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Clinical paper

Serum GFAP and UCH-L1 for the prediction of neurological outcome in comatose cardiac arrest patients

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Abstract

Objective: Neurological outcome prediction is crucial early after cardiac arrest. Serum biomarkers released from brain cells after hypoxic-ischaemic injury may aid in outcome prediction. The only serum biomarker presently recommended in the European Resuscitation Council prognostication guidelines is neuron-specific enolase (NSE), but NSE has limitations. In this study, we therefore analyzed the outcome predictive accuracy of the serum biomarkers glial fibrillary acidic protein (GFAP) and ubiquitin C-terminal hydrolase-L1 (UCH-L1) in patients after cardiac arrest.

Methods: Serum GFAP and UCH-L1 were collected at 24, 48 and 72 h after cardiac arrest. The primary outcome was neurological function at 6-month follow-up assessed by the cerebral performance category scale (CPC), dichotomized into good (CPC1-2) and poor (CPC3-5). Prognostic accuracies were tested with receiver-operating characteristics by calculating the area under the receiver-operating curve (AUROC) and compared to the AUROC of NSE.

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Results: 717 patients were included in the study. GFAP and UCH-L1 discriminated between good and poor neurological outcome at all time-points when used alone (AUROC GFAP 0.88–0.89; UCH-L1 0.85–0.87) or in combination (AUROC 0.90–0.91). The combined model was superior to GFAP and UCH-L1 separately and NSE (AUROC 0.75–0.85) at all time-points. At specificities ≥95%, the combined model predicted poor outcome with a higher sensitivity than NSE at 24 h and with similar sensitivities at 48 and 72 h.

Conclusion: GFAP and UCH-L1 predicted poor neurological outcome with high accuracy. Their combination may be of special interest for early prognostication after cardiac arrest where it performed significantly better than the currently recommended biomarker NSE.

Keywords: Out-of-hospital cardiac arrest, Neurological outcome prognostication, Serum biomarkers of neurological injury

Introduction

An estimated 56 individuals per 100,000 are treated by emergency medical services for out-of-hospital cardiac arrest annually across European countries.[1] Similar statistics apply to the United States, Australia and New Zealand.[2,3] Successfully resuscitated patients frequently suffer from hypoxic-ischaemic brain injury which continues to be the principal cause of adverse outcome and death in this group of patients after admission to intensive care.[4] Death often follows withdrawal of life sustaining therapy (WLST), guided by ethical principles due to presumed poor prognosis.[5] In order to minimize the risk of erroneous pessimistic prognoses, the European Resuscitation Council (ERC) and the European Society of Intensive Care Medicine (ESICM) recommend a multimodal prognostication model including the serum biomarker of brain injury neuron-specific enolase (NSE).[6] Several other serum biomarkers, e.g., S100 calcium-binding protein (S100), tau protein and neurofilament light chain (NFL), have recently been investigated as prognostic markers after hypoxic-ischaemic brain injury following cardiac arrest, but only NSE is recommended in the ERC/ESICM algorithm and then as a part of a multimodal approach.[6–10]

However, NSE is not an ideal biomarker, since it is also expressed in red blood, neuroendocrine and small-cell lung cancer cells, increasing the risk of falsely increased serum levels in cases of haemolysis or in the presence of neuroendocrine tumours.[11] Moreover, NSE shows an acceptable, but still only moderate, sensitivity at high specificity and performs poorly before 48 h after cardiac arrest.[10] An ideal serum biomarker should show a specificity close to 100% (low false positive rates) in combination with a high sensitivity early after cardiac arrest to provide meaningful clinical cut-offs. This is difficult in practice, since the most critical metric in cardiac arrest, specificity, often has to be prioritized at the cost of sensitivity to prevent a possible incorrect decision on prognosis. The serum biomarkers tau protein and NFL display higher specificity, sensitivity and better stability ex vivo than NSE, but both lack robust validation and the analytical methodology used is presently not available for clinical practice.[7,8]

Glial fibrillary acidic protein (GFAP) is a 50 kilodalton (kDa) monomeric intermediate-filament component of the astrocytic cytoskeleton almost exclusively expressed in the central nervous system (CNS) with a plasma half-life ($t_{1/2}$) of 24–48 h.[12–14] Serum GFAP is upregulated after brain injury and has been found to predict neurological outcome in patients after cardiac arrest.[12,15–18] Previous analyses have shown GFAP to be less sensitive than S100 and NSE, but those trials were underpowered for several endpoints and GFAP was analyzed using a prototype kit.[16,17] Ubiquitin C-terminal hydrolase-L1 (UCH-L1) is a 26 kDa neuronal deubiquitinase with a plasma $t_{1/2}$ of 6–12 h, primarily expressed in neurons and neuroendocrine cells.[14,19,20] UCH-L1 is important for neuro-axonal stability and repair after brain injury and has not previously been investigated in adult cardiac arrest.[21–23] The combination of GFAP and UCH-L1 has in a previous analysis shown to be a useful aid in determining the need for CT scanning of conscious patients with suspected traumatic brain injury (TBI) and has for this indication been approved for use in clinical practice.[24]

In this study, we evaluated the prognostic accuracy of the serum biomarkers GFAP and UCH-L1 for neurological outcome after cardiac arrest and compared the prognostic performance of serum GFAP and UCH-L1 with NSE.

Methods

This study is an explorative analysis of serum biomarkers in the international multicentre Target Temperature Management after out-of-hospital cardiac arrest (TTM)-trial, randomizing 939 unconscious (GCS < 8), adult (≥18 years of age) patients with out-of-hospital cardiac arrest of presumed cardiac origin from November 2010 to January 2013 to two different temperature managements (33 and 36 °C, respectively).[25] Resuscitation data in the TTM-trial was reported using the Utstein-style criteria and ethical committees in each participating country approved the TTM-trial protocol.[26] Informed consent was waived or obtained from all participants/relatives according to national legislations, in line with the Helsinki declaration. Of the 36 TTM-trial sites 29 participated in the collection of biobank material, resulting in a cohort of 819 patients for this study. The present study was conducted following the STARD criteria.[27]

Serum biomarker analyses

We investigated serum GFAP and UCH-L1 levels measured in samples collected prospectively per an a priori defined protocol at 24, 48 and 72 h after cardiac arrest as previously published.[7–10] The samples were processed at the respective site, aliquoted and frozen to −80 °C before shipment to the Integrated Biobank of Luxembourg for storage. All serum biomarker analyses were performed by board-certified laboratory technicians blinded to clinical data after completion of the TTM-trial. GFAP and UCH-L1 were analyzed in November 2018 with a commercially available method (Banyan BTI) by in vitro diagnostic chemiluminescent enzyme-linked immunosorbent assay (Banyan Biomarkers) (eMethods 1).[24] NSE, measured with a Cobas e601 instrument with electrochemiluminescent immunoassay (Roche Diagnostics), was used for comparison.[10] All serum samples were tested for haemolysis using the Roche haemolysis index with measurements at 600 and 570 nm with a haemolysis index (≥500 ng/ml of haemoglobin) being regarded as positive.[28]

Outcome

The primary outcome was overall neurological function according to cerebral performance category scale (CPC) assessed at 6-month

follow-up after cardiac arrest by face-to-face interview with the patient (86%) or telephone interview with the patient, care provider or relative (14%) by an assessor blinded to the target temperature and the to the biomarkers.[29] Neurological function was dichotomized into good and poor outcome with CPC1 (good cerebral performance, mild neurological disability) and CPC2 (moderate cerebral disability) considered as good outcome, and CPC3 (severe cerebral disability), CPC4 (vegetative state) and CPC5 (brain death) considered as poor outcome.[30,31]

Statistical methods

Bivariate associations were tested by Mann–Whitney *U*-test or by Kruskal–Wallis *H*-Test. Associations between UCH-L1 or GFAP and neurological outcome were tested by logistic regression, where all GFAP and UCH-L1 data were log 10-transformed before statistical analysis, because of skewed distribution. However, for clarity we include presentation of data (median and inter-quartile range, IQR) on the original scales in the results. Diagnostic performance for poor outcome was tested with receiver-operating characteristics (ROC) analysis, by calculating the area under the ROC curve (AUROC). The AUROC over all specificities (100–0%) was denominated total AUROC. In addition to the total AUROC, we also calculated the area under the ROC curve depicting the specificities ≥95% only, because this area of the AUROC is of high interest for prognostication purposes (partial AUROC). In order to increase comparability, the partial AUROC values were normed to 1.0 (eFigure 4). Comparisons between paired ROC curves (comparing diagnostic performance of UCH-L1, GFAP and the combination of both (GFAP + UCH-L1)) with NSE were done by a bootstrap procedure ($n = 2000$ iterations). We determined cut-off points for each serum biomarker at specificities ≥95% and compared the sensitivities for specificities ≥95% for UCH-L1, GFAP and GFAP + UCH-L1 with the previously published sensitivities of NSE for the corresponding specificities.[10] The confidence intervals for sensitivities and specificities were provided using Wilsons score method when only one variable was analyzed. For models including more than one variable the confidence intervals were provided out of 2000 bootstrap replicates of each ROC curve. We also compared different models where we added GFAP + UCH-L1 to clinical information (age, sex, time to ROSC, bystander cardiopulmonary resuscitation (CPR) [yes/no], initial rhythm shockable [yes/no] and serum lactate on admission), and neurological examination (bilaterally absent corneal reflexes and bilaterally absent pupillary reflexes) since these findings were highly reliable markers for poor outcome after cardiac arrest.[32] The Akaike information criterion (AIC) was used as a measure of overall model fit for model comparisons, with a lower AIC indicating a better model fit. A difference ≥2 in AIC favours the model with the smallest AIC. Diagnostics of regression models included standard inspection of residuals, q-q plots, and correlations between residuals and predicted and observed data. True missing data, due to sampling errors and technical issues, was missing completely at random. Systematically missing data was due to patients dying before the first sampling or between sampling time-points (eFigure 1A and B). Since we analyzed samples of

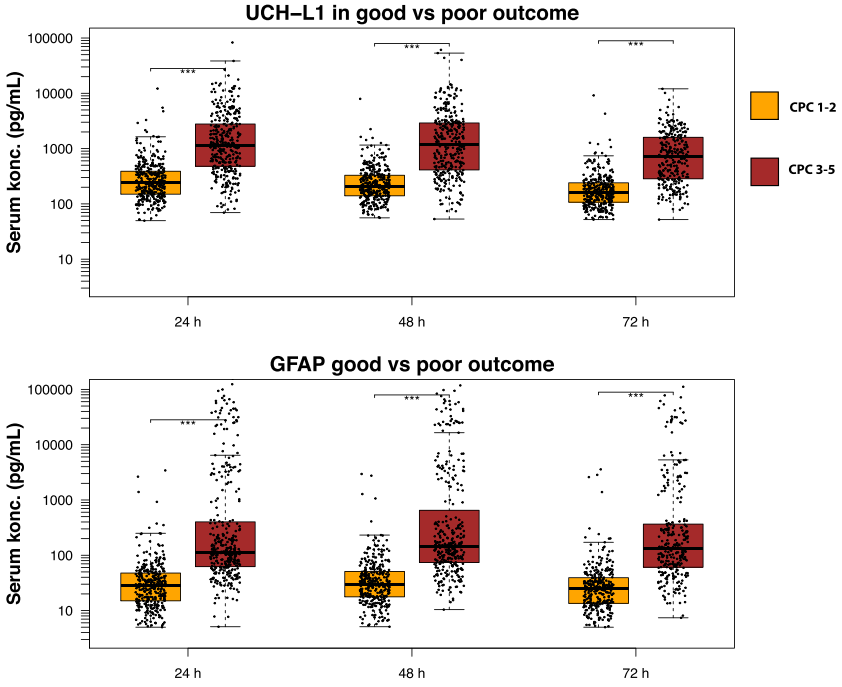

Fig. 1 – Levels of GFAP and UCH-L1 as boxplots with median and first and third quartile. Whiskers depict the smallest and largest non-outliers. All measuring points outside the whiskers are outliers. Measurements are shown as unadjusted data in pg/ml at 24, 48 and 72 h after cardiac arrest in the groups of good (CPC 1 and 2) and poor (CPC 3–5) outcome. Median UCH-L1 serum levels were significantly higher in poor outcome patients at all time-points ($P < 0.001$); 24 h, 241.1 (150.1–388.5) vs 1132.2 (479.6–2784.4) pg/mL; 48 h, 305.6 (140.0–329.4) vs 1180.4 (411.3–2884.9) pg/mL; and 72 h, 160.9 (107.2–240.6) vs 712.7 (284.2–1601.0) pg/mL. Median GFAP levels were also significantly higher in poor outcome patients at all time-points ($P < 0.001$); 24 h, 27.9 (15.0–47.6) vs 143.8 (74.0–588.8) pg/mL; 48 h, 30.0 (17.7–50.7) vs 143.8 (74.0–588.8) pg/mL; and 72 h, 25.3 (13.5–39.3) vs 132.4 (60.9–364.2) pg/mL. GFAP, glial fibrillary acidic protein; UCH-L1, ubiquitin C-terminal hydrolase-L1; pg, picogram; mL, millilitre.

patients alive, the population size diminished over time. We did not impute missing GFAP or UCH-L1 data points and all patients included had neurological outcome data registered. Significance was set at $P<0.05$. However, our results were not corrected for multiple hypothesis testing. R-version 3.5.1 (The R-Foundation for Statistical Computing) was used for statistical analyses.[33]

Results

Biobank material was collected of 819 patients, of those 717 had at least one GFAP or UCH-L1 measurement at 24, 48 or 72 h after cardiac arrest and were included into the study (eFigure 1A). Baseline characteristics of the included patients are displayed in eTable 1. Three hundred and sixty (50.2%) patients had a poor outcome at 6 months (eFigure 1A). The primary ROC analyses were derived from a cohort of patients with all serum biomarkers available at the different time-points (Fig. 2A–C and eTable 2). A subsequent ROC sensitivity analysis investigated all patients with serum biomarker levels measured and yielded similar results (eTable 2).

GFAP levels were higher in patients with poor outcome compared to patients with good outcome at all time-points ($P<0.001$) (Fig. 1). GFAP predicted poor outcome with a total AUROC of 0.88–0.89 (Fig. 2A–C), which was significantly greater than total AUROC for NSE at all time-points ($P<0.001$, $P=0.03$, $P=0.02$) (Table 1). There was no difference in partial AUROC between GFAP and NSE at 24 or 72 h ($P=0.16$, $P=0.05$, respectively) but partial AUROC NSE was significantly greater than GFAP at 48 h ($P=0.004$) (Table 1). Sensitivities and cut-off values of GFAP at ≥95% are described in Table 2. GFAP levels were significantly lower at 48 and 72 h for patients in the 36 °C group compared to the 33 °C group ($P=0.04$) (eTable 3). GFAP was significantly lower at 72 h in patients with haemolysis ($P=0.004$). However, removing all samples with haemolysis ($n=31$, $n=23$, $n=31$ at 24, 48 and 72 h, respectively) did not affect our main results (eFigure 2).

UCH-L1 levels were higher in patients with poor outcome compared to patients with good outcome at all time-points ($P<0.001$) (Fig. 1). UCH-L1 predicted poor outcome with a total AUROC of 0.85–0.87 (Fig. 2A–C) which was significantly greater than total AUROC for NSE at 24 ($P<0.001$) and 48 h ($P=0.03$), but not at 72 h ($P=0.34$) (Table 1). The partial AUROC of UCH-L1 was significantly greater than the partial AUROC of NSE at 24 h but not at 48 and 72 h ($P=0.04$, $P=0.76$, $P=0.19$) (Table 1). Sensitivities and cut-off values of UCH-L1 at specificities ≥95% are described in Table 3. There was no difference in UCH-L1 values between the temperature groups (eTable 3). There were also no effects of haemolysis on UCH-L1 concentrations (eFigure 3).

eTable 4 shows the prognostic accuracies of GFAP, UCH-L1 and GFAP + UCH-L1 at 24, 48 and 72 h. The total AUROC of the GFAP + UCH-L1 model was significantly greater than the total AUROC of GFAP or UCH-L1 separately at all time-points ($P<0.001$–0.01) and also significantly greater than the total AUROC of NSE at all time-points ($P<0.001$). The partial AUROC of GFAP + UCH-L1 was significantly greater than the partial AUROC of NSE at 24 h ($P<0.001$) but not at 48 and 72 h ($P=0.88$, $P=0.54$) (Table 1). Sensitivities of GFAP + UCH-L1 at specificities ≥95% compared to UCH-L1, GFAP separately and NSE are displayed in Table 4.

The associations between GFAP, UCH-L1, and GFAP + UCH-L1 and neurological outcome remained significant when adjusting for age, sex, and temperature arm (eTable 5 and 6).

Clinical information (see above under Statistical Methods) had moderate performance for predicting poor outcome at 24, 48 and 72 h after cardiac arrest (AUROC 0.81–0.79). AUROC increased to 0.93 at all measuring time-points when GFAP + UCHL-1 was introduced into the model. The partial AUROC revealed a similar pattern with increase of partial AUROC 0.59–0.57 to partial AUROC 0.78–0.76 over the measuring-points when GFAP + UCH-L1 was added. Adding bedside neurological testing (see above under Statistical Methods) to the models with clinical information and GFAP + UCHL-1, changed the AUROCs only marginally. However, the AIC favoured the model with all three modalities (eTable 7).

Discussion

In this explorative study we investigated the astroglial serum biomarker GFAP and the neuronal serum biomarker UCH-L1 as predictors of neurological outcome in a large cohort of targeted temperature management treated cardiac arrest patients. The overall performance to predict poor neurological outcome for each of these two serum biomarkers alone and their combination measured as total AUROC was, with the exception of UCH-L1 at 72 h, superior to NSE. However, the prediction of outcome after cardiac arrest with a serum biomarker requires a test with high specificity in order to avoid false-positive predictions of poor outcome. Therefore, we also compared

Table 1 – Comparison of total AUROC and partial AUROC between UCH-L1, GFAP, GFAP + UCH-L1 and NSE.

Time-point	Serum biomarker	Total AUROC (95% CI)	*P*-value	Partial AUROC 100–95% (95% CI)	*P*-value
24 h	UCH-L1 vs NSE	0.85 (0.82–0.88) vs 0.75 (0.72–0.79)	<0.001***	0.67 (0.61–0.73) vs 0.60 (0.57–0.64)	0.042*
	GFAP vs NSE	0.88 (0.85–0.91) vs 0.75 (0.72–0.79)	<0.001***	0.64 (0.60–0.71) vs 0.60 (0.57–0.64)	0.164
	GFAP + UCH-L1 vs NSE	0.90 (0.88–0.92) vs 0.75 (0.72–0.79)	<0.001***	0.72 (0.67–0.77) vs 0.60 (0.57–0.64)	<0.001***
48 h	UCH-L1 vs NSE	0.87 (0.84–0.90) vs 0.84 (0.81–0.88)	0.028*	0.75 (0.70–0.80) vs 0.75 (0.71–0.80)	0.757
	GFAP vs NSE	0.88 (0.86–0.91) vs 0.84 (0.81–0.88)	0.028*	0.67 (0.62–0.72) vs 0.75 (0.71–0.80)	0.004**
	GFAP + UCH-L1 vs NSE	0.91 (0.88–0.93) vs 0.84 (0.81–0.88)	<0.001***	0.75 (0.70–0.80) vs 0.75 (0.71–0.80)	0.884
72 h	UCH-L1 vs NSE	0.86 (0.83–0.89) vs 0.85 (0.81–0.88)	0.338	0.70 (0.63–0.76) vs 0.75 (0.69–0.80)	0.193
	GFAP vs NSE	0.89 (0.86–0.92) vs 0.85 (0.81–0.88)	0.024*	0.67 (0.62–0.74) vs 0.75 (0.69–0.80)	0.050
	GFAP + UCH-L1 vs NSE	0.91 (0.88–0.93) vs 0.85 (0.81–0.88)	<0.001***	0.72 (0.66–0.79) vs 0.75 (0.69–0.80)	0.539

Comparison of the prognostic accuracies for the prediction of poor neurological outcome (CPC 3–5) at 6-month post cardiac arrest for the total Area Under the Receiver Operating Characteristics curve (Total AUROC) with specificities from 100–0% and for the partial AUROC at high specificities ≥95%. AUROC of UCH-L1, GFAP and the combination of GFAP and UCH-L1, respectively, were compared with AUROC of NSE at 24, 48 and 72 h after cardiac arrest. 95% CI = 95% confidence interval. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.

Table 2 – Serum GFAP specificity, cut-off levels and sensitivity.

GFAP	Specificity (95% CI)[a]	Cutoff Level (pg/mL)[b]	Sensitivity (95% CI)[a]	Patients no.
24 h	1.00 (0.99–1.00)	3425	0.17 (0.13–0.21)	689
	0.99 (0.97–1.00)	677	0.23 (0.19–0.28)	689
	0.98 (0.96–0.99)	256	0.29 (0.25–0.34)	689
	0.97 (0.94–0.98)	232	0.31 (0.26–0.36)	689
	0.96 (0.93–0.98)	152	0.40 (0.35–0.45)	689
	0.95 (0.92–0.97)	117	0.48 (0.43–0.53)	689
48 h	1.00 (0.99–1.00)	2952	0.19 (0.15–0.24)	654
	0.99 (0.97–1.00)	849	0.25 (0.20–0.30)	654
	0.98 (0.96–0.99)	227	0.36 (0.31–0.41)	654
	0.97 (0.95–0.98)	187	0.40 (0.35–0.45)	654
	0.96 (0.93–0.97)	153	0.48 (0.43–0.54)	654
	0.95 (0.92–0.97)	142	0.50 (0.45–0.55)	654
72 h	1.00 (0.99–1.00)	3581	0.12 (0.09–0.17)	598
	0.99 (0.97–1.00)	1238	0.21 (0.16–0.26)	598
	0.98 (0.95–0.99)	208	0.38 (0.33–0.44)	598
	0.97 (0.94–0.98)	165	0.41 (0.36–0.47)	598
	0.96 (0.93–0.98)	131	0.51 (0.45–0.56)	598
	0.95 (0.92–0.97)	118	0.52 (0.47–0.58)	598

[a] Sensitivity and specificity for serum GFAP levels, measured at 24, 48 or 72 h after cardiac arrest to separate poor outcome (CPC 3–5) from good outcome (CPC 1–2) at 6-month follow-up.
[b] Cut-off levels were identified at specificities of ≥95%. GFAP = glial fibrillary acidic protein, h = hour, pg = picogram, mL = millilitre, CI = confidence interval, no. = number.

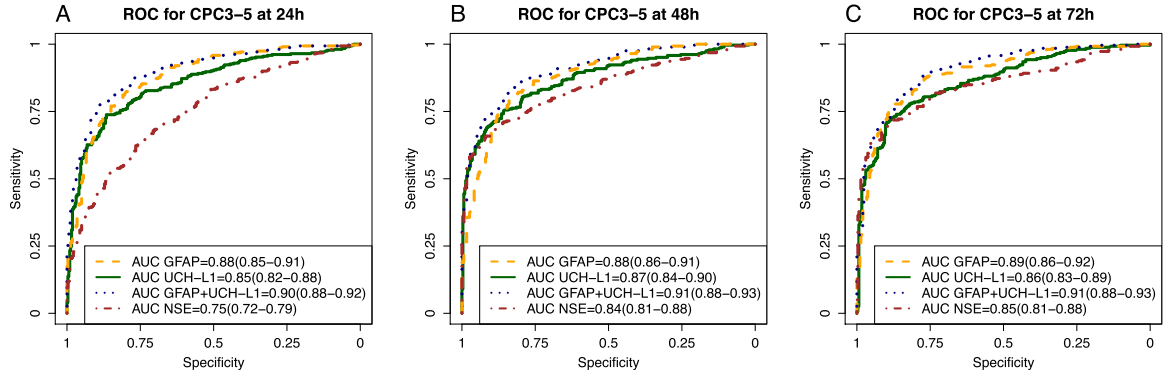

Fig. 2 – (A–C) Receiver-operating characteristic (ROC) analyses for prediction of Cerebral Performance Category Scale (CPC) 1–2 vs. CPC3-5 at 6-months follow-up for serum samples collected at 24, 48 and 72 h. Area under the ROC curve (AUROC) for UCH-L1 (green curve) was significantly greater than AUROC NSE (brown curve) at 24 h ($P < 0.001$) and 48 h ($P = 0.03$), but not at 72 h ($P = 0.34$). AUROC for GFAP (yellow curve) was significantly greater than AUROC NSE at all time-points ($P < 0.001$, $P = 0.03$, $P = 0.02$). AUROC for GFAP + UCH-L1 (blue curve) was significantly greater than AUROC NSE at all time-points ($P < 0.001$). These tests were done on patients for whom data were available for all markers (24 h, $n = 633$; 48 h, $n = 597$; 72 h, $n = 558$). GFAP, glial fibrillary acidic protein; UCH-L1, ubiquitin c-terminal hydrolase-L1, NSE Neuron-specific enolase. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

the partial AUROC of the serum biomarkers at specificities ≥95% and found that UCH-L1 and GFAP + UCH-L1 continued to be significantly more accurate than NSE at 24 h but not at 48 and 72 h. This was also reflected in the sensitivities of GFAP, UCH-L1 and GFAP + UCH-L1 at specificities ≥95% which were higher at 24 h than the corresponding sensitivities of NSE, but at later time-points, only the GFAP + UCH-L1 model yielded results similar to NSE, whereas GFAP and UCHL-1 separately performed worse. Compared over all measuring time-points, the partial AUROC of the GFAP + UCH-L1 model showed less variation than the partial AUROC of NSE and adding GFAP + UCH-L1 to a model with clinical and bedside neurological information improved diagnostic accuracy significantly.

The target temperature level did not affect UCH-L1, but GFAP values at 48 and 72 h were significantly lower in the 36 °C group compared to the 33 °C group, which might be due to decreased elimination of GFAP-sized molecules through hepatic metabolism in patients with lower body temperature.[14,34] Hemolysis had no influence on UCH-L1 serum levels, but GFAP levels were lower in samples with haemolysis at 72 h. However, removing all samples with haemolysis did not influence prognostic accuracies significantly.

Table 3 – Serum UCH-L1 specificity, cut-off levels and sensitivity.

UCH-L1	Specificity (95% CI)[a]	Cutoff Level (pg/mL)[b]	Sensitivity (95% CI)[a]	Patients no.
24 h	1.00 (0.99–1.00)	12175	0.04 (0.02–0.07)	693
	0.99 (0.97–1.00)	3113	0.24 (0.20–0.29)	693
	0.98 (0.96–0.99)	1698	0.38 (0.33–0.43)	693
	0.97 (0.94–0.98)	1476	0.42 (0.37–0.47)	693
	0.96 (0.93–0.98)	1274	0.46 (0.40–0.51)	693
	0.95 (0.92–0.97)	994	0.56 (0.50–0.61)	693
48 h	1.00 (0.99–1.00)	7945	0.09 (0.06–0.12)	663
	0.99 (0.97–1.00)	1509	0.46 (0.41–0.52)	663
	0.98 (0.96–0.99)	1137	0.51 (0.46–0.56)	663
	0.97 (0.94–0.98)	972	0.55 (0.50–0.60)	663
	0.96 (0.93–0.98)	824	0.58 (0.52–0.63)	663
	0.95 (0.92–0.97)	739	0.61 (0.56–0.66)	663
72 h	1.00 (0.99–1.00)	9170	0.01 (0.00–0.03)	609
	0.99 (0.97–1.00)	1255	0.31 (0.26–0.37)	609
	0.98 (0.96–0.99)	839	0.46 (0.40–0.52)	609
	0.97 (0.94–0.98)	661	0.51 (0.45–0.57)	609
	0.96 (0.93–0.97)	613	0.53 (0.47–0.58)	609
	0.95 (0.92–0.97)	590	0.54 (0.48–0.59)	609

[a] Sensitivity and specificity for serum UCH-L1 levels, measured at 24, 48 or 72 h after cardiac arrest to separate poor outcome (CPC 3–5) from good outcome (CPC 1–2) at 6-month follow-up.
[b] Cut-off levels were identified at specificities ≥95%. UCH-L1 = ubiquitin carboxy-terminal hydrolase L1, h = hour, pg = picogram, mL = millilitre, CI = confidence interval, no. = number.

Table 4 – Sensitivities for ≥95% specificity of NSE, UCH-L1, GFAP and GFAP + UCH-L1.

Time-point[a]	Specificity[b]	Sensitivity[c] NSE (95% CI)	Sensitivity[c] UCH-L1 (95% CI)	Sensitivity[c] GFAP (95% CI)	Sensitivity[c] GFAP + UCH-L1
24 h	1.00	0.09 (0.06–0.12)	0.04 (0.02–0.07)	0.17 (0.13–0.21)	0.24 (0.18–0.37)
	0.99	0.15 (0.11–0.19)	0.24 (0.20–0.29)	0.23 (0.19–0.28)	0.35 (0.24–0.50)
	0.98	0.21 (0.16–0.25)	0.38 (0.33–0.43)	0.29 (0.25–0.34)	0.44 (0.32–0.57)
	0.97	0.24 (0.19–0.28)	0.42 (0.37–0.47)	0.31 (0.26–0.36)	0.50 (0.37–0.62)
	0.96	0.29 (0.24–0.34)	0.46 (0.40–0.51)	0.40 (0.35–0.45)	0.55 (0.43–0.65)
	0.95	0.33 (0.28–0.38)	0.56 (0.50–0.61)	0.48 (0.43–0.53)	0.59 (0.49–0.71)
48 h	1.00	0.27 (0.22–0.32)	0.09 (0.06–0.12)	0.19 (0.15–0.24)	0.31 (0.26–0.44)
	0.99	0.47 (0.42–0.53)	0.46 (0.41–0.52)	0.25 (0.20–0.30)	0.41 (0.29–0.58)
	0.98	0.58 (0.52–0.64)	0.51 (0.46–0.56)	0.36 (0.31–0.41)	0.51 (0.37–0.64)
	0.97	0.59 (0.53–0.64)	0.55 (0.50–0.60)	0.40 (0.35–0.45)	0.59 (0.43–0.69)
	0.96	0.60 (0.55–0.66)	0.58 (0.52–0.63)	0.48 (0.43–0.54)	0.62 (0.50–0.72)
	0.95	0.61 (0.55–0.67)	0.61 (0.56–0.66)	0.50 (0.45–0.55)	0.65 (0.56–0.74)
72 h	1.00	0.52 (0.46–0.58)	0.01 (0.00–0.03)	0.12 (0.09–0.17)	0.23 (0.17–0.34)
	0.99	0.54 (0.48–0.60)	0.31 (0.26–0.37)	0.21 (0.16–0.26)	0.30 (0.20–0.48)
	0.98	0.58 (0.52–0.64)	0.46 (0.40–0.52)	0.38 (0.33–0.44)	0.35 (0.26–0.62)
	0.97	0.63 (0.57–0.68)	0.51 (0.45–0.57)	0.41 (0.36–0.47)	0.56 (0.29–0.67)
	0.96	0.62 (0.56–0.68)	0.53 (0.47–0.58)	0.51 (0.45–0.56)	0.60 (0.38–0.69)
	0.95	0.63 (0.57–0.69)	0.54 (0.48–0.59)	0.52 (0.47–0.58)	0.64 (0.54–0.72)

[a] Biomarker sampling time-point after cardiac arrest.
[b] Specificities ≥95%.
[c] Sensitivities with 95% CI of NSE, UCH-L1, GFAP and GFAP + UCH-1 at the 24, 48 and 72 h sampling time-points. 95% CI's for GFAP, UCH-L1 and NSE were calculated by Wilson score method, for GFAP + UCH-L1 a bootstrapping procedure was employed. The number of patients differ between the serum biomarkers and the sampling time-points, at 24 h (NSE $n = 650$, UCH-L1 $n = 693$, GFAP $n = 689$, GFAP + UCH-L1 $n = 685$) at 48 h (NSE $n = 617$, UCH-L1 $n = 663$, GFAP $n = 654$, GFAP + UCH-L1 $n = 650$) at 72 h (NSE $n = 578$, UCH-L1 $n = 609$, GFAP $n = 598$, GFAP + UCH-L1 $n = 595$). The sensitivities of NSE in the TTM-trial were previously published by Stammet et al.[10] h = hours.

UCH-L1 is in the context of adult cardiac arrest a novel serum biomarker, while GFAP has been investigated previously.[12,16,17] However, both have been extensively investigated as outcome predictors in TBI patients.[35–37] After TBI, serum levels are indicative of injury severity and similar to the results of this study, both serum biomarkers have been shown to reliably differentiate good from poor outcome.[36] Also in line with our analyses, sensitivity and specificity increased when GFAP and UCH-L1 were used in combination, however, this effect was most prominent when distinguishing TBI patients from healthy controls.[38] A test employing both serum biomarkers has been validated and approved for clinical use in 2018 as an aid in the assessment of conscious TBI patients.[24]

Although not unique to GFAP and UCH-L1, the release of serum biomarkers of brain injury due a different aetiology than hypoxic-ischaemic, e.g., a recent TBI, has to be regarded as a potential source of error after cardiac arrest as well as erroneous sampling technique or poor timing.

In a previous analysis, investigating the same cohort as in the present study, NFL measured 24, 48 and 72 h after cardiac arrest has shown 69−73% sensitivity at 98% specificity, which is higher than any other biomarker investigated so far or in this study.[8] However, in contrast to GFAP, UCH-L1 and NSE, the NFL analysis was performed with a novel ultrasensitive assay not widely available, and without current use in clinical practice.[8,39]

In summary, our findings indicate that the combination of GFAP and UCH-L1 provides a more accurate overall prediction of neurological outcome after cardiac arrest than each biomarker separately and NSE. At specificities ≥95%, the GFAP + UCH-L1 model performed superior to NSE at 24 h and showed little variation over time which may aid early prognostication and may increase safety with single measurements. Adding GFAP + UCH-L1 to clinical and bedside information increased diagnostic accuracy. Haemolysis did not interfere with prediction of outcome. However, due to the potential sources of error and the lack of high sensitivity at specificity ≥95%, none of the investigated serum biomarkers in this study, alone or in combination, can be considered being sufficiently robust to be used outside a multimodal prognostication model for the prediction of poor outcome after cardiac arrest if introduced into clinical practice.

There are several limitations to this study. Sampling commenced 24 h after cardiac arrest and stopped at 72 h, therefore, we cannot make statements concerning the predictive value of the investigated serum biomarkers prior or after these time-points. Missing data was not compensated for and the populations compared with each other at the different time-points differ in size, mainly due to patients dying between 24 and 72 h post cardiac arrest, which may increase the risk of bias. The lack of defined cut-off values and standardized assays in previous analyses, make comparison of our results with the existing studies difficult. Furthermore, before a possible introduction into clinical practice, the results of our analyses and possible cut-off values for the biomarker models need to be validated in a different cohort of cardiac arrest patients. The main strengths of our study are a large sample size, few missing patients and the prospective, blinded multicentre design and the strict prognostication protocol of the TTM-trial. Moreover, GFAP, UCH-L1 and NSE were sampled at the same time-points in the same cohort of patients, reducing the influence of external factors and increasing internal validity. All samples were analyzed after completion of the TTM-trial, excluding a direct WLST bias due to access to serum biomarker information, leaving, however, a risk for an indirect bias based on other pathological findings co-varying with elevated serum biomarker levels. GFAP and UCH-L1 were analyzed using a well-established method and a commercially available kit with defined calibration standards, which may facilitate validation of our results in future studies.

Conclusion

GFAP and UCH-L1 separately and in combination accurately predicted poor neurological outcome after cardiac arrest. Compared to NSE, the combined GFAP + UCH-L1 model showed higher sensitivities at specificities ≥95% at 24 h after cardiac arrest. GFAP and UCH-L1 may be useful to inform neurological prognosis after cardiac arrest.

Authors' contribution

Drs. Ebner, Moseby-Knappe and Nielsen had full access to all of the data in the study, and take responsibility for the integrity of the data and the accuracy of the data analysis. Drs. Ebner, Moseby-Knappe, Cronberg, Undén, Mattsson, Nielsen were responsible for the concept and design of the study. Drs. Ebner, Moseby-Knappe, Cronberg, Undén, Nielsen wrote the first draft of the manuscript. Drs. Moseby-Knappe and Ullén performed the statistical analyses. All authors critically revised, contributed intellectual content and approved the final version of the manuscript.

Conflict of interest

Dr. Friberg is scientific advisor at QuickCool. No other disclosures were reported.

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Biomarker analysis: Banyan Biomarkers provided the GFAP and UCH-L1 analyses free of charge.

Role of the funder/sponsor: The funding organizations or Banyan Biomarkers had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional information: Samples used in this study were stored and processed at the Integrated BioBank of Luxembourg in compliance with ISO 9001:2008, NF S96-900:2011, and ISO 17025: 2005 standards and International Society for Biological and Environmental Repositories Best Practices.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.resuscitation.2020.05.016.

REFERENCES

1. Grasner JT, Wnent J, Herlitz J, et al. Survival after out-of-hospital cardiac arrest in Europe – results of the EuReCa TWO study. Resuscitation 2020.
2. Benjamin EJ, Muntner P, Alonso A, et al. Heart disease and stroke statistics – 2019 update: a report from the American Heart Association. Circulation 2019;139:e56–e528.
3. Beck B, Bray J, Cameron P, et al. Regional variation in the characteristics, incidence and outcomes of out-of-hospital cardiac arrest in Australia and New Zealand: results from the Aus-ROC Epistry. Resuscitation 2018;126:49–57.
4. Sekhon MS, Ainslie PN, Griesdale DE. Clinical pathophysiology of hypoxic ischemic brain injury after cardiac arrest: a "two-hit" model. Crit Care 2017;21:90.
5. Cronberg T, Kuiper M. Withdrawal of life-sustaining therapy after cardiac arrest. Semin Neurol 2017;37:81–7.
6. Nolan JP, Soar J, Cariou A, et al. European Resuscitation Council and European Society of Intensive Care Medicine Guidelines for Post-resuscitation Care 2015: Section 5 of the European Resuscitation Council Guidelines for Resuscitation 2015. Resuscitation 2015;95:202–22.
7. Mattsson N, Zetterberg H, Nielsen N, et al. Serum tau and neurological outcome in cardiac arrest. Ann Neurol 2017;82:665–75.
8. Moseby-Knappe M, Mattsson N, Nielsen N, et al. Serum neurofilament light chain for prognosis of outcome after cardiac arrest. JAMA Neurol 2019;76:64–71.
9. Stammet P, Dankiewicz J, Nielsen N, et al. Protein S100 as outcome predictor after out-of-hospital cardiac arrest and targeted temperature management at 33 degrees C and 36 degrees C. Crit Care 2017;21:153.
10. Stammet P, Collignon O, Hassager C, et al. Neuron-specific enolase as a predictor of death or poor neurological outcome after out-of-hospital cardiac arrest and targeted temperature management at 33 degrees c and 36 degrees c. J Am Coll Cardiol 2015;65:2104–14.
11. Callaway CW, Donnino MW, Fink EL, et al. Part 8: post-cardiac arrest care: 2015 American Heart Association guidelines update for cardiopulmonary resuscitation and emergency cardiovascular care. Circulation 2015;132:S465–82.
12. Kaneko T, Kasaoka S, Miyauchi T, et al. Serum glial fibrillary acidic protein as a predictive biomarker of neurological outcome after cardiac arrest. Resuscitation 2009;80:790–4.
13. Zoltewicz JS, Scharf D, Yang B, Chawla A, Newsom KJ, Fang L. Characterization of antibodies that detect human GFAP after traumatic brain injury. Biomark Insights 2012;7:71–9.
14. Thelin EP, Zeiler FA, Ercole A, et al. Serial sampling of serum protein biomarkers for monitoring human traumatic brain injury dynamics: a systematic review. Front Neurol 2017;8:300.
15. Pelinka LE, Kroepfl A, Schmidhammer R, et al. Glial fibrillary acidic protein in serum after traumatic brain injury and multiple trauma. J Trauma 2004;57:1006–12.
16. Larsson IM, Wallin E, Kristofferzon ML, Niessner M, Zetterberg H, Rubertsson S. Post-cardiac arrest serum levels of glial fibrillary acidic protein for predicting neurological outcome. Resuscitation 2014;85:1654–61.
17. Helwig K, Seeger F, Holschermann H, et al. Elevated serum glial fibrillary acidic protein (GFAP) is associated with poor functional outcome after cardiopulmonary resuscitation. Neurocrit Care 2017;27:68–74.
18. Huang L, Nakamura Y, Lo EH, Hayakawa K. Astrocyte signaling in the neurovascular unit after central nervous system injury. Int J Mol Sci 2019;20.
19. Andersson FI, Jackson SE, Hsu ST. Backbone assignments of the 26 kDa neuron-specific ubiquitin carboxyl-terminal hydrolase L1 (UCH-L1). Biomol NMR Assign 2010;4:41–3.
20. Day IN, Thompson RJ. UCHL1 (PGP 9.5): neuronal biomarker and ubiquitin system protein. Prog Neurobiol 2010;90:327–62.
21. Bishop P, Rocca D, Henley JM. Ubiquitin C-terminal hydrolase L1 (UCH-L1): structure, distribution and roles in brain function and dysfunction. Biochem J 2016;473:2453–62.
22. Liu H, Povysheva N, Rose ME, et al. Role of UCHL1 in axonal injury and functional recovery after cerebral ischemia. Proc Natl Acad Sci USA 2019.
23. Liu MC, Akinyi L, Scharf D, et al. Ubiquitin C-terminal hydrolase-L1 as a biomarker for ischemic and traumatic brain injury in rats. Eur J Neurosci 2010;31:722–32.
24. Bazarian JJ, Biberthaler P, Welch RD, et al. Serum GFAP and UCH-L1 for prediction of absence of intracranial injuries on head CT (ALERT-TBI): a multicentre observational study. Lancet Neurol 2018;17:782–9.
25. Nielsen N, Wetterslev J, Cronberg T, et al. Targeted temperature management at 33 degrees C versus 36 degrees C after cardiac arrest. N Engl J Med 2013;369:2197–206.
26. Perkins GD, Jacobs IG, Nadkarni VM, et al. Cardiac arrest and cardiopulmonary resuscitation outcome reports: update of the Utstein Resuscitation Registry Templates for Out-of-Hospital Cardiac Arrest: a statement for healthcare professionals from a task force of the International Liaison Committee on Resuscitation (American Heart Association, European Resuscitation Council, Australian and New Zealand Council on Resuscitation, Heart and Stroke Foundation of Canada, InterAmerican Heart Foundation, Resuscitation Council of Southern Africa, Resuscitation Council of Asia); and the American Heart Association Emergency Cardiovascular Care Committee and the Council on Cardiopulmonary, Critical Care, Perioperative and Resuscitation. Circulation 2015;132:1286–300.
27. Bossuyt PM, Reitsma JB, Bruns DE, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. BMJ 2015;351:h5527.
28. Petrova DT, Cocisiu GA, Eberle C, et al. Can the Roche hemolysis index be used for automated determination of cell-free hemoglobin? A comparison to photometric assays. Clin Biochem 2013;46:1298–301.
29. Abramson NS, Safar P, Detre KM, et al. Neurologic recovery after cardiac arrest: effect of duration of ischemia. Brain Resuscitation Clinical Trial I Study Group. Crit Care Med 1985;13:930–1.
30. Jennett B, Bond M. Assessment of outcome after severe brain damage. Lancet 1975;1:480–4.
31. Phelps R, Dumas F, Maynard C, Silver J, Rea T. Cerebral Performance Category and long-term prognosis following out-of-hospital cardiac arrest. Crit Care Med 2013;41:1252–7.
32. Dragancea I, Horn J, Kuiper M, et al. Neurological prognostication after cardiac arrest and targeted temperature management 33 °C versus 36 °C: results from a randomised controlled clinical trial. Resuscitation 2015;93:164–70.
33. Team RC. R: A language and environment for statistical computing. Austria: R Foundation for Statistical Computing Vienna; 2017 https://wwwR-projectorg/.
34. Zhou J, Poloyac SM. The effect of therapeutic hypothermia on drug metabolism and response: cellular mechanisms to organ function. Expert Opin Drug Metab Toxicol 2011;7:803–16.
35. Takala RS, Posti JP, Runtti H, et al. Glial fibrillary acidic protein and ubiquitin C-terminal hydrolase-L1 as outcome predictors in traumatic brain injury. World Neurosurg 2016;87:8–20.
36. Wang KK, Yang Z, Zhu T, et al. An update on diagnostic and prognostic biomarkers for traumatic brain injury. Expert Rev Mol Diagn 2018;18:165–80.
37. Shemilt M, Boutin A, Lauzier F, et al. Prognostic value of glial fibrillary acidic protein in patients with moderate and severe traumatic brain injury: a systematic review and meta-analysis. Crit Care Med 2019;47:e522–9.
38. Diaz-Arrastia R, Wang KK, Papa L, et al. Acute biomarkers of traumatic brain injury: relationship between plasma levels of ubiquitin C-terminal hydrolase-L1 and glial fibrillary acidic protein. J Neurotrauma 2014;31:19–25.
39. Wilson DH, Rissin DM, Kan CW, et al. The Simoa HD-1 Analyzer: a novel fully automated digital immunoassay analyzer with single-molecule sensitivity and multiplexing. J Lab Autom 2016;21:533–47.