156)
UCH-L1 72h	242 (141-692)	GFAP 72h	45 (21-137)

pg/ml= picogram per milliliter, h= hours, GFAP= Gliary fibrillary acidic protein, UCH-L1= Ubiquitin Carboxy-terminal Hydrolase L1.

5.2.1 Primary analyses, prediction of poor outcome

Dividing the median GFAP and UCH-L1 levels according to good and poor outcome showed that median GFAP and UCH-L1 levels were significantly higher in poor outcome patients at all time-points (P<0.001, for all measurements) (Figure 12).

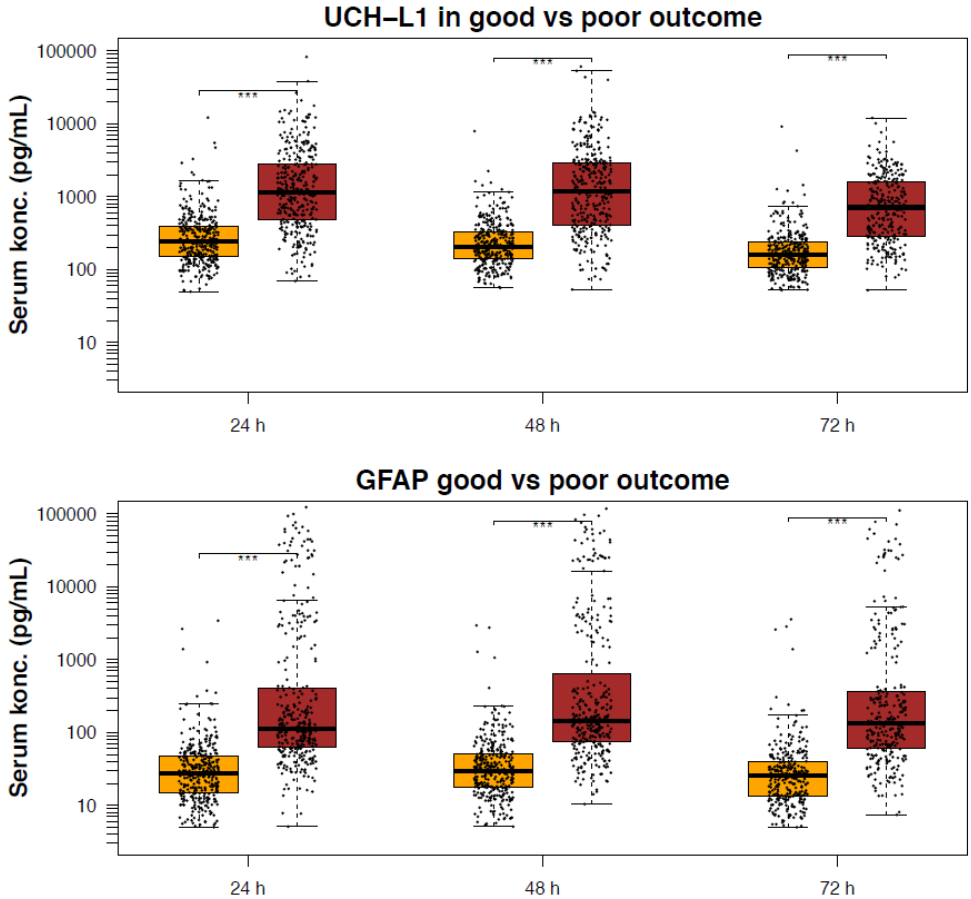

Figure 12. GFAP and UCH-L1 levels in patients with good (CPC 1 and 2) (yellow boxplots) and poor (CPC 3-5) (red boxplots) outcome at 24, 48 and 72 hours after OHCA. Levels of GFAP and UCH-L1 as boxplots with median and first and third quartile. Whiskers depict the smallest and largest non-outliers. All measuring points outside the whiskers are outliers. Median GFAP levels at 24 hours, 27.9 (15.0-47.6) vs 143.8 (74.0-588.8) pg/mL, at 48 hours, 30.0 (17.7-50.7) vs 143.8 (74.0-588.8) pg/mL, and at 72 hours, 25.3 (13.5-39.3) vs 132.4 (60.9-364.2) pg/mL (P <0.001 for all measurements). Median UCH-L1 levels at 24 hours, 241.1(150.1-388.5) vs 1132.2 (479.6-2784.4) pg/mL, at 48 hours 305.6 (140.0-329.4) vs 1180.4 (411.3-2884.9) pg/mL, and at 72 hours 160.9 (107.2-240.6) vs 712.7 (284.2-1601.0) pg/mL, (P <0.001 for all measurements). GFAP= Gliary fibrillary acidic protein, UCH-L1= Ubiquitin Carboxy-terminal Hydrolase L1, pg/mL= picogram per mililiter, h= hours.

The ROC analyses for prediction of poor outcome at 6-month follow-up for serum samples collected at 24, 48 and 72 hours after OHCA, are depicted in Figure 13 together with the AUROC of the serum biomarkers.

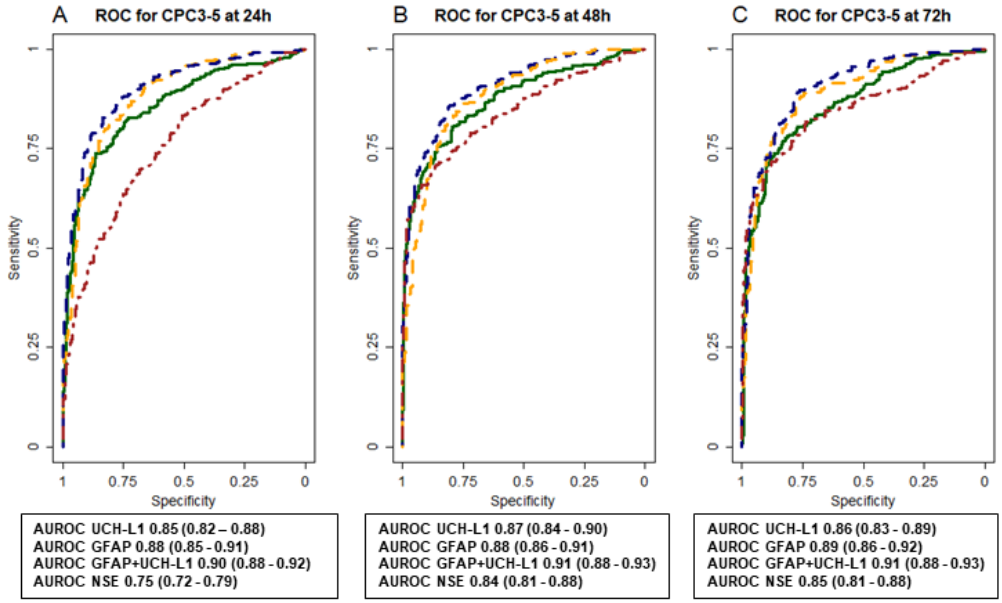

Figure 13. A-C, Receiver-operating characteristic (ROC) analyses for prediction of Cerebral Performance Category Scale (CPC) 1-2 vs. CPC3-5 at 6-months follow-up for serum samples collected at 24, 48 and 72 hours after OHCA. Area Under the ROC curve (AUROC) for UCH-L1 (green curve) was significantly greater than AUROC NSE (brown curve) at 24 hours ($p<0.001$) and 48 hours ($p=0.03$), but not at 72 hours ($p=0.34$). AUROC for GFAP (yellow curve) was significantly greater than AUROC NSE at all time-points ($p<0.001$, $p=0.03$, $p=0.02$). AUROC for GFAP+UCH-L1 (blue curve) was significantly greater than AUROC NSE at all time-points ($p<0.001$). These tests were performed on patients for whom data were available for all serum biomarkers (24 hours, n=633; 48 hours, n= 597; 72 hours, n=558). GFAP= Gliary fibrillary acidic protein, UCH-L1= Ubiquitin Carboxy-terminal Hydrolase L1, NSE= Neuron specific enolase.

Compared to NSE at the same time-points, the AUROC of GFAP was significantly greater than the AUROC of NSE at all time-points. The AUROC of UCH-L1 was also significantly greater than the AUROC of NSE at 24 and 48 but not at 72 hours after OHCA. The AUROC of GFAP+UCH-L1 was at all time-points significantly greater than the AUROC of NSE (Table 6).

Table 6. AUROC of UCH-L1, GFAP, GFAP+UCH-L1 versus NSE at 24, 48 and 72 hours after OHCA.			
Serum biomarker	Time-point	AUROC (95% CI)	P-value AUROC
UCH-L1 vs NSE	24h	0.85 (0.82–0.88) vs 0.75 (0.72–0.79)	<0.001
GFAP vs NSE	24h	0.88 (0.85–0.91) vs 0.75 (0.72–0.79)	<0.001
GFAP+UCH-L1 vs NSE	24h	0.90 (0.88–0.92) vs 0.75 (0.72–0.79)	<0.001
UCH-L1 vs NSE	48h	0.87 (0.84–0.90) vs 0.84 (0.81–0.88)	0.028
GFAP vs NSE	48h	0.88 (0.86–0.91) vs 0.84 (0.81–0.88)	0.028
GFAP+UCH-L1 vs NSE	48h	0.91 (0.88–0.93) vs 0.84 (0.81–0.88)	<0.001
UCH-L1 vs NSE	72h	0.86 (0.83–0.89) vs 0.85 (0.81–0.88)	0.338
GFAP vs NSE	72h	0.89 (0.86–0.92) vs 0.85 (0.81–0.88)	0.024
GFAP+UCH-L1 vs NSE	72h	0.91 (0.88–0.93) vs 0.85 (0.81–0.88)	<0.001

GFAP= Gliary fibrillary acidic protein, UCH-L1= Ubiquitin Carboxy-terminal Hydrolase L1, NSE= Neuron specific enolase, h= hours, 95% CI= 95% confidence interval.

The partial AUROC 100–95% normed to 1.0 of GFAP, UCH-L1, GFAP+UCH-L1 compared to NSE at 24, 48 and 72 hours after OHCA are shown in table 7. These analyses revealed that at high specificities, the AUROC of UCH-L1 and the AUROC of GFAP+UCH-L1 were significantly greater at 24 hours than the AUROC of NSE, but not at 48 and 72 hours. The partial AUROC of GFAP was significantly smaller than the AUROC of NSE at 48 hours and showed a borderline P-value at 72 hours.

Table 7. Partial AURCOC of GFAP, UCH-L1, GFAP+UCH-L1 versus NSE at 24, 48 and 72 hours after OHCA			
Serum biomarker	Time-point	Partial AUROC 100 - 95% (95% CI)	P-value
UCH-L1 vs NSE	24h	0.67 (0.61–0.73) vs 0.60 (0.57–0.64)	0.042
GFAP vs NSE	24h	0.64 (0.60–0.71) vs 0.60 (0.57–0.64)	0.164
GFAP+UCH-L1 vs NSE	24h	0.72 (0.67–0.77) vs 0.60 (0.57–0.64)	<0.001
UCH-L1 vs NSE	48h	0.75 (0.70–0.80) vs 0.75 (0.71–0.80)	0.757
GFAP vs NSE	48h	0.67 (0.62–0.72) vs 0.75 (0.71–0.80)	0.004
GFAP+UCH-L1 vs NSE	48h	0.75 (0.70–0.80) vs 0.75 (0.71–0.80)	0.884
UCH-L1 vs NSE	72h	0.70 (0.63–0.76) vs 0.75 (0.69–0.80)	0.193
GFAP vs NSE	72h	0.67 (0.62–0.74) vs 0.75 (0.69–0.80)	0.050
GFAP+UCH-L1 vs NSE	72h	0.72 (0.66–0.79) vs 0.75 (0.69–0.80)	0.539

GFAP= Gliary fibrillary acidic protein, UCH-L1= Ubiquitin Carboxy-terminal Hydrolase L1, NSE= Neuron specific enolase, h= hours, 95% CI= 95% confidence interval.

At high specificities the sensitivities of GFAP, UCH-L1 and GFAP+UCH-L1 were similar to that of NSE after OHCA at the different time-points, with frequently overlapping 95% CI. The maximum sensitivity, for example, at 98% specificity, for GFAP was 38% (95% CI 33–44) at 72 hours, UCH-L1 showed a maximum sensitivity at 48 hours of 51% (95% CI 46–56) and the maximum sensitivity for GFAP+UCH-L1 was 51% (95% CI 37-64) also at 48 hours. NSE showed a maximum sensitivity of 58% (95% Cl 52–64) at 48 hours.

5.2.2 Secondary analyses, hemolysis

Hemolysis had no effect on GFAP sampled at 24 or 48 hours, but GFAP was lower at 72 hours in patients with hemolysis (median 22 (14–58) vs 47 (23–137) pg/mL), P= 0.004). However, a sensitivity analysis excluding 24 hour samples with hemolysis (n= 31), 48 hour samples with hemolysis (n= 23) and 72 hour samples with hemolysis (n= 31) showed that the main results were not affected by removing these samples. UCH-L1 concentrations were not affected by the presence of hemolysis.

5.2.3 Secondary analyses, TTM

GFAP levels did not differ between patients in the 33°C and 36°C groups at 24 hours, but GFAP levels were significantly lower at 48 and 72 hours for patients in the 36°C group compared to the 33°C group (P= 0.04). A similar pattern was revealed when we compared the good outcome temperature groups with each other, again, we found lower GFAP values in the 36°C group at 48 and 72 hours (P <0.001). However, there was no difference between the poor 33°C and 36°C poor outcome groups. UCH-L1 values did not differ between the 33°C and the 36°C groups.

6 Discussion

OHCA occurs suddenly and unexpectedly, and without immediate care, chances of survival with good neurological outcome deteriorate rapidly. Substantial efforts have been made during recent decades to improve survival and neurological outcome after OHCA. National and international committees have raised public awareness, illustrated by a significant increase in bystander CPR, the development of dispatcher assisted CPR, and more recently, the availability of automated external defibrillators in public places. The efforts have also led to the implementation of automatic CPR devices in advanced cardiac life support algorithms and higher standards of prehospital care and monitoring, as well as current research in highly resource and cost intensive approaches like extra-corporeal cardiopulmonary resuscitation.

As shown previously, admission to hospital as well as 30-day survival after out of hospital cardiac arrest OHCA has increased in recent years and the majority of the 30-day survivors after OHCA are discharged with good neurological function. However, survival to discharge is still an exception since the proportion of patients dying after hospital admission is more than fifty percent. The major causes of death and poor neurological outcome are the primary anoxic-ischemic cerebral injury sustained during the no-flow time of the OHCA and the additional secondary cerebral reperfusion injury that commences at ROSC.

TTM to 32-34°C has been suggested as an intervention to attenuate the anoxic-ischemic injury sustained during OHCA and to improve neurological outcome. After initial optimism, more recent studies are inconclusive and the effectiveness of TTM has been questioned. Current studies indicate varying use internationally.[241] The ongoing TTM-2 trial randomizing 1900 patients to either 33°C or strict normothermia investigates the effectiveness of TTM treatment further and results will be presented in 2021.

In this doctoral thesis, we investigated data of OHCA patients collected in two high resolution databases. In paper I, II and III we investigated the association of PaO_2 and $PaCO_2$ in the period following ROSC with neurological outcome. In paper I and II we also investigated the association of PaO_2 and $PaCO_2$ with a serum biomarker of neurological injury. In paper IV we analyzed two new serum biomarkers of neurological

injury, GFAP and UCH-L1 and compared their accuracy to predict poor neurological outcome with the current standard serum biomarker, NSE.

6.1 Paper I, II and III

6.1.1 Paper I and III – $PaCO_2$ analyses

We investigated the exposure to hypercapnemia and hypocapnemia in paper I and III. In both papers, exposure to abnormal $PaCO_2$ was common but more frequent in patients included in the TTM database (paper I). The difference in prevalence might be attributed to the difference in the definition of normocapnemia; 4.5–6.0 kPa in paper I and 4.0–6.7 kPa in paper III, different observation periods between paper I and paper III (37 vs 24 hours) and possibly, more frequent blood gas sampling in the cohort of paper I in accordance with the TTM-trial study protocol. We studied the exposure to abnormal $PaCO_2$ as single value exposure, the difference between the extreme values, longitudinal exposure over time and the association between abnormal $PaCO_2$ and serum tau levels at 48 or 72 hours after OHCA. Neither in paper I nor in paper III did we find hypercapnemia or hypocapnemia to be independently associated, at the 0.05 threshold level with poor neurological outcome after correction of our logistic regression analyses for in the context of OHCA important covariates. However, the estimates for hypercapnemia exposure in paper I and III point uniformly towards a lesser probability for poor neurological outcome, compared to normocapnemia or no hypercapnemia exposure. Moreover, the widths of our 95% CI indicate that we cannot exclude a possible type II error in our analyses (accepting a false null hypothesis) and that, had we investigated a larger cohort of patients, we may have found a significant association between $PaCO_2$ or peak tau levels and neurological outcome.

There are a number of studies that have, in contrast to our analyses, found an association between exposure to abnormal $PaCO_2$ and neurological outcome in patients following cardiac arrest. However, the results are inconsistent. Hypocapnemia has frequently been associated with poor neurological outcome, [138,139,242,243] whereas hypercapnemia has been associated with poor outcome,[139,242] beneficial outcome,[134,137,138,244] or no difference in outcome.[245] In an observational single center study, including a cohort of 193 IHCA and OHCA patients, Roberts et al. investigated the association between exposure to $PaCO_2$ under 4.0 kPa and over 6.7 kPa during the first 24 hours after cardiac arrest and found both, exposure to $PaCO_2$ under 4.0 kPa and over 6.7 kPa to be associated with poor outcome at discharge defined as CPC ≥3.[139] Schneider et al. analyzed a large binational registry including 16542 patients with

cardiac arrest and found hypocapnemia, defined as $PaCO_2$ <4.7 kPa, to be associated with increased mortality and a lesser likelihood to be discharged home among survivors. In contrast, exposure to hypercapnemia ($PaCO_2$ >6.7 kPa) was associated with increased likelihood of being discharged home among survivors.[138] In another observational multicenter database analysis of 9176 adult OHCA patients in the north American ROC-network, Wang et al. showed that hypercapnemia ($PaCO_2$ >6.7 kPa) at any time-point within the first 24 hours after OHCA and hypocapnemia ($PaCO_2$ <4.0 kPa) towards the end of the first 24 hours was associated with increased in-hospital mortality.[242] A recent study including blood gases sampled per protocol within the first 6 hours after cardiac arrest, found that in a mixed group of 280 IHCA and OHCA patients, $PaCO_2$ had an inverted "U" shaped association with good outcome and a mean $PaCO_2$ of 9.1 kPa had the highest predicted probability of good neurological outcome. Even in the presence of metabolic acidosis (BE ≤-6) a $PaCO_2$ of 6.8 kPa was found to have the highest predicted probability of good neurological outcome.[246]

Following observational studies that suggested better neurological outcome in patients exposed to hypercapnemia, targeted therapeutic mild hypercapnemia (TTMH) has been hypothesized to attenuate the detrimental effects of the anoxic-ischemic insult sustained during the cardiac arrest. In a randomized pilot study (n= 86) that used NSE as a surrogate marker for brain injury, Eastwood et al. showed lower NSE values, measured at 24, 48 and 72 hours after ROSC and a pattern of more favorable GOSE scores at 6 months after cardiac arrest, in the group exposed for 24 hours after ROSC to the elevated TTMH $PaCO_2$ levels.[137] In a further pilot study, Jakkula et al. randomized 123 patients resuscitated from OHCA to low-normal (4.5–4.7 kPa) or high-normal (5.8–6.0 kPa) $PaCO_2$ exposure groups, but was not able to show a reduction in NSE values at 48 hours or a difference in neurological outcome at 6-month follow-up.[209] We compared $PaCO_2$ exposure groups similar to Eastwood et al., but were not able to show a favorable neurological outcome or lower serum tau levels in this group, in paper I. However, the studies had significant differences, most importantly, Eastwood et al. randomized patients into targeted $PaCO_2$ ranges whereas our group was observational and nontargeted. Nevertheless, our analyses did not show an adverse effect of mild hypercapnemia either. The ongoing phase III Multi-Centre Randomized Controlled TAME cardiac arrest trial (NCT03114033), randomizing 1700 OHCA patients into either targeted normocapnemia (TN) ($PaCO_2$ 4.7–6.0 kPa) or TTMH ($PaCO_2$ 6.7–7.3 kPa) for 24 hours after sustained ROSC investigates the effectiveness of TTMH further.[223] Trial conclusion is estimated for December 2022.

$PaCO_2$ and PaO_2 exert different effects on cerebral physiology. Exposure to abnormal $PaCO_2$ and PaO_2 separately, has been shown to be common after cardiac arrest.[138,139,230,247,248] Our and other studies indicate that exposure to the joint presence

might also be frequent, but the association with functional neurological outcome has rarely been investigated. A study by Vahersaalo et al.,[244] showed that exposure to moderate hypercapnemia combined with mild hyperoxemia was associated with improved neurological outcome at 12-month follow-up. In paper III we investigated the combination of hypercapnemia with normoxemia, and hyperoxemia with hypocapnemia, but we were not able to show a statistical significant association with neurological outcome in these groups. However, there were significant differences between the investigations, most notably, Vahersaalo et al. analyzed multiple $PaCO_2$ and PaO_2 values to calculate mean exposure ranges which were subsequently compared to each other, whereas we analyzed single exposure to abnormal $PaCO_2$ and PaO_2.

The reasons for the divergence in outcome of the different studies are not entirely clear but there are several factors that may contribute to the variety of results; the foremost being that except for two pilot studies,[137,209] all other studies are, to the author´s knowledge, observational. Furthermore, inclusion criteria differ between the investigations. The majority of the studies include mixed groups of IHCA and OHCA patients,[137-139,243,245,249,250] but some restrict their analyses to OHCA patients only.[209,244] Previous studies show that IHCA and OHCA differ significantly in cardiac arrest etiology, bystander proficiency, first rhythm and outcome and therefore, analyzing IHCA and OHCA patients in one cohort may increase the risk for study bias.[5-8] Information on the cardiac arrest circumstances is of importance and the lack of variables highly associated with outcome, most importantly, age, time to ROSC, first rhythm and bystander CPR might affect results.[71,251] Moreover, there are physiological parameters at admission that are also highly associated with outcome, e.g., level of consciousness, shock on admission, pH, lactate and base excess.[71,250,252] Failure to correct for these confounders might represent a source of error, as shown, in the $PaCO_2$ and pH analysis in paper I.[242] TTM treatment is a further potential source of error, firstly, if beneficial at all, the adequate target temperature is still unknown and the use of different temperature targets in study cohorts may increase study bias. Secondly, the CO_2 solubility and consequently the $PaCO_2$ changes with temperature, which might also lead to study bias, if different arterial blood gas analysis methods (alpha stat or pH stat) are used in the same cohort.[51,154,241,253] Different strategies for the selection of arterial blood gases have been employed in the above mentioned studies, for example, the most extreme $PaCO_2$ exposure within 24 hours after admission,[139] the $PaCO_2$ of the blood gas with the lowest PaO_2/FiO_2 ratio,[138] mean $PaCO_2$ values over time according to protocolized blood gas sampling,[134] or mean values over the first 24 hours.[244] The inconsistent selection criteria for arterial blood gas sampling represent an additional source of heterogeneity between investigations. Furthermore, since most

blood gases were sampled at the discretion of the treating physician, the risk for sampling bias also seems high in the presented studies.

6.1.2 Paper II and III – PaO_2 analyses

In paper II and III we investigated the exposure to hyperoxemia and hypoxemia. Definition of abnormal PaO_2 was similar in paper II and III, and exposure to hyperoxemia and hypoxemia was frequent in both studies, but similar to the PaCO2 investigations of paper I and III, more frequent in the TTM-trial cohort described in paper II.

We analyzed the single most extreme exposure, time weighted mean exposure over 12 and 37 hours and maximum PaO_2 difference. We also investigated cut-off values for the onset of the association with poor neurological outcome, across increasing and decreasing PaO_2 values, and the association between abnormal PaO_2 and serum tau levels at 48 or 72 hours after OHCA. None of our analyses revealed statistically significant results. However, the point estimates in our primary analyses indicate a higher probability for poor outcome in the cohort exposed to hyperoxemia and after evaluating the 95% CI´s we concluded that in the analyses of paper II and III we cannot rule out a possible type II error, indicating that we might have found statistically significant results, had we investigated a larger cohort of patients.

Animal cardiac arrest models comparing the administration of 100% oxygen for up to 60 minutes after ROSC, to lower concentrations of oxygen, almost uniformly report worse neurological deficit scores in the groups of animals exposed to 100% oxygen and in subsequent histological evaluation, increased neurological damage was reported in the exposure group.[254] These pre-clinical findings indicate that hyperoxemia in the phase after OHCA is potentially harmful. A subsequent retrospective multicenter cohort study including 6326 cardiac arrest patients by Kilgannon et al., showed an independent association between hyperoxemia exposure after OHCA, defined as a PaO_2 of ≥40 kPa and decreased in-hospital survival, and thus, confirmed the findings of the animal studies in a human cohort.[246] This study also reported that exposure to hyperoxemia was a common occurrence in patients after OHCA. Following this investigation, other retrospective studies further corroborated the findings of Kilgannon et al.[255-257] However, a retrospective cohort study by Bellomo et al, analyzing data of 12806 cardiac arrest patients found hypoxemia to be associated with increased risk of in-hospital mortality, but showed only a weak relationship between hyperoxemia and death, which was significantly reduced by the addition of illness severity scores into the model and disappeared after correction for FiO_2.[230] The authors of this study suggest that a high PaO_2 might be the result of a high FiO_2, reflecting an attempt to

compensate for greater physiological instability and that PaO_2 is rather a marker of illness severity instead of a mediator of injury.[258] In a subsequent study, Ihle et al. investigated a group of OHCA patients (n= 584) with detailed pre-hospital information and found no association between oxygenation in the first 24 hours after admission and survival at discharge.[259] The authors of this investigation point out that pre-hospital information, e.g., bystander CPR and time to ROSC is highly associated with outcome and therefore of importance when investigating OHCA patients. Similar to Ihle et al., we found pre-hospital data also to be predictive of long-term outcome in our cohort in paper II. In a later prospective observational multicenter study evaluating arterial blood gas samples according to an *a priori* defined protocol at 1 and 6 hours after ROSC, Roberts et al. found a weak correlation between FiO_2 and PaO_2, debasing the assumptions of Bellomo et al.,[258] however, they confirmed that FiO_2 was a predictor of hyperoxemia exposure. The authors also showed that hyperoxemia exposure occurs predominantly early after ROSC and that the that the risk of poor neurological outcome begins at a PaO_2 ≥40 kPa.[229] In paper II, 99% of the hyperoxemia exposures occurred within 5 hours, confirming the findings of Roberts et al. that hyperoxemia exposure occurs predominantly early. In paper II we also found the correlation between FiO_2 and PaO_2 to be weak, similar to Roberts et al., but correcting our results for FiO_2 did not change the outcome. We investigated a possible cut-off point for the onset of the association of PaO_2 with poor neurological outcome in paper II and III, but we were not able to confirm a defined cut-off point for the onset of poor neurological outcome, neither at 40 kPa nor at higher or lower cut-off points. Nevertheless, further examination of the point estimates of our cut-off point analysis in paper III showed a lower probability for the association with poor outcome at 20 kPa compared to higher cut-off points. A similar pattern is visible in paper II and in the study by Roberts et al.[229] Previous studies have also shown lower sequential organ failure assessment (SOFA) scores and the lowest probability of in-hospital death in PaO_2 ranges between 13 and 40 kPa.[245,256] However, it is of importance to point out, that these findings lack statistical significance and that the only study randomizing patients to normoxemia (PaO_2 10–15 kPa) versus moderate hyperoxemia (PaO_2 20–25 kPa) by Jakkula et al. did not show a difference in NSE values at 48 hours or in their secondary end point, functional neurological outcome.[209] In accordance with Jakkula et al. we were also not able to show an association between hyperoxemia and peak serum biomarker levels after 48 or 72 hours in paper II.

In contrast to the results yielded by animal studies, showing harmful effects of hyperoxemia, the results of clinical studies do not uniformly confirm an association between abnormal PaO_2 and poor outcome. However, there are several differences between the animal cardiac arrest models conducted in a controlled laboratory

environment and an IHCA or OHCA; most noteworthy, the animals were all anaesthetized, intubated and mechanically ventilated before the cardiac arrest and the cardiac arrest was most commonly induced by an electric shock. Moreover, the animals were young, had no co-morbidities and no prior pharmacological treatment for chronic ailments.[254] In contrast, cardiac arrest patients are commonly older than 60-years of age and have numerous co-morbidities, in particular previous cardio-vascular, metabolic and respiratory ailments which have been shown to be associated with decreased 30 day survival and poor neurological outcome after cardiac arrest.[260,261] Also the incidence of polypharmacy increases with age in this group and might affect outcome.[262,263] The predominant underlying cause for the cardiac arrest in humans is ischemia due to coronary disease or a primary malignant arrhythmia. Both conditions lead to a build-up of metabolic end-products and the depletion of ATP stores in cardiomyocytes before the onset of arrest,[264] which is a major contrast to a cardiac arrest induced by an electric current in a heart that was normally perfused and oxygenated prior to the arrest. Furthermore, CPR in the laboratory animals was conducted by professionals and oxygen was applied almost immediately, in contrast to clinical practice, especially in OHCA, where the majority of the patients would receive bystander CPR and no or expired air ventilation until the arrival of emergency medical services.

Clinical studies investigating the effects of abnormal PaO_2 in cardiac arrest patients show different outcomes and there are several possible reasons for this. Most importantly, there are no sufficiently large clinical randomized trials with mortality or neurological functional outcome as primary outcome, available. The majority of investigations conducted are hypothesis generating retrospective or prospective observational studies with significant differences in study design and potential sources of bias. For example, the available studies do not use uniform definitions of abnormal PaO_2 values, in particular the definition of hyperoxemia differs in-between analyses. Most studies define a threshold value of 40 kPa for the onset of hyperoxemia,[229,243,244,246] but a few analyses investigate PaO_2 as a continuous variable.[248,255] Furthermore, the selection criteria for the PaO_2 values included varies from study to study, some investigations include the PaO_2 of the first blood gas available,[243,246] while others chose the highest PaO_2 or the worst PaO_2 during the first 24 hours.[230,248,257] More recent studies have also included multiple PaO_2 values.[229,244,250,265]

In clinical routine, arterial blood gas sampling is conducted to monitor the patient, and sampling frequency is commonly higher in unstable patients than in stable patients. This sampling bias represents a potential source of error that applies to the majority of the previously mentioned studies. Arterial blood gas sampling according to an *a priori* defined protocol may reduce this risk, but has, prior to the investigation that we describe in paper II, only been conducted by Roberts et al.[229] For varying reasons, the

majority of studies include mixed IHCA and OHCA cohorts in their investigations. However, as previously discussed, there are significant differences between IHCA and OHCA patients, and thus, analyzing mixed cohorts represents a further source of bias. Scant reporting of cardiac arrest background information, most importantly, omitting outcome modifiers of the Utstein style of reporting, such as time to ROSC and first rhythm are also sources of bias in several investigations.[230,246,248]

To the author´s knowledge, there are two randomized controlled trials, investigating the effects of hyperoxemia following ROSC published. Kusima et al. randomized OHCA patients after ROSC to ventilation with either 30% FiO_2 or 100% FiO_2 for 60 minutes, whereas Jakkula et al. randomized OHCA patients after ROSC into normoxemia or moderate hyperoxemia for 36 hours. Both trials used levels of serum biomarkers of neurological injury as primary outcome and were pilot studies including 28 and 123 patients respectively.[209,266] In both studies, the overall levels of serum biomarkers did not differ between the 100% O_2/hyperoxemia and 30% O_2/normoxemia groups. However, in a subgroup analysis of the study by Kuisma et al., patients (n= 15) exposed to 100% FiO_2 and not treated with mild induced hypothermia showed significantly higher NSE levels at 48 hours after ROSC.[266]

A further study, the ICU-ROX trial, testing a conservative (SpO_2 90%–97%) against a usual oxygen therapy (SpO_2 ≥91%), in a cohort of 965 ICU patients, applied for the ICU stay of the patient or 28 days after randomization, is also worth mentioning here. In this multicenter randomized trial, the investigators detected better survival and neurological outcome at 180 days, and fewer ventilator days in a subgroup of patients with suspected hypoxic–ischemic encephalopathy (n= 164), when included in the conservative oxygen therapy group (n= 86). Besides the potential benefits of a conservative oxygen therapy shown in this study, the results also raise the question whether elevated oxygen levels may be harmful to the brain after hypoxic-ischemic injury for a much longer period than previously investigated. However, despite the randomized design and the comparably large group size, the results of the post-hoc analyses of this trial have to be regarded as hypothesis generating.[267]

6.1.3 Conclusion, paper I, II and III

In conclusion, in paper I, II, and III we investigated PaO_2 and $PaCO_2$ and their association with neurological outcome after OHCA. Our analyses revealed in contrast to several previous studies, no independent association between abnormal $PaCO_2$ and PaO_2 values and neurological outcome or a serum biomarker of neurological injury. However, the nature of our investigations is such that we cannot exclude a type II error.

The existing body of studies displays significant heterogeneity and the risk for study bias in the investigations seems very high, possibly explaining the variety in outcome. A plausible next step to further investigate the influence of $PaCO_2$ and PaO_2 on outcome following cardiac arrest would be adequately sized clinical randomized trials with reporting standards according to the Utstein style, that investigate homogenous groups of cardiac arrest patients and evaluate outcome according to a comparable standard across all the investigations

6.2 Paper IV

In paper IV, we investigated the release pattern and the accuracy to predict neurological outcome at 6-month follow-up of the two serum biomarkers of neurological injury GFAP and UCH-L1, sampled at 24, 48 and 72 hours after OHCA. We also investigated the outcome predictive accuracy of the serum biomarkers in combination (GFAP+UCH-L1). Furthermore, we compared the predictive accuracy of GFAP, UCH-L1 and GFAP+UCH-L1 to that of the current standard serum biomarker in clinical use, NSE.

The predictive accuracy of GFAP for neurological outcome has previously been investigated in adult cardiac arrest patients, while UCH-L1 has only been analyzed in pediatric cardiac arrest studies.[185,188,189] GFAP and UCH-L1 have also been investigated as an aid to determine the need for a CT in conscious TBI patients and a commercially available blood test combining both serum biomarkers has been approved in the United States for clinical use. [268,269] This blood test was employed for the analysis of GFAP and UCH-L1 in this study.

In our analyses, GFAP peaked after 48 hours while UCH-L1 was highest at 24 hours. Both serum biomarkers were significantly higher in the poor outcome group compared to the good outcome group, confirming results of previous studies.[185,186,188,189,270] In our analyses, the overall performance in terms of predicting poor neurological outcome of these two serum biomarkers alone, and in combination, measured by AUROC, was excellent and with the exception of UCH-L1 at 72 hours, significantly better than that of NSE. This stands in contrast to previous studies reporting lower AUROCs, in particular for GFAP.[185,188,189,271] However, these investigations included significantly fewer patients, sampled at different time-points and also showed lower AUROCs for NSE.[188,189]

For outcome prediction after cardiac arrest, it is essential that a biomarker performs well at high specificity to avoid false positive predictions. Therefore, we analysed the

partial AUROC at 100% to 95% specificity. In this analysis, UCH-L1 and GFAP+UCH-L1 were significantly more accurate than NSE at 24 hours, but not at 48 and 72 hours after OHCA. Moreover, the predictive accuracy of NSE was significantly better than GFAP at 48 hours. The sensitivities differed depending on sampling time-point and level of specificity, but were overall similar. These results stand in contrast to a previous study showing NSE to be significantly more accurate and sensitive in predicting poor outcome after OHCA than GFAP, at high specificities and at all time-points.[188]

Adding GFAP+UCH-L1 to clinical information about the OHCA (age, sex, time to ROSC, bystander CPR [yes/no], initial rhythm shockable [yes/no] and serum lactate on admission) increased predictive accuracy significantly. Interestingly, adding bedside information, (bilaterally absent corneal reflexes and bilaterally absent pupillary reflexes) commonly highly associated with poor outcome, changed prognostic accuracy only marginally.[75] Nevertheless, the AIC preferred the model with all three modalities.

Hemolysis was associated with lower GFAP levels at 72 hours, opposing a previous study reporting no influence of hemolysis.[272] However, removing samples with hemolysis did not affect outcome in our study in a subsequent sensitivity analysis.

We also found that patients in the TTM33 group had higher GFAP levels than patients in the TTM36 group whereas UCH-L1 was not sensitive to TTM. The reason for this finding is unknown, but may be caused by reduced hepatic metabolism in patients treated with induced hypothermia.[273] However, little is known about the metabolism of serum biomarkers but renal clearance has been reported for lower molecular weight serum biomarkers such as S100B and is therefore also likely to apply to UCH-L1, whereas higher molecular weight serum biomarkers, e.g., GFAP and NSE might undergo hepatic metabolism.[274]

Neurological examination of patients in particular during the first 72 hours after cardiac arrest, is hampered by sedation and mechanical ventilation. Levels of serum biomarkers of neurological injury are unaffected by patient treatment and therefore present a quantitative prognostic tool early after cardiac arrest. Several serum biomarkers have been shown to predict neurological outcome with high accuracy,[82,162,176,201] but the only serum biomarker in routine clinical use is NSE. However, NSE is not exclusive to the CNS and increased levels may also be seen in the setting of hemolysis and in patients with neuroendocrine tumors.[171] Moreover, NSE levels might vary with different assays.[196] Due to the high variability of the analysis methods, recent international guidelines have ceased to supply defined thresholds for poor neurological outcome but recommend that NSE levels should be significantly elevated according to locally established standards and increase in value from 24 to 48 hour or between any time

points within the first 72 hours. Furthermore, NSE may only be used in combination with other prognostication modalities like SSEP, imaging and clinical examination.[51] In comparison to NSE, GFAP and UCH-L1 and their combination, predict poor neurological outcome with a higher overall accuracy and UCH-L1 and GFAP+UCH-L1 are superior at high specificity early after OHCA. GFAP and UCH-L1 show very little variation over the three measuring points, which may increase robustness. However, neither GFAP nor UCH-L1, separately or in combination, are sufficiently accurate to predict outcome independently. Moreover, GFAP and UCH-L1 are also prone to error, e.g., in patients with recent head trauma, and if they were to be introduced into clinical practice, they would, like NSE, only be used in a multimodal prognostication model.

A recent investigation has shown NFL to prognosticate poor neurological outcome at 6-month follow-up with higher accuracy than any other serum biomarker or any other clinical, imaging or neurophysiological method. However, in contrast to our analyses, in which we employed a well-established method with a commercially available ELISA kit for analysis, the method employed to detect NFL is novel and at the present time not available for use in clinical practice.[162,275]

The study described in paper IV had several limitations, we analyzed GFAP and UCH-L1 at 24, 48 and 72 hours after OHCA and we are therefore unable to make statements about the predictive accuracy at an earlier or later time-point. Due to different study design and analysis methods, comparing our results with previous studies can only be made with caution. However, the strengths are a large sample size, few missing patients, and the prospective, double-blinded multicenter design and the strict prognostication protocol of the TTM-trial. We employed a commercially available blood test that we analyzed with a well-established method facilitating future validation of our results.

6.2.1 Conclusion, paper IV

In conclusion, our analyses indicate that GFAP, UCH-L1 and, in particular their combination predict poor neurological outcome at 6-month follow-up better than NSE, especially at 24 hours after OHCA. Hemolysis was associated with lower GFAP values but removing samples with hemolysis did not affected outcome. However, GFAP may be sensitive to TTM. Future analyses, for example, the TTM-2 trial will provide an opportunity to validate our results. Future analyses might also offer an opportunity to establish common analysis methods and cut-off values.

6.3 Conclusions

- Exposure to extreme PaO_2 and $PaCO_2$ values was common after OHCA.
- Exposure to extreme $PaCO_2$ values occurred most frequently early after OHCA and exposure to extreme PaO_2 values almost exclusively early after OHCA.
- Exposure to extreme $PaCO_2$ or PaO_2 values after OHCA was not independently associated with neurological outcome at discharge or at 6-month follow-up.
- Peak serum tau levels at 48 or 72 after OHCA were not significantly associated with exposure to extreme PaO_2 or $PaCO_2$ values.
- There was no demonstrable numerical threshold for the onset of the association of PaO_2 or $PaCO_2$ with poor neurological outcome.
- GFAP and UCH-L1 levels differ between good and poor outcome in OHCA patients.
- The GFAP+UCH-L1 model predicts neurological outcome better than GFAP and UCH-L1 separately.
- GFAP, UCH-L1 and GFAP+UCH-L1 predicted poor neurological outcome at 6-month follow-up with higher overall accuracy than NSE.
- At high specificity GFAP+UCH-L1 predicted outcome at 6-month follow-up with higher accuracy than NSE at 24 hours but not at 48 and 72 hours.
- GFAP, UCH-L1 and GFAP+UCH-L1 showed little variation over all measuring points.
- GFAP+UCH-L1 may improve outcome prediction in a model including bedside and clinical information.

6.4 Future aspects

As pointed out under 6.1.3, the available studies investigating $PaCO_2$ and PaO_2 after cardiac arrest are heterogeneous in many aspects, which may contribute to the variety of results. It seems important that future studies become more homogenous, especially concerning patient cohorts and the utilization of background information and

outcome. Future studies should preferably investigate either OHCA or IHCA patients and not mixed cohorts. A standard for reporting of cardiac arrest data should be defined, for example the Utstein style criteria as well as a standardized measure of outcome, e.g., the COSCA. Furthermore, protocolized blood gas sampling may reduce study bias, and the same can be said for employing a uniform arterial blood gas analysis method. However, the studies presented in paper I and II were conducted according to the above outlined standards and we did not find an association between $PaCO_2$ or PO_2 and neurological outcome, but we yielded moderately wide 95% CIs. There is a risk that further studies of the same type may experience the same outcome, possibly with somewhat narrower 95% CIs. In order to ensure conclusive results, randomized trials of adequate size are necessary. These future randomized trials should incorporate the information gained by the observational studies, like the ongoing phase III multi-center, randomized, parallel-group, controlled TAME cardiac arrest trial. The results from this trial will provide high quality information and may establish a causality between elevated $PaCO_2$ levels and outcome after OHCA. If the TAME cardiac arrest trial establishes a favorable effect of TTMH, further trials would have to investigate the most beneficial $PaCO_2$ level.

Future studies may also be used to investigate the potential protective or outcome modulating properties of $PaCO_2$. For example, the possible suppression of seizures which could be investigated by comparing reported seizures or aEEG data within the first days after OHCA and time weighted mean $PaCO_2$ values during the same time period. Furthermore, the alteration of CBF by different $PaCO_2$ levels may also be of interest, but although a number of techniques are available to measure CBF, the most feasible analysis method in patients admitted to ICU after OHCA would be the indirect estimation of CBF by bedside transcranial Doppler-ultrasound.[118,276] Furthermore, the intensity of the inflammatory response after OHCA has been shown to be associated with outcome.[277] Therefore, investigating increased $PaCO_2$ as an intervention to reduce inflammation, measured with inflammatory serum biomarkers or primary transcription factors such as NF-κB would also be of interest in future studies.

As mentioned in the discussion, randomizing OHCA patients into different PaO_2 groups would be a plausible approach to establish possible outcome modifying effects. However, there are ethical aspects to be considered, since animal studies indicate that very high levels of PaO_2 are possibly harmful and there are observational studies corroborating this finding. Moreover, no observational study on patients has so far indicated beneficial effects of very high PaO_2 values. However, there are observational studies suggesting that moderate hyperoxemia may be associated with improved outcome. It would therefore be a sensible approach, in a future randomized trial, to assign OHCA patients to a moderate hyperoxemia group and to a group exposed to a

PaO_2 range considered clinically normal, as done in a pilot study by Jakkula et al.[206] Alternatively, a future investigation could replicate the ICU-ROX trial for OHCA patients only.[267]

Serum biomarkers present a quantitative measure of neurological injury after OHCA, but possible pitfalls are faulty sampling, very recent cerebral insults and extracerebral sources of the serum biomarker. A variety of serum biomarkers have been studied but so far, no single serum biomarker or combinations of serum biomarkers have shown sufficient robustness to predict outcome independently.[80] A possible design for future studies might be to further investigate combinations of serum biomarkers, preferably originating from different cells or regions of the CNS which may provide increased safety against sampling errors, higher predictive accuracy, and possibly, an indication for the location of the anoxic-ischemic injury. Serum biomarkers have been shown to be indicative of poor outcome and are used as an aid to the clinician when termination of care is being considered. However, studies investigating the prognostic accuracy of serum biomarkers for good outcome are rare and results of such studies may be useful in clinical practice, to help identify patients who are very likely to have a good outcome, early after OHCA, ensuring more aggressive treatment measures are taken in their management. As pointed out in the ERC guidelines the variability of different analysis methods is large, which makes the determination of general cut-off points for good or poor outcome currently impossible.[51] Future studies may also be used to develop standardized assays so that results may be reproducible and comparable.

7 Swedish summary

Bakgrund

Ett hjärtstopp innebär att hjärtats pumpförmåga och därmed cirkulation av syrerikt blod till kroppens alla organ upphör. I många fall är hjärtstoppet ett förväntat och naturligt avslut på livet. Ett plötsligt hjärtstopp kommer oförväntat och kroppens organ, framförallt hjärnan, tar snabbt skada om inte effektiv hjärt- och lungräddning (HLR) startas inom kort. Utan HLR sjunker överlevnaden med 7–10 % per minut. Men även effektiv HLR levererar inte mer än en tredjedel av hjärtats normala pumpförmåga och det är avgörande att hjärtat kommer igång med egenaktivitet (ROSC) inom en rimlig tid. Denna är individuell, men risken att svåra hjärnskador uppstår ökar efter 20–30 minuter även med god HLR. Hjärtstopp utanför sjukhus (OHCA) där HLR påbörjas har en incidens kring 56/100000/år i Europa. Omkring en tredjedel av patienterna återfår hjärtaktivitet och ROSC efter hjärtstopp, men inte medvetande, och behöver vård på intensivvårdsavdelning (IVA) i respirator. Risken att avlida efter inläggning på IVA, trots att hjärtat har kommit igång, är över femtio procent, framförallt på grund av hjärnskador som har uppstått under hjärtstoppet och direkt efter ROSC. Patienter som överlever och vaknar upp drabbas i stor utsträckning av en bestående neurologisk funktionsnedsättning som kan innebära allt från mindre kognitiva störningar till svåra funktionsnedsättningar.

Djurexperimentella hjärtstoppsmodeller under sent nittiotal och i början av tvåtusentalet har visat att djur som inte utsatts för höga syrgaspartialtryck (PaO_2) efter ROSC hade bättre neurologisk funktionsnivå och mindre strukturella hjärnskador jämfört med djur som utsattes för höga PaO_2. Ett flertal efterföljande observationella kohortstudier på människa visade att exponering för mycket höga PaO_2 efter hjärtstopp var vanligt. Mycket höga och mycket låga PaO_2 kunde förknippas med sämre neurologisk funktionsnivå efter hjärtstopp i ett antal studier. Alla studier kunde dock inte reproducera dessa resultat.

Ofysiologiskt höga eller låga koldioxidpartialtryck ($PaCO_2$) har också visat sig vara mycket vanliga efter hjärtstopp i ett antal observationella kohortstudier. En stor del av dessa studier visar en association mellan höga $PaCO_2$ och låga $PaCO_2$ och neurologisk funktion efter hjärtstopp, men även här pekar studieresultaten åt olika håll.

PaO_2 och $PaCO_2$ påverkar blodcirkulationen i hjärnan hos friska försökspersoner, liksom hos patienter efter hjärtstopp. $PaCO_2$ har dessutom kramp- och inflammationshämmande egenskaper. Modifiering av PaO_2 och $PaCO_2$ tidigt efter ROSC kan vara en möjlighet att förbättra den neurologiska återhämtningen hos patienter som har överlevt ett OHCA. Befintliga studier uppvisar dock brister i metodik, patient rekrytering, dokumentation samt analyser av blodgaser. Betydelsen av PaO_2 och $PaCO_2$ efter hjärtstopp avseende den neurologiska återhämtningen är oklar.

Prognostisering av det neurologiska utfallet sker tidigast tre dygn efter hjärtstoppet och baseras på klinisk neurologisk undersökning, datortomografi, elektroencefalografi samt en serum-hjärnskademarkör, neuron specific enolase (NSE). Många patienter med dålig prognos går inte att identifiera vid den första bedömningen eftersom de mest robusta negativa prediktorerna i kliniskt bruk har begränsad sensitivitet och bedömningen försvåras ofta av sederande läkemedel. Hjärnskademarkörer frisätts till blodet från hjärnans nervceller vid ischemisk eller hypoxisk skada och är kvantitativa markörer oberoende av sedering. NSE är den enda hjärnskademarkör i kliniskt bruk och har blivit en viktig del i klinisk prognostisering av patienter som har överlevt ett OHCA. NSE uppvisar dock enbart moderat sensitivitet vid hög specificitet och på grund av förekomst utanför det centrala nervsystemet finns det risk för falskt höga värden i samband med t ex. hemolys. Ett flertal nya hjärnskademarkörer (t ex tau och neurofilament light chain, NFL) har under de senaste åren undersökts och några har uppvisat bättre sensitivitet vid hög specificitet än NSE, men är inte kommersiellt tillgängliga och saknar extern validering. Två nya hjärnskademarkörer, glial fibrillary acidic protein (GFAP) och ubiquitin carboxy terminal hydrolase L1 (UCH-L1), är sedan 2018 kommersiell tillgängliga i ett analys-kit. Detta är i USA godkänt för att avgöra behovet av CT undersökning i samband med lätta till moderata traumatiska hjärnskador.

Metoder

I arbete I, II och III analyserade vi PaO_2- och $PaCO_2$-värden i OHCA patienter ur två hjärtstoppsdatabaser. I arbete I och II undersökte vi data från 939 patienter ur TTM-databasen som skapades under Targeted Temperature Management (TTM) studien mellan 2010 och 2013. I arbete III analyserade vi PaO_2- och $PaCO_2$- data från 2162 patienter ur International Cardiac Arrest Registry (INTCAR) 2.0-databasen som samlades in mellan 2008 och 2018. TTM-databasen inkluderade färre patienter men samlade in fler mätpunkter per patient än INTCAR 2.0-databasen.

I arbete I studerade vi sambandet mellan $PaCO_2$-värden utanför normalområdet och neurologiskt utfall 6 månader efter hjärtstopp. Vi undersökte extremt höga eller låga

$PaCO_2$-värden, exponering över tid, variation av $PaCO_2$ samt om det finns ett samband mellan extrema $PaCO_2$-värden och en serum hjärnskademarkör, serum tau.

I arbete II prövade vi sambandet mellan onormalt höga och låga PaO_2-värden och neurologiskt utfall efter 6 månader. Vi undersökte extremvärden, PaO_2 över tid och svängningar i PaO_2. Vi undersökte också om det finns ett tröskelvärde där sambandet mellan höga PaO_2 och dåligt neurologiskt utfall börjar, i en modell av successivt stigande PaO_2, och om det finns ett samband mellan ofysiologisk höga eller låga PaO_2 värden och serum tau.

I arbete III analyserade vi associationen mellan exponering för onormalt höga eller låga PaO_2 och $PaCO_2$ och det neurologiska utfallet vid utskrivning från sjukhus. Dessutom testade vi associationen mellan kombinationer av PaO_2 och $PaCO_2$ och neurologiskt utfall vid utskrivning och om det finns ett tröskelvärde där association mellan höga eller låga PaO_2 eller låga $PaCO_2$ och dåligt neurologiskt utfall börjar.

I studie IV undersökte vi hur GFAP och UCH-L1 frisätts 24, 48 och 72 timmar efter OHCA, om GFAP och UCH-L1 skiljer sig åt mellan patienter med bra och dåligt utfall samt hur exakt GFAP och UCH-L1 och deras kombination (GFAP+UCH-L1) prognostiserar det neurologiska utfallet 6 månader efter hjärtstopp. Vi jämförde våra resultat med NSE. Jämförelsen av utfallsprediktionen mellan de olika hjärnskademarkörerna gjordes genom receiver-operating characteristics (ROC) kurvor och ytan under ROC kurvan (AUROC). Vi jämförde också utfallsprediktionen av de olika hjärnskademarkörerna vid hög specificitet (lågt falsk positiv prediktion) mellan 100 och 95%.

Utfallsmått

Det primära utfallsmåttet i alla studier var neurologisk funktionsnivå enligt cerebral performance category (CPC)-skala som bedömdes 6 månader efter hjärtstopp i TTM-kohorten eller vid utskrivning från sjukhuset i INTCAR 2.0-kohorten. I CPC skalan delas patientens funktionsnivå in i 5 kategorier. CPC 1 - god cerebral funktion. Patienten har förmåga att arbeta och leva ett normalt liv. Kan ha mindre psykologiska eller neurologiska svårigheter. CPC 2 - måttlig cerebral funktionsnedsättning. Kan arbeta i skyddad omgivning, klarar vardagslivet självständigt, men kan ha epilepsi, talsvårigheter eller minnesstörningar. CPC 3 - svår cerebral funktionsnedsättning. Patienten är vid medvetande men är beroende av andra för alla dagliga aktiviteter på grund hjärnskadorna och oftast i behov av vård i institution. CPC 4 - medvetslös. Patienten är omedveten om omgivningen och har ingen uppfattningsförmåga. CPC 5 – Patient är död. CPC 1 – 2 definierades som bra neurologiskt utfall och CPC 3 – 5

som dåligt. I studie I och II använde vi också nivån på serum tau 48 eller 72 timmar efter hjärtstopp som utfallsmått.

Resultat

Oavsett $PaCO_2$ modalitet, kunde vi inte påvisa ett samband mellan $PaCO_2$ och det neurologiska utfallet efter 6 månader i arbete I. Vi kunde inte heller påvisa ett samband mellan $PaCO_2$ värden och nivån på serum tau 48 eller 72 timmar efter hjärtstopp. I den statistiska analysen visade sig dock att våra resultat hade måttligt stora 95% konfidens intervall (CI), vilket betyder att vi inte med säkerhet kan utesluta signifikanta resultat i en större grupp av patienter (typ II fel)

I arbete II kunde vi inte påvisa ett oberoende samband mellan ofysiologiska PaO_2 och neurologiskt utfall efter 6 månader. Inte heller kunde vi påvisa ett tröskelvärde för början av associationen med dåligt utfall. Serum tau nivåerna 48 eller 72 timmar efter hjärtstopp visade sig inte vara förknippad med onormal höga eller låga PaO_2 värden. Liksom i arbete I visade den statistiska analysen på måttligt stora 95% CI och vi kan inte heller i PaO_2 analysen utesluta att vi hade hittad signifikanta resultat om vi hade undersökt en större grupp av patienter

Inte heller i arbete III kunde vi påvisa ett statistiskt signifikant samband mellan onormala PaO_2 eller $PaCO_2$ och neurologiskt utfall, eller identifiera ett tröskelvärde för början av associationen med dåligt neurologiskt utfall.

Trots att vi inte kunde hitta statistiska samband med ett p-värde under 0.05 nivån pekar punktestimat från arbete I och III på mindre sannolikhet för dåligt neurologiskt utfall hos patienter som exponerades för förhöjda $PaCO_2$ och från arbete II och III på högre sannolikhet för dåligt neurologiskt utfall hos patienter som exponerades för höga PaO_2.

Analysen av GFAP och UCH-L1 i arbete IV visade att GFAP, UCH-L1 och GFAP+UCH-L1 predikterar dåligt neurologiskt utfall med hög precision. GFAP+UCH-L1 har högre precision än GFAP och UCH-L1 separat och NSE. Vid höga specificiteter predikterar GFAP+UCH-L1 utfallet signifikant bättre än NSE 24 timmar efter hjärtstoppet men inte vid 48 och 72 timmar. GFAP värden var lägre i samband med hemolys, 72 timmar efter OHCA, men utfallet påverkades inte när hemolyserade blodprover exkluderades i en sensitivitetsanalys. UCH-L1 påverkades inte av hemolys.

Slutsats

Sammanfattningsvis visar våra resultat inte på ett samband mellan $PaCO_2$ nivåer eller PaO_2 nivåer efter OHCA och neurologisk utfall, men våra analyser indikerar att vi inte kan utesluta ett statistiskt signifikant samband i en större patientgrupp. Frågan om

huruvida PaO_2 och $PaCO_2$ efter ROSC påverkar utfallet efter hjärtstopp bör vidare undersökas i framtiden, företrädesvis med randomiserade studier med tillräcklig statistisk styrka för att kunna fastställa en eventuell kausalitet. GFAP, UCH-L1 och GFAP+UCH-L1 predikterar dåligt neurologiskt utfall efter hjärtstopp med högre precision än NSE. Framförallt GFAP+UCH-L1 skulle kunna förbättra prediktion av dåligt utfall efter hjärtstopp i en multimodal prediktionsmodell, men våra resultat måste valideras först innan GFAP+UCH-L1 kan tas i kliniskt bruk.

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About the Author

Florian Ebner is an anesthetist and intensivist and works as a senior consultant at Helsingborg Hospital, Sweden. He is pauciloquent when it comes to talking about himself. However, it is known that he has a weak spot for Renaissance art and South Africa.

According to his supervisor, this is what he looks likes after being on-call.

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