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Clinical paper

Biomarker prognostication of cognitive impairment may be feasible even in out-of hospital cardical arrest survivors with good neurological outcome

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Abstract

Background: Patients surviving out-of hospital cardicac arrest, with good neurological outcome according to Cerebral Performance Category, frequently have neuropsychological impairment. We studied whether biomarker data (S-100b and neuron-specific enolase) obtained during the ICU stay predicted cognitive impairment 6 months after resuscitation.

Methods: Patients (N = 79) with a CPC-score $\leq$2 were recruited from two trial sites taking part in the TTH48 trial comparing targeted temperature management (TTM) for 48 h vs. 24 h at 33 $\pm$ 1 °C. We assessed patients 6 months after the OHCA. We measured biomarkers S-100b and NSE at arrival and at 24, 48 and 72 h after reaching the target temperature of 33 $\pm$ 1 °C.

Four cognitive domain z-scores were calculated, and global cognitive impairment was defined as $z < -1.67$ on at least 3 out of 13 cognitive tests. Non-parametric correlations were used to assess the relationship between cognitive domain and biomarkers. ROC curves were used to assess prediction of cognitive impairment from the biomarkers. Logistic regression was used to investigate whether TTM duration moderated biomarker prediction of cognitive impairment.

Results: Cognitive impairment was present in 22% of the patients with memory impairment being the most common. The biomarkers correlated significantly with several cognitive domain scores and NSE at 48 h predicted cognitive impairment with 100% sensitivity and 56% specificity. The predictive properties of NSE at 48 h was unaffected by duration of TTM.

Conclusions: Early biomarker prognostication of cognitive impairment is feasible even in OHCA survivors with good neurological outcome as defined by CPC. NSE at 48 h predicted cognitive impairment.

Keywords: Neurological outcome, Out of hospital cardiac arrest, Neuropsychology, Hypothermic treatment

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https://doi.org/10.1016/j.resuscitation.2021.02.025

Introduction

In Europe, most survivors of out-of-hospital cardiac arrest (OHCA) have a good neurological outcome, often expressed as Cerebral Performance Category (CPC) of 1 or 2.[1] Nevertheless, about 50% of these OHCA survivors suffer from cognitive sequela.[2] As cognitive deficits may severely affect functional capacity and render return to work and self-sufficiency impossible,[3] prediction of future cognitive impairment is clinically important as it may help planning the rehabilitation process.

Most studies on neurological prognosis in the early phase after OHCA have employed crude measures of functioning such as the CPC[4] which is problematic regarding sensitivity, as considerable cognitive impairment may be present even in patients with good CPC scores of $<=2$.[5] Even though formal neuropsychological tests should ideally be employed in order to measure cognitive outcome after OHCA, this is often not feasible. Hence, there is a need for early predictors of neuropsychological outcome that can be employed regardless of other clinical variables and that are relatively independent from TTM. Two such predictors are the biomarkers S-100b and neuron-specific enolase (NSE.[6] S-100b is a calcium binding protein that is found in Schwann-cells and glial cells and neuron specific enolase (NSE) is found inside neurons and plays a role in axonal transport.[6] Survival as well as superficially and subjectively evaluated neurological outcome can be predicted from S-100b and NSE in patients undergoing TTM.[7,8] However, these studies on NSE and S100b using CPC as outcome mainly demonstrated prediction of survival, as very few surviving patients had a CPC score >2. Further, many patients with CPC $\leq$2 may suffer from significant cognitive sequelae that are detected when sensitive neuropsychological tests are used.[5,9]

Thus, we analyzed S-100b and NSE regarding prediction of cognitive impairment, using neuropsychological data from a sub-study of the RCT "TTH48" comparing prolonged targeted temperature management (TTM) for 48 h vs. standard 24 h at 33 ± 1 °C, and where we found that prolonged TTM was associated with improved memor.[9]

The research questions were whether cognitive impairment in OHCA survivors six months after resuscitation can be predicted from NSE and S-100b obtained during their ICU stay and whether length of TTM moderates this prediction. We first explored cognitive impairment prevalence as well as cognitive domain impairment. Finally, we evaluated which cognitive domains were most strongly associated with NSE and S-100b six months after OHCA and whether length of TTM moderated such associations.

Methods

This study is a post hoc follow-up-study based on the TTH48 trial, an investigator-initiated, blinded-outcome-assessor, parallel controlled multicenter trial in which patients with OHCA were randomized to receive TTM at 33 ± 1 °C for either 24 or 48 h.[10,11]

Patients

The sample was patients recruited from the TTH48 trial with a CPC score $\leq$ 2. They consented to a follow-up neuropsychological assessment six months following resuscitation from OHCA. We have previously described the present sample,[9] hence we only briefly summarize the study sampling here.

The TTH48 trial enrolled 355 patients, and 159 patients participated in the sub-study at the intensive care units (ICUs) at Aarhus University Hospital, Denmark and Stavanger University Hospital, Norway. Every unconscious OHCA patient was screened for eligibility at ICU admission. A legal surrogate and a relative gave written informed consent before randomization of the patient to 24 or 48 h TTM. The inclusion criteria were: Age over 17 and below 80 years, OHCA with a presumed cardiac origin, sustained (>20 min) spontaneous circulation after resuscitation, Glasgow Coma Score[12] (GCS) <8 on admission. Detailed inclusion and exclusion criteria can be found in the original study protocol.[13] Included patients were recruited for neuropsychological assessment six months post OHCA. This resulted in the inclusion of 79 patients as described in Table 1.

The study was approved by the Danish Data Protection Agency and the Central Denmark Region Committees on Health Research Ethics (case number 20110022) and the Regional Ethics Committee of Western Norway (ref 2013/1486).

Measures

S-100b and NSE

Blood samples for measuring S-100b and NSE were drawn upon patient admission, 24 h, 48 h and 72 h after the patient reached the target temperature. Details of the lab procedures have been published previously.[8] Measurement ranges were .3−740 μg/l for NSE and .02 −39 μg/l for S-100b.

Neuropsychological variables

Six months after OHCA, tests of memory, attention, and executive functions were administered by trained and assessor-blinded research assistants. The following tests were performed: Rey Auditory-Verbal Learning Test (RAVLT),[12] Rey−Osterreith Complex Figure Test (ROCFT),[13,14] WAIS-IV Digit Span and Vocabulary,[15] Trail Making Test A & B (TMT-A & B),[16,17] and D-KEFS Verbal Fluency.[18] Normative data for the WAIS-IV and D-KEFS subscales were obtained from the test manuals. Normative data for TMT-A & B, RAVLT and ROCFT were obtained from Mitrushina[19] based on regression equations derived by meta-analysis of multiple normative datasets. Thus, cognitive raw-scores were converted to z-scores based on normative data and adjusted for age as estimated from the norms. The cut-off limit for impairment based on the z-scores was set at $z<=-1.67$ as the standardized scaled scores of WAIS-IV and D-KEFS subscales jump in one-third SD increments and this z-value most closely resembles the fifth percentile (4.75%). Since we included 13 different test scores in assessing cognitive impairment, this increases the risk of false positive cases (type 1 error). We assessed the number of observed significant findings and took into account the likelihood of a single score below cut-off occurring by chance, e.g. the probability of one or more significant findings in 13 comparisons (corresponding to $p = .49$), two or more findings ($p = .14$), three or more ($p = .03$), and so forth. Consequently, patients with performance below the cut-off score on three or more cognitive tests were considered cognitively impaired (corresponding to $p < .02$).

Further, in order to investigate impairment in different cognitive domains, we calculated average z-scores in 4 domains and validated the domain scales using Cronbach alpha as a measure of internal consistency: Episodic memory domain (RAVLT learning, recall, recognition and RCFT immediate and delayed recall; $\alpha = .748$), Working memory domain (Digit span forwards, backwards, sequencing and RAVLT trial 1; $\alpha = .765$), Verbal fluency domain (Category

Table 1 – Descriptive statistics.

	TTM24	TTM48	p	Total
N (Male/Female)	36 (34/2)	43 (37/6)	=.280	79 (71/8)
Cognitively impaired (%)	12 (33%)	5 (12%)	=.028	17 (22%)
	Median (IQR)	*Median (IQR)*		*Median (IQR)*
Age	59 (10)	60 (19)	=.672	60.00 (15.00)
ROSC time	22 (15)	17 (12)	=.969	18.50 (13.00)
Glasgow coma scale arrival*	3 (0)	3 (0)	=.113	3 (0)
Glasgow coma svare 64 h**	14 (6)	11 (11)	=.108	14 (9)
Glasgow coma scale ICU***	14.5 (1)	14.0 (1)	=.636	14.0 (1)
S100b arrival (N = 54)	.75 (1.43)	.90 (1.89)	=.332	.88 (1.43)
S100b 24 h (N = 59)	.89 (.78)	.72 (.67)	=.813	.83 (.06)
S100b 48 h (N = 58)	.88 (.13)	.73 (.47)	=.777	.82 (.06)
S100b 72 h (N = 56)	.45 (.65)	.08 (.06)	=.012	.75 (.08)
NSE arrival (N = 54)	17.67 (16.96)	22.22 (23.26)	=.164	20.23 (18.26)
NSE 24 h (N = 59)	8.33 (6.41)	10.51 (6.47)	=.192	9.07 (7.35)
NSE 48 h (N = 57)	9.56 (7.61)	7.96 (8.11)	=.872	9.31 (7.89)
NSE 72 h (N = 55)	7.27 (7.84)	7.59 (5.85)	=.301	7.43 (6.56)

IQR: Interquartile range; ROSC time: Time from cardiac arrest to return of spontaneous circulation.
S100b and NSE units: μg/l.
* Three patients scored >3 (Scores: 4/6/7).

** Measured 64 h after arrival, thus 8 h after end of hypothermia in the TTM48 group.

*** Measured at ICU discharge.

fluency, semantic, phonological, category shift; $\alpha = .840$) and Visuo-motor domain (RCFT copy, TMT A, TMT B; $\alpha = .680$). Impairment in each domain was defined as an average z-score $<= -1.67$.

Statistical methods

S-100b and NSE were severely right-skewed, hence non-parametric statistics were chosen throughout. Continuous data are presented as medians and interquartile ranges and categorical data as counts and percentages.

Categorical data were analyzed using Fisher's exact test, as some of the cell-counts were below 5. Continuous data were analyzed using Mann–Whitney *U* for group comparisons, and Spearman rho non-parametric correlations for assessing relationship between variables.

In order to investigate the predictive values of S-100b and NSE, we performed non-parametric ROC-analyses, reporting areas under the curve as well as optimal cut-off points with regard to sensitivity and specificity, choosing the cut-off with the maximum Youden's index value[20] and additionally showing the range of values from a sensitivity of 100% to a specificity of 100%.

Finally, in order to assess whether duration of TTM affected the predictive properties of S-100b and NSE, sequential logistic regression analyses were performed with the binary variable indicating cognitive impairment as dependent variable. In the first block, the biomarker was entered as predictor and in the second block, the variable indicating TTM condition was entered. Finally, in the third block, the interaction term between TTM condition and the biomarker was entered.

All data were analyzed using SPSS® Version 25 for Windows®.

Results

The sample derived from the TTH48 trial is the same as we have reported previously[9] and details on patient recruitment can be found there. In Table 1, we describe the patients enrolled in the TTM24 vs TTM48 conditions as well as the total sample with complete neuropsychological data. The Consort flow-chart is included as supplementary figure S1. In addition, the number of patients with NSE and/or S100b data at each measurement time is shown in Table 1.

The prevalence of cognitive impairment was higher in the TTM24 group. The difference in NSE 48 h values was not significant according to a Bonferrroni-corrected alpha limit of .005.

Prognostication of cognitive impairment among OHCA survivors

In Fig. 1 we present the s100b values at arrival to the hospital, and at 24, 48 and 72 h after the target temperature of 33+−1 °C was reached. s100b was elevated at admittance, but rapidly declined.

As seen in Table 1, s100b was significantly ($p = .012$) higher in TTM48 than in TTM24 at 72 h, even after applying a Bonferroni-corrected alpha limit of $p < .0125$ (4 comparisons).

In Fig. 2, we show the NSE values at arrival to the hospital, and at 24, 48 and 72 h after the target temperature of 33+−1 °C was reached. NSE was elevated in the impaired group as compared to the non-impaired group, most pronounced in the TTM24 group at 48 h.

In Table 2, we show the non-parametric correlation coefficients between the neuropsychological domain scores and S100b/NSE.

NSE at 48 h covaried with the cognitive domain scores and the effect was strongest for verbal fluency and visuo-motor performance. None of the NSE scores correlated significantly with the memory domain scores, whereas the S100b score at 48 h did.

In Fig. 3, we show ROC-curves for S100B and NSE with regard to cognitive impairment (yes/no) as presented in the groups in Table 1. The results of the ROC-analyses are shown in detail in Table 3.

None of the S100b variables were significant predictors of cognitive impairment. However, NSE at 48 h was a predictor of

cognitive impairment at six months. The optimal cut-off was an NSE value of 8.41, which resulted in a sensitivity of 100% and a specificity of 56%. NSE values of 13.68, 18.87 and 29.31 resulted in sensitivity/specificity of 50/83, 30/97 and 20/100 respectively.

To investigate whether the TTM intervention at 48 vs 24 h moderated the predictive value of NSE at 48 h, we performed a sequential logistic regression analysis with NSE at 48 h as predictor and cognitive impairment as dependent variable in block 1 (Odds ratio: 1.122, p = .019). In block 2, we entered allocation to TTM24 or TTM48 treatment and both NSE48 (Odds ratio: 1.154, p = .014) and treatment condition (Odds ratio: 1.113, p = .012) predicted cognitive impairment. In block 3, the interaction between NSE48 and treatment was non-significant (p = .485). Thus, NSE based prediction at 48 h was independent of length of TTM.

Discussion

NSE at 48 h was a predictor of cognitive impairment six months after out-of-hospital cardiac arrest among patients with seemingly good outcome (CPC $\leq$ 2) and the predictive value was independent of length of TTM. NSE at 48 correlated with verbal fluency measures and visuo-motor measures and NSE at 24 h correlated with verbal fluency measures. Further, S100b at 48 h correlated with memory and verbal fluency and S-100b at arrival also correlated with verbal fluency six months later. Thus, S-100b was also related to cognitive outcome although it was not a predictor of global cognitive impairment.

The cognitive domain most affected by OHCA was memory, as has been found in most other studies,[2] and the present study is consistent

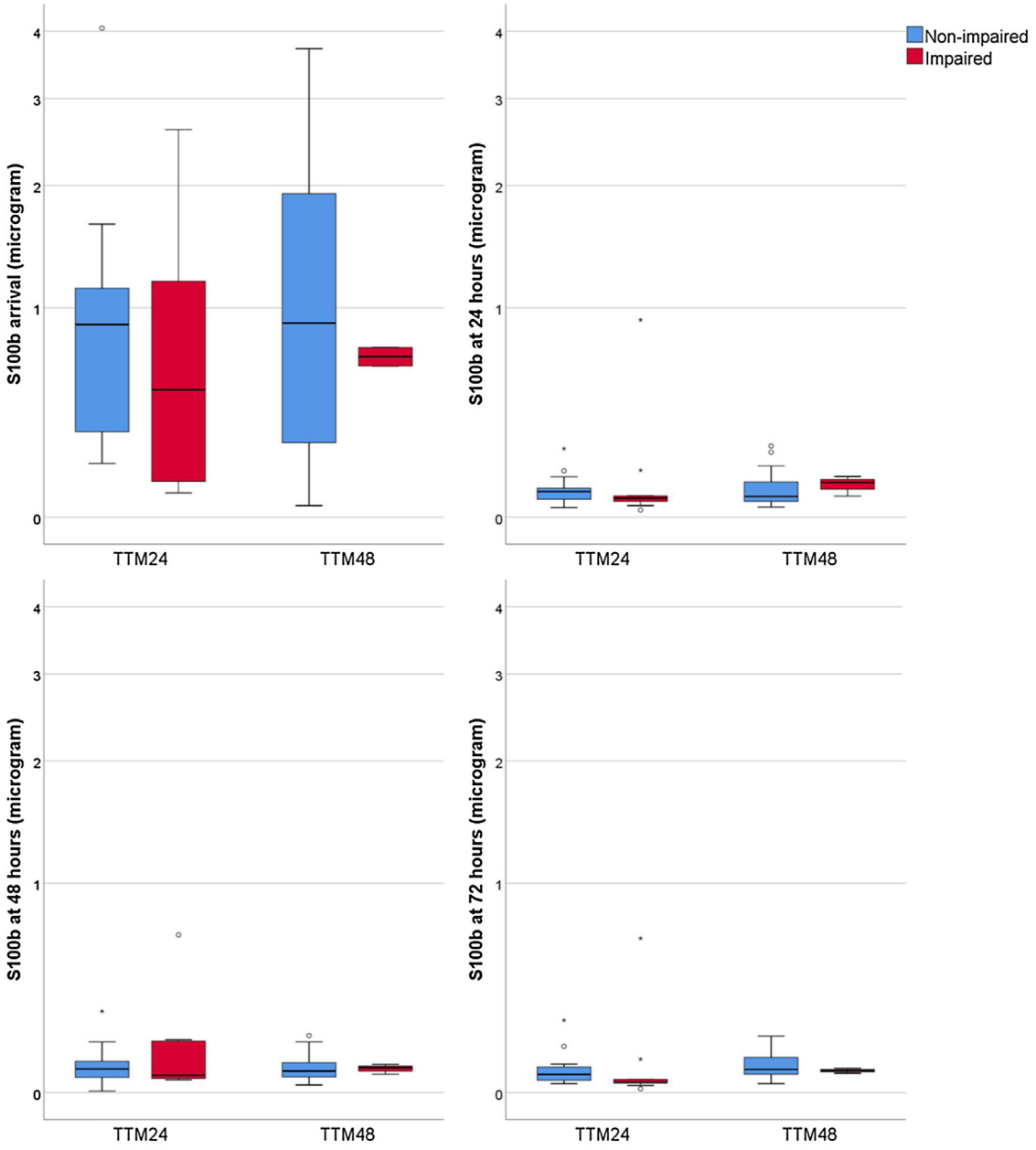

Fig. 1 – Boxplots of s100b values for impaired vs non-impaired subjects at 4 different times.

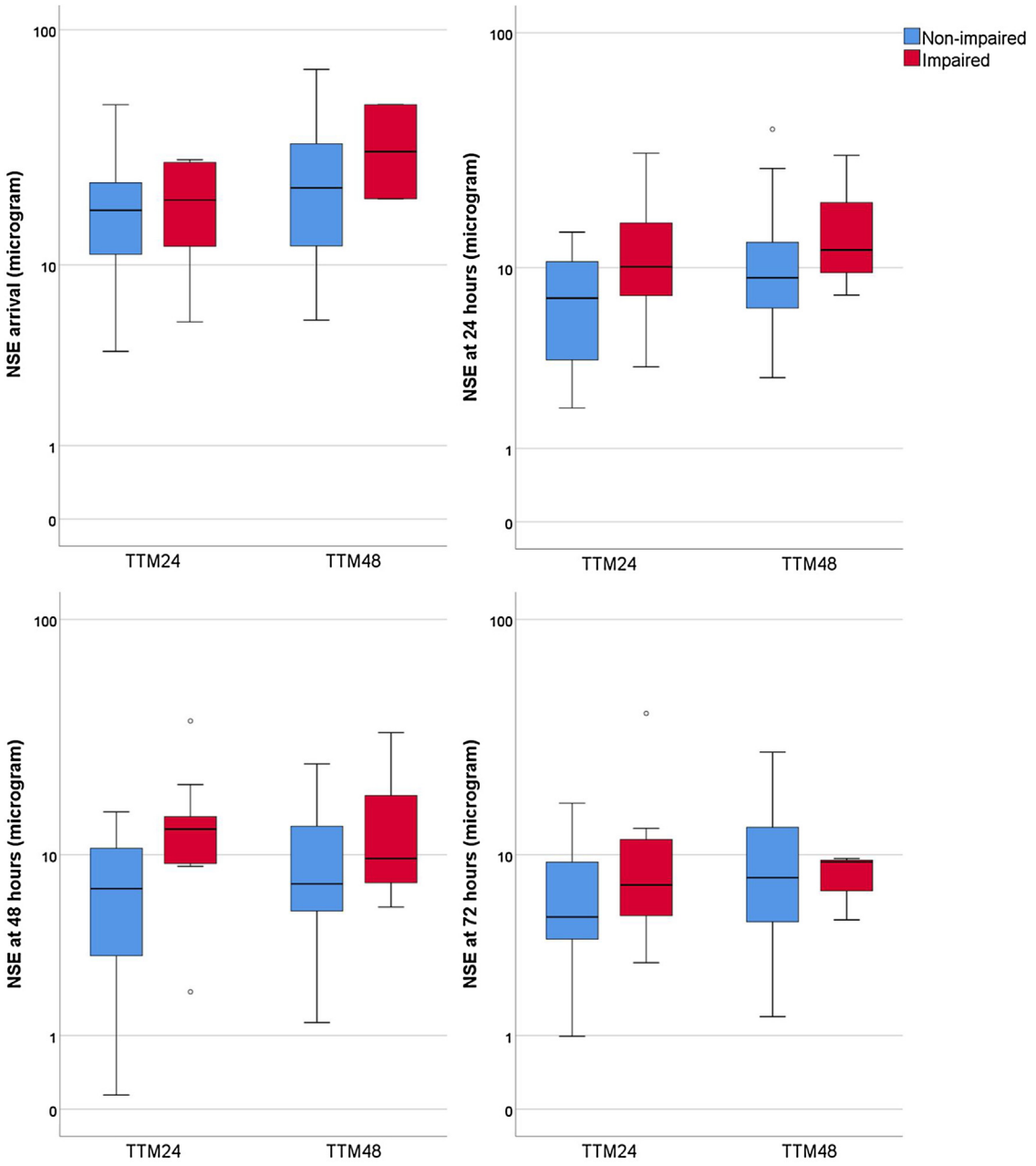

Fig. 2 – Boxplots of NSE values for impaired vs non-impaired subjects at 4 different times.

Table 2 – Correlations between the neuropsychological domain scores and S100b/NSE.

Neuropsychological domain z score		Arrival	24 h	48 h	72 h
Memory domain	**S100b**	.229	−.158	−.276*	.151
	NSE	.164	−.002	−.239	−.126
Verbal fluency domain	**S100b**	−.341*	−.179	−.309*	−.054
	NSE	−.215	−.265*	−.381**	−.107
Visuo-motor domain	**S100b**	.139	.003	.140	.249
	NSE	−.236	−.133	−.349**	−.190
Working memory domain	**S100b**	−.030	−.056	−.053	.194
	NSE	−.233	−.185	−.242	−.101

* $p < .05$.

** $p < .01$.

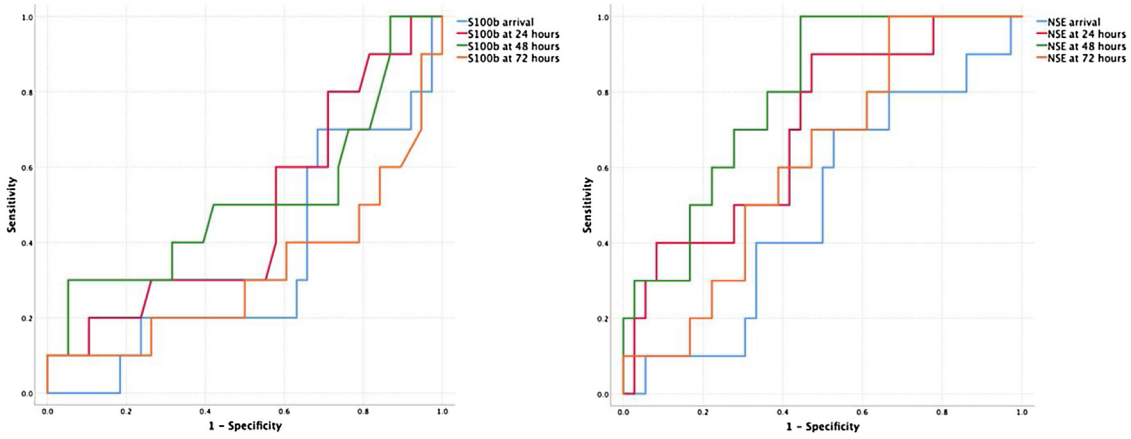

Fig. 3 – ROC-curves for S100B and NSE with cognitive impairment as predicted variable.

Table 3 – Area Under the Curve: S100b/NSE.

Test Result Variable(s)		Area	Std. Error[a]	p[b]	95% Confidence Interval	
					Lower Bound	Upper Bound
Arrival	S100b	.342	.096	.128	.154	.530
	NSE	.494	.099	.958	.300	.689
24 h	S100b	.478	.105	.829	.273	.683
	NSE	.700	.090	.055	.524	.876
48 h	S100b	.513	.116	.899	.285	.741
	NSE	.789	.070	.006	.652	.926
72 h	S100b	.318	.108	.080	.108	.529
	NSE	.619	.089	.252	.445	.794

The test result variable(s): S100b at 24 h, S100b at 48 h, S100b at 72 h has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.

[a] Under the nonparametric assumption.

[b] Null hypothesis: true area = .5.

with earlier research which has shown memory deficits in patients with CPC< = 2.[5]

The optimal cut-off in the present study was 8.41 μg/L for NSE at 48 h, with a sensitivity of 100% and a specificity of 56%. This is a lower cut-off than the cut-off found by Choi et al.[7] and Duez et al.[8] These previously identified cut-offs are quite different in that they are based on all admitted patients of which for instance only 2 patients with "poor" outcome survived in the Duez et al. study. Thus, the high cut-off is primarily a predictor of survival, not a prognostic factor among those who survived. The low cut-off on the present study should however be interpreted as a within-survivor predictor of cognitive outcome and future studies should explore and validate the cut-offs.

The main limitations in the present study are related to the fact that this was a post-hoc study based on the TTH48 trial which focused on the effects of prolonged TTM. Thus, the present sample is highly selected with a good neurological outcome, defined as CPC ≤ 2 and willingness to come to the test. This leads to restriction of range and weakens the predictive properties of the biomarkers with regard to cognitive outcome. Further, as mentioned above, we did not have information concerning premorbid cognitive functioning and this may have attenuated the prediction properties of the biomarkers. Finally, our sample was small, also limiting the generalizability of the study. Thus, these results should be viewed as tentative and exploratory and future studies should be done to validate the findings.

This is the first study which has demonstrated the prognostic properties of S-100b and NSE in OHCA survivors with CPC ≤ 2 using a comprehensive neuropsychological battery and which also have showed that length of TTM did not affect these prognostic properties. Thus, the study is an important first step, both clinically and theoretically, in establishing prognostication of cognitive impairments among OHCA survivors.

Future studies should replicate these results and establish two different cut-off scores for NSE and S100B for clinically meaningful prognostication. A high cut-off score for prognostication of survival and a lower for prognostication of intact cognitive outcome. Patients with biomarker levels between those two cutoff levels would most likely survive with cognitive impairment and hence have need for special attention and support.

Conclusions

Biomarker prognostication of cognitive impairment may to some extent be feasible among OHCA survivors with good outcome as defined by CPC ≤ 2. Many of these patients will suffer from cognitive impairment. NSE at 48 h predicted overall cognitive impairment with an optimal cut-off at 8.41 μg/L resulting in sensitivity of 100% and specificity of 56%.

Conflicts of interest

None.

CRediT authorship contribution statement

Kolbjørn Brønnick: Conceptualization, Methodology, Data curation, Writing - original draft, Writing - review & editing. **Lars Evald:** Methodology, Data curation, Writing - review & editing. **Christophe Henri Valdemar Duez:** Methodology, Data curation, Writing - review & editing. **Anders Morten Grejs:** Methodology, Writing - review & editing. **Anni Nørgaard Jeppesen:** Methodology, Writing - review & editing. **Hans Kirkegaard:** Conceptualization, Methodology, Writing - review & editing. **Jørgen Feldbæk Nielsen:** Conceptualization, Methodology, Writing - review & editing. **Eldar Søreide:** Conceptualization, Methodology, Writing - review & editing.

Acknowledgements

We wish to thank the research assistants at the Hammel Neurorehabilitation and Research Centre and the Department of Psychiatry at the Stavanger University Hospital.

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.2021.02.025.

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