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Lauri Wihersaari

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ABSTRACT

Hypoxic-ischemic brain injury (HIBI) is the main cause of severe neurological disability and death after cardiac arrest (CA). The assessment of HIBI is critical in prognostication after CA, and it remains challenging. Neurobiomarkers are one part of the multimodal prognostication of neurological outcome. Current guidelines recommend using neuron-specific enolase (NSE) as a neurobiomarker, together with clinical examination, imaging, and neurophysiological studies. However, NSE levels can misleadingly increase because of haemolysis and after other brain injuries. NSE can also be released from extracerebral sources, altering its prognostic ability. Also, the predictive value of NSE in the oldest patients and in those with a short resuscitation time is unclear. Furthermore, sedative medications and muscle relaxants can confound the interpretation of clinical and neurophysiological examinations, even if they have no effect on blood biomarkers. Given these challenges, accurate prognostication after CA requires methods that can reliably detect or exclude severe HIBI. Ubiquitin c-terminal hydrose L1 (UCH-L1) and neufofilament light (NfL) are promising novel biomarkers that can have potential in prognostication after CA.

Aims

The aims of this study were to: 1) determine the ability of serum NSE to predict unfavourable long-term functional outcome after out-of-hospital cardiac arrest (OHCA) in subgroups (divided into quartiles according to the patient's age and time from collapse to the return of spontaneous circulation [ROSC]); 2) determine the ability of serum UCH-L1 to predict long-term functional outcome after OHCA and compare it to that of NSE; and 3) determine the value of plasma NfL in predicting unfavourable long-term outcome after OHCA and compare it to NSE, and to assess the impact of two different arterial blood carbon dioxide tension ($PaCO_2$), arterial blood oxygen tension (PaO_2), and mean arterial pressure (MAP) targets on NfL concentrations.

Materials and methods

This study includes four post-hoc laboratory studies (numbered I–IV). The blood samples used were collected and stored during the original studies this work is based on. The patients for Studies I, II, and IV came from the FINNRESUSCI study, which included adult patients resuscitated from OHCA and treated in 21 Finnish intensive care units (ICUs) in 2010–2011. The patients in Study III came from the Carbon dioxide, Oxygen, and Mean arterial pressure After Cardiac Arrest and REsuscitation (COMACARE) study (NCT02698917). This randomised, controlled trial studied the effect of low-normal and high-normal $PaCO_2$, PaO_2, and MAP levels on the outcome of adult OHCA patients resuscitated from shockable initial rhythms.

The primary outcome was assessed at one year (Studies I, II, and IV) and at six months (Study III) based on the Cerebral Performance Category (CPC). A score of CPC 1–2 indicates favourable outcome, and CPC 3–5 is unfavourable outcome (death or severe disability). Hospital survival was used as a secondary outcome.

Blood samples for Studies I, II, and IV were obtained at 24 and 48 h after CA in a total of 249 patients. We analysed serum concentrations of NSE (Studies I and IV), serum concentrations of UCH-L1 (Study II), and plasma concentrations of NfL (Study IV) at 24 and 48 h. The blood samples for Study III were collected at the time of ICU admission and at 24, 48, and 72 h

after CA, and we analysed the NfL and NSE concentrations of 112 patients at those time points. To assess all the biomarkers' ability to predict unfavourable outcome, we calculated the area under the receiver operating characteristic curve (AUROC).

Main results

In total, 121 of 249 patients (48.6%) in Studies I and II, 39 of 112 patients (34.8%) in Study III, and 120 of 248 patients (48.4%) in Study IV had unfavourable outcome. In Study I, NSE had a satisfactory prognostic ability (AUROC 0.72). The prognostic ability of NSE was excellent in patients with young age (18-56 years; AUROC 0.91) and good in patients with long time from collapse to ROSC (≥29 min; AUROC 0.84). The prognostic ability of NSE was poor in patients with a high age (≥72 years; AUROC 0.53) and a short time from collapse to ROSC (≤13 min; AUROC 0.45). In Study II, UCH-L1 had a moderate prognostic ability (AUROC 0.66) and offered no benefit compared to NSE.

In Study III, NfL had an excellent ability to predict unfavourable outcome at 24–72 h after OHCA (AUROCs 0.98; 95% confidence interval [CI] 0.95–1.00), superior to that of NSE. NfL concentrations were significantly lower in the higher blood pressure group (MAP 80-100 mmHg) than in the lower blood pressure group (MAP 65-75 mmHg) at 48 h (p=0.041) and 72 h (p=0.007). In Study IV, which included unselected OHCA patients, NfL at 24 and 48 h had a better prognostic value (AUROC 0.90 and 0.88, respectively) than NSE (AUROC 0.65 and 0.72, respectively).

Conclusion

NSE had poor prognostic value in the oldest patients and in those with the shortest time from collapse to ROSC, whereas the prognostic ability of NfL was satisfactory in those patients. UCH-L1 did not demonstrate any benefits in prognostication compared to NSE. NfL had an excellent ability to predict long-term functional outcome after OHCA in both a select population with only cardiogenic CA (COMACARE) and a larger population with various types of CA (FINNRESUSCI). The prognostic ability of NfL was

superior to that of NSE. NfL levels were lower in the higher MAP group than in the lower MAP group.

Keywords: Out-of-hospital cardiac arrest, OHCA, resuscitation, prognostication, neurological outcome, neurobiomarkers, neuron-specific enolase, NSE, ubiquitin c-terminal hydrolase L1, UCH-L1, neurofilament light, NfL

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TIIVISTELMÄ

Hapenpuutteesta johtuva aivovaurio (hypoxic-ischemic brain injury, HIBI) on yleisin elvytyksen jälkeinen kuolemaan ja vakavaan neurologiseen vammautumiseen johtava syy. Hapenpuutteesta johtuvan aivovaurion vaikeuden arvioiminen on keskeistä mutta haastavaa ennustearviossa. Aivovaurion merkkiaineet ovat osa useaan menetelmään perustuvaa neurologista ennustearviota. Nykyiset ohjeet suosittelevat neuronispesifisen enolaasin (NSE) käyttöä yhdessä kliinisen tutkimisen, kuvantamisen ja neurofysiologisten tutkimusten kanssa. NSE:n pitoisuudet veressä voivat kuitenkin virheellisesti nousta muiden aivovammojen tai hemolyysin seurauksena. Lisäksi muualta kuin aivoista voi vapautua NSE:a, mikä heikentää sen ennustekykyä. Lisäksi NSE:n ennustekyky iäkkäillä on epäselvä. Sedatoiva lääkitys ja lihasrelaksanttien käyttö voi vaikeuttaa kliinisen ja neurofysiologisen tutkimisen tulkintaa, mutta ne eivät vaikuta merkkiaineisiin. Hapenpuutteesta johtuvan aivovaurion tunnistamiseen kykeneviä menetelmiä tarvitaan elvytyksen jälkeiseen ennustearvion tekemiseen. Ubikitiinin hiilipään hydrolaasi L1 (UCH-L1) ja neurofilamentin kevytketju (NfL) ovat lupauksia herättäviä uusia merkkiaineita, jotka saattaisivat olla käyttökelpoisia sydänpysähdyksen ja elvytyksen jälkeisessä ennustearviossa.

Tavoitteet

Tutkimuksen tavoitteina olivat: 1) määrittää seerumin NSE:n kyky ennustaa sairaalan ulkopuolella elvytettyjen neurologista pitkäaikaisennustetta eri alaryhmissä (iän ja elvytysajan mukaisiin kvartiileihin jaettuna); 2) selvittää seerumin UCH-L1:n ennustekyky sairaalan ulkopuolisen sydänpysähdyksen jälkeen ja verrata sitä NSE:n ennustekykyyn; 3) määrittää plasman NfL:n ennustekyky sairaalan ulkopuolisen sydänpysähdyksen jälkeen ja verrata sitä NSE:n ennustekykyyn, ja määrittää kahden eri happi- ja hiilidioksiditavoitteen ja keskiverenpainetavoitteen vaikutus NfL-pitoisuuksiin.

Aineisto ja menetelmät

Tutkimus koostuu neljästä jälkikäteisanalyysinä tehdystä laboratoriotutkimuksesta (tutkimukset I-IV). Verinäytteet kerättiin ja varastoitiin alkuperäisten tutkimusten aikana, tämä tutkimus perustuu näihin alkuperäistutkimuksiin. Potilaat tutkimuksiin I, II ja IV tulivat FINNRESUSCI-tutkimuksesta, jossa kerättiin tietoa 21:llä suomalaisella teho-osastolla vuosina 2010-2011 hoidetuista sairaalan ulkopuolella elvytetyistä aikuispotilaista. Tutkimuksen III potilaat ovat Carbon dioxide, Oxygen, and Mean arterial pressure After Cardiac Arrest and Resuscitation (COMACARE) -tutkimuksesta (NCT02698917). Tämä satunnaistettu ja kontrolloitu tutkimus tutki normaalin matalan ja normaalin korkean happi- ja hiilidioksidipitoisuuden ja verenpaineen vaikutuksia iskettävistä rytmeistä sairaalan ulkopuolella elvytetyillä.

Ensisijaiset päätemuuttujat määriteltiin vuoden kohdalla (tutkimukset I, II, IV) ja puolen vuoden (tutkimus III) kohdalla elvytyksestä Cerebral Performance Category:n (CPC) mukaisesti. CPC 1-2 tarkoittaa hyvää toipumista, CPC 3-5 huonoa lopputulosta (kuolema tai vaikea vammautuminen). Sairaalakuolleisuus valittiin toissijaiseksi päätemuuttujaksi.

Verinäytteet tutkimuksiin I, II ja IV kerättiin 24 ja 48 tuntia elvytyksestä 249 potilaalta. Analysoimme seerumin NSE-pitoisuudet (tutkimukset I ja IV), seerumin UCH-L1-pitoisuudet (tutkimus II) ja plasman NfL-pitoisuudet (tutkimus IV) 24 ja 48 tuntia elvytyksestä. Verinäytteet tutkimukseen III

kerättiin teho-osastolle saapuessa ja 24, 48 ja 72 tuntia elottomuudesta, ja 112 potilaan NfL-pitoisuudet kyseisinä aikoina analysoitiin. Määritimme ROC-käyrän alle jäävän pinta-alan (the area under the receiver operating characteristic curve, AUROC) arvioidaksemme biomarkkerien ennustekykyä.

Tärkeimmät tulokset

Huono lopputulos todettiin 121:llä potilaalla 249:stä (48,6 %) tutkimuksessa I ja II, 39:llä potilaalla 112:sta (34,8 %) tutkimuksessa III ja 120:llä potilaalla 248:sta (48,4 %) tutkimuksessa IV. Tutkimuksessa I NSE:llä oli tyydyttävä ennustekyky (AUROC 0,72). Iäkkäillä (≥ 72 vuotta) ja lyhyen aikaa elvytetyillä (≤13 minuuttia) NSE:n ennustekyky oli huono. Tutkimuksessa II UCH-L1:llä todettiin keskinkertainen ennustekyky (AUROC 0,66), joka ei tuo lisäarvoa NSE:iin verrattuna. Tutkimuksessa III 24–72 tuntia elottomuudesta mitatulla NfL:lla todettiin erinomainen kyky ennustaa huonoa lopputulosta (AUROC 0,98, 95 %:n luottamusväli 0,95-1,00). Tämä oli selvästi parempi kuin NSE:n ennustekyky. NfL-pitoisuudet olivat matalampia potilailla, jotka hoidettiin korkeammalla keskiverenpainetavoitteella (MAP 80-100 mmHg) kuin niillä, jotka hoidettiin matalammalla tavoitteella (MAP 65-75 mmHg) 48 tunnin kohdalla (p=0.041) ja 72 tunnin kohdalla (p=0.007). Tutkimuksessa IV, jonka aineistona oli valikoimaton joukko sairaalan ulkopuolella elvytettyjä potilaita, 24 tai 48 tunnin kohdalla mitattu NfL oli ennustekyvyltään parempi (AUROC 0,90 ja 0,88) kuin NSE (AUROC 0,65 ja 0,72).

Yhteenveto

NSE:n ennustekyky oli heikko iäkkäillä ja lyhytkestoisesta elottomuudesta elvytetyillä potilailla, kun taas NfL:n ennustekyky oli tyydyttävä näissäkin tapauksissa. UCH-L1 ei osoittautunut ennustekyvyltään NSE:a paremmaksi. NfL:lla todettiin erinomainen kyky ennustaa sairaalan ulkopuolella elvytettyjen toipumista sekä COMACARE-tutkimuksen sydänperäisistä elottomuuksista elvytettyjen kohdalla, että laajemmassa FINNRESUSCI-aineistossa, joka sisälsi erilaisia sydänpysähdyksen syitä. NfL:n

ennustekyky oli parempi kuin NSE:n. NfL-pitoisuudet olivat matalampia korkeamman kuin matalamman keskiverenpainetavoitteen ryhmässä.
Avainsanat: Sairaalan ulkopuolinen elottomuus, elvytys, ennustearvio, neurologinen ennuste, aivovaurion merkkiaineet, neuronispesifinen enolaasi, NSE, ubikitiinin hiilipään hydrolaasi L1, UCH-L1, neurofilamentin kevytketju, NfL

To my family

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This dissertation is based on the following original publications:

I Wihersaari L, Tiainen M, Skrifvars MB, Bendel S, Kaukonen KM, Vaahersalo J, Romppanen J, Pettilä V, Reinikainen M; FINNRESUSCI study group. Usefulness of neuron specific enolase in prognostication after cardiac arrest: Impact of age and time to ROSC. Resuscitation. 2019;139:214-221.

II Wihersaari L, Reinikainen M, Tiainen M, Bendel S, Kaukonen KM, Vaahersalo J, JRomppanen J, Pettilä V, Skrifvars MB, the FINNRESUSCI study group. Ubiquitin C-terminal hydrolase L1 after out-of-hospital cardiac arrest. Acta Anaesthesiol Scand. 2023;67:964-971.

III Wihersaari L, Ashton NJ, Reinikainen M, Jakkula P, Pettilä V, Hästbacka J, Tiainen M, Loisa P, Friberg H, Cronberg T, Blennow K, Zetterberg H, Skrifvars MB; COMACARE STUDY GROUP. Neurofilament light as an outcome predictor after cardiac arrest: a post hoc analysis of the COMACARE trial. Intensive Care Med. 2021;47:39-48.

IV Wihersaari L, Reinikainen M, Furlan R, Mandelli A, Vaahersalo J, Kurola J, Tiainen M, Pettilä V, Bendel S, Varpula T, Latini R, Ristagno G, Skrifvars MB. Neurofilament light compared to neuron-specific enolase as a predictor of unfavourable outcome after out-of-hospital cardiac arrest. Resuscitation. 2022;174:1-8.

CONTENTS

ABBREVIATIONS

ACNS American Clinical Neurophysiology Society's

ADC Apparent diffusion coefficient

APACHE II Acute Physiology And Chronic Health Evaluation

ASY Asystole

AUROC Area under the receiver operating characteristic curve

BBB Blood-brain barrier

BS Burst suppression

CA Cardiac arrest

CBF Cerebral blood flow

CI Confidence interval

CNS Central nervous system

COPD Chronic obstructive pulmonary disease

CPC Cerebral Performance Category

CPP Cerebral perfusion pressure

CPR Cardiopulmonary resuscitation

CSF Cerebrospinal fluid

CT	Computed tomography
DWI	Diffusion-weighted imaging
EEG	Electroencephalography
ELISA	Enzyme-linked immunosorbent assay
ERC	European Resuscitation Council
ESICM	European Society of Intensive Care Medicine
FPR	False positive rate
GCS	Glasgow Coma Scale
GFAP	Glial fibrillary acidic protein
GM	Grey matter
GOS	Glasgow Outcome Scale
GWR	Grey–white matter ratio
HIBI	Hypoxic-ischemic brain injury
ICH	Intracerebral haemorrhage
ICP	Intracranial pressure
ICU	Intensive care unit
IDI	Integrated discrimination improvement

IHCA In-hospital cardiac arrest

IL6 Interleukin 6

kDa Kilodalton

LR+ Positive likelihood ratio

LR- Negative likelihood ratio

MAP Mean arterial pressure

MGOS Modified Glasgow Outcome Scale

MRI Magnetic resonance imaging

mRS Modified Rankin Scale

NfL Neurofilament light

NPV Negative predictive value

NRI Net reclassification improvement

NSE Neuron-specific enolase

OHCA Out-of-hospital cardiac arrest

OR Odds ratio

$PbtO_2$ Partial oxygen pressure of brain tissue

PEA Pulseless electronic activity

PPV	Positive predictive value
ROSC	Return of spontaneous circulation
SAH	Subarachnoid haemorrhage
SAPS II	Simplified Acute Physiology Score
SIMOA	Single Molecule Array
SM	Status myoclonus
SOFA	Sequential Organ Failure Assessment
SSEP	Somatosensory evoked potential
TBI	Traumatic brain injury
TH	Therapeutic hypothermia
TTM	Targeted temperature management
UCH-L1	Ubiquitin C-terminal hydrolase L1
VF	Ventricular fibrillation
VT	Ventricular tachycardia
WM	White matter
WLST	Withdrawal of life-sustaining therapies

1 INTRODUCTION

Sudden out-of-hospital cardiac arrest (OHCA) affects at least 500,000 people each year in Europe [1]. Mortality after OHCA remains high, over 90%, and it is the third most common cause of death in Europe [2]. In the early phase of treatment, resuscitated patients are typically unconscious, and prognostication is a challenge [3,4]. OHCA is already catastrophic for individuals with cardiac arrest (CA) and their loved ones, and an uncertain prognosis is an extra burden.

The main cause of unfavourable outcome after CA is the brain injury caused by circulatory arrest and tissue hypoxia—i.e., hypoxic-ischemic brain injury (HIBI), which causes two thirds of the resulting deaths [4,5]. For clinicians, it is important to carry out a multimodal prognostication process for patients who remain unconscious, since an accurate neurological prognosis is difficult to obtain in the first 1–3 days [6]. Making the correct prognosis is crucial for avoiding futile care of individuals with a severe disability and no chance for meaningful recovery—and conversely, to prevent the termination of life-sustaining care for patients who may be able to have a satisfactory neurological recovery [6].

Prehospital patient- and resuscitation-related factors that can affect a patient's outcome include an unwitnessed collapse, a lack of bystander cardiopulmonary resuscitation (CPR), a nonshockable initial rhythm, a severe comorbidity, a very advanced age, and a long time from collapse to the return of spontaneous circulation (ROSC) [7]. Individual factors that can weaken the prognosis and probability of survival are not specific for unfavourable outcome, and multimodal prognostication is necessary [6].

As HIBI is the key factor for neurological outcome and death after CA, its assessment is the main issue in predicting long-term outcome [8]. In addition to predicting unfavourable outcome, is necessary to avoid incorrect withdrawal of life-sustaining therapies (WLST) [9]. The ability to predict the probability of favourable outcome by excluding the presence of severe HIBI is a rising topic in prognostication [10].

The latest guidelines of the European Resuscitation Council (ERC) and the European Society of Intensive Care Medicine (ESICM) on prognostication after CA recommend multimodal prognostication [6]. It should include several highly specific tests and should be performed after a length of time sufficient to minimise a falsely pessimistic prognosis [6]. Multimodal prognostication includes a clinical neurological examination, brain imaging with computed tomography (CT) or magnetic resonance imaging (MRI), neurophysiological studies (e.g., somatosensory evoked potential [SSEP] and electroencephalography [EEG]), and the measurement of blood neurobiomarkers [6].

Neurobiomarkers have several benefits: their blood concentrations can be easily and inexpensively measured, and they are not confounded by sedative medications and muscle relaxants. The optimal neurobiomarker is brain-specific, is released within 1–3 days after CA due to brain injury, and has minimal confounding extracerebral sources. Neuron-specific enolase (NSE) is the most-studied and the only biomarker recommended in recent prognostication guidelines. However, NSE has some limitations, as haemolysis [11], small-cell lung carcinoma [12], and non-HIBI brain injuries [13,14] can misleadingly increase its blood concentration. To minimise false positive results (FPR), the recommended cutoff level of NSE for predicting unfavourable outcome is high, but this can worsen the sensitivity of the test [6]. Moreover, NSE is not a very sensitive biomarker for excluding severe HIBI [15], and its prognostic accuracy in the oldest patients and in those with a short time from collapse to ROSC is unclear. To improve the accurate prognostication of resuscitated patients, it is necessary to investigate novel, easily obtained biomarkers that are better than NSE at discriminating patients regarding HIBI.

2 REVIEW OF THE LITERATURE

2.1 OVERVIEW OF OUT-OF-HOSPITAL CARDIAC ARREST

2.1.1 Incidence of OHCA

OHCA is a major health issue worldwide; the exact incidence of OHCA is unknown because not all arrests are identified or registered with emergency medical services [16,17]. The global registered incidence from 1976–2019 was 55/100,000 people per year in a meta-analysis that included 4.6 million OHCA patients with the initiation of CPR [18]. In Europe, the estimated annual incidence of OHCA is 67–170/100,000 [19]. A large European register study (EuReCa TWO) included data from 28 countries covering 180 million inhabitants; it reported an annual incidence of 56/100,000 OHCAs with the initiation of CPR [1].

The majority of OHCA patients die before hospital admission; only 30% of resuscitated individuals worldwide achieve ROSC, and only 22% survive until admitted to hospital [18]. The number of patients resuscitated from both OHCA and in-hospital cardiac arrest (IHCA) and treated in ICUs has increased in recent years, as has the length of ICU treatment [2]. In Finland, the annual incidence of OHCA with attempted CPR, according to the FINNRESUSCI study, was 51/100,000 inhabitants in 2010–2011 [20].

2.1.2 Aetiologies of OHCA

The underlying disease or condition that causes CA has a strong impact on achieving ROSC and on survival. Cardiac causes, especially coronary artery disease and acute myocardial infarction, are the most common causes of OHCA, and they typically result in shockable initial rhythms of ventricular fibrillation (VF) and ventricular tachycardia (VT) [7,21,22]. These cause blood pressure and cardiac output to collapse, leading to CA. VF is the most common arrythmia causing cardiogenic CA [23]. VF and VT are shockable rhythms because they can be returned to pulsative rhythms with defibrillation in optimal circumstances.

Other rhythms that cause CA are nonshockable, including pulseless electronic activity (PEA) and asystole (ASY). Conditions that can cause nonshockable rhythms include asphyxia, hypovolemia, sepsis and other distributive shocks, major trauma, and drug overdose, which are also defined as non-cardiac aetiologies [24]. When the treatment of the underlying condition is delayed, cell death and irreversible tissue damage can occur in many cases, finally leading to CA.

2.1.3 Survival

Overall survival did not improve between the 1980s and 2009 [18,25], but short- and long-term survival trends improved after that from 2010–2019 [18]. Survival rates of patients resuscitated from OHCA until hospital discharge remain low (8–9%), and one-year survival is below 8% [18,19]. In the register of the International Liaison Committee on Resuscitation (ILCOR), favourable neurological recovery at hospital discharge or at 30 days after arrest varies between 2.8–18.2% [17]. In a recent Swedish register study including 55,000 OHCA patients, 4 out of 5 patients died before hospital admission, and a favourable one-year neurological outcome was seen in 7.4% of the resuscitated patients [7]. In Finland, according to the FINNRESUSCI study in 2010–2011, one-year survival was 13%, and 91% of the survivors were independent in their basic activity of daily living [26]. The proportion of survivors who were able to live independently was comparable in a Swedish register study from 1990–2020 [22]. Incidence and survival strongly vary among countries, partly explained by differences in emergency medical services, treatment in hospitals, and case mix [19]. It is recommended that studies involving CA report outcome and definitions according to the Utstein criteria [27] to enable more precise comparisons.

2.2 FACTORS ASSOCIATED WITH OUTCOME

2.2.1 Factors related to resuscitation.

Overall, patients with shockable initial rhythms have better survival rates than those with nonshockable initial rhythms [5,25]. In recent years, the incidence of nonshockable rhythms has increased, negating the improvement of survival rates [22]. In a large meta-analysis of OHCA patients over 3 decades, the factors that had the greatest positive impact on survival were EMS-witnessed collapse, bystander CPR, and a shockable initial rhythm [25].

The shockability of initial rhythm is a robust predictor of survival; the survival rate was 14.8–23% for individuals with a shockable rhythm but only 0.4–7.2% for those with nonshockable rhythms [25]. When the first detected rhythm is asystole, the survival rate decreases to 0.2–4.7% [25]. Survival is lowest in patients whose CA is caused by trauma (2.8%), drowning (5.4%), and asphyxia (5.6%); those typically lead to nonshockable rhythms [1]. However, some patients with nonshockable rhythms still survive. In a large analysis of 17,000 resuscitated patients in Japan, favourable overall neurological recovery was seen in 21.8%; for patients with shockable rhythms, the rate was 52.1%, and for those with ASY it was 4.5% [28].

The large variation in survival in different register-based studies probably reflects differences throughout the whole “chain of survival”, which means rapid recognition of CA, activation of emergency medical services, CPR, and defibrillation [29]. In the EuReCa study, there were significant variations among countries in the proportions of bystander CPR (13–82.6%), shockable initial rhythm (11.4–36.8%), and achievement of ROSC (6.9–43.3%) [1]. Other factors in addition to initial rhythm can decrease survival rates. Unwitnessed collapse and a lack of bystander CPR significantly decreases survival [25]. Unwitnessed collapse, nonshockable rhythms, duration of CPR and higher total dose of adrenaline (likely due to prolonged CPR and nonshockable rhythms) are also strongly associated

with brain oedema, which dramatically decreases the probability of survival [30].

Shockability of the rhythm had also strong impact on survival in the FINNRESUSCI study, where 31% of the patients had a shockable initial rhythm [20]. Of these, 65% achieved ROSC, which was almost twice as many as patients with a nonshockable initial rhythm. The one-year survival difference was even greater: 33% of the patients with a shockable rhythm survived, compared to only 5% with a nonshockable rhythm [20].

2.2.2 Underlying diseases and comorbidities

The worsening of an underlying disease can be a cause of CA. Moreover, in cases where the cause of CA is different from a significant underlying disease, the underlying disease can reduce the probability of survival. Cardiac diseases, including hypertension (the most common one), are the most frequent underlying diseases, followed by type 2 diabetes mellitus [7]. Cardiac causes of CA typically lead to shockable rhythms, and survival can be more likely if the cause of OHCA is cardiogenic [22]. However, the proportion of cardiogenic causes is decreasing in line with incidence of severe coronary artery disease, so the significance of other causes will increase in the future [22].

The accumulation of illnesses decreases the probability of survival [31]. Existing liver cirrhosis and the development of severe acute-on-chronic liver failure significantly reduces survival after CA [31]. In addition, malignancy and chronic obstructive pulmonary disease weakens the probability of survival after OHCA [31,32]. In a study by Terman et al. that included 588 OHCA patients, the presence of dementia, but not the overall comorbidity index, was independently associated with worse outcome [33]. A parallel result was found in a study by Beesems et al. looking at an older OHCA population, though with a nonsignificant trend toward worse outcome in individuals with more comorbidities [34]. It is plausible that severe underlying diseases and high comorbidity reduce survival after OHCA.

2.2.3 Age

Aging of the population is a worldwide trend, so more elderly patients will be resuscitated from OHCA in the future. With increasing age, frailty increases and comorbidities accrue [35], which can worsen survival after CA. In a large review of patients ≥70 years old who were resuscitated from OHCA, the overall survival rate was low (4.1%) [36]. In that review, the older age was associated with worse survival, but comorbidities have not been separated from age itself in many studies. In a large register study by Hessulf et al., the patient's age was the most significant predictor of survival among patient-related factors [7]. A later review focusing on resuscitated patients ≥70 years old reported a survival after OHCA to hospital discharge ranging from 0–11% that declined with age [37]. However, this review demonstrated an improvement in the survival of elderly resuscitated patients over the last decade, and among those who survived to hospital discharge, 77–92% had favourable neurological outcome.

Many single studies have proven that increasing age reduces survival after CA. In a recent multi-centre observational study, a younger age was associated with favourable neurological outcome among patients with prolonged (>30 min) resuscitation [38]. In a retrospective cohort of OHCA patients, each decade of a patient's age decreased the odds of favourable neurological outcome (the Cerebral Performance Category [CPC] 1–2 at 6–12 months after CA) by 21% [33]. In a study of OHCA patients ≥70 years old, the overall long-term outcome was favourable in 10.6% [34]. When those patients were separated into two categories, favourable outcome occurred in 14.2% of the patients aged 70–79 years, and favourable outcome was less common (6.6%) in patients ≥80 years. In another study of OHCA patients, increasing age was associated with higher mortality and unfavourable long-term outcome [39].

It is possible that a lower intensity of care has a worse impact on survival in the oldest resuscitated patients, perhaps revealing a self-fulfilling prophecy. Roselló et al. studied outcome and treatment of elderly OHCA patients and found that interventions were used less frequently in elderly individuals (≥80 years) compared to younger individuals (<80 years),

giving rise to a hypothesis that interventions were restricted due to old age [40]. Older age was an independent predictor of in-hospital mortality in that study but not a predictor of unfavourable neurological outcome. Though higher age worsens survival after CA, age itself should not be used as a justification to restrict therapies if a probability of sufficient recovery exists [37].

2.2.4 Time from collapse to ROSC

Resuscitation time is a significant factor influencing survival. During CA and CPR, the blood and oxygen supplies to the brain and other vital organs are critically impaired. Björklund et al. found a clear correlation between resuscitation time and the severity of HIBI in autopsies of resuscitated individuals who died thereafter [41]. Large analyses provide great impact on ROSC time to survival after CA. In a study of over 55,000 OHCA patients, the time from collapse to ROSC was the second most significant patient-related factor (after age) that had an impact on survival [7] and in a large analysis of 17,000 patients by Goto et al., a longer resuscitation time impaired survival [28].

The optimal time to stop unsuccessful resuscitation is unclear. In a study of 64,000 patients who were resuscitated from in-hospital CA in 435 hospitals in the USA, the median resuscitation time was 12 min for those who achieved ROSC, whereas the median resuscitation time of those who did not achieve ROSC was 20 min [42]. In those hospitals that continued resuscitation longer, compared to those with the shortest resuscitation attempts, the likelihood of survival to hospital discharge was higher and the neurological recovery among survivors was not statistically worse in those who had a longer time to ROSC. Less than 1% of the patients in a Japanese study had a good recovery when CPR lasted more than 35 min (for shockable rhythms and PEA) or 42 min (ASY) (28). A resuscitation time of 20 min or more indicates a higher probability of unfavourable outcome in several studies [31,40,43].

A longer resuscitation time is strongly associated with unfavourable recovery, but some individuals can still achieve a meaningful functional

recovery after a remarkably long resuscitation time. In Japan, EMS personnel are not allowed to terminate CPR, which is typically continued until the achievement of ROSC or until the decision of termination of CPR upon hospital arrival. In a nationwide observational study of OHCA patients in Japan, the number of patients with favourable outcome decreased with a longer CPR duration, especially when CPR lasted for longer than 20 minutes [38]. A small number of patients, after a prolonged resuscitation (over 30 min), can have favourable outcome; it is more likely in those with a shockable rhythm, a witnessed collapse, and a younger age [38]. The target of resuscitation is not only the achievement of ROSC but also a satisfactory neurological and functional recovery.

2.3 TREATMENT IN INTENSIVE CARE UNITS AFTER CARDIAC ARREST

2.3.1 General principles

The ERC–ESICM issued guidelines for post-resuscitation care in 2015 [44], and the guidelines were updated in 2021 [6]. After CA, several targets must be addressed in intensive care treatment to optimise the probability of survival. At the very beginning of care, it is important to prevent recurrent CA. Patients are typically unconscious, so they need to be intubated and mechanically ventilated to secure the airway and maintain sufficient oxygenation and normocapnia [6]. After CA, many patients have low blood pressure that requires vasopressors (mainly noradrenaline) to maintain sufficient blood perfusion to the vital organs [6]. The possible disease or condition related to CA should be treated, if possible. Overall, the most important aims in post-resuscitation care are: 1) ensuring sufficient oxygenation and circulation to provide oxygen to the vital organs; 2) definitive treatment of the underlying cause of CA (cardiac catheterisation in ST-elevation myocardial infarction as a top priority); and 3) multimodal neurological prognostication. Otherwise, the general principles for treating critically ill patients are to maintain homeostasis, preserve organ function, and prevent additional injuries. In CA patients, these practices include, for

example, maintaining normoglyceamia, normal gas exchange (normoxia and normocapnia), and normal blood pressure (mean arterial pressure >65 mmHg, systolic arterial pressure <140–180 mmHg); preventing fever; and treating electrolyte imbalances [6].

2.3.2 The role of temperature management

The first reports of hypothermia as a preventive treatment of brain damage after CA were published in 1950–60 [45]. The theory was that hypothermia could protect the brain from secondary damage in multiple ways, such as reducing brain metabolism and enabling neuronal recovery [46]. Later, hypothermia-based treatment (defined as 30–33°C) developed into mild therapeutic hypothermia, which seemed to increase the proportion of patients with favourable neurological outcome after CA [47,48]. Mild therapeutic hypothermia (32-36°C) held a firm place in post-resuscitation care for years [44,49]. However, two targeted temperature management (TTM) studies [50,51] demonstrated that hypothermic treatment does not improve neurological recovery compared to normothermia (defined as ≤37.8°C). The ERC-ESICM guidelines were modified accordingly, now recommending to avoid fever (>37.7°C) during the first 72 h after CA [52].

2.3.3 The roles of carbon dioxide, oxygen, and blood pressure

Carbon dioxide (CO_2) has a substantial effect on the regulation of cerebral blood flow**:** low CO_2 tension in arterial blood (hypocapnia) causes vasoconstriction that decreases blood flow, and high tension (hypercapnia) causes vasodilation that increases blood flow [53]. In a study by Eastwood et al., the effect of mild hypercapnia (6.7-7.3 kPa) among resuscitated patients showed the attenuation of NSE release to be a possible indirect sign of milder hypoxic brain injury in those patients, compared to those with normocapnia [54]. Regarding oxygen, severe hyperoxia (defined as ≥40 kPa) was associated to increased in-hospital mortality compared to hypoxia (<8 kPa) and normoxia (8.1-39.9 kPa) after CA in a large database analysis [55]. Low blood pressure is associated with unfavourable outcome after CA [56],

and elevated blood pressure was more common among survivors than non-survivors in a one observational study [31]. The effect of higher blood pressure should be considered with caution, as most of the studies are observational, and the definitions of blood pressure vary between studies [56]. Two prospective studies of Ameloot et al. and Kjaergaard et al. have assessed the impact of different blood pressure targets to outcomes after CA, however they found no differences in outcomes[57].

To measure the effectiveness of the aforementioned factors, Jakkula et al. conducted a randomised, controlled Carbon dioxide, Oxygen, and Mean arterial pressure After Cardiac Arrest and Resuscitation (COMACARE) pilot trial. It utilised a 2^3 factorial design to assess the effectiveness of two different CO_2 and oxygen (O_2) tension targets in arterial blood and two different mean arterial blood pressure targets on outcome in 120 OHCA patients [58,59]. For CO_2, the groups were low-normal ($PaCO_2$ 4.5–4.7 kPa) and high-normal ($PaCO_2$ 5.8–6.0 kPa), and there were no differences in primary or secondary outcomes between the groups. Mild hyperoxia (arterial oxygen tension [PaO_2] 20–25 kPa), as compared to normoxia (PaO_2 10–15 kPa), did not affect NSE concentrations or neurological outcome. Neither low-normal mean arterial pressure (65–75 mmHg) nor high-normal (80–100 mmHg) demonstrated any difference in outcome.

2.4 REASONS FOR UNFAVOURABLE OUTCOME AFTER CARDIAC ARREST

2.4.1 Hypoxic-ischemic brain injury (HIBI)

The human brain weighs only 2% of total body weight but has high oxygen and energy demands; at rest, it consumes 20% of all oxygen and glucose delivery [60,61]. High oxygen and energy demand make the brain vulnerable, and collapse of blood pressure and cardiac output during CA causes HIBI. HIBI causes most deaths in OHCA patients, showing its importance in post-resuscitation care [5,62].

Brain injury caused by CA can be described by a two-hit model that includes primary and secondary injuries [4,63]. The primary injury occurs

during CA because oxygen and energy delivery to the brain are interrupted simultaneously with the cessation of cerebral blood flow (CBF), leading to neuronal ischemia in minutes [4]. The immediate initiation of CPR can restore some oxygen delivery to the brain [64], partly attenuating the developing primary brain injury. Yet, CPR can sustain only half the minimal (approximately 40–50% of normal) CBF that is required to avoid ischemic injury [65]. Neurons in the brain have an inherent lack of energy stores, and they are not capable of withstanding a stoppage of energy delivery [66]. The result is the cessation of aerobic metabolism: adenosine triphosphate (ATP) production runs out and halts ion channel function [67]. Hence, huge amounts of Na^{+} ions shift into brain cells along with water, causing cytotoxic intracellular oedema [4,63]. The cell membranes shortly depolarise, causing an influx of Ca^{++} ions that activates enzymatic lysis [68], and finally, apoptosis begins with the activation of proteases and lipases [69]. The mechanism of HIBI is presented in Figure 1.

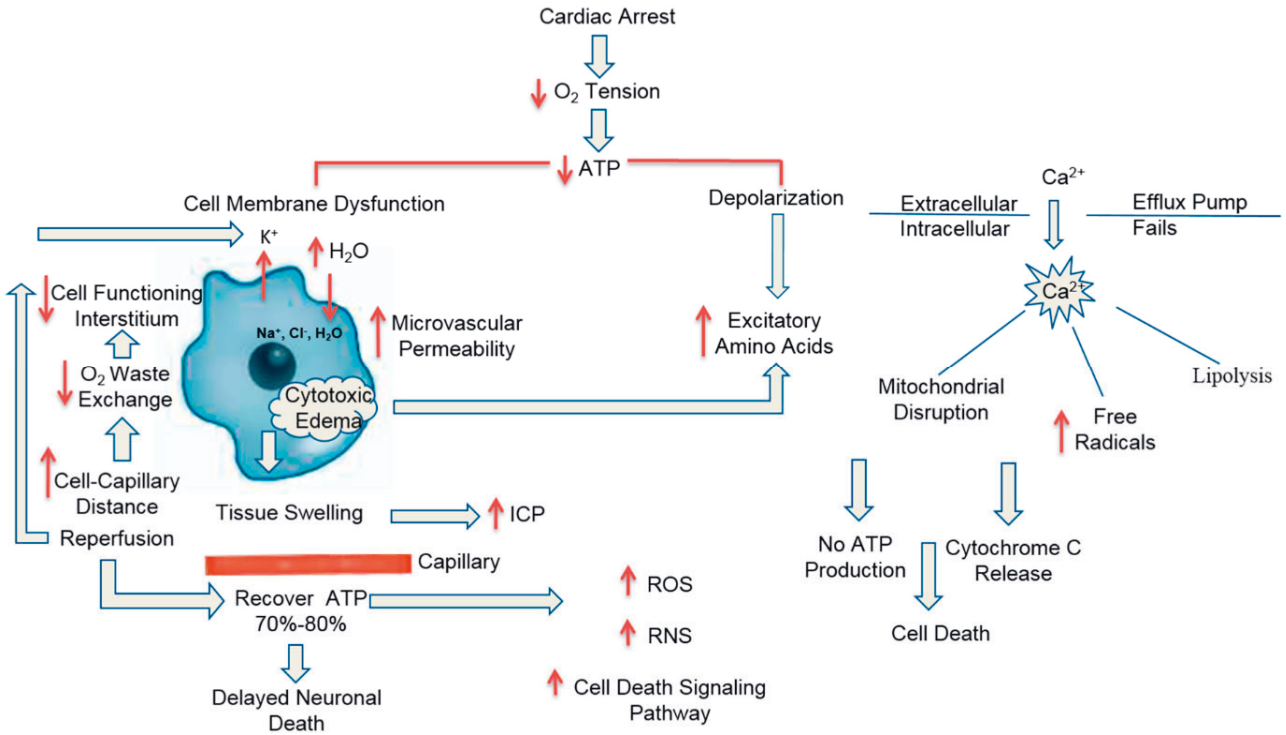

Figure 1. The cascade of effects after cardiac arrest that leads to hypoxic-ischaemic brain injury (HIBI). Abbreviations: ATP, adenosine triphosphate; ICP, intracranial pressure; ROS, reactive oxygen species; RNS, reactive nitrogen species. Reis C et al. Pathophysiology and the Monitoring Methods for Cardiac Arrest Associated Brain Injury. Int J Mol Sci. 2017 Jan 11;18(1):129. Adapted with the permission of MDPI.

The achievement of ROSC restores circulation and CBF, stopping the further generation of primary brain injury. However, the secondary reperfusion brain injury begins soon after ROSC [63]. In patients with HIBI, CBF becomes MAP-dependent because of impaired vascular autoregulation. Too low cerebral perfusion pressure (CPP) can lead to ischaemia and too high CPP can lead to oedema [70]. The mechanisms that cause secondary injury after reperfusion are microvascular dysfunction, impaired vascular autoregulation, and cerebral oedema [4,63]. One possible mechanism beyond these is neuroinflammation, which is caused by cytokine release, the activation of neuroglia, and the migration of leucocytes to the neuronal tissue [4,71]. Endothelial dysfunction can also lead to the formation of microthrombi, disturbances in vascular autoregulation, and dysfunction of the blood-brain barrier (BBB) [63]. Reperfusion causes free radical release and the accumulation of intracellular calcium ions [72], further activating proteases and phospholipases and causing mitochondrial dysfunction [4].

Cerebral oedema can be a consequence of cytotoxic and vasogenic mechanisms [4]. Cytotoxic oedema occurs within hours, whereas vasogenic oedema appears in 1–2 days and is more uncommon. Cytotoxic oedema is a result of a metabolic crisis in neurons that is caused by a shortage of adenosine triphosphate (ATP) and an influx of sodium and water. In vasogenic oedema, fluids shift from blood vessels into the interstitium. Aquaporin 4-protein has an important role in this phenomenon [73]. Leucocyte migration can cause endothelial disturbance, BBB breakdown, and further vasogenic oedema [74].

Brain oedema as a part of HIBI after CA is not uncommon. When primary or secondary brain injury causes oedema, that can increase intracranial pressure (ICP), decrease CPP, and finally, cause brain death [75]. In a cohort of 1,340 resuscitated patients, the incidence of brain oedema on CT scans within 4 h after CA was 22% [30]. Massive oedema worsened survival significantly in that study; only 2% of those with massive oedema survived, whereas 36% of those without brain oedema survived. A nonshockable initial rhythm, a long low-flow time, a low sodium concentration, and a neurological cause of CA are associated with an

increased number of brain deaths after CA [76,77]. Kang et al. found significantly higher ICP values in resuscitated patients with unfavourable outcome (16 mmHg) than in those with favourable outcome (11 mmHg) and found also more severe BBB disruption in those with unfavourable outcome [78]. In addition, cerebral autoregulation after CA is right-shifted (requiring a higher blood pressure to maintain CBF) or narrowed in approximately 30–50% of patients [79,80].

HIBI can occur in many structures and cells in hypoxia-sensitive brain areas. The neurovascular unit contains neurons, axons, synapses, astrocytes, and glial cells that are surrounded by blood vessels, and the BBB is between neuronal and vascular structures [81]. The neurovascular unit is presented in Figure 2, page 70.

HIBI causes widespread neuroglial injury in neurovascular unit structures that can be measured as the release of specific biomarkers after BBB damage; this was revealed in a small study by Hoiland et al. [82]. Secondary brain injury was associated with inflammation in that study and was associated with permanently lower partial oxygen pressures of brain tissue ($PbtO_2$) [82]. In another exploratory study that found low brain $PbtO_2$ caused by HIBI, the high oxygen gradient between brain tissue and venous blood (determined with a jugular bulb catheter) was a continuous phenomenon and not correlated with ICP, indicating a permanent oxygen diffusion deficit, and suggesting irreversible brain injury in those patients [83].

In CA patients that remain unconscious after 7 days, the white matter (i.e., axon) lesions on MRI scans have demonstrated excellent discriminative ability, suggesting a crucial role of axonal injury in prognosis [84]. The same study also suggested that white matter injury may be involved in secondary HIBI. In an autopsy study of resuscitated individuals, axonal degeneration was the major underlying process in acute ischemic leucoencephalopathy [85]. That study also found microglial activation and astrocytosis in the deceased.

The brain structures related to secondary injury are the cerebral cortex, hippocampus, thalamus, corpus striatum, and cerebellar vermis—i.e., structures that are highly metabolically active [63]. The hippocampus is the

brain structure most sensitive to the damage caused by hypoxia [41]. In autopsy studies of HIBI after CA, the hippocampus and cerebellum have been found to be the most vulnerable brain structures, followed by the cerebral cortex, whereas the brainstem is the structure most tolerant to ischemia [41,86]. After ischemia, injuries can occur in both grey and white brain matter. However, white matter is more resistant to irreversible injuries; this has been studied with positron emission tomography [87].

The clinical manifestations of severe HIBI include coma, seizures, myoclonus, and—if massive oedema occurs—brain death [88]. In optimal circumstances, with effective CPR and a fast enough ROSC, HIBI will not occur. The severity of HIBI is a continuum rather than a dichotomous phenomenon, and less severe but still notable HIBI can result in problems with memory, executive function, and subtle motor function [89].

2.4.2 Post-resuscitation syndrome

Post-resuscitation syndrome includes myocardial dysfunction and a global ischemia–reperfusion injury in addition to HIBI [88]. The causes of death can be divided into 1) post-CA brain injury and 2) post-CA injury to other parts of the body [5]. The pathophysiology of post-resuscitation syndrome can be understood as a whole-body ischemia–reperfusion injury [88], and in many individuals, pathologies in the brain and other organs happen simultaneously. Likewise, in secondary HIBI, the other organs can suffer additional ongoing injury after ROSC [90]. The timeline of possible ongoing extracerebral injuries starts immediately after ROSC. In the first two days, post-CA shock is the most prominent cause of death in resuscitated patients [5]. HIBI, which is typically found in a prognostication process after 72 h, is the main cause of all deaths after CA [4,8].

The most prominent clinical features of extracerebral post-resuscitation syndrome are haemodynamic instability, myocardial stunning, microvascular damage, and multiorgan failure [88,90,91]. Overlap with features of post-CA injury and underlying conditions related to CA, as well as baseline comorbidities, is common [88]. Persistent precipitating factors, such as acute myocardial infarction, pulmonary embolism, haemorrhage, sepsis,

and drug overdose confound the assessment of post-CA syndrome [88] and can complicate its treatment. However, many of those conditions are treatable.

Cardiovascular instability [92] and low cardiac output are associated with multiorgan failure and are the most common causes of early death after CA [91]. Among survivors, haemodynamic instability can be reversed and typically improves during the first 72 h after CA. The neurological outcome seems not to be affected by haemodynamic instability, possibly reflecting the different mechanisms of an ischemia–reperfusion injury in the brain as compared to other organs [91]. Myocardial stunning is a typical finding and is different from irreversible lethal shock in that it appears as a reversible haemodynamic instability (low cardiac output) in ca. 7 h after CA and is typically attenuated within 24 h [91].

The ischemia–reperfusion injury mimics septic shock, comprising impaired vasoregulation [88], activation of the inflammatory response [93], and coagulopathy [94]. Higher concentrations of inflammatory markers are associated with increased mortality after CA, and inflammation is associated to shock, long CPR time and high lactate levels, reflecting more severe primary ischemic injury beyond severe post-CA inflammation [95,96]. Ischemia–reperfusion and post-CA shock can cause multiorgan failure just like sepsis, including ischemic hepatitis, acute kidney injury, and intestinal injury, which are associated with unfavourable outcome [97,98].

2.4.3 Withdrawal of life-sustaining therapies (WLST)

In countries where WLST decisions are less common (e.g., Japan and South Korea), survival with a poor neurological recovery is more common, and as many as one third of survivors are in a vegetative state [16], which is not a desirable result of resuscitation. Poor neurological recovery is also possible in countries where WLST decisions because of an unfavourable predicted outcome are made: in a Swedish register, 7.5% of OHCA survivors had poor neurological function [7]. As expected, WLST increases mortality in those who are comatose, apnoeic, and dependent on ventilatory support. WLST due to an unfavourable expected neurological outcome is a common cause

of death after CA [4,5,9]. In a study on 16,875 OHCA patients, Elmer et al. found that early WLST (≤72 h after CA) occurred in one third of the patients who died in the hospital. Based on adjusted analyses, the authors estimated that 16% of these patients might have had a favourable neurological outcome if early WLST had not occurred [9]. This estimated excess mortality can possibly be reduced by carrying out multimodal prognostication according to post-CA treatment guidelines.

In comatose OHCA individuals, making a correct prognosis of neurological recovery is challenging in the early phase, and recent guidelines instruct the implementation of prognostication no earlier than 72 h after CA [6]. In a post-hoc analysis, 33% of the OHCA patients were comatose 72 h after CA and underwent multimodal prognostication; a WLST decision was made for 45% of them because of an unfavourable predicted prognosis [99]. A few patients who are comatose at 72 h after CA can still reach favourable neurological outcome [100–102]. However, when severe HIBI is defined according to guidelines, or the patient has a severe comorbidity or irreversible multiorgan failure, WLST can be justified.

To avoid futile care, reasonable arguments for early WLST include dementia, significant frailty, and severe comorbidity (e.g., terminal malignancy). In a study by Dumas et al., the proportion of patients who died after WLST was higher when comorbidity was more severe [43]. In a cohort including OHCA and IHCA individuals who died in hospital, WLST for presumed unfavourable neurological prognosis was more common among OHCA individuals (73% vs 27%), whereas WLST due to comorbidities was more common in the IHCA population (36% vs 4%) [103]. Mortality due to refractory haemodynamic shock and sudden cardiac death was also higher in IHCA patients than OHCA patients. This characterisation underlines the differences in the OHCA and IHCA populations, including shorter ROSC time in IHCA individuals, which is reflected as a lower percentage of neurological deaths. Parallel findings have been described earlier [104].

In regions where WLST decisions are not typically used, the continuation of therapies when unfavourable outcome is likely can result in an increased number of neurological deficits in survivors. For example, in a Korean study, the percentage of brain deaths in resuscitated patients was

29% [105]. In case of brain death after CA, organ donation can be possible, and it is important to remember this possibility [6].

2.5 OUTCOME PREDICTION AFTER CARDIAC ARREST

2.5.1 Outcome definitions

The treatment target of patients resuscitated from CA, starting with CPR and ending with hospital discharge, is to maintain the functional ability that those individuals had before CA. Many patients have severe HIBI and/or post-CA syndrome that can cause death, obviously an unfavourable outcome. The opposite category of patients is those who survived without HIBI and can recover back to normal life. Between these two outcomes, there is a grey zone with varying levels of neurological deficits, weakness, memory loss, cognitive impairment, and need of assistance in daily living.

In resuscitated patients, several outcome definitions are used to define the neurological function. The Glasgow Outcome Scale (GOS) was published in 1975 to classify the neurological function of patients with traumatic brain injury (TBI), but the GOS has also been used to classify patients with other types of brain injuries [106]. The Pittsburgh Cerebral Performance Category (CPC) is the most used classification in resuscitated patients [107]. The Modified Glasgow Outcome Scale (MGOS) has been modified from the GOS to improve the classification of resuscitated patients [108]. The MGOS differs from other classifications by separately considering deaths that are caused by HIBI (MGOS 1) and deaths where brain status is unknown (MGOS 0). In the most recent guidelines, the modified Rankin Scale (mRS) [109] is recommended for use in classification after CA [110]. The benefit of the mRS is that it includes three categories for good outcome, indicating at least independent living and only slight disability (mRS 0–2,). In other classifications, typically only two categories are offered for a favourable outcome definition—e.g., 1–2 in the CPC classification. However, there is variation between studies in the use of classifications and in the time of outcome assessments, making

comparisons somewhat difficult. Outcome definitions are compared in Table 1. according to original studies [106-109].

Table 1. Outcome definitions according to original studies [106-109].

Value	CPC	mRS	GOS	MGOS
0		No symptoms		Patient died with unknown cerebral status
1	Good cerebral performance. Conscious. Able to work and to live a normal life. May have minor neurological or psychological deficits	No significant disability, despite symptoms. Able to perform all usual duties and activities	Death	Patient died with documented hypoxic brain damage
2	Moderate cerebral disability. Conscious. Sufficient for part-time work in a sheltered environment or independent activity of daily life. May have hemiplegia, seizures, ataxia, dysartria, dysphasia of permanent memory or mental changes	Slight disability. Able to look after own affairs without assistance, but unable to perform all previous activities	Persistent vegetative state. Unable to interact with environment	Persistent vegetative state. Unable to interact with environment
3	Severe cerebral disability: conscious. Dependent for daily support on others because of impaired brain function. Has limited cognition	Moderate disability. Able to walk without assistance, but requires some help	Severe disability. Unable to live independently, but able to follow commands	Severe disability. Unable to live independently, but able to follow commands

4	Coma or vegetative state: unconscious. Unaware of surroundings, no cognition. No verbal or psychological interactions with environment	Moderately severe disability. Unable to walk without assistance and unable to attend to own bodily needs without assistance	Moderate disability. Able to live independently, but unable to return to work	Moderate disability. Able to live independently, but unable to return to work
5	Brain death or death	Severe disability. Bedridden, incontinent, and requires constant nursing care and attention	Mild or no disability. Able to return to work	Mild or no disability. Able to return to work
6		Death		

Abbreviations: CPC, Cerebral Performance Category; mRS, modified Rankin Scale; GOS, the Glasgow Outcome Scale; MGOS, modified Glasgow Outcome Scale

Neurological function can recover after hospital discharge in many patients, and it is reasonable to assess a long-term rather than a short-term outcome [111]. On the other hand, a study by Taccone et al. showed that 4.2% of resuscitated individuals recovered consciousness but died thereafter for noncerebral reasons and were categorised into unfavourable outcome [112]. For patients who regain consciousness but die because of other reasons than HIBI, reporting the best cerebral status achieved in addition to the final long-term outcome is informative in studies focusing on neurological prognostication.

2.5.2 Accuracy of tests used in predicting unfavourable outcome

When predicting unfavourable outcome with prognostic tests, there is a risk of false positive results. The key point regarding diagnostic tests is to define their prognostic accuracy, typically done by calculating the areas under the receiver operating characteristic curves (AUROC). For a given test, the ROC curve describes the sensitivities corresponding to different specificities. Generally, an AUROC value of 0.5 says that a test has no discriminative ability at all; for a 100% specific and sensitive test, the AUROC value is 1. No prognostic test can have a specificity of 100% in real life and still have a good sensitivity, but specificities of 100% can be assessed in clinical studies [6].

When using a test to detect a likely unfavourable outcome in a resuscitated patient, a positive test result (i.e., an unfavourable predicted outcome) can lead to termination of life-sustaining care, and death will probably follow. Therefore, a prognostic test should have a high specificity. Specificity means the likelihood of a negative test result among those who are truly negative. In other words, specificity = (true negatives) / (true negatives + false positives) [113]. When a test has a specificity of 95%, the false positive rate (FPR) is 0.05, meaning that, on average, 5 of 100 individuals who do not have the tested condition will get a false positive test result. Many studies present different thresholds for continuous tests so that the most useful threshold for clinical decision-making can be chosen.

Demanding a very high specificity can result in low sensitivity when the discriminative ability of the test is not very good [114]. Poor sensitivity means a lot of false negative results, and many individuals who truly have a poor prognosis are not identified [113]. Tests that give a dichotomous answer (having or not having the condition) with a high specificity (for example, SSEP) typically have limited sensitivity [62]. A continuous test or a test that can be divided into several categories offers more possibilities for optimal threshold selection. Moreover, such tests can offer the possibility of making a bidirectional prognostication, using higher cutoff levels for defining a positive test result and lower cutoff levels for defining a negative test result.

A highly sensitive test can reliably find most individuals with the condition of interest, but there can be false positive test results if the specificity is low. Sensitivity means the likelihood of a positive test result among those who are truly positive. In other words, sensitivity = (true positives) / (true positives + false negatives) [113]. If a test has a sensitivity of 100%, a negative result means that for the tested individual, the condition of interest has been ruled out with certainty. However, a very high sensitivity typically results in low specificity with a considerable number of false positive results [113]. Prognostication strategies that utilise tests with high levels of sensitivity have still not been adopted into the post-resuscitation guidelines because so far few studies support this.

Many physicians think that a prognostic test used for resuscitated patients should have a very high specificity. According to a survey, the majority of respondents thought that when making decisions about WLST after CA, an acceptable FPR is ≤ 0.1% [115]. This would mean a specificity exceeding 99.9%, and these kinds of thresholds are not useful for single diagnostic tests, because the corresponding sensitivity would be so low that the test would be useless in clinical practice. High specificity is required for predicting unfavourable outcome, but finding tests with high discriminative ability is more important than only finding thresholds that give 100% specificity. This is because threshold values can be adjusted to achieve suitably high levels of both specificity and sensitivity.

Additionally, the prior probability (pre-test probability) of an outcome of interest has a key role in the use of diagnostic tests. When the pre-test probability of a condition is high, a positive result of a highly specific test reasonably indicates the outcome of interest. However, pre-test probabilities vary widely according to different resuscitation features [7], and according to current guidelines prognostication tests are conducted without utilising pre-test probability.

2.5.3 Predicting favourable outcome

Almost all studies of prognostication methods after CA focus on finding HIBI and assessing unfavourable outcome, which is necessary to avoid futile care among those with severe HIBI and no reasonable chance of sufficient recovery [6]. However, multimodal prognostication gives an indeterminate outcome prediction for many comatose individuals, and there is also a risk of false positive results [10]. When predictive methods that are highly sensitive for finding HIBI are used, a negative test result with a high negative predictive value (NPV) can exclude severe brain injury, meaning that favourable outcome is probable. That would likely prevent an incorrect WLST in patients who remain comatose for a long time, among whom there may be individuals capable of reaching sufficient neurological function [102,116,117].

Moseby-Knappe et al. studied the utility of biomarkers in the prognostication of unfavourable outcome according to ERC-ESICM guidelines, resulting in over 50% of patients with indeterminate outcome (e.g., patients who were comatose at day three and did not fulfil the criteria of unfavourable outcome) [15]. Normal biomarker concentrations, especially for GFAP, Tau, and NfL, demonstrated very high sensitivities and NPVs with low levels of false negatives for all patients and for those with an indeterminate prognosis. When the upper limits of normal concentrations were used as cutoff levels, the sensitivities were 96–97%, with the highest specificities for NfL (39%) and GFAP (26%). The study demonstrates that favourable outcome can be reliably predicted with low/normal concentrations of sensitive neurobiomarkers, suggesting a wait-and-see

strategy instead of WLST if unfavourable outcome is not defined according to ERC-ESICM criteria.

Two different cutoff values for two-sided prognostication can be set for biomarkers because they are continuous variables. For clinical examination, electrophysiological studies, and imaging, this is not as straightforward because their cutoff settings are coarser. In a recent review focusing on the prognostication of favourable outcome after CA, Sandroni et al. assessed the abilities of various tests to predict favourable outcome [10]. Continuous and normal voltage EEG can exclude severe HIBI, and in one study, the specificity for predicting favourable outcome was 91% [118]. In another recent EEG study, a modified classification of "benign EEG" characteristics resulted in high (97%) sensitivity in the prediction of unfavourable outcome [119]. SSEP and other evoked potentials are not as sensitive as EEG in assessing probability to favourable outcome [10]. Regarding imaging, in a study that used a semiquantitative assessment of ischemic areas in brain CTs, favourable outcome was predicted with 89% specificity and sufficient sensitivity [120]. In another study utilising diffusion-weighted imaging (DWI) in MRI, the specificities for favourable outcome prediction were 92–95% for single or no lesions, with rather good sensitivities [121].

Although novel biomarkers, MRI, and EEG offer suitable accuracy for predicting favourable outcome, unfavourable outcome is still more likely in those patients who do not wake up during the first days, so prognostication and decision making in CA patients is challenging. In an observational study of 228 patients who woke up after CA, late awakening occurred in 34% of the patients, and the median time to awakening was 5 days after sedation withdrawal, ranging from 3–23 days [122]. The delirium rate was nearly two-fold greater, and an unfavourable 3-month outcome was over two-fold greater when awakening was prolonged.

2.6 PROGNOSTICATION STRATEGY AND METHODS

2.6.1 Multimodal prognostication

Prognostication is an essential part in the recently published ERC-ESICM guidelines on post-resuscitation care, which contain the latest evidence-based knowledge [6]. HIBI remains the most common cause of unfavourable outcome in OHCA patients. This is the rationale for prognostication that focuses on brain examinations to detect or rule out severe HIBI. Overall mortality after OHCA remains high, but pre-hospital factors (e.g., comorbidity, age) and features of CA are not yet able to give a sufficient estimation of outcome. Thus, there is a need for thorough prognostication algorithms. In the early phase of treatment, individuals are typically unconscious, and a lack of diagnostic evidence makes the situation uncertain for the treating physicians and the patient's family [88]. No currently used diagnostic test can give 100% specificity in real-life patient care. The rationale for using more than one prognostic test, even when the tests are robust, is that using several tests decreases the risk of an incorrect pessimistic prognosis.

The most significant changes from the 2015 guidelines [44] to the 2021 prognostication guidelines are: 1) to use the motoric response to pain stimuli based on Glascow Coma Scale (GCS-M) ≤ 3 (i.e., flexion, extension, or no response) to identify patients needing neurological prognostication, compared to the previous cutoff of GCS-M ≤2 (extension or no response); 2) to interpret EEG waveforms according to the American Clinical Neurophysiology Society's (ACNS) guidelines [123] and interpret suppression, burst suppression, and nonreactivity as malign signs; and 3) to use an NSE cutoff value of >60 µg/L at 48 and/or 72 h after CA as a sign of poor prognosis [6]. Additional topics include paying attention to the follow-up time, being aware of the risk of a self-fulfilling prophecy, and remembering the possibility of organ donation in case brain death occurs.

The guidelines include five different tests and offer cutoffs for unfavourable outcome for continuous or nominal variables; they recommend using at least two examinations with high specificity to assess

unfavourable outcome. Multimodal prognostication strategies have been demonstrated to predict unfavourable outcomes with 0% FPR, but in some studies, at least several individuals with unfavourable outcome went undetected because of limited sensitivity in the tests used [15].

Different prognostication methods are compared in Table 2.

Table 2. Prognostication methods.

	Advantages	Weaknesses	Specificity	Sensitivity
Motor response	Feasibility GCS-M≤3 ≥72 h after CA has high specificity	Residual sedation and relaxation confounds	62-96%	55-94%
Pupillary reflex	Feasibility High specificity ≥24 h after CA	Differences in examination standards Residual sedation confounds Limited sensitivity in several studies	90-100% (standard) 95-100 (automated)	17-63% (standard) 5-59% (automated)
Corneal reflex	Feasibility High specificity ≥24 h after CA Overall moderate sensitivity	Differences in examination standards Residual sedation can confound Limited sensitivity in several studies	89-100%	23-67%
Myoclonus	Very high specificity for status myoclonus (SM)	Difficulties in interpretation EEG needed to avoid falsely pessimistic prognosis	99-100% (SM) 78-100% (myoclonus)	6-49% (SM) 18-44 (myoclonus)
MRI	Good discriminative ability. Informative in mid-brain and brain stem pathologies. Promising accuracy in assessing white matter injury regarding HIBI	Variations in measurement techniques Not feasible in the early phase	66-100%	13-90%
CT	Informative on underlying conditions Can assess cerebral oedema Fast, feasible	Limited sensitivity Variations in measurement techniques	85-100%	3-83%

EEG	Good discriminative ability at 12-24 h Extensively studied method Useful in assessment of probability to favourable outcome,assessment of myoclonies	Differences in interpretation Sedation confounds	98-100% (BS) 62-100% (Discont) 91-100% (Highly malignant) 83-100% (SE)	14-41 (BS) 4-33% (Discont) 9-97% (Highly malignant) 2-36% (SE)
N20 SSEP	Very high specificity in most studies	Electronical devices can confound. Limited sensitivity	50-100%	18-69%
NSE	Feasibility, inexpensive. High specificity for cut-off >60 µg/L Sedation and muscle relaxants have no effect	Extracerebral sources can confound Variations between laboratory methods Haemolysis affects results	88-95% (>33 µg/L) 95-96% (>40-50 µg/L) 100% (>50-120 µg/L)	30-69% (>33 µg/L) 60-74% (>40-50 µg/L) 27-52% (>50-120 µg/L)

Abbreviations: MRI, magnetic resonance imaging; CT, computed tomography; EEG, electroencephalography; N20 SSEP, N20 sometosensory evoked potential; NSE, neuron-specific enolase; GCS-M, motoric Glasgow coma score; CA, cardiac arrest; SM, status myoclonus; BS, burst suppression; Discont, discontinuous EEG, SE, status epilepti

2.6.2 Clinical examination

Evaluating the prognosis of a resuscitated patient always starts with clinical examination. It includes the motor response for pain stimulus, brainstem reflexes (pupillary light reaction and corneal reflex), and the evaluation of possible myoclonus [6]. When a meaningful motor response is detected or the patient wakes up, HIBI is unlikely, and a prognostication of unfavourable neurological outcome is not necessary.

In the very early phase of treatment, resuscitated patients are typically unconscious and under sedation [3]. Therefore, a clinical examination, especially for poor motor response, cannot be used in the early assessment of HIBI. Rather high FPR for early clinical examination to predict unfavourable outcome has been found: 38% for GCS-M ≤ 2 and 32% for the absence of bilateral corneal reflexes at one day after CA [124]. This suggests against using early clinical examination in prognostication.

Sedative medications are often necessary to maintain ventilatory support and to enable and facilitate a patient's treatment in the ICU. The use of sedation confounds the prognostic ability of clinical examination, and patients treated with therapeutic hypothermia are more likely to receive sedatives [125]. Currently, the use of therapeutic hypothermia is not recommended [52], so the use of sedative medication has decreased but not stopped. Benzodiazepines can accumulate in the body and can prolong the time to awakening. Overall, the median awakening time after CA in patients with favourable outcome is 2–4 days [126,127]. In a study by Levito et al., the median awakening time was longer, 5 days, for patients who received high-dose benzodiazepines, compared to 3 days for those who did not [126].

Muscle relaxants impair the function of skeletal muscles, and residual relaxation must be excluded before a correct response to pain stimulus can be assumed. In addition, severe liver failure or renal failure, either as underlying diseases or due to an ischemia–reperfusion injury, can confound the clinical examination, as hepatic encephalopathy and uraemia alter brain function [128]. Severe metabolic disorders should be treated before accurate clinical examination results can be obtained. Also,

definitions of “awakening” vary, confounding comparisons among studies [127].

The earliest study of clinical examination after CA was conducted in 1985 by Levy et al.; it found the absence of pupillary light reflexes and a motor response of GCS-M ≤ 3 after CA in almost all patients with unfavourable outcome [129]. Some individuals with GCS-M ≤ 3 and ≤2 at 72 h can still reach favourable outcome [130]. In a post-hoc analysis of a TTM population, GCS-M ≤ 3 at ≥72 h after CA demonstrated 93% specificity, but a Norwegian study focusing on WLST and outcome showed a high FPR of 27% [102,131].

The absence of pupillary or corneal reflexes in the very early phase do not predict unfavourable outcome with reasonable specificity, probably because they are confounded by deeper sedation, sympathomimetic drugs, and TTM during this period [62]. In the current guidelines, a brainstem examination should be performed ≥72 h after CA [6]. At 48–72 h after CA, absent pupillary or corneal reflexes can indicate HIBI with a low FPR [132,133]. In a TTM substudy, the bilateral absence of pupillary reflexes resulted in 97% specificity but poor sensitivity (24%), leading to a high number of false negative results [134]. Automatic pupillometry has been shown to be accurate in prognostication after CA [135]. Sedative medications, especially opioids, weaken pupil reactivity, and clinical interpretation of this reflex can be difficult.

In terms of testing the corneal reflex, techniques vary greatly, and suboptimal stimulation to activate the reflex has been noted, weakening its diagnostic credibility [136]. Overall, the accuracy of the corneal reflex seems comparable to that of the pupillary reflex [62].There are few studies on the prognostic value of absence of other brainstem reflexes [62]. Admiraal et al. found a 0% FPR with 27% sensitivity for absent bilateral corneal and pupillary reflexes [132]. In two small studies, absent oculocephalic or gag/cough reflexes showed 100% specificity and comparable sensitivity compared to pupillary and corneal reflexes [133,137]. As the brainstem can better resist ischemia than other brain structures [86], it is likely that very severe HIBI occurs when the brainstem is injured as well. Thus, it is

reasonable that tests showing the absence of brainstem function would show high specificity.

Myoclonus is visible as sudden, short jerks and muscle contractions and thus is usually included in clinical examinations. Myoclonus can be focal, multifocal, or generalised, and the generalised form is mostly associated with unfavourable outcome [138]. Overall, myoclonus is strongly associated with unfavourable outcome [139,140]. However, false positives are detected in 4–22% of resuscitated patients with myoclonus [133,137,141]. Status myoclonus (SM) is the most severe form. Though there is no real consensus on the definition of SM, it can be defined as myoclonia that lasts more than 30 min [142]. SM is strongly associated with unfavourable outcome [138,141,143].

In very rare cases, patients with SM can reach favourable outcome [141]. A study by Sivaraju et al. found that unfavourable outcome occurred in 85% of resuscitated patients who had myoclonus, and all of those with unfavourable outcome showed burst suppression or low voltage on an EEG [133]. Some patients with myoclonus or SM can still have a favourable outcome when EEG is continuous or presents normal voltage or background [144]. Most of the studies that reported outcome according to detected myoclonus did not define myoclonus exactly [62]. These findings underline the importance of a precise definition and assessment of myoclonus and SM and suggest the use of EEG when estimating the relevance of myoclonus to prognosis [8].

2.6.3 Imaging: MRI and CT

Computed tomography (CT) and magnetic resonance imaging (MRI) of the brain are the only methods that can give information on different brain areas and structures among resuscitated patients in addition to predicting HIBI and outcome. In particular, MRI has good discriminative ability, and it can quite reliably assess pathologies of the whole brain, including the middle brain and brainstem [145]. Another advantage of imaging is that sedation and relaxation have no effect on its interpretation, and it can also offer information on possible underlying neurological pathologies (e.g., intracranial haemorrhages or strokes) [146]. CT is easier to perform, whereas

MRI requires more resources. Also, MRI is often unsuitable in the early phase because patients are often haemodynamically unstable, and MRI takes longer than CT.

CT is often performed upon hospital admission to find the possible intracranial cause of CA [147]. Possible intracranial pathologies that can cause CA include subarachnoid haemorrhage (SAH) and other types of intracranial bleeding and ischaemic stroke. Somewhat later, CT can reveal cerebral oedema. Cytotoxic oedema is rather rare, but the most relevant finding related to severe HIBI in CT imaging [148]. Vasogenic oedema is even rarer [148]. Cytotoxic oedema occurs within highly metabolically active neurons of grey matter (GM) within hours. Oedema can be seen in CT as a loss of density and blurring in the GM/white matter (WM) interface [148]. The GM/WM density ratio (GWR) can be assessed, and a lower GWR is associated with unfavourable outcome [149]. GM density can be assessed within the basal ganglia and cerebrum and WM density within the corpus callosum, capsula interna, centrum semiovale, and high convexity. Using the GWR, unfavourable outcome can be found with high specificity and moderate sensitivity [150]. However, in OHCA patients with a cardiac cause of CA, the prognostic ability is worse, probably because of the lower incidence of severe brain swelling [151,152]. The GWR threshold that can predict unfavourable outcome with a 0% FPR differs among studies, affecting its sensitivity [62].

In a TTM substudy, generalised brain oedema was found in 10% of patients who underwent CT within the first 24 h, and in 46% when imaging was performed between 1–7 days [146]. Generalised oedema predicted unfavourable outcome overall with 98% specificity and 34% sensitivity, with increasing prognostic ability when performed after 24 h [146]. Abnormalities found in early brain CT can result in early WLST, and therefore, may predispose self-fulfilling prophecies [153]. Early brain CT can still predict unfavourable outcome with a low FPR, and when HIBI occurs within the first two hours, the brain injury is more severe. Brain death was detected in one third of those individuals [152].

The baseline MRI technique for detecting ischemic brain injury is diffusion-weighted imaging (DWI), which investigates changes in diffusion

of water in the brain. The diffusion of water is reduced in cells compared to the extracellular space. In brain ischemia, diffusion in the ischemic area first decreases due to cytotoxic oedema, which causes the intracellular water volume to increase (158). After that, starting at 2–3 days after reperfusion and lasting for few days, vasogenic oedema can occur and can cause a pseudonormalisation in diffusion [154]. Hypoxic-ischemic injuries can be seen as strong signal changes in DWI with good sensitivity, even 1–3 days after CA [145]. In a study by Ryoo et al., the most common brain areas with pathological DWI were the parietal (81%) and occipital (77%) cortex and the basal ganglia and thalamus area (47%), whereas DWI detected brainstem injury in only 3% of the patients [145]. When using one DWI high-signal area as a cutoff for unfavourable outcome, the sensitivity is excellent, but specificity decreases significantly [155].

The weakness of using DWI is that its definition is somewhat subjective and can result in differences in prognostic ability across radiologists. The apparent diffusion coefficient (ADC) is a more quantitative method calculated using DWI and provides information on the severity and extent of the injury [156]. Regarding HIBI, ADC values are different for cytotoxic and vasogenic oedemas that are potentially found in different time windows [156]. In two small studies, brain injury assessment with ADC demonstrated 20–90% sensitivity (depending on the definition method) with a threshold of a 0% FPR to predict unfavourable outcome [157,158]. Although there is variation in MRI timing and HIBI definition between studies, MRI using DWI in the assessment of HIBI at 3–7 days after CA has been found to be an accurate prognostic tool [62,159,160].

The role of WM (i.e., axons) has not been so well studied in HIBI as the role of GM. Velly et al. studied whole-brain MRI with WM anisotropy in CA patients who were comatose after 7 days, and this method provided 90% sensitivity without false positives [84]. This finding suggests a remarkable role of WM injury in individuals who remain comatose after a long following period, possibly reflecting secondary HIBI in some individuals that had unfavourable outcome.

2.6.4 Electrophysiological studies: EEG and SSEP

Electroencephalography (EEG) can measure brain electrical activity and waveforms with electrodes placed on the scalp. It is especially useful in unconscious individuals [161]. In addition to use in epileptic seizures, EEG is recommended for use in prognostication for comatose resuscitated patients [6]. Currently, EEG findings are recommended to be interpreted according to ACNS terminology for critical care in order to standardise the findings [123]. Highly malignant EEG contains suppression, suppression with periodic discharges, and burst suppression [123,162] and is a robust predictor of unfavourable outcome after CA with a 0–3% FPR [162]. Some individuals with EEG interpretation of burst suppression or low voltage during the first 12 h can still have a favourable outcome [133]. Regarding different interpretations of EEG findings, discontinuous EEG has a worse ability to predict unfavourable outcome with an FPR of 10–38% [163,164] whereas epileptiformic EEG is a reliable sign of unfavourable outcome [139,164].

The absence of EEG reactivity can discriminate outcomes of OHCA patients with good accuracy. In a study by Admiraal et al., the sensitivity was 73% and the specificity 82% for a prediction of unfavourable outcome, leading to an opportunity for favourable outcome prediction using reactivity as a positive signal [132]. Similar sensitivities for EEG reactivity in predicting unfavourable outcome have been found by others [133,165]. However, the specificity of absent EEG reactivity for ruling out false positive results is not high enough for decision making. In a large cohort of 850 OHCA patients, continuous EEG patterns during the first 12 h predicted favourable outcome with a specificity of 91% [164]. Overall, the predictive value of EEG is high at 12–24 h after CA [163–165].

Somatosensory evoked potentials (SSEPs) can present information on the function of thalamocortical connections [166]. Thalamic relay neurons and their interactions in somatosensory tracks are vulnerable during global ischemia, which can lead to thalamocortical dissociation [167]. In a severe thalamocortical injury, consciousness may remain impaired. The short-term latency N20 peak is the most studied method among evoked

potentials [62]. The N20 peak can be detected at the contralateral brain cortex with a scalp sensor 20 ms after the median nerve stimulus [168].

If the thalamocortical track or brainstem is injured, or a large cortical injury exists, the N20 wave will not proceed to the sensor. An absent N20 SSEP after brainstem injury is strongly associated with severe injury [169]. The brainstem is the brain structure most resistant to hypoxic-ischemic injury, [41] and unfavourable outcome is very likely when a brainstem injury is caused by hypoxic-ischemic insult. As the absence of bilateral N20 SSEP indicates severe thalamocortical and/or brainstem injury, those patients are not likely to reach consciousness. Hence, it is reasonable that bilateral absent N20 SSEP is a robust predictor of unfavourable outcome after CA, as proven in studies with FPRs near 0% [135,163,164]. Only in one small study did N20 SSEP demonstrate a clearly worse predictive ability [170].

As a confounder, electrical or technical artefacts may disturb the interpretation of SSEP and, in very rare cases, can give false positive results [171]. N20 SSEP can still be normal in milder brain injuries that may lead to significant functional deficits, however, and this can be seen as a limitation of the sensitivity of SSEP. Other less-studied evoked potentials involved in unfavourable outcome after CA are pain-related middle-latency SSEP, brainstem auditory-evoked potential, and visual-evoked potential, and their prognostic ability is comparable to N20 SSEP [172–174].

2.7 NEUROBIOMARKERS IN PROGNOSTICATION AFTER CARDIAC ARREST

Biomarkers are parts of structures (e.g., proteins) that can be measured in body fluids or secretions and can give information about the condition or disease being sought [175]. Neurobiomarkers are parts of the injured central nervous system that are released into the circulation, where their concentrations can be measured. In the earliest studies, biomarkers were measured from cerebrospinal fluid (CSF) [176]. Obtaining CSF is, however, a laborious and invasive procedure, and blood neurobiomarkers are currently being investigated and used in prognostication [6,62].

As HIBI remains the cause of most unfavourable outcomes after CA, neurobiomarkers that are potentially able to predict unfavourable outcome should be brain specific. The optimal biomarker can reliably distinguish individuals with severe HIBI from those without HIBI and have only minimal sources of error. Moreover, early biomarker release (within 1–3 days) into the blood as a mark of severe HIBI is beneficial for avoiding prolonged futile care. Depending on the biokinetics of each biomarker, elevated concentrations in patients with HIBI can be found in the blood even within the first hours after CA, whereas maximal concentrations are typically found 24–72 h after CA [177], which is also time when other prognostic tests are mainly conducted [6].

As biomarker concentrations are continuous variables, the definition of cutoff levels with suitable false positive and false negative thresholds is very important in clinical decision making. Different discriminative abilities and cutoff concentrations of neurobiomarkers have been studied, and variation among studies is prominent. The differences can be caused by differences in laboratory methods, outcome definitions, WLST practices, and patient case mix [62]. As the predicted event is severe HIBI that may lead to termination of life-sustaining therapies, it is necessary for the biomarkers used to have high specificity with a minimal number of false positive results [62]. Cutoffs with a 100% specificity are mainly chosen, but slightly lower specificities of 95–99% are also suggested to improve the sensitivity using the selected cutoff concentration [114]. High concentrations of neurobiomarkers that are not related to HIBI alter test specificity and result in false positives. Optimal cutoff values have been most widely studied for NSE because it is used in clinical prognostication across the world [6]. Comparison between features of neurobiomarkers are presented in Table 3.

In addition to neurons (GM), the brain contains glial cells, including astrocytes, oligodendrocytes, and ependymal cells, and axons, which make up the WM [177]. The area of the brain containing all the structures that have been found to be related to HIBI is called the neurovascular unit [177]. Hypoxic-ischemic injury and subsequent reperfusion injury can damage parts of neurovascular units, causing parts of neurons, axons, and glial

cells to be released. Because swelling and ischemia alter BBB function, the injured parts can leak into the circulation and can be measured in blood samples. The neurovascular unit and release of biomarkers after HIBI are presented in Figure 2.

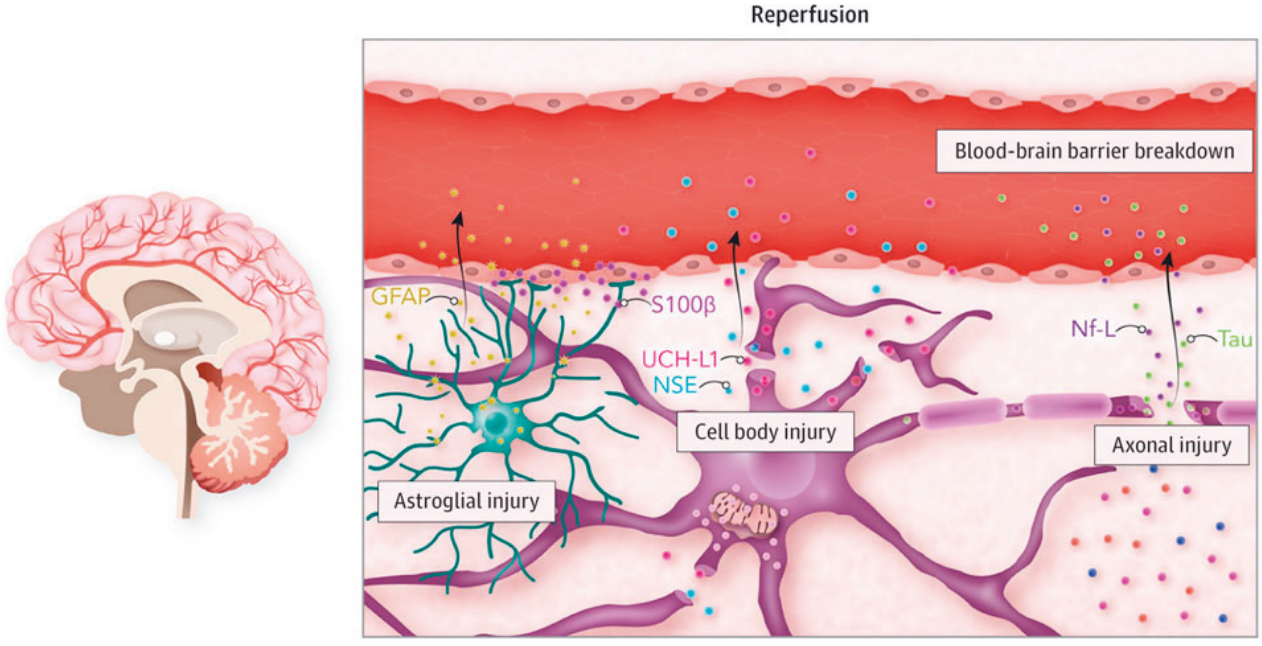

Figure 2. The Neurovascular Unit and Brain Injury Biomarker Release.

The neurovascular unit represents the principal anatomical and functional unit of the brain parenchyma where complex interplay occurs among neurons, the cerebral microvasculature, and surrounding glial cells to maintain homeostasis. The microvasculature is composed of the blood-brain barrier, which is partially formed by adjoining projections from astrocytes. Surrounding neuron cell bodies give rise to myelinated axons, which conduct signal transduction and facilitate communication with distinct anatomical locations in the brain. Following return of spontaneous circulation, ischemia-reperfusion injury pathophysiology occurs, and widespread injury across the neurovascular unit is reflected in the release of brain injury biomarkers into the bloodstream, which is facilitated by blood-brain barrier breakdown. Biomarkers reflecting astrocyte injury include glial fibrillary acidic protein (GFAP) and serum 100 calcium-binding protein β (S100β). Neuron cell body injury is reflected by release of neuron-specific enolase (NSE) and ubiquitin carboxyl hydrolase L1 (UCH-L1). In addition, axonal injury is reflected by release of neurofilament light (Nf-L) and tau. As such, the relative concentrations of the various biomarkers seen in the bloodstream can allude to signatures of damage to the neurovascular unit and its specific components.
Hoiland RL et al. Neurologic Prognostication After Cardiac Arrest Using Brain Biomarkers: A Systematic Review and Meta-analysis. JAMA Neurol. 2022 Apr 1;79(4):390-398. Adapted with the permission of American Medical Association (license 5640770957287)

Thus far, the neuronal biomarker NSE has been used as a “standard” marker of HIBI. Recent studies suggest that injury to WM is also a significant factor in HIBI, making axonal/WM neurobiomarkers promising prognostic factors [82,178].

The evaluation of ongoing secondary HIBI is a novel issue, whereas assessing the neurological prognosis by evaluating the severity of HIBI according to guidelines is a fundamental practice in the use of neurobiomarkers [10]. Secondary HIBI is clinically important and may be an independent process that is not related to the severe primary brain injury [82], potentially offering opportunities to attenuate the developing injury and improve outcome. Thus far, specific therapies for this purpose have not been found. Neurobiomarkers may have potential in the assessment of secondary brain injury, but this area is still unclear.

Table 3. Features of different neurobiomarkers.

	NSE	**S100B**	**UCH-L1**	**GFAP**	**Tau**	**NfL**
Origin	Grey matter: neurons	White and grey matter Astrocytes, glial cells, choroid plexus	Mostly grey matter: neurons	White matter: astrocytes	White matter: microtubules in neuronal axons	White matter: myelinated neuronal axons and synapses
Half-life	24-72 h	0.5-24h	6-13 h	24-48 h	~10 h	Days-weeks
Molecular size	45-78 kDa	10-21 kDa	25-27 kDa	50 kDa	33-67 kDa	68-70 kDa
Prognostic ability: AUROC	0.63-94	0.77-0.85	0.85-0.94	0.67-0.89	0.85-0.91	0.79-0.99
Time to best predictive ability	48-72 h	(3-)24-48 h	24-72 h	48-72 h	72-96 h	24-72 h
Sources of error	Haemolysis, small-cell lung carcinoma, neuroendocrine tumour, other brain injuries	Many extracerebral tissues, other brain injuries	Neuroendocrine cells, pancreas, endothelial and smooth muscles, many tumors, lupus nephritis, other brain injuries.	Glioblastoma. Peripherally Schwann cells, chondrocytes, osteocytes, Leydig cells (testis). Many acute brain injuries	Neurodegenerative diseases, peripheral axons. Many acute brain injuries	Neurodegenerative diseases, peripheral axons. Aging and renal dyscunction increases. Many acute brain injuries
Advantages	Extensively studied accuracy and cut-offs. Recommended in guidelines. Clinical experience in prognostication.	Fast release after CA. Short half-life may be beneficial in secondary HIBI assessment. Commercial laboratory method.	Promising prognostic accuracy at 24-72 h. Short half-life may be beneficial in secondary HIBI assessment.	Very brain-specific. Good prognostic accuracy. Normal values can reliably exclude HIBI	High prognostic accuracy. Widely already used in diagnostics among neurodegenerative diseases.	Fast release after injury. Best prognostic accuracy (stabile over 1-3 days). Normal values can reliably exclude HIBI

Mechanisms under investigation	Accuracy to predict favourable outcome	The role in astroglial activation, neurionflammation and BBB injury	Prognostic accuracy after CA as neuronal biomarker	The role in astroglial activation, neurionflammation and BBB injury. Potential to exclude severe HIBI.	The role of axonal biomarkers in prognostication after CA.	Widely studied in many neurological disorders. Feasible cut-offs for prognostication.

Abbreviations: NSE, neuron-specific enolase; S100B, protein S100B; UCH-L1, ubiquitin c-terminal hydrolase L1; GFAP, glial fibrillary acidic protein; NfL, neurofilament light; AUROC, the area under the receiver operating characteristic curve; HIBI, hypoxic-ischemic brain injury; CA, cardiac arrest; BBB, blood-brain barrier

Several neurobiomarkers have been studied in prognostication after CA. The most studied are NSE, calcium-binding protein S100B (S100B), ubiquitin C-terminal hydrolase L1 (UCH-L1), neurofilament light (NfL), glial fibrillary acidic protein (GFAP), and Tau [62]. NSE and S100B are the older and most widely studied neurobiomarkers, and UCH-L1, GFAP, Tau, and NfL are more novel biomarkers. Of these, NSE and UCH-L1 are mostly located in neurons, and high concentrations of them after CA reflects mainly neuronal injury in GM [177]. S100B and GFAP can reflect injury of astrocytes and glial cells, and Tau and NfL are more likely to signify white matter injury as axonal biomarkers [177].

Hoiland et al. studied neurobiomarkers in prognostication after CA in a recent meta-analysis including 10,567 individuals in 86 studies and created summaries of receiver operating characteristic curves to assess the prognostic values of neurobiomarkers [177]. The best prognostic abilities belonged to NfL, Tau, and UCH-L1, but the number of studies demonstrating the superiority of those novel biomarkers over NSE remains limited. Studies focusing on prognostication after CA using neurobiomarkers are presented in Table 4.

Table 4. Characteristics of studies focusing on neurobiomarkers in prognostication after cardiac arrest.

	Number of patients, location of CA	**Assessment of unfavourable outcome**	**Cut-off (definition time). False positive rate, FPR (%)**	**Discriminative ability: AUROC**	**Main findings**
NSE			Cut-off µg/L		
Tiainen 2003 (190)	70, OHCA	6 mth CPC 3-5	25 (48 h), TH+ 9 (48 h), TH- FPR 0 for both	0.80–0.89 (48 h)	Decreasing NSE associated to favourable outcome in therapeutic hypothermia (TH) patients
Zandbergen 2006 (188)	231, OHCA and IHCA	Unconsciousness at 1 mth	33 (24–72 h) FPR 0	Not specified	NSE and SSEP were robust predictors of outcome
Oksanen 2009 (189)	90, OHCA	6 mth CPC 3-5	33 (48 h) FPR 0	0.82 (48 h) 0.84 (48–24 h)	Increasing NSE >6 µg/L 24-48h predicted unfavourable outcome (100% spec)
Pfeifer 2014 (219)	201, OCHA and IHCA	1 mth CPC 4-5	40 (72 h) FPR 5	0.60 (24 h) 0.83 (48 h) 0.89 (3–5 days)	Best prognostic ability in 3-5 days. TH had no effect
Huntgeburth 2014 (173)	73, OHCA	2 mth CPC 4-5	112 (48 h) 65 (72 h) FPR 0 for both	0.63 (24 h) 0.87 (48 h) 0.94 (72 h)	Increasing NSE >4 µg/L 24-48h predicted unfavourable outcome (100% spec)
Stammet 2015 (179)	686, OHCA	6 mth CPC 3-5	76 (48 h) 53 (72 h) FPR 0	0.75 (24 h) 0.85 (48 h) 0.86 (72 h)	High prognostic ability at 48-72 h, TH did not influence NSE
Streitberger 2017 (184)	1053, OHCA and IHCA	CPC 4-5 at ICU discharge	90 (72 h) FPR 0.5	0.85–0.9 (72 h, OHCA) 0.79 (72 h, IHCA)	High ability to predict outcome at ICU discharge, better for OHCA vs IHCA patients

Helwig 2017 (186)	100, OHCA	4-week MGOS 0-3	34 (48 h) FPR 0	0.63 (48 h)	Moderate prognostic ability, better for TH
Nakstad 2020 (102)	259, OHCA	6 mth CPC 3-5	80 (≥24 h) FPR 0	Not reported	Median awakening time 6 days in patients with favourable outcome, high NSE a strong predictor of unfavourable outcome
Lissner Östlund 2021 (185)	368, OHCA	6 mth CPC 3-5	112 (48 h) 83 (72 h) FPR 0 for both	0.90	NSE cut-off 60 µg/L resulted in 4% of FPR
S100B			Cut-off µg/L		
Zandbergen 2006 (188)	231, OHCA and IHCA	Unconsciousness at 1 mth	0.7 (24 h) FPR 3 0.7 (48 h) FPR 2 0.7 (72 h) FPR 0	Not specified	S100B had inferior prognostic ability than NSE
Pfeifer 2014 (219)	201, OCHA and IHCA	1 mth CPC 4-5	1.03 (72 h) FPR 7	0.77 (24 h) 0.85 (48 h) 0.88 (72 h)	Best prognostic ability at 72 h, higher FPR vs NSE.
Stammet 2017 (114)	687, OHCA	6 mth CPC 3-5	2.6 (24 h) FPR 0 3.7 (48 h) FPR 0 1.8 (72 h) FPR 0	0.80 (24 h) 0.79 (48 h) 0.77 (72 h)	Best prognostic ability at 24 h, ovarell worse vs NSE
Duez 2018 (218)	115, OHCA	6 mth CPC 3-5	16.6 (0 h) FPR 0 1.1 (24 h) FPR 0 0.95 (48 h) FPR 0 0.7 (72 h) FPR 0	0.66 (0 h) 0.81 (24–48 h) 0.74 (72 h)	Prognostic ability worse vs NSE, prolonged TH had no effect
Deye 2020 (215)	330, OHCA	3 mth CPC 3-5	0.5 (3.5h) FPR 21 0.3 (24 h) FPR 36 0.1 (48 h) FPR 24	0.83 (0 h) 0.83 (24 h) 0.82 (48 h)	Best prognostic ability at admission, decreasing concentrations in all patients at 24-48 h
UCH-L1			Cut-off pg/mL		

Ahn 2020 (205)	38, OHCA	6 mth CPC 3-5	40 (0 h) FPR 50 40 (24-48 h) FPR 9 50 (72 h) FPR 4	0.71 (0 h) 0.85 (24 h) 0.90 (48 h) 0.94 (72 h)	Excellent prognostic ability at 48-72 h, measurement in CSF had better ability
Ebner 2020 (206)	717, OHCA	6 mth CPC 3-5	12175 (24 h) 7945 (48 h) 9170 (72 h) FPR 0 for all	0.85 (24 h) 0.87 (48 h) 0.86 (72 h)	Good prognostic ability 24-72 h, better vs NSE at 24-48 h but nor at 72 h
Huesgen 2021 (207)	22, OHCA	CPC 3-5 at hospital discharce	4670 (0 h) FPR 0 1031 (6 h) FPR 0 2488 (24 h) FPR 0 3214 (48 h) FPR 0 1228 (72 h) FPR 0	Not specified	Good sensitivities with FPR 0% starting at 12-18 h
GFAP			Cut-off ng/mL		
Kaneko 2009 (231)	44, OHCA	6 mth GOS 1-3	0.1 (12–48 h) FPR 0	Not specified	Best predictive ability at 24-48 h
Larsson 2014 (232)	125, location of CA not identified	6 mth CPC 3-5	1.1 (24 h) FPR 0 0.3 (48 h) FPR 0 0.5 (72 h) FPR 0 0.04 (96 h) FPR0	0.59 (24 h) 0.63 (48 h) 0.67 (72 h) 0.65 (96 h)	Not sufficient prognostic ability, poor sensitivity
Helwig 2017 (186)	100, OHCA	4 week MGOS 0-3	0.08 (48 h) FPR 0	0.65 (48 h)	Moderate prognostic ability, comparable vs NSE
Ebner 2020 (206)	717, OHCA	6 mth CPC 3-5	3.4 (24 h) FPR 0 3.0 (48 h) FPR 0 3.6 (72 h) FPR 0	0.88 (24–48 h) 0.89 (72 h)	Good prognostic ability 24-72 h, significantly better vs NSE
Humaloja 2022 (234)	112, OHCA	6 mth CPC 3-5	3.3 (0h) FPR 1 8 (24h) FPR 1 6.3 (48h) FPR 1	0.65 (0 h) 0.87 (24 h) 0.91 (48–72 h)	Excellent prognostic accuracy at 48-72 h

			4.2 (72 h) FPR 1		
Tau			Cut-off pg/mL		
Randall 2013 (247)	25, location of CA not identified	6 mth CPC 3-5	500 (sum across all time points) FPR 0	0.67 (24 h) 0.86 (for sum across all time points)	FPR 0% and 90% of sensitivity for cut-off 500 mg/mL (sum of all)
Mattsson 2017 (237)	689, OHCA	6 mth CPC 3-5	875 (24 h) FPR 0 149 (48 h) FPR 0 73 (72 h) FPR 0	0.81 (24 h) 0.90 (48 h) 0.91 (72 h)	Excellent prognostic ability at 48-72 h. Good sensitivity for FPR 1-2%
Hasslacher 2020 (248)	132, OHCA	CPC 3-5 at hospital discharge	1274 (0-24 h) 1102 (24-48h) 1443 (48-72h) 1218 (72-96h) FPR 0 for all	0.65 (0–24 h) 0.78 (24–48 h) 0.78 (48–72 h) 0.85 (72–96 h)	Good prognostic ability at 72-96h. Stabile cut-offs for FPR 0%
Huesgen 2021 (207)	22, OHCA	C 3–5 at hospital discharce	1479 (0h) FPR 0 32 (6h) FPR 0 148 (12h) FPR 0 34 (24h) FPR 0 88 (48h) FPR 0 399 (72 h) FPR 0	Not specified	Good sensitivity at 24-48 h. Small pilot study, not focused on prognostic ability.
Humaloja 2022 (234)	112, OHCA	6 mth CPC 3-5	206 (0h) FPR 1 40 (24h) FPR 1 16 (48h) FPR 1 10 (72 h) FPR 1	0.58 (0 h) 0.82 (24 h) 0.93 (48 h) 0.95 (72 h)	Excellent prognostic ability at 48-72 h
NfL			Cut-off pg/mL		
Rana 2014 (267)	85, OHCA	6 mth MGOS 1-2	323 (24 h) FPR 0 405 (48 h) 309 (72 h) 383 (5 d) 252 (7 d)	0.93 (24 h) 0.85 (48 h) 0.92 (72 h) 0.97 (5 d) 0.99 (7 d)	Excellent prognostic accuracy, best at 5-7 days. High 75-94 sensitivities

					(FPR 0%) at 3-7 days
Moseby-Knappe 2019 (134)	717, OHCA	6 mth CPC 3-5 mRS (secondary)	12317 (24h) 1539 (48h) 1756 (72h) FPR 0 for all	0.94 (24 ,48, 72 h)	Excellent prognostic ability at 24-72h. 64-65% of sensitivity for FPR 0% at 48-72h.
Hunziker 2021 (268)	164, OHCA	CPC 3-5 at hospital discharge	75 (0–24 h) FPR 11	0.82 (0–24 h)	Single measurement within 24h, good prognostic ability
Wurm 2022 (269)	70, OHCA	6 mth CPC 3-5	Not specified	0.79 (48 h)	Single measurement at 48 h. Moderate prognostic ability, better for NfL vs NSE
Pouplet 2022 (270)	49, OHCA	90 d CPC 3-5	500 (48 h) FPR 0	0.87 (48 h)	Sensitivity of ERC-ESICM-guided prognosticatio n improved with NfL

Abbreviations: Spec, specificity; CA, cardiac arrest; OHCA, out-of-hospital cardiac arrest; IHCA, in-hospital cardiac arrest; AUROC, the area under the receiver operating characteristic curve; TH, therapeutic hypothermia; TH+, therapeutic hypothermia treatmen was given; TH-, therapeutic hypothermia treatment was not given; FPR, false positive rate; CPC, cerebral performance category, GOS, the Glasgow Coma Scale, MGOS, modified Glasgow Coma Scale; mRS, modified Rankin Scale; CSF, cerebrospinal fluid; NSE, neuron-specific enolase; S100B, protein S100B; UCH-L1, ubiquitin c-terminal hydrolase L1, GFAP, glial fibrillary acidic protein; NfL, neurofilament light; ERC, European Resuscitation Council, ESICM, European Society of Intensive Care Medicine.

2.8 NEURONAL BIOMARKERS: NSE AND UCH-L1

2.8.1 NSE

Neuron-specific enolase (NSE) is the most extensively studied neurobiomarker in prognostication after CA and also the biomarker

included in current prognostication guidelines [6,177]. NSE is a glycolytic enzyme with a molecular weight of 78 kDa and is located mostly in GM (i.e., in neurons of the brain cortex); it is a biomarker of neuronal injury [179]. It can also be found in neuroendocrine cells, peripheral nerves, and small-cell lung carcinoma [12,180]. Red blood cells and thrombocytes contain NSE, and elevated serum concentrations can be found in cases of haemolysis [11]. Other conditions can misleadingly raise NSE serum concentrations and confound their interpretation. TBI, ischemic stroke, and other brain cerebrovascular diseases such as SAH, can increase blood NSE concentrations and confound the assessment of HIBI [13,14,181,182]. Moreover, NSE values can vary widely between different types of assays, and this has an impact on differences in cutoff values [183]. However, NSE is a stable enzyme when frozen, so freezing does not affect the reliability of post-hoc analyses [183].

NSE is released into the circulation after CA within 24–48 h after neuronal injury, and overall, it can predict unfavourable outcome after CA at 48–72 h [62]. In two largest NSE studies of almost 2000 patients by Streitberger et al.[184] and Stammet et al., [179] the prognostic abilities were good to excellent with AUROCs of 0.85-0.90. In contrast to other studies, the unfavourable outcome definition in the study by Streitberger et al. was CPC 4–5 at ICU discharge; it is more often defined as CPC 3–5 at 6 months [131,179]. Many other studies have presented comparable prognostic abilities [173,185]. A significantly worse discriminative ability of NSE was reported in one study, where the prognostic ability was remarkably higher in patients treated with TH than in those who were not, possibly reflecting some level of bias [186]. In a meta-analysis by Hoiland et al., when emphasising specificity (97%), the optimal cutoff for NSE was close to that recommended in the guidelines, and the AUROC was 0.84 [187]. The overall prognostic value of NSE is good, but its sensitivity in clinically relevant threshold values remains only moderate.

An NSE cutoff >33 μg/L demonstrated 100% specificity for predicting unfavourable outcome after CA in studies conducted in 2006 and 2009 [188,189]. This cutoff has been widely used to predict unfavourable outcome thereafter. Increasing NSE concentrations have been found to predict

unfavourable outcome; likewise, decreasing NSE levels have been found to predict favourable outcome [173,189,190]. In a later study, the use of NSE >33 µg/L to predict unfavourable outcome after OHCA in a post-hoc analysis clearly resulted in a worse specificity of 90% [131]. The cutoff value has been updated in current ERC-ESICM guidelines to >60µg/L at 48–72 h [6]. Favourable neurological outcome has also been detected in individuals who exceeded this cutoff [173,179,184,185]. In a validation of the latest ERC-ESICM guidelines, the cutoff of 60 µg/L demonstrated a 4% FPR, and to achieve a 2% FPR, the cutoff demand for NSE rose to 101 µg/L [185].

In predicting favourable outcome (i.e., by excluding severe HIBI), Streitberger et al. found that only 14 patients with NSE < 17 µg/L had unfavourable outcome, and that was mostly caused by extracerebral factors [184]. Moseby-Knappe et al. used the same cutoff for patients whose prognostication according to ERC-ESICM guidelines resulted in indeterminate outcome and found a sensitivity of 90% and an NPV of 79%, demonstrating that some individuals with normal NSE can still have unfavourable outcome [15].

2.8.2 UCH-L1

Ubiquitin c-terminal hydrolase L1 (UCH-L1) is a novel biomarker of neuronal injury. It is a small protein whose molecular weight is 27 kDa, and it is rather brain-specific, mostly located in the cytoplasm of neurons in the brain cortex [191,192]. Additionally, smaller amounts of UCH-L1 have been found in axons, neuroendocrine cells, and endothelial and smooth muscle cells [191,192]. UCH-L1 can also be found in the pancreas and in some kidney diseases, and it is involved in many cancers and neurodegenerative disorders [193–196]. UCH-L1 has been studied in TBI patients and can accurately discriminate patients according to severity of injury [197,198]. In one study comparing UCH-L1, S100B, and GFAP in TBI patients, UCH-L1 was the most robust marker in discriminating according to injury and reached the highest sensitivity [199], and in a cohort of 2283 TBI patients, UCH-L1 demonstrated the best incremental prognostic value among all studied biomarkers, when added to the prediction model [200]. Elevated UCH-L1

concentrations can also be found after SAH, intracerebral haemorrhage (ICH), and stroke [201,202].

The half-life of UCH-L1 is 6–13 h, and in newborns with hypoxic brain injury, the highest concentrations were measured at 0–6 h after delivery [198,203]. There is only a small number of studies regarding UCH-L1 in prognostication after CA [187]. In a small 2016 study that included paediatric CA patients, UCH-L1 showed good accuracy in predicting unfavourable outcome [204]. The first study in adults in 2020 included 38 OHCA patients; it found that serum UCH-L1 predicted unfavourable outcome with good-to-excellent accuracy (AUROCs of 0.85–0.94 at 24–72 h) [205].

In a study of 717 OHCA patients by Ebner et al., UCH-L1 predicted unfavourable outcome with good ability (AUROCs of 0.85–0.87) [206]. The prognostic ability of UCH-L1 was superior to that of NSE at 24–48 h, but at 72 h, the prognostic abilities were not different. The sensitivities of UCH-L1 in using cutoff values for a 0% FPR were worse than those of other studied biomarkers (NSE and GFAP). However, sensitivities of all biomarkers were low, and for specificities of 95–99% at 24 h, UCH-L1 demonstrated better sensitivities than NSE. This is probably reflected by the poorer prognostic accuracy of NSE at the 24 h time point. In a small pilot study, UCH-L1 demonstrated good sensitivities at 12–18 h after CA and at 72 h, even with 100% of specificity [207]. Overall, UCH-L1 has demonstrated sufficient prognostic accuracy at 24–72 h after CA, but the number of studies is very small.

2.9 GLIAL AND ASTROCYTE MARKERS: S100B AND GFAP

2.9.1 S100B

Calcium-binding protein S100B (S100B) is a traditional and widely studied neurobiomarker like NSE. It is a small (21 kDa) molecular weight calcium-binding protein involved in many functions, such as neuronal differentiation, proliferation, and apoptosis [208]. It can induce neuronal cell death through nitric oxide release from astrocytes [209]. S100B is located mostly in neurons, astrocytes, and dendrites, but is also widely found in

peripheral Schwann cells, other neural cells, and the choroid plexus [210]. Moreover, S100B can be found in neuroectodermal tumour cells, cardiomyocytes, adipocytes, muscle cells, and chondrocytes, thus confounding its interpretation regarding prognostication after CA [211]. High blood concentrations of S100B can be also found after TBI and ICH [212,213].

Stammet et al., in the largest study of S100B in prognostication after CA, examined 687 patients; the best ability to predict unfavourable outcome was noted at 24 h with an AUROC of 0.80 [114]. The prognostic ability of S100B was better than that of NSE at 24 h, but NSE overcame S100B at later time points. In one study, S100B at 24 h predicted unfavourable outcome after CA with an excellent AUROC of 0.92 [214].

S100B has a very short half-life of <2 h, which could explain its rapidly falling concentration, but which also offers the opportunity for use in very early predictions after CA. In a study by Deye et al., S100B that was obtained at a median time of 3.5 h after CA in 330 patients showed a good ability to predict unfavourable outcome, with an AUROC of 0.83—clearly better than NSE [215]. At later time points, the prognostic ability slightly decreased, and the concentrations of S100B decrease greatly. The fast release of S100B is hypothesised to reflect glial activation and injury to the BBB rather than the brain itself, and in TBI patients, S100B is associated with disruption of the BBB and increased vascular permeability [216,217].

Regarding ultra-early sampling time, in another study, S100B had a clearly inferior prognostic ability at ICU admission compared to later time points [218]. That study, together with other studies, demonstrated that concentrations of S100B are high in the early phase of treatment in all resuscitated patients but decrease over 24 h. Contrary to the findings of Stammet et al. and Deye et al., Pfeifer et al. found that the prognostic ability of S100B was better at 48–72 h than at 24 h, perhaps confounded by different definitions of unfavourable outcome [219]. Overall, high S100B concentrations in the early phase (24 h) offer sufficient but not excellent prognostic value, being somewhat comparable to NSE [187].

2.9.2 GFAP

Glial fibrillary acidic protein (GFAP) is a 50 kDa cytoskeletal protein of astrocytes and is highly brain-specific [220]. GFAP is involved in the normal structure of GM and WM as part of astrocytes and neuroglia [221]. Unlike another glial protein, S100B, it is more astrocyte-specific [210]. It has a half-life of 24–48 h. Elevated GFAP serum concentrations are found in the brain cancer glioblastoma multiforme but not in other brain tumours [222]. Peripherally, GFAP is expressed in Schwann cells, chondrocytes, osteocytes, and Leydig cells of the testes [220]. Astrocytes execute many important functions in the brain, including ionic homeostasis and the prevention of excitotoxicity, and they interact with microglia and endothelial cells. In multiple sclerosis (MS) patients, higher serum GFAP concentrations are associated with a loss of GM volume, but this association has not been found in WM, suggesting that GFAP is more involved in GM inflammation and injury [223].

GFAP is a robust predictor of outcome after TBI [224,225]. Elevated concentrations can be also found after ICH, SAH, and ischemic stroke [226–228]. The reactivity of astrocytes increases together with BBB breakdown after TBI [229], and it is likely that GFAP as an astrocyte-specific structure is involved in BBB disturbances. In addition, GFAP is upregulated in astrocytes after ischemia, suggesting a remarkable role in the reactivity of astrocytes, and probably in oedema due to BBB breakdown when secondary HIBI occurs [230]. One study suggests that higher GFAP concentrations reflect disruption of the BBB [231].

The predictive ability of GFAP in CA patients is not unequivocal, however. In one small study, GFAP had a poor predictive ability after CA, and in another study of 125 CA patients, its sensitivity and overall prognostic ability was inferior to that of NSE and S100B [232,233]. Only moderate prognostic accuracy (AUROC 0.65 at 48 h) was found in another study, though that study also found a moderate prognostic ability of NSE [186]. In a meta-analysis by Hoiland et al. summarising prognostic abilities of neurobiomarkers, the AUROC was also moderate 0.77 [187]. However, in the largest study of GFAP in prognostication after CA, Ebner et al. found good

prognostic accuracy [206]. The AUROCs were 0.88-0.89, significantly better than those of NSE. In contrast to other studies, Ebner et al. utilised a commercial laboratory method in GFAP assessment, which may explain the remarkable prognostic differences when compared to previous studies. In a post-hoc analysis of the COMACARE trial, GFAP predicted unfavourable outcome at 48–72 h with AUROCs of 0.91; however, the ability of GFAP was not significantly better than that of NSE [234].

GFAP has also been studied in a CA population with secondary HIBI, and significant ongoing GFAP release (defined by the concentration gradient across arterial and jugular vein blood) was found, suggesting ongoing astroglial injury [82]. Astroglial injury has been hypothesised to be a mechanism of GFAP release after CA, as is true for another neuroglial marker, S100B [216].

2.10 AXONAL MARKERS: TAU AND NFL

2.10.1 Tau

Tau is a structural protein of 33–67 kDa molecular weight that stabilises microtubules in axons of the CNS [235]. It can be found in unmyelinated axons and astrocytes and is mostly located in WM [236]. Tau is also expressed in the muscles, kidneys, liver, testes, and peripheral nerves. Haemolysis does not increase its blood levels [237]. Tau is involved in many brain diseases and has been extensively studied in the past. Tauopathies are a result of abnormal Tau phosphorylation or levels, or gene mutations [235]. Hyperphosphorylation and accumulation of Tau can disturb axonal functions and can cause neurodegeneration [238]. Higher concentrations of Tau measured in cerebrospinal fluid are associated with neurodegenerative disorders (e.g., cognitive impairment, Alzheimer's disease, Parkinson's disease) [239–241]. Elevated plasma Tau concentrations can be found in Alzheimer's disease, which is the most studied tauopathy, and can be used to discriminate ill from healthy individuals [239,242].

Tau can cross the normal BBB, so it is reasonable that elevated blood concentrations can be measured in brain injuries [243]. Blood concentrations

of Tau increase rapidly after ischemic stroke and TBI [244,245]. After CA, Tau fragments are associated with inflammatory markers, suggesting that axonal inflammation is one mechanism in secondary HIBI [246]. Overall, Tau has not been studied as extensively in acute brain injuries as other neuronal biomarkers.

There have not been many studies of Tau in prognostication after CA, either. Randall et al. conducted a pilot study in 2013 of 25 CA patients and found significantly higher Tau concentrations in patients with unfavourable outcome [247]. In a TTM substudy of 689 patients, Tau predicted unfavourable outcome with AUROCs of 0.90–0.91 at 48–72 h, demonstrating excellent prognostic ability, better than that of NSE [237]. That study also demonstrated good sensitivity (66%) achieving a low FPR of 2%. Tau concentrations also demonstrated such linear association with outcome scales (CPC and mRS), indicating that Tau could predict the grade of HIBI in addition to discriminating according to a dichotomic outcome. In another TTM substudy based on the same population, but focusing on NfL, another axonal marker, Tau presented the second-best prognostic ability [134]. In another study, Tau measured at 72–96 h had a slightly worse but still good prognostic accuracy (AUROC 0.85) [248]. In a post-hoc analysis of the COMACARE trial, the prognostic ability of Tau was excellent at 48–72 h with AUROCs of 0.93–0.95 [234].

The studies focusing on Tau after CA indicate a later timeline (≥72 h) for the best prognostication [248]. Randall et al. found a delayed peak of Tau to be a strong predictor of unfavourable outcome, and as Tau has a short biological half-life of <10 h, a new or continuing rise of blood Tau levels may be a signal of ongoing secondary HIBI [234,247].

2.10.2 NfL

Neurofilament light (NfL) is one subunit of three intermediate filaments containing light, medium, and heavy chains of neurofilaments [249]. It is a cytoskeletal protein of neurons that is abundant in myelinated axons and is mainly white matter-specific. NfL has a molecular weight of 70 kDa, like albumin [250]. The function of NfL is to be a structural support for axons and

maintain their size and shape [251]. In addition to the CNS, NfL is also expressed in peripheral nerves [252]. Renal dysfunction and ageing increase NfL blood concentration and can confound its interpretation [253]. NfL is a marker of axonal injury, and elevated concentrations can be found in CSF and serum in patients with demyelinating MS [251,254].

NfL concentrations that can be measured in plasma are very low, defined as picograms/mL, so a highly sensitive and stable assay is needed. Novel single molecular array (SIMOA) offers the possibility of a highly sensitive analysis of NfL in plasma [255]. Elevated plasma NfL concentrations can be found in many neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, all cortical dementias, amyotrophic lateral sclerosis, and Down's syndrome; however, the concentrations are not remarkably higher than in healthy individuals [256–258]. NfL concentrations also rise after spinal cord injury and in CNS injury caused by HIV [259,260].

As NfL is mostly expressed in myelinated axons, it is a marker of WM injury. In a recent study of MS patients, increasing NfL was associated with a loss of WM volume on MRI, but NfL did not reflect GM loss [223]. In addition to neurodegenerative diseases, NfL has been increasingly studied in many acute brain diseases. After acute ischemic stroke, ICH, and SAH, NfL concentrations rise significantly and can predict long-term outcome [261]. In a study of TBI patients, NfL concentrations measured in plasma and in damaged WM tissue using microdialysis correlated significantly, and NfL concentrations also correlated with WM injury on MRI scans [262]. The study also found that plasma NfL concentrations predicted long-term outcome and WM degeneration, and interestingly, elevated NfL concentrations were measured in a majority of TBI patients after a 6–12 month follow-up. In another study of TBI patients, elevated NfL concentrations at 8 months after trauma were associated with a loss of WM during a 5-year follow-up, suggesting that ongoing axonal damage was causing NfL release [263]. The biological half-life of NfL is unknown, but it seems to be the longest among neurobiomarkers, and half-lives of several days or weeks have been suggested [264,265].

The first study of NfL in prognostication after CA was conducted by Rosén et al. in 2004; it assessed NfL concentrations in CSF at 12 days after

CA and found NfL to be a significant predictor of unfavourable outcome with high specificity and sensitivity [266]. The first study of plasma NfL after CA was conducted in 2014 by Rana et al. [267]. The predictive ability was excellent: AUROCs were 0.93 at 24 h and 0.92–0.99 at 3–7 days after CA. The laboratory method in this study was enzyme-linked immunosorbent assay (ELISA)-based. A comparable prognostic ability was found in a TTM substudy by Moseby-Knappe et al. It measured the NfL concentrations of 717 OHCA patients using the novel ultrasensitive SIMOA platform [134]. The concentration differences between patients with unfavourable and favourable outcome were greater than in the earlier study, and the AUROCs were 0.94-0.95. NfL demonstrated the best predictive accuracy among all the neurobiomarkers and had 29–49% better sensitivities (with similar specificities) than EEG, SSEP, CT, and pupillary and corneal reflexes [134]. Importantly, NfL levels were not affected by haemolysis. Overall, the study suggests that NfL is the best prognostic test among biomarkers, imaging, and neurophysiological studies.

In two other studies on OHCA patients, the prognostic ability of NfL was lower (AUROCs of 0.82 and 0.79), but still clearly better than that of NSE [268,269]. Those studies included only single NfL measurements, and in one of those studies, the outcome was defined at hospital discharge. In a third study, NfL had good prognostic accuracy, and after adding NfL to the prognostication model that was made according to ERC-ESICM guidelines, the sensitivity of the model improved significantly without false positives [270]. Even though those three studies presented good but not excellent accuracy, NfL demonstrated benefits compared to NSE and to clinical model. Together with excellent ability that was found in the TTM study [134] and in study by Rana et al. [267], NfL seems to be very promising in prognostication after CA.

In a study that measured biomarker release during secondary HIBI, the release of NfL and Tau from brain to circulation was more significant than the release of NSE, UCH-L1, GFAP, suggesting the superiority of axonal markers in the assessment of secondary HIBI [82]. The importance of WM in the assessment of HIBI and neurological prognosis was also suggested in an MRI study [84]. Taken together, axonal neurobiomarkers have

demonstrated the best ability among all neurobiomarkers to predict unfavourable outcome after CA, and NfL is the most promising biomarker overall. However, the evidence on NfL in prognostication after CA and importance of axonal injury in HIBI is still limited.

3 AIMS OF THE STUDY

The aims of this study were:

1) To assess the ability of NSE to predict unfavourable long-term outcome after OHCA in subgroups divided into quartiles according to patient's age and time from collapse to ROSC (Study I).
2) To assess the ability of UCH-L1 to predict unfavourable long-term outcome after OHCA and compare it to that of NSE (Study II).
3) To assess the ability of NfL to predict unfavourable long-term outcome after OHCA and compare it to that of NSE (Studies III and IV), and to assess the impact of two different PaO_2, $PaCO_2$, and mean arterial pressure (MAP) targets on NfL concentrations (Study III).

4 SUBJECTS AND METHODS

4.1 STUDY DESIGN AND PATIENTS

4.1.1 FINNRESUSCI population: Studies I, II, and IV

Studies I, II, and IV are post-hoc laboratory analyses of the FINNRESUSCI study, which was a nationwide observational prospective study conducted between 1 March 2010 and 28 February 2011 in 21 Finnish ICUs [48]. All 5 university hospitals and 14 of 15 central hospitals in Finland participated in the study, and over 98% of Finnish people live in the referral area of these ICUs. The FINNRESUSCI initially included 548 adult (≥18 years) individuals who were resuscitated from OHCA and treated in ICUs and finally included those 504 patients who were unconscious. The study included individuals with shockable (VF and VT) and nonshockable (ASY and PEA) initial rhythms, and with various causes of cardiac arrest. The FINNRESUSCI evaluated the post-resuscitation care of OHCA patients and examined the effect of TH on 12-month outcome. The patients were not treated according to any study-specific protocols; they were treated according to the prevailing guidelines and the local protocols of each ICU [88]. TH treatment was recommended in patients whose arrest was witnessed, whose initial rhythm was shockable, and who were comatose at ICU admission. Informed consent was obtained from the patients' next-of-kin. NSE analyses were available in 6 of the participating hospitals that treated 35.3% of the study patients, and NSE was used in prognostication in some of those patients. Blood samples were collected, frozen, and stored during the original study for later use. All those patients whose blood samples were available were included in Studies I, II, and IV. The flowchart is presented in Figure 3.

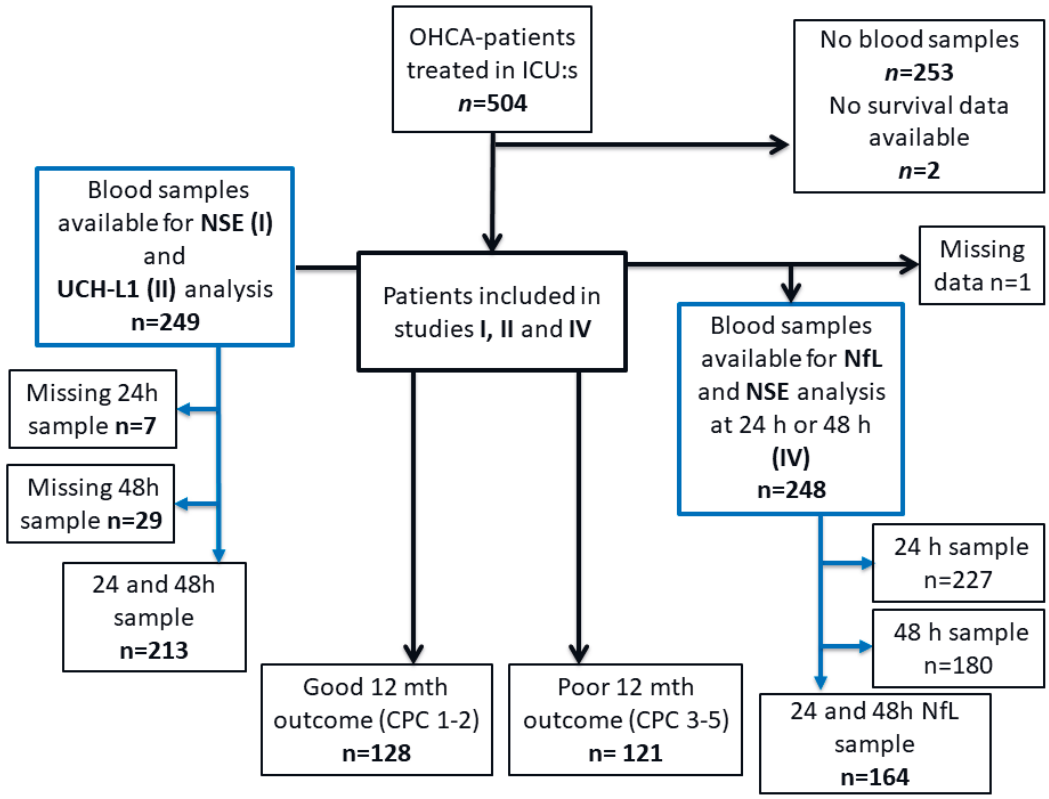

Figure 3. Flowchart of the FINNRESUSCI population (studies I, II and IV). Abbreviations: OHCA, out-of-hospital cardiac arrest; NfL, neurofilament light; NSE, neuron-specific enolase; UCH-L1, ubiquitin c-terminal hydrolase L1; CPC, Cerebral Performance Category.

Studies I and II included 249 of 504 comatose OHCA patients of the FINNRESUSCI study that were treated in ICUs. We analysed NSE and UCH-L1 concentrations of those patients at 24 and 48 h after CA. We excluded 255 patients whose blood samples were not available. At 24 h, written consent was not available for seven patients, so those NSE and UCH-L1 samples are missing.

In Study I, we defined the prognostic value of NSE at 24 and 48 h after CA to predict unfavourable long-term neurological outcome. We defined the prognostic ability of NSE and assessed the additional value of NSE to the clinical prognostic model in all patients pooled together and separately in age and ROSC quartiles. To separate the possible effect of NSE values previously used in prognostication on the current analysis, we performed a sensitivity analysis on the predictive value of NSE separately in patients treated in hospitals where NSE measurement was available and in patients treated in hospitals where it was not. We did not use the same NSE samples that were used during the FINNRESUSCI study; all the biomarkers in this study were analysed post hoc from stored blood samples.

In Study II, we measured the prognostic ability of UCH-L1 to predict unfavourable long-term neurological outcome at 24 h and 48 h after CA. We compared the prognostic ability of UCH-L1 to that of NSE. We assessed the ability of UCH-L1 to find patients with a high probability of favourable outcome by using cutoff values with high sensitivities.

In study IV, we included a total of 248 patients for whom blood samples were available for NSE and NfL analysis at 24 or 48 h after CA. Overall, the patient cohort was almost the same as in Studies I and II (one patient with CPC 3–5 at 12 months is missing). We examined the prognostic ability of NfL to predict unfavourable neurological outcome at 24 and 48 h after CA and compared it to that of NSE. Moreover, we assessed the additional value that NSE and NfL give to the clinical prognostic model in all patients. We assessed the prognostic value of NfL in age and ROSC quartiles. We evaluated the ability of NfL and NSE to find patients with a high probability of favourable outcome by using cutoffs with high sensitivities, and the highest normal biomarker concentrations as cutoff values.

4.1.2 COMACARE population: Study III

Study III was a post-hoc laboratory analysis of the randomised, controlled pilot trial Carbon dioxide, Oxygen and Mean arterial pressure After Cardiac Arrest and REsuscitation (COMACARE) (NCT02698917) that included 120 comatose adult OHCA individuals [58,59]. The COMACARE study was conducted in six Finnish and one Danish ICUs between March 2016 and November 2017. COMACARE examined the effect of low-normal and high-normal arterial oxygen (PaO_2 10–15 kPa or 20–25 kPa) and carbon dioxide ($PaCO_2$ 4.5–4.7 kPa or 5.8–6.0 kPa) tensions and mean arterial pressures (MAP 65–75 mmHg or 80–100 mmHg) within the first 36 h in ICU on outcome. The primary outcome definition was NSE concentration at 48 h, and secondary outcome included NSE, S100B, and cardiac troponin (at 24, 48, and 72 h after CA); cerebral oxygenation; and epileptic activity and neurological outcome (at 6 months). The interventions were conducted using a 2^3 factorial design. All the patients were treated with TTM (33°C or 36°C). Laboratory samples for later analyses were collected, frozen, and stored only in Finnish hospitals that were participating in the current study. COMACARE included only comatose (GCS-M < 5) patients 18–80 years old with a shockable (VF or VT) initial rhythm, with a suspected or confirmed cardiac origin of arrest, and a time from collapse to ROSC of 10–45 min. Individuals who were pregnant or had assumed or confirmed intracranial pathology (e.g., haemorrhage), severe chronic obstructive pulmonary disease (COPD), or severe hypoxaemia (PaO_2/FiO_2 < 100 mmHg), were excluded. Informed consent from the patients' next-of-kin was obtained as soon as possible, and consent was also obtained afterwards from those patients who recovered sufficient neurological function. Neurological prognostication was performed according to current guidelines, including the assessment of NSE [44,49].

Study III focused on NfL neurobiomarker concentrations and their ability to predict unfavourable long-term outcome at ICU admission and at 24, 48, and 72 h after CA. An additional subject of interest was the possible effect of low-normal and high-normal PaO_2, $PaCO_2$, and MAP on NfL levels. We compared the prognostic ability of NfL to NSE and S100B, as they are

widely studied and NSE is included in the current guidelines. To find patients with a high probability of favourable outcome, we defined the cutoff values for NfL to predict unfavourable outcome with high sensitivity. We included all the patients whose blood samples were available; study III included a total of 112 patients out of the original 120 COMACARE patients from whom blood samples were available. The blood samples from all four time points were available in 103 (94.5%) of the patientsThe flofchart of study population is presented in Figure 4.

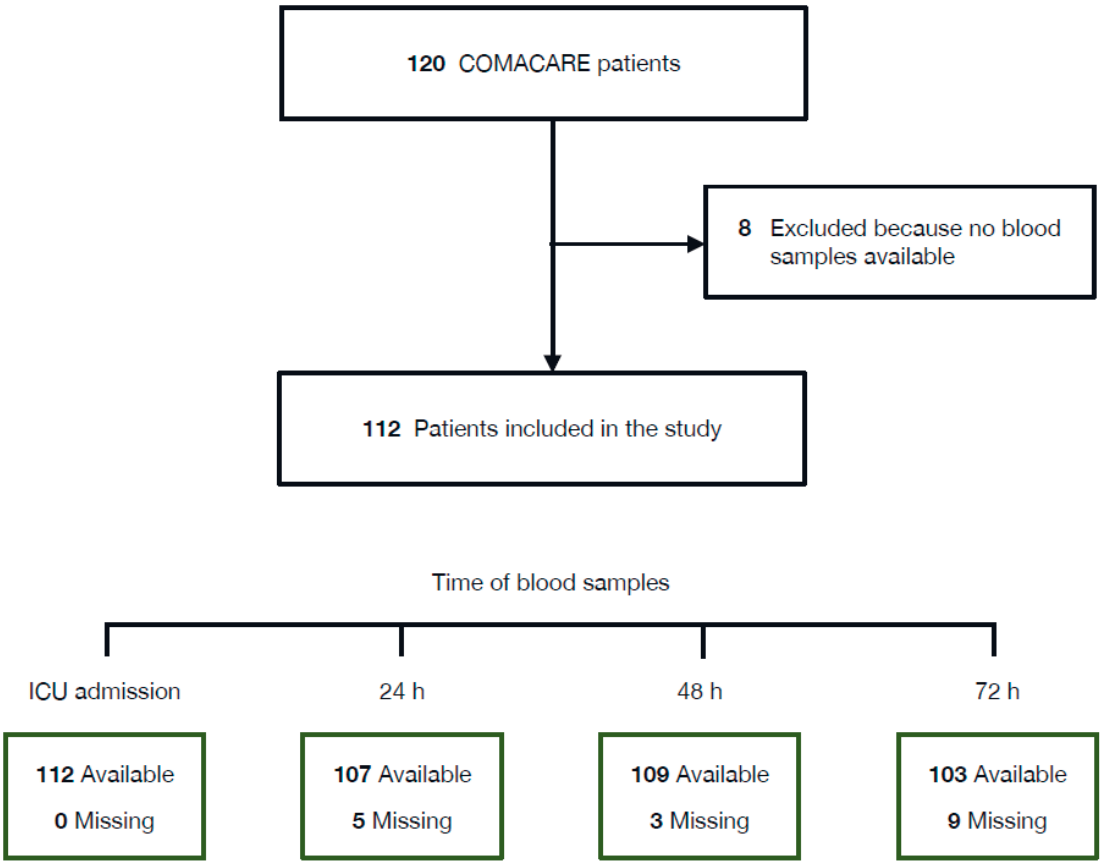

Figure 4. Flowchart of the COMACARE population (study III).
Abbreviations: ICU, intensive care unit

4.2 DATA COLLECTION

4.2.1 Studies I, II, and IV

Patient data from the FINNRESUSCI study were collected using Internet-based case report forms. Data on previous health status and diseases were obtained from patients' medical history. The aetiology of CA (cardiogenic or other) was defined according to the Utstein criteria [27]. The patient data recorded during hospital treatment, including hospital mortality, were collected to the Finnish Intensive Care Consortium (FICC) database. Mortality data were obtained from Statistic Finland. Blood samples in the FINNRESUSCI study were collected at 24 and 48 h after CA from patients whose next-of-kin had provided written consent.

4.2.2 Study III

Patient data from COMACARE study patients (including age and previous health status) were collected in an electronic database (Absolute Imaginary Software, Finland). Patient data during the first 48 h (including arterial blood pressure, $EtCO_2$, and SpO_2) were added every 10 min to a medical tablet computer connected to a patient monitor. The blood samples for biomarker analysis were taken at ICU admission (0 h) and at 24, 48, and 72 h after CA from patients whose next-of-kin had provided by written consent.

4.3 OUTCOMES

In Studies I, II, and IV, neurological outcome was assessed at 12 months after CA via phone contact between the patient and a specialist in neurology, who was blinded to patient care and laboratory results. The neurological outcome was defined according to CPC classification (Table 1.), and a structured interview was used in its assessment. In all studies, we defined favourable outcome as CPC 1–2 and unfavourable outcome as CPC 3–5. We chose death in hospital as a secondary outcome (Studies I and II).

In Study III, a specialist in neurology who was blinded to study treatments and biomarker results made phone contact with patients 6 months after CA and defined their neurological outcome according to CPC criteria. We chose CPC 1–2 as favourable outcome and CPC 3–5 as unfavourable (Table 1).

4.4 LABORATORY METHODS

In Studies I, II, and IV, the blood samples were obtained at 24 and 48 h after CA. They were allowed to clot at room temperature for 60 min. Then, the blood samples were centrifuged and frozen at -70°C for later analysis. In Study III, the blood samples were obtained at the time of ICU admission and at 24, 48, and 72 h after CA. The blood samples were centrifuged for 10 min at 2000 G and frozen at -70°C for later analysis. All blood samples were thawed immediately before analysis.

4.4.1 Study I

Serum NSE concentrations were measured with a commercial electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). All analyses were conducted in the same laboratory in April 2015. The laboratory staff were blinded to clinical patient data. The measurement range of NSE was 0.05–740 µg/L, and the samples were diluted two-fold if necessary. The inter- and intra-assay coefficients of variation (CV) were <3.9% and <3.2%. Significant haemolysis was defined by assessing the amount of free haemoglobin and considered values above 500mg/L to be significant [179]. In case of significant haemolysis, we decided to analyse NSE concentrations in a way that imitated a real-life clinical situation in Finland where laboratories typically do not report haemolysis. We reported NSE results and outcomes of each individual whose blood sample was significantly haemolysed.

4.4.2 Study II

Serum UCH-L1 concentrations were measured using a commercial ELISA kit (USCN, Wuhan, China) in March 2015. All analyses were conducted in the same laboratory with the staff blinded to clinical patient data. All samples were analysed in duplicate, and the intra- and inter-assay CVs were <7.5% and <11.5%. The samples were diluted 4-fold, or if necessary, 20-fold.

4.4.3 Study III

Plasma NfL concentrations were measured using a commercial SIMOA Nf-Light immunoassay (Quanterix, Billerica, MA, USA). The analyses were performed in September 2019 at the Clinical Neurochemistry Laboratory of the University of Gothenburg, whose staff were blinded to clinical data and outcomes. All blood samples were diluted four-fold, and to complete the analysis, a single batch of reagents was utilised for eight analytical runs. In the low-concentration control sample, NfL was 6.9 ng/L and inter- and intra-assay CVs were 8.9% and 7.4%, respectively. For the high-concentration control sample (NfL 55.1 ng/L), the corresponding CVs were 10.4% and 7.1%.

Serum NSE and S100B concentrations were measured using a COBAS e601 line (Hitachi High Technology Co, Tokyo, Japan) with an electrochemiluminescent immunoassay kit (Roche Diagnostics GmbH, Mannheim, Germany). Analyses were conducted in January 2018 in one laboratory (ISLAB, Kuopio, Finland). The laboratory staff were blinded to clinical patient data and outcomes. Possible haemolysis was tested for in all the samples using the Roche haemolysis index. We considered a haemolysis level of >500mg of free haemoglobin per litre to be significant, and we excluded those samples from the calculation of prognostic ability.

4.4.4 Study IV

Plasma NfL concentrations were measured using a commercial two-step digital immunoassay using the single-molecule array Quanterix SIMOA™

NF-Light® Kit and SIMOA™ HD-1 analyser (SIMOA™, Quanterix Corporation, Lexington, MA, USA). Analyses were conducted Milan, Italy, in January 2020. Laboratory staff were blinded to clinical patient data and outcomes. Serum NSE concentrations for comparison were the same as in Study I.

4.5 STATISTICAL METHODS

We present all categorical patient data as absolute numbers with percentages (95% CIs) and all continuous data (e.g., biomarker concentrations) as medians (with interquartile ranges [IQRs]). For categorical data, we used Pearson's Chi test or Fisher's exact test (as appropriate) for comparison. We tested the normality of distribution with the Kolmogorov-Smirnov test. For a comparison of normally distributed variables, we used an independent sample *t*-test, and for variables that were not normally distributed, we used a Mann-Whitney *U* test or Kruskall-Wallis test as appropriate. We considered $p < 0.05$ as significant for Studies I–IV. We conducted statistical analyses with SPSS (SPSS, Chicago, IL, USA) version 21 (Study I), version 25 (Study III), and version 27 (Studies II and IV); and R (R Foundation, Vienna, Austria) version 3.1.1 (Study I), version 4.0.4 (Study II), version 3.5.1 (Study III), and 4.0.0 (Study IV).

To determine the ability of the biomarkers to predict unfavourable outcome, we calculated the AUROCs [271] with 95% CIs. We compared the AUROCs with the bootstrap method, which is within the roc.test function (R program, https://www.rdocumentation.org). We assessed the cutoff values for biomarkers from the ROCs. In Studies I–IV, we defined the optimal cutoffs to predict unfavourable outcome using the Youden method, which maximises specificity and sensitivity [272,273].

In Studies I and IV, we divided patients into quartiles according to their age and time to ROSC. We determined the prognostic ability of NSE (Study I) and NfL (Study IV) in those quartiles by calculating the AUROCs to predict unfavourable outcome, then compared them with the bootstrap method.

We assessed cutoff values for high (95–100%) specificities to minimise the number of patients incorrectly categorised into the unfavourable outcome category (targeting a low FPR; Studies I–IV). For the cutoffs

targeting a low FPR with high specificities, we also calculated sensitivities, specificities, positive and negative predictive values (PPVs and NPVs), and positive likelihood ratios (LR+). To find patients with a high probability of favourable neurological outcome, we defined the cutoffs corresponding to high sensitivities to minimise the number of false negative results (Studies II–IV). In Study IV, we used the highest normal biomarker concentrations, 17 µg/mL for NSE [184] and 55 pg/mL for NfL [15], to assess their ability to identify patients with a high probability of favourable outcome. For the cutoffs of low/normal biomarker concentrations, we calculated specificities, sensitivities, and NPVs.

We created baseline multivariable models with logistic regression to predict unfavourable outcome by using clinical variables such as age, initial rhythm, time to ROSC, and witnessed collapse. We calculated the odds ratios (ORs) for each variable, then added the biomarkers to the model, and then calculated the ORs for the biomarkers if they were significant variables in the model as defined by backward stepping in logistic regression (Studies I–IV). To find whether the addition of a biomarker could improve the predictive ability of the clinical multivariable model, we separately calculated the AUROCs for the model alone and the model with the biomarker, and then compared the AUROCs (Studies I and III).

We also assessed the net reclassification improvement (NRI) by adding biomarkers (NSE and NfL) to the model (Studies I and III). We defined event NRI (NRIe) and non-event NRI (NRIne). NRIe is ([the number of patients with the predicted event given a higher risk after the addition of a biomarker] – [the number of patients with the event given a lower risk]) / (the number of patients with the event). NRIne is the net proportion of patients without the event given a lower risk. The sum of the NRIe and the NRIne is the overall NRI. The range of values for NRIe and NRIne is –1 to +1, and accordingly, for overall NRI it is –2 to +2 [274,275].

In Studies I and III, we calculated the integrated discrimination improvements (IDI) reached by adding biomarkers (NSE and NfL) to the multivariable models. We calculated event IDI (IDIe) for patients with unfavourable outcome as (mean probability of unfavourable outcome with baseline model + biomarker) – (mean probability of unfavourable outcome

with baseline model) and non-event IDI (IDIne) for individuals with favourable outcome as (mean probability of unfavourable outcome with baseline model) – (mean probability of unfavourable outcome with baseline model + biomarker). IDI is the sum of IDIe and IDIne. The range of IDIe and IDIne is –1 to +1, and for IDI the range is –2 to +2 [275,276].

4.6 ETHICS

In Studies I, II, and IV, the FINNRESUSCI study protocol (5070210) was approved by the ethics committee of each participating hospital (decision no. 137/2009). The first amendment to permit the collection of blood samples for later use was approved by the Ethics Committee of HUS in October 2010. The amendment for Studies I and II was accepted by Siun Sote/North Karelian Central Hospital (decision no. 2141/12.00.01.01/2017). The amendment for Study IV was accepted by Kuopio University Hospital in November 2020 (decision no. 148/2020).

The original COMACARE study protocol was accepted by the Ethics Committee of Northern Savo Hospital District, Finland (decision no. 295/2015). The first amendment, including the plan for the current analysis of Study III, was approved in December 2017, and the second amendment, including post-hoc analysis of NfL, was approved in February 2019 by the Ethics Committee of Northern Savo Hospital District.

4.7 PERMISSIONS

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5 RESULTS

5.1 STUDY PATIENTS AND BASELINE CHARACTERISTICS

5.1.1 Studies I, II, and IV

Unfavourable outcome, defined as CPC 3–5 at 12 months, occurred in 121 patients (48.6%). In Study II, the secondary outcome was hospital survival, and in total, 86 patients (34.5%) died during hospital treatment. The flowchart of the study population is presented in Figure 3. in the Subjects and Methods section.

The aetiology of CA was assessed as cardiogenic in 79.1% of the patients, being slightly more common in patients with favourable outcome (82.8%) than in those with unfavourable outcome (75.0%). The initial rhythm was shockable in 71% of the patients.

Based on the availability of NSE during the FINNRESUSCI study, NSE was available in 88 of the study patients (35.3%) in 9 of 21 hospitals. In those individuals who were treated in hospitals that utilised NSE in prognostication, the numbers of witnessed collapses, bystander CPR, and shockable initial rhythms were higher, the median Simplified Acute Physiology Score (SAPS II) points were higher, and the percentage of patients with unfavourable outcome lower (38.6% vs 54 %, $p = 0.020$).

The study population significantly differed from the other FINNRESUSCI study patients in number of male patients (84% vs 69%, $p < 0.001$), shockable rhythms (71.4% vs 44.7%, $p < 0.001$), and TTM (77.4% vs 39.7%, $p < 0.001$). The baseline characteristics of the patients in Studies I, II, and IV are presented in Table 5.

Table 5. Baseline characteristics of the FINNRESUSCI patients (studies I, II and IV).

	All	Hospital mortality			12 mth outcome		
	n=249	Survivors n=163	Non-survivors n=86	p	CPC 1-2 n=128	CPC 3-5 n=121	p
Age, years (IQR)	63.0 (56.5-71)	62.0 (56.0-70.0)	66.0 (59.0-72.0)	0.019	61.5 (55.3-67.0)	67.0 (59.0-72.0)	0.001
Sex, males, n (%)	209 (83.9)	139 (85.3)	70 (81.4)	0.428	107 (83.6)	102 (84.3)	0.88
Witnessed CA, n (%)	227 (91.2)	152 (93.3)	75 (87.2)	0.11	123 (96.1)	104 (86.0)	0.005
Bystander CPR, n (%)	146 (58.6)	92 (56.4)	54 (62.8)	0.333	78 (60.9)	68 (56.2)	0.448
Shockable rhythm, n (%)	177 (71.1)	128 (78.5)	49 (60.0)	<0.001	106 (82.8)	71 (58.7)	<0.001
Time to ROSC, min (IQR)	20.0 (13.5-28.0)	17.0 (11.0-23.0)	26.0 (20.0-31.3)	<0.001	16.0 (11.0-23.0)	24.0 (19.0-31.0)	<0.001
TTM, n (%)	193 (77.5)	126 (77.3)	67 (77.9)	0.913	100 (78.1)	93 (76.9)	0.811
SAPS II score (IQR)	58.0 (42.0-69.0)	52.0 (36.0-63.0)	67.0 (58.8-73.0)	<0.001	47.0 (34.0-60.8)	65.0 (55.5-71.0)	<0.001
SOFA score (IQR)	9 (7-11)	8 (6-10)	11 (9-11)	<0.001	8 (6-10)	10 (8-11)	0.004
CA aetiology, n (%)				0.111			0.556
Cardiogenic		134 (82.2)	63 (73.3)		106 (82.8)	91 (75.0)	
Hypoxia		6 (3.7)	5 (5.8)		4 (3.1)	7 (5.8)	
Drowning		2 (1.2)	3 (3.5)		2 (1.6)	3 (2.5)	
Hypothermia		1 (0.6)	0 (0)		1 (0.8)	0 (0)	
Intoxication		4 (2.5)	2 (2.3)		3 (2.3)	3 (2.5)	
Trauma		1 (0.6)	0 (0)		1 (0.8)	0 (0)	
Other etiologies		2 (1.2)	6 (7.0)		2 (1.6)	6 (5.0)	
Unknown		8 (4.9)	1 (1.2)		5 (3.9)	4 (3.3)	
Missing		5 (3.1)	6 (7.0)		4 (3.1)	7 (5.8)	

Abbreviations: CPC, Cerebral Performance Category; VF, ventricular fibrillation; VT, ventricular tachycardia, PEA, pulseless electronic activity; ASY, asystole; CPR, cardiopulmonary resuscitation; ROSC, return of spontaneous circulation; IQR, interquartile range; CA, cardiac arrest, SAPS II, Simplified Acute Physiology Score; SOFA, Sequential Organ Failure Assessment (based on the first 24 h in the intensive care unit); TTM, targeted temperature management.

The baseline characteristics of the patients in Studies I and IV according to age and time to ROSC are presented in Table 6.

Table 6. Baseline characteristic of FINNRESUSCI patients according to age and ROSC quartiles. Abbreviations: ROSC, return of spontaneous circulation; IQR, interquartile range; Witnessed, witnessed collapse; CPR, cardiopulmonary resuscitation; SAPS II, Simplified Acute Physiology Score; CPC, Cerebral Performance Category.

	Age, years					**Time to ROSC, min**				
	18-56	**57-63**	**64-71**	**≥72 years**	**p**	**1-13 min**	**14-20 min**	**21-28 min**	**≥29 min**	**p**
Number of patients, n	62	69	59	59		62	69	58	60	
Age, median (IQR), years	48.0 (42.0-54.0)	60.0 (59.0- 62.0)	67.0 (66.0- 70.0)	77.0 (73.0-81.0)		63.0 (57.0-74.3)	63.0 (55.5-70.5)	64.5 (57.0-71.0)	62.0 (54.0-69.0)	0.608
Gender, males, n, (%)	50 (80.6)	60 (87.0)	52 (88.1)	47 (79.7)	0.469	53 (85.5)	55 (79.7)	50 (86.2)	51 (85.0)	0.730
Witnessed, n (%)*	56 (90.3)	68 (98.6)	51 (86.4)	52 (88.1)	0.071	58 (93.5)	63 (91.3)	51 (87.9)	55 (91.7)	0.750
Bystander CPR, n (%)	34 (54.8)	43 (62.3)	41 (69.5)	28 (47.5)	0.084	31 (50.0)	40 (58.0)	40 (69.0)	35 (58.3)	0.215
Cardiogenic reason of arrest, n (%)	41 (66.5)	57 (82.6)	50 (84.7)	51 (86.4)	0.018	50 (80.6)	56 (81.2)	47 (81.0)	46 (76.7)	0.913
Time to ROSC, median (IQR), min	20.0 (14.0-29.3)	21.0 (13.0- 30.0)	23.0 (16.0- 28.0)	19.0 (10.0-24.0)	0.138	10.0 (6.0- 11.0)	17.0 (15.0-19.5)	24.0 (22.8-26.0)	34.0 (30.3-40.0)	
Shockable rhythm, n (%)	42 (67.7)	49 (71.0)	45 (76.3)	41 (69.5)	0.757	43 (69.4)	50 (72.5)	40 (69.0)	44 (73.3)	0.934
SAPS II, median (IQR), points	52.0 (32.0-61.0)	57.0 (40.0- 64.0)	60.0 (42.0- 72.0)	67.0 (52.0-73.0)	<0.001	47.5 (33.0-60.8)	56.0 (37.5-65.5)	60.5 (45.8-69.0)	64.5 (52.3-71.8)	<0.001
CPC 3-5, n (%)	25 (40.3)	26 (37.7)	34 (57.6)	36 (61.0)	0.014	16 (25.8)	24 (34.8)	38 (65.5)	43 (71.7)	<0.001

5.1.2 Study III

The 6 months outcome was unfavourable in 39 patients (35%). Of the patients with unfavourable outcome, two were classified to CPC 3, and 37 died. The flowchart of Study III is presented in Figure 4. in the Subjects and Methods section.

HIBI was the cause of death in 86% of those who died. WLST occurred for 32 of the patients during ICU treatment. In the prognostication for those patients, EEG was used in 25 cases (78.1%), SSEP in 5 (15.6%), brain CT in 20 (62.5%), and brain MRI in 7 (21.9%); at least one method was used in 100% of patients with WLST. Baseline characteristics of the patients in Study III are presented in Table 7.

Table 7. Baseline characteristics of the study patients.

	All patients n=112	**CPC 1-2 n=73**	**CPC 3-5 n=39**	**p**
Age, median (IQR), y	62 (53-68)	58 (51-66)	66 (58-75)	0.004
Male sex, n (%)	92 (82.1)	61 (83.6)	31 (79.5)	0.592
Weight, median (IQR), kg	85.0 (72.3-93)	85.0 (72.5-94)	83.0 (70.0-90.0)	0.646
Neurological function before cardiac arrest				1
Normal, CPC 1, n (%)	103 (92)	67 (91.8)	36 (92.3)	
Some disability, CPC 2, n (%)	9 (8)	6 (8.2)	3 (7.7)	
Medical history				
Hypertension, n (%)	56 (50)	33 (45.2)	23 (59)	0.165
Chronic heart failure (NYHA 3 or 4), n (%)[a]	9 (8)	4 (5.5)	5 (12.8)	0.151
Smoker, n (%)[b]	35 (31.3)	22 (30.1)	13 (33.3)	0.235
Resuscitation factors				
Bystander life support, n (%)	93 (83)	66 (90.4)	27 (69.2)	0.004

Time to ROSC, median (IQR), min	21.1 (16.2-26)	17.2 (14.9-22.3)	25.0 (22-31.6)	<0.001
Clinical status on ICU admission				
GCS, median, (IQR)[c]	3 (3-3)	3 (3-5)	3 (3-3)	<0.001
APACHE II score, median (IQR)	28 (24-31)	27 (24-29)	31 (26-35)	<0.001
TTM				0.003
33°C, n (%)	75 (67)	56 (76.7)	19 (48.7)	
36°C, n (%)	37 (33)	17 (23.3)	20 (51.3)	

Abbreviations: CPC, Cerebral Performance Category; IQR, interquartile range; NYHA, New York Heart Association; ROSC, return of spontaneous circulation; ICU, intensive care unit; GCS, Glasgow Coma Scale; SD, standard deviation; APACHE II; Acute Physiology And Chronic Health Evaluation.
[a] data missing for 2 patients; [b] data missing for 13 patients; [c] data missing for 9 patients

5.2 PROGNOSTIC ACCURACY AND CONCENTRATIONS OF BIOMARKERS

5.2.1 NSE, UCH-L1, and NfL in the FINNRESUSCI population

In Study I, NSE concentrations were significantly higher for patients with unfavourable outcome than for those with favourable outcome at 24 and 48 h ($p < 0.001$ at both time points). At 24 h, the median NSE concentration was 12.9 µg/L (IQR 7.6–23.6) in patients with unfavourable outcome and 8.9 µg/L (5.9–13.4) in those with favourable outcome. At 48 h, the concentrations were 17.9 µg/L (8.1–56.4) and 8.2 µg/L (5.9–12.1), respectively.

The AUROCs to predict unfavourable outcome were 0.65 (0.58–0.72) at 24 h and 0.72 (0.65–0.80) at 48 h. Prognostic ability was better at 48 h than at 24 h ($p = 0.005$). For the change in NSE between 24 and 48 h, the AUROC to predict unfavourable outcome was 0.70 (0.63–0.78), not significantly different from the single NSE measurement at 48 h ($p = 0.489$). We found

significant haemolysis (≥500mg/L) in three patients that were included in the NSE analysis; two of them had CPC 1–2 (48 h NSE 36 µg/L and 37 µg/L), and one patient with an NSE at 48 h of 6.8 µg/L died (CPC 5). After excluding those individuals, the prognostic ability of NSE did not change (AUROC 0.73 [0.66–0.81] at 48 h).

In Study II, concentrations of UCH-L1 were significantly higher at 24 and 48 h in patients with unfavourable outcome compared to those that reached favourable outcome ($p < 0.001$ at both time points). At 24 h, the median concentration of UCH-L1 was 10.8 ng/mL (IQR 7.5–18.5) in patients with unfavourable outcome and 7.8 (5.9–11.8) in those with favourable outcome. At 48 h, the concentrations were 16.2 ng/mL (12.2–27.7) and 11.5 ng/mL (9.0–17.2), respectively. The AUROC to predict unfavourable outcome was 0.66 (0.60–0.73) at 24 h and 0.66 (0.59-0.74) at 48 h. The prognostic ability of UCH-L1 was not significantly different from that of NSE at 24 h ($p = 0.82$) or at 48 h ($p = 0.827$).

Regarding secondary outcome (death in hospital), the UCH-L1 concentrations were higher in those who died than in those who survived ($p < 0.001$ at 24 h and 48 h). At 24 h, the median UCH-L1 concentration was 12.6 ng/mL (IQR 8.5–20.6) in patients who died and 7.9 ng/mL (6.1–12.1) for those who survived. The concentrations were 17.1 ng/mL (12.6–30.0) and 12.1 (9.1–18.4), respectively, at 48 h. The AUROC for UCH-L1 to predict death in hospital was 0.69 (0.62–0.76) at 24 h and 0.68 (0.60–0.78) at 48 h. For NSE, the corresponding AUROCs were 0.70 (0.63–0.77) and 0.76 (0.68–0.83).

In Study IV, the NfL concentrations were up to 20-fold higher for patients with unfavourable outcome compared to those that reached favourable outcome. At 24 h, the median NfL concentration was 688.9 pg/mL (IQR 146.1–1803.8) for individuals with unfavourable outcome and 30.9 pg/mL (16.9–61.2) for those with favourable outcome ($p < 0.001$). At 48 h, the concentrations were 1162.4 pg/mL (146.8–4360.5) and 35.6 pg/mL (21.3–86.7), respectively ($p < 0.001$). The aetiology of CA (cardiogenic vs others) had no effect on NfL levels according to outcome. NfL had a good/excellent ability to predict unfavourable outcome; at 24 h, the AUROC was 0.90 (0.86–0.94), and at 48 h, the AUROC was 0.88 (0.83–0.94). The prognostic

ability of NfL was significantly better than that of NSE at 24 h and 48 h ($p < 0.001$ for both).

Concentrations of NSE, UCH-L1, and NfL at 24 and 48 h according to outcome definition at 12 months are presented in Figure 5.

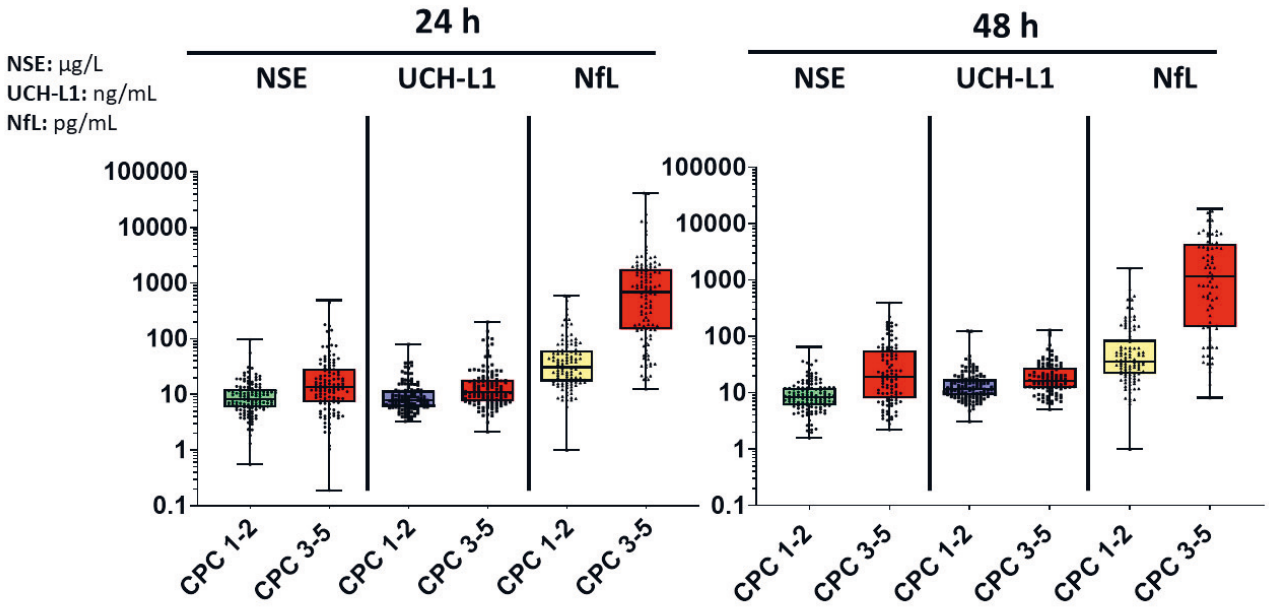

Figure 5. Concentrations of NSE, UCH-L1 and NfL at 24 h and 48 h according to 12-month neurological outcome (CPC 1-2 indicating favourable outcome and CPC 3-5 unfavourable outcome). The scale is logarithmic. Each box represents the interquartile range, the line inside the box shows the median value and the whiskers show minimal and maximal concentrations. Circles, triangles, and squares presents concentrations of each individual.
Abbreviations: NSE, neuron-specific enolase; UCH-L1, ubiquitin c-terminal hydrolase L1; NfL, neurofilament light; CPC, Cerebral Performance Category.

The AUROCs for NfL to predict unfavourable outcome were higher than those of UCH-L1 and NSE. The AUROCs for UCH-L1 to predict unfavourable outcome were slightly (not significantly) lower than those of NSE, which were lower than those of NfL. A condensed comparison of the prognostic abilities of biomarkers, AUROCs (95% CIs), and ROC curves for the biomarkers studied in the FINNRESUSCI patients is presented in Figure 6.

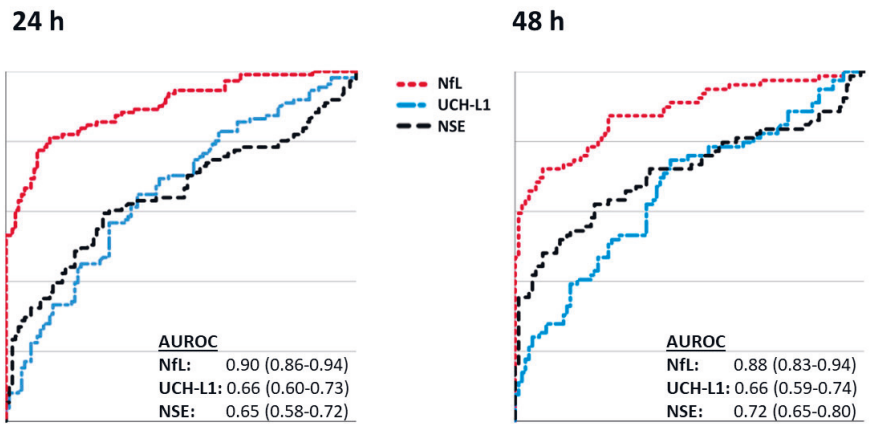

Figure 6. AUROCs (with 95% confidence intervals) for NfL, UCH-L1 and NSE at 24 h and 48 h after cardiac arrest as predictors of unfavourable neurological outcome at 12 months.
Abbreviations: AUROC, the area under the receiver operating characteristic curve; NfL, neurofilament light; UCH-L1, ubiquitin c-terminal hydrolase L1; NSE, neuron-specific enolase.

5.2.2 NfL, NSE, and S100B in the COMACARE population

This study contained a highly selected cohort of OHCA patients with shockable initial rhythms. NfL concentrations were significantly higher for patients with unfavourable outcome at 6 months than for those with favourable outcome at ICU admission and at 24, 48, and 72 h after CA. At 48 h, the difference in NfL concentration between patients with

unfavourable and favourable outcome was 100-fold. The ability of NfL to predict unfavourable outcome was excellent and stable at 24–72 h: the AUROC was 0.98 (0.97–1.00) at 24 and 48 h and 0.98 (0.95–1.00) at 72 h. Moreover, NfL had an excellent ability to discriminate patients with HIBI, with the highest AUROC of 0.97 (0.95–1.00) at 48 h. The TTM target had no impact on the prognostic ability of NfL: the AUROCs at 24–72 h were 0.97–0.98 (95% CI 0.93–1.00) in the group that targeted 33°C and 0.99 (0.96–1.00) in the group that targeted 36°C. The NfL concentrations at all time points according to outcome are presented in Figure 7.

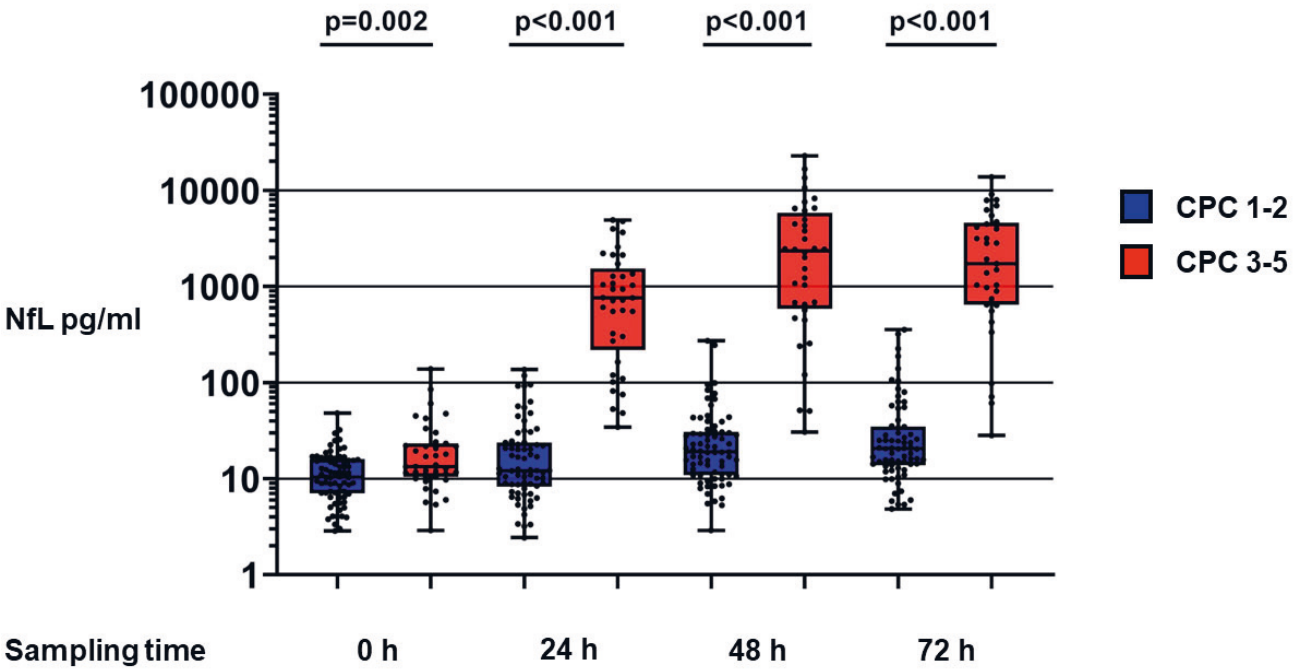

Figure 7. NfL concentrations at ICU admission and 24, 48 and 72 h after cardiac arrest for patients with favourable (CPC 1-2) and unfavourable (CPC 3-5) outcome. The scale is logarithmic. Each box represents the interquartile range, the line inside the box shows the median value, the whiskers show minimal and maximal concentrations and dots show concentrations of each individual.
Abbreviations: NfL, neurofilament light; CPC, Cerebral Performance Category.

NSE presented a good and S100B a satisfactory ability to predict unfavourable outcome. For NSE, the best predictive ability was at 72 h (AUROC 0.89 [IQR 0.81–0.96]). For S100B, the best accuracy was at 24 h (AUROC 0.77 [0.67–0.88]). The AUROCs for NfL to predict unfavourable outcome were significantly higher than those of NSE ($p < 0.001$ at 24 and 48 h; $p = 0.012$ at 72 h) and S100B ($p < 0.001$ at 24–72 h). At ICU admission, all biomarkers showed unfavourable prognostic ability. The ROC curves and corresponding AUROCs are presented in Figure 8.

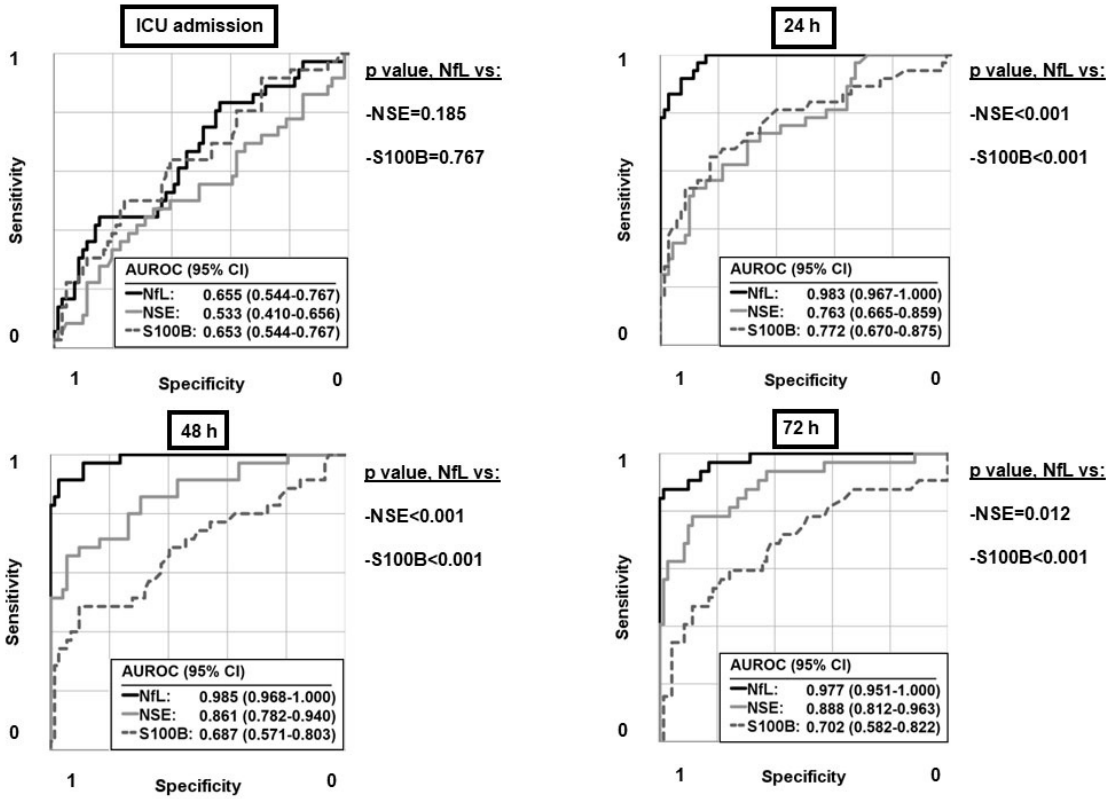

Figure 8. Receiver operating characteristic (ROC) curves and areas under the ROC curves (AUROC) with 95% confidence intervals (CI) for NfL, NSE and S100B at time of intensive care unit admission and at 24 h, 48 h and 72 h after cardiac arrest as predictors of unfavourable outcome (CPC 3-5) at six months.
Abbreviations: NfL, neurofilament light; NSE, neuron-specific enolase; S100B, protein S100B; ICU, intensive care unit.

5.2.3 Impact of haemolysis

Haemolysis can significantly affect NSE concentrations. We measured the amount of free haemoglobin in all blood samples and assessed haemolysis indices. Significant haemolysis (≥500 mg/L) was scarce and was detected in totally seven samples at all time points. Detectable haemolysis (≥100mg/L) was more common and was found in 153 (35%) of the samples. NfL and NSE concentrations at different time points in relation to haemolysis are presented in Table 8.

Table 8. Numbers (with percentages) of haemolysed samples at ICU admission (0 h) and 24, 48 and 72 h after cardiac arrest for detectable (≥100mg of free haemoglobin per litre) and for significant (≥500mg of free haemoglobin per litre) haemolysis (**A**). Concentrations (median, IQR) of NfL and NSE at ICU admission (0h) and at 24, 48 and 72 h after cardiac arrest for samples with detectable haemolysis and for those without (**B**).

A		**0h**	**24h**	**48h**	**72h**
	Haemolysis index ≥100, n (%)	71 (63.4)	36 (32.1)	23 (20.5)	27 (24.1)
	Haemolysis index ≥500, n (%)	4 (3.6)	0 (0)	2 (1.8)	1 (0.9)
	Haemolysis index, median (IQR)	170 (120-260)	145 (120-228)	140 (120-280)	150 (110-250)

B			**No haemolysis**	**Haemolysis**	**p**
	0h	NfL	12.9 (8.9-18.3)	11.4 (7.2-16.9)	0.199
		NSE	19.2 (16.2-24.7)	27.2 (21.8-33.5)	<0.001
	24h	NfL	34.5 (11.5-644.2)	16.2 (6.9-66.4)	0.019
		NSE	22.7 (18.2-34.2)	30.9 (25.3-36.7)	0.003
	48h	NfL	28.8 (15.5-586.9)	43.8 (13.8-680.9)	0.614
		NSE	18.7 (13.5-33.2)	28.2 (24.7-36.6)	0.002
	72h	NfL	48.0 (16.1-912.3)	25.0 (14.3-62.0)	0.115
		NSE	16.5 (11.4-25.5)	21.4 (15.1-27.5)	0.08

Abbreviations: ICU, intensive care unit; IQR, interquartile range; NfL, neurofilament light; NSE, neuron-specific enolase.

NSE levels were significantly higher in samples with detectable haemolysis at ICU admission and at 24 and 48 h, whereas NfL was not affected by haemolysis. Moreover, NSE and free haemoglobin were significantly correlated (Spearman's Rho 0.354, $p < 0.001$). NfL had no correlation with free haemoglobin (Spearman's Rho 0.007, $p = 0.93$).

5.3 IMPACT OF AGE AND TIME TO ROSC ON THE CONCENTRATIONS AND PROGNOSTIC ABILITIES OF NSE AND NFL

5.3.1 Impact of age

We divided patients into quartiles according to age. For NSE at 48 h (Study I), the concentrations were significantly affected by higher age. In the oldest age quartile (≥72 years), the NSE concentrations did not differ between patients with unfavourable and favourable outcome ($p = 0.687$). In other age groups, NSE was higher for patients with unfavourable outcome than for those with favourable outcome. The distribution of NSE concentrations in age quartiles was different for patients with unfavourable outcome ($p = 0.033$) but not for those with favourable outcome ($p = 0.858$). The AUROC to predict unfavourable outcome was poor for the oldest patients at 0.53 (0.37–0.70). For the youngest patients (18–56 years), NSE had excellent prognostic accuracy with an AUROC of 0.91 (0.81–1.00). The availability of NSE during the original study did not affect the impact of age on the prognostic ability of NSE. NSE concentrations at 48 h in different age quartiles according to outcome are presented in Figure 9.

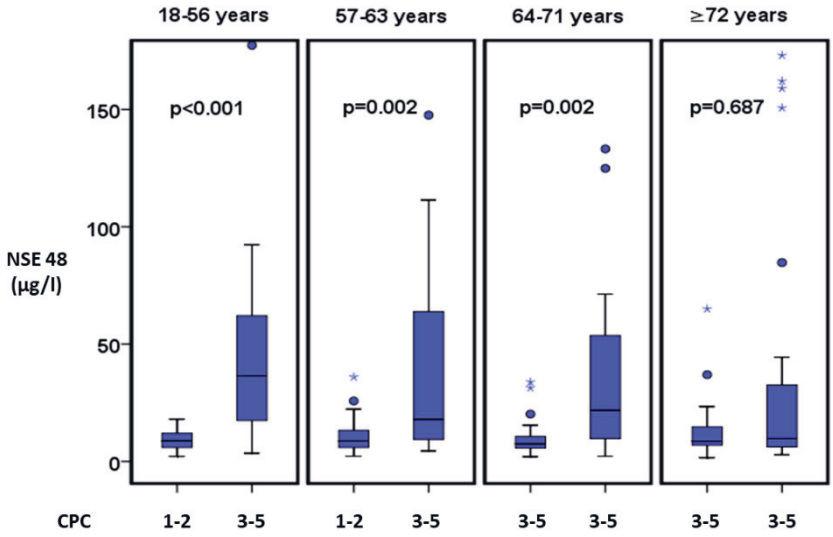

Figure 9. Distribution of NSE concentrations at 48 h for the patients with favourable (CPC 1–2) and unfavourable (CPC 3–5) outcome in quartiles according to age. Boxplot figures; each box showing the interquartile range, with a horizontal line inside the box showing the median value; bars showing the range of values except outliers (circles and stars), defined as values more than 1.5 box lengths from the edge of the box.
Abbreviations: NSE, neuron-specific enolase; CPC, cerebral performance category.

In Study IV, we assessed the concentrations and prognostic ability of NfL in corresponding age quartiles. NfL was significantly higher at 24 and 48 h for patients with unfavourable outcome than for those with favourable outcome in all age groups (24h: p<0.001 in all groups; 48h: p=0.005 for ≥72 years, p<0.001 for others). For patients with favourable outcome, NfL concentration increased with increasing age, and concentrations were different across age groups at 24 h (p < 0.001) and 48 h (p = 0.001). For patients with unfavourable outcome, NfL concentrations in different age quartiles were not different (p = 0.132 at 24 h, p = 0.363 at 48 h). NfL concentrations at 24 h and 48 h in different age quartiles relative to outcome are presented in Figure 10.

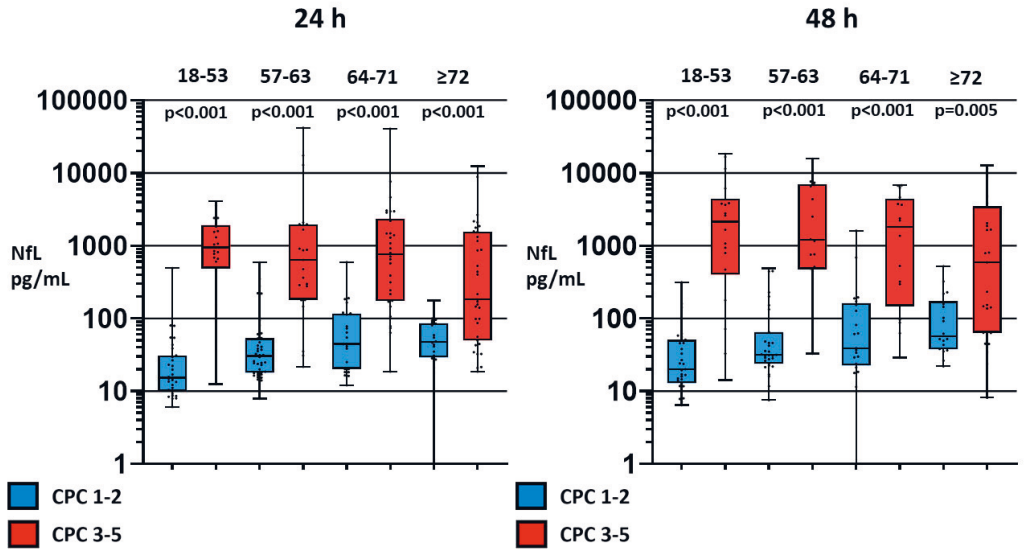

Figure 10. Boxplots for NfL concentrations at 24 h and 48 h after cardiac arrest for patients with favourable (CPC 1–2) and unfavourable (CPC 3–5) outcomes, according to different age quartiles. The scale is logarithmic. Each box presents the interquartile range, the line inside the box shows the median value, the whiskers show the lowest and the highest concentrations, and the dots show the concentrations for each individual. Age intervals (years) with p values (for differences in concentrations for patients with favourable [CPC 1–2] and unfavourable [CPC 3–5] outcomes in each quartile) are presented above each figure.
Abbreviations: NFL, neurofilament light; CPC, Cerebral Performance Category.

The AUROCs for NfL to predict unfavourable outcome were significantly higher in all age groups compared to those of NSE at 24 h. At 48 h, NfL had better prognostic ability in patients aged 57–63 years and in the oldest group (≥72 years). AUROCs according to different age groups are presented in Table 9.

Table 9. AUROCs (with 95% CIs) for NfL and NSE at 24 h and 48 h after cardiac arrest to predict unfavourable outcome, according to age quartiles.

	NfL AUROC (95% CI)	NSE AUROC (95% CI)	p for difference
24 h			
18-56 years	0.96 (0.90-1.00)	0.66 (0.50-0.81)	<0.001
57-63 years	0.90 (0.82-0.99)	0.75 (0.62-0.87)	0.005
64-71 years	0.91 (0.83-0.98)	0.70 (0.56-0.84)	0.002
≥72 years	0.79 (0.67-0.91)	0.53 (0.37-0.68)	0.002
48 h			
18-56 years	0.93 (0.85-1.00)	0.91 (0.81-1-00)	0.791
57-63 years	0.94 (0.87-1.00)	0.75 (0.61-0.89)	0.005
64-71 years	0.87 (0.75-0.99)	0.77 (0.63-0.92)	0.143
≥72 years	0.75 (0.61-0.89)	0.56 (0.40-0.73)	0.020

Abbreviations: CI, confidence interval; NfL, neurofilament light; NSE, neuron specific enolase; AUROC, area under the receiver operating characteristic curve

5.3.2 Impact of ROSC

In Study I, NSE levels and their prognostic ability at 48 h were significantly different in different ROSC quartiles. For patients with the shortest time to ROSC (1–13 min), the AUROC to predict unfavourable outcome was 0.45 (0.30–0.61). The AUROC was highest (0.84 [0.74–0.95]) for those with the longest resuscitation time (≥29 min). The availability of NSE during the original study did not affect the prognostic ability of NSE according to time to ROSC. NSE concentrations at 48 h in different ROSC quartiles according to outcome are presented in Figure 11.

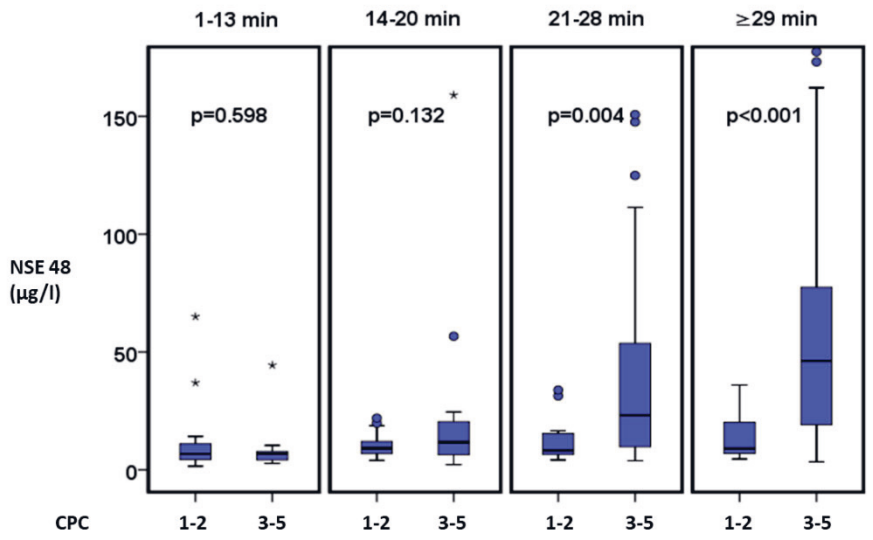

Figure 11. Distribution of NSE concentrations at 48 h for the patients with favourable (CPC 1–2) and unfavourable (CPC 3–5) outcome in quartiles according to time from collapse to ROSC. Boxplot figures; each box showing the interquartile range, with a horizontal line inside the box showing the median value; bars showing the range of values except outliers (circles and stars), defined as values more than1.5 box lengths from the edge of the box.
Abbreviations: NSE, neuron speficif enolase; CPC, Cerebral Performance Category.

In Study IV, we assessed NfL levels and their prognostic ability in ROSC quartiles. NfL concentration was significantly higher for patients with unfavourable outcome than for those with favourable outcome at 24 h and 48 h in all ROSC groups. Moreover, the AUROCs for NfL were significantly higher at 24 and 48 h compared to those of NSE in all ROSC groups. The AUROCs according to different ROSC time groups are presented in Table 10.

Table 10. AUROCs (with 95% CIs) for NSE and NfL at 24 h and 48 h after cardiac arrest to predict unfavourable outcome, according to ROSC quartiles.

	Time to ROSC	NfL AUROC (95% CI)	NSE AUROC (95% CI)	p for difference
24 h				
	≤13 min	0.70 (0.52-0.87)	0.43 (0.26-0.59)	0.010
	14-20 min	0.86 (0.77-0.95)	0.49 (0.32-0.66)	0.001
	21-28 min	0.93 (0.86-1.00)	0.70 (0.56-0.85)	0.002
	≥29 min	0.93 (0.87-1.00)	0.65 (0.50-0.80)	<0.001
48 h				
	≤13 min	0.72 (0.53-0.91)	0.46 (0.29-0.62)	0.042
	14-20 min	0.84 (0.73-0.94)	0.62 (0.44-0.80)	0.031
	21-28 min	0.89 (0.78-0.99)	0.75 (0.61-0.89)	0.042
	≥29 min	0.97 (0.92-1.00)	0.86 (0.75-0.96)	0.028

Abbreviations: CI, confidence interval; NfL, neurofilament light; NSE, neuron specific enolase; AUROC, the area under the receiver operating characteristic curve

NfL levels rose in both patients with favourable (p = 0.034 at 24 h; p = 0.004 at 48 h) and unfavourable (p < 0.001) outcome together with resuscitation time. NfL levels in different ROSC groups according to outcome are presented in Figure 12.

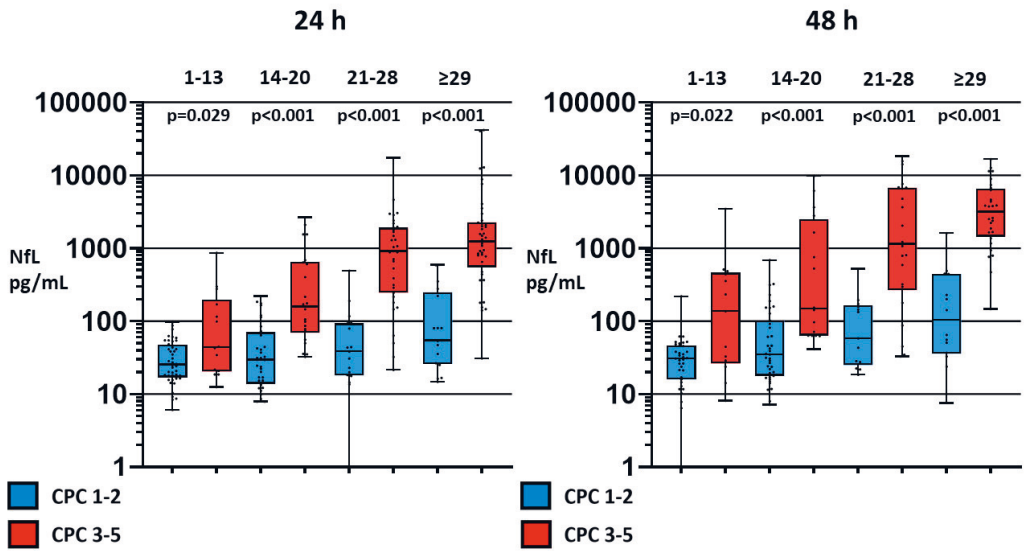

Figure 12. Boxplots for NfL concentrations at 24 h and 48 h after cardiac arrest for patients with favourable (CPC 1–2) and unfavourable (CPC 3–5) outcomes, according to different ROSC quartiles. The scale is logarithmic. Each box presents the interquartile range, the line inside the box shows the median value, the whiskers show the lowest and the highest concentrations, and the dots show the concentrations for each individual. ROSC intervals (minutes) with p values (for differences in concentrations for patients with favourable [CPC 1–2] and unfavourable [CPC 3–5] outcomes in each quartile) are presented above each figure. Abbreviations: NfL, neurofilament light; CPC, Cerebral Performance Category.

5.4 ADDED VALUE OF BIOMARKERS IN PROGNOSTICATION MODELS

In Study I, we constructed a baseline clinical model including age, time to ROSC, initial rhythm, and SAPS II points to predict unfavourable outcome. The model had an AUROC of 0.81 (0.75–0.86). NSE was a significant predictor of unfavourable outcome in the model, with an OR of 1.055 (1.025–1.085, $p < 0.001$). After adding NSE at 48 h to the model, the AUROC increased to 0.84 (0.79–0.89; $p = 0.021$). As Study I focuses on the

prognostic value of NSE in age and ROSC groups, the addition of NSE to the baseline model improved the AUROC only in the youngest patients (p = 0.013) and in those with the longest time to ROSC (p < 0.001). Also, continuous NRI and IDI were highest in those patients. For individuals with the highest age and shortest time to ROSC, the addition of NSE worsened the predictive ability of model, demonstrated as negative NRI and IDI. Table 11.

Table 11. Multivariable model to predict unfavourable 12-months outcome. AUROC, IDI and NRI for all patients and stratified according to age and time to ROSC.

1.	OR (95% CI)	p
Age[a]	1.045 (1.018-1.074)	0.001
ROSC[a]	1.076 (1.042-1.112)	<0.001
Witnessed[a]	0.319 (0.101-1.011)	0.052
Shockable[a]	0.309 (0.152-0.627)	0.001
***SAPSII**[a]	1.041 (1.019-1.064)	<0.001
NSE48[b]	1.055 (1.025-1.085)	<0.001

			Age				**ROSC**			
2.		**ALL**	**18-56**	**57-63**	**64-71**	**≥72**	**1-13**	**14-20**	**21-28**	**≥29**
AUROC (95% CI)	Baseline model	0.81 (0.75-0.86)	0.82 (0.71-0.93)	0.84 (0.74-0.95)	0.82 (0.71-0.94)	0.68 (0.52-0.84)	0.72 (0.58-0.86)	0.80 (0.68-0.91)	0.83 (0.71-0.95)	0.71 (0.54-0.87)
	Baseline model + NSE 48h	0.84 (0.79-0.89)	0.88 (0.79-0.97)	0.88 (0.79-0.97)	0.84 (0.73-0.95)	0.67 (0.52-0.82)	0.68 (0.54-0.83)	0.82 (0.71-0.93)	0.85 (0.75-0.96)	0.90 (0.82-0.99)
	P value for difference	0.021	0.013	0.226	0.665	0.819	0.100	0.453	0.561	0.001
NRI	Continuous	0.394	0.773	0.516	0.457	-0.332	-0.491	0.244	0.628	1.140

	NRI_e	-0.082	0.217	-0.143	0.040	-0.379	-0.467	-0.444	-0.067	0.257
	NRI_{ne}	0.476	0.556	0.659	0.417	0.047	-0.024	0.688	0.695	0.883
IDI	IDI	0.032	0.141	0.035	0.017	-0.077	-0.059	0.000	0.039	0.228
	IDI_e	-0.010	0.078	-0.017	-0.035	-0.041	-0.039	-0.046	-0.013	0.036
	IDI_{ne}	0.041	0.063	0.052	0.051	-0.035	-0.020	0.047	0.051	0.192

Abbreviations: AUROC, the area under the receiver operating characteristic curve; IDI, integrated discrimination improvement; NRI, net reclassification improvement; ROSC, return of spontaneous circulation; SAPS II, Simplified Acute Physiology Score; NSE, neuron-specific enolase.

[a] Variables in baseline model. [b] NSE 48 h added to baseline model. *SAPS II without age points

In Study II, we constructed a multivariable prognostication model and added UCH-L1 to it. At 24 h, UCH-L1 was a significant predictor of hospital mortality and CPC 3–5 at 12 months. At 48 h, UCH-L1 predicted hospital mortality but not unfavourable outcome at 12 months. Table 12.

Table 12. Logistic regression model for clinical variables and serum UCH-L1 to predict hospital mortality and unfavourable outcome (CPC 3-5) at 12 months.

	Hospital mortality		**12-mth CPC 3-5**	
Variable	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Age (years)	1.037 (1.011-1.064)	0.005	1.050 (1.022-1.078)	<0.001
Unwitnessed collapse	-	-	3.574 (1.169-10.925)	0.025
Nonshockable rhythm	3.688 (1.946-6.989)	<0.001	4.609 (2.354-9.022)	<0.001
ROSC (minutes)	1.092 (1.060-1.126)	<0.001	1.095 (1.062-1.129)	<0.001
UCH-L1 24 h	1.035 (1.010-1.059)	0.005	1.024 (1.000-1.047)	0.047
UCH-L1 48 h	1.020 (1.002-1.038)	0.029	-	-

Abbreviations: CPC, Cerebral Performance Category; OR, odds ratio; ROSC, return of spontaneous circulation.

In Study III, we made a multivariable clinical model using age, time to ROSC, and lack of basic life support that had an AUROC of 0.86 (0.79–0.93) to predict unfavourable outcome. The predictive accuracy of the model improved significantly after adding NfL to it; the achieved AUROC was 0.98 (0.97–1.00) at 24 h and 0.99 (0.98–1.00) at 48 and 72 h. NRI and IDI were highest for NfL addition at 48 h; NRI was 1.78 (95% CI 1.58–1.97) and IDI was 0.45. Table 13.

Table 13. Net reclassification improvement (NRI) and integrated discrimination improvement (IDI) for the improvement of prognostic value (B) in baseline model (A) achieved with the addition of NfL.

A	Variables in baseline model (BM)	OR	CI (95%)	p
	[a] **ROSC, min**	1.201	1.108-1.301	<0.001
	[a] **Age, years**	1.059	1.011-1.109	0.016
	[a] **BLS, no (-)**	5.018	1.370-18.377	0.015

B		AUROC BM	[b] AUROC BM+NfL	NRI	NRIe	NRIne	IDI	IDIe	IDIne
	24h	0.860 (0.792-0.928)	0.983 (0.965-1.000)	1.514 (1.237-1.790)	0.514	1.000	0.405	0.285	0.120
	48h	0.860 (0.792-0.928)	0.992 (0.982-1.000)	1.779 (1.583-1.974)	0.833	0.945	0.448	0.345	0.104
	72h	0.859 (0.790-0.927)	0.992 (0.981-1.000)	1.736 (1.533-1.939)	0.879	0.857	0.447	0.359	0.089

Abbreviations: OR, odds ratio; CI, confidence interval; ROSC, return of spontaneous circulation; BLS, basic life support; AUROC, area under the receiver operating characteristic curve; BM, baseline model, NfL, neurofilament light; NRI, net reclassification improvement; NRIe, NRI event; NRIne, NRI non-event; IDI, integrated discrimination improvement; IDIe, IDI event; IDIne, IDI non-event.
[a] Variables with OR:s for baseline clinical predicting model (BM) achieved by logistic regression with backward stepping method.
[b] NfL data was added to baseline model (BM) with logistic regression Enter method

In Study IV, we made a clinical multivariable model to predict unfavourable outcome with age, time to ROSC, nonshockability of initial rhythm, and unwitnessed collapse. We separately added NfL at 24 h and NSE at 48 h to the model, and both were significant predictors of unfavourable outcome ($p < 0.001$). After backward stepping, only age, shockability, and 24 h NfL were significant variables in the model. Table 14.

Table 14. Multivariable model to predict unfavorable outcome (CPC 3-5) at 12 months. Clinical information[1] was added with the enter method, then we separately added NfL at 24 h and NSE at 48 h to the model with the enter method[2] (A). Significant variables were obtained to the final model with backward stepping[3] (B).

A	**OR**	**95% CI**	**p**
ROSC delay, minutes[1]	1.096	1.063-1.131	<0.001
Age, years[1]	1.052	1.025-1.080	<0.001
Unwitnessed [1]	3.682	1.199-11.308	0.023
Nonshockable[1]	4.648	2.359-9.156	<0.001
NSE 48 h, µg/L[2]	1.070	1.034-1.107	<0.001
NfL 24 h, pg/mL[2]	1.007	1.004-1.010	<0.001
B			
Age, years[3]	1.063	1.021-1.107	0.003
Nonshockable[3]	2.181	0.902-5.274	0.083
NfL 24 h, pg/mL[3]	1.007	1.004-1.010	<0.001

Abbreviations: OR, odds ratio; CI, confidence interval; ROSC, return of spontaneous circulation; unwitnessed, unwitnessed collapse; nonshockable, nonshockable initial rhythm; NfL, neurofilament light; NSE, neuron-specific enolase

5.5 EFFECT OF LOW-NORMAL AND HIGH-NORMAL CARBON DIOXIDE, OXYGEN, AND BLOOD PRESSURE TARGETS ON NFL LEVELS

In the original COMACARE study, NSE levels were not affected by different $PaCO_2$, PaO_2, and MAP levels. We examined NfL concentrations in those intervention groups, as NfL is more accurate in identifying individuals with HIBI. NfL concentrations did not differ between low-normal and high-normal $PaCO_2$ groups or between normoxia and moderate hyperoxia groups. Regarding MAP targets, NfL was significantly lower in patients with high-normal MAP than in those with low-normal MAP at 48 h (23 pg/mL [11–251] vs 43 pg/mL [19–1066]; $p = 0.041$) and at 72 h (23 pg/mL [13–152] vs 63 pg/mL [21–1609], $p = 0.007$). NfL concentrations according to intervention targets are presented in Table 15.

Table 15. NfL concentrations (median IQR) at ICU admission (baseline) and 24, 48 and 72h after cardiac arrest, according to whether patients were treated with high/low normal arterial blood carbon dioxide tension ($PaCO_2$), arterial blood oxygen tension (PaO_2) and mean arterial pressures (MAP).

		Lower target	Higher target	p
$PaCO_2$	**Baseline**	12.2 (8.0-17.0)	11.6 (7.4-20.0)	0.972
	24h	22.2 (11.2-96.7)	34.5 (8.9-665.9)	0.695
	48h	28.8 (15.6-133.3)	39.3 (14.6-1221.5)	0.521
	72h	25.4 (15.5-152.1)	55.4 (15.9-1209.4)	0.203
PaO_2	**Baseline**	11.7 (7.1-17.8)	11.7 (8.4-17.2)	0.660
	24h	20.1 (9.1-129.8)	44.9 (11.1-549.8)	0.176
	48h	27.9 (14.6-272.2)	47.2 (15.5-1298.5)	0.260
	72h	26.8 (25.8-225.1)	57.6 (15.4-1375.9)	0.233
MAP	**Baseline**	11.9 (9.1-17.0)	11.0 (6.6-18.5)	0.303
	24h	31.6 (11.6-323.5)	20.1 (8.9-157.2)	0.143
	48h	43.2 (18.9-1066.2)	23.1 (10.8-250.7)	0.041
	72h	63.0 (20.6-1608.9)	22.9 (12.8-152.1)	0.007

5.6 CUTOFF VALUES IN PREDICTING UNFAVOURABLE OUTCOME

We calculated cutoffs for biomarkers by targeting high (95–99%) specificities to minimise false positive results. To facilitate comparability across biomarkers, we calculated Youden-based cutoff values. For NSE at 48 h, the cutoff by targeting 99% specificity was 37 µg/L in the FINNRESUSCI study population (Studies I and IV) and almost the same (36.5 µg/L) in the COMACARE study population (Study III). However, the corresponding sensitivity was better in Study III than Study IV: 51% (95% CI 35–68%) vs 36% (26–45%).

For UCH-L1 at 48 h, a cutoff of 46 ng/mL resulted in a specificity of 98% (96–100) and a low sensitivity of 7% (2–12), with a PPV of 78% (51–100; p = 0.041).

For NfL at 24 h and 48 h, the cutoffs for 99% specificity were higher in Study IV (FINNRESUSCI) compared to those of Study III (COMACARE). At 24 h, the cutoff for 99% specificity was 589 pg/mL in Study IV and 127 pg/mL in Study III, and at 48 h, the cutoffs were 721 pg/mL and 263 pg/mL, respectively. For 99% specificity, the sensitivities of NfL at 24 and 48 h were higher than the sensitivity for NSE at 48 h in the FINNRESUSCI population (54–60% for NfL vs 36% for NSE) and in the COMACARE population (78–83% vs 51%). The cutoffs for UCH-L1, NSE, and NfL across studies are presented in Table 16.

Table 16. Cut-off values with sensitivities, PPVs, NPVs and LR+ for UCH-L1 (ng/mL), NSE (µg/L) and NfL (pg/mL) to predict unfavourable outcome, defined by targetin specificities of 95% and 99%, and by the Youden method.

	Target	Cut off	Specificity (95% CI)	Sensitivity (95% CI)	PPV (95% CI)	NPV (95% CI)	LR+ (95% CI)	p
UCH-L1 48 h	99%	46	0.98 (0.96-1.00)	0.07 (0.02-0.12)	0.78 (0.51-1.00)	0.57 (0.50-0.64)	4.4 (0.9-20.5)	0.041
FINN-RESUSCI	95%	32	0.95 (0.91-0.99)	0.18 (0.11-0.26)	0.75 (0.58-0.92)	0.59 (0.52-0.66)	3.7 (1.5-9.0)	0.001
	Youden	12	0.53 (0.44-0.62)	0.78 (0.69-0.86)	0.57 (0.49-0.66)	0.75 (0.66-0.84)	1.7 (1.3-2.1)	<0.001
NSE 48 h	99%	37	0.99 (0.98-1.00)	0.36 (0.26-0.45)	0.97 (0.92-1.00)	0.66 (0.59-73)	43.6 (6.1-312.4)	<0.001
FINN-RESUSCI	95%	24	0.95 (0.91-0.99)	0.44 (0.34-0.54)	0.88 (0.79-0.97)	0.68 (0.60-0.75)	8.9 (4.0-20.1)	<0.001
	Youden	20	0.93 (0.88-0.97)	0.50 (0.40-0.60)	0.85 (0.75-0.94)	0.70 (0.63-0.77)	6.8 (3.5-13.1)	<0.001
	99%	36.5	0.97 (0.93-1.00)	0.51 (0.35-0.68)	0.90 (0.77-1.00)	0.80 (0.72-0.89)	18.5 (4.5-75.4)	<0.001
COMA-CARE	95%	35	0.94 (0.89-99.7)	0.54 (0.38-0.71)	0.83 (0.67-0.98)	0.81 (0.72-0.89)	9.8 (3.6-26.6	<0.001
	Youden	33	0.93 (0.87-0.99)	0.66 (0.50-0.81)	0.82 (0.68-0.96)	0.85 (0.77-0.93)	9.5 (3.9-22.8)	<0.001

NfL 24 h	99%	589	0.99 (0.97-1.00)	0.54 (0.45-0.63)	0.98 (0.95-1.00)	0.69 (0.61-0.76)	61.5 (8.7-436.4)	<0.001
FINN-RESUSCI	95%	232	0.96 (0.92-0.99)	0.66 (0.57-0.74)	0.94 (0.88-0.99)	0.74 (0.67-0.81)	14.9 (6.3-25.5)	<0.001
	Youden	97	0.87 (0.81-0.93)	0.82 (0.74-0.89)	0.86 (0.79-0.93)	0.83 (0.76-0.89)	6.2 (3.8-10.0)	<0.001
	99%	127	0.99 (0.96-1.00)	0.78 (0.65-0.92)	0.97 (0.90-1.00)	0.90 (0.83-0.96)	54.9 (7.8-386.9)	<0.001
COMA-CARE	95%	92	0.93 (0.87-0.99)	0.86 (0.75-0.98)	0.86 (0.75-0.98)	0.93 (0.87-0.99)	12.1 (5.2-28.5)	<0.001
	Youden	69	0.93 (0.87-0.99)	0.92 (0.83-1.00)	0.87 (0.77-0.98)	0.96 (0.91-1.00)	12.9 (5.5-30.1)	<0.001
NfL 48 h	99%	721	0.99 (0.97-1.00)	0.60 (0.49-0.70)	0.98 (0.94-1.00)	0.76 (0.68-0.83)	60.1 (8.5-426.0)	<0.001
FINN-RESUSCI	95%	445	0.95 (0.91-0.99)	0.66 (0.55-0.76)	0.91 (0.84-0.99)	0.78 (0.71-0.85)	13.3 (5.6-31.7)	<0.001
	Youden	231	0.92 (0.87-0.97)	0.72 (0.62-0.82)	0.88 (0.80-0.96)	0.81 (0.74-0.88)	9.1 (5.6-18.0)	<0.001
	99%	263	0.99 (0.96-1.00)	0.83 (0.71-0.96)	0.97 (0.91-1.00)	0.92 (0.86-0.98)	60.8 (8.6-428.4)	<0.001
COMA-CARE	95%	89	0.95 (0.89-1.00)	0.92 (0.83-1.00)	0.89 (0.79-0.99)	0.96 (0.91-1.00)	16.7 (6.4-43.6)	<0.001
	Youden	109	0.97 (0.94-1.00)	0.92 (0.83-1.00)	0.94 (0.87-1.00)	0.96 (0.91-1.00)	33.5 (8.5-131.7)	<0.001

Abbreviations: CI, 95% condifence interval; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; UCH-L1, ubiquitin c-terminal hydrolase L1; NSE, neuron-specific enolase; NfL, neurofilament light.

5.7 BIOMARKERS IN PREDICTING FAVOURABLE OUTCOME

We evaluated cutoffs corresponding to very high sensitivity in identifying unfavourable outcome. When a very sensitive test is used, a negative test result (i.e. a biomarker concentration below the cutoff level) has a high negative predictive value. Thus, these cutoffs can be used to identify patients with a high likelihood of favourable outcome.

In Study II, when targeting 99% sensitivity for UCH-L1, the cutoff was 4.1 ng/mL at 24 h (p = 0.063) and 6.4 ng/mL at 48 h (p = 0.039). The corresponding specificities were 7% (95% CI 2–11%) at both time points, and the NPVs were 80% (55–100 %) at 24 h and 89% (68–100 %) at 48 h.

In the COMACARE population (Study III), an NfL cutoff of 30 pg/mL at 24–48 h and 27 pg/mL at 72 h resulted in 100% sensitivity, meaning that no individual below those cutoffs had unfavourable outcome (p < 0.001 for all time points). The corresponding specificities were 79% (69–88%) at 24 h, 74% (64–84%) at 48 h, and 69% (58–79%) at 72 h, and the NPVs were 100% at 24–72h.

In the FINNRESUSCI population (Study IV), NfL cutoff of 18.5 pg/mL at 24 h and 14 pg/mL at 48 h resulted in 99% sensitivity (p<0.001 at 24h, p=0.006 at 48h). The corresponding specificities were 32% (23-40%) and 12% (6-18%), and the NPVs were 97% (92-100%) and 92% (78-100%), respectively. For NSE at 48 h, the cutoff for 99% sensitivity was 2.7 µg/L, with a resulting specificity of 6% (2-10%) and a NPV of 88% (65-100%) (p=0.062). When using the highest normal biomarker values (55 pg/mL for NfL; 17 µg/L for NSE), the sensitivity for NfL was 86% at 24 h and 87% at 48 h, and for NSE, it was 54% (p<0.001 for all). The corresponding specifities were 74% (66-82%) and 67% (58-76%) for NfL and 90% (85-95%) for NSE.

6 DISCUSSION

6.1 SUMMARY OF MAIN FINDINGS

In the FINNRESUSCI patients, we found that the ability of NSE to predict unfavourable 12-month outcome after OHCA was dependent on the patient's age and time from collapse to ROSC. NSE had an excellent prognostic ability in the youngest patients and in those with the longest resuscitation time. However, for the oldest patients and for those with shortest ROSC time, the prognostic ability of NSE was poor. UCH-L1 demonstrated only moderate prognostic ability in the same population and did not present any benefits compared to NSE. In the third study of the same population, NfL had superior prognostic ability compared to that of NSE and UCH-L1. The prognostic ability of NfL was satisfactory even for the oldest patients and for those with the shortest time from collapse to ROSC; it was also superior to that of NSE.

In the COMACARE population, NfL predicted unfavourable 6-month neurological outcome with excellent accuracy. The AUROCs were 0.98 at 24-72 h, significantly better than those of NSE and S100B. The median NfL concentrations were 100-fold greater for patients with unfavourable outcome compared to those with favourable outcome. Moreover, NfL levels were unaffected by haemolysis, whereas NSE concentrations were significantly higher in haemolysed samples. Interestingly, NfL levels were significantly lower in the higher MAP group than in the lower MAP group at 48 and 72 h. NfL demonstrated sufficient ability in predicting favourable outcome in both study populations.

6.2 ASSESSING NEUROLOGICAL PROGNOSIS WITH BIOMARKERS

Several studies have provided evidence that the neuronal injury biomarker NSE is suitable for predicting neurological outcome after CA [179,184,188–190], as recommended in the ERC-ESICM guidelines [6]. Overall, the prognostic ability of NSE in the largest studies was good to excellent, with AUROCs of 0.85–

0.90 [179,184]. We found a clearly worse AUROC of 0.72 at 48 h for NSE in the FINNRESUSCI patients. In one previous study, NSE had even poorer prognostic ability than that found in our study, but different outcome definitions confound the comparability [186]. In the highly selected COMACARE population that included only patients with shockable rhythms, NSE predicted unfavourable outcome with a higher AUROC of 0.89 at 72 h. This is comparable to the prognostic accuracy of NSE in the FINNRESUSCI patients in the youngest age quartile (18–53 years) and in those with the longest resuscitation time (≥29 min; i.e., in the patients we assume to be less likely to have restricted treatments and whose cause of death was more likely to be HIBI than extracerebral causes). Our findings are in line with other studies, suggesting good prognostic ability for NSE, however with some caution in the oldest patients and in those with short resuscitation time.

For UCH-L1, we found only limited prognostic value that was not significantly different to that of NSE. Contrary to our findings, some previous studies have reported good to excellent prognostic accuracy for UCH-L1.[205,206]. In a recent study of OHCA patients, where analyses were made in both CSF and serum, UCH-L1 had an excellent ability to predict six-mont outcome [277]. We used ELISA for UCH-L1 analyses unlike two of these studies that used the novel SIMOA platform [205]. In addition, the commercial ELISA kit in the study by Ebner et al. was different than in our study [206]. This may reduce the comparability of studies. The worse prognostic ability of UCH-L1 in the FINNRESUSCI population compared to that of the TTM population is, however, in line with the worse ability of NSE in the current study [179]. Studies suggesting the usefulness of UCH-L1 in prognostication after CA are few, and our results do not support the use of UCH-L1 in prognostication after OHCA.

In comparison to the traditional biomarker NSE, S100B in the COMACARE population had satisfactory prognostic ability (AUROC 0.77). That prognostic ability is poorer than what has been found in some other studies [114,214]. Several features do not support the use of S100B. Firstly, S100B may reflect glial activation and BBB disruption rather than HIBI [216,217]. Secondly, it is expressed in cardiomyocytes, muscle cells, and

chondrocytes, which can be extracerebral sources of error after CPR [211]. Thirdly, its overall prognostic ability is not superior to that of NSE. Hence, our findings do not support the use of S100B in assessing long-term outcome after OHCA.

Among the biomarkers in the current study, the axonal marker NfL clearly had the best prognostic accuracy in both study populations. In the FINNRESUSCI study, the AUROCs were 0.88–0.90, and in the COMACARE study, the AUROCs were 0.98 between 24–72 h, which means close to optimal discriminative ability. The prognostic accuracy of NfL in the FINNRESUSCI population was slightly poorer than in the TTM population [134], however. Our results in both study populations support the superiority of NfL over other biomarkers in assessing neurological prognosis after OHCA and strengthen the evidence in line with findings of the TTM study. [134]. Moreover, we found excellent prognostic accuracy of NfL in the unselected FINNRESUSCI population, which is an important addition to the findings of the TTM substudy and the current COMACARE substudy that both included selected OHCA patients.

Some recent studies on NfL have also demonstrated excellent prognostic ability. In a study by Levin et al., NfL predicted unfavourable outcome with an AUROCs of 0.93 at 12 h and 0.97 at 48 h for OHCA patients [278]. In another study in Korea by Song et al. on OHCA patients whose life-sustaining therapies were not restricted, decreasing the risk of self-fulfilling prophecy, NfL had the best prognostic accuracy compared to NSE, S100B, GFAP, Tau, and UCH-L1, with AUROCs of 0.90–0.95 at 24–72 h [277]. Ultra-early measurement of NfL presented worse prognostic ability in both of those studies, in accordance with our findings. The kinetics of NfL seem to be that significant release into the circulation starts about 12 h after CA and continues to 24–48 h until the stable phase.

As a comparison between axonal biomarkers, Tau had an AUROCs of 0.93–0.95 at 48–72 h in the same COMACARE population than in the current study [234], also inferior to what we assessed for NfL. In accordance, a meta-analysis by Hoiland et al. on neurobiomarkers after CA summarised that the prognostic ability of NfL was superior to that of Tau [187].

Accordingly, NfL had better prognostic ability than Tau in the TTM patients [134,237], and so far, has presented superior accuracy among axonal markers.

In the TTM substudy, brainstem reflexes, N20 SSEP, EEG, and brain CT were compared to NfL with cutoff values with similarly high specificities [134]. NfL was the most robust predictor of unfavourable outcome, with 29–49% higher sensitivity than other methods. High specificities can be defined for almost all methods, but simultaneous high sensitivity is possible only for the methods that have high discriminative ability. The AUROCs near 1.00 at three time points in the current COMACARE substudy and the significantly better prognostic accuracy of NfL compared to NSE in the FINNRESUSCI patients are consistent with the TTM results suggesting that NfL has great potential to complement prognostication of CA patients when used alongside recommended methods.

6.2.1 Comparison of the populations

For the biomarkers evaluated in this study, the prognostic abilities were weaker in the FINNRESUSCI population than in the COMACARE population. Likewise, the prognostic abilities in the FINNRESUSCI population were weaker than those in the TTM population [134], whereas the prognostic abilities in the COMACARE population were comparable to those in the TTM population. The worse prognostic ability of neurobiomarkers in the FINNRESUSCI population probably reflects differences in the study populations, as FINNRESUSCI included patients with all types of OHCA, while COMACARE and TTM included only those with a presumed cardiac cause of OHCA. The proportion of patients with unfavourable outcome was 39% in the COMACARE study, which included a highly selected OHCA population of adult patients ≤80 years with shockable rhythms, a confirmed or suspected cardiac cause of CA, and a resuscitation time between 10–45 min. FINNRESUSCI included OHCA patients with shockable and nonshockable rhythms and all types of CA. In the current analysis that included 249 of the 504 FINNRESUSCI patients, the percentage of patients with unfavourable outcome was higher than in the COMACARE study (49%). However, in the original FINNRESUSCI study, the proportion of

patients with unfavourable outcome was even greater (69%), probably due to the lower percentage of shockable rhythms (56% in the whole population compared to 71% in the current post hoc analysis) [48]. This selection was necessitated by the greater availability of blood samples from patients with shockable rhythms, making our population somewhat different than a real-life population. Another possible explanation for lower prognostic abilities of biomarkers in the FINNRESUSCI study compared to the COMACARE and some other studies is the time of outcome assessment: in many other studies [102,179,185,189] and in the COMACARE study, outcome was assessed at six months, whereas in FINNRESUSCI, outcome was assessed at 12 months.

6.2.2 Cutoff values

For use in clinical decision making, a cutoff value must have high specificity so that false positive results (i.e., incorrect conclusions of an unfavourable prognosis) are avoided. However, clinical usefulness also necessitates a reasonably good sensitivity, and the requirement of a very high specificity tends to lead to low sensitivities. The variation among the optimal cutoffs of NSE in a wide variety of studies is high [179,184,185,188,190]. Extensive information on NSE and its cutoffs exists, and it is clear that optimal cutoffs can vary widely among studies and populations; also, the validation of cutoffs may be necessary in different countries.

In the FINNRESUSCI analysis, by targeting 99% specificity, we defined an NSE cutoff of 37 µg/L at 48 h to predict unfavourable outcome, which had 36% sensitivity. This cutoff is close to that described earlier [188,189] but lower than that in the ERC-ESICM guidelines [6] and some other studies [184,185]. In the COMACARE analysis, the NSE cutoff for 100% specificity was 40 µg/L with a sensitivity of 51% (data not published before). When we used a demand of 0% FPR (i.e., 100% specificity) in the FINNRESUSCI study, the cutoff rose to 68 µg/L, and the sensitivity decreased to 17% (data not published before). Very few patients with an NSE level over 40 µg/L survived in both studies, but when we targeted an FPR of 0%, the sensitivity of NSE decreased significantly, and this cutoff could identify only some of

the patients with favourable outcome. Furthermore, this cutoff resulted in a high number of false negatives. Thus, some patients whose NSE was between 40–67 µg/L had favourable outcome (false positives), but many of those with NSE under 68 µg/L had unfavourable outcome (false negatives).

When increasing the cutoff by targeting 100% specificity, the sensitivity deteriorates, and cutoffs of even 80–112 µg/L have been defined for NSE [102,184,185]. Our study also demonstrates how requiring a specificity of 100% instead of 99% increases the cutoff level remarkably. Moreover, as previously shown, too high a specificity requirement for a method with limited accuracy results in limited sensitivity, and slightly lower (98–99%) specificity thresholds can be more useful from a clinical aspect [114], especially when the method in question is part of multimodal prognostication. The recommended NSE cutoff of 60 µg/L in the ERC-ESICM guidelines corresponds to 99–100% specificity in unfavourable outcome prediction in both populations of the study, though with rather low sensitivity. In Study II, UCH-L1 showed how a method with limited discriminative ability can have a 95–99% specificity for a cutoff but an unusable 9–18% sensitivity.

The superior discriminative ability of NfL over NSE offers the possibility of utilising a cutoff with a very high specificity (99–100%) and a simultaneously high sensitivity. Few studies offer cutoff values for NfL in prognostication after CA, and the TTM study is the best one to compare our results with. For cutoffs defined with a specificity of 99%, we assessed sensitivities of 54–60% in the FINNRESUSCI study and even 78–85% in the COMACARE study, showing that NfL can identify many of the true positive cases with a minimal number of false positive results. We defined much lower cutoffs for 99% specificity in the COMACARE study (127–344 pg/mL) than in the corresponding levels in the FINNRESUSCI study (589–721 pg/mL); the FINNRESUSCI levels are more comparable to those in the TTM study (641–1,122 pg/mL) [134] and a recent study by Song et al. (521–690 pg/mL) [277]. In two small studies, the cutoffs with 100% specificity were 323–405 pg/mL [267] and 500 pg/mL [270]. Lowering the target specificity to 95% enabled the cutoffs to be 154–590 pg/mL in the TTM study. The COMACARE population was highly selected and rather small, lowering the possibility of

outliers and probably resulting in better discriminative ability, that can be seen as lower cutoffs for a high specificity.

To date, the use of NfL is not recommended in the current guidelines because of a lack of evidence, and useful cutoffs with very high specificities have been not provided. According to our findings and current knowledge, it appears that levels above 600–700 pg/mL can find many true positive individuals (high NfL and unfavourable outcome) with only a minimal number of false positives (high NfL and favourable outcome). When using NfL, the cutoff can be set at a level corresponding to very high specificity, and the sensitivity is still rather good. However, the number of published studies about the association of NfL with prognosis of resuscitated patients is still limited, and more research is needed before decisions about adequate cutoffs can be made.

6.2.3 Pitfalls in prognostication

The prognostication guidelines do not suggest using pre-hospital parameters (e.g., shockability of the rhythm and resuscitation time) in the assessment of outcome [6,49]. Clinical models could help in assessing pre-test probability [7] but this kind of prognostication model has not been adopted in prognostication after CA and is not suggested in the guidelines. However, pre-hospital resuscitation and patient features can be used intuitively, though this creates a risk of care restriction and self-fulfilling prophecy when the “gut feeling” indicates unfavourable outcome. For as many as 21–25% of resuscitated patients, life support may be withdrawn early, and these patients have a very high mortality. However, matched cohorts with similar resuscitation and comorbidity features suggest probabilities of 16-21% for favourable functional outcome in this kind of patient [9,117].

Another problem is deviation from the guideline-recommended multimodal prognostication, as demonstrated in a recent study of IHCA patients in the United States by Elmer et al. They found that adequate neurological prognostication was performed for only a minority of the patients [279]. In the European TTM study, contrary to the findings of Elmer et

al., protocol-driven prognostication was significantly more common [99]. Moreover, all the COMACARE patients in the current study that had WLST because of a presumed unfavourable outcome had been examined utilising at least one imaging or electrophysiological exam, and the interquartile range of time to death among these patients was 3–7 days, suggesting that early WLST was not common in our study. This is likely decreasing the risk of self-fulfilling prophecy and biased results.

However, the weakness of the ERC-ESICM guidelines that gives indeterminate outcome predictions occurs in Europe, too. One problem with prognostication performed according to the ERC-ESICM guidelines is the high number of patients who do not wake up but who do not fulfil the criteria of unfavourable outcome; they are categorised into indeterminate outcome [15]. This obviously reflects the limited sensitivity of recommended methods, and the indeterminate prognosis in comatose patients is likely to increase the risk of WLST. However, highly sensitive NfL has potential to reduce proportion of indeterminate prognoses if adopted to clinical prognostication strategies.

An additional area of uncertainty is the optimal follow-up time for patients who do not wake up until day 3. The avoidance of WLST can result in a considerable number of patients who survived with unfavourable neurological function, but severe neurological deficits are not uncommon in countries that make WLST decisions [7]. The balance between avoiding unnecessary WLST and futilely continuing care in patients with indeterminate outcome is difficult.

An early, highly sensitive method could facilitate decision making regarding the continuation of the care until multimodal prognostication at day 3 if test results indicate some probability of favourable outcome. In the TTM analysis, NfL had a very high sensitivity and the lowest false negative rate among all neurobiomarkers (including NSE) for normal values in prediction of unfavourable outcome [15]. Similarly, we found that NfL as a sensitive marker with early release into the circulation after HIBI can predict favourable outcome. In the COMACARE analysis, NfL levels below 27–30 pg/mL had 100% sensitivity and NPV in predicting unfavourable outcome (i.e., no patients with low NfL levels had unfavourable outcome).

In the FINNRESUSCI analysis, NfL below 18.5 pg/mL at 24 h and 24 pg/mL at 48 h resulted in a sensitivity of 99%, and when using the highest normal NfL level (55 pg/mL) as the cutoff, the sensitivities were still useful at 86–87%. Targeting a high sensitivity of NSE resulted in a very low cutoff level and low specificity, making the test useless for this purpose. Our findings suggests that NfL is capable to detect individuals with a high probability of favourable outcome, whereas NSE seems not to be sensitive enough.

Interestingly, we found a rising trend of NfL levels with increasing resuscitation time among patients with favourable outcome in the FINNRESUSCI group. Moreover, NfL levels were two-fold greater in patients with CPC 2 than those with CPC 1 in the COMACARE study (data not shown) and three- to four-fold greater in the FINNRESUSCI study (data not shown). Higher NfL levels in CPC 2 patients and in those with a long resuscitation time suggest that NfL is a sensitive enough biomarker to separate asymptomatic individuals from those with mild HIBI. Mildly elevated NfL may be a way to detect patients who survive but are likely to suffer neurological and psychiatric symptoms and need rehabilitation. A novel strategy with two-dimensional prognostication utilising sensitive methods could narrow the gap between certainty and uncertainty of prognosis [10]. So far, the NfL analyses are not yet available in daily clinical practice, but in the future NfL will probably improve the prognostication of CA patients, and it may also become cost-saving if the lengths of ICU stay of patients with hopeless prognosis become shorter. However, our findings on NfL predicting favourable outcome need further validation, as does the role of NfL in assessing milder neurological deficits.

6.3 CONFOUNDING ASPECTS OF BIOMARKERS

6.3.1 Age

We found NSE to be a poor predictor of neurological outcome in patients who were ≥72 years old. To date and to the best of our knowledge, no other studies focusing on the impact of age on NSE levels after CA exist. The studies examining NSE in different ages is controversial, however with

a trend towards increasing levels with aging [280]. In the current study, as NfL levels in the same population presented similar age-dependence for prognostic ability, it is possible that the phenomenon merely reflects the FINNRESUSCI study population. Because neurobiomarkers can reliably discriminate individuals according to HIBI [187] but not according to extracerebral reasons for unfavourable outcome, we can hypothesise that some of the oldest patients died for reasons other than HIBI. Unfortunately, data on causes of death were not collected from the FINNRESUSCI patients. Nonetheless, our findings suggest in some caution when using NSE in prognostication among the oldest.

We found no differences in ROSC time or proportion of cardiac causes of CA in different age quartiles. In the oldest patient quartile (median age 77 years), unfavourable outcome was more common: 61% compared to 38–40% in two younger quartiles. This probably reflects the poorer overall capacity of the elderly to maintain homeostasis after CA. In the oldest old (≥85 years) patients that were treated in ICUs in Finland, Pietiläinen et al. found that premorbid poor functional status almost doubled the one-year mortality [281], which likely increased the proportion of unfavourable outcome in the oldest age group in our study. We did not report comorbidities that can worsen survival after CA [43], but elderly individuals are more likely to have a greater accumulation of severe underlying diseases [35], which possibly affected our results, presented as weaker discriminative ability of the biomarkers that release into the circulation after HIBI.

Like NSE, NfL had worse discriminative ability in the oldest patients. Aging is a quite well-known factor that increases NfL blood concentrations by about 2% per year [253,282]. This is supposedly associated with brain degeneration during aging. However, the differences in NfL concentrations between younger (20–40 years) and older (70–80 years) people were not very prominent in a study by Fitzgerald et al. that included individuals without neurological disorders. For younger individuals, the concentrations were about 10 pg/mL, and for older, the concentrations were 20–30 pg/mL [253]. This difference was significant but clearly not as great as what we found between OHCA patients with unfavourable and favourable outcome. The

median NfL concentrations in both study populations were 10–35 pg/mL in patients with favourable neurological outcome, which is comparable to concentrations in individuals with no neurological disorders.

Many neurodegenerative disorders slightly increase NfL concentrations [256–258], and dementia is more common in the elderly. Dementia also worsens survival after OHCA [33]. Thus, dementia provides an additional explanation for worse survival in the oldest patients and worse discriminative ability of neurobiomarkers in the oldest individuals. We hypothesise that the narrow difference in NfL of the oldest patients according to outcome reflects the increasing effect of age on NfL levels and the assumed greater burden of comorbidities and frailty that results in an increased number of deaths caused by reasons other than HIBI. However, NfL had moderate prognostic value in the oldest patients contrary to NSE, making those confounders less significant for NfL than for NSE.

One novel, clinically relevant finding in OHCA patients in the current study was the significantly greater NfL level together with greater age in patients with favourable neurological outcome. Our findings on the impact of age on NfL levels supports earlier studies and suggests caution in interpreting mildly/moderately elevated NfL concentrations when assessing HIBI after CA in elderly patients. Age correction may be helpful when using NfL in prognostication, but this topic needs more investigation.

6.3.2 Other confounding factors

Many other brain injuries cause blood concentrations of brain-specific biomarkers to rise [13,14,181,182,201,202,262], and UCH-L1 is used as a marker of TBI [197,199]. Hence, previous brain injury can possibly affect concentrations of neurobiomarkers. NfL seems to have a very long biological half-time, and significantly elevated concentrations after TBI have been measured several months after trauma [262,263]. NfL levels decrease between 1–3- and 6-month measurement times after TBI and HIBI, being still five-fold greater than in healthy controls [283]. Previous brain injury within weeks—or, in the case of NfL, within months—before CA can probably affect biomarker levels and

possibly weaken their prognostic accuracy. However, no studies so far have reported data about this.

Chronic renal failure can increase concentrations of both NSE and NfL and is an additional source of error in prognostication after CA [253,284]. This is probably caused by the large molecular weight of NSE and NfL (about 70 kDa). Biomarkers that are released into the blood may be eliminated mostly via the kidneys, but hepatic and intravascular clearance may also occur [265]. The clearance rate of blood protein biomarkers depends on their molecular weight—faster for smaller molecular weights and slower for higher molecular weights; up to 70 kDa, the slowing is linear [265]. For molecules larger than 70 kDa, the clearance rate decreases more rapidly, likely because this is the molecular weight of plasma proteins (albumin), and kidney glomeruli retain those larger molecules in the blood [285]. UCH-L1 has a low molecular weight (25–27 kDa) which means rapid renal clearance. However, it is expressed in the podocytes in kidneys, making higher concentrations possible in patients with kidney disorders [193]. UCH-L1 concentrations in the FINNRESUSCI patients was correlated with creatinine levels and urine output (results not published), supporting the impact of kidney diseases on UCH-L1 levels as potential confounders.

In addition to age and kidney diseases, other factors that can increase NfL levels and confound the assessment of HIBI after CA are higher blood haemoglobin A1c (HbA1c) and overall comorbidity burden [253]. Diabetic polyneuropathy also increases NfL levels [286] and is likely associated with higher HbA1c.

6.3.3 Haemolysis

Red blood cells and thrombocytes contain NSE, and haemolysis is a significant confounder of NSE, causing its blood levels to rise. Mechanical stress on red blood cells and thrombocytes in haemodialysis and mechanical circulatory support causes haemolysis [287,288]. Elevated NSE levels have been found in patients treated with mechanical circulatory support [219,289] and haemodialysis [290,291] due to haemolysis. Many other conditions, transfusion [292], and even blood sampling technique can cause

haemolysis [293]. Thus, the risk of misleadingly elevated NSE levels due to haemolysis in resuscitated patients remains real, likely affecting its prognostic reliability. We found a significant correlation between haemolysis and higher NSE levels in the COMACARE patients [11]. Detectable haemolysis was common (35% of the samples), but significant haemolysis was rare. Importantly, NfL was not altered by haemolysis, confirming the findings of the TTM substudy [134]. It is unknown how haemodialysis or circulatory support affects NfL concentrations. However, as NfL is not sensitive to haemolysis, it is likely that those treatments have no effect on NfL.

6.4 HIBI AND NEUROBIOMARKERS

6.4.1 Axonal injury

In addition to neuronal injury, axonal injury markers can be released into the circulation after HIBI [187]. Neurons use three-fold more energy per unit weight than WM (mainly axons), and it is understandable that the neurons, as structures consuming more energy and being highly metabolically active, are less tolerant to ischemia than WM [61]. The difference in energy consumption can be explained by an 80-fold greater amount of synapses, which are responsible for the majority of energy consumption [61]. Neurofilaments are involved in neuronal synapses [294] and of them, NfL is expressed in the GABAergic and glutaminergic synapses in the brain cortex and more likely in postsynaptic axons [295]. The elimination of neurofilaments disturbs synaptic plasticity [296].

NfL levels after TBI and HIBI can be elevated months after injury, and levels are higher in those with HIBI [283]. The mechanism of prolonged NfL release and suspected WM neurodegeneration after HIBI is unclear. Traumatic diffuse axonal injury can cause secondary neurodegeneration and WM loss [297], and it is possible that HIBI can similarly trigger neurodegeneration, thus explaining long-standing highly elevated NfL levels. A 5-year follow-up of older adults demonstrated a clear association between NfL and degeneration of WM on brain MRI [298]. In CA patients, WM

anisotropy of the brain on MRI shows excellent prognostic ability at 7–21 days after CA, supporting the importance of WM injury in prognosis [84]. The findings of the current study on high release of NfL after HIBI, in accordance with other studies [134,270,277,278], support the impact of axonal injury in HIBI. Moreover, great release of NfL in patients with HIBI, together with previous physiological knowledge regarding NfL expression in metabolically active synapses, suggest the hypothesis that synaptic injury can occur in HIBI. This may be an additional explanation for the superior discriminative ability of the axonal marker NfL.

6.4.2 Secondary HIBI

Hoiland et al. studied the arterial and jugular vein gradients of several neuroglial biomarkers of patients with ongoing (secondary) HIBI, which was defined with low $PbtO_2$ [82]. Neuroglial biomarkers and the inflammatory marker Interleukin 6 (IL6) were high in patients with low $PbtO_2$ and secondary HIBI compared to those with normal $PbtO_2$ and HIBI, whereas global ischemic markers were not different, suggesting that neuroinflammation participates in a process that can cause secondary HIBI [82]. In the COMACARE population, inflammatory markers were associated with a disturbed 48 h continuous EEG, suggesting some level of association between neuroinflammation and secondary HIBI; however, those markers were not significantly associated with unfavourable outcome when compared to NfL [299]. In the current study, S100B, which can reflect neuroinflammation and BBB injury, did not have a good prognostic ability, whereas the highly predictive nature of NfL makes it more likely that axonal injury plays a significant role in HIBI.

The exact mechanism of secondary HIBI, its impact on neurological prognosis, and the role of inflammation remain somewhat unclear. Regarding different oxygen and carbon dioxide targets, COMACARE did not find any differences in outcome [58]. The recent analysis of the TTM2 trial also demonstrated no impact of carbon dioxide on outcome, whereas hypo- and hyperoxaemia increased mortality [300,301]. Similarly, NfL levels did

not differ in the current study in patients with different oxygen and carbon dioxide targets.

6.4.3 Blood pressure in HIBI

Interestingly, we found a possible indirect demonstration of the effects of blood pressure on secondary HIBI in the COMACARE analysis, as there were lower NfL levels at 48–72 h after CA in patients who were treated with higher blood pressure targets. The median NfL concentration was 63 pg/mL at 72 h in patients who were treated with lower blood pressure targets (65–75 mmHg), whereas the median NfL concentration was 23 pg/mL when higher blood pressure (80–100 mmHg) was targeted. The increase of NfL levels from baseline (ICU admission) to 72 h was two-fold in the higher blood pressure group and almost six-fold in those with lower blood pressure. Although NSE levels in the original study did not differ in those groups, and neurological outcome was similar [59], NfL as a very sensitive marker may reflect milder HIBI in the higher blood pressure group, suggesting that higher blood pressure may be beneficial.

The association between higher MAP and lower NfL concentrations must be understood as a hypothesis-generating finding that is also clinically important. The differences between NfL and NSE according to MAP targets can be explained by the superiority of NfL over NSE in discriminating patients according to neurological outcome. Moreover, NfL is a more sensitive marker of neurological injury than NSE and has demonstrated a high capacity to exclude severe HIBI.

Other studies that have examined the effect of higher blood pressure after CA have not demonstrated differences in outcomes. In a study by Ameloot et al., no differences in brain injuries on MRI were detected; however, they did not assess WM injuries [302]. In a large study by Kjaergaard et al., the MAP targets of 63 mmHg and 77 mmHg had no impact on either neurological outcome or NSE levels [57]. A notable issue in this study was the small MAP difference between groups, making significant differences in the outcomes more difficult to detect.

6.5 LIMITATIONS

This study has some limitations. Firstly, post-hoc studies on resuscitated patients run a risk of self-fulfilling prophecy if the clinicians responsible for decision making are aware of the results of the biomarker analyses. The prognostication methods examined here focus on detecting unfavourable neurological outcome, and there is always a risk that findings from secondary analyses will repeat the errors in the primary analyses, as prognostication methods typically correlate strongly. However, NSE analyses were not available in prognostication during the FINNRESUSCI in 12 of 21 participating hospitals, which treated 64.7% of the patients.

Moreover, all NfL analyses in the FINNRESUSCI and COMACARE have been made post hoc, and these data were not available during the prognostication of the patients. Secondly, the FINNRESUSCI study was conducted in 2010–2011, and both post-CA treatment and prognostication have changed since then, possibly having some impact on generalisability of the results to the current practice. However, this is not affecting the observed differences between prognostic abilities of biomarkers. Thirdly, we did not have data on the cause of death of the FINNRESUSCI patients, and we did not know the exact prognostication methods used for each patient. Fourth, we had blood samples from about half the FINNRESUSCI patients, and Studies I, II, and IV include more patients with a shockable initial rhythm compared to other FINNRESUSCI patients, affecting other characteristics and causing a risk of bias. Fifth, the blood samples after the FINNRESUSCI study were thawed and analysed several years after freezing. However, the studied biomarkers are stable [303–305]. Sixth, the age and ROSC subgroups were rather small. Seventh, the number of patients in Study III was small, especially in the intervention groups. However, the outcomes were reliably assessed, strengthening the results of biomarker use in assessing HIBI and making comparison to other studies meaningful.

6.6 FUTURE PERSPECTIVES

The main finding of this study is the strong prognostic accuracy and potential of NfL in the assessment of neurological prognosis after OHCA. NSE is an established part of multimodal prognostication. NfL demonstrated superior prognostic ability in this study, as it has done in some other studies, but the identification of optimal cutoff values needs more research before NfL can be recommended in guidelines. Additionally, prognostication according to the current ERC-ESICM results in several individuals being assessed as having indeterminate prognosis, as prognostication focuses on finding unfavourable outcome with highly specific methods and their cutoffs. As the prediction of favourable outcome is a novel approach, it needs validation, but it would narrow the gap between certainty and uncertainty of prognosis if adapted to the prognostication guidelines in the future. In addition, clinical models utilising prehospital resuscitation and patient-related features and machine learning have the potential to complement prognostication [7]. So far, there is little evidence about the usefulness of artificial intelligence (AI) in decision-making concerning patients resuscitated from CA, but the prospects are fascinating [306–308]. As NfL improved the prognostic ability of clinical models in this study and has great prognostic accuracy, its additional value could be beneficial in other models. A recent study suggests that combining clinical and and biomarker data (including NfL as the most promising biomarker) with AI may be useful [309]. In the near future, the pre-planned biomarker analysis of the TTM2 trial will be published, offering more results with NfL in addition to other biomarkers [310].

One interesting aspect was the effect of higher blood pressure on NfL levels, which is still a single observation. In the future, the multi-centre factorial Sedation, Temperature, and Pressure after Cardiac Arrest and REsuscitation (STEPCARE) trial (NCT05564754) is planned to recruit over 3,000 resuscitated participants and will investigate the effects of sedation, temperature, and blood pressure on outcome. This large study will hopefully give conclusive answers on blood pressure targets. Moreover, it

will utilise NfL levels. When the results of STEPCARE and the TTM-2 are completed, our knowledge of NfL as a potential neurobiomarker will be much greater.

7 CONCLUSIONS

The four studies in this work assessed the usefulness of three neurobiomarkers (NSE, UCH-L1, and NfL) in prognostication in two different OHCA populations (the FINNRESUSCI and the COMACARE). According to our findings, the following conclusions can be drawn:

1) NSE predicted unfavourable long-term outcome with excellent accuracy in the youngest patients (18–53 years) and with good accuracy in those with the longest time from collapse to ROSC (≥ 29 min). NSE had a poor ability to predict outcome after OHCA in the oldest patients (≥ 72 years) and in those with the shortest time from collapse to ROSC (≤ 13 min; Study I).
2) UCH-L1 had a moderate ability to predict unfavourable long-term outcome after OHCA and did not offer any benefits in prognostication compared to NSE (Study II).
3) NfL predicted unfavourable long-term outcome with excellent accuracy, which was significantly better than that of NSE (Studies III and IV). Neither low-normal PaO_2 or $PaCO_2$ targets had an impact on NfL concentrations. NfL concentrations were significantly lower in the higher MAP group (80–100 mmHg) than in the lower MAP group (65–75 mmHg; Study III).

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ORIGINAL PUBLICATIONS (I – IV)

I

Usefulness of neuron specific enolase in prognostication after cardiac arrest: Impact of age and time to ROSC

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Clinical paper

Usefulness of neuron specific enolase in prognostication after cardiac arrest: Impact of age and time to ROSC☆

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Abstract

Aim of the study: We evaluated the impact of patient age and time from collapse to return of spontaneous circulation (ROSC) on the prognostic accuracy of neuron specific enolase (NSE) after out-of-hospital cardiac arrest (OHCA).

Methods: Using electrochemiluminescence immunoassay, we measured serum concentrations of NSE in 249 patients who were admitted to intensive care units after resuscitation from OHCA. In each quartile according to age and time to ROSC, we evaluated the ability of NSE at 48 h after OHCA to predict poor outcome (Cerebral Performance Category 3–5) at 12 months.

Results: The outcome at 12 months was poor in 121 (49%) patients. The prognostic performance of NSE was excellent (area under the receiver operating characteristic curve, AUROC, 0.91 [95% confidence interval, 0.81–1.00]) in the youngest quartile (18–56 years), but worsened with increasing age, and was poor (AUROC 0.53 [0.37–0.70]) in the oldest quartile (72 years or more). The prognostic performance of NSE was worthless (AUROC 0.45 [0.30–0.61]) in the quartile with the shortest time to ROSC (1–13 min), but improved with increasing time to ROSC, and was good (AUROC 0.84 [0.74–0.95]) in the quartile with the longest time to ROSC (29 min or over).

Conclusion: NSE at 48 h after OHCA is a useful predictor of 12-month-prognosis in young patients and in patients with a long time from collapse to ROSC, but not in old patients or patients with a short time to ROSC.

Keywords: Neuron specific enolase (NSE), OHCA, Resuscitation, Cardiac arrest, Neurological outcome, Biomarkers

☆ Some of the results were presented as an abstract at the 37th International Symposium on Intensive Care and Emergency Medicine, 21–24 March 2017, Brussels, Belgium.

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Introduction

After cardiac arrest and resuscitation, prognostication is challenging.[1,2] In addition to clinical neurological examination, imaging and neurophysiological studies, certain biomarkers, particularly neuron specific enolase (NSE), are considered useful.[3–7] Sedative medications affect clinical examination and electroencephalography (EEG) but not biomarkers.[7] Hypoxic brain injury increases blood NSE concentrations[8] and international guidelines recommend the use of NSE as one part of multimodal prognostication.[6]

However, also other factors than hypoxic brain damage may elevate the NSE concentration[4,8–14] and a good outcome is possible despite high concentrations.[8,15–17] In addition, NSE concentrations can remain low despite severe brain damage.[15]

It is not known whether age affects the prognostic ability of NSE after cardiac arrest. Moreover, the time from collapse to the return of spontaneous circulation (ROSC) probably affects the severity of hypoxic-ischemic brain injury, a typical cause of death after cardiac arrest,[18] but it is unknown if this affects the prognostic value of NSE.

We aimed to evaluate the impact of the patient's age and time from collapse to ROSC on the ability of NSE to predict poor long-term outcome in patients resuscitated from out-of-hospital cardiac arrest (OHCA).

Methods

Study population

This study is a sub-study of the FINNRESUSCI study.[19] In brief, the FINNRESUSCI study prospectively collected data on 504 adult patients who were treated in 21 Finnish intensive care units (ICUs) after OHCA between March 1st, 2010, and February 28th, 2011. In the current study we included 249 unconscious patients, for whom blood samples were available. The FINNRESUSCI study protocol was approved by the Ethics Committee of Helsinki University Hospital and by each participating hospital.

We assessed neurological outcome according to the Cerebral Performance Category (CPC)[20] at 12 months after cardiac arrest. We determined good outcome as sufficient neurological function for managing activities of daily living independently (CPC 1–2) and poor outcome as severe neurological deficits or death (CPC 3–5). The cause of cardiac arrest (cardiogenic or other) was determined with clinical criteria. We chose death in hospital as a secondary outcome.

Data collection

Patient data were collected by using Internet-based case report forms. Data on previous state of health was collected from the patient's medical history and mortality data were obtained from Statistics Finland. Neurological status of all patients at 12 months after cardiac arrest was assessed by phone contact between the patient and a specialist in neurology who was blinded to treatment details. A structured interview to determine the Pittsburgh Cerebral Performance Category (CPC) was used.

Blood sampling and biomarker analysis

Blood samples were taken at 24 and 48 h after cardiac arrest. The blood sample was allowed to clot at room temperature for 60 min, after which it was centrifuged and the obtained serum stored at −70 °C. Serum concentrations of NSE were measured with a commercially available electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany) in April 2015. All analyses were made in the same laboratory. The range of measurements was 0.05–370 μg/l (or up to 740 μg/l for 2-fold diluted samples) and the range of normal values was 0–16.3 μg/l. The intra and inter assay coefficients of variation were < 3.9% and < 3.2%, respectively. We considered a concentration of 500 mg/l or higher of free haemoglobin as an indicator of significant haemolysis.[3] In line with a real-life situation in Finland, we included all blood samples to the study regardless of the amount of haemolysis.

Statistical analysis

We present continuous data as medians with interquartile ranges (IQRs) and categorical data as absolute numbers with percentages (95% confidence intervals [CIs]). We tested normality of distribution with the Kolmogorov–Smirnov test. We used the independent samples t test to compare continuous data with normal distributions. When the distribution was not normal, we used the Mann–Whitney U test or the Kruskal–Wallis test, as appropriate. We compared categorical variables by using Pearson's Chi test or Fisher's exact test, as appropriate.

To assess the ability of NSE to discriminate between patients with poor outcome (CPC 3–5) and those with good outcome (CPC 1–2), we calculated areas under the receiver operating characteristic curves (AUROCs)[21] with 95% CIs. We defined values <0.7 as poor, values of 0.7–0.8 as satisfactory, 0.8–0.9 as good and values >0.9 as excellent. In addition to NSE levels at 24 h and 48 h after cardiac arrest, we also studied the change in NSE between 24 h and 48 h after cardiac arrest. We determined IQRs for patient' ages and the times from collapse to ROSC, and for every quartile we calculated the AUROC for NSE at 48 h. We compared AUROCs by using the bootstrap method. Based on the sensitivity and specificity for different cut-off values, we selected cut-offs using the Youden index.[22,23] We also determined the cut-off value for 99% specificity. We calculated the sensitivity, specificity, positive predictive value (PPV) and positive likelihood ratio (LR+) for these cut-off values.

We used logistic regression analysis to create a baseline multivariate model to predict poor outcome. We evaluated the predictive value of this model by determining the AUROC. We also assessed the continuous Net Reclassification Improvement (NRI) achieved by the addition of NSE into the baseline model. We assessed event NRI (NRIe) and non-event NRI (NRIne). NRIe is calculated as [(the number of individuals with the predicted event, i.e. poor outcome, given a higher risk after addition of NSE) — (the number of individuals with the event given a lower risk)]/[the number of individuals with the event]. Likewise, NRIne is the net proportion of individuals without the event given a lower risk. The overall NRI is the sum of NRIe and NRIne. The theoretical range of values for both NRIe and NRIne is −1 to +1, and that of the overall NRI is −2 to +2. [24,25]

In addition, we determined the Integrated Discrimination Improvement (IDI) achieved by the addition of NSE into the baseline multivariate model. IDI measures not only the direction of the change in probability with the addition of new information, but also the magnitude of the change. We calculated event IDI (IDIe) for patients with poor outcome as [(mean probability of poor outcome with baseline model + NSE)− (mean probability of poor outcome with baseline model)] and non-event IDI (IDIne) for patients with good outcome as [(mean probability of poor outcome with baseline model) − (mean probability of poor outcome with baseline model + NSE)]. IDI is the sum of IDIe and IDIne. The theoretical range of IDIe and IDIne is −1 to +1 and that of IDI is −2 to +2. [24,26]

We considered p values <0.05 as significant. We made the analyses with SPSS version 21 (SPSS, Chicago, IL, USA) and R version 3.1.1.

Results

In total, 249 OHCA patients were included in the study (Fig. 1). Blood samples at 48 h were available for 220 patients. Because the consent was not available for all patients at 24 h, samples from this time point are missing for seven patients. The initial rhythm was shockable in 177 (71%) patients. The aetiology of the arrest was cardiac in 199 (79.9%) patients. Targeted temperature management was used in 193 (77.5%) patients. The baseline characteristics of the study population are presented in Table 1.

Patient outcomes and prognostic ability of NSE

Overall, 121 patients (49%) had a poor outcome at 12 months. The median NSE concentration at 24 h was 12.9 μg/l (IQR, 7.6-23.6) in patients with poor outcome and 8.7 μg/l (5.9–13.4) in those with good outcome ($p < 0.001$). The median NSE concentration at 48 h was 17.9 μg/l (8.1–56.4) in patients with poor outcome and 8.2 μg/l (5.9–12.1) in those with good outcome ($p < 0.001$). The ability to predict poor outcome was better for NSE at 48 h (AUROC 0.72 [0.65–0.80]]) than NSE at 24 h (AUROC 0.65 [0.58–0.72]), $p = 0.005$.

The AUROC for the change in NSE concentration between 24 h and 48 h after cardiac arrest was 0.70 (0.63–0.78[$p < 0.001$]), which was not significantly different from the AUROC of NSE at 48 h ($p = 0.489$).

Among those 29 patients, for whom blood samples at 48 h were not available, poor outcome occurred in 23 patients (79.3%), 13 of whom died before the 48 h time point. For these 29 patients, the concentrations of NSE at 24 h were 15.3 μg/l (4.8-47.9) for patients with poor outcome and 6.3 μg/l (2.9–12.5) for those with good outcome.

For three patients, significant haemolysis was found. For two of those patients, with NSE concentrations 36.0 μg/l and 37.0 μg/l, respectively, at 48 h, CPC was 1–2. For one patient, with an NSE concentration of 6.8 μg/l at 48 h, CPC at 12 months was 5 (death).

Cut-off values

Based on the Youden index, the cut-off value for NSE at 48 h as a predictor of poor outcome at 12 months was 20 μg/l. With this cut-off, sensitivity was 50%, specificity 92.6%, PPV 84.5% and LR + 6.8 (3.5–13.1) ($p < 0.001$). When we required a 99% threshold for specificity, we obtained the cut-off 37 μg/l (with sensitivity 35.7%, PPV 97.2%, LR + 43.6 [6.1–312.4])

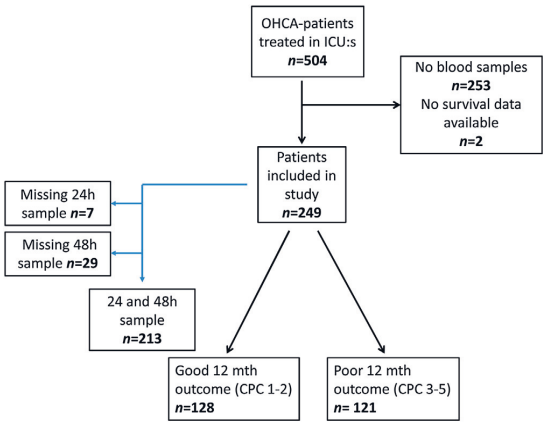

Fig. 1 – Flowchart of the study population.

Table 1 – Baseline characteristics for all patients and stratified according to age and time to ROSC.

	All	Age				p	Time to ROSC				p
		18–56 years	57–63 years	64–71 years	≥72 years		11–13 min	14–20 min	21–28 min	≥29 min	
Number of patients, n	249	62	69	59	59		62	69	58	60	
Age, median (IQR), years	63 (56.5–71.0)	48.0 (42.0–54.0)	60.0 (59.0– 62.0)	67.0 (66.0– 70.0)	77.0 (73.0–81.0)		63.0 (57.0–74.3)	63.0 (55.5– 70.5)	64.5 (57.0–71.0)	62.0 (54.0–69.0)	0.608
Gender, males, n, (%)	209 (83.9)	50 (80.6)	60 (87.0)	52 (88.1)	47 (79.7)	0.469	53 (85.5)	55 (79.7)	50 (86.2)	51 (85.0)	0.730
Witnessed, n (%)*	227 (91.2)	56 (90.3)	68 (98.6)	51 (86.4)	52 (88.1)	0.071	58 (93.5)	63 (91.3)	51 (87.9)	55 (91.7)	0.750
Bystander CPR, n (%)	146 (58.6)	34 (54.8)	43 (62.3)	41 (69.5)	28 (47.5)	0.084	31 (50.0)	40 (58.0)	40 (69.0)	35 (58.3)	0.215
Cardiogenic reason of arrest, n (%)	199 (79.9)	41 (66.5)	57 (82.6)	50 (84.7)	51 (86.4)	0.018	50 (80.6)	56 (81.2)	47 (81.0)	46 (76.7)	0.913
Time to ROSC, median (IQR), min	20.0 (13.5–28.0)	20.0 (14.0–29.3)	21.0 (13.0–30.0)	23.0 (16.0–28.0)	19.0 (10.0–24.0)	0.138	10.0 (6.0–11.0)	17.0 (15.0–19.5)	24.0 (22.8–26.0)	34.0 (30.3–40.0)	
Shockable rhythm, n (%)	177 (71.1)	42 (67.7)	49 (71.0)	45 (76.3)	41 (69.5)	0.757	43 (69.4)	50 (72.5)	40 (69.0)	44 (73.3)	0.934
SAPS II, median (IQR), points	58.0 (42.0–69.0)	52.0 (32.0–61.0)	57.0 (40.0– 64.0)	60.0 (42.0– 72.0)	67.0 (52.0–73.0)	<0.001	47.5 (33.0–60.8)	56.0 (37.5– 65.5)	60.5 (45.8–69.0)	64.5 (52.3–71.8)	<0.001
Poor outcome (CPC 3-5), n (%)	121 (48.6)	25 (40.3)	26 (37.7)	34 (57.6)	36 (61.0)	0.014	16 (25.8)	24 (34.8)	38 (65.5)	43 (71.7)	<0.001

IQR, interquartile range; Witnessed, witness for collapse; CPR, cardiopulmonary resuscitation; ROSC, return of spontaneous circulation; Shockable rhythm, ventricular fibrillation or ventricular tachycardia; SAPSII, Simplified Acute Physiology Score, CPC, Cerebral Performance Category.

($p < 0.001$). A specificity of 100% was obtained with the cut-off value 68 μg/l (corresponding sensitivity 17%). Cut-off values for specificities 95–100% for NSE at 48 h are presented in Supplementary material, Table S1.

The Youden index-based cut-off values for NSE 48 h according to age and time to ROSC are presented in Supplementary material, Table S2.

The value of NSE in different age groups

The difference in NSE concentrations between patients with poor outcome and those with good outcome was most remarkable in the youngest quartile, whereas there was no statistically significant difference in the oldest quartile. Distributions of NSE concentrations at 48 h for patients with poor outcome and for those with good outcome, stratified according to age quartiles, are presented in Fig. 2.

The ability of NSE at 48 h to predict poor outcome in different age groups is presented in Table 2. The ability of NSE at 48 h to predict death in hospital is presented in the Supplementary material, Table S3.

The value of NSE in different groups according to time to ROSC

Distributions of NSE concentrations at 48 h for patients with poor and for those with good outcome, stratified according to time to ROSC quartiles, are presented in Fig. 3. The ability of NSE at 48 h to predict poor outcome in different groups according to time to ROSC is presented in Table 2. The prognostic value was poor in the first quartile (1–13 min), but improved with increasing time to ROSC, and was good for patients with the longest time to ROSC (≥29 min).

The ability of NSE at 48 h to predict death in hospital in different quartiles according to time to ROSC is presented in the Supplementary material, Table S3.

The main results remained essentially unchanged after exclusion of the patients with haemolytic blood samples (Supplementary material, Table S4).

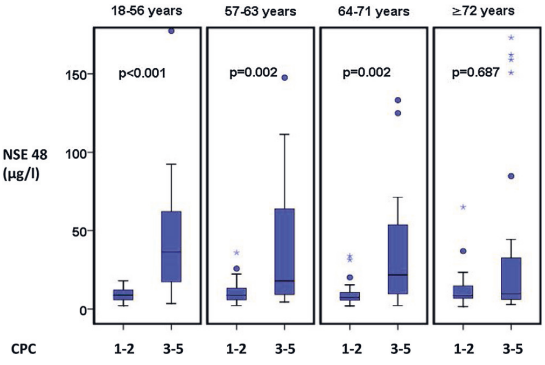

Fig. 2 – Distribution of NSE concentrations (μg/l) at 48 h for the patients with good (Cerebral Performance Category, CPC 1–2) and poor (CPC 3–5) outcome in quartiles according to age.
Boxplot figures; each box showing the interquartile range, with a horizontal line inside the box showing the median value; bars showing the range of values except outliers (circles and stars), defined as values more than 1.5 box lengths from the edge of the box.

Table 2 – The ability of NSE at 48 h to predict poor outcome at 12 months, for all patients and in quartiles according to age and time to ROSC.

		All	Age				Time to ROSC			
			18–56 years	57–63 years	64–71 years	≥72 years	1–13 min	14–20 min	21–28 min	≥29 min
NSE 48 h	AUROC	0.72	0.91	0.74	0.76	0.53	0.45	0.62	0.75	0.84
	CI (95%)	0.65–0.80	0.81–1.00	0.60–0.88	0.62–0.90	0.37–0.70	0.30–0.61	0.44–0.80	0.61–0.89	0.74–0.95
	p	<0.001	<0.001	0.002	0.002	0.687	0.598	0.132	0.004	<0.001
NSE48 h >20 μg/l	Sensitivity	50.0	69.6	47.6	56.0	31.0	6.7	27.8	56.7	74.3
	Specificity	92.6	100	90.2	91.7	85.7	95.1	97.8	89.5	76.5
	PPV	84.5	100	71.4	87.5	75.0	33.3	83.3	89.5	86.7
	LR+ (95% CI)	6.8 (3.5–13.1)	∞	4.9 (1.7–13.7)	6.7 (1.7–26.5)	2.2 (0.7–7.1)	1.4 (0.1–14.0)	12.5 (1.6–99.7)	5.4 (1.4–20.7)	3.2 (1.3–7.6)
	p	<0.001	<0.001	0,003	0,001	0,201	1.00	0.006	0.002	0.001
NSE 48 h >37 μg/l	Sensitivity	35.7	47.8	33.3	40.0	24.1	6.7	11.1	36.7	60.0
	Specificity	99.2	100	100	100	95.2	97.6	100	100	100
	PPV	97.2	100	100	100	87.5	50.0	100	100	100
	LR+ (95% CI)	43.6 (6.1–312.4)	∞	∞	∞	5.1 (0.7–38.2)	2.7 (0.2–41.0)	∞	∞	∞
	p	<0.001	<0.001	<0.001	0.001	0.117	0.468	0.078	0.003	<0.001

ROSC, return of spontaneous circulation; AUROC, area under the receiver operating characteristic curve; PPV, positive predictive value; LR+, positive likelihood ratio.

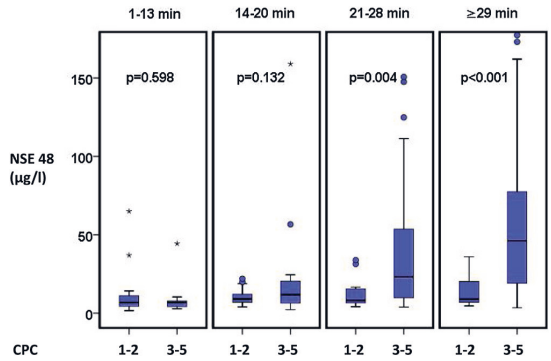

Fig. 3 – Distribution of NSE concentrations (µg/l) at 48 h for the patients with good (Cerebral Performance Category, CPC 1–2) and poor (CPC 3-5) outcome in quartiles according to time from collapse to ROSC.
Boxplot figures; each box showing the interquartile range, with a horizontal line inside the box showing the median value; bars showing the range of values except outliers (circles and stars), defined as values more than 1.5 box lengths from the edge of the box.

The value of NSE in addition to other prognostic data

Our baseline risk prediction model including age, time to ROSC, initial rhythm and Simplified Acute Physiology Score (SAPS) II points[27] without age points had an AUROC of 0.81 (0.75-0.86) for predicting poor outcome at 12 months. When 48 h NSE was added to this model, the AUROC increased to 0.84 (0.79-0.89) ($p = 0.021$).

Regarding different age groups, adding NSE to the baseline risk prediction model improved the AUROC only for the youngest patients (18–56 years) ($p = 0.013$).

Considering different times to ROSC, adding NSE to the baseline model improved the AUROC only for patients with longest time to ROSC (29 min or more) ($p < 0.001$).

AUROC, NRI and IDI data for all patients and according to age and time to ROSC quartiles are presented in Table 3.

NSE analyses were available in 9 of 21 participating hospitals at the time of the FINNRESUSCI study. These hospitals treated 35.3% of the study patients. The prognostic ability of NSE was dependent on age and time to ROSC in both hospital groups (Supplementary Tables S5 and S6).

Discussion

The main finding of our study is that the ability of NSE to predict one-year outcome was dependent on both the patient's age and the time from collapse to ROSC. In young patients, NSE at 48 h had an excellent predictive value, whereas the predictive value was poor in the oldest patients. For patients with a short time from collapse to ROSC, NSE at 48 h was not able to predict outcome, but it showed a good predictive ability for patients with a long time to ROSC. These findings are important as NSE is one of the parameters commonly used in prognostication of patients resuscitated from cardiac arrest. Further refinement of the use of NSE by identifying appropriate and inappropriate patient groups is of great importance.

We suggest that there may be a plausible explanation for our findings: NSE is a marker of neurological injury, but it may not be able to predict a poor long-term prognosis that is caused by other factors than hypoxic brain injury. For young patients, poor outcome after cardiac arrest is often associated with hypoxic brain damage, whereas poor long-term outcome in elderly patients may often be influenced by other factors (e.g. heart failure, pulmonary or renal disease, infirmity), i.e. factors that may not be reflected by post-resuscitation NSE levels. For patients who die after initially successful resuscitation, hypoxic brain injury is the most common cause of death, but deaths because of circulatory failure also occur frequently.[28]

In the normal population, NSE levels do not vary significantly in different ages.[29] However, in patients with Alzheimer's disease, NSE concentrations in serum tend to decrease with increasing severity of brain atrophy.[30] It might be possible that Alzheimer's disease and other neurodegenerative disorders that are more common among the old than in younger people may cause loss of neuronal tissue, which might decrease the response of increasing NSE concentrations after hypoxic brain injury.

In addition to age, the time from collapse to ROSC influenced the ability of NSE to predict poor outcome. NSE at 48 h showed good predictive ability for patients with a long time to ROSC, but not for those with a short time to ROSC. A possible explanation is that for cardiac arrest patients with a long time to ROSC, the cause of poor outcome is often hypoxic brain injury that typically causes high NSE concentrations, whereas a poor outcome despite a short time to ROSC may not be caused by hypoxic encephalopathy, but rather the underlying conditions responsible for the cardiac arrest. In the study by Streitberger et al. on 1053 resuscitated patients, the cause of death was other than hypoxic brain injury for the majority of patients who died even though the NSE concentration was 17 µg/l or lower.[4]

In our study, the cut-off obtained with the Youden method was 20 µg/l, whereas it was 29 µg/l in the study by Stammet et al. For a 99% threshold of specificity, the cut-off was 37 µg/l, as compared to 68 µg/l in the study by Stammet et al. Requiring 100% specificity results in low sensitivity, which limits the clinical use of biomarkers, and a lower specificity for cut-off values has been proposed by Stammet et al.[31]

Optimal cut-off values for NSE at 48 h to predict poor neurological outcome have varied between 25 and 97 µg/l in different studies.[4,11,15] There are several possible explanations for the large variation: there are differences in laboratory methods,[32] in patient case-mix,[4,15] in definitions of poor outcome[15] and in the time between the cardiac arrest and the assessment of neurological outcome.[3-4,16,33-36] Commonly, outcome has been determined at six months after cardiac arrest,[3,16,36] whereas we assessed outcome at 12 months.

Table 3 – Multivariate models to predict poor outcome at 12 months (A). Area under receiver operating characteristic curve (AUROC), Net Reclassification Improvement (NRI) and Integrated Discrimination Improvement (IDI) for all patients and stratified according to age and time to ROSC (B).

	OR (95% CI)	p
Age[a]	1.045 (1.018–1.074)	0.001
ROSC[a]	1.076 (1.042–1.112)	<0.001
Witnessed[a]	0.319 (0.101–1.011)	0.052
Shockable[a]	0.309 (0.152–0.627)	0.001
*SAPSII[a]	1.041 (1.019–1.064)	<0.001
NSE48[b]	1.055 (1.025–1.085)	<0.001

			AGE, years				Time to ROSC, min			
		ALL	18–56	57–63	64–71	≥72	1–13	14–20	21–28	≥29
AUROC (95% CI)	Baseline model	0.81 (0.75–0.86)	0.82 (0.71–0.93)	0.84 (0.74–0.95)	0.82 (0.71–0.94)	0.68 (0.52–0.84)	0.72 (0.58–0.86)	0.80 (0.68–0.91)	0.83 (0.71–0.95)	0.71 (0.54–0.87)
	Baseline model + NSE 48 h	0.84 (0.79–0.89)	0.88 (0.79–0.97)	0.88 (0.79–0.97)	0.84 (0.73–0.95)	0.67 (0.52–0.82)	0.68 (0.54–0.83)	0.82 (0.71–0.93)	0.85 (0.75–0.96)	0.90 (0.82–0.99)
	p value for difference	0.021	0.013	0.226	0.665	0.819	0.100	0.453	0.561	0.001
NRI	Continuous	0.394	0.773	0.516	0.457	−0.332	−0.491	0.244	0.628	1.140
	NRI_e	−0.082	0.217	−0.143	0.040	−0.379	−0.467	−0.444	−0.067	0.257
	NRI_{ne}	0.476	0.556	0.659	0.417	0.047	−0.024	0.688	0.695	0.883
IDI	IDI	0.032	0.141	0.035	0.017	−0.077	−0.059	0.000	0.039	0.228
	IDI_e	−0.010	0.078	−0.017	−0.035	−0.041	−0.039	−0.046	−0.013	0.036
	IDI_{ne}	0.041	0.063	0.052	0.051	−0.035	−0.020	0.047	0.051	0.192

ROSC, return of spontaneous circulation; Witnessed, witnessed cardiac arrest, Shockable, shockable initial rhythm (ventricular fibrillation or ventricular tachycardia); SAPSII, Simplified Acute Physiology Score II; NSE48, NSE concentration at 48 h after cardiac arrest; AUROC, the area under the receiver operating characteristic curve; NRI, net reclassification improvement; NRI_e, event NRI; NRI_{ne}, non-event NRI; IDI, integrated discrimination improvement; IDI_e, event IDI; IDI_{ne}, non-event IDI.

*SAPSII without age points.

[a] Variables in baseline model. Calculated with logistic regression for 220 patients for whom NSE at 48 h was available.

[b] NSE at 48 h added to the baseline model.

For some individuals, NSE concentrations after cardiac arrest and resuscitation may be high although their prognosis is good.[8,15–17] Also, ischaemic or haemorrhagic stroke or traumatic intracerebral bleeding increase the serum NSE values, but high levels do not exclude the possibility of a good outcome.[12–14] In addition, extracerebral sources of NSE may cause bias: high NSE concentrations have been found in association with several diseases, including small cell lung cancer[9] and many neuroendocrine tumors.[37] Therefore, it is advisable to avoid decisions about futility of care on the basis of NSE concentrations alone. Nevertheless, NSE is a useful part of multimodal prognostication based on repeated clinical examination, electrophysiological studies and brain imaging.[6–7] However, it is important to realise that haemolysis may increase NSE concentrations,[4,10] and NSE measurements from haemolytic blood samples must not be used for prognostication.

Strengths and limitations

Our study has a number of strengths. This was a nationwide multicentre study with 249 patients. All blood samples were analysed in the same laboratory at one time and long-term neurological outcome was defined by an experienced neurologist blinded to the NSE results.

There are also limitations. Firstly, we did not have blood samples from all FINNRESUSCI study patients. In fact, there was a difference in the proportion of shockable rhythms between our study (71.1%) and the original FINNRESUSCI study (56.8%) and in the proportion of cardiac aetiology of CA (79.9% vs. 66.3). Accordingly, the proportion of patients with good outcome was higher in our study (51%) than in the original FINNRESUSCI study (38.5%), indicating some degree of selection bias. Secondly, we do not know the best CPC or the cause of death of our study patients. Third, the number of patients in the subgroups was rather small.

Conclusions

In this observational study, we found that the ability of NSE at 48 h to predict long-term outcome after resuscitation from OHCA was good for young patients and for patients with a long time from collapse to ROSC, but poor for the oldest patients and for those with a short time to ROSC. If these findings are confirmed in other studies, they should be taken into account when prognostication guidelines are updated.

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Conflicts of interests

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.resuscitation.2019.04.021.

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II

Ubiquitin C-terminal hydrolase L1 after out-of-hospital cardiac arrest

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RESEARCH ARTICLE

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Ubiquitin C-terminal hydrolase L1 after out-of-hospital cardiac arrest

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Abstract

Background: We studied the prognostic ability of serum ubiquitin C-terminal hydrolase L1 (UCH-L1) after out-of-hospital cardiac arrest (OHCA), compared to that of neuron-specific enolase (NSE).

Methods: In this post-hoc analysis of the FINNRESUSCI study, we measured serum concentrations of UCH-L1 in 249 OHCA patients treated in 21 Finnish intensive care units in 2010–2011. We evaluated the ability of UCH-L1 to predict unfavourable outcome at 12 months (defined as cerebral performance category 3–5) by assessing the area under the receiver operating characteristic curve (AUROC), in comparison with NSE.

Results: The concentrations of UCH-L1 were higher in patients with unfavourable outcome than for those with favourable outcome: median concentration 10.8 ng/mL (interquartile range, 7.5–18.5 ng/mL) versus 7.8 ng/mL (5.9–11.8 ng/mL) at 24 h ($p < .001$), and 16.2 ng/mL (12.2–27.7 ng/mL) versus 11.5 ng/mL (9.0–17.2 ng/mL) ($p < .001$) at 48 h after OHCA. For UCH-L1 as a 12-month outcome predictor, the AUROC was 0.66 (95% confidence interval, 0.60–0.73) at 24 h and 0.66 (0.59–0.74) at 48 h. For NSE, the AUROC was 0.66 (0.59–0.73) at 24 h and 0.72 (0.65–0.80) at 48 h. The prognostic ability of UCH-L1 was not different from that of NSE at 24 h ($p = .82$) and at 48 h ($p = .23$).

Conclusion: Concentrations of UCH-L1 in serum were higher in patients with unfavourable outcome than in those with favourable outcome. However, the ability of UCH-L1 to predict unfavourable outcome after OHCA was only moderate and not superior to that of NSE.

KEYWORDS
biomarkers, cardiac arrest, neurological outcome, OHCA, prognostication, resuscitation, ubiquitin C-terminal hydrolase L1

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Editorial Comment

In this post-hoc analysis of the FINNRESUSCI study, serum ubiquitin C-terminal hydrolase L1 (UCH-L1), an enzyme mostly located in neurons of the cerebral cortex, was measured at 24 and 48 h following out-of-hospital cardiac arrest of any cause in 249 patients. The levels of UCH-L1 showed moderate predictive performance for unfavourable neurological outcome (cerebral performance category 3–5) at 12 months follow up and was not superior to the more commonly used neuron-specific enolase assay.

1 | INTRODUCTION

Hypoxic-ischemic brain injury (HIBI) is the most common reason for severe disability and death after cardiac arrest (CA).[1,2] Identifying imminent severe HIBI that results in poor prognosis is an essential part of patient management.[3] Biomarkers are one part of multimodal prognostication along with imaging and clinical and neurophysiological examinations.[4] Biomarkers have some special benefits: they are non-invasive and inexpensive, and the results are not confounded by sedative medications.[3]

Neuron-specific enolase (NSE) is the biomarker recommended in the latest ERC-ESICM guideline.[4] However, when high NSE cut-off values are used to minimize the risk of falsely pessimistic prognosis, the sensitivity of NSE remains rather low, and many individuals with poor prognosis are not identified correctly.[5,6] In addition, haemolysis,[7,8] extracerebral sources,[9,10] stroke and traumatic brain injury (TBI)[11–13] may increase NSE concentrations. Moreover, we have previously shown that the prognostic value of NSE is dependent on the patient's age and the duration of CA.[14]

Ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1) is an enzyme mostly located in neurons of the cerebral cortex.[15,16] After TBI, UCH-L1 concentrations are associated with severity of injury.[17–19] Results from a previous study suggested that UCH-L1 may be a rather good predictor of unfavourable outcome in OHCA patients with a presumably cardiac cause of arrest.[20]

To assess the prognostic ability of UCH-L1 in an unselected OHCA population, we analysed UCH-L1 concentrations in serum of patients included in the FINNRESUSCI study.[21]

2 | METHODS

2.1 | Patient selection and data collection

Patients for this post-hoc study were included in the prospective FINNRESUSCI study[21] that collected data on adult patients who were resuscitated from OHCA, irrespective of aetiology of CA. The study was performed in 21 Finnish intensive care units (ICUs) between 2010 and 2011. Blood samples from 249 of the original 548 FINNRESUSCI study patients were available, and we included all of them to the study (Figure 1). Clinical criteria were used to determine the cause (cardiogenic or other) of CA.

The study protocol of the FINNRESUSCI study was approved by the Ethics Committee of Helsinki University Hospital and by each participating hospital.

Internet-based case report forms were used to record patient data. Data on previous health and diseases were collected from the patient's medical history. Mortality data were obtained from Statistics Finland. A structured interview was used to define the neurological recovery at 12 months after CA. A specialist in neurology, who was blinded to the treatment of the patients and test results, performed all assessments of patient's neurological status by phone contacts.

2.2 | Outcome definitions

We defined favourable outcome as cerebral performance category (CPC) 1–2 and unfavourable outcome as CPC 3–5 at 12 months after CA. CPC 1–2 means sufficient neurological function for at least independently managing basic activities of daily living, whereas CPC 3–5 describes severe disability, persistent vegetative state or death.[22] As short-term outcome, we used death during index hospital treatment.

2.3 | Laboratory analyses

Blood samples were drawn from FINNRESUSCI study patients, for whom written consent was provided by a next of kin. The samples were allowed to clot at room temperature for 60 min, after which they were centrifuged, and serum was stored at −70°C. We analysed serum concentrations of UCH-L1 in March 2015 by a commercial enzyme-linked immunosorbent assay kit (USCN, Wuhan, China). We analysed all samples in duplicate. The intra- and inter-assay coefficients of variation (CV) were <7.5% and <11.5%, respectively, for UCH-L1. Intra-assay CVs were determined in 16 aliquots of two serum pools analysed in the same run and inter-assay CVs were determined in 10 aliquots of two serum pools analysed in the consecutive runs. The calibrators covered the range 0.16–10 ng/mL for UCH-L1. Serum samples were diluted 4-fold or up to 20-fold when needed prior to assay of UCH-L1.

We have previously published the concentrations and prognostic ability of NSE in this same study population.[14,23] In the current study, we measured the UCH-L1 concentrations and additionally compared the prognostic ability of UCH-L1 to that of NSE.

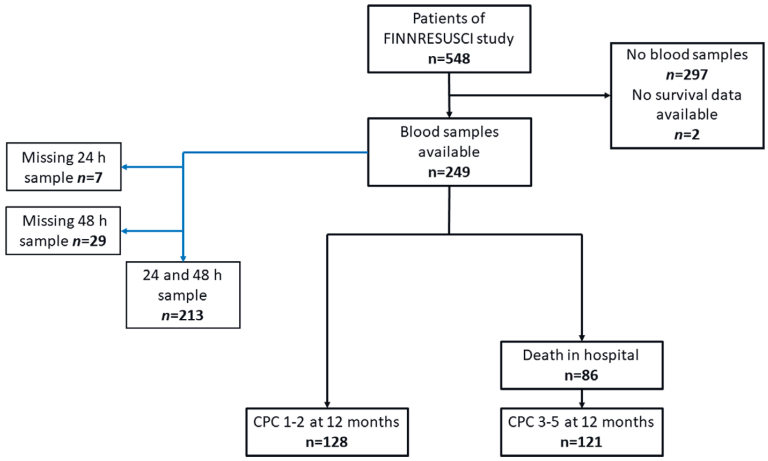

FIGURE 1 Flowchart of the study population. CPC, cerebral performance category.

2.4 | Statistical analysis

We present categorical data as absolute numbers with percentages (95% confidence intervals [CIs]). For continuous data, like biomarker concentrations, we present medians with interquartile ranges. We tested normality of distribution with the Kolmogorov–Smirnov test. For categorical data, we used a chi-square test or Fisher's exact test, as appropriate. For continuous variables, we used the independent samples *t*-test for data with normal distribution and the Mann–Whitney U test or Kruskal–Wallis test for data that were not normally distributed.

To assess prognostic ability, we determined the area under the receiver operating characteristic curve (AUROC)[24] with 95% CI for UCH-L1 and compared it with the previously determined AUROC of NSE. We used the bootstrap method to compare AUROCs.

We determined cut-off values for UCH-L1 at 24 and 48 h after CA to predict unfavourable outcome. We used the Youden method[25] to determine the optimal cut-off. To minimize the number of patients with false positive results, we determined cut-off values with high specificities (95% and 99%). We also calculated positive predictive values (PPVs), negative predictive values (NPVs) and positive likelihood ratios (LR+) with 95% CIs for those cut-off values.

We also evaluated the ability of UCH-L1 to identify patients with a high probability of favourable outcome. For this, we determined cut-off values with high (90%–99%) sensitivities. We calculated PPVs, NPVs and negative likelihood ratios (LR−) with 95% CIs for these cut-offs.

We used multivariable logistic regression analysis to analyse the independent association of UCH-L1 with unfavourable 12-month outcome and with risk of in-hospital death. We selected significant clinical variables to the models. To predict unfavourable outcome at 12 months, the variables included were patient's age, time from collapse to return of spontaneous circulation (ROSC), witnessed collapse and initial rhythm (shockable or non-shockable). Data on whether the arrest was witnessed were non-significant in the model predicting death in hospital and thus were not included. We calculated odd ratios (OR) (with 95% CIs) for these variables. Then we separately added UCH-L1 at 24 and 48 h to the models and calculated ORs.

We used SPSS version 27 (SPSS, Chicago, IL, USA) and R (version 4.0.4) for the statistical analyses and GraphPad Prism (version 9.4.1) for drawing figures.

3 | RESULTS

Of 249 patients, 177 (71.1%) had a shockable initial rhythm. The cause of CA was assessed as cardiogenic in 199 (79.9%) patients. During hospital treatment, 86 (35.5%) patients died. Twelve-month outcome was unfavourable for 121 (48.6%) patients. Data on patient characteristics are presented in Table 1.

Concentrations of UCH-L1 were significantly higher for patients with unfavourable 12-month outcome than for those with favourable outcome: the median UCH-L1 concentration was 10.8 ng/mL (IQR 7.5–18.5 ng/mL) versus 7.8 ng/mL (5.9–11.8 ng/mL), $p < .001$, at 24 h, and 16.2 ng/mL (12.2–27.7 ng/mL) versus 11.5 ng/mL (9.0–17.2 ng/mL), $p < .001$, at 48 h. The UCH-L1 concentrations were also higher for those who died during hospital treatment than for those who survived: 12.6 ng/mL (8.5–20.6 ng/mL) versus 7.9 ng/mL (6.1–12.1 ng/mL) at 24 h ($p < .001$) and 17.1 ng/mL (12.6–30.0 ng/mL) versus 12.1 ng/mL (9.1–18.4 ng/mL) at 48 h ($p < .001$). The UCH-L1 concentrations according to primary and secondary outcomes are presented in Figure 2.

3.1 | Prognostic ability

For UCH-L1 at 24 h as a predictor of unfavourable 12-month outcome, the AUROC was 0.66 (0.60–0.73). For UCH-L1 at 48 h, the AUROC was 0.66 (0.59–0.74), $p < .001$.

TABLE 1 Baseline characteristics of the study population.

		Hospital mortality			12-month outcome		
	All ($n = 249$)	Survivors $n = 163$	Non-survivors $n = 86$	p	CPC 1–2 $n = 128$	CPC 3–5 $n = 121$	p
Age, years (IQR)	63.0 (56.5–71)	62.0 (56.0–70.0)	66.0 (59.0–72.0)	.019	61.5 (55.3–67.0)	67.0 (59.0–72.0)	.001
Sex, males, *n* (%)	209 (83.9)	139 (85.3)	70 (81.4)	.428	107 (83.6)	102 (84.3)	.88
Witnessed CA, *n* (%)	227 (91.2)	152 (93.3)	75 (87.2)	.11	123 (96.1)	104 (86.0)	.005
Bystander CPR, *n* (%)	146 (58.6)	92 (56.4)	54 (62.8)	.333	78 (60.9)	68 (56.2)	.448
Shockable rhythm, *n* (%)	177 (71.1)	128 (78.5)	49 (60.0)	<.001	106 (82.8)	71 (58.7)	<.001
Cardiogenic aetiology of CA, *n* (%)	199 (79.9)	136 (83.4)	63 (73.3)	.057	110 (85.9)	89 (73.6)	.015
Time to ROSC, min (IQR)	20.0 (13.5–28.0)	17.0 (11.0–23.0)	26.0 (20.0–31.3)	<.001	16.0 (11.0–23.0)	24.0 (19.0–31.0)	<.001
TTM, *n* (%)	193 (77.5)	126 (77.3)	67 (77.9)	.913	100 (78.1)	93 (76.9)	.811
SAPS II score (IQR)	58.0 (42.0–69.0)	52.0 (36.0–63.0)	67.0 (58.8–73.0)	<.001	47.0 (34.0–60.8)	65.0 (55.5–71.0)	<.001
SOFA score (IQR)	9 (7–11)	8 (6–10)	11 (9–11)	<.001	8 (6–10)	10 (8–11)	.004

Abbreviations: CA, cardiac arrest; CPC, cerebral performance category; CPR, cardiopulmonary resuscitation; IQR, interquartile range; ROSC, return of spontaneous circulation; SAPS II, Simplified Acute Physiology Score II; SOFA, Sequential Organ Failure Assessment Score based on first 24 h in the ICU; TTM, targeted temperature management.

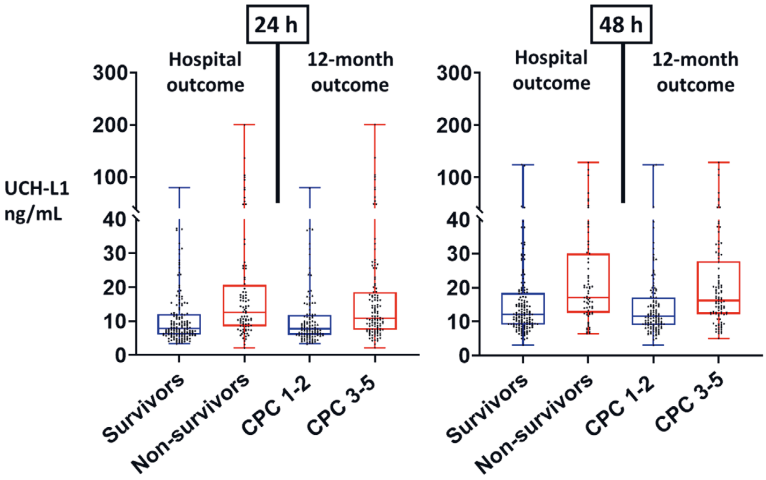

FIGURE 2 Concentrations of UCH-L1 in serum at 24 and 48 h after out-of-hospital cardiac arrest for hospital survivors and for non-survivors, and for patients with favourable outcome (CPC 1–2) and for those with unfavourable outcome (CPC 3–5) at 12 months. Each box presents the interquartile range, the line inside the box shows the median value, the whiskers show the lowest and the highest concentrations and dots show concentrations for each patient. CPC, cerebral performance category; UCH-L1, ubiquitin carboxyl-terminal hydrolase L1.

For UCH-L1 as a predictor of in-hospital death, the AUROC was 0.69 (0.62–0.76) at 24 h and 0.68 (0.60–0.78) at 48 h.

In the multivariable logistic regression model, UCH-L1 at 24 h was a significant predictor of hospital mortality (OR 1.035 [1.010–1.059], $p = .005$) and unfavourable 12-month outcome (OR 1.024 [1.000–1.047], $p = .047$). At 48 h, UCH-L1 predicted hospital mortality (OR 1.020 [1.002–1.038], $p = .029$) but not unfavourable outcome at 12 months. The ORs for UCH-L1 and clinical variables are presented in Table 2.

3.2 | Comparison with NSE

The AUROCs for NSE as a predictor of unfavourable outcome at 12 months were 0.66 (0.59–0.73) at 24 h and 0.72 (0.65–0.80) at 48 h. There was no difference between the AUROC of NSE at 24 h and that of UCH-L1 at 24 h ($p = .82$) or 48 h ($p = .827$). Likewise, there was no difference between the AUROC of NSE at 48 h and that of UCH-L1 at 24 h ($p = .184$) or 48 h ($p = .230$).

To predict death in hospital, the AUROCs for NSE were 0.70 (0.63–0.77) at 24 h and 0.76 (0.68–0.83) at 48 h. These were not statistically significantly different from the corresponding AUROCs of UCH-L1.

3.3 | Cut-off values

We determined cut-off values for UCH-L1 to predict unfavourable outcome at 12 months. For UCH-L1 at 24 h, the Youden-based cut-off value was 9.1 ng/mL, which provided a specificity of 62.9% (95% CI, 54.4–71.4%) and a sensitivity of 66.1% (95% CI, 57.6–74.6%). At 48 h, the Youden-based cut-off value was 12.0 ng/mL, with a specificity of 53.3% (95% CI, 44.4–62.1) and sensitivity of 77.6% (95% CI,

TABLE 2 Logistic regression model for clinical variables and serum UCH-L1 to predict hospital mortality and unfavourable outcome (CPC 3–5) at 12 months.

	Hospital mortality			12-month CPC 3–5		
Variable	**OR**	**95% CI**	***p***	**OR**	**95% CI**	***p***
Age (years)	1.037	1.011–1.064	.005	1.050	1.022–1.078	<.001
Witnessed collapse (NO)	–	–	–	3.574	1.169–10.925	.025
Shockable rhythm (NO)	3.688	1.946–6.989	<.001	4.609	2.354–9.022	<.001
ROSC (min)	1.092	1.060–1.126	<.001	1.095	1.062–1.129	<.001
UCH-L1 24 h	1.035	1.010–1.059	.005	1.024	1.000–1.047	.047
UCH-L1 48 h	1.020	1.002–1.038	.029	–	–	–

Abbreviations: CPC, cerebral performance category; OR, odd ratios; ROSC, return of spontaneous circulation.

TABLE 3 Cut-off values for UCH-L1 to predict unfavourable outcome (CPC 3–5) at 12 months with the Youden method-based cut-off and with high specificities.

	Cut-off definition	Concentration ng/mL	Specificity %	Sensitivity %	PPV %	NPV %	LR+	*p*
24 h	Youden	9.1	62.9 (54.4–71.4)	66.1 (57.6–74.6)	62.9 (54.4–71.4)	66.1 (57.6–74.6)	1.8 (1.4–2.3)	<.001
	95% specificity	30.5	95.2 (91.4–98.9)	11.9 (6.0–17.7)	70.0 (49.9–90.1)	53.2 (46.6–59.7)	2.5 (1.0–6.2)	.047
	99% specificity	37.2	98.4 (96.2–100)	9.3 (4.1–14.6)	84.6 (65.0–100)	53.3 (46.8–59.7)	5.8 (1.3–25.5	.008
48 h	Youden	12.0	53.3 (44.4–62.1)	77.6 (69.3–85.8)	57.1 (48.7–65.6)	74.7 (65.6–83.8)	1.7 (1.3–2.1)	<.001
	95% specificity	32.1	95.1 (91.2–98.9)	18.4 (10.7–26.0)	75.0 (57.7–92.3)	59.2 (52.3–66.1)	3.7 (1.5–9.0)	.001
	99% specificity	45.5	98.4 (96.1–100)	7.1 (2.0–12.2)	77.8 (50.6–100)	56.9 (50.2–63.6)	4.4 (0.9–20.5)	.041

Abbreviations: CPC, cerebral performance category; LR+, positive likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

TABLE 4 Cut-off values for UCH-L1 to predict CPC 3–5 at 12 months with high sensitivities.

	Cut-off definition	Concentration ng/mL	Specificity %	Sensitivity %	PPV %	NPV %	LR−	*p*
24 h	90% sensitivity	5.7	21.0 (13.8–28.1)	90.7 (85.4–95.9)	52.2 (45.4–59.0)	70.3 (55.5–85.0)	0.44 (0.23–0.86)	.012
	95% sensitivity	4.9	13.7 (7.7–19.8)	94.9 (91.0–98.9)	51.1 (44.5–57.8)	73.9 (56.0–91.9)	0.37 (0.15–0.91)	.022
	99% sensitivity	4.1	6.5 (2.1–10.8)	98.3 (96.0–100)	50.0 (43.6–56.4)	80.0 (55.2–100)	0.26 (0.06–1.21)	.063
48 h	90% sensitivity	8.1	17.2 (10.5–23.9)	89.8 (83.8–95.8)	46.6 (39.4–53.7)	67.7 (51.3–84.2)	0.59 (0.29–1.20)	.138
	95% sensitivity	7.0	10.7 (5.2–16.1)	94.9 (90.5–99.3)	46.0 (39.2–52.9)	72.2 (51.5–92.9)	0.48 (0.18–1.30)	.135
	99% sensitivity	6.4	6.6 (2.2–11.0)	99.0 (97.0–100)	46.0 (39.3–52.7)	88.9 (68.4–100)	0.16 (0.02–1.22)	.039

Abbreviations: CPC, cerebral performance category; LR−, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

69.3–85.8). The cut-off values for UCH-L1 at 24 h and 48 h with corresponding sensitivities, specificities, PPVs, NPVs and LR+s are presented in Table 3.

To evaluate the ability of UCH-L1 to identify patients with a high probability of favourable outcome at 12 months, we determined cut-off values to predict unfavourable outcome with sensitivities of 90%–99%. These high sensitivities resulted in low specificities (Table 4).

4 | DISCUSSION

In this post-hoc study on ICU-treated OHCA patients included in the observational FINNRESUSCI study, we found that UCH-L1 concentrations were elevated for patients with unfavourable outcomes. However, the power of UCH-L1 to discriminate between patients with poor prognosis and those with good prognosis was only moderate, as reflected by AUROCs below 0.7. The prognostic ability of UCH-L1 was not better than that of NSE. Moreover, the sensitivities of UCH-L1 corresponding to high (95%–99%) specificities were lower (9%–18%) compared to those we found for NSE (37%–46%) in our previous study on the same study population.[23] These results do not support the use of UCH-L1 to aid in prognostication and clinical decision-making in patients resuscitated from OHCA.

In some previous studies, the prognostic abilities of UCH-L1 and NSE have been somewhat better than what we found.[26] For UCH-L1, AUROCs as high as 0.85–0.87 have been reported.[20] For NSE, some previous studies have reported AUROCs of 0.85–0.90.[7,27] This is in line with our earlier finding that the prognostic ability of

neurofilament light in FINNRESUSCI study patients[23] was, albeit very good, somewhat poorer than that found in some other studies.[28,29]

The most likely explanation for these differences is the unselected cohort of patients included in the observational FINNRESUSCI study compared to studies focusing on cardiogenic OHCA with most patients having a shockable initial rhythm. Neuronal biomarkers are quite good in predicting unfavourable outcome caused by HIBI, which is the most common reason of death after CA.[30] However, some deaths are the result of extracerebral causes, and neuronal biomarkers may not be able to predict those. We have previously shown that the prognostic ability of NSE is poor in old patients and in those with a short time from collapse to ROSC,[14] and a plausible explanation is that in these patient groups the cause of death may often be another cause than HIBI.

In our study outcome was assessed at 12 months after OHCA, whereas some other studies have assessed outcomes at 6 months.[20,27] Some of our patients may have initially recovered but later died of causes unrelated to the CA. It is also possible that there may be differences in laboratory methods.[31]

In present-day prognostication after CA, not only identifying imminent unfavourable outcome but also identifying a high likelihood of favourable outcome is an important topic.[32–34] There is a need for reliable prognostic tools to detect individuals with good prognosis. In our study, cut-off values of UCH-L1 yielding high sensitivities resulted in low specificities. Thus, UCH-L1 demonstrated only limited value in identifying patients with good prognosis, in accordance with the recent study by Moseby-Knappe et al.[35]

The timing of blood samples is a relevant issue. In our study, blood samples were drawn at 24 and 48 h after ROSC. In one study, the concentrations of UCH-L1 in CA patients with unfavourable outcome were high already at the time of ICU admission.[36] In a study on TBI patients, the mean half-life of UCH-L1 in serum was 13 h and median half-life 9 h.[19] After hypothermic circulatory arrest in canines, serum UCH-L1 at 8 h was able to predict brain damage.[37] In a study on hypoxic-ischemic encephalopathy in newborns, the highest UCH-L1 concentrations were detected at 0–6 h after delivery, and concentrations decreased during first 24 h.[38] As samples for biomarker measurements can be easily taken in the early phase of care, a sensitive biomarker with concentrations increasing rapidly after CA might be useful. The usefulness of UCH-L1 measured from very early samples may be worth investigating.

4.1 | Strengths and limitations

This study has several strengths. It is a multi-centre nationwide study with all five Finnish university hospitals and 14 of the 15 non-university central hospitals participating. The study population was unselected, including patients with shockable and non-shockable initial rhythms and all types of CA. The outcome assessor was blinded for clinical data and biomarker results. However, there are also limitations. First, the blood samples were from patients included in the original study more than 10 years ago, and it is likely that there have been changes in the treatment of resuscitated patients. Second, we have no data on methods used for prognostication when considering withdrawal of life-sustaining treatments.

5 | CONCLUSION

In this post-hoc laboratory study on OHCA patients, UCH-L1 measured at 24 or 48 h after CA had limited ability to predict outcome. UCH-L1 was not superior to NSE.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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III

Neurofilament light as an outcome predictor after cardiac arrest: a post hoc analysis of the COMACARE trial

Wihersaari L, Ashton NJ, Reinikainen M, Jakkula P, Pettilä V, Hästbacka J, Tiainen M, Loisa P, Friberg H, Cronberg T, Blennow K, Zetterberg H, Skrifvars MB; COMACARE STUDY GROUP.

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ORIGINAL

Neurofilament light as an outcome predictor after cardiac arrest: a post hoc analysis of the COMACARE trial

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Abstract

Purpose: Neurofilament light (NfL) is a biomarker reflecting neurodegeneration and acute neuronal injury, and an increase is found following hypoxic brain damage. We assessed the ability of plasma NfL to predict outcome in comatose patients after out-of-hospital cardiac arrest (OHCA). We also compared plasma NfL concentrations between patients treated with two different targets of arterial carbon dioxide tension ($PaCO_2$), arterial oxygen tension (PaO_2), and mean arterial pressure (MAP).

Methods: We measured NfL concentrations in plasma obtained at intensive care unit admission and at 24, 48, and 72 h after OHCA. We assessed neurological outcome at 6 months and defined a good outcome as Cerebral Performance Category (CPC) 1–2 and poor outcome as CPC 3–5.

Results: Six-month outcome was good in 73/112 (65%) patients. Forty-eight hours after OHCA, the median NfL concentration was 19 (interquartile range [IQR] 11–31) pg/ml in patients with good outcome and 2343 (587–5829) pg/ml in those with poor outcome, $p < 0.001$. NfL predicted poor outcome with an area under the receiver operating characteristic curve (AUROC) of 0.98 (95% confidence interval [CI] 0.97–1.00) at 24 h, 0.98 (0.97–1.00) at 48 h, and 0.98 (0.95–1.00) at 72 h. NfL concentrations were lower in the higher MAP (80–100 mmHg) group than in the lower MAP (65–75 mmHg) group at 48 h (median, 23 vs. 43 pg/ml, $p = 0.04$). $PaCO_2$ and PaO_2 targets did not associate with NfL levels.

Conclusions: NfL demonstrated excellent prognostic accuracy after OHCA. Higher MAP was associated with lower NfL concentrations.

Keywords: Cardiac arrest, Prognostication, Biomarkers, Neurofilament light (NfL)

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Introduction

Many patients resuscitated from out-of-hospital cardiac arrest (OHCA) suffer from hypoxic–ischaemic brain injury (HIBI) and die in the intensive care unit (ICU) without regaining consciousness [1, 2]. Accurate prognostication is of paramount importance to prevent prolonged futile intensive care and, on the other hand, to avoid withdrawal of care in those who have a chance to recover [3, 4]. Current guidelines recommend a multimodal approach in the prognostication of cardiac arrest (CA) patients including clinical examination, radiological imaging, neurophysiological assessment, and biomarkers [5]. Unfortunately, some of these methods are not universally available [6, 7] and others can be affected by sedative medication and muscle paralysis often needed with targeted temperature management (TTM) [8].

Blood biomarkers are considered potential tools for prognostication because they are easy to obtain, and the results are not affected by the use of medication. Currently, neuron-specific enolase (NSE) is the most widely used biomarker in cardiac arrest (CA) patients [5]. NSE concentration at 48–72 h after CA has demonstrated moderate accuracy with areas under the receiver operating characteristic curves (AUROC) between 0.85 and 0.90 in predicting neurological outcome, but optimal cut-off values vary widely between studies [9–12]. Unfortunately, NSE is sensitive to blood sample haemolysis [13] and its prognostic ability is reduced in the elderly and in patients with shorter CA duration [14]. Therefore, novel biomarkers with superior performance to predict neurological outcome after OHCA are urgently needed to guide clinical management of this group of patients. Neurofilament light (NfL) is a 68 kD cytoskeletal neuron-specific protein showing high promise as a clinically useful biomarker for acute brain conditions such as traumatic brain injury [15]. Recently, serum NfL was also shown to have excellent prognostic accuracy at 24–72 h from CA [16], but further external validation in independent patient cohorts is required before the routine use of NfL can be incorporated into clinical practice.

In the Carbon dioxide, Oxygen and Mean arterial pressure After Cardiac Arrest and REsuscitation (COMACARE) trial, we recently showed that targeting high-normal or low-normal carbon dioxide tension ($PaCO_2$), normoxia or moderate hyperoxia, and low-normal or high-normal mean arterial pressure (MAP) did not affect NSE concentrations in comatose OHCA patients [17, 18]. Given the possible superior prognostic accuracy of NfL and its different sensitivity to axonal brain injury, we performed a post hoc sensitivity analysis and measured NfL concentrations in patients included in the COMACARE trial. We hypothesised first that compared to NSE, NfL would be an earlier and more accurate biomarker of neurological outcome and that targeting high-normal $PaCO_2$, moderate hyperoxia, and, second, high normal MAP would result in lower levels of NfL when compared with the lower targets.

Take-home message

Neurofilament light (NfL) appears to be a very accurate and early marker of long term neurological outcome after out-of-hospital cardiac arrest. Targeting a higher mean arterial blood pressure is associated with lower NfL levels than a standard blood pressure target.

Methods

Study population and research approvals

The COMACARE trial (NCT02698917) was a prospective, randomised pilot study of 120 comatose OHCA patients resuscitated from an initial shockable rhythm. The trial was a 2^3 factorial trial exploring the effects of low-normal vs. high-normal arterial carbon dioxide tension (4.5–4.7 vs. 5.8–6.0 kPa), normoxia vs. moderate hyperoxia (arterial oxygen tension 10–15 vs. 20–25 kPa) and low-normal vs. high-normal mean arterial pressure (65–75 vs. 80–100 mmHg) for 36 h post-resuscitation on markers of neurological damage, assessed primarily with neuron-specific enolase (NSE) concentrations in serum at 48 h after cardiac arrest. The study was conducted in seven ICUs in Finland and Denmark between March 2016 and November 2017. Randomisation was stratified according to TTM (33 °C or 36 °C, according to site-specific protocols). Neurological prognostication was performed according to European Resuscitation Council and European Society of Intensive Care Medicine guidelines [19]. The study protocol and the main results have been published previously [17, 18, 20]. In the current post hoc analysis, we measured the NfL concentrations in the blood samples of 112 Finnish patients included in the COMACARE trial. The original COMACARE study protocol was approved by the Ethics committee of Northern Savo Hospital District, Finland (decision no. 295/2015), and an amendment including the plan for the current analysis was approved in December 2017.

Data collection

We obtained blood samples at the time of ICU admission (0 h) and 24, 48, and 72 h after CA. The samples were centrifuged (2000 G, 10 min) and stored at − 70 °C for later analysis. Plasma NfL concentration was measured using the commercially available Single Molecule Array (Simoa) NF-Light immunoassay (Quanterix, Billerica, MA, United States) in the Clinical Neurochemistry Laboratory of the University of Gothenburg (Mölndal, Sweden) in September 2019. Staff who conducted the

analysis were blinded to all clinical data. The samples were diluted fourfold, and a single batch of reagents was utilised for all eight analytical runs needed to complete the analyses. For the low-concentration control sample (LCS; 6.9 ng/l), the intra-assay coefficient of variation was 7.4% and the inter-assay coefficient of variation was 8.9%, whilst for the high concentration quality control sample (HCS; 55.1 ng/l), the corresponding coefficients of variation were 7.1% and 10.4%, respectively.

Serum NSE concentration was measured using a COBAS e601 line (Hitachi High Technology Co, Tokyo, Japan) with an electrochemiluminescent immunoassay kit (Roche Diagnostics GmbH, Mannheim, Germany) by ISLAB laboratories (Kuopio, Finland) in January 2018. Because haemolysis can significantly affect NSE results, all samples were tested using the Roche haemolysis index, and the samples with a haemolysis level of more than 500 mg of free haemoglobin per litre were excluded from the analyses [21].

Patient data regarding comorbidities, functional status, and resuscitation-associated factors were collected into an Internet-based database (Absolute Imaginary Software, Helsinki, Finland). A neurologist blinded to the results of the laboratory analysis, study group allocations, and treatment during hospital stay evaluated the neurological outcome using the Cerebral Performance Category (CPC) scale at six months after CA via a telephone interview. We considered CPC 1–2 as good outcome and CPC 3–5 as poor outcome.

Statistical analysis

We present categorical variables as counts and percentages, including 95% confidence intervals (CI) where applicable, and continuous variables as medians and interquartile ranges (IQR). We compared categorical variables with Pearson's Chi squared test or Fisher's exact test. We tested the normality of distribution of continuous variables with the Kolmogorov–Smirnov test and then compared normally distributed variables using the independent samples t test, and non-normally distributed variables using the Mann–Whitney U test or the Kruskal–Wallis test.

We assessed the ability of NfL and NSE to predict poor neurological outcome at 6 months by calculating the area under the receiver operating characteristic curve (AUROC). We compared the discriminative ability of NfL and NSE by comparing their AUROC values at ICU admission and at 24, 48 and 72 h after CA with a bootstrap method. The used bootstrap function is within the roc.test function (R program, https://www.rdocumentation.org). We assessed optimal cutoff values for NfL at 24–72 h after CA from the receiver operating characteristic curves (ROC). Because high specificity is important in prognostication, we assessed cutoff values with a specificity higher than 95%. In addition, we determined cutoff values with the Youden method [22, 23]. We also determined the sensitivity, specificity, positive (PPV) and negative predictive values (NPV), and positive likelihood ratios (LR +) for these cutoff values. We also report cutoff values for the prediction of good functional outcome with high specificity and sensitivity.

To predict poor 6-month neurological outcome, we created a multivariable model, including patient age, receipt of bystander-given basic life support, and the time from collapse to the return of spontaneous circulation (ROSC). We then added NfL concentrations measured at 24, 48, and 72 h into this model and assessed the improvement of prognostic accuracy by comparing the AUROCs between the baseline model and the models after the addition of NfL. We also calculated the Net Reclassification Improvement (NRI) achieved with the addition of NfL into the baseline model. Event NRI (NRIe) is calculated as [(the number of patients with poor outcome for whom the predicted probability of poor outcome increases with addition of NfL to the baseline prediction model) − (the number of patients with poor outcome for whom the predicted probability decreases)]/[the number of all patients with poor outcome]. Similarly, non-event NRI (NRIne) is the net proportion of patients with good outcome given a lower probability of poor outcome after addition of NfL to the baseline prediction model. The overall NRI is the sum of NRIe and NRIne. The theoretical range of values for both NRIe and NRIne is − 1 to + 1, and the range of values for overall NRI is – 2 to + 2 [24, 25].

In addition, we determined the integrated discrimination improvement (IDI) that was obtained by adding NfL into the baseline prediction model. Event IDI (IDIe) is calculated for patients with poor outcome as [(mean probability of poor outcome given by the model including NfL) − (mean probability of poor outcome given by the baseline model)] and non-event IDI (IDIne) for patients with good outcome as [(mean probability of poor outcome given by the baseline model) − (mean probability of poor outcome given by the model including NfL)]. IDI is the sum of IDIe and IDIne. The theoretical range of values for both IDIe and IDIne is − 1 to + 1, and the range of values for IDI is – 2 to + 2 [25, 26].

We conducted all statistical analyses with SPSS (SPSS, Chicago, IL, USA) version 25.0 and R version 3.5.1. We used two-tailed p values with the level of significance set at a p value less than 0.05, and made no correction to the p value despite the multiple testing.

Results

Blood samples for NfL analysis were available for 112 of the 120 patients included in the COMACARE trial (Fig. 1). The baseline characteristics and resuscitation-associated factors are presented in Table 1. All patients were unconscious on arrival to the ICU and treated with TTM either in 33 °C or 36 °C. The 6-month neurological outcome was poor in 39 (35%) patients, with 37 deaths (33%) and two patients recovering to CPC 3. In 32 (86%) of the deceased, the cause of death was HIBI. The investigations used to determine the prognosis in patients with a poor functional outcome are shown in the Electronic Supplementary Material (ESM) (Tables S1).

Prognostic accuracy of NfL

NfL concentrations were significantly higher in patients with poor outcome than in those with good outcome at all studied time points (Table 2, Fig. 2). The difference in NfL concentration between patients with poor and those with good outcome was at its greatest 48 h after cardiac arrest, when the median (IQR) NfL concentrations were 2343 (587–5829) pg/ml and 19 (11–31) pg/ml, respectively ($p < 0.001$).

The ability of NfL to discriminate patients with poor outcome from those with good outcome was excellent at 24, 48, and 72 h after CA with AUROC values of 0.98 (95% CI 0.97–1.00), 0.98 (95% CI 0.97–1.00), and 0.98 (95% CI 0.95–1.00), respectively (Fig. 3). The NfL concentration at ICU admission had a poor prognostic ability with an AUROC of 0.66 (95% CI 0.54–0.77), which was clearly inferior compared to the AUROCs of NfL concentrations at 24, 48 and 72 h after CA ($p < 0.001$). NfL had an excellent ability to predict death from HIBI (ESM, Fig. S1). There was no difference in the prognostic accuracy of NfL depending on TTM target used during ICU care (ESM, Fig. S2).

NfL compared with NSE and S100B

Compared with NSE, NfL had a markedly better ability to discriminate patients with poor outcome from those with good outcome ($p < 0.001$ for measurements at 24 and 48 h; $p = 0.012$ at 72 h]) (Fig. 3). NfL was also better than S100B to discriminate between patients with good outcome and those with poor outcome at 24–72 h ($p < 0.001$) (Fig. 3). At ICU admission, the prognostic performance of NfL was poor, and the AUROCs were not significantly different between NfL and NSE ($p = 0.185$) and NfL and S100B ($p = 0.767$) (Fig. 3).

Cutoff values

The cutoff values at 24, 48 and 72 h after CA for obtaining a specificity of 99% in predicting poor outcome were 127, 263, and 344 pg/ml, respectively. The corresponding sensitivities and positive and negative predictive values are presented in Table 2. Cutoff

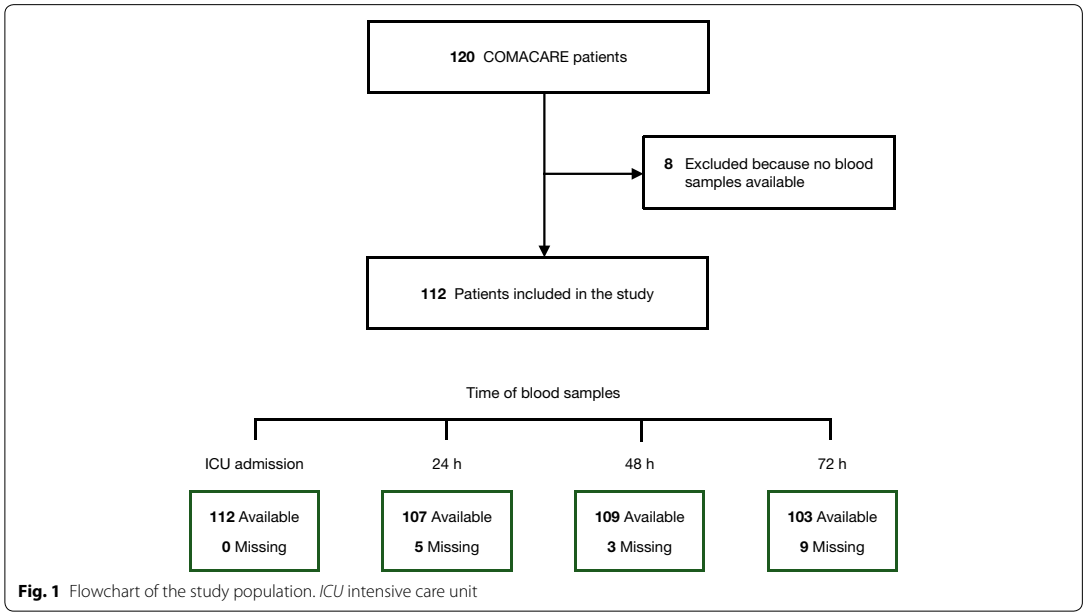

Fig. 1 Flowchart of the study population. *ICU* intensive care unit

Table 1 Baseline characteristics of the study patients

	All patients (*N* = 112)	CPC 1–2 (*N* = 73)	CPC 3–5 (*N* = 39)	*p* value
Age, median (IQR) (years)	62 (53–68)	58 (51–66)	66 (58–75)	0.004
Male sex [*n* (%)]	92 (82.1)	61 (83.6)	31 (79.5)	0.592
Weight [median (IQR), kg]	85 (72.3–93)	85 (72.5–94)	83 (70–90)	0.646
Neurological function before cardiac arrest				
Normal, CPC 1 [*n* (%)]	103 (92)	67 (91.8)	36 (92.3)	1
Some disability, CPC 2 [*n* (%)]	9 (8)	6 (8.2)	3 (7.7)	
Medical history				
Hypertension [*n* (%)]	56 (50)	33 (45.2)	23 (59)	0.165
Chronic heart failure (NYHA 3 or 4) [*n* (%)][a]	9 (8)	4 (5.5)	5 (12.8)	0.151
Smoker [*n* (%)][b]	35 (31.3)	22 (30.1)	13 (33.3)	0.235
Resuscitation factors				
Bystander life support [*n* (%)]	93 (83)	66 (90.4)	27 (69.2)	0.004
Time to ROSC [median (IQR), min]	21 (16–26)	17 (15–22)	25 (22–32)	< 0.001
Clinical status on ICU admission				
GCS [median, (IQR)][c]	3 (3–3)	3 (3–5)	3 (3–3)	< 0.001
APACHE II score, median (IQR)	28 (24–31)	27 (24–29)	31 (26–35)	< 0.001
TTM				
33 °C [*n* (%)]	75 (67)	56 (76.7)	19 (48.7)	0.003
36 °C [*n* (%)]	37 (33)	17 (23.3)	20 (51.3)	

CPC Cerebral Performance Category, *IQR* interquartile range, *NYHA* New York Heart Association, *ROSC* return of spontaneous circulation, *ICU* intensive care unit, *GCS* Glasgow Coma Scale, *SD* standard deviation, *APACHE II* Acute Physiology and Chronic Health Evaluation

[a] Data missing for two patients

[b] Data missing for 13 patients

[c] Data missing for nine patients

Table 2 NfL concentrations (medians with IQRs) at ICU admission and 24, 48 and 72 h after cardiac arrest for patients with good outcome (CPC 1–2) and for those with poor outcome (CPC 3–5), and cutoff values with sensitivities, PPVs and NPVs at 24–72 h according to 99% of specificity

NfL concentration pg/ml (IQR)				Cutoff for 99% specificity				
Time	CPC 1–2	CPC 3–5	*p* value	NfL pg/ml	Sensitivity	PPV (95% CI)	NPV (95% CI)	*p* value
ICU admission	10.4 (7.1–16)	13.4 (10.5—23.2)	0.002					
24 h	12.1 (8.3–23.7)	761.9 (217.6–1534.9)	< 0.001	127	0.78 (0.65–0.92)	0.97 (0.90–1.00)	0.90 (0.83–0.96)	< 0.001
48 h	19.1 (11–30.7)	2342.6 (586.9–5828.8)	< 0.001	263	0.83 (0.71–0.96)	0.97 (0.91–1.00)	0.92 (0.86–0.98)	< 0.001
72 h	20.5 (13.8–34.8)	1727.9 (643.1–4583.5)	< 0.001	344	0.85 (0.73–0.97)	0.97 (0.90–1.00)	0.93 (0.88–0.99)	< 0.001

CPC Cerebral Performance Category, *NfL* neurofilament light, *IQR* interquartile range, *ICU* intensive care unit, *PPV* positive predictive value, *NPV* negative predictive value, *CI* confidence interval

values determined according to the Youden method and according to specificities of 95–100%, together with the corresponding sensitivities and positive and negative predictive values are presented in the ESM (Table S2). Cutoff values for identification of patients with good functional outcome are presented in the ESM (Table S3).

NfL and clinical prognostication data

The baseline multivariable model predicted a poor 6-month outcome with an AUROC of 0.86 (95% CI 0.79–0.93) (Table S4). After NfL concentration at 24 h was added to this model, the AUROC improved to 0.98 (0.97–1.00), $p < 0.001$. After adding NfL concentration at 48 h, the AUROC increased to 0.99 (0.98–1.00) ($p < 0.001$), and after adding NfL concentration at 72 h, the AUROC was 0.99 (0.98–1.00) ($p < 0.001$). With the

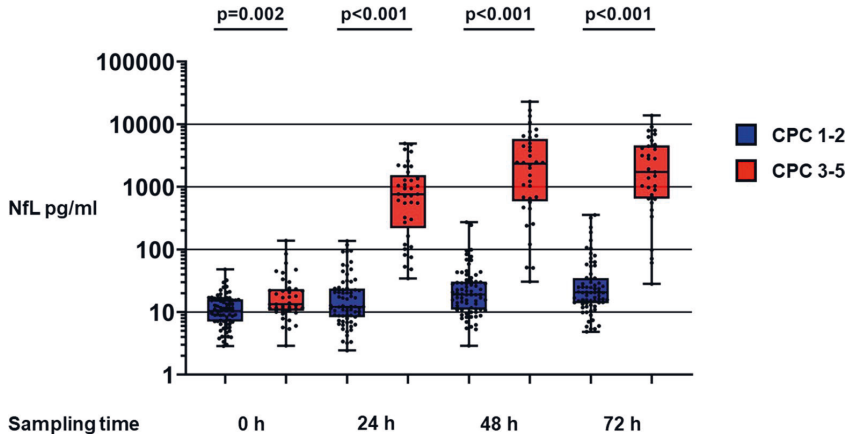

Fig. 2 Scatter plots and box plots presenting neurofilament light (NfL) concentrations at intensive care unit admission (0 h) and 24, 48 and 72 h after cardiac arrest for patients with good outcome (Cerebral Performance Category [CPC] 1–2) and those with poor outcome (CPC 3–5) with a 10-based logarithmic scale. Dots present concentrations for individual patients. Each box depicts the interquartile range, the line inside the box shows the median value, and the whiskers show the range of values

inclusion of NfL to the baseline model at 24 h, NRI was 1.51 (95% CI 1.24–1.79); at 48 h, NRI was 1.78 (95% CI 1.58–1.97); and at 72 h, NRI was 1.74 (1.53–1.94). The corresponding IDI values were 0.41 at 24 h, 0.45 at 48 h, and 0.45 at 72 h (ESM, Table S4).

Haemolysis

Detectable haemolysis (more than 100 mg of free haemoglobin per litre) was observed in 157 (35%) of all samples. Haemolysis did not increase NfL concentrations, whereas NSE concentrations were markedly higher in samples with haemolysis (ESM, Table S5, Figs. S3 and S4).

NfL concentrations according to carbon dioxide, oxygen, and blood pressure targets

There were no significant differences in NfL concentrations between the groups targeting low-normal or high-normal $PaCO_2$ and normoxia or moderate hyperoxia at any of the studied time points (ESM Table S7). In patients assigned to the high-normal MAP group, NfL concentrations were lower than in patients in the low-normal MAP group at 48 h (median 23 [IQR 11–251] pg/ml vs. 43 [19–1066] pg/ml, [$p=0.04$]) and at 72 h (23 [13–152] pg/ml vs 63 [21–1609] pg/ml [$p=0.007$]) (ESM Table S6). NfL levels according to outcome in all intervention groups are presented in the ESM (Figs. S5–S7).

Discussion

Our findings provide evidence of the excellent ability of NfL to predict long-term neurological outcomes in a homogenous population of cardiac arrest patients resuscitated from a shockable rhythm and treated with TTM. Moreover, the results of this post hoc analysis offer additional information on the effects of carbon dioxide, oxygen, and blood pressure on the development of neurological injury after OHCA [17, 18]. Using NfL as a marker of neurological injury, we found that a higher MAP target of 80–100 mmHg was associated with lower NfL levels when compared with the conventional target of MAP 65–75 mmHg. We did not observe any significant difference in the NfL concentrations between the groups targeting low-normal and high-normal $PaCO_2$, or normoxia and moderate hyperoxia.

The predictive accuracy of NfL observed in the current study is well in line with the findings of a previous study based on the TTM trial [16], whereas the cutoff values in our study were much lower. The patient populations in these two studies were different. The TTM trial included patients without any age limit and included patients with a non-shockable initial rhythm, whereas the COMACARE trial only included patients younger than 80 years, with a shockable initital rhythm and patients with time to ROSC less than 45 min. Moreover, in the current study we have used a new commercially available kit for measuring NfL, while the Moseby-Knappe study used a custom-made assay [27, 28].

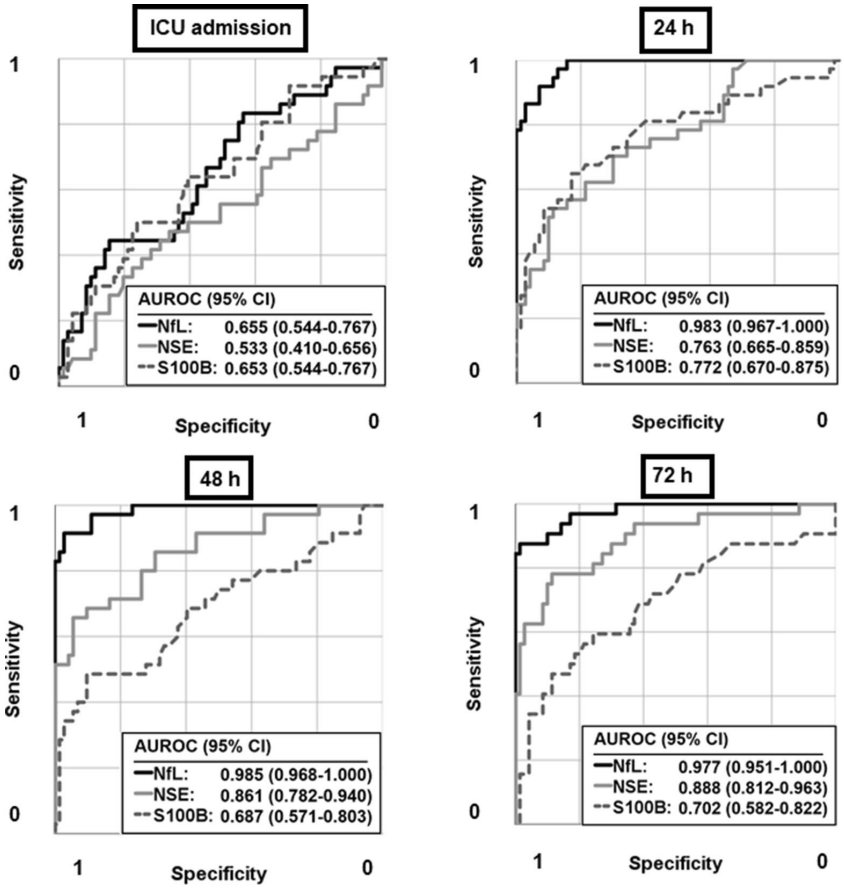

Fig. 3 Receiver operating characteristic curves and areas under the curves (AUROC) with 95% confidence intervals (CI) for NfL, NSE and S100B at intensive care unit (ICU) admission and 24, 48 and 72 h after cardiac arrest, presenting these biomarkers' ability to discriminate between patients with good outcome (Cerebral Performance Category [CPC] 1–2) and those with poor outcome (CPC 3–5) at six months. *NfL* neurofilament light, *NSE* neuron-specific enolase

The AUROC values of NfL are very high at 24 h from CA. This level of accuracy has not been demonstrated for any other neurological biomarker [1], and our results reinforce the superiority of NfL compared to NSE [16]. One possible explanation for this can be the different distribution of these biomarkers in the grey and white matter of the brain. NSE is a neuronal enzyme that is more abundant in the grey matter [29]. NfL, in contrast, is a structural protein that can be found especially in large axons in the white matter [30]. Interestingly, radiological studies have suggested that white matter is particularly susceptible to ischaemic damage and that the extent of white matter injury seems to be associated with CA outcome [31]. In addition, it needs to be recognized that NSE, S100B and NfL have very different half-lives and this will influence the time trajectory of these biomarkers in brain injury patients [15]. The very long half-life of NfL may explain why it appeared, in contrast with NSE, to rise in the patients with good functional outcome as well.

Thus far, no biomarkers can reliably predict the outcome of OHCA patients at the time of hospital admission [1]. In the current study, we observed that the NfL levels at the time of admission were slightly higher in patients with poor outcome, but because of considerable overlap, this is not likely to be useful in clinical practice. At later time points, high NfL concentrations were predictive of

poor outcome: no patients with concentrations higher than 390 pg/ml at any time point had a good outcome. However, our study population is too small to enable a threshold value to be proposed for prognostication.

One of the known limitations of NSE is that even mild haemolysis in the sample can result in erroneously elevated NSE levels [13]. Haemolysis is especially common in patients receiving continuous renal replacement therapy, intra-arterial balloon pump support, or extracorporeal membrane oxygenation, which are not uncommon interventions after OHCA. Importantly, the study by Moseby-Knappe et al. did not show any association between the level of haemolysis and NfL concentrations [16]. Our study also suggests that haemolysis does not influence NfL concentrations. Other conditions such as amyotrophic lateral sclerosis, HIV-associated dementia, and extensive traumatic brain injury (TBI) can affect NfL levels, but they are very uncommon in OHCA patients [16].

This additional analysis of the COMACARE trial shows an association between higher MAP and lower NfL concentrations, which may reflect the degree of brain injury. Given the small sample size and the post hoc design of the current study, this finding should be interpreted with caution and considered as hypothesis generating. Moreover, the absolute difference in NfL concentrations between the different MAP groups was small when compared with the 100-fold difference between the patients with good and poor outcomes. The possible beneficial effect of the higher MAP on clinical outcomes may be small compared to other factors, such as age, delay in resuscitation, and ROSC, and larger randomised trials are needed before the optimal blood pressure target after OHCA can be defined. Regarding carbon dioxide and oxygen, the results of the additional NfL analyses of the current study support the neutral results of the COMACARE trial on NSE.

Strengths and limitations

The current study has several strengths. First, the NfL results were not available to clinicians during patient care and could thus not have influenced treatment decisions. Second, the NfL concentrations were measured concurrently by the same laboratory. Third, we studied patients treated in multiple centres with TTM at both 33 °C and 36 °C.

A major limitation of the current study is the relatively small sample size. Moreover, our patient cohort was rather selected, including only patients with witnessed cardiac arrest with a shockable initial rhythm from a presumed cardiac origin and with time from collapse to ROSC between 15 and 45 min. Future studies are needed to clarify the accuracy of NfL in unselected patient populations. The analysis was conducted in frozen samples, but the evidence suggests the stability of NfL in freeze–thaw samples [32]. Regarding the effect of carbon dioxide, oxygen, and MAP on NfL levels after OHCA, we acknowledge that the current study was designed post hoc, increasing the possibility of chance findings.

Conclusions

In unconscious OHCA patients treated with TTM, NfL had excellent prognostic accuracy already at 24 h. Compared to NSE, NfL seems to be a more accurate biomarker for prognostication after CA, and if validated in further samples, it has potential to replace NSE in the multimodal prognostication algorithms. Targeting a higher MAP of 80–100 mmHg was associated with lower levels of NfL, generating a hypothesis that higher blood pressure after CA could attenuate brain injury.

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s00134-020-06218-9) contains supplementary material, which is available to authorized users.

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Compliance with ethical standards

Conflicts of interest

Markus Skrifvars reports speakers' fees and travel grants from BARD Medical (Ireland) and a research grant from GE Healthcare. Kaj Blennow has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. Henrik Zetterberg has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

Protocol

The protocol of the COMACARE study has been previously published [20].

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IV

Neurofilament light compared to neuron-specific enolase as a predictor of unfavourable outcome after out-of-hospital cardiac arrest

Wihersaari L, Reinikainen M, Furlan R, Mandelli A, Vaahersalo J, Kurola J, Tiainen M, Pettilä V, Bendel S, Varpula T, Latini R, Ristagno G, Skrifvars MB.

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Clinical paper

Neurofilament light compared to neuron-specific enolase as a predictor of unfavourable outcome after out-of-hospital cardiac arrest ☆

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Abstract

Aim: We compared the prognostic abilities of neurofilament light (NfL) and neuron-specific enolase (NSE) in patients resuscitated from out-of-hospital cardiac arrest (OHCA) of various aetiologies.

Methods: We analysed frozen blood samples obtained at 24 and 48 hours from OHCA patients treated in 21 Finnish intensive care units in 2010 and 2011. We defined unfavourable outcome as Cerebral Performance Category (CPC) 3–5 at 12 months after OHCA. We evaluated the prognostic ability of the biomarkers by calculating the area under the receiver operating characteristic curves (AUROCs [95% confidence intervals]) and compared these with a bootstrap method.

Results: Out of 248 adult patients, 12-month outcome was unfavourable in 120 (48.4%). The median (interquartile range) NfL concentrations for patients with unfavourable and those with favourable outcome, respectively, were 689 (146–1804) pg/mL vs. 31 (17–61) pg/mL at 24 h and 1162 (147–4360) pg/mL vs. 36 (21–87) pg/mL at 48 h, $p < 0.001$ for both. The corresponding NSE concentrations were 13.3 (7.2–27.3) μg/L vs. 8.5 (5.8–13.2) μg/L at 24 h and 20.4 (8.1–56.6) μg/L vs. 8.2 (5.9–12.1) μg/L at 48 h, $p < 0.001$ for both. The AUROCs to predict an unfavourable outcome were 0.90 (0.86–0.94) for NfL vs. 0.65 (0.58–0.72) for NSE at 24 h, $p < 0.001$ and 0.88 (0.83–0.93) for NfL and 0.73 (0.66–0.81) for NSE at 48 h, $p < 0.001$.

Conclusion: Compared to NSE, NfL demonstrated superior accuracy in predicting long-term unfavourable outcome after OHCA.

Keywords: Neurofilament light (NfL), Neuron-Specific Enolase (NSE), Out-of-hospital cardiac arrest, OHCA, Resuscitation, Cardiac arrest, Neurological outcome, Biomarkers

☆ Some results were presented as abstracts at the 34th ESICM LIVES Digital Annual Congress, 3–6 October 2021 and at Operatiiviset Päivät 17–19 November 2021, Helsinki, Finland.

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Introduction

Prognostication after cardiac arrest (CA) should be performed using a multimodal approach, including clinical assessment, neurophysiology, radiological investigations and biomarkers.[1–3] The updated European Resuscitation Council (ERC)-European Society of Intensive Care Medicine (ESICM) guidelines recommend using neuron-specific enolase (NSE) as one component of multimodal prognostication.[1] However, the recommended high NSE cut-off values that are necessary to achieve high specificity may result in low sensitivity in detecting patients with poor prognosis.[4,5] One example is the decreased prognostic accuracy in elderly patients and patients with a short time from collapse to return of spontaneous circulation (ROSC).[6] NSE also has well-known sources of error, resulting in falsely elevated levels further weakening its prognostic accuracy.[7–12]

A novel axonal biomarker, neurofilament light (NfL), can be measured in plasma with an ultrasensitive novel single molecule array (SIMOA) method.[13] NfL demonstrated a very high capacity to predict unfavourable six-month outcome after out-of-hospital cardiac arrest (OHCA) with a presumed cardiac cause.[14,15] NfL also appeared to have the best ability among a group of neurobiomarkers, including NSE, to find patients with a favourable outcome despite the indeterminate prognosis given by examinations recommended in the ERC-ESICM guidelines.[16] Before wider adoption, the utility and presumed superiority of NfL over NSE should be validated also in unselected CA populations. Accordingly, we analysed NfL concentrations and its prognostic capacity in an unselected OHCA population, including patients with shockable and non-shockable initial rhythms and resuscitated from different CA aetiologies. We hypothesised that NfL would be superior to NSE in predicting unfavourable long-term outcome in patients treated in the intensive care unit (ICU) following OHCA. The secondary hypothesis was that NfL would have better prognostic value in those patient subgroups (high age, short time from collapse to ROSC) where NSE has demonstrated poor prognostic accuracy.

Methods

Study population and definitions

This was a post hoc analysis of the prospective multicentre FINNRESUSCI study of 548 adult patients resuscitated after OHCA and treated in 21 Finnish ICUs between 2010 and 2011.[17] All five university hospitals and 14 out of 15 non-university central hospitals participated in FINNRESUSCI. Over 98% of the Finnish population live in the referral areas of these hospitals. The FINNRESUSCI study protocol was approved by the Helsinki University Hospital Ethics Committee and by each participating hospital. A post-hoc substudy of NSE values was published earlier.[6] In this post-hoc study, we included 248 patients whose blood samples were stored (Fig. 1). We defined outcome according to the Cerebral Performance Category (CPC)[18] at 12 months after CA: CPC 1–2 indicates favourable outcome and CPC 3–5 indicates unfavourable outcome. The CA cause was defined with clinical criteria.

Data collection

The patient data were collected using Internet-based forms. Data on previous health status were collected from the patients' medical history and mortality data from Statistics Finland. The outcome according to CPC classification was assessed 12 months after CA with phone interviews conducted by a neurology specialist blinded to the hospital treatment and the laboratory analysis.

Blood samples

The blood samples were from patients in the FINNRESUSCI study for whom the next of kin had provided written informed consent. The plasma samples were collected at 24 and 48 hours from OHCA, stored at −80 °C and thawed for this analysis. We measured the NfL levels quantitatively using a commercially available two-step digital immunoassay using the single molecule array Quanterix SIMOA™ NF-light® Kit and SIMOA™ HD-1 Analyzer (SIMOA™, Quanterix Corporation, Lexington, MA, USA). The plasma NfL concentrations were expressed in picograms per millilitre (pg/mL). For comparison, we used NSE samples from the same time points determined according to previously described methods.[6] The obtained serum samples were stored at −70 °C during the original study and analysed with a commercially available electrochemiluminescence immunoassay (Roche Diagnostics GmbH, Mannheim, Germany) in April 2015. We excluded all NSE samples with significant haemolysis, ≥ 500 mg/L.[19]

Statistical analysis

We present categorical data as absolute numbers with percentages (95% confidence intervals [CIs]) and continuous data as medians with interquartile ranges (IQRs). For continuous data, we used Student's t test (normal distribution) and the Mann-Whitney U test or the Kruskal-Wallis test (skewed distribution) for comparison. We compared the categorical variables with the Chi square test or Fisher's exact test. We divided the study population into quartiles according to patients' age and time to ROSC[6] to detect differences in prognostic values between NfL and NSE.

We calculated the areas under the receiver operating characteristic curves (AUROCs) with 95% CIs to assess the ability of NfL and NSE to discriminate between patients in favourable (CPC 1–2) and those in unfavourable (CPC 3–5) outcome groups. We compared the AUROCs of NfL to NSE at 24 and 48 h after CA using the bootstrap method. We constructed a multivariable model with clinical factors such as age, initial rhythm, delay to ROSC and witnessed collapse for the prediction of poor functional outcome. Into this model, with a backward stepwise approach, we subsequently inserted NfL and NSE and report results with odds ratios and 95% CIs.

We defined the NfL cut-off values to predict unfavourable outcome at 24 and 48 h after CA from the receiver operating characteristic curve and for NSE at 48 h, accordingly. The cut-off values for NSE at 24 h were not calculated because of its poor prognostic accuracy.[6] We determined biomarker concentrations for high specificity (low false positive rate, [FPR]) to detect patients with a high probability for unfavourable outcome and concentrations for high sensitivity to detect those with a high probability for favourable outcome (low false negative rate). We calculated the Youden-based[20,21] cut-off values to assess the concentrations that simultaneously have as high specificity and sensitivity as possible, to promote their comparability. Furthermore, we defined cut-offs for high sensitivity (95% and 99%) and used normal levels of NfL to detect patients with favourable outcome. We used concentrations of 55 pg/mL for NfL[16] and 17 μg/mL for NSE[7] as the highest normal value. We also calculated the sensitivity, specificity, positive predictive value (PPV), negative

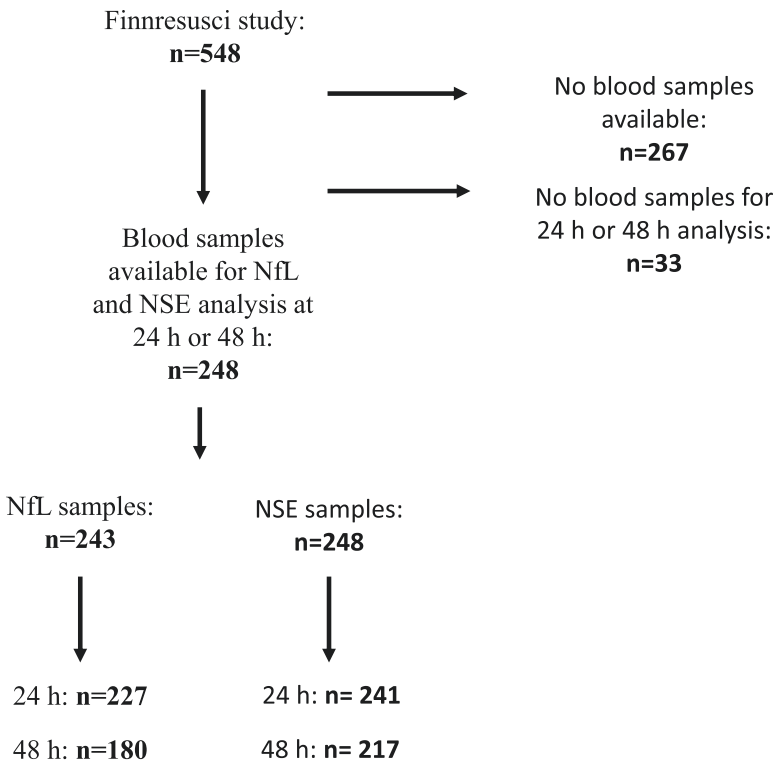

Fig. 1 – Flowchart of the study population. Abbreviations: NfL: neurofilament light. NSE: neuron-specific enolase.

predictive value (NPV), positive likelihood ratio (LR+) or negative likelihood ratio (LR-), if suitable, for these cut-off values. We considered *p* values < 0.05 as significant. We performed statistical analyses with SPSS version 27 (SPSS, Il, Chicago, USA) and R program, version 4.0.0.

Results

The 12-month outcome was unfavourable in 120/248 (48.4%) of the patients. Of these patients, 177 (71.4%) had a shockable initial rhythm. Blood samples enabled NfL analysis in 243 patients and NSE analysis in 248 patients (Fig. 1). Table 1 shows the outcome data and patient characteristics. The comparison of the study patients to the FINNRESUSCI patients in whom blood samples were unavailable are shown in Table S1.

NfL and NSE concentrations and prognostic ability

The NfL concentrations were significantly higher for the patients with unfavourable outcome than for those with favourable outcome at all time points. At 24 h, the median concentrations (IQR) were 688.9 pg/mL (146.1–1803.8) for the patients with unfavourable outcome vs. 30.9 pg/mL (16.9–61.2) pg/mL for those with favourable outcome ($p < 0.001$). Accordingly, the concentrations at 48 h were 1162.4 pg/mL (146.8–4360.5) vs. 35.6 pg/mL (21.3–86.7), $p < 0.001$. Fig. 2 shows the concentrations indexed by outcome. The NSE concentrations were higher for the patients with unfavourable outcome than for those with favourable outcome; at 24 h, the concentrations were 13.3 μg/L (7.2–27.3) for the patients with unfavourable outcome vs. 8.5 μg/L (5.8–13.2) for those with favourable outcome, $p < 0.001$. At 48 h, the concentrations were 20.4 μg/L (8.1–56.6) vs. 8.2 μg/L (5.9–12.1), respectively ($p < 0.001$) (Fig. 2). The NfL and NSE concentrations were not different for the patients with a cardiac aetiology of arrest compared to those with a non-cardiac aetiology, according to outcome Table S2.

The prognostic ability assessed with AUROC (with 95% CI) was significantly higher at 24 h after CA to predict unfavourable outcome for NfL (0.90 [0.86–0.94]) than NSE (0.65 [0.58–0.72]), $p < 0.001$. At 48 h, the AUROC was higher for NfL (0.88 [0.83–0.94]) than NSE (0.72 [0.66–0.81]), $p < 0.001$. The AUROC for NfL at 24 h was also higher than NSE at 48 h, $p < 0.001$. NfL at 24 h was a significant predictor of unfavourable outcome in the multivariable model, whereas NSE at 48 h was not (Table S3). The AUROCs for NfL and NSE according to the CA aetiology are presented in Table S2.

Cut-off values

The NfL cut-off values to predict unfavourable outcome using the Youden method (maximising sensitivity and specificity) were 97 pg/mL at 24 h and 231 pg/mL at 48 h. For those cut-offs, the specificities (with 95% CIs) were 86.8% (80.6–93.0) and 92.1% (86.8–97.3), and the sensitivities were 81.8% (74.2–88.6) and 72.2 (62.3–82.0), respectively. For 99% specificity, the cut-offs were 589 pg/mL and

Table 1 – Characteristics of the study patients according to Cerebral Performance Category classification.

		CPC 1-2	CPC 3-5
Number of patients, n (%)		128 (51.6)	120 (48.4)
Initial rhythm[a]			
Shockable rhythms, n (%)	VF	104 (81.3)	70 (58.3)
	VT	2 (1.6)	1 (0.8)
Non-shockable rhythms, n (%)	PEA	9 (7.0)	23 (19.2)
	ASY	13 (10.2)	25 (20.8)
Witnessed, n (%)		123 (96.1)	103 (85.8)
Bystander CPR, n (%)		78 (60.9)	67 (55.8)
ROSC, min (IQR)		16 (11–23)	24 (19–31)
CA aetiology, n (%)			
	Cardiogenic	106 (82.8)	90 (75.0)
	Hypoxia	4 (3.1)	7 (5.8)
	Drowning	2 (1.6)	3 (2.5)
	Hypothermia	1 (0.8)	0 (0)
	Intoxication	3 (2.3)	3 (2.5)
	Trauma	1 (0.8)	0 (0)
	Other etiologies	2 (1.6)	6 (5.0)
	Unknown	5 (3.9)	4 (3.3)
	Missing	4 (3.1)	7 (5.8)
SAPS II, points (IQR)		47 (34–60.8)	64.5 (55.3–71)
Male gender, n (%)		107 (89.2)	101 (84.2)
TTM, n (%)		100 (78.1)	92 (76.7)

Abbreviations: ASY: asystole. CA: cardiac arrest. CPC: Cerebral Performance Category. CPR: cardiopulmonary resuscitation. IQR: interquartile range. PEA: pulseless electrical activity. ROSC: return of spontaneous circulation. SAPS II: Simplified acute physiology score. TTM: targeted temperature management. VF: ventricular fibrillation. VT: ventricular tachycardia.

[a] Data missing in 1 (0.8%) of the patients with CPC 3-5.

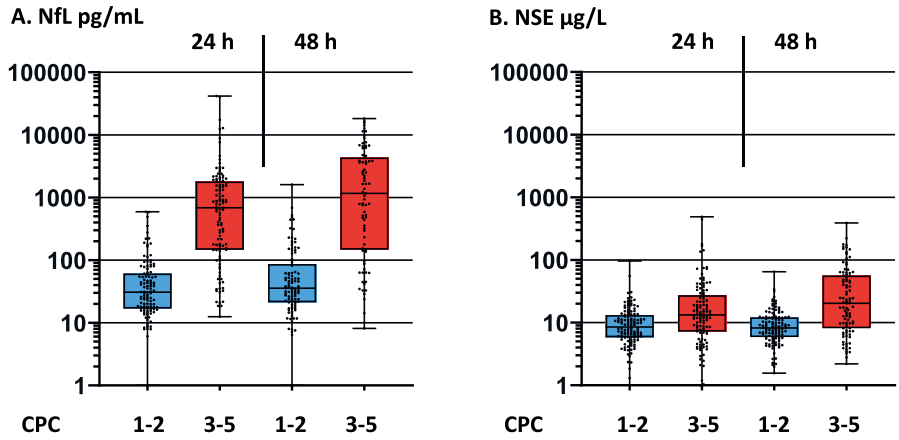

Fig. 2 – Boxplots for NfL (A) and NSE (B) concentrations at 24 h and 48 h after cardiac arrest for patients with favourable (CPC 1–2) and unfavourable (CPC 3–5) outcomes with a 10-based logarithmic scale. Each box presents the interquartile range. The line inside the box shows the median value, the whiskers show the lowest and the highest concentrations, and the dots show the concentrations for each individual. Abbreviations: CPC: Cerebral Performance Category. NfL: neurofilament light. NSE: neuron-specific enolase.

721 pg/mL, respectively with sensitivities of 54.0% (44.8–63.2) and 59.5% (48.7–70.3), respectively (Table 2).

Regarding NSE at 48 h, using a 35 µg/L cut-off value with 99% specificity resulted in a 37.1% (27.5–46.7) sensitivity. Table 2 shows the cut-off values with corresponding characteristics for NfL at 24 h and 48 h and for NSE at 48 h using the Youden method and 95% and 99% specificity. The cut-off values for NfL and NSE to predict favourable outcome are presented in Table S4.

Combining NfL and NSE, 0.3% of the patients who exceeded the cut-offs for 95% specificity had a favourable outcome (Table S5).

Table 2 – Characteristics (with 95% CIs) of cut-off values for NfL at 24 h and 48 h and for NSE at 48 h after cardiac arrest for high demand of specificities to predict unfavourable outcome.

	Basis for cut-off setting	Cut-off	Specificity (%)	Sensitivity (%)	PPV (%)	NPV (%)	LR+	p
NfL 24 h	Youden	97 pg/mL	86.8 (80.6–93.0)	81.8 (74.2–88.6)	86.0 (79.4–92.6)	82.5 (75.7–89.3)	6.2 (3.8–10.0)	<0.001
	95% specificity	232 pg/mL	95.6 (91.9–99.4)	65.5 (56.7–74.3)	93.7 (88.3–99.0)	73.6 (66.6–80.7)	14.9 (6.3–35.5)	<0.001
	99% specificity	589 pg/mL	99.1 (97.4–100)	54.0 (44.8–63.2)	98.4 (95.3–100)	68.5 (61.4–75.6)	61.5 (8.7–436.4)	<0.001
NfL 48 h	Youden	231 pg/mL	92.1 (86.8–97.3)	72.2 (62.3–82.0)	87.7 (79.7–95.7)	80.9 (73.7–88.1)	9.1 (5.6–18.0)	<0.001
	95% specificity	445 pg/mL	95.1 (90.8–99.3)	65.8 (55.4–76.3)	91.2 (83.9–98.6)	78.0 (70.7–85.4)	13.3 (5.6–31.7)	<0.001
	99% specificity	721 pg/mL	99.0 (97.1–100)	59.5 (48.7–70.3)	97.9 (93.9–100)	75.8 (68.4–83.1)	60.1 (8.5–426.0)	<0.001
NSE 48 h	Youden	20 μg/L	94.2 (90.0–98.4)	50.5 (40.6–60.5)	87.5 (78.8–96.2)	70.2 (63.1–77.3)	8.7 (4.1–18.2)	<0.001
	95% specificity	22 μg/L	95.0 (91.1–98.9)	46.4 (36.5–56.3)	88.2 (79.4–97.1)	68.7 (61.6–75.7)	9.3 (4.1–20.8)	<0.001
	99% specificity	35 μg/L	99.2 (97.5–100)	37.1 (27.5–46.7)	97.3 (92.1–100)	66.1 (59.2–73.0)	44.5 (6.2–319.0)	<0.001

Abbreviations: CI: confidence interval. LR+: positive likelihood ratio. NfL: neurofilament light. NPV: negative predictive value. NSE: neuron-specific enolase. PPV: positive predictive value.

NfL in different subgroups

Age quartiles

In all age groups, the NfL concentrations were significantly higher for the patients with unfavourable outcome than for those with favourable outcome at 24 h and 48 h after CA (Fig. 3). The prognostic ability of NfL was significantly better at 24 h than that of NSE in all age subgroups (Table S6). At 48 h, the prognostic ability of NfL was better than that of NSE in the patients aged 57–63 years (p = 0.005) and in the oldest subgroup, ≥72 years (p = 0.020) (Table S6). The AUROC for NfL to predict unfavourable outcome was lower in the oldest quartile compared to the youngest quartile (18–56 years) both at 24 h (p = 0.016) and 48 h (p = 0.032). The AUROC was also lower in the fourth quartile (≥72 years) at 48 h than in the second quartile (57–63 years), p = 0.020. The NfL concentrations in the patients with favourable outcome were significantly different according to age group at 24 h (p < 0.001) and at 48 h (p = 0.001).

ROSC quartiles

The NfL concentrations were significantly higher for the patients with unfavourable outcome compared to those with favourable outcome at all times from collapse to ROSC quartiles at 24 h and 48 h (Fig. 4). The prognostic ability of NfL was also better than that of NSE in all ROSC subgroups at 24 h and 48 h after CA (Table S7). The AUROC for NfL to predict unfavourable outcome was lower in the quartile with the shortest time from collapse to ROSC (1–13 min) than in the quartile with the longest time to ROSC (≥29 min) at 24 h (p = 0.014) and at 48 h (p = 0.019). The AUROC was also lower for the patients in the second quartile (ROSC 14–

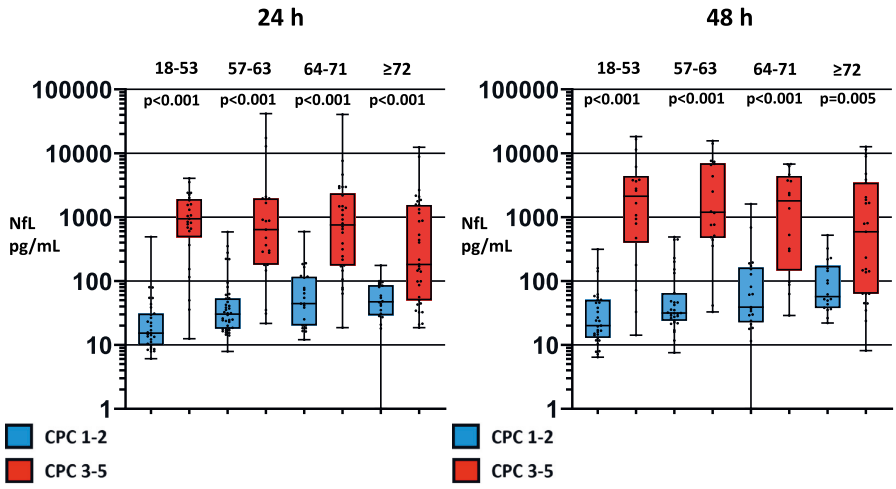

Fig. 3 – Boxplots for NfL concentrations at 24 h and 48 h after cardiac arrest for patients with favourable (CPC 1–2) and unfavourable (CPC 3–5) outcomes with a 10-based logarithmic scale, according to different age quartiles. Each box presents the interquartile range. The line inside the box shows the median value, the whiskers show the lowest and the highest concentrations, and the dots show the concentrations for each individual. Age intervals (years) with p values (for differences in concentrations for patients with favourable [CPC 1–2] and unfavourable [CPC 3–5] outcomes in each quartile) are presented above each figure. Abbreviations: CPC: Cerebral Performance Category. NfL: neurofilament light.

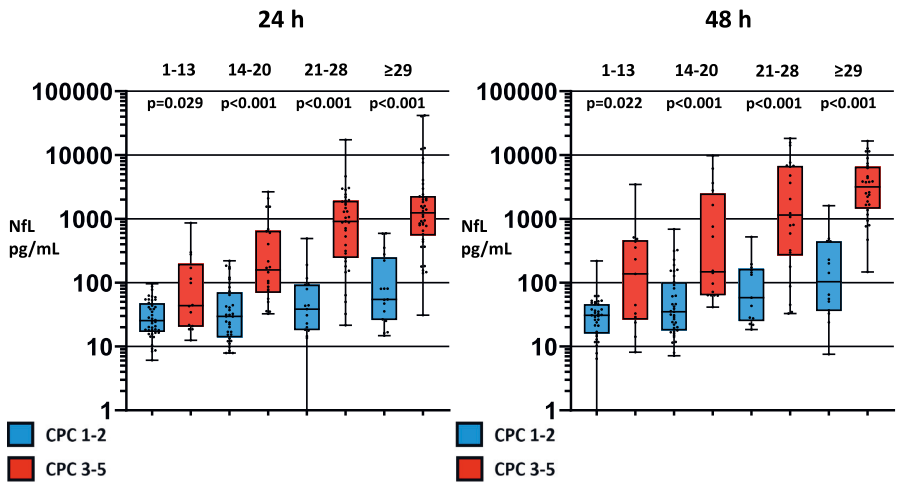

Fig. 4 – Boxplots for NfL concentrations at 24 h and 48 h after cardiac arrest for patients with favourable (CPC 1–2) and unfavourable (CPC 3–5) outcomes with a 10-based logarithmic scale, according to different ROSC quartiles. Each box presents the interquartile range. The line inside the box shows the median value, the whiskers show the lowest and the highest concentrations, and the dots show the concentrations for each individual. ROSC intervals (minutes) with p values (for differences in concentrations for patients with favourable [CPC 1–2] and unfavourable [CPC 3–5] outcomes in each quartile) are presented above each figure. Abbreviations: CPC: Cerebral Performance Category. NfL: neurofilament light. ROSC: return of spontaneous circulation.

20 min) than for those in the fourth quartile (≥29 min) at 48 h, p = 0.032. The distributions of NfL concentrations were significantly different according to outcome in the ROSC subgroups: for the patients with favourable outcome (at 24 h p = 0.034; at 48 h p = 0.004) and for those with an unfavourable outcome (p < 0.001 at 24 h and 48 h).

Discussion

In this post-hoc analysis of OHCA patients resuscitated from various arrest aetiologies, NfL was significantly more accurate than NSE in predicting unfavourable 12-month outcome. The prognostic ability of NfL was already excellent at 24 hours after CA. The median concentrations for the patients with unfavourable outcome were about 20-fold greater than for those with favourable outcome. Importantly, NfL was also accurate in the patients resuscitated from a likely non-cardiac cause of arrest. We also found a less clear association between age and time to ROSC and predictive accuracy than we previously showed with NSE.[6] As our sample presents heterogeneous OHCA patients, our findings support wider utilisation of NfL in clinical prognostication after CA.

The lack of wider adoption of NfL thus far may have been due to the unavailability of a commercial assay, but given the introduction of the ultrasensitive SIMOA method, this is likely to change.

However, few studies exist about prognostication after CA using the ultrasensitive SIMOA method. NfL measurement within the first 24 h after ROSC demonstrated an AUROC of 0.82 to predict in-hospital death.[22] In a Targeted Temperature Management (TTM) substudy including 782 OHCA patients with a likely cardiac aetiology of arrest, the AUROCs at 24–72 h to predict poor six-month outcome were 0.94–0.95.[14] In our study of OHCA patients with VF as the initial rhythm, the AUROCs were very high at 0.98.[15] The present study, including an unselected population with both shockable and non-shockable rhythms, found AUROCs to predict CPC 3–5 at 12 months of 0.88–0.90, demonstrating slightly worse but still excellent discriminative ability. Pouplet et al demonstrated AUROC of 0.87 to predict CPC 3–5 at 90 days after CA in patients with shockable rhythms using different but comparable commercial laboratory method.[23] In Stammet et al.'s TTM substudy,[19] NSE had an AUROC of 0.85–0.86 at 48–72 h, and Streitberger et al. found an AUROC of 0.85–0.90 at 72 h.[7] In summary, studies conducted to date suggest better accuracy for NfL compared to NSE.[14,15] We found a slightly lower discriminative ability, especially for NSE, than previously reported. The likeliest explanation is the inclusion of different types of CA patients in whom the reason for the unfavourable outcome may not only be due to post-cardiac arrest brain injury (PCABI), which is the most common cause of death after CA.[24] Clearly, NfL and NSE can only work for predicting death or poor outcome related to brain injury.

The levels of NSE for patients with unfavourable outcome were somewhat lower in this study compared to some previous studies. There are several possible explanations for this. Firstly, the laboratory methods used may be important.[25] Secondly, it is possible that the lower levels and prognostic ability of NSE seen in the present study compared to previous studies are related to differences in the definition of unfavourable outcome[7] and follow-up time.[7,19]

Our secondary finding was that NfL's prognostic ability was better than NSE in subgroups where the prognostic value of NSE was poor, such as the elderly and those with a shorter arrest duration. In our study, the NfL levels were higher in those with longer time from collapse to ROSC, and the accuracy was highest in those with the longest time to ROSC. However, even in the group with a short time to

ROSC, the discriminative ability was satisfactory. This may suggest that NfL is more sensitive even in detecting milder hypoxic brain injury. Importantly, for patients with a short time from collapse to ROSC and patients aged ≥72 years, the prognostic value of NfL was superior to NSE. Increasing age is one confounding factor of NfL; the concentrations increase about 2% per year,[26,27] and for individuals over 60 years of age, the variability of NfL levels increases.[28] We also found a rising trend of NfL levels in CPC 1–2 patients with increasing age. This finding may provide an additional explanation for the worse discriminative ability of NfL in the oldest patient group.

The ERC-ESICM guidelines recommend a 60 μg/L NSE cut-off.[1] In this study, the 35 μg/L NSE cut-off at 48 h yielded 99% specificity but 37% sensitivity. Generally, demanding a very high specificity results in low sensitivity if the diagnostic method's performance is insufficient.

Targeting specificities of 95% and 99%, the cut-off values for NfL at 24 h were 232 pg/mL and 589 pg/mL, respectively; the cut-off values for NfL at 48 h were 445 pg/mL and 721 pg/mL, respectively.

Those cut-off concentrations are comparable to corresponding values in a TTM substudy.[14] Lower NfL cut-off values with higher sensitivities were presented in our study of a highly selected population with shockable rhythms.[15] The Youden-based NfL cut-offs showed 72–82% sensitivities and 87–91% specificities. In this study population, NfL presented better sensitivity than NSE, even with clinically useful specificities. The combination of cut-offs of NfL and NSE for 95% specificity resulted in a 0.3% FPR.

Recent studies have raised the concern that there might be CA patients with potentially favourable outcome despite poor prognosis given by prognostic methods.[29,30] Targeting 95% and 99% specificity to find patients with favourable outcome, the NfL cut-offs were 14–29 pg/mL, which are in the normal range. NSE demonstrated insufficient capacity to detect patients with favourable outcome. NfL has a better ability than NSE to find patients with favourable outcome using normal or lower values.

Strengths and limitations

Our study has several strengths. It was a nationwide multicentre study with a large patient sample from many ICUs. Importantly, we included CA patients of various arrest aetiologies. The treating clinicians were blinded to the NfL results. Neurological outcome was defined by an experienced neurologist blinded to the biomarker results. However, some limitations exist. First, the original study is 10 years old, and prognostication and clinical care of resuscitated patients are likely to have changed. Second, our study population was selected by consent availability, and, consequently, the proportion of patients with bystander cardiopulmonary resuscitation, shockable rhythm and TTM was significantly higher in those included than those excluded. Third, we do not have conclusive data on the patients' cause of death or prognostication; the patients were managed according to protocols available at the time. Fourth, the numbers of patients in the subgroups were small.

Conclusion

NfL is more valuable than NSE in prognostication of unfavourable outcome after OHCA, also in cases with non-cardiac aetiologies. Contrary to NSE, NfL retained its accuracy in the elderly and those with a short delay to ROSC, suggesting the ability of NfL to also identify milder forms of hypoxic brain injury.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

L. Wihersaari: Writing – original draft, Formal analysis, Visualization, Software, Data curation. **M. Reinikainen:** Conceptualization, Methodology, Writing – original draft, Supervision. **R. Furlan:** Resources, Validation. **A. Mandelli:** Resources, Validation. **J. Vaahersalo:** Validation, Investigation. **J. Kurola:** Validation, Conceptualization. **M. Tiainen:** Validation, Investigation. **V. Pettilä:** Validation, Supervision. **S. Bendel:** Validation, Supervision. **T. Varpula:** Validation, Conceptualization. **R. Latini:** Resources, Validation. **G. Ristagno:** Resources, Validation. **MB. Skrifvars:** Writing – original draft, Conceptualization, Methodology, Project administration, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.resuscitation.2022.02.024.

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Neurobiomarkers are a part of multimodal prognostication after cardiac arrest. This dissertation assessed the prognostic value of three biomarkers, neuron-specific enolase (NSE), ubiquitin C-terminal hydrolase L1 (UCH-L1) and neurofilament light (NfL), to predict outcome after out-of-hospital cardiac arrest. NSE demonstrated weak prognostic ability in the oldest patients and in those with a short resuscitation time. NfL had excellent prognostic ability, which was better than that of NSE and UCH-L1. Higher blood pressure target resulted in lower NfL concentrations.

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