Prediction of neurological outcome after cardiac arrest and targeted temperature management

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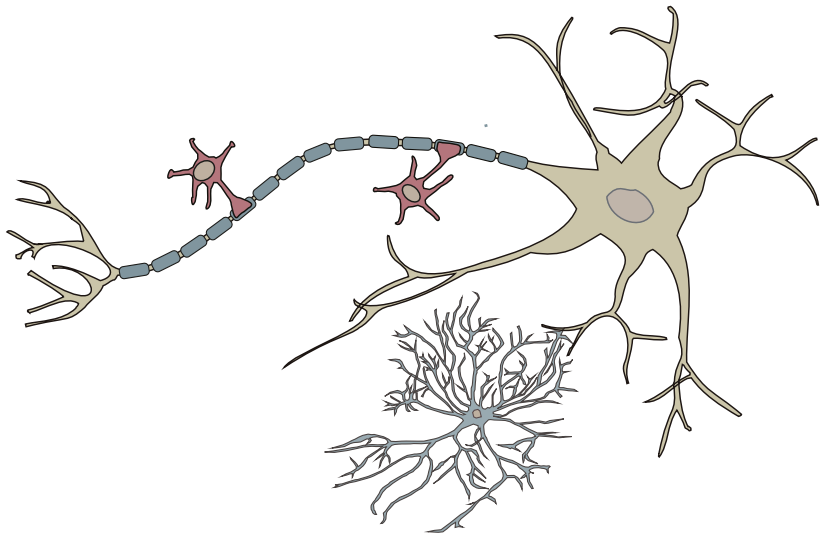

Marion Moseby-Knappe is a clinical neurologist at Skåne University Hospital. Her PhD-thesis from the Center for Cardiac arrest at Lund University in Sweden is focusing on routine and novel methods for prediction of neurological outcome in patients treated after out-of-hospital cardiac arrest. During the time as a doctoral student, she has discovered an interest for designing medical illustrations.

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Prediction of neurological outcome after cardiac arrest and targeted temperature management

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DOCTORAL DISSERTATION

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Faculty opponent
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Title: Prediction of neurological outcome after cardiac arrest and targeted temperature management

Abstract

Background: Prediction of neurological outcome in unconscious patients after cardiac arrest (CA) forms the basis for decisions on further level-of-care based on results from clinical neurological examinations, neuroimaging (CT or MRI), neurophysiology (EEG or SSEP) and blood biomarkers of brain injury. Methods must be highly specific to avoid misclassifying patients with possibilities of a good outcome. In 2015, the European Resuscitation Council (ERC) and the European Society of Intensive Care Medicine (ESICM) published an algorithm to identify poor outcome patients after CA. Novel methods to analyse biomarkers of brain injury have become available and may prove superior to the recommended marker Neuron-specific enolase (NSE).

Purpose: I) To describe findings on head computed tomography (CT) after CA, and evaluate the prognostic accuracy of generalised oedema. II & III) To explore the prognostic accuracies of 3 novel blood biomarkers after CA: the axonal marker serum Neurofilament light (NFL), the astrocytic Glial fibrillary acidic protein (GFAP) and the neuronal marker Ubiquitin C-terminal hydrolase (UCH-L1). IV) To assess the predictive performance of the ERC/ESICM algorithm and modifications thereof. To describe prognostic accuracies of single and combined prognostic methods as recommended by the ERC/ESICM.

Methods: All patients participated in the Target Temperature after Out-of-hospital cardiac arrest (TTM) trial, a prospective international multicentre trial randomising adult patients with CA of presumed cardiac origin to targeted temperature managements of 33°C or 36°C for 24 hours. Papers I and IV are retrospective studies utilizing information from the TTM database. Serum samples studied in Papers II and III were collected prospectively at 24, 48 and 72 hours post-arrest, and stored in a biobank for batch analysis after trial completion. Primary outcome was poor neurological outcome, defined as Cerebral Performance Category Scale 3-5 (severe cerebral disability, vegetative state or death) at 6 months follow-up.

Results: I) Early CT examinations ≤24h were usually normal. Subacutely, generalised oedema was the most common finding, and strongly associated with poor outcome. II) Already at 24 hours post-arrest, serum NFL analysed with an ultrasensitive assay (Simoa) predicted poor neurological outcome with higher prognostic accuracy than any prognostic method currently recommended in the ERC/ESICM algorithm. NFL also differentiatied between various levels of brain injury. II) GFAP and UCH-L1 may be useful as early markers 24h after CA, yet at 48 and 72 hours their prognostic accuracies were not superior to neuron specific enolase. GFAP and UCH-L1 were not elevated in hemolysis, which may prove an advantage compared to NSE. IV) The ERC/ESICM algorithm predicted poor outcome without false positive predictions (100% specificity) and identified approximately fourty percent of patients with poor outcome. Any two pathological findings according to the ERC/ESICM criteria predicted poor outcome without false positive predictions, regardless of level of unconsciousness. Withdrawal of life-sustaining-therapy (WLST) was common in the TTM trial and may have influenced our results.

Conclusion: Prognostication after CA should always be multimodal. The current ERC/ESICM algorithm safely predicted poor outcome, but could benefit from minor modifications. Serum NFL has the potential to guide treatment decisions, both to predict poor outcome and to identify patients with a presumed good neurological prognosis where further treatment is life-saving.

Key words neurological prognostication, brain injury, cardiac arrest, biomarkers, outcome, CT, guideline

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“The important thing is not to stop questioning”

Albert Einstein

Table of Contents

List of publications

This thesis is based on the following papers, which in the text will be referred to by their Roman numerals.

I. **Moseby-Knappe M**, Pellis T, Dragancea I, Friberg H, Nielsen N, Horn J, Kuiper M, Roncarati A, Siemund R, Undén J, Cronberg T; TTM-trial investigators. (2017) Head computed tomography for prognostication of poor outcome in comatose patients after cardiac arrest and targeted temperature management. *Resuscitation* (2017) **119**: 89-94.

II. **Moseby-Knappe M***, Mattsson N*, Nielsen N, Zetterberg H, Blennow K, Dankiewicz J, Dragancea I, Friberg H, Lilja G, Insel PS, Rylander C, Westhall E, Kjaergaard J, Wise MP, Hassager C, Kuiper MA, Stammet P, Wanscher MCJ, Wetterslev J, Erlinge D, Horn J, Pellis T and Cronberg T (2018) Serum Neurofilament Light Chain for Prognosis of Outcome After Cardiac Arrest, *JAMA Neurol.* (2019) **76**: 64-71. *Authors contributed equally.

III. Ebner F, **Moseby-Knappe M**, Mattsson-Carlgren N, Undén J, Dragancea I, Friberg H, Lilja G, Kjaergaard J, Wise MP, Hassager C, Kuiper M, Jaeger Wanscher MC, Wetterslev J, Erlinge D, Horn J, Stammet P, Ullén S, Cronberg T, Nielsen N (2020) Serum GFAP and UCH-L1 for the prediction of neurological outcome in comatose cardiac arrest patients, *Resuscitation*. (2020) **154**: 61-68.

IV. **Moseby-Knappe M**, Westhall W, Backman S, Mattsson-Carlgren N, Dragancea I, Lybeck A, Friberg H, Stammet P, Lilja G, Horn J, Kjaergaard J, Rylander C, Hassager C, Ullén S, Nielsen N and Cronberg T. (2020) Performance of a guideline recommended algorithm for prognostication of poor neurological outcome after cardiac arrest, *Intensive Care Med.* (2020) **46**:1852-1862.

Abbreviations

ACNS	American Clinical Neurophysiology Society
ADC	Apparent diffusion coefficient
AIC	Akaike information criterion
AUROC	Area under the receiver operating characteristics curve
CA	Cardiac arrest
CI	Confidence interval
CNS	Central nervous system
CMT	Charcot-Marie-Tooth
CPC	Glasgow-Pittsburgh Cerebral Performance Category Scale
CPR	Cardiopulmonary resuscitation
CT	Computed tomography
DWI	Diffusion-weighted magnetic resonance imaging
ECG	Electrocardiogram
ECLIA	Electrochemiluminescent immunoassay
EEG	Electroencephalogram
ELISA	Enzyme-linked immunosorbent assay
ERC	European Resuscitation Council
ESICM	European Society of Intensive Care Medicine
FLAIR	Fluid-attenuated inversion recovery
FOUR	Full Outline of Unresponsiveness Scale
FPR	False positive rate
GCS	Glasgow Coma Scale
GCS-M	Glasgow Coma Scale motor score
GFAP	Glial fibrillary acidic protein
GWR	Grey white matter ratio
HU	Hounsfield unit
ICU	Intensive care unit
ILCOR	International Liaison Committee on Resuscitation

IQR	Interquartile range
kDa	Kilo dalton
MRI	Magnetic resonance imaging
mRS	Modified Rankin Scale
NFL	Neurofilament light chain protein
NFH	Neurofilament heavy chain protein
NSE	Neuron specific enolase
OHCA	Out-of-hospital cardiac arrest
PAUROC	Partial area under the receiver operation characteristics curve
PCAS	Post-cardiac arrest syndrome
ROI	Region of interest
ROSC	Return of spontaneous circulation
SIMOA	Single molecule array
SSEP	Short-latency somatosensory evoked potentials
TAME-trial	Targeted therapeutic mild hypercapnia after resuscitated cardiac arrest trial
TTM	Targeted temperature management
TTM-trial	Target temperature management 33°C versus 36°C after out-of-hospital cardiac arrest trial
TTM2-trial	Targeted hypothermia versus targeted normothermia after out-of-hospital cardiac arrest trial
UCH-L1	Ubiquitin carboxy-terminal hydrolase L1
WLST	Withdrawal of life-sustaining therapy

1. Populärvetenskaplig sammanfattning

Plötsligt hjärtstillestånd är en av de vanligaste dödsorsakerna i västvärlden. När hjärtat stannar behövs omedelbar behandling för att förhindra bestående hjärnskador eller död. Överlevnaden efter hjärtstopp har närmare fördubblats de senaste tjugo åren. Fortfarande avlider varannan patient som behandlas på intensivvårdsavdelning efter hjärtstillestånd. Den vanligaste anledningen till att patienten dör är att man avslutar livsuppehållande behandling på grund av ett förmodat dåligt utfall, baserat på resultaten från olika undersökningsfynd. I tillägg till neurologiska undersökningar av patienten, används ofta neurofysiologiska metoder vilka testar hjärnfunktioner, datortomografi eller magnetkamera-undersökningar som ger en digital bild av hjärnan eller ett blodprov som mäter nivån av hjärnskademarkören neuron specifik enolase (NSE). För att minimera risken för felbedömningar är det helt avgörande att metoderna som används är tillförlitliga. Internationella riktlinjer underlättar bedömningen och bidrar till att ge en jämlik vård till patienter i Sverige och i Europa. En algoritm från det europeiska förbundet för hjärt-lung-räddning (European Resuscitation Council, ERC) och det europeiska sällskapet för intensivvård (European Society of Intensive Care Medicine, ESICM) definierar kriterier för att identifiera patienter med dåligt utfall med hjälp av ovannämnda undersökningsmetoder. Nya analysmetoder möjliggör utvärdering av hjärnskademarkörer som hittills ej undersökts hos hjärtstoppspatienter.

Denna avhandling undersöker olika metoder för att förutspå sannolikt utfall efter hjärtstopp. Patientdata från 939 vuxna deltagare i världens största hjärtstoppsstudie har analyserats; the Target temperature management 33°C versus 36°C after out-of-hospital cardiac arrest trial, TTM-studien. Denna studie var ett samarbete mellan 36 sjukhus i Sverige, övriga Europa och Australien. Patienterna som deltog lottades till kylbehandling vid 33°C eller 36°C. Från en stor del av patienterna finns blodprover tagna 24, 48 och 72 timmar efter hjärtstopp, vilket har möjliggjort analyser av olika hjärnskademarkörer och andra relevanta markörer. TTM-studien hade strikta regler för temperaturbehandlingen och tydligt definierade kriterier för när man fick avsluta livsuppehållande behandling. Sex månader efter insjuknandet registrerades patients utfall, oftast vid ett återbesök på sjukhuset som behandlat patienten. Dåligt utfall klassades som död, koma eller ett behov av hjälp till att sköta sina dagliga aktiviteter.

De viktigaste fynden från denna avhandling är:

- De flesta datortomografier av hjärnan (DT) som utfördes inom 24 timmar efter hjärtstopp bedömdes som normala. Efter det första dygnet bedömde man att varannan patient hade uttalad svullnad av hjärnan.
- Nästan alla patienter med uttalad hjärnsvullnad på DT hade dåligt utfall och förhöjda halter av hjärnskademarkören NSE i blodet. Ingen patient med bra utfall hade både uttalad hjärnsvullnad på DT och höga halter av NSE.
- Nivåer av hjärnskademarkören neurofilament light (NFL) i blodet analyserat med en ny och särskilt känslig metod skiljde betydligt bättre mellan patienter med bra och dåligt utfall än andra hjärnskademarkörer. Dessutom identifierade NFL fler patienter med dåligt utfall jämfört med alla metoder som rekommenderas enligt nuvarande riktlinjer. NFL nivåerna ökade med graden av hjärnskada, vilket är unikt för denna markör.
- Två hjärnskademarkörer UCH-L1 och GFAP från blodprov analyserades med rutinmetoder. Jämfört med rutinmarkören NSE var UCH-L1 och GFAP enbart bättre i att skilja mellan bra och dåligt utfall under det första dygnet efter hjärtstopp.
- Alla patienter som uppfyllde ERC/ESICM algoritmens kriterier för dåligt utfall identifierades rätt. Algoritmen missade dock mer än hälften av patienterna med dåligt utfall vilket visar att det finns förbättringspotential.

Patientsäkerheten för bedömning av sannolikt utfall efter hjärtstopp kan förbättras genom att använda minst två olika undersökningsmetoder. Genom att bedöma alla medvetslösa patienter kan flera patienter med dåligt utfall fångas upp av ERC/ESICM kriterierna utan att patientsäkerheten påverkas. I framtiden kan ett blodprov med hjälp av NFL tidigt skilja mellan sannolikt bra och dåligt neurologiskt utfall. Genom att identifiera medvetslösa patienter med låga NFL värden och förväntat bra neurologiskt utfall kan en fortsatt intensivvårdsbehandlingen rädda liv.

2. Deutsche Zusammenfassung

Plötzlicher Herzstillstand ist eine der häufigsten Todesursachen in westlichen Ländern. Wenn das Herz aufhört zu schlagen, kann nur eine unmittelbare Behandlung schwerste Gehirnschäden oder den Tod verhindern. Die Anzahl der Überlebenden nach einem plötzlichem Herzstillstand hat sich in den letzten zwanzig Jahren verdoppelt. Trotzdem stirbt jeder zweite Patient, der auf einer Intensivstation nach einem Herzstillstand behandelt wird. Die häufigste Todesursuche ist der frühzeitige Abbruch von lebenserhaltenden Maßnahmen, weil ausgeprägte Hirnschäden vermutet werden. Um das Risiko für Fehleinschätzungen zu minimieren, müssen die zur Verfügung stehenden neurologischen Prognosezeichen äusserst sicher sein. Bei der Beurteilung kommen unterschiedliche Untersuchungsmethoden zur Anwendung: 1. die neurologische Untersuchung des Patienten, 2. neurophysiologische Verfahren, die die Gehirnfunktion testen, 3. digitale Bilder (Computertomographie oder Magnetresonanztomographie) und 4. Blutprobe zur Bestimmung von Markern für Nervenschädigungen (Neuronen-spezifische enolase, NSE).

Internationale Leitlinien sollen diese Beurteilung erleichtern und standardisieren. Ein Algorithmus der Europäischen Gesellschaft für Herz-Lungen-Wiederbelebung (European Resuscitation Council, ERC) und der Europäischen Gesellschaft für Intensivmedizin (European Society of Intensive Care Medicine, ESICM) definiert Kriterien, um Patienten mit vermeintlicher schlechter Prognose mit Hilfe der oben genannten Untersuchungsmethoden zu erkennen. Neue technische Verfahren, die Analysen von neuen Markern der Hirnschädigung ermöglichen, stehen seit einiger Zeit zur Verfügung

Diese Promotionsarbeit beinhaltet unterschiedliche Methoden zur Beurteilung der neurologischen Prognose von Patienten nach einem Herzstillstand. Es wurden Informationen von 939 erwachsensen Teilnehmern der bisher größten Herzstillstandstudie, der TTM-Studie, analysiert. Die TTM-Studie wurde von dem Zentrum für Herzstillstand an der Lund Universität in Schweden organisiert und hatte 36 teilnehmende Krankenhäuser in Europa und in Australien. Alle Patienten erhielten eine Kühlbehandlung: entweder bei 33°C oder bei 36°C. Von den meisten Patienten wurden Blutproben 24, 48 und 72 Stunden nach Herzstillstand entnommen. Die TTM-Studie hatte strikte Regeln für die Kühlbehandlung und vordefinierte Kriterien für den Abbruch von lebenserhaltenden Maßnahmen. Sechs

Monate nach dem Ereignis wurde erfasst, ob die Patienten überlebt hatten, und in welchem gesundheitlichem Zustand sie sich befanden.

Die wichtigsten Ergebnisse dieser Promotionsarbeit sind:

- Innerhalb der ersten 24 Stunden nach einem Herzstillstand ergaben die meisten Computertomographien (CT) des Schädels einen Normalbefund. Nach dem ersten Tag hatte jeder zweite untersuchte Patient eine ausgedehnte Schwellung des Gehirns (Hirnödem).
- Fast alle Patienten mit ausgedehntem Hirnödem waren nach 6 Monaten verstorben oder hatten schwerwiegende neurologische Ausfälle. Das Hirnödem ging häufig mit erhöhten Blutwerten des Nervenzellmarkers NSE einher.
- Mit Hilfe einer neuen und besonders empfindlichen Messmetode zeigte sich, dass das Eiweiß „Neurofilament light“ (NFL) im Blut besser zwischen Patienten in gutem neurologischem Zustand oder in schlechtem neurologischem Zustand nach Herzstillstand trennen konnte als andere Marker.
- NFL erkannte einen größeren Anteil an Patienten mit ungünstiger Prognose, als alle Standardmethoden, die von den Leitlinien empfohlen werden. Die NFL Werte stiegen mit der Ausprägung an Hirnschädigung an.
- Zwei weitere Marker für Hirnschädigung, UCH-L1 und GFAP im Blut wurden mit Standardmethoden analysiert. Im Gegensatz zu NSE konnten UCH-L1 und GFAP nur innerhalb der ersten 24 Stunden besser zwischen guter und schlechter Prognose unterscheiden.
- Alle Patienten, die die Kriterien des ERC/ESICM Algorithmus für eine schlechte Prognose erfüllten, wurden richtig erkannt. Allerdings wurden mehr als die Hälfte der Patienten mit schlechter Prognose nicht erkannt.

Mindestens zwei Untersuchungsmethoden sollten kombiniert werden, um die Zuverlässigkeit der Prognose nach Herzstillstand zu verbessern. Wenn alle bewusstlosen Patienten beurteilt würden, könnten mehr Patienten mit schlechter Prognose erkannt werden. In Zukunft könnte eine Blutprobe zur Bestimmung des NFL frühzeitig zwischen Patienten mit guter und schlechter neurologischer Prognose unterscheiden. Eine Fortsetzung der Intensivbehandlung von bewusstlosen Patienten mit niedrigen NFL Werten und damit guter Prognose könnte deutlich mehr Leben retten als bisher.

3. Background

3.1 Epidemiology and survival

Cardiac arrest (CA) is a life-threatening condition with abrupt loss of heart function resulting in cessation of blood circulation. Cardiopulmonary resuscitation (CPR) within minutes is necessary to prevent extensive brain injury or death. This thesis will focus on out-of-hospital cardiac arrest (OHCA). The incidence, survival and quality of documentation of CA varies throughout the world. A systematic review estimated a global incidence of OHCA treated by emergency medical services of 62 cases per 100.000 persons annually, of which 75-85% had a primary cardiac cause of arrest.[1] In Sweden, approximately 6.100 OHCA patients were treated by emergency medical services in 2018, the median age was 71 years and the majority of patients were male.[2] Main causes of OHCA in adults ≥40 years in Sweden were cardiac, ischaemia was the most common subtype.[2] In younger adults intoxication, trauma, pulmonary diseases and near-drowning accounted for most cases of OHCA, whilst in infants, sudden infant death syndrome was one of the main causes.[2] Cardiac arrhythmias often progress from a shockable rhythm (ventricular tachycardia or ventricular fibrillation) to non-shockable rhythms (asystole or pulseless electrical activity) in the minutes after CA. Prognosis is more often unfavourable in patients with non-shockable rhythms.[3] The average survival of OHCA in Sweden has nearly doubled in the last two decades (Fig. 1).[2]

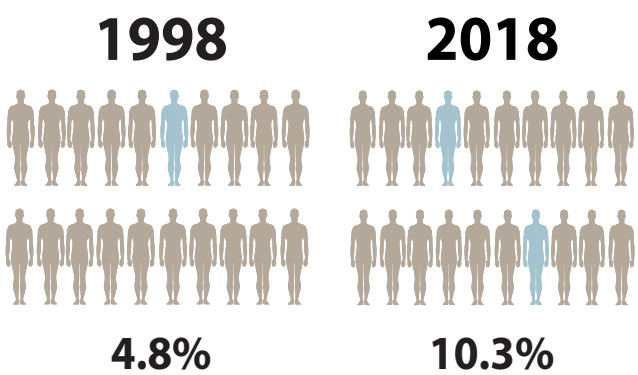
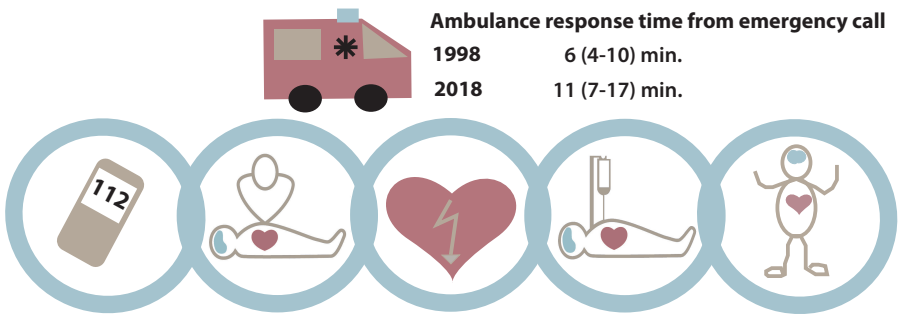


Fig. 1 Symbolic illustration of the average 30 day survival in OHCA patients treated by emergency medical services in Sweden in years 1998 and 2018. Even though the average survival has nearly doubled in recent years, the total mortality of OHCA in Sweden is still ~90% [2]. Blue figures indicate patients alive, brown figures are non-survivors.

The increased incidence in bystander CPR, and availability of early defibrillators in public locations are most likely contributing to this effect. [4-6] In Sweden, bystander CPR is now performed in 3/4 OHCA patients and the median time from OHCA to CPR is 1 minute (Fig. 2).[2]



	Emergency call	CPR	Defibrillation	ICU admission	Survival CPC 1-2
1998	4 (2-7) min.	10 (5-15) min.	11 (9-16) min.	14.2%	?
2018	2 (1-4) min.	1 (0-7) min.	10 (6-16) min.	20.4%	407/437 (93.1%)

Fig. 2 is a modified version of the so called "Chain of Survival" after OHCA also including data from the Swedish Heart-Lung-Registry [2,7]. The figure demonstrates the reported change within the last 20 years of the median time in minutes, lower and upper quartiles to: emergency call, time to start of CPR and first defibrillation as well as time from emergency call to arrival of the ambulance. ICU admission indicates percentage of patients admitted alive on the Intensive Care Unit (ICU). Survival CPC 1-2 indicates number of patients with no or mild cerebral disability.

The 30-day survival for males was almost twice as high compared to females (12.1% versus 6.9%).[2] Women more often have CA at home, with an initial non-shockable rhythm on ECG, both factors associated with poor outcome. [3,8]

Within the first 2-3 days after CA, the main causes of mortality are circulatory failure, cardiac failure or multi organ failure.[9] After 3-4 days, death is often a result of withdrawal of life-sustaining-therapy (WLST) due to a presumed poor neurological prognosis.[9]

3.2 Brain cells and ischaemia

Neurons and glial cells form the basis of the nervous system, with an overall ratio of approximately 1:1, depending on anatomical location and developmental stage.[10] In brief, neurons are electrically excitable cells that communicate with other cells via synapses. Glial cells do not produce electrical impulses but display numerous supportive and regulative functions: oligodendrocytes produce myelin sheaths that function as insulation speeding up nerve pulse conduction and provide metabolic support to axons. Microglia are the brain's macrophages, destroying pathogens and removing dead neurons. Astrocytes are a heterogenous group of the remaining glial cells with different functions and morphology. They may be star-shaped with thousands of processes and have the ability to interact with all cells of the central nervous system, blood vessels and regulate various processes including regulation of synapse functions, metabolic and homeostatic support.

Prolonged cardiac arrest initially causes a whole-body ischaemia resulting in global tissue and organ injuries. Some are immediate, while others progress within hours or days post-arrest. The brain cells have very high metabolic demands (glucose and oxygen) and are especially vulnerable to deprivations in blood supply. Cessation of blood-flow rapidly causes malfunctioning of the energy dependent Na^+/K^+-pumps in the cell membranes resulting in a loss of ion gradients (anoxic depolarisation) (Fig. 3).

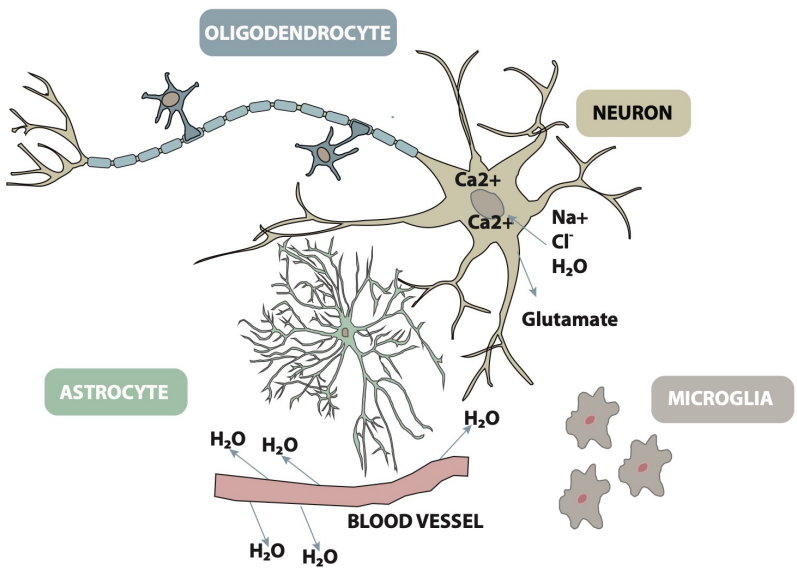


Fig. 3 Schematic illustration of a neuron surrounded by various glial cells: an astrocyte, myelin producing oligodendrocytes on the neuronal axon and microglia. Osmotic ions (sodium and chloride) and water enter the cells causing cytotoxic/ionic oedema. The neurons "power plants", the mitochondria, are irreversibly damaged, leading to a further depletion of energy (ATP) production, lactate accumulation and cell necrosis. Calcium accumulates intracellularly causing cytotoxicity and release of glutamate. Water accumulates extracellularly as vasogenic oedema due to an increased permeability of the blood-brain barrier.

3.3 Post-cardiac arrest syndrome

Re-establishing spontaneous circulation does not necessarily reverse the injuries caused by cessation of blood-flow. In fact, reperfusion may cause additional damage through release of free radicals, excitotoxicity, inflammation and microvascular damage. The "post-cardiac arrest syndrome" (PCAS) is the term used to describe these complex mechanisms and symptoms that may occur in the hours and days post-arrest (Fig. 4).[11,12] The PCAS has four defined components: 1) brain injury, 2) myocardial dysfunction, 3) systemic ischaemia/reperfusion response and 4) persistent precipitating pathology.[11]

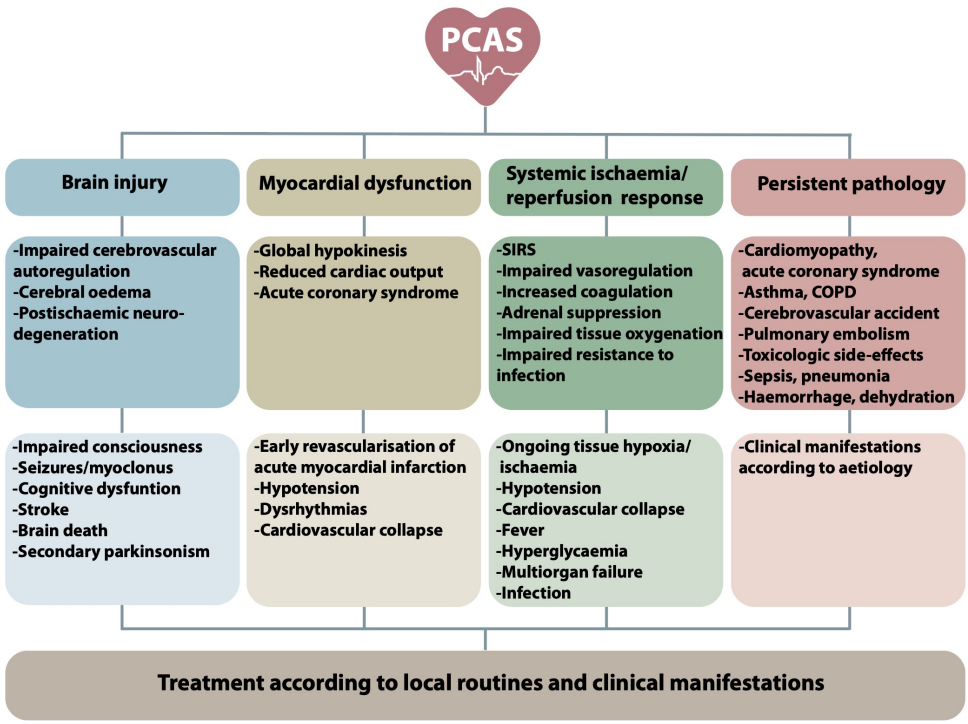


Fig. 4 Schematic illustration of the four components of the post-cardiac arrest syndrome (PCAS), their pathophysiology and clinical manifestations according to the International Liaison Committee on Resuscitation, ILCOR.[11] SIRS, systemic inflammatory response syndrome; COPD, chronic obstructive pulmonary disease.

Despite return of perfusion and restoration of energy levels, brain cells may not survive. Certain anatomical regions such as the pyramidal cells in the cortical layers III, V and VI, the hippocampi, the thalami, cerebellar Purkinje cells and watershed-regions between two arteries are more vulnerable to ischemic injury, often demonstrating a delayed selective neuronal death several days post-arrest.[13-16] Other regions are less vulnerable, possibly due to lower energy demands.

The brain stem usually demonstrates initial recovery within days post-arrest, leading to a return of brain-stem reflexes, sometimes despite extensive ischemic injuries in the basal ganglia, cortex or cerebellum.[15] Glial cells may not seem as vulnerable to injury as neurons; however, the ischaemia/reperfusion also impacts the oligodendrocytes and astrocytes which may become damaged. The pathophysiology behind the delayed demyelination of neurons is not fully understood. This late subacute non-inflammatory process becomes visible weeks post-arrest.[13] In the chronic phase, months to years' post-arrest, brain volume loss due to neuronal death and atrophy is sometimes seen.[13]

3.4 Targeted temperature management

Targeted temperature management (TTM) describes a strategy to reduce the core temperature of the body in patients after CA using techniques such as ice packs or cold infusions, surface cooling devices, intravascular devices or by nasal or oesophageal cooling. Reduction of the core temperature has long believed to have neuroprotective effects, probably by reduced cerebral blood flow and brain metabolism and by reducing excitotoxicity. The use of mild induced hypothermia after CA was recommended by guidelines [17,18] after two smaller randomised trials had reported that TTM resulted in higher survival rates [19,20] and increased favourable neurological outcome.[20] Both studies included patients with OHCA and initial shockable rhythms, targeted temperatures were between 32-34°C for a duration of 12-24 hours.[19,20] A meta-analysis including 478 patients from these two trials and three other randomised controlled trials found that there was a lack of firm evidence for a benefit of TTM after CA.[21] The authors concluded that the quality of evidence for mild induced hypothermia was low when using GRADE-methodology, downrating was due to high risk of bias and concerns with directness.[21] The Target temperature management after out-of-hospital cardiac arrest (TTM) trial found no difference in survival nor neurological outcome between TTM 33°C and 36°C in patients with OHCA of presumed origin when TTM was initiated in hospital.[22] The TTM trial is described further in the methods section.

Currently, ERC/ESICM guidelines recommend TTM, maintaining a constant target temperature between 32°-36°C based on low quality of evidence for patients with initial shockable rhythms for at least 24 hours.[23] TTM is suggested with very low-quality evidence for patients with OHCA and initially non-shockable rhythms and for in-hospital CA of any initial rhythm.[24] A recent trial randomising CA patients with an initial non-shockable rhythm to intrahospital TTM 33°C or targeted normothermia (37°C) found no difference in mortality at 90 days, but reported a higher percentage of patients with favourable neurological outcome in the 33°C group.[25]

An extensive meta-analysis of animal models of CA concluded that TTM was favoured compared to controls when evaluating neurobehavioral outcome, brain histology or mortality, regardless of the timing of TTM (pre-, intra- or post-arrest).[26] These results may not apply to the clinical setting in humans, since animal models mainly use young animals (often rodents) without comorbidities, applying different mechanisms of circulatory cessation, with shorter time to ROSC and TTM, shorter time to follow-up, and simpler tests of neurobehavioral outcome in overall extremely well controlled environments.[26] Several clinical trials have tried to examine whether intra-arrest cooling may improve survival or neurological outcome, yet no such overall benefit has been found.[27-31] Two studies reported that infusion of cold saline was significantly associated with complications such as re-arrest in patients with initial shockable rhythms and a higher number of patients with clinically suspected pulmonary oedema.[28,30] A recent sub analysis using propensity matching of the PRINCESS trial reported that good neurological outcome was more common in patients with initial shockable rhythms treated rapidly with transnasal intra-arrest cooling.[32]

A failure to treat fever in control groups has been discussed as a potential source of bias in previous TTM trials. In addition, neurological prognostication within trials examining the benefit of TTM rarely followed a pre-specified protocol,[20,27-31] and information on WLST within treatment groups was sometimes lacking.[27-29,31] The Targeted hypothermia versus targeted normothermia after out-of-hospital cardiac arrest (TTM2) trial randomised 1900 patients with a presumed cardiac or unknown cause of arrest to TTM 33°C or normothermia with early treatment of fever ≤37.8°C.[33] Results are pending and the TTM2-trial will be discussed further in the "future perspectives" section. Another large ongoing international multicentre OHCA trial, the TAME-trial, aims to assess whether targeted mild hypercapnia improves neurological outcome compared to standard care.[34,35]

3.5 Neurological prognostication

The prediction of neurological outcome in unconscious patients after CA should be delayed until any residual effects of muscle relaxation or sedation have been ruled out. Patients treated with TTM require sedation to tolerate the lower body temperature, and TTM may reduce the clearance of drugs.

Various methods should be combined to evaluate the extent of brain injury and subsequently the presumed prognosis including clinical neurological examinations, structural neuroimaging, neurophysiological examinations and serum biomarkers (Fig. 5). The results of this neuroprognostication, together with cardiac function, pre-existing medical comorbidities, ethical aspects and a patient's presumed wishes, are all taken into account when deciding on the level of care. All factors together influence neurological outcome.

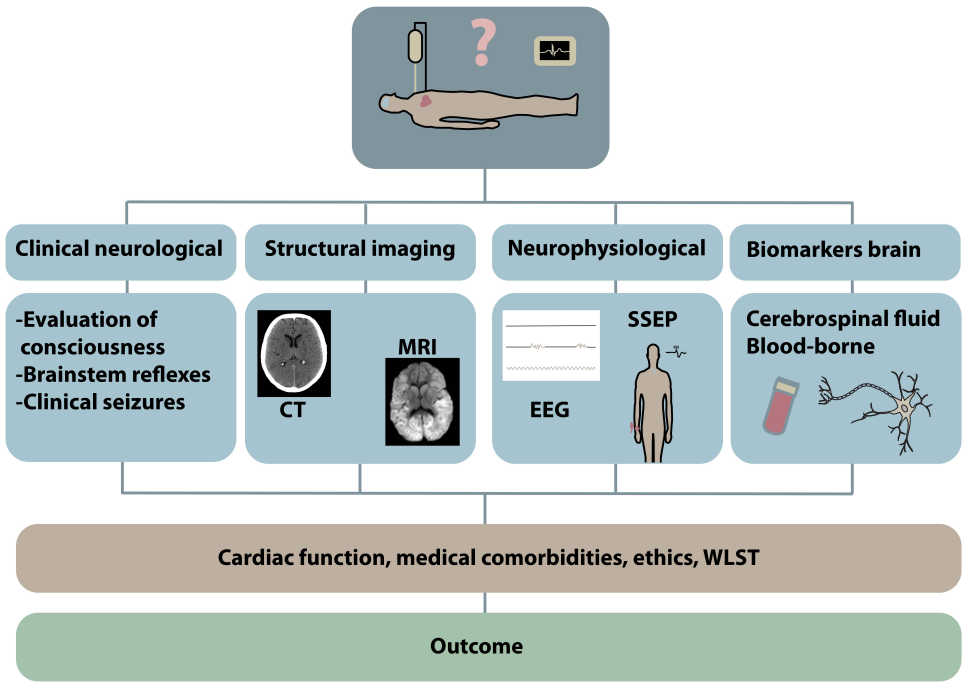


Fig. 5 Illustrates the various methods often used within neuroprognostication. CT; head computed tomography, MRI; head magnetic resonance imaging, SSEP; short-latency somatosensory evoked potentials, EEG; electroencephalogram, WLST; withdrawal of life-sustaining therapy.

3.5.1 ERC/ESICM guideline recommendations

In 2015, the European Resuscitation Council (ERC) and the European Society of Intensive Care Medicine (ESICM) published joint guideline recommendations for neurological prognostication after CA based on the current level of evidence together with a stepwise algorithm based on expert opinion.[24,36-38] Paper IV in this thesis aims to assess the prognostic performance of this algorithm. The algorithm consists of four separate steps, which for clarity we have named Step 0-3 (Fig. 6). Prognostication should be performed >72 hours after CA and only after major confounding factors such as metabolic derangements and residual effects of sedation or muscle relaxants have been excluded (Step 0).

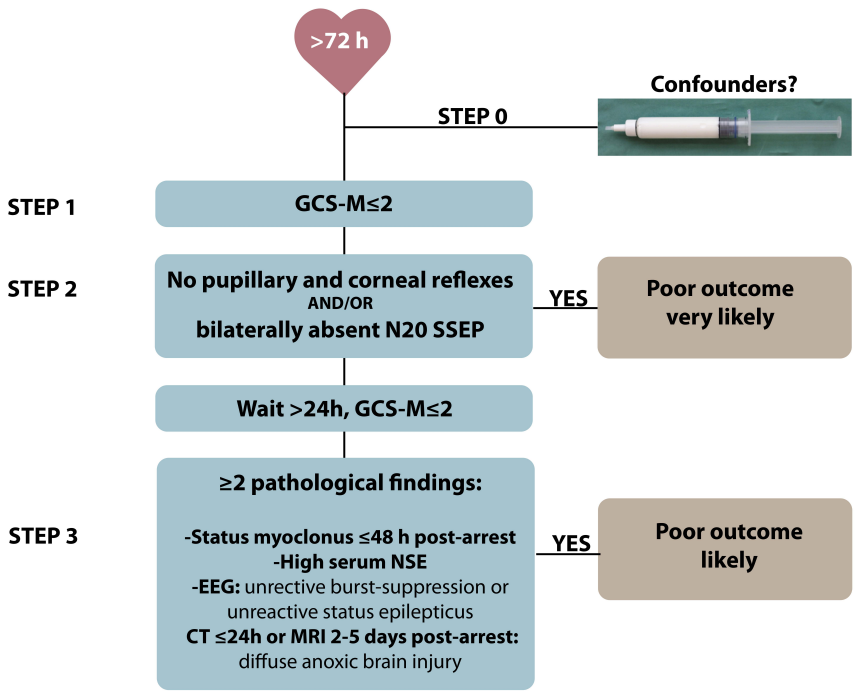


Fig. 6 A simplified illustration of the ERC/ESICM algorithm for prognostication of poor outcome after cardiac arrest [24,36]. GCS-M; Glasgow Coma Scale Motor Score, SSEP; short-latency somatosensory evoked potentials, CT; head computed tomography, MRI; head magnetic resonance imaging.

In Step 1, the patient's best motor response to a centrally given painful stimulus is used as a screening criterion. Patients with no motor reaction or extension posture (GCS-M ≤2), should be examined further. A poor outcome is considered "very likely" if both pupillary and corneal reflexes are bilaterally absent, or if N20 potentials on short-latency somatosensory evoked potentials (SSEP) are bilaterally absent (Step 2).

Patients who do not fulfil the criteria in Step 2 should be re-examined ≥24 hours later. In Step 3, a poor outcome is considered "likely" if the patient is still deeply unconscious and at least two of the following characteristic findings are present: early status myoclonus (≤48 hours), generalised oedema on neuroradiology (CT ≤24 h or MRI performed 2-5 days post-arrest), unreactive status epilepticus or unreactive burst-suppression on electroencephalogram (EEG) or elevated neuron specific enolase (NSE) in blood at 48 or 72 hours post-arrest according to locally established cut-off values.[24] Each prognostic method included in this algorithm will be explained below.

3.5.2 Clinical neurological examination

Evaluation of consciousness

Various methods and scales exist to evaluate a patient's level of consciousness. In addition to auditory stimuli (e.g. calling out the patient's name), response to a painful stimulus is used to evaluate patients who are not awake. Common locations for noxious stimuli are the supraorbital ridge, the temporomandibular joints, the sternum and the nail beds of fingers and toes. Various quantitative scales exist. In this thesis, the Glasgow Coma Scale (GCS) consisting of three subcategories (eye, motor and verbal) was used to assess level of consciousness (Table 1).[39] Another quantitative scale, the FOUR Score (Full Outline of Unresponsiveness) includes evaluation of brain stem reflexes and respiratory function, in addition to motor response and eye-response, and may be better suited for evaluation of mechanically ventilated patients than the GCS.[40]

Table 1 The Glasgow Coma Scale (GCS)

Subcategory	Points	Criteria
Eye response (E)	4	Eyes open spontaneously
	3	Eye opening to verbal command
	2	Eye opening to pain
	1	No eye opening
Motor response (M)	6	Obeys commands
	5	Localizing pain
	4	Withdrawal from pain
	3	Flexion response to pain
	2	Extension response to pain
	1	No motor response
Verbal response (V)	5	Oriented
	4	Confused
	3	Inappropriate words
	2	Incomprehensible words
	1	No verbal response

Motor response

A patients best motoric response is often reported by the GCS-M. Absence of motor response may be confounded by sedation and muscle relaxants, whilst extension or flexion postures may be considered more specific to brain injury. Due to the sensitivity to pharmacological agents and reported false positive ratios of 10-20% in TTM-treated patients after CA,[37,41] GCS-M should not be considered a prognostic marker *per se*, but more as a screening criterion for identifying patients requiring further neuroprognostication.[24,42-44] The ERC/ESICM algorithm recommends GCS-M≤2 as a screening criterion in order to fulfil criteria for "poor outcome likely".[24]

Brainstem reflexes

Pupillary light reflexes and corneal reflexes are routinely examined to assess brain stem function in unconscious patients. The pupillary light reflex (hence called "pupillary reflex") is used to test the integrity of the sensory and motor functions of the eye. Corneal reflexes are used to assess the integrity of the trigeminal (afferent) and facial (efferent) nerves. Bilaterally absent pupillary or corneal reflexes on day 1-3 post-arrest have been described as predictors of poor outcome with 0% FPR (0-3) previous to the TTM era.[45]

Sedatives and muscle relaxants may give a false cause the impression of absent brainstem reflexes in patients with good neurological outcome.[37,41,44] Nevertheless, bilaterally absent pupillary and corneal reflexes together predicted poor neurological outcome with no false positive predictions in TTM-treated patients, with individual sensitivities of 24.1% and 34.5%, respectively.[41] The brainstem is more resistant to brain injury after CA[15,16] and brainstem reflexes may recover, explaining why present pupillary or corneal reflexes are no reliable predictors of good outcome.[46]

Clinical seizures

Clinical seizures after CA are common, occurring in one third of patients.[47] Their cerebral origin may be focalised or generalised, and the clinical seizures may be classified as myoclonic, tonic-clonic, or a combination of both. Myoclonic seizures are the most common and will be described below. Tonic-clonic seizures and combined seizures are less commonly observed and are not necessarily associated with poor outcome.[47]

Status myoclonus

Myoclonus is a brief and involuntary twitching of muscles or muscle groups observed in approximately 20% of patients treated with TTM after CA.[48] A severe generalised form of acute myoclonus, often called "status myoclonus" or "myoclonic status epilepticus" has been associated with poor outcome if present ≤48 hours post-arrest.[45,48,49] However, definitions and terminology vary between studies and WLST may have influenced outcome.[44]

In this thesis, the term "status myoclonus" will be used to describe a clinically observed generalised myoclonus with twitching in the face and extremities bilaterally persisting >30 minutes in unconscious patients, irrespective of EEG findings.

The exact pathophysiology of neuronal injuries that causes acute post-hypoxic myoclonus is unclear. Myoclonus is sometimes dichotomised by its presumed origin in cortical or subcortical, even though the clinical features may be similar.[50,51] Clinical and electrophysiological studies of association with the presumed anatomical localisation of injury are inconclusive.[50-53]

Patients regaining consciousness despite early status myoclonus have demonstrated a continuous background on EEG, present N20 potentials on SSEP and present brainstem reflexes, emphasising on the importance of a multimodal approach to prognostication.[49,54,55] These patients probably have an early form of the Lance-Adams syndrome, a rare condition involving chronic action myoclonus, which can sometimes be seen after hypoxic brain injury.[56] Radiological lesions in the cerebellum and thalami have been described in patients with Lance-Adams syndrome.[57]

3.5.3 Structural neuroimaging

Computed tomography

Computed tomography is a widely available imagining procedure in which a narrow beam of x-rays is aimed at a patient whilst rotating around the body. The resulting signals are computer-processed into cross-sectional images. Head computed tomography (CT) after CA is often performed early after hospital admission to exclude intracranial haemorrhages or other cerebral causes of unconsciousness. The ERC/ESICM recommend CT within 24 hours after CA.[24]

Post-arrest brain injury is associated with development of generalised oedema, which is considered an indicator of poor neurological prognosis, but with low level of evidence.[37] The initial swelling of neurons and glial cells is caused by an influx of osmotically active ions (cytotoxic/ionic oedema) (Fig. 3). Subsequently, increased microvascular permeability increases extracellular oedema, "vasogenic oedema", but both types of oedema likely coexist. On CT, generalised oedema can be seen as a reduced differentiation between the grey and white matter and effacement of the cortical sulci (Fig. 7A-B).

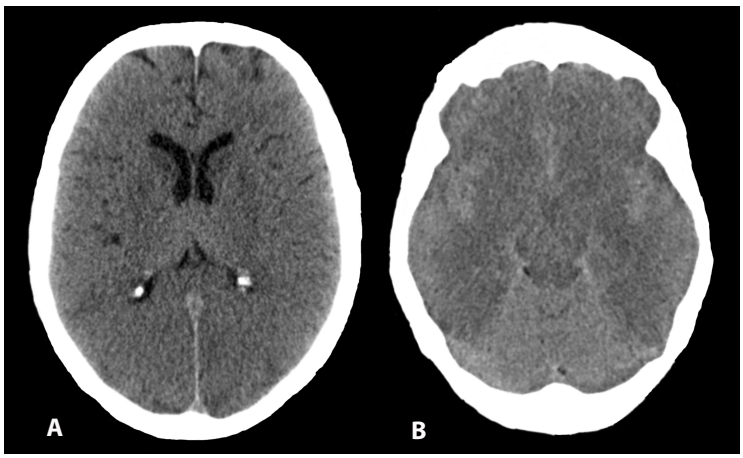


Fig. 7A-B Head computed tomography illustrating generalised oedema after cardiac arrest.

The majority of research on CT after CA has been investigating quantitative measurements of absolute densities (in Hounsfield units, HU) of the grey and white matter in so called "regions of interest" (ROI), often in the basal ganglia or cortical structures. These HU measurements can be used to calculate a grey-white matter density ratio (GWR), where a lower GWR has been found to correspond with unfavourable outcome.[58-61] A decreased GWR after CA mainly results from a loss of grey matter density due to oedema, but may become further enhanced due to a slight gain in white matter density due to distension of medullary veins.[59]

The placement and number of ROIs used to calculate GWR vary between studies and currently there is no consensus on method, nor optimal cut-off value to identify unfavourable outcome with high specificity.[24,37] The majority of published studies are retrospective, with varying timing of examination and outcome assessment. Reported cut-off values for prediction of poor outcome with maximal specificity range from 1.08-1.23, and highly varying sensitivities.[58,59,61-65]

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a widely available radiological technique where strong magnetic fields, radio frequency currents and a computer are used to create detailed images. Although applying MRI scans on ICU patients demands specific planning and may be unfeasible for unstable patients, brain MRI can contribute with valuable prognostic information. Various MRI sequences exist to visualise cerebral oedema in CA patients. Diffusion weighted imaging (DWI) detects cytotoxic/ionic oedema in the early phase within the first 7-10 days post-arrest, visible as hyperintense signals (Fig. 8A).[66] A reduced apparent diffusion coefficient (ADC) can be used to quantify the restricted diffusion caused by the cytotoxic/ionic oedema (Fig. 8B).[13] The fluid attenuation inversion recovery (FLAIR) sequence can detect both cytotoxic/ionic and vasogenic oedema, usually from 1-2 days post-arrest, remaining visible for up to several weeks (Fig. 8C). Early T2-FLAIR hyperintensities represent vasogenic oedema, while hyperintensities weeks post-arrest represent gliosis.[66]

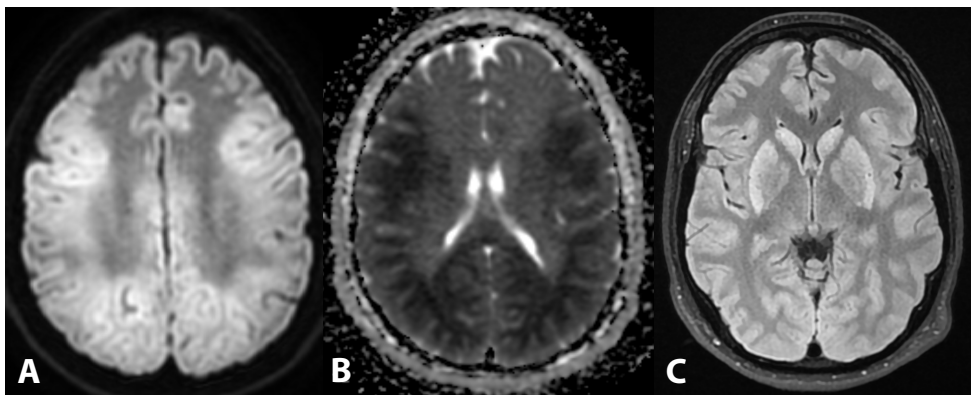


Fig. 8A-C Magnetic resonance imaging with generalised oedema on DWI (A) and reduced diffusion on ADC (B) cortically and subcortically. The T2 FLAIR (C) at 4.5 days post-arrest demonstrates oedema in the basal ganglia (mainly the Caudate nucleus and the Putamen).

The ERC/ESICM include “the presence of diffuse ischaemic changes on brain MRI 2-5 days after ROSC” as one criterion in their multimodal algorithm for prediction of poor neurological outcome.[23] Neuroradiology is presumably not affected by TTM or sedation, but other conditions such as epileptic seizures, hypoglycaemia or hyponatraemia may mimic the presence of cytotoxic oedema om MRI.[13]

3.5.4 Neurophysiology

Short-latency somatosensory evoked potentials

The short-latency somatosensory evoked potentials (SSEP) are examined by repeatedly stimulating a peripheral nerve, usually the median nerve. Averaged afferent signals result in characteristic *negative (N)* and *positive (P)* deflections registered by electrodes above the contralateral primary somatosensory area and other anatomical structures along the signal pathway (Fig. 9). A cortical *negative* deflection approximately 20 milliseconds after stimulation of the median nerve, *N20*, is generated by depolarisation of the pyramidal cells in the post-central gyrus.

Bilaterally absent N20 potentials have been associated with bilateral thalamic damage, sometimes in combinations with cortical damage.[67] The absence of N20-potentials should only be diagnosed if the subcortical brainstem responses are normal, especially to avoid misinterpreting high cervical injuries as cerebral injuries.

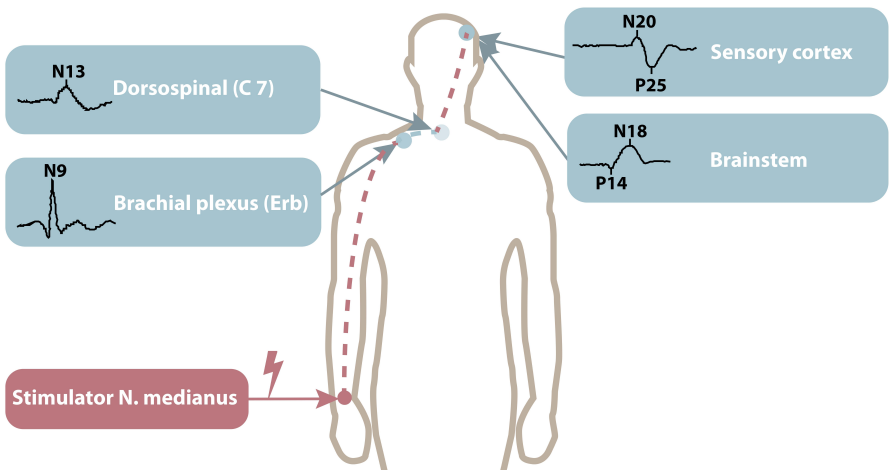


Fig. 9 Illustrates the afferent signal pathway of SSEP when stimulated at the right median nerve and registered at Erb (N9/10), at the dorsal cervical column (N13) and at the contralateral somatosensory cortex (P14, N18, N20 and P25).

Bilaterally absent N20 potentials have been robust predictors of poor outcome after CA in several trials (specificity >99%) both in studies allowing WLST and in studies where WLST was not performed.[41,68-70] SSEP results could be confounded by muscle activity, interference of electronic devices in the ICU or by inter-rater variability.[71] Moderate sedation or metabolic disturbances are not believed to affect cortical SSEP responses, but very high levels of barbiturates/sodium thiopental or intoxication with heroine or cocaine could increase latency and decrease the amplitude of cortical responses.[71,72] According to ERC/ESICM guideline recommendations, SSEP is regarded robust to effects of TTM when performed after rewarming.[24]

Electroencephalogram

EEG is the most commonly used prognostic method after CA,[73] offering real-time assessment of synaptic function of cortical pyramidal cells. The electrical activity is synchronised by subcortical neurons in the thalamus, but also by cortico-cortical connections. The EEG-activity is modulated by the reticular activating system in the brainstem, responsible for regulating the sleep-wake cycle. EEG can be performed intermittently by using a full-montage of electrodes for a short period of time (20-30 minutes) a so called "routine-EEG", or by continuous monitoring (cEEG) of the patient. The registered EEG is evaluated according to the background activity, presence of discharges and electrographic reactivity to external stimuli. Unfortunately, definitions of EEG patterns vary between studies, complicating comparisons of results. The American Clinical Neurophysiology Society (ACNS) has suggested a standardised EEG terminology for critical care patients, which has been increasingly used in recent years.[74]

In CA patients, the initial background activity is often suppressed (very low amplitudes). EEG-patterns after CA are dynamic, and within hours or days post-arrest, the synaptical activity may recover and develop into a normal continuous background, or with varying degrees of discontinuous or suppressed background activity. Early return of a continuous background or reactivity to external stimuli has been described as indicative of a favourable outcome within 12-24 hours post-arrest.[46,75-77]

Westhall et al. have suggested a standardised terminology of "highly malignant patterns" after rewarming based on the ACNS criteria that accurately predicted poor neurological outcome after CA with substantial interrater agreement.[46,78-80] The ERC/ESICM guidelines includes two EEG patterns considered indicative of poor neurological outcome; "unreactive status epilepticus" and "unreactive burst-suppression".[24] Burst-suppression is defined as suppression periods with amplitudes below 10 μV for $\geq$50% of the EEG recording with alternating bursts of cortical activity.[24] Definitions of electrographic status epilepticus and criteria for reactivity testing is not specified.[24]

Studies have demonstrated that patients with a continuous background prior to status epilepticus may be compatible with a good neurological outcome.[81] CA patients are usually receiving sedation with short-acting drugs such as propofol to tolerate TTM. A study concluded that although propofol did reduce amplitude, continuity and dominant EEG frequency, the prognostic value was not significantly affected by routine TTM and sedation with propofol or midazolam,[82] which is in accordance with results from other studies.[79] However, there have been reports of false pathological classifications of EEG's in sedated patients, therefore sufficient time without sedation should be allowed before assessing EEG for prognostic purposes.[78]

3.5.5 Biomarkers of brain injury

Neurons and glial cells consist of different biochemical components that represent potential markers of cerebral injury (Fig. 10).

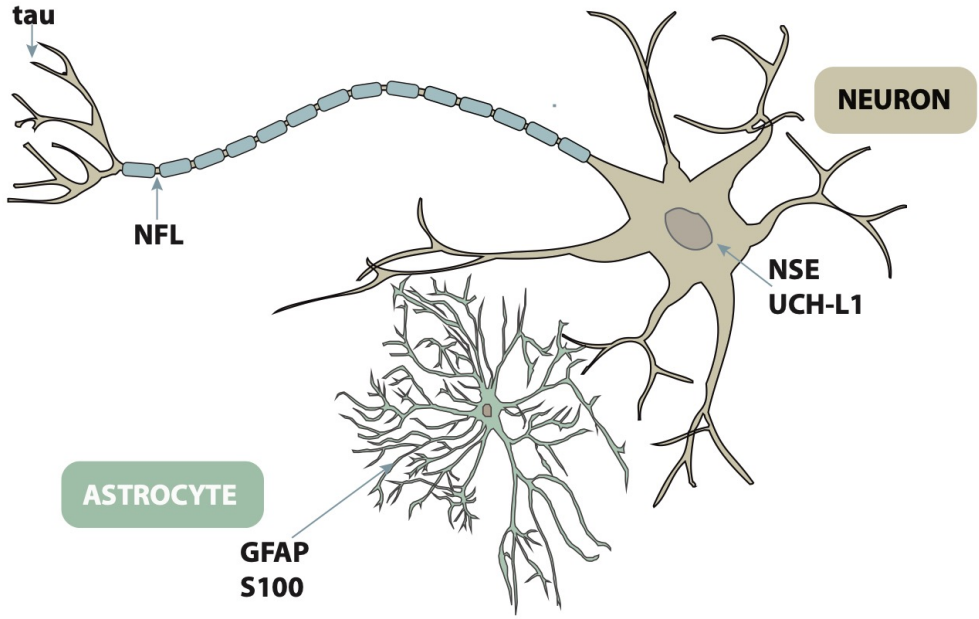


Fig. 10 Illustrates a neuron and an astrocyte together with the main structural locations of biomarkers included in this thesis.

These breakdown products from brain cells presumably reach the blood either 1) by diffusion through the blood-brain barrier, or 2) possibly thorough cerebrospinal fluid drainage into the blood via perivenous pathways ("glymphatic system").[83,84]

Absolute biomarker concentrations may be higher if measured directly in the cerebrospinal fluid than when measured in blood, but blood is collected more easily, and sampling is considered less invasive.[85,86]

An optimal biomarker of cerebral injury should be highly specific for the central nervous system, be unaffected by haemolysis and be able to discriminate between various degrees of brain injury with high accuracy.

3.5.5.1 Neuronal body markers

NSE

Neuron specific enolase (NSE), also known as enolase 2 (ENO2) or Gamma-enolase, has neuroprotective properties on a broad spectrum of central nervous system neurons promoting cell survival.[87] NSE is present in the plasma membrane and cytosol of neurons, but also in oligodendrocytes, neuroendocrine cells, platelets, erythrocytes and serves as a marker for several cancer types (Table 2, page 36).[86,87]

Even invisible haemolysis will contaminate the sample due to the very high concentration of NSE in erythrocytes, representing an important limitation to its use within neuroprognostication.[86] Several studies found that serum NSE is higher in CA patients with poor neurological outcome.[68,88,89] NSE at 24 hours post-arrest is not a reliable predictor of poor outcome, but prognostic accuracy improves at 48 and 72 hours.[44,88]

Serum NSE is the only biomarker recommended for neuroprognostication in the ERC/ESICM guidelines, however specific cut-off values are not defined and should be established locally due to a lack of a calibration standard.[24] Since NSE is not specific for the central nervous system and could be elevated due to for example neuroendocrine tumours or haemolysis, evaluation of increasing serum levels between 24-48 hours post-arrest is recommended.[42]

UCH-L1

Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) mainly shows enrichment in the cytoplasm and nucleoplasm in the cell body, axon and synapses of neurons.[87] UCH-L1 is also expressed in neuroendocrine cells, in endothelial and smooth muscle cells and is a prognostic marker in endometrial and urothelial cancer (Table 2).[86,87]

UCH-L1 is important for neuro-axonal stability and repair after brain injury and has commonly been studied after traumatic brain injury.[86,90] Together with glial fibrillary acidic protein (GFAP), UCH-L1 has recently been approved as a biomarker aimed at reducing head CT examinations in patients with mild traumatic brain injury.[90] In an animal model, UCH-L1 correlated with the severity of neuronal hippocampal apoptosis in piglets.[91] A pilot-study found that UCH-L1 was significantly elevated in paediatric CA patients compared to controls, and predictive of poor outcome at approximately 10 and 72 hours post-arrest.[92] UCH-L1 has not previously been investigated in adult cardiac arrest patients.

3.5.5.2 Axonal injury markers

NFL

Neurofilaments are structural proteins highly expressed in large-caliber myelinated axons and may also play a role in intracellular transport to axons and dendrites.[86] There are five different neurofilaments of which three are named after their molecular weight: neurofilament heavy chain, medium chain and light chain (NFL), in addition to internexin-α and peripherin. The expression of the neurofilaments vary between different nerve cells and different stages of development.[87,93] Neurofilaments are also present in peripheral nerves and is enhanced in renal cancer.[87] The gene encoding NFL is located on chromosome 8, and mutations cause peripheral neuropathies of Charcot-Marie-Tooth disease types CMT1F and CMT2E, and elevated plasma NFL concentrations can therefore also be found in CMT patients.[87,94]

One small pilot study found elevated levels of NFL in cerebrospinal fluid of CA patients.[95] Two small pilot studies found that elevated blood levels of neurofilaments corresponded with poor neurological outcome after CA, when samples were analysed with standard immunoassays.[96,97] Analysed with a novel ultrasensitive assay (Simoa), serum NFL correlated strongly with CSF NFL[98] and was elevated in a range of neurological conditions including traumatic brain injury, parkinsonian disorders, HIV-dementia, amyotrophic lateral sclerosis (ALS) and ischaemic stroke.[85,99-102] Serum NFL analysed with an ultrasensitive assay had not been investigated as a prognostic marker after CA.

Tau

Tau (also called MAPT) is a microtubule-associated protein predominantly present in the plasma membrane and nucleus of thin unmyelinated axons of the cortex, in astrocytes and oligodendrocytes.[103] Tau is expressed in several organs outside the CNS and is enhanced in several cancer types (Table 2).[87] Tau pathologies are present in various neurodegenerative disorders such as dementias and parkinsonian disorders.

In a small cohort of CA patients, CSF total-tau (t-tau) analysed with an immunoassay was increased approximately 2 weeks post-arrest compared to healthy controls.[104] Analysed with an ultrasensitive assay, serum t-tau was higher in poor outcome patients treated with TTM after CA.[103,105] Tau release after CA has been described as bimodal in a small study, with one early peak within the first hours after CA and a delayed peak after 24-48 hours, with higher tau levels corresponding with neurological outcome.[103]

3.5.5.3 Glial cell markers

S100B

S100 proteins are a family of at least 26 human Ca^{2+}-binding subtypes with a large variety of different tissue-specific regulating effects, either intracellularly, extracellularly or both.[106,107] The S100 proteins are not CNS specific and are present in most types of tissue (Table 2). Serum S100B has been extensively studied as a marker of traumatic brain injury or cardiac arrest. Analysis of S100B usually includes both dimers with at least one B monomer, representing the sum of S100A1B and S100BB concentrations.[108] A small study found no significant difference between the prognostic accuracy of using either one of these dimers or the sum of both in patients with traumatic brain injury.[108] In this thesis, the term S100B describes the sum of S100A1B and S100BB.

S100A1B is found in the cytoplasm, cytoskeletal components and mitochondria of neurons, but also in skeletal muscle fibres and cardiomyocytes and is mostly studied as a marker of cardiac injury.[106]

S100BB is highly expressed in astrocytes, and to a lesser extent in certain neurons, Schwann cells, dendritic cells, as well as in numerous cells outside the nervous system (Table 2). Increased expression represents astrocytic activation and not necessarily astrocytic injury. In low/physiological concentrations S100BB stimulates cell proliferation and in high concentrations, it may worsen inflammatory responses by acting as a cytokine itself and by causing oxidative damage to neurons.[106] S100BB is elevated after CNS injury such as ischaemic stroke, subarachnoid haemorrhage, traumatic brain injury, but also in patients with bacterial meningitis, multiple sclerosis, schizophrenia and mood disorders.[106]

After CA, S100B was higher in poor outcome patients than in good outcome patients at baseline until approximately 72 hours post-arrest, with highest prognostic accuracy at 24-48 hours (AUROC 0.80 (95% CI 0.77-0.83).[109-113] Median serum concentrations are high already at baseline, both in good and poor outcome patients and decrease significantly within the first three days post-arrest, probably explained by the low molecular weight and short half-life of approximately 2 hours.[109,111-115] S100B is mentioned as a possible predictor of neurological outcome by the American Heart Association´s guidelines for post-cardiac arrest care, but no cut-off values are defined.[43]

Glial fibrillary acidic protein (GFAP)

Glial fibrillary acidic protein (GFAP) is an intermediate-filament component of the astrocytic cytoskeleton and Schwann cells of the peripheral nervous system. GFAP is mainly expressed in the CNS, but is also present in other tissue (Table 2).[87] There are regional differences in GFAP distribution with higher presence in the hypothalamus, hippocampus, olfactory bulb, amygdala, substantia nigra, periaqueductal grey matter and the molecular layer of the cerebellum.[87] Serum GFAP is has been increased in paediatric and adult poor outcome patients post-arrest, but with varying abilities of predicting poor neurological outcome.[92,116-118] GFAP has been extensively studied as a marker of traumatic brain injury, and a test-kit with GFAP and UCH-L1 has recently been approved in the USA for clinical use to reduce the number of head CT examinations in patients with mild traumatic brain injury.[90]

Table 2 Characteristics of biomarkers of brain injury

Biomarker	Location CNS	Other locations	Size	Half-life
NSE	Plasmamembrane and cytosol of neurons, oligodendrocytes	Neuroendocrine cells, platelets and erythrocytes. Enhanced in small-cell lung cancer, renal cancer, liver cancer and colorectal cancer	78 kDa[119]	24 h[119]
UCH-L1	Cytoplasm and nucleoplasm in the cell body, axon and synapses of neurons	Peripheral nervous system, neuroendocrine cells, endothelial and smooth muscle cells. Enhanced in endometrial and urothelial cancer.	26 kDa	6-12 h
NFL	Large myelinated axons of neurons	Peripheral nerves, (Charcot Marie Tooth), renal cancer	70 kDa	Days to weeks?
Tau	Unmyelinated axons of the cortex, astrocytes, oligodendrocytes	Muscles, kidneys, liver, testes and peripheral nerves. Enhanced in breast cancer, glioma, prostate cancer, renal cancer.	48-67 kDa[103]	<10 h[105]
S100A1B	Cytoplasm, cytoskeletal components and mitochondria of neurons	Skeletal muscle fibres and cardiomyocytes	21 kDa[119]	2 h[119]
S100BB	Astrocytes, neurons and dendritic cells	Schwann cells, melanocytes, chondrocytes, adipocytes, skeletal myofibers, dendritic cells, macrophages, Langerhans cells, keratinocytes, breast epithelial cells, myocardial tissue and lymphocytes	10.7 kDa[107]	30 min[107]
GFAP	Cytoskeleton of astrocytes	Schwann cells, Leydig cells of testis, keratinocytes, osteocytes, chondrocytes, in animal models even in kidney, pancreas and liver.	50kDa	24-48 h

This table displays the six biomarkers included in this thesis, together with their location within the central nervous system (CNS) and other locations outside the CNS, approximate molecular weight in kilo Dalton (kDa) and half-life. Renal elimination is presumed possible for all markers with molecular weights up to 60-70 kDa. The table may not be complete, but is meant for basic comparisons of these biomarkers.

3.6 Assessment of functional outcome

Survivors of CA can have various neurological outcomes, ranging from no symptoms to unresponsive wakefulness syndrome. Outcome is often reported using crude scales such as the Glasgow-Pittsburgh Cerebral Performance Category Scale (CPC)[120] (Table 3) or the modified Rankin Scale (mRS)[121] (Table 4). These scales are clinician-reported and are often dichotomized into "good" and "poor" outcome to simplify statistical analysis.

There is debate on which scores and cut-offs should be preferred.[122] In countries where WLST is commonly performed, most surviving CA patients are classified as having "good neurological outcome".[123,124] When WLST is not commonly performed, half of patients survive with substantial brain injuries, in conditions such as a minimally conscious state or an unresponsive wakefulness syndrome.[65,125]

Table 3 Cerebral Performance Category Scale

Definitions of binary outcome in this thesis	Cerebral Performance Category Scale
Good outcome	**CPC 1** Good cerebral performance. May have mild neurologic or psychological deficit.
	CPC 2 Moderate cerebral disability. Independent activities of daily life. Able to work in a sheltered environment.
Poor outcome	**CPC 3** Severe cerebral disability. Wide range of neurological conditions where patient is dependent on others in activities of daily life.
	CPC 4 Coma or vegetative state/unresponsive wakefulness syndrome.
	CPC 5 (Brain) death

Table 4 Modified Rankin Scale

Definitions of binary outcome in this thesis	Modified Rankin Scale
Good outcome	**mRS 0** No symptoms
	mRS 1 No significant disability. Able to carry out all usual activities despite some symptoms.
	mRS 2 Slight disability. Able to look after own affairs without assistance, but unable to carry out all previous activities.
	mRS 3 Moderate disability. Requires some help, but able to walk unassisted.
Poor outcome	**mRS 4** Moderately severe disability. Unable to walk without assistance and unable to attend to own bodiliy needs without assistance.
	mRS 5 Severe disability. Bedridden, incontinent and requiring constant nursing care and attention.
	mRS 6 Dead

Can the condition of a poor-outcome patient improve?

Most cognitive recovery is observed within the first 3 months post arrest.[126] Some studies have observed the natural course of recovery in poor outcome patients when WLST is not performed.

A small prospective study from Italy found that all patients with unresponsive wakefulness syndrome more than one month post-arrest either remained severely impaired (at best achieving a minimally conscious state), or had died by the 2-year follow-up.[127]

Another study from South-Korea described a cohort of patients from 1 month post-arrest, and found that only 4% of poor outcome patients had improved in six months, namely from CPC 3 (severe cerebral disability) to CPC 2 (moderate cerebral disability).[125] None of the poor-outcome patients showed further improvement after this time-point, and no improvement was observed in the CPC 4 patients.[125] The same study found, that 25% of the patients with unresponsive wakefulness syndrome were still alive after 3 years, remaining in the same condition.[125]

Is "good outcome" also considered good by the patient?

Despite having been classified on clinician-rated scales as having a "good outcome", survivors of CA often report symptoms such as fatigue, headache, emotional instability, lack of concentration, anxiety, post-traumatic stress disorder and difficulties with communication.[126,128] Reported quality of life is good, and does not seem affected by TTM.[124] However, cognitive impairment is common in CA survivors. Patients often suffer from problems with attention and reduced processing speed, affecting participation in social activities and their ability to return to work.[124,129]

Cognitive impairment and psychological distress diagnosed post-arrest may not always be related to the CA itself. Similar problems have been described in an age-matched control group with ST-segment-elevation myocardial infarction.[128,129]

According to the ERC/ESICM guidelines, follow-up after CA should always include screening for cognitive impairment and emotional problems.[24] In addition, patients and caregivers should receive both written and oral information regarding commonly experienced symptoms after CA (neurological and cardiological symptoms, information on general issues, advise on how to return to daily activities etc.). If problems are identified during screening, patients should be referred to the appropriate rehabilitation facility.

3.7 Ethical considerations

Patients admitted to hospital for treatment after CA are usually unconscious and unable to consent to treatment or to participation in clinical trials. As always, physicians should adhere to ethical recommendations and jurisdiction when treating patients.

The Declaration of Helsinki and its amendments, published by the World Medical Association, is a statement of ethical principles for medical research involving human subjects.[130] General principles of the Declaration include putting the patient's health first, acting in the best interests of the patient and seeking the guidance and approval of the appropriate ethics committee before starting studies.[130] Within clinical decision-making, four ethical principles were defined by Beauchamp and Childress: autonomy, beneficence, non-maleficence and justice (Fig. 11).[131]

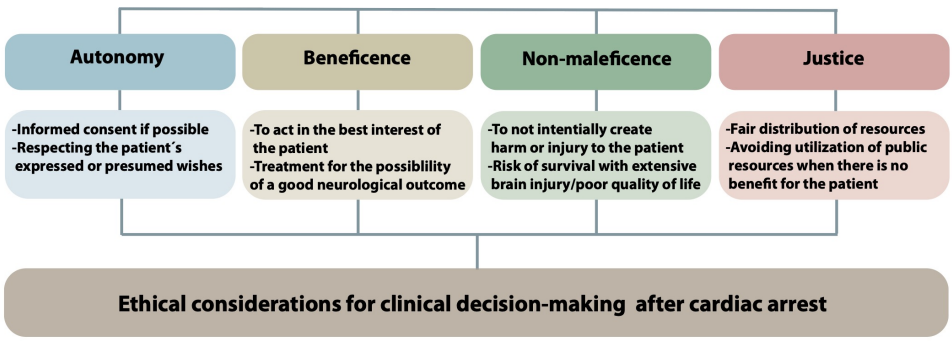


Fig. 11 These four principles of biomedical ethics should be considered and balanced when treating cardiac arrest patients and recruiting patients for clinical trials, but also when making decisions on level-of-care.

Respect for the patient's previously expressed or presumed wishes should be maintained even if the patient is unable to consent. Beneficence and non-maleficence "aim at producing net benefit over harm" for each patient.[131] The principles of justice calls for a fair distribution of resources, respect for the patient's rights and respect for morally acceptable laws.[131] According to this ethical principle, continuing to treat a patient whose prognosis is deemed futile, would be considered unethical if another patient may, as a consequence, miss out on life-saving treatment.

In Sweden, if continued intensive-care treatment is no longer considered beneficial for the patient, WLST is legally permitted. In patients for whom treatment is deemed futile, WLST is regarded as balancing the principles of beneficence, non-maleficence and justice. In other countries, cultures and religions, however, WLST may be considered unethical or even illegal.

4. Aims of the thesis

This thesis is focused on prediction of neurological outcome in unconscious patients after cardiac arrest treated with targeted temperature management (TTM). The main aim was to evaluate and compare prognostic accuracies of routine and novel methods of neuroprognostication for which evidence from clinical trials are insufficient.

The specific aims were:

- To describe typical findings on head computed tomography after CA.
- To examine the prognostic value of generalised oedema on head computed tomography alone, and together with serum levels of neuron specific enolase (NSE) for predicting poor outcome.
- To evaluate the prognostic accuracy of a novel serum biomarker, neurofilament light chain protein (NFL) analysed with an ultrasensitive assay for predicting poor neurological outcome.
- To evaluate the prognostic accuracies for poor outcome of two novel serum biomarkers, glial fibrillary acidic protein (GFAP) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) analysed with commercially available assays.
- To assess the prognostic performance of the 2015 ERC/ESICM algorithm by examining the number of correctly and incorrectly identified poor outcome patients.
- To identify strengths and weaknesses of the ERC/ESICM algorithm by examining the recommended diagnostic tests separately and in combinations, and to explore possible modifications of the algorithm.

5. Methods

5.1 Overview

This thesis consists of four studies (Table 5). All are based on data from the prospective randomized international multicentre Target Temperature Management 33°C versus 36°C after out-of-hospital cardiac arrest (TTM)-trial with 36 participating intensive care units in Europe and Australia (Clinicaltrials.gov NCT01020916).

Between November 2010 and January 2013, 950 adult CA patients were randomized to targeted temperature managements of either 33°C or 36°C, with the aim of comparing survival and neurological outcome after 6 months. The modified intention-to-treat population consisted of 939 patients.

Table 5 Studies included in this thesis

Paper	I	II	III	IV
Method	Head CT and peak serum NSE	Serum NFL	Serum GFAP and UCH-L1	Performance of ERC/ESICM algorithm
Design	Multicentre retrospective study	Multicentre batch analysis of prospectively collected and frozen serum samples	Multicentre batch analysis of prospectively collected and frozen serum samples	Multicentre retrospective study
Study population	≥18 year old patients with OHCA of presumed cardiac origin treated with TTM 33°C or 36°C between 2010-2013			
Participants	n= 357	n= 717	n= 717	n= 585

5.2 The TTM-trial

Inclusion and exclusion criteria

Eligible were adults ≥18 years with a presumed cardiac origin of arrest, remaining unconscious (GCS <8) after at least 20 minutes of sustained ROSC.

Exclusion criteria were unwitnessed CA with asystole on the initial ECG, ≥ 4 hours from ROSC to screening, limitations in therapy including do-not-resuscitate orders or known illness making survival to 180 days unlikely, previous bleeding diathesis, known or suspected intracranial haemorrhage or stroke, body temperature ≤30°C on admission, pregnancy, persistent cardiogenic shock despite medical interventions and mechanical assist, and pre-existing neurological disability (CPC3-4).[132]

Randomisation

Patients were randomised 1:1 to a strict targeted temperature management regimen of either 33°C or 36°C, using a web-based application. Randomisation was stratified by center in permuted blocks with varying block-sizes.[132]

Informed consent and ethical approval

Ethical consent was obtained by the ethics board of each participating country and followed the principles of the Declaration of Helsinki and its amendments.[130] The TTM trial was approved by the Regional Ethical Review Board at Lund University (324/2009). Informed consent was obtained from surviving patients. A patient's next of kind was informed of the inclusion in the study during the first contact with the hospital.

Targeted temperature management

The target core temperature in both groups (33°C/36°C) was achieved as soon as possible after randomisation using an intravascular or external system .[132] Core body temperature was measured by a urine catheter. Patients were actively rewarmed to 37°C at a maximum speed of 0.5°C /hour from 28 hours after randomisation (Fig. 12). Both TTM groups were sedated, intubated and mechanically ventilated throughout the 36-hour intervention period. Centres were instructed to follow local protocols for sedation and muscle relaxants, and to provide equal treatment for both intervention groups.[133]

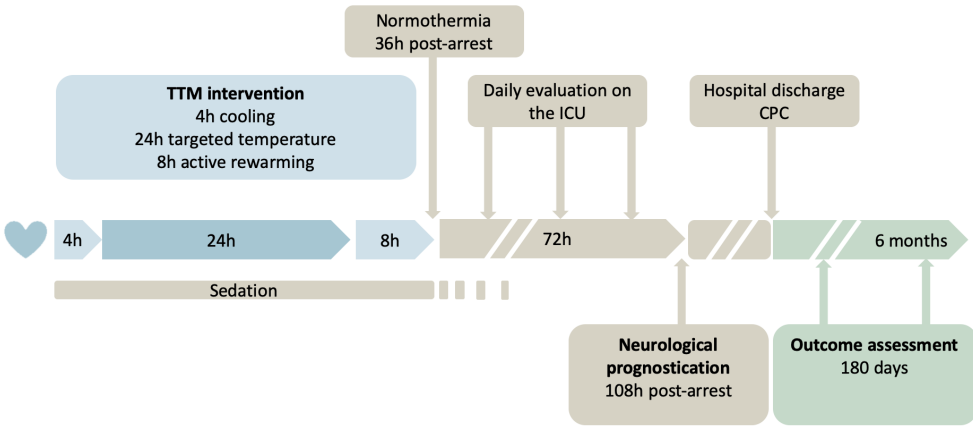


Fig. 12 TTM trial study timeline from cardiac arrest to assessment of neurological outcome after six months. *Illustration by Sofia Backman. Reprinted with permission.*

Neurological prognostication

According to protocol, all patients should be actively treated until formal neurological prognostication at least 72 hours after rewarming (108 hours post-arrest).[134] Unconscious patients, defined as GCS-M ≤2 were neurologically assessed by a physician blinded to TTM and not being part of the ICU team treating the patient.

GCS-M and the presence of clinical seizures were evaluated daily. Brainstem reflexes were reported at formal neurological prognostication. A routine EEG was performed 48-72 hours post-arrest in patients remaining unconscious. Original EEG data was evaluated retrospectively by examiners blinded to clinical information, based on the ACNS terminology[74] and classified into *unreactive burst-suppression* and *unreactive status epilepticus* (abundant rhythmic/periodic discharges) according to ERC/ESICM.[24] In an exploratory analysis, the standardised highly malignant EEG patterns as proposed by Westhall et al. was applied.[79] If available, median nerve SSEP was recommended at 84-108 hours after CA. Neuroimaging (CT and MRI) were performed on clinical indication and evaluated for presence of generalised oedema by local radiologists. Serum samples from 29/36 sites participating in the biobank-substudy were collected prospectively at 24, 48 and 72 hours post-arrest, aliquoted, frozen to -80 °C and stored at the Integrated BioBank of Luxembourg before batch analysis after trial completion.[88] For prognostic accuracies of NSE, we used our clinically established values ≥48 pg/mL at 48 hours and/or ≥38 pg/mL at 72 hours post-arrest, accepting a 2 % FPR according to previously published results by Stammet et al.[88] Peak-NSE was defined as the highest available serum concentration at either 48 or 72 hours after CA.

The physician performing neurological prognostication would make a recommendation on whether to *continue active intensive care, not to escalate intensive care* or to *withdraw intensive care*.[134] However, the decision of whether or not to act on these recommendations was made by the physician responsible for patient care.

WLST

Withdrawal of life-sustaining therapy before formal prognostication was permitted only due to the following pre-specified criteria to minimise the risk of bias due to premature WLST:

- Early status myoclonus ≤ 24 hours post arrest and bilaterally absent N20 potentials after rewarming
- Persisting coma defined as GCS-M≤ 2 and bilaterally absent N20 potentials
- GCS-M≤ 2 and treatment refractory status epilepticus at ≥ 108 hours post arrest
- Brain death due to cerebral herniation according to national legislation
- Ethical reasons (also including treatment refractory shock or end-stage multi-organ failure)

Outcome

Neurological outcome was evaluated by examiners blinded to TTM. In all four papers, primary outcome was poor neurological outcome 6 months after CA, classified as CPC 3-5 (severe cerebral disability, vegetative state/coma or death) (Table 3). In Paper IV, we also reported prognostic accuracies of the ERC/ESICM algorithm by using an alternative definition of poor outcome (CPC 4-5). In Paper II, we reported serum NFL concentrations both according to CPC, and according to mRS, with poor outcome defined as mRS 4-6 (Table 4).

Data collection

Baseline data, information on medical comorbidities, neurological prognostication, presumed cause of death or neurological outcome was registered pseudonymised by each hospital in an electronic case report form. All electronic case report forms were evaluated systematically regarding the findings and timing of prognostic examinations. Site investigators were contacted for clarification of incomplete or incoherent data, which was corrected accordingly.

5.3 Analysis of serum biomarkers

Enzyme-linked immunosorbent assay (ELISA)

Serum UCH-L1 and GFAP were analysed with a sandwich ELISA by Banyan Biomarkers, Inc., San Diego, CA (Fig. 13).[135] Samples were tested in duplicate, and high and low positive controls were included with each plate.

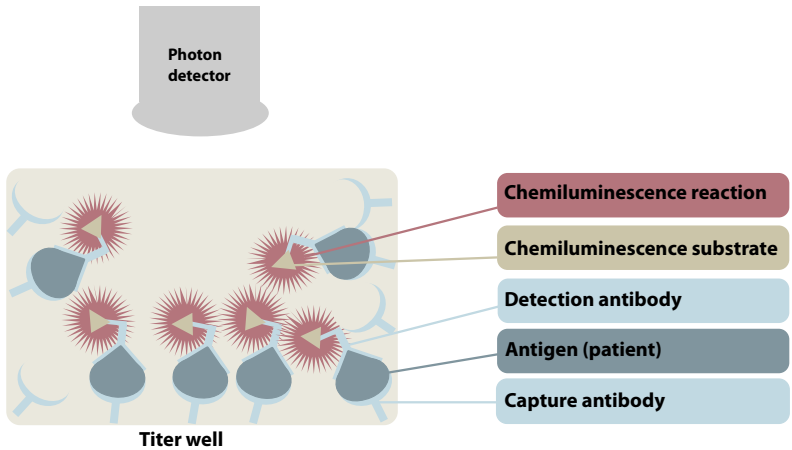


Fig. 13 Example of sandwich ELISA. Wells of a microtiter plate are coated with specific caputure antibodies for GFAP or UCH-L1. Detection antibodies bind to antigens and form a complex with the capture antibody attached to the wells. Excess detection antibodies are washed out before adding a chemiluminescent substrate to the well. With help of a horseradish peroxidase enzyme as catalyst, the chemiluminescent substrate produces light detected with a luminometer. The amount of light signal is compared to a standard calibration curve to determine GFAP or UCH-L1 concentrations.[135]

Electrochemiluminescent immunoassay (ECLIA)

Serum NSE and S100B (S100A1B and S100BB) were analysed using a COBAS e601 line with an ECLIA kit (Roche Diagnostics, Rotkreuz, Switzerland) (Fig. 14).[88] Haemolysis was tested using the Roche hemolysis index with measurements at 600 and 570 nm. All samples with a positive hemolysis index (≥500 mg/l of haemoglobin) were discarded.[88]

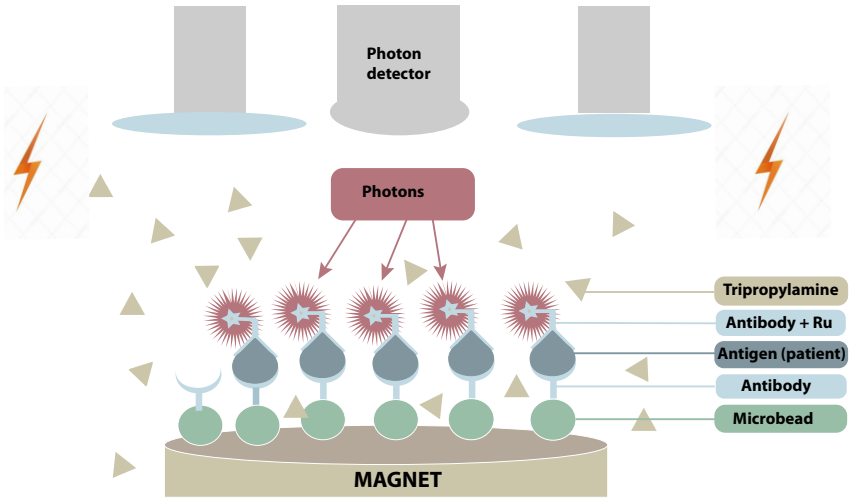


Fig. 14 Schematic illustration of the principles of the electrochemiluminescent assay (ECLIA). Two antibodies, one with Ruthenium (Ru) and the other with a magnetic microbead attached, form a sandwich-complex with the patient´s antigen (NSE or S100B). The complex is secured onto a magnet with help of the microbeads. When voltage is applied, Tripropylamine (TPA) reduces Ru^{3+} to Ru^{2+}. A photon detector measures the resulting light signal, which is equivalent to the concentration of the target analyte. This illustration was inspired by a demo-video by Roche Diagnostics LTD.[136]

Single Molecule Array (Simoa)

Serum NFL was analysed using an ultrasensitive Single Molecule Array (Simoa™) by Quanterix Lexington, MA, with a Homebrew kit. Serum tau was also measured on the Simoa analyser with the Human Total Tau kit, Quanterix, Lexington, MA.[105] The Simoa technique is sometimes referred to as a digital ELISA, and only a single molecule is required to reach the detection limit, reducing the signal-to-noise ratio (Fig. 15).[137]

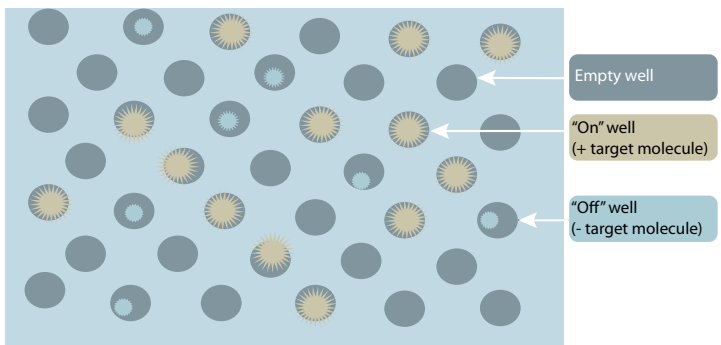


Fig. 15 Illustration of the so called digitial ELISA of the Simoa™ Technology. In a first step, a specific antibody capture agents (in this case the Homebrew kit or the Human Total Tau kit) is attached to paramagnetic beads. Each bead has approximately 250.000 attachment sites and the beads outnumber the target molecules (NFL or tau). The beads quickly form a complex with the target molecules, and left over beads are washed out. Only beads attached to a target molecule is labelled with an enzyme and are loaded into tiny wells that hold no more than one bead each before being sealed with oil. Beads that successfully formed an immunocomplex with NFL/tau are converted to a fluorescent product ("on" well). The number of "on wells" are digitally compared to single beads without target molecules ("off" wells). [137]

5.4 Statistical analysis

Continuous variables are presented as mean (standard deviation) or as median (interquartile range). Categorical variables are presented in numbers and percentages. Bivariate associations were calculated by t-test, Mann-Whitney-U-test, Kruskal-Wallis-H-test or Chi-square test as appropriate. Tests were two-sided and p-values <0.05 were considered statistically significant. Linear relationships of continuous non-parametric data were examined using Spearman´s rho.

Sensitivities and specificities of diagnostic methods are presented with 95% confidence intervals calculated with Wilson's method. To avoid confusion, the terminology will be briefly explained below using generalised oedema on CT as a prognostic test for prediction of poor neurological outcome. *Negative (N)* describes a test with *normal* findings, whilst *positive (P)* describes a *pathological* test. The term *true (T)* indicates that predicted and reported outcome are identical (identical colour on findings and outcome), whilst *false (F)* indicates that the outcome prediction was contrary to the reported outcome (non-identical colours). For example, *true negative (TN)* indicates a good outcome patient correctly identified as such by having a negative (normal) test result (Fig. 16).

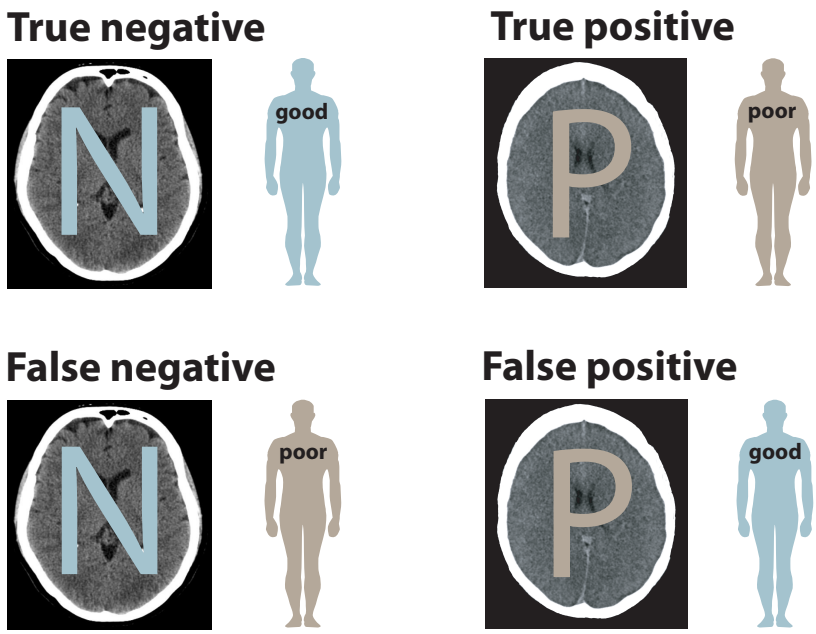


Fig. 16 Example of a diagnostic test evalutating generalized oedema on head computed tomography. Blue color is used for a negative/normal CT or a good outcome (figure), brown signifies a positive/pathological CT or a poor outcome (figure).

Sensitivity

The sensitivity of a test describes how well a test identifies poor outcome patients (Fig. 17).

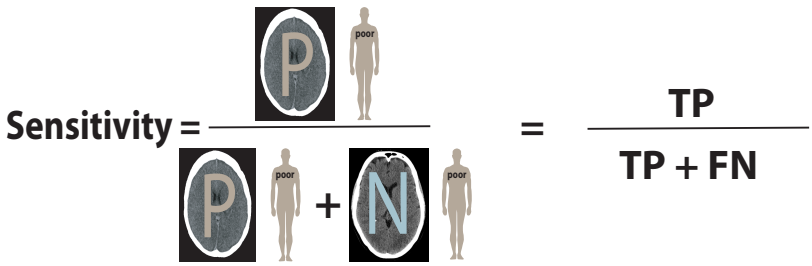


Fig. 17 Generalised oedema on CT as a prognostic test for identifying the percentage of poor outcome patients with a pathological test. P, positive/ pathological test, N, negative/normal test. Only poor outcome patients are included when calculating sensitivity.

Specificity

The specificity describes how many good outcome patients examined had a normal test result (Fig. 18).

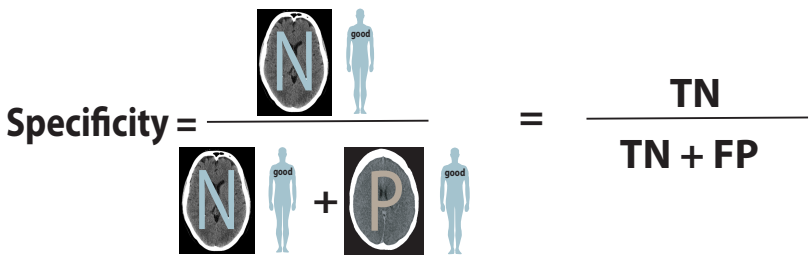


Fig. 18 Generalised oedema on CT as a prognostic test indicating the percentage of good outcome patients with a negative test result. N, negative/normal test, P, positive/ pathological test, only good outcome patients are included when calculating specificity.

Sometimes the term *False Positive Ratio (FPR)* is used instead of specificity, which is calculated as FPR= 100% - specificity. A specificity of 100% is identical to a FPR of 0, a specificity of 99% is identical to FPR 1 et cetera. Within neurological prognostication after CA, a high specificity, or a low number of false positive predictions of poor outcome is the most important measure, because any FP test results could in the worst-case scenario lead to WLST and death of the patient.

ROC analysis

Overall diagnostic performance for poor outcome (CPC 3-5 at 6 months) was determined by calculating the area under the receiver operating characteristics curve (AUROC). Comparisons between paired ROC-curves of biomarkers were done by a bootstrap procedure with n = 2000 iterations. Sensitivities and cut-off values for biomarkers were determined at specificities 100% - 95%. In Paper III, prognostic accuracies of the biomarkers were also compared using partial AUROC for specificities between 100% - 95% (the left part of the ROC-curve, Fig. 19). In order to increase comparability with overall AUROC, the partial AUROC was normed to represent 100%.

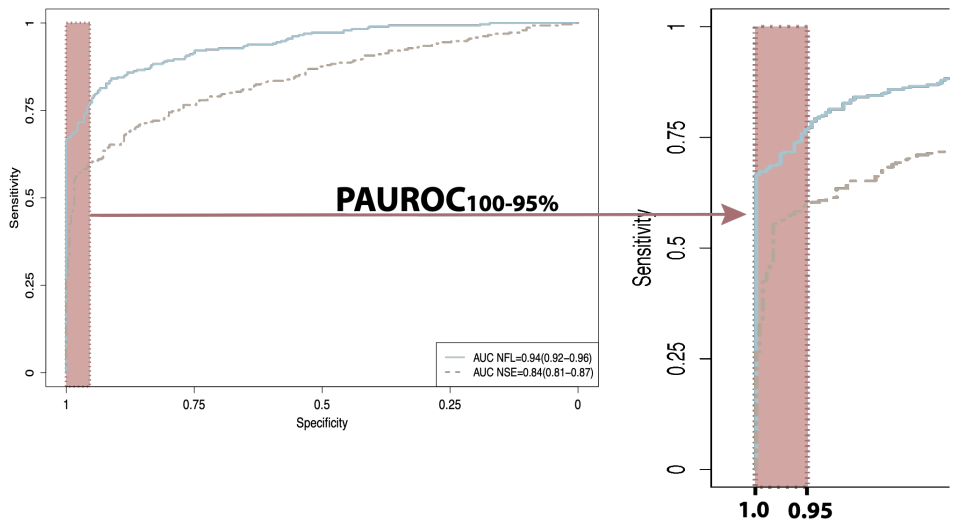


Fig. 19 Demonstrates the partial area under the ROC curve (PAUROC) between 100-95% specificities. The dotted rectangle is the adapted area of which the PAUROC is calculated which is then normed to 1.

Regression models for prediction of neurological outcome were compared using biomarkers, baseline and clinical data. The Akaike information criterion (AIC) was used as a measure of overall model fit for model comparisons.[138] A lower AIC indicates a better model fit, a difference of 2 or more favouring the model with the lowest AIC. Statistical analyses were performed with SPSS, versions 23.0 and 24.0 (IBM Corp.), and R, version 3.3.2 (The R Foundation for Statistical Computing).

6. Results

6.1 CT and NSE (Paper I)

Patients

In this study, 357 patients (38.0%) underwent ≥1 CT examination of which 36 patients (~10%) were examined twice. Included patients less frequently had an initial shockable ECG rhythm ($p<0.001$) and more often had poor neurological outcome after 6 months than excluded patients ($p<0.001$) (Fig. 20). Time to ROSC and TTM allocation did not differ significantly between included and excluded patients.

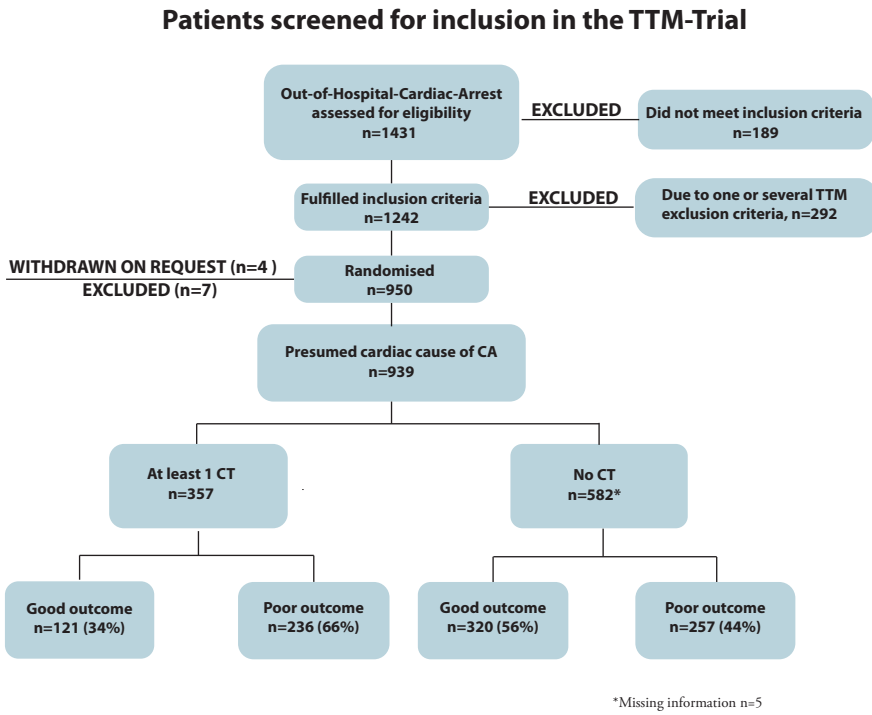


Fig. 20 Flowchart of patient inclusion in the TTM trial and patients examined with head CT in numbers or percentages. Neurological outcome was dichotomised into good (CPC 1-2) and poor (CPC 3-5) at 6 months.

Timing of examinations and findings

More than sixty percent of examinations were performed within 24 hours after CA. The median time to CT was 2 hours (IQR: 1-4 hours) for early examinations (Fig. 21A). CT for neuroprognostication (Fig. 21B) was performed at median 77 hours post-arrest (IQR: 53-114) and median time to CT in Fig. 21C was 245 hours post-arrest (IQR: 193-348).

Almost 80% of all examinations performed ≤ 24 hours post-arrest were described as "normal" by local radiologists. Only 9.6% of early CT´s were diagnosed with "generalised oedema", compared to CT´s performed during the phase of neuroprognostication where generalised oedema was diagnosed in nearly 50% of examinations. "Acute/subacute ischaemia" was also a common finding within one week of CA. The third group was a small and heterogenous group which will therefore not be discussed further in this thesis.

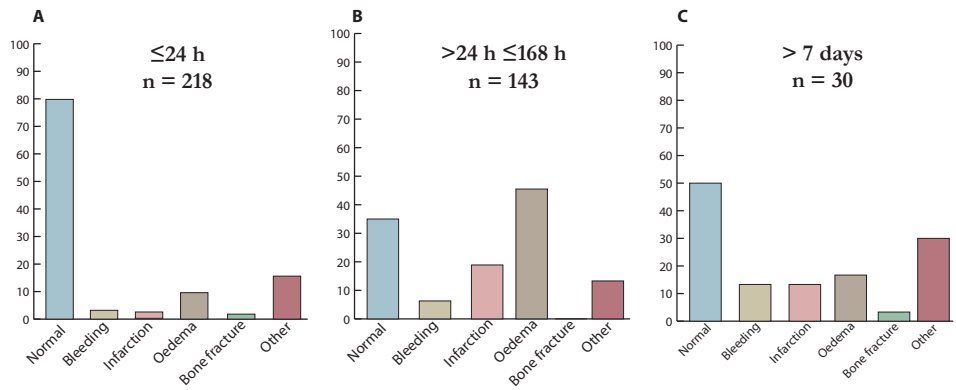


Fig. 21A-C Findings on head CT after CA as described by local radiologists in (%). Multiple options possible.

Progress of oedema

Fifteen patients with "normal" early CT scans were diagnosed with generalised oedema on a second CT scan at a median of 77 hours (IQR: 41-121 hours) post-arrest. Only one of these patients were previously diagnosed with generalised oedema on the first scan.

Peak-NSE

Median peak-NSE levels were significantly higher in patients diagnosed with generalised oedema on CT than in patients without generalised oedema both in early ≤ 24h and later examinations > 24h ≤ 7 days post-arrest ($p = 0.012$ and $p < 0.001$, respectively) (Fig. 22). The median peak-NSE levels did not differ significantly between patients with generalised oedema on early or later CT´s.

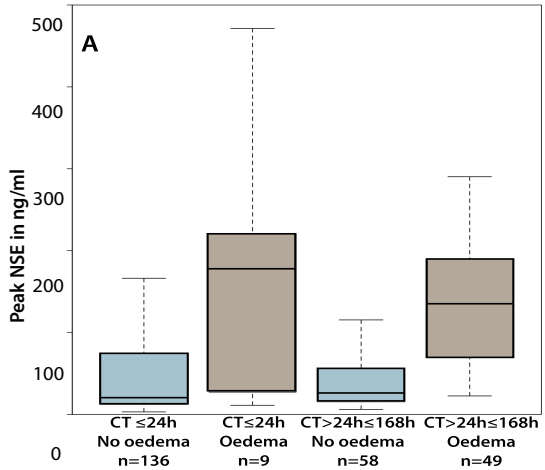


Fig. 22. Peak serum neuron specific enolase levels in ng/mL at 48 or 72 hours after CA. NSE was available for n=276.

Prognostic accuracies

Neurological outcome was poor in 89/91 (97.8%) of all patients diagnosed with generalised oedema on CT at any time-point. Overall, generalised oedema identified approximately one third of poor outcome patients, with a specificity of 98.4%, due to two false positive predictions of poor outcome (Table 6). Sensitivity increased from 14.4% in early examinations, to 56.5% within the first weeks post-arrest, but there was a selection of poor outcome patients in the later group (60 % versus 80%, respectively, $p < 0.001$). Combining generalised oedema with peak-NSE above our locally established cut-off values predicted poor outcome without false positive predictions.

Table 6 Prediction of poor outcome CT and Peak-NSE

Prognostic examination	All	Sensitivity (95% CI)	Specificity (95% CI)	FP
All CT´s	392*	33.6 (28.1-39.5)	98.4 (94.3-99.6)	2
Early CT ≤ 24h	218*	14.4 (9.4-21.4)	97.6 (91.8-99.4)	2
Neuroprogn CT >24h ≤ 7days	143	56.5 (47.3-65.3)	100.0 (87.9-100.0)	0
CT >7 days	30	27.8 (12.4-50.9)	100.0 (75.7-100.0)	0
Peak-NSE >38ng/ml	276	64.8 (57.6-71.5)	95.7 (89.5-98.4)	4
Peak-NSE >48ng/ml	276	61.5 (54.3-68.3)	95.7 (89.5-98.4)	4
CT+ Peak-NSE >38 ng/ml	185	46.0 (36.5-55.8)	100.0 (95.6-100.0)	0
CT + Peak-NSE >48ng/ml	194	45.9 (36.8-55.3)	100.0 (95.6-100.0)	0

Data in numbers and percentages with 95% confidence intervals. CT: head computed tomography, Peak-NSE: maximum Neuron-specific enolase at either 48h or 72h; cut-off values for NSE ≥ 48 ng/ml at 48h; and ≥ 38 ng/ml at 72h accepting a 2% false positive ratio according to Stammet et al. FP: false positive. *Missing data outcome for 1 patient. 36 patients underwent two CT examinations and were reported twice.

6.2 NFL (Paper II)

Patients

Seven hundred and seventeen patients had NFL analysed on at least one time-point (24, 48 or 72 hours) (Fig 23).

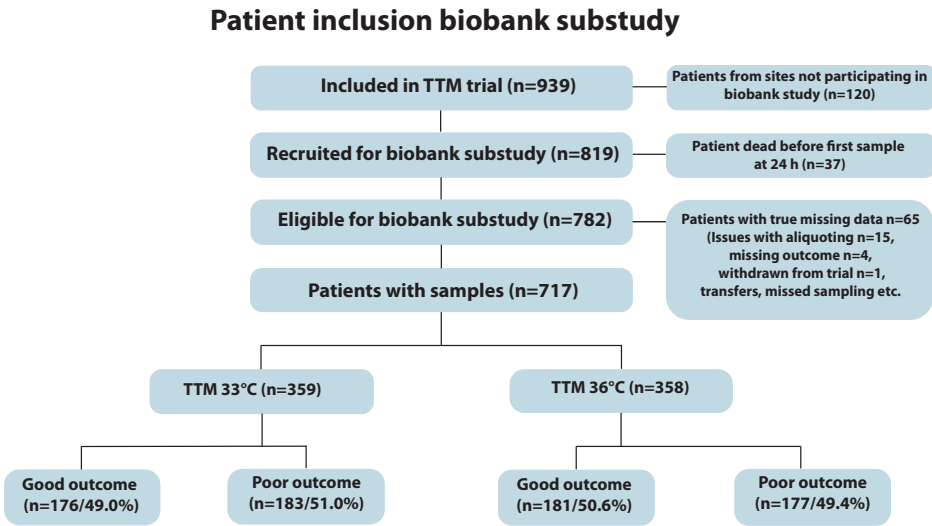


Fig. 23 Flowchart of patients included in TTM biobank substudy. Results are presented in numbers or percentages and are identical for Papers II (NFL) and III (GFAP and UCH-L1). Poor outcome was defined as CPC 3-5 at 6 months.

Baseline data did not differ between included and excluded patients, between patients included at each time-point, nor between TTM groups (Paper II, eTable 1). Mean age of included patients was 64 years (SD 12.2), the majority (80%) were male with an estimated median time to ROSC of 25 minutes (IQR: 17-39). Initial rhythm on ECG was shockable in almost 80% of patients, bystander CPR was performed in approx. 70%. At six months follow-up, neurological outcome was poor (CPC 3-5) in 50% of patients.

Serum NFL and outcome

NFL was significantly higher in poor outcome patients than in good outcome patients at all time-points ($p < 0.001$) (Fig. 24). Median serum concentrations in poor outcome patients nearly doubled between 24 and 48 hours and remained high at 72 hours. Higher serum NFL levels were associated with older age ($\rho = 0.28$, $p < 0.001$), longer time to ROSC ($\rho = 0.39$, $p < 0.001$) and absence of bystander CPR ($p < 0.001$). NFL concentrations were not elevated in the presence of haemolysis.

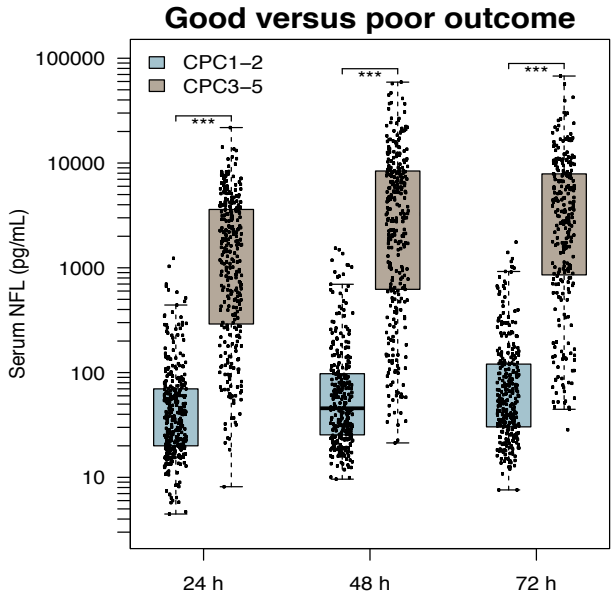


Fig. 24 Boxplots comparing median serum concentrations between patients with good and poor outcome at 24, 48 and 72 hours. CPC, Cerebral Performance Category Scale at 6 months after CA.

Serum NFL was significantly higher when compared to the next lower level of the CPC scale at all time-points (CPC 4 vs CPC 3, CPC 3 vs CPC 2, CPC 2 vs CPC 1) (Fig. 25). There was no such difference between CPC 4 and CPC 5.

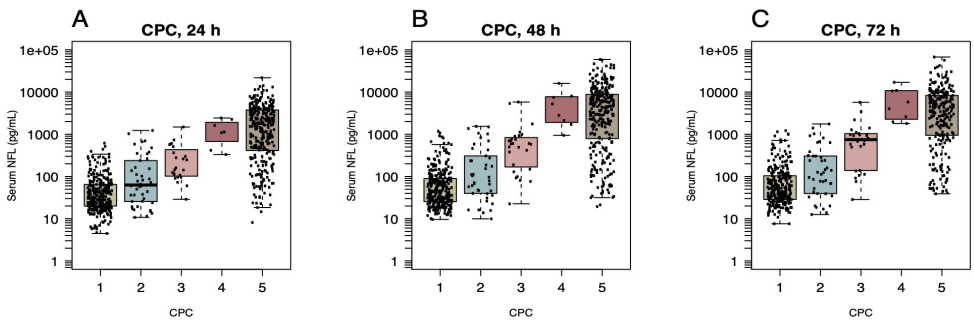


Fig. 25 Boxplot with median serum NFL levels in pg/mL for each level of the CPC scale at 24, 48 and 72 hours post-arrest.

Prognostic accuracies

Serum NFL had significantly higher prognostic accuracies at all time-points for prediction of poor outcome, compared to the biomarkers NSE, tau and S100 (Fig. 26A-C). Combining time-points did not increase prognostic accuracy of NFL (Paper II, eTable 3).

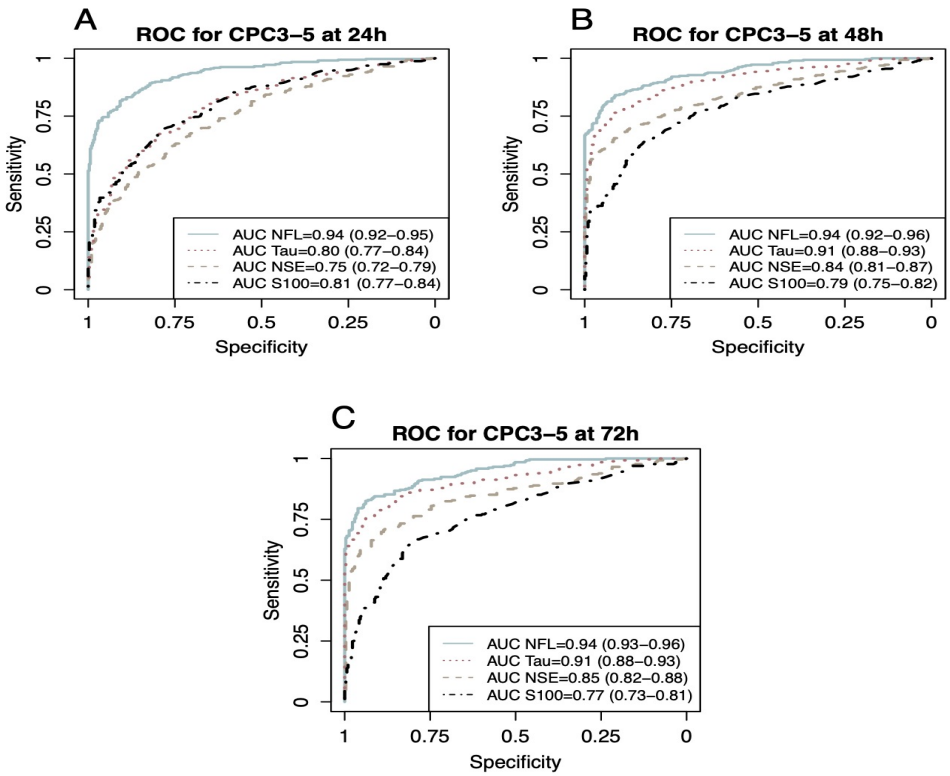


Fig. 26A-C Receiver-operating characteristic (ROC) for prediction of CPC 1-2 vs. CPC 3-5 at 6 months after CA. Only patients with available data for all four biomarkers at the respective time-point were included in these comparisons. (24h, n=641; 48h, n= 608; 72h, 573).

Adding NFL to clinical information (age, sex, time to ROSC, bystander CPR and serum lactate on admission) or bedside examination of brainstem reflexes significantly improved prognostic performance of poor outcome (Paper II, eTables 4 and 5). Serum NFL at 24 hours identified a larger number of poor outcome patients than any routine method for prognostication (CT, SSEP, routine EEG or brainstem reflexes) at matched specificities and within the same cohort of patients (Paper II, Table 3).

Temperature effects

There was no difference in NFL concentrations between poor outcome patients between the 33°C and 36°C groups at 24 and 48 hours. At 72 hours, median NFL levels were significantly higher in the 33°C poor outcome patients than in 36°C poor outcome patients (4205 pg/mL (IQR: 959-10193) vs. 2693 pg/mL (IQR: 683-6660), $p = 0.02$).

6.3 GFAP and UCH-L1 (Paper III)

Patients

Included and excluded patients are identical to those reported in Paper II NFL (Fig. 23, page 56).

Serum concentrations and outcome

Both GFAP and UCH-L1 were significantly higher in poor outcome patients than in good outcome patients at all time-points ($p < 0.001$) (Fig. 27). On a group level in poor outcome patients, median serum concentrations were similar between 24 and 48 hours, but with a decreasing trend at 72 hours. GFAP and UCH-L1 were not elevated in the presence of haemolysis (Paper III, eFig. 2 and 3).

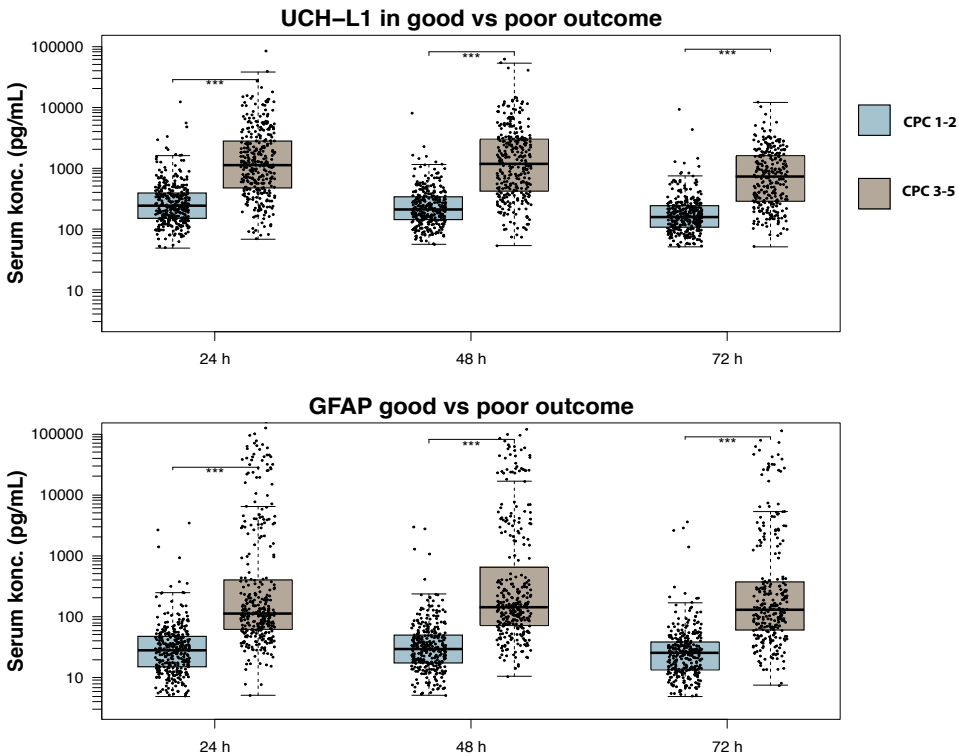


Fig. 27 Boxplots with median (IQR) serum concentrations at 24, 48 and 72 hours after CA for good (CPC 1-2) and poor (CPC 3-5) outcome patients for UCH-L1 (upper figure) and for GFAP (lower figure).

Prognostic accuracies overall

When comparing overall prognostic accuracies, AUROC for GFAP was significantly greater than NSE at all time-points (Fig 28A-C). Overall AUROC for UCH-L1 was significantly greater than NSE at 24 and 48 hours only. The combination of GFAP and UCH-L1 demonstrated significantly higher prognostic accuracy than NSE at all time-points.

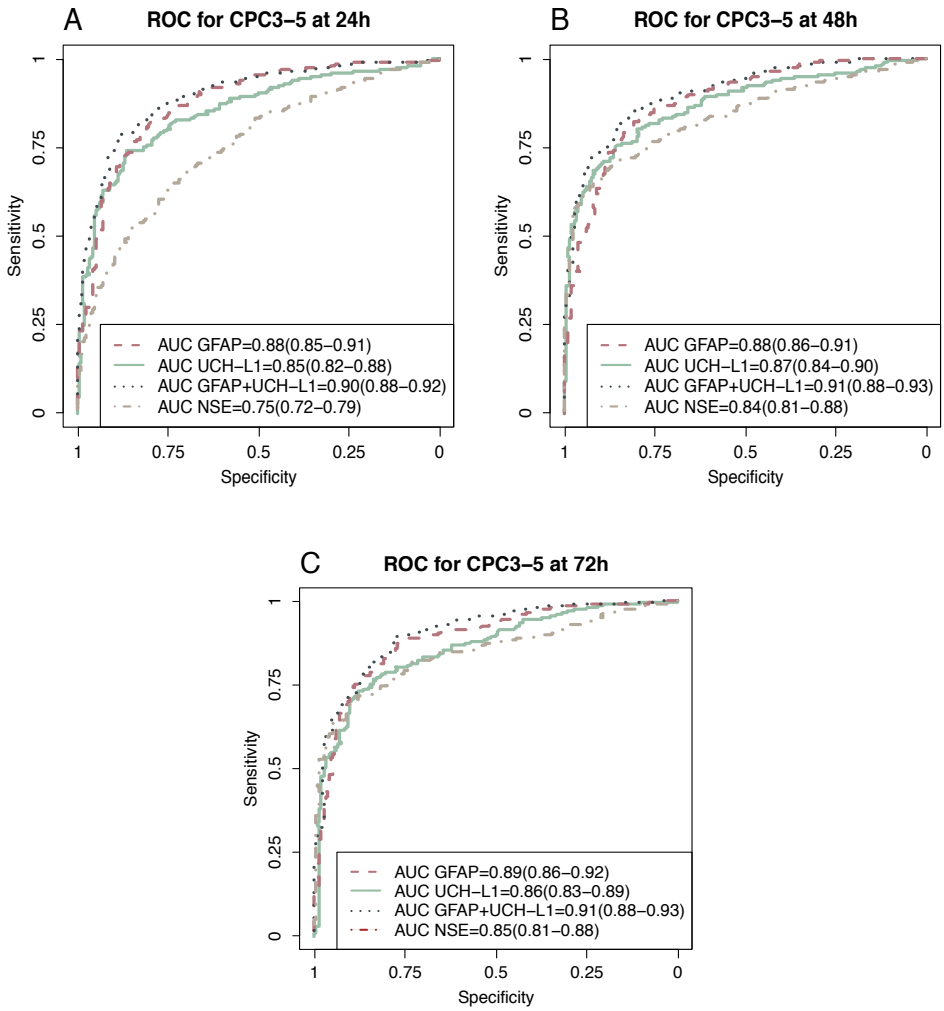


Fig. 28A-C Receiver-operating characteristic (ROC) curves for prediction of poor outcome (CPC3-5) at 6-months post-arrest at 24, 48 and 72 hours. Analyses are restricted to patients with available data all biomarkers at the respective time-points (24 hours, n=633; 48 hours, n= 597; 72 hours, n=558).

Prognostic accuracies at high specificities

When comparing prognostic accuracies for specificities between 100% - 95% (the left side of the ROC curve only), we see that sensitivities and hence the ROC curves overlap each other for some markers (Fig. 28A-C). When limiting comparisons of prognostic accuracies to specificities between 100% and 95% only, UCH-L1 had the highest partial AUROC at 24 hours and was significantly better than NSE (Table 7). At 48 and 72 hours, UCH-L1 and NSE were comparable, and NSE was superior to GFAP. The combination of GFAP and UCH-L1 was significantly better than NSE at 24 hours, but not at 48 or 72 hours. Both biomarkers contributed significantly in the combined models (Paper III, eTable 4).

Table 7 Partial AUROC at high sensitivities

	Serum biomarker	PAUROC 100% – 95% (95% CI)	p-value
24h	UCH-L1 vs NSE	0.67 (0.61 – 0.73) vs 0.60 (0.57 – 0.64)	0.042*
	GFAP vs NSE	0.64 (0.60 – 0.71) vs 0.60 (0.57 – 0.64)	0.164
	GFAP+UCH-L1 vs NSE	0.72 (0.67 – 0.77) vs 0.60 (0.57 – 0.64)	<0.001***
48h	UCH-L1 vs NSE	0.75 (0.70 – 0.80) vs 0.75 (0.71 – 0.80)	0.757
	GFAP vs NSE	0.67 (0.62 – 0.72) vs 0.75 (0.71 – 0.80)	0.004**
	GFAP+UCH-L1 vs NSE	0.75 (0.70 – 0.80) vs 0.75 (0.71 – 0.80)	0.884
72h	UCH-L1 vs NSE	0.70 (0.63 – 0.76) vs 0.75 (0.69 – 0.80)	0.193
	GFAP vs NSE	0.67 (0.62 – 0.74) vs 0.75 (0.69 – 0.80)	0.050*
	GFAP+UCH-L1 vs NSE	0.72 (0.66 – 0.79) vs 0.75 (0.69 – 0.80)	0.539

Comparison of prognostic accuracies of serum biomarkers for the partial AUROC at specificities 100%–95% for predicting poor neurological outcome (CPC 3-5) at 6 months post-arrest for samples taken at 24, 48 and 72 hours after cardiac arrest. 95% CI = 95% confidence interval. *p<0.05; **p<0.01; ***p<0.001.

Temperature effects

Median UCH-L1 levels did not differ between the 33°C and the 36°C groups. GFAP, however, was significantly higher at 48 and 72 hours in the 33°C group (Paper III, eTable 3).

6.4 ERC/ESICM algorithm (Paper IV)

Patients

Five hundred and eighty-five patients were examined with GCS-M on day 4 post-arrest and were included when assessing the prognostic performance of the ERC/ESICM algorithm (Fig. 29). Median age was 64 years, 82% were male, the majority had an initial shockable rhythm on ECG and median time to ROSC was 24 minutes (Paper IV, Table 1).

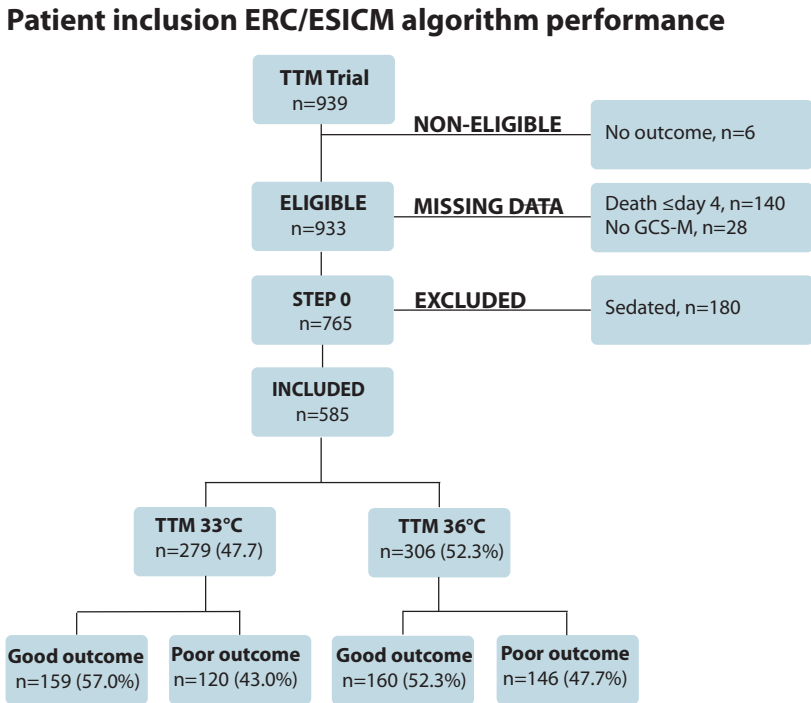


Fig. 29 Flowchart of included and excluded patients when assessing the prognostic performance of the ERC/ESICM algorithm in numbers and percentages. Neurological outcome at 6 months was dichotomised according to the Cerebral Performance Category Scale (CPC) into "good" (CPC 1-2) and "poor" (CPC 3-5).

Overall prognostic performance of the ERC/ESICM algorithm

In the first step of the algorithm, 380 patients had GCS-M ≥ 3 (flexion response to pain or better), of which 305 patients had good outcome (TN) and 75 had poor outcome (FN) (Fig. 30). Patients with GCS-M ≥ 3 and good outcome were significantly younger, had shorter time to ROSC and more often an initial shockable rhythm on ECG compared to those with a poor outcome. Patients with GCS-M ≤ 2 (extension or no reaction), were examined further.

In the second step, 67 patients had bilaterally absent pupillary AND bilaterally absent corneal reflexes AND/OR bilaterally absent N20 potentials on SSEP. In the third step, 36 patients had ≥ 2 pathological findings (high NSE, pathological EEG according to ERC/ESICM criteria, early status myoclonus ≤ 48 hours, or generalised oedema on CT or on MRI).

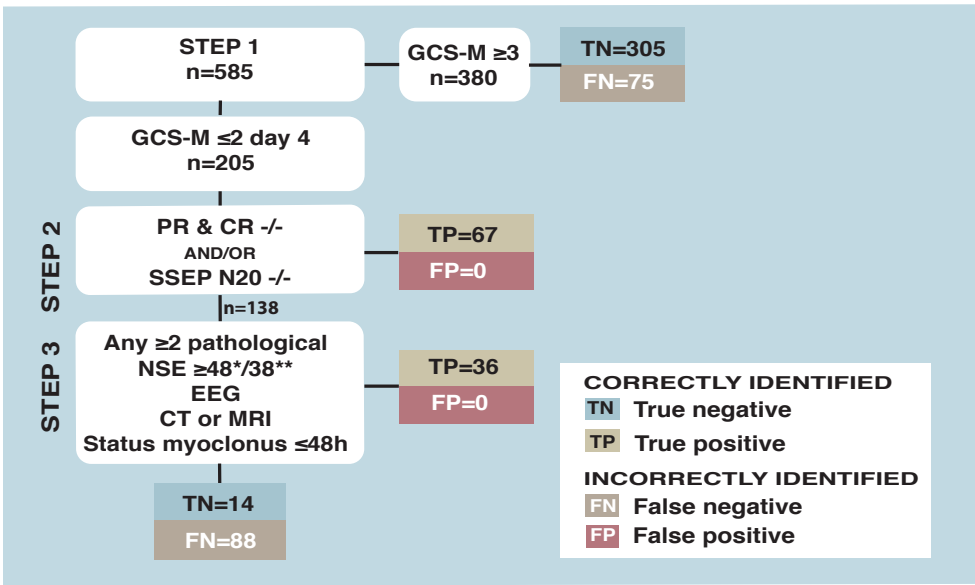


Fig. 30 Prognostic performance of the ERC/ESICM algorithm in n = 585 patients after cardiac arrest. TN, True Negative; predicted and reported outcome good (CPC 1-2), TP, True Positive; predicted and reported outcome poor (CPC 3-5), FN, False Negative; predicted good and reported poor outcome, FP, False Positive; predicted poor and reported good outcome.

Poor outcome

All patients fulfilling Step 2 or Step 3 criteria had poor outcome after 6 months (CPC 3-5). There were no false positive predictions of poor outcome in good outcome patients (100.0% specificity, 95% CI 98.8-100.0). In this cohort, the ERC/ESICM algorithm identified 38.7% of the poor outcome patients (95% CI 33.1-44.7). Applying an alternative definition of poor outcome (CPC 4-5), one patient with CPC 3 fulfilled Step 3 criteria. Overall specificity was 99.7% and sensitivity 41.8% (Paper IV, eFig. 3).

Misclassified poor outcome patients

More than sixty percent of poor outcome patients were not identified by the algorithm. The majority had died at 6 months (84.0%). Treating physicians estimated that the presumed cause of death was neurological in 79/137 (57.7%) of FN patients who were deceased at follow-up.

Alternative screening criteria

The ERC/ESICM Step 2 and 3 criteria maintained 100% specificity regardless of the level of unconsciousness used as a screening criterion in Step 1, increasing overall sensitivity slightly (from 38.7% to maximum 42.5%). All patients fulfilling Step 2 or Step 3 criteria had GCS-M ≤ 4 on day 4.

Combining Steps 2 and 3

When allowing any 2 pathological findings or more recommended in Steps 2 and 3, all patients fulfilling criteria had poor outcome at 6 months, regardless of level of unconsciousness (100% specificity) (Fig. 31). All TP patients had GCS-M ≤ 4 on day 4. Fewer patients fulfilled the criteria of ≥ 2 pathological findings than the regular ERC/ESICM algorithm criteria, decreasing overall sensitivities.

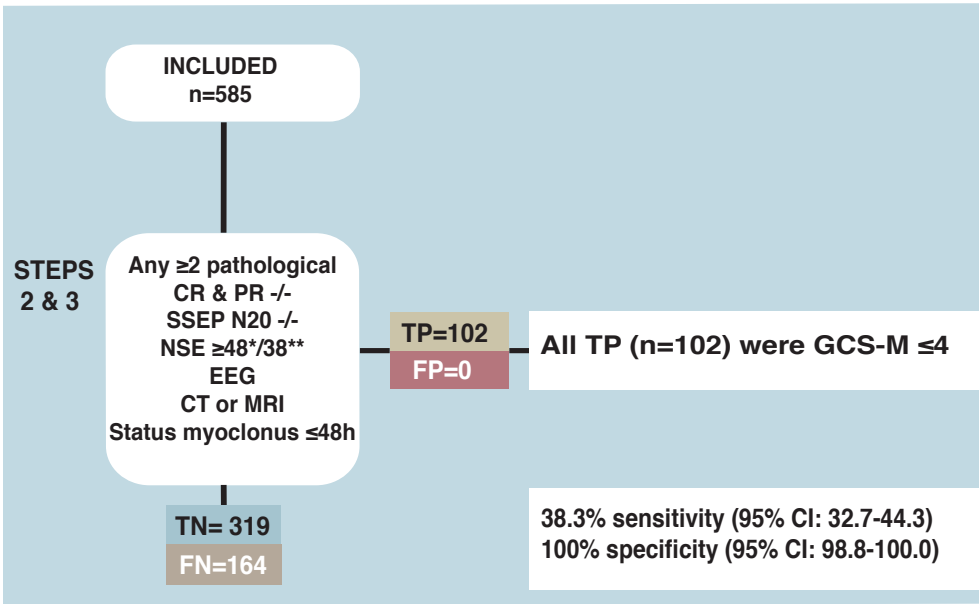


Fig. 31 Explorative analysis in the simplest version: any ≥ 2 pathological findings in Steps 2 and 3 were considered indicative of poor outcome irrespectable of GCS-M.

Good outcome

The majority of good outcome patients, 310/319 (97.2%) had no pathological findings according to the definitions used in this study, and almost 70% were awake and obeying commands on day 4 post-arrest. Less than three percent of patients with good outcome had single pathological findings (n = 9): one had early status myoclonus, one fulfilled ERC/ESICM criteria for unreactive status epilepticus on EEG 75 h after CA and the remaining 7 patients had elevated NSE levels at 48 h and/or 72 h, of which 3 had decreasing levels of NSE from 24-72h.

Most patients (6/9) with single FP findings were awake and obeying commands (GCS-M 6) on day 4. All fourteen good outcome patients with GCS-M ≤ 2 were male, most had ventricular fibrillation as the initial rhythm on ECG and their NSE-levels were below the cut-off for poor outcome applied in this study in all but one patient.

Withdrawal of life-sustaining therapy

WLST due to neurological futility was performed in 59.2-87.7% of poor outcome patients with pathological prognostic findings.

Prognostic accuracies

Data from all patients with available 6-month-outcome (n = 933) was used when calculating prognostic accuracies (Table 8).

Table 8 Prognostic accuracies of single methods

Method	Sensitivity (95% CI)	Specificity (95% CI)	FP	N	Poor outcome
GCS-M ≤2	71.8 (66.1-76.9)	95.6 (92.8-97.4)	14	585	266 (45.5)
GCS-M ≤3	77.1 (71.7-81.7)	92.8 (89.4-95.2)	23	585	266 (45.5)
GCS-M ≤4	85.7 (81.0-89.4)	83.7 (79.3-87.4)	52	585	266 (45.5)
PR/CR	20.1 (15.6-25.4)	100.0 (92.4-100.0)	0	301	254 (84.4)
SSEP	45.3 (37.9-53.1)	97.4 (86.8-99.6)	1	200	161 (80.5)
NSE ≥ 33*/**	67.3 (61.9-72.3)	89.9 (86.2-92.7)	34	646	309 (47.8)
NSE ≥48*/≥38**	60.2 (54.6-65.5)	96.4 (94.0-98.0)	12	646	309 (47.8)
EEG ERC/ESICM	31.7 (25.9-38.1)	98.8 (93.6-99.8)	1	305	221 (72.5)
EEG "highly malignant"	38.0 (31.9-44.6)	98.8 (93.6-99.8)	1	305	221 (72.5)
S. Myoclonus ≤ 48h	6.9 (5.0-9.5)	99.8 (98.7-100.0)	1	933	493 (53.8)
CT	32.3 (26.7-38.4)	98.3 (94.2-99.6)	2	356	235 (66.0)
MRI	13.0 (4.5-32.1)	100.0 (75.8-100.0)	0	35	23 (65.7)

The table describes variations in defintions of prognostic methods included in the ERC/ESICM algorithm, overall sensitivities, specificities, false positive predictions of poor outcome in good outcome patients (FP), number of examined patients and number and percentages of poor outcome (CPC 3-5 at 6 months). GCS-M; Glasgow Coma Scale Motor Score on day 4 post-arrest, PR/CR; bilaterally absent pupillary and corneal reflexes, SSEP; bilaterally absent N20 potentials, NSE; serum Neuron-specific enolase at *48 and **72 hours post-arrest, EEG; pathological EEG according to ERC/ESICM[23], EEG "highly malignant" according to definitions by Westhall et al.[79], S. myoclonus; generalised status myoclonus, CT/MRI; generalised oedema.

7. Discussion

7.1 Summary

Cardiac arrest is a common cause of death worldwide. Every day, treatment decisions are guided by the results of prognostic examinations. Premature or incomplete assessments may lead to withdrawal of life-sustaining therapy based on insufficient information, ultimately risking patient lives. However, not only "life" or "death" is relevant, but also being able to predict the extent of brain injury, the level of functioning and the quality of life that can be achieved for each CA survivor. Systematic reviews and meta-analyses have demonstrated knowledge gaps within neuroprognostication, some of which we have tried to narrow with the studies included in this thesis.

In the largest retrospective CT-study after CA, we concluded that the presence of generalised brain oedema is almost always associated with poor outcome, irrespective of timing, and that sensitivity for identifying poor outcome patients increases within the first days post-arrest.

The ERC/ESICM guidelines recommend a prognostic algorithm based on expert opinion, which had only been validated in two single center studies.[23,139,140] Using patient data from the largest published CA trial to date we have concluded that the current ERC/ESICM algorithm safely predicts poor neurological outcome. Nonetheless, the algorithm failed to identify nearly sixty percent of patients with poor outcome. We have therefore taken the liberty to suggest modifications to improve future algorithms.

Furthermore, we explored three novel biomarkers, of which NFL demonstrated the most impressive results. In our cohort, serum NFL was a superior predictor of poor outcome compared to any routine prognostic method already at 24 hours post-arrest. It's ability to differentiate between various levels of brain injury may help when making decisions on level-of-care. In addition, NFL has potential as a quantitative predictor for good outcome; low serum levels may help identify unconscious patients with a good neurological prognosis where continued treatment is life-saving.

7.2 ERC/ESICM algorithm (Paper IV)

A perfect specificity is always the main goal when predicting poor neurological outcome after CA, since false pathological findings may put the patient at risk of a self-fulfilling prophecy with death following WLST. If, and how often these situations occur, cannot be determined directly from our data. However, except for the criteria in Step 2 (bilaterally absent SSEP N20 and/or bilaterally absent corneal and pupillary reflexes), prognostication is always multimodal in the ERC/ESICM algorithm to reduce the risk of self-fulfilling prophecies. In this algorithm, bilaterally absent N20 in unconscious patients (GCS-M $\leq$ 2) is indicative of "poor outcome very likely", whilst the same findings in the TTM trial was one of the possible criteria for neurological WLST.[24,134]

We therefore examined if there seemed to be a high risk of self-fulfilling prophecies in patients with bilaterally absent N20, thereby fulfilling one TTM-WLST-criterium in our cohort.[134] Reassuringly, we found that almost ninety percent of patients with bilaterally absent N20 fulfilled at least one remaining ERC/ESICM criterium for poor outcome, and more than half fulfilled two or more additional criteria (Paper IV, eFig. 2). This indicates, that patients with bilaterally absent SSEP findings in Step 2 did indeed have signs of severe brain injury. Our results are supported by trials where WLST was not commonly performed, in which no good outcome patient had bilaterally absent N20.[65,70]

Bilaterally absent brainstem reflexes only identified 20% of poor outcome patients in our study, but in combination their specificity was 100% in the TTM trial.[41] The current ERC/ESICM algorithm permits combining motor score with two other criteria of the category clinical neurological examination (bilaterally absent pupillary and corneal reflexes), all methods that could be affected by sedation in TTM-treated patients.[37] Therefore, it may be advisable to also combine these results with additional prognostic methods from Steps 2 or 3. In the ongoing TTM2- and TAME-trials, the stepwise approach was abandoned, allowing any two pathological findings to be indicative of poor outcome in unconscious patients at 96 hours post-arrest.[33,35] We found that any two prognostic methods recommended by the ERC/ESICM algorithm predicted poor outcome with 100% specificity.

In general, it seems reasonable to always examine unconscious cardiac arrest patients with several entities of prognostic methods by combining clinical examinations with neurophysiological information, imaging and/or biomarkers. Clinical neurological examination of unconscious patients is mandatory and should be performed daily by the physicians treating the patient. In our study we found that seventy percent of good outcome patients were awake and obeying commands on day 4. Two thirds of patients with single false pathological findings were also awake and following commands on the fourth day post-arrest. Patients demonstrating the ability to understand and execute verbal commands are likely to have a good

neurological outcome, at least when evaluated by clinician-rated scales. Although most patients deeply unconscious on day 4 did have poor neurological outcome, the level of unconsciousness in itself is not a reliable predictor of poor outcome.[41,44] Our results indicate, that the ERC/ESICM criteria were reliable predictors of poor outcome in all unconscious patients regardless of motor score, and that patients unconscious on day 4 and not fulfilling ERC/ESICM criteria could still wake up with a favourable outcome.

Neurophysiological examination techniques and skills may not always be available, but if performed, it can deliver important information on cerebral function. Continuous EEG may give a good impression of the dynamics of recovery, where an early continuous background or reactivity may be indicators of a favourable outcome.[78,141] In case of a continuous EEG background, SSEP may not give any additional prognostic information, since N20 is usually present in these patients.[141,142] Most importantly, present N20 potentials are not a good predictor of poor outcome.[141] The presence of an early status myoclonus is often, but not always associated with a poor prognosis. A continuous EEG background in patients with myoclonus has been associated with a good prognosis.[49] ERC/ESICM recommends a multimodal approach for poor outcome prediction in patients with status myoclonus. In contrast to the American Heart Association's recommendations, the ERC/ESICM criteria do not clearly define that status myoclonus should be evaluated together with EEG.[24,43]

Despite specificity being the most important measure for prediction of poor outcome, the sensitivity of a test or an algorithm is still relevant. Ideally, a test should identify all patients with poor outcome, without misclassifying good outcome patients. We found that the ERC/ESICM guideline algorithm identified approximately 40% of poor outcome patients without false positive predictions. Two previous single-center studies validated the ERC/ESICM algorithm and also found a 100% specificity for poor outcome prediction, but with slightly lower sensitivities of 18-26%.[139,140] However, sensitivities are not only determined by the test's prognostic ability, but as seen in our study, the timing of examination and the selection and number of patients examined also influence sensitivities.

When comparing our sensitivities with the results from Zhou et al. and Bongiovanni et. al, we see that neuroimaging and EEG was more commonly used than NSE with its higher single sensitivity, probably contributing to the lower overall sensitivities of the ERC/ESICM algorithm in these studies.[139,140] Yet, the overall sensitivity of the algorithm could also have been affected by other factors.

The algorithm missed sixty percent of poor outcome patients, and there are several possible explanations for this:

1) The screening criterium for prognostication may be too conservative by only including GCS-M$\leq$ 2, since our results indicate that the exact level of unconsciousness did not impact specificity in this cohort.

2) Patients may not have been examined with sufficient prognostic methods to fulfil criteria, especially if there were other conditions that led to decisions on WLST, limiting the value of further prognostication.

3) Patients may have died of non-neurological causes, in which case neurological examinations may have demonstrated normal findings despite for example cardiac or systemic complications being indicative of a poor prognosis.

Timing of prognostication

The ideal time-point for prognostication has yet to be determined. The ERC/ESICM recommend to delay prediction of neurological outcome until at least 72 hours post-arrest,[24] whilst the American Heart Association recommends waiting 72 hours after normothermia in TTM treated patients when evaluating clinical examinations of patients.[43] The Swedish CPR Council Expert Group recommend the same time-limit as the ERC/ESICM.[42] The ongoing TTM2- and TAME-trials postponed formal neuroprognostication to 96 hours post-arrest.[33,35]

There may be perceived pressure to perform prognostication as early as possible in patients with a presumed poor neurological prognosis, arguing that it is unethical to continue treatment which is not beneficial for the patient, and which may take resources from other patients requiring life-saving treatment. However, some studies with matched controls have reported that early WLST in patients with a presumed poor neurological prognosis may have cost unnecessary lives in 16-19% of patients who are believed to could have survived with a good outcome.[143,144]

A study from the TTM-trial found that TTM at 33°C was significantly associated with later awakening despite having received similar doses of sedation.[133] However, median time to awakening was 4 days in both the 33°C and 36°C groups, corresponding to 72-96 hours post-arrest, which was the time-point used in our study.[133] Thirty percent of our patients with good outcome were still not awake on day 4 post-arrest.

Results from examinations included in the formal neurological prognostication are collected within the first days after CA, and it may seem frustrating to wait until a formal deadline when WLST may be performed based on these results. However, the timing of the various prognostic methods may anyhow indicate that at least 72 hours should be awaited prior to prognostication to confirm poor prognosis. Possibly even longer in patients treated with TTM 33°C, since TTM affects renal clearance and may prolong effects of sedation in patients with an injured brain.[145] Unconscious patients with no clear indicators of a poor prognosis should be given additional time for renewed evaluations.

7.3 Head computed tomography (Paper I)

In our retrospective study, more than sixty percent of CT examinations were performed within the first 24 hours after CA, usually to exclude cerebral causes of unconsciousness such as major intracranial haemorrhage or stroke. We found, that the majority of early CT examinations were evaluated as "normal" by local radiologists. The high number of examined patients, together with the low number of patients where generalised oedema was diagnosed may partly explain why sensitivity was only 14.4% for early CT's. We presume, that patients examined with CT at a later time-point (after the first day, but within one week after CA) were examined for another reason, mainly for its use within neuroprognostication.

Patients who are awake within the first days post-arrest usually have a favourable outcome and are less frequently examined.[133] In contrast, patients still unconscious at 72 hours or later post-arrest should be considered for prognostication according to guidelines.[24,42,43] That later CT examinations were preferably performed in sicker patients is also reflected in the increased number of patients with poor outcome in our study. Eighty percent of patients examined after day one, but within one week post-arrest had poor outcome. This selection of poor outcome patients presumably contributes to the increased sensitivity of later CT's compared to CT performed upon hospital admission.

Brain injury post-arrest is a dynamic process that may progress, and in rare cases possibly even regress within the first days after CA. Initially, there may be more cytotoxic/ionic oedema within the brain cells.[146] With increasing injury and permeability of the blood-brain barrier, the extracellular vasogenic oedema increases, a process that may proceed for days. In addition, there are parallel mechanisms of injury, such as initial necrosis, delayed neuronal cell death, mechanisms of excitotoxicity and other symptoms of the post-cardiac-arrest syndrome influencing the brain.[11,12]

Forty percent of our patients who were re-examined with CT progressed from normal findings on admission, to generalised oedema on the second scan, supporting this theory. The selection of sicker patients for the prognostic CT examinations, taken together with a progression of oedema are the likely explanations for a nearly 4-fold increase in sensitivity between CT on hospital admission to CT for prognostic purposes.

Another contributing factor may be hesitancy from radiologists to diagnose generalised oedema early, knowing that this diagnosis may lead to withdrawal of life-sustaining therapy and subsequently to death of the patient. Nonetheless, we did not collect original CT data and do not know how much interrater variability there may be for radiologic eye-balling of cerebral oedema. Various quantitative methods for evaluating the grey-white matter ratio (GWR) exist, but there is currently no

consensus on method nor on cut-off values, and the prognostic performance may vary with technical equipment or examiners.[44,58,66,70,147,148]

Our patients with generalised oedema had significantly higher peak-NSE serum levels than patients where generalised oedema was not diagnosed. The two good outcome patients where CT was classified as "with generalised oedema" were examined within the first 24 hours post-arrest, both having peak-NSE levels below our locally established cut-off values for poor outcome. Combining CT and NSE in a multimodal approach as recommended by guidelines identified poor outcome patients without false positive predictions.

7.4 Serum biomarkers (Papers II and III)

Neuron specific enolase is the only biomarker recommended by the ERC/ESICM guidelines.[24] However, cut-off values must be established locally and there is no calibration standard, limiting comparisons. Furthermore, NSE is present in erythrocytes, and is therefore sensitive to haemolysis, risking falsely elevated serum levels.[86] The best prognostic performance for NSE is at 48 or 72 hours post-arrest, with increasing serum levels between 24 and 48 hours.[44,88,89] The American Heart Association also mention S100 as a possible biomarker in CA patients, but with limited evidence.[43]

NFL

Studies using material from the TTM-trial biobank have examined the prognostic accuracies of the cerebral markers NSE, S100B and tau, of which the axonal marker tau had the highest overall prognostic accuracies.[88,105,109] However, when comparing these three markers with NFL within the same group of patients, we found significantly higher prognostic accuracies for NFL at all time-points between 24-72 hours post-arrest.

Another small study with 14 adult CA patients also found significantly higher NFL concentrations in poor outcome patients when analysed with an ultrasensitive assay.[149] Results from a small paediatric CA study on NFL for prognostication were inconclusive.[150] Compared to routine methods at matched specificities, NFL at 24 hours demonstrated higher sensitivities in our study. The high prognostic accuracy of NFL from 24-72 hours was recently confirmed in the COMACARE trial.[151] In this study, NFL on hospital admission was not a good predictor of neurological outcome, supporting the theory that neuroaxonal injury develops over hours and days post-arrest.[151] The exact half-life of NFL is not known, but seems longer than those reported for the other biomarkers (Table 2), giving the impression of a robust marker over time.

NFL is also the only biomarker that has demonstrated the ability to differentiate between the extent of brain injury represented by the lower levels of the CPC scale (Fig. 25). NFL could not differentiate between CPC 4 (coma or vegetative state) or CPC 5 (death). When examining the wide range of distribution of NFL levels in CPC 5 patients, this may not be surprising; many patients with low NFL levels may have died from non-primary neurological causes, and WLST may have been performed in potential CPC 4 patients with similarly high NFL levels as patients surviving in an unresponsive wakefulness syndrome. NFL was not elevated in samples with haemolysis, which represents another advantage compared to NSE.

Most importantly, NFL also represents a potential marker for identifying patients were low levels indicates that there is little or no sign of brain injury. This could help reduce the risk of self-fulfilling prophecies in patients who were otherwise subjected to premature WLST due to a presumed poor neurological prognosis.

UCH-L1

Similar to NSE, UCH-L1 is another neuronal marker which has not yet been examined in adult CA patients. It has often been studied as a marker of traumatic brain injury, usually in combination with the astrocytic marker GFAP.[86,90] Its molecular weight is similar to S100 and smaller than NFL, GFAP, NSE or tau, which may be a theoretical benefit for passing the blood-brain-barrier. Yet its short half-life of 6-12 hours gives UCH-L1 a theoretical disadvantage for its use within neuroprognostication compared to markers with longer half-life. Serum UCH-L1 levels were elevated in our poor outcome patients and did in fact decrease after 48 hours. UCH-L1 was only superior to NSE at 24 hours, possibly reflecting the longer half-life of NSE. In contrast to NSE, UCH-L1 levels were not influenced by haemolysis. Based on our results, UCH-L1 may be an acute marker of brain injury, yet probably not robust enough for prediction of neurological outcome after CA.

GFAP

The astrocytic structural protein GFAP is highly expressed in the CNS. Glial cells have numerous supportive functions and have demonstrated increasing activity after brain injury. Increased serum levels of GFAP can be indicative of both astrocytic injury and increased astrocytic activity. This differentiation may not be clinically relevant however, as long as GFAP serum levels correspond with neurological outcome.

In our cohort, GFAP as a single marker, never had significantly better prognostic accuracy than NSE at high specificities (Table 7). On the contrary; at high specificities, GFAP was inferior to NSE both at 48 and 72 hours. Despite higher overall prognostic accuracies, there is little tolerance for false positive predictions within neuroprognostication after CA, and for this reason partial AUROC at high

specificities is clinically more relevant than total AUROC. The commonly used combination for traumatic brain injury, GFAP together with UCH-L1 at high specificities was only superior to NSE at 24 hours. Both biomarkers contributed significantly to the overall prognostic accuracies of the models (Paper III, eTable 4). The mechanism of injury in mild traumatic brain injury has often been described as diffuse axonal injury, which differs from the complex mechanism of brain injury after CA.[11,86] The combination of GFAP and UCH-L1 have found their clinical use to reduce unnecessary radiation in patients with mild traumatic brain injury.[90] The short half-life of UCH-L1 represents a theoretical disadvantage compared to for example NFL, and it is possible that prognostic accuracy of the glial marker GFAP could be improved further by combining it with other neuronal markers such as NFL.

Which is the perfect biomarker of brain injury?

Our results indicate that the most likely answer to this question is neurofilament light. The smaller molecular size simplifies the passage through the blood-brain-barrier compared to the almost three times heavier neurofilament heavy chain (NFH) which is probably the main reason why the prognostic accuracy of NFL is superior to NFH since both neurofilaments originate from the same localisation within neurons.[97] In this thesis, both axonal markers NFL and tau had superior prognostic accuracies compared to those reported for the neuronal body markers NSE and UCH-L1, and the glial markers S100B and GFAP.[88,105,109,152] We cannot conclude whether these axonal markers are in fact superior for post-arrest injury, or to which extent the biochemical methods used influenced our results. In the present study, only tau and NFL were analysed with the ultrasensitive Simoa assay, whilst S100B and NSE were analysed with ECLIA, and GFAP and UCH-L1 were analysed with ELISA. Simoa has been described as 126-fold more sensitive than ELISA and 25-fold more sensitive than ECLIA for NFL detection in serum.[98] The same study found that NFL in CSF and serum were highly correlated both for the Simoa and ECLIA, with better correlation for Simoa.[98] It is possible that prognostic accuracies could be improved by using ultrasensitive assays.

Future research may exclude the influence of different techniques on the prognostic performance of biomarkers by comparing markers head-to-head. Theoretically, the longer half-life indicates that NFL may still remain a superior marker of post-arrest brain injury compared to S100B or UCH-L1, with their extremely short half-lives.[153] In addition, the optimal time-point for the highest prognostic accuracy differs between the various markers and cut-off values may be equally dynamic. These effects may be difficult to demonstrate statistically within the same cohorts due to patients dying between sampling et cetera but could possibly be assessed with the help of machine learning.

When determining if levels of a biomarker is "high" or "normal", it is very important to remember that none of the six biomarkers discussed in this thesis are exclusively expressed in the central nervous system, and several potential sources of falsely elevated serum levels exist (Table 2).[87] Neither NFL, GFAP nor UCH-L1 were elevated in haemolytic samples since they are not present in erythrocytes, demonstrating a potential benefit compared to the currently used marker NSE.[88]

It is not clear if or how much serum levels are influenced by renal insufficiency, which can sometimes occur post-arrest. All brain biomarkers analysed in this thesis have similar or smaller molecular weights than albumin (66.5 kDa), which indicates that since albumin can pass the glomerular filtration barrier, these biomarkers probably also can. However, larger molecules are not as easily filtrated as smaller ones. The molecular weight alone, however, does not seem to explain the variations in half-life between markers. Further research is required to evaluate the impact of elimination on serum levels in patients with renal insufficiency post-arrest.

The lack of a calibration standard for most biomarkers of brain injury, together with the absence of normal values and cut-off values to identify poor outcome patients still limits their clinical utility.[154] Two recent studies have attempted to describe normal serum NFL concentrations and variation with patient age, and found increased NFL concentrations in older patients.[155,156] A large Chinese study with over 86.000 participants reported that 95% of normal NSE values were below 20.5 ng/mL within a presumably healthy adult population when analysed with the same ECLIA method as NSE within our study.[157] In patients with mild traumatic brain injury, the serum levels of UCH-L1 and GFAP analysed with the same ELISA method as in our study were within the range of good outcome patients in our study.[90] Direct comparisons of exact serum levels between neurological disorders should be avoided at this point. However, on a group-level, poor outcome patients in our study demonstrated serum NFL concentrations considerably higher than patients with ischaemic stroke, Alzheimer's disease, parkinsonian disorders or traumatic brain injury when analysed with the same ultrasensitive assay.[85,99,100,158] In contrast, patients with ALS or HIV-dementia demonstrated very high levels of NFL similar to those in our study.[101,102] Further research is required to estimate which neurological conditions may interfere with cerebral biomarkers after CA.

7.5 Strengths

The TTM-trial is the largest cardiac arrest trial published to date and was the first trial with a strict and conservative protocol for neurological prognostication and with pre-defined criteria for WLST. Detailed clinical information and a structured approach to follow-up of patients has given unique possibilities of studying various aspects of intensive care treatment, neurological assessment and outcome after CA.

The TTM-biobank serum samples were collected prospectively and analysed after trial completion, excluding the risk of results directly influencing clinical decisions. The international design of the trial allows for a greater generalisability of the results compared to single center studies.

7.6 Limitations

Patient data for all four papers included in this thesis were collected in the TTM-trial. Thus, groups for statistical analyses sometimes overlap and our results are therefore not completely independent from each other. Also, the ERC/ESICM algorithm is partly based on results from the TTM-trial. Patients were adult OHCA survivors with a presumed cardiac origin of arrest, and our results are primarily representative for this patient group.

The TTM-trial compared the influence of two temperature regimens on mortality and neurological outcome, and despite its conservative protocol for neurological prognostication and WLST, neuroprognostication was not the main purpose of this trial. Papers I and IV are limited by their retrospective nature where some examinations such as neuroimaging or SSEP were performed on clinical indication, causing a selection of sicker patients. However, this reflects clinical practice, where patients who are awake and obeying commands will not require the same prognostic examinations as unconscious patients. WLST was permitted if fulfilling specific criteria of the TTM-protocol, and we cannot exclude the risk of self-fulfilling prophecies. Also, there is some overlap between the TTM protocol and the ERC/ESICM guideline algorithm, and some co-authors have participated in both committees.

Interrater variability in evaluations of clinical neurological examinations, neuroimaging and neurophysiology cannot be excluded, yet this also reflects the pragmatic approach of the trial which is similar to clinical practice.

8. Conclusions

The most important findings in each of the papers included in this thesis are summarized as follows:

I. Generalised brain oedema on CT is associated with higher levels of serum NSE and is almost always associated with poor neurological outcome after cardiac arrest, irrespective of timing of the examination.

II. Serum NFL analysed with a novel ultrasensitive assay demonstrated prognostic abilities superior to several other markers of brain injury, and to all routine methods currently recommended in guidelines.

    NFL has the potential to prevent premature WLST in patients with a presumed good prognosis.

III. Serum GFAP and UCH-L1 may represent early markers of brain injury but were not robust predictors of poor outcome in our study.

IV. The ERC/ESICM algorithm safely predicted poor neurological outcome, but sensitivity was limited.

9. Future perspectives

9.1 Prognostic algorithms

Based on the results from Paper IV, the following adaptations are proposed for future neuroprognostic guideline algorithms:

1) Broadening the screening criterion to include patients with GCS-M ≤ 3 or even GCS-M ≤ 4 on day 4 post-arrest should be considered for selecting which patients should undergo neurological prognostication. According to our results, the exact level of unconsciousness does not influence specificity of prognostic methods included in the ERC/ESICM algorithm.
2) It seems reasonable to always combine at least two prognostic methods from different entities in a multimodal approach, with the goal of reducing the risk of false positive predictions which are sometimes reported with single prognostic methods. Further research examining all patients with all prognostic methods is required to identify ideal combinations of methods.
3) Establishing standardised criteria for evaluation of imaging, neurophysiology, or defining calibration standards and cut-off values for biomarkers may improve prognostication and reduce the risk of interrater variability. Results on new quantitative methods such as pupillometry or blood biomarkers should be validated and may become useful additions in future algorithms.
4) Prediction of outcome after cardiac arrest is limited by non-neurological complications, which is why the potential benefit of a combined neurological, cardiac and circulatory prognostic model should be explored. The current guidelines for post-resuscitation care focusses on identifying patients with a presumed poor neurological outcome, however further research is also needed to identify patients with a presumed good prognosis to prevent premature decisions on WLST.

9.2 Neuroimaging

Prospective trials limiting the risk of selective examinations of poor outcome patients are required to validate qualitative and quantitative radiological methods after CA. The TTM2-trial is an international prospective trial which randomised 1900 adult OHCA patients to a targeted temperature management of 33°C or to targeted normothermia (Clinicaltrials.gov NCT02908308).[33] Results from the main trial are pending, and several registered substudies exist.

The TTM2 CT-substudy is an ongoing international multicentre trial with 13 participating centres in Sweden, Germany, UK and France and has further international collaborators (Clinicaltrials.gov NCT03913065). Patients from participating sites who were still unconscious at 48 hours post-arrest were prospectively examined with non-contrast head CT. Images will be collected and evaluated centrally by radiologists blinded to clinical information.

Analyses will include "eye-balling" for generalised oedema, manual measurements of grey-white matter ratio (GWR) within regions of interest, an exploratory scoring system and automated measurements. Prognostic accuracies and interrater variability of these methods for prediction of poor neurological outcome at 6 months will be presented. Our study aims to validate different previously published methods for determining GWR. Exploratory analysis will include correlations with markers of brain injury, neurocognitive outcomes and automated measurements of generalised oedema on CT.

Future studies in neuroimaging should also include targeted examinations using for example MRI in patients with indeterminate outcome according to guideline recommendations to better classify the prognosis of this patient group.

9.3 Biomarkers of brain injury

Large prospective studies should be performed to fully understand and validate the potential of blood biomarkers after cardiac arrest. Ideally, sampling should be performed from baseline to several weeks or months post-arrest to monitor the dynamics of brain injury and recovery.

Comparing various brain injury markers using identical platforms for analysis within the same patient group may demonstrate each marker's true potential for identifying the extent of brain injury and for both good and poor outcome prediction.

Establishment of international calibration standards are necessary to define age related normal values and to allow for comparisons of absolute values of biomarkers between neurological conditions.

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